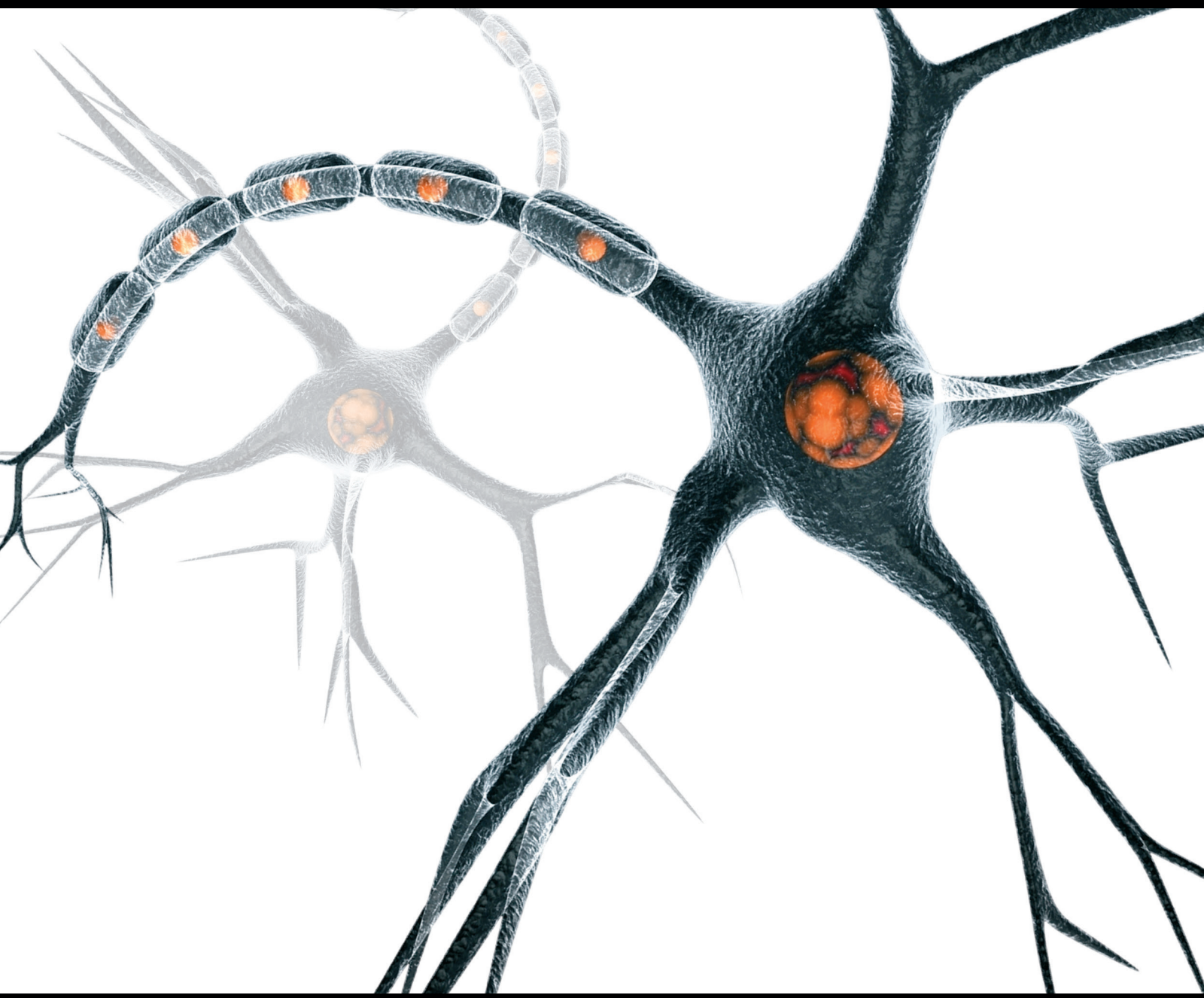
Neural Plasticity

The Many Faces of Stress: Implications for Neuropsychiatric Disorders

Guest Editors: Laura Musazzi and Jordan Marrocco



The Many Faces of Stress: Implications for Neuropsychiatric Disorders

Neural Plasticity

The Many Faces of Stress: Implications for Neuropsychiatric Disorders

Guest Editors: Laura Musazzi and Jordan Marrocco

Editorial Board

Contents

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 8389737, 2 pages
http://dx.doi.org/10.1155/2016/8389737

Editorial

The Many Faces of Stress: Implications for Neuropsychiatric Disorders

Laura Musazzi[1] and Jordan Marrocco[2]

[1]*Laboratorio di Neuropsicofarmacologia e Neurogenomica Funzionale, Dipartimento di Scienze Farmacologiche e Biomolecolari and CEND, Università degli Studi di Milano, 20133 Milano, Italy*
[2]*Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10065, USA*

Correspondence should be addressed to Laura Musazzi; laura.musazzi@unimi.it
and Jordan Marrocco; jordan.marrocco@rockefeller.edu

Received 23 February 2016; Accepted 23 February 2016

Environmental stress is widely recognized as one of the main risk factors in neuropsychiatric diseases, including mood and anxiety disorders. Stress, with the ensuing activation of the hypothalamic-pituitary-adrenal axis, deeply affects neurotransmission and synaptic morphology in brain areas associated with behavioral responses and mental states. Clinical and preclinical studies have demonstrated that the impact of stressful life events on emotional and cognitive behaviors may vary depending on the nature of stress, its intensity or duration, and the time window of development during which stress exposure occurs (perinatally, adolescence, adulthood, or advanced age). The many-sided faces of stress also depend on brain region, sex, and individual differences.

When the stress response is efficient, it can induce adaptive neuroplasticity and improve cognition; however, when the stress response is overused, it can have toxic effects. A maladaptive stress response can lead to epigenetic changes associated with impaired brain functions and may ultimately trigger the development of neuropsychiatric disorders. Thus, the identification of neural mechanisms underlying resilience and vulnerability to stress is of crucial importance in understanding the pathophysiology of neuropsychiatric disorders and in developing improved treatments.

Four papers within the special issue deal with the long-term impact of early-life stress on neurotransmission, behavior, and coping strategies in the adulthood. In our review paper, we discuss the recent literature on the remodeling of excitatory neurotransmission and brain morphology, induced by acute and chronic stress in animal models. We also discuss that the integration between perinatal and late-life experiences may induce long-lasting consequences on neuronal excitatory transmission and morphology. We propose that reprogramming mechanisms may underlie the reorganization of excitatory neurotransmission in the short- and long-term response to stressful stimuli. The review by A. Ashokan et al. focuses on "stress inoculation," suggesting that previous exposure to stress may improve coping strategies and stress resilience. The authors report experimental evidence indicating that moderate stressors, especially during early life, lead to enhanced health outcomes and stress resilience, while more severe stress may have deleterious consequences on health. G. Grigoryan and M. Segal contributed a review describing opposite functional and structural changes in dorsal versus ventral hippocampus induced by prenatal stress. This review summarizes the recent literature with emphasis on the importance of the septotemporal axis of the hippocampus in stress effects. Finally, an article by J. Maguire and I. Mody demonstrates that a mouse model of postpartum depression increases depressive- and anxiety-like behaviors in the offspring.

The issue contains three review papers highlighting stress as a major risk factor in the etiopathogenesis of stress-related disorders. First, D. Ness and P. Calabrese discuss the influence of stress on multiple memory systems and their contributions to the learning process leading to behavioral alterations. I. Negrón-Oyarzo et al. review the specific role

of the prefrontal cortex in the maladaptive response to stress. The authors suggest that the impairment of functional connectivity within the prefrontal cortex induced by chronic stress has a mechanistic role in the etiopathogenesis of psychopathologies. The link between chronic stress and systemic illness (including neurological disorders, substance and alcohol abuse, neurodegenerative disorders, cardiovascular diseases, and gastrointestinal and metabolic disorders) is discussed by V. Duric et al. The authors emphasize that stress is a common risk factor in the pathophysiology of both psychiatric and systemic disorders.

Within the special issue, we have the honor to include a provocative review by E. R. de Kloet and M. L. Molendijk. The authors discuss the use of the forced-swim test as a reliable model for measuring depressive-like behavior. They report that the test is generally accepted to assess the antidepressant potential of drugs. However, rodents quickly activate coping strategies to adapt to the stressor, suggesting that the test could be used in the study of mechanisms of adaptation, rather than assessing depressive-like behavior.

Three research articles describe molecular and behavioral changes induced by stress in animal models of mood disorders. Y. Dwivedi and H. Zhang demonstrate reduced activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) cascade in the brain of learned helplessness rats, highlighting the role of ERK1/2 signaling in stress vulnerability. The paper by A. Ieraci et al. describes behavioral deficits and changes in gene expression induced by social isolation in adulthood. In particular, the authors propose that increased anxious- and depressive-like behavior is associated with reduction of several neuroplasticity-related genes induced by social isolation stress. R. Molteni et al. show changes in activity-dependent transcription levels of Brain-Derived Neurotrophic Factor (BDNF) in the hippocampus of chronic mild stressed rats, a model that recapitulates the phenotypic hallmarks of depression.

Other research papers focus on both the immediate and delayed effects of different protocols of acute stress. D. Bonini et al. demonstrate that acute footshock stress induces time-dependent changes in glutamate receptor subunits in prefrontal and frontal cortex. Of note, this is consistent with stress-induced enhancement of glutamatergic synaptic transmission. H. A. Vecchiarelli et al. present a paper analyzing the time-dependent changes induced by acute stress. Here, selective time-dependent changes in enzymes belonging to the kynurenine metabolic pathway were found in corticolimbic areas of rats subjected to acute restraint stress. This suggests that the dysregulation of kynurenine metabolites may alter excitatory signaling. Another paper examines the long-term behavioral consequences of transient middle cerebral artery occlusion in rats. J. Kasahara et al. use a model of poststroke depression induced by transient ischemia. They demonstrate a spontaneous depressive-like phenotype 20 weeks after the induction of cerebral ischemia, which is associated with loss of granular neurons in the ipsilateral hippocampus, increased apoptosis, and changes in neurogenesis. Interestingly, these behavioral and cellular deficits were prevented by treatment with imipramine.

The present special issue also includes two papers focusing on the involvement of energy metabolism in the stress response. F. Jeanneteau and M. Arango-Lievano review the link between mitochondrial efficiency and synaptic activity. Mitochondria are key regulators of synaptic plasticity and changes in synaptic metabolism are involved in both adaptive and maladaptive mechanisms in response to stress. T. Larrieu et al. present evidence of molecular and morphological changes within the prefrontal cortex of mice fed with n-3 polyunsaturated fatty acid deficient diet. In particular, n-3 polyunsaturated fatty acid deficient diet induces alterations in the glucocorticoid receptor signaling pathway and reduces dendrite arborization.

Finally, we have the pleasure to include in this special issue a clinical research paper by C. Raymond et al., who report a pilot study on the effect of reading self-help books on stress reactivity and depression. The results demonstrate that consumers of growth-oriented self-help books showed higher cortisol reactivity to stress, whereas consumers of problem-focused self-help books manifested higher depressive symptomatology compared with nonconsumers. The authors suggest that reading self-help books might be associated with increased stress and/or mental disorders.

Collectively, this special issue includes 17 exciting papers that span diverse aspects of the multifaceted effects of stress, which involves either adaptive responses or increased vulnerability to neuropsychiatric disorders. We believe that this collection of manuscripts offers the latest insights into the molecular and neurochemical foundations of stress-related diseases, especially mood and anxiety disorders.

Laura Musazzi
Jordan Marrocco

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 6752193, 14 pages
http://dx.doi.org/10.1155/2016/6752193

Review Article

Stress Response and Perinatal Reprogramming: Unraveling (Mal)adaptive Strategies

Laura Musazzi[1] and Jordan Marrocco[2]

[1]*Laboratorio di Neuropsicofarmacologia e Neurogenomica Funzionale, Dipartimento di Scienze Farmacologiche e Biomolecolari and CEND, Università degli Studi di Milano, 20133 Milano, Italy*
[2]*Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10065, USA*

Correspondence should be addressed to Laura Musazzi; laura.musazzi@unimi.it
and Jordan Marrocco; jordan.marrocco@rockefeller.edu

Received 1 December 2015; Accepted 17 February 2016

Academic Editor: Menahem Segal

Environmental stressors induce coping strategies in the majority of individuals. The stress response, involving the activation of the hypothalamic-pituitary-adrenocortical axis and the consequent release of corticosteroid hormones, is indeed aimed at promoting metabolic, functional, and behavioral adaptations. However, behavioral stress is also associated with fast and long-lasting neurochemical, structural, and behavioral changes, leading to long-term remodeling of glutamate transmission, and increased susceptibility to neuropsychiatric disorders. Of note, early-life events, both *in utero* and during the early postnatal life, trigger reprogramming of the stress response, which is often associated with loss of stress resilience and ensuing neurobehavioral (mal)adaptations. Indeed, adverse experiences in early life are known to induce long-term stress-related neuropsychiatric disorders in vulnerable individuals. Here, we discuss recent findings about stress remodeling of excitatory neurotransmission and brain morphology in animal models of behavioral stress. These changes are likely driven by epigenetic factors that lie at the core of the stress-response reprogramming in individuals with a history of perinatal stress. We propose that reprogramming mechanisms may underlie the reorganization of excitatory neurotransmission in the short- and long-term response to stressful stimuli.

1. Introduction

Life experiences often produce uncertainty or threat and trigger a physiological response, the so-called "stress response," aimed at promoting adaptation and improving survival [1]. The stress response, including fast and transient activation of the autonomic nervous system and of the hypothalamic-pituitary-adrenocortical (HPA) axis, implies release of catecholamines and corticosteroids (mainly cortisol in humans and corticosterone in rodents). Corticosteroids exert their function through the activation of the high-affinity mineralocorticoid receptor (MR) and the low-affinity glucocorticoid receptor (GR), which are widely expressed both at peripheral level and in the brain. Corticosteroids, together with regulating metabolism, food intake, and the immune system, modulate brain function, neuronal transmission, and plasticity, especially in corticolimbic areas [2, 3].

In the high majority of individuals, the stress response is able to activate coping strategies to adverse environmental changes, promoting stress resilience. However, in vulnerable subjects, the stress response may become dysregulated and induce maladaptive changes, which in turn underlie increased susceptibility to stress-related neuropsychiatric diseases [4–6]. Daskalakis and collaborators [7] proposed the 3-hit concept (hit 1: genetic predisposition, hit 2: early-life environment, and hit 3: later-life environment) to explain why some individuals can cope with adverse events and remain resilient while others are vulnerable and succumb to stress-related disorders. This concept readapts the cumulative hypothesis of stress [8], which indicates that accumulating failures to cope with stressors lead to dramatic consequences on the individuals, consistent with increased vulnerability to psychiatric disorders [1]. Of note, the 3 hits also endorse the mismatch hypothesis of psychiatric disorders, suggesting that

early-life adversities can prepare the individuals to cope with future life similar challenges; conversely, the coping strategies are compromised when the later-life events exhibit mismatch with the early-life environment [9, 10].

In this context, during the last decades, there have been a growing number of studies on short- and long-term consequences of early-life stress (including manipulations during the prenatal period and/or the early phase of postnatal development), suggesting an increased interest in the impact of early-life adversities on stress response and susceptibility to neuropsychiatric disorders in the adulthood [11–24].

In the first sections of the present review, we will summarize functional, morphological, and epigenetic changes in adults induced by stress exposure during adulthood, or during early life. Finally, in order to recapitulate the interplay between life adversities at early stages and in the adulthood, we will introduce the concept of "reprogramming," a process whereby a stimulus or insult, during a sensitive period of development, has lasting and/or lifelong significance, inducing readaptation of the stress response.

2. Acute and Chronic Stress at Synapses: Corticosterone-Dependent Effects of the Stress Response

A growing body of literature has analyzed the multifaceted effects of different stress protocols and of corticosteroids (mainly corticosterone) on neurotransmission, neuronal plasticity, and behavior (see below). MR and GR are nuclear receptors, acting as transcription factors, ultimately leading to regulation of gene expression. However, more recently, compelling evidence has reported fast effects of corticosteroids on neuronal excitability, in line with early nongenomic mechanisms that are likely dependent on membrane-located receptors (for recent reviews, see [25–27]).

In the next sections, we will review the most recent findings on fast and delayed effects of acute and repeated stress, mediated by genomic and nongenomic action of corticosteroids in the brain.

2.1. Corticosterone-Dependent Effects of Stress on Excitatory Neurotransmission. A number of studies have been performed to unravel the time-dependent and brain area-specific effects of stress on neuronal excitability and cognitive processes (for recent reviews, see [2, 26–28]). The changes in neuronal excitability and synaptic plasticity induced by stress are the result of an imbalance of excitatory (glutamatergic) and inhibitory (GABAergic) transmission, leading to long-lasting (mal)adaptive functional modifications [28–34]. Although both glutamate and GABA transmission are critically associated with stress-induced alteration of neuronal excitability [32, 34], the present review will focus on the modulation of glutamate release and transmission induced by stress and glucocorticoids.

Genomic and nongenomic effects of acute stress were characterized in both the hippocampus and the prefrontal cortex. Acute stress was consistently reported to rapidly enhance the frequency of miniature excitatory currents (mEPSCs) at hippocampal synapses, thus suggesting increased probability of glutamate release, through nongenomic action of corticosterone, and activation of membrane-located pre- and postsynaptic MR [29, 35–37]. On the other hand, slower genomic effects of acute stress in the hippocampus are mainly mediated by GR, which prevents synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor potentiation and enhances voltage-dependent calcium currents and mEPSCs amplitude. This leads to steady depolarization and attenuation of firing activity [29, 38, 39] and impaired long-term potentiation (LTP) [30, 40].

Partially different effects of acute stress were reported in the prefrontal cortex, where nongenomic mechanisms, despite being involved in the priming of excitatory synapses, are not sufficient to induce changes in glutamate release and transmission [41–43]. Indeed, it was found that corticosterone rise induced by acute footshock stress increases the size of the readily releasable pool of glutamatergic vesicles in the prefrontal cortex, through completely nongenomic mechanisms involving both MR and GR located at synaptic sites. However, in the same brain area, slower genomic mechanisms are required to enhance presynaptic glutamate release and mEPSCs amplitude [43–45]. In line with these observations, different acute stress protocols, as well as acute corticosterone *in vivo* and *in vitro* treatments, were shown to induce delayed and long-lasting increase of excitatory transmission in prefrontal cortex pyramidal neurons [44–46].

In recent studies, fast and slow effects of acute stress were also analyzed in the amygdala [47]. In basolateral amygdala, a brain area responsible for the control of emotion and fear memory, different and partially opposite effects of stress compared to hippocampus and prefrontal cortex were reported. Indeed, the early nongenomic increase of mEPSCs frequency induced by corticosterone is accompanied by a fast decrease in synaptic potentiation (LTP), while genomic and delayed effects include enhancement of neuronal excitability and synaptic plasticity.

Long-term adaptive changes induced by repeated stress exposure include impairments in neuronal transmission and synaptic activity (extensively reviewed in [27, 31, 32, 48]). Indeed, in both hippocampus and prefrontal cortex, chronic stress was associated with defects in the ability to induce or maintain LTP, and increased long-term depression (LTD), together with enhancing basal transmission, while opposite changes were measured in basolateral amygdala. The changes in neuronal activity induced by chronic stress in brain areas involved in the negative feedback of the HPA axis are consistent with impairment and dysregulation of the stress response [31]. It has been suggested that functional connectivity between amygdala and ventral hippocampus plays a key role in stress-induced changes in synaptic plasticity (for a recent review, see [49]). In particular, Ghosh and coworkers showed that, in animals subjected to repeated restraint stress, the directional connection from the amygdala to the hippocampus is gradually and persistently potentiated [50]. The authors suggest that this mechanism could be involved in

the long-term emotional and cognitive impairments induced by chronic stress.

2.2. Morphological and Cytoarchitectural Changes Induced by Stress. Stress and corticosterone cause structural alterations, including dendritic remodeling and changes in spine density, mostly in brain areas implicated in the regulation of the emotional state (for recent reviews, see [27, 51, 52]).

Chronic stress was reported to reduce dendritic arborization and synaptic contacts both in hippocampus and in prefrontal cortex, whereas in basolateral amygdala, both chronic and acute exposure to stressors significantly increased dendritic complexity (for recent reviews, see [27, 48–53]). These long-lasting structural changes occur together with increased anxious- and depressive-like behaviors, strongly suggesting that dendritic atrophy induced by chronic stress may induce severe behavioral deficits [48, 53, 54].

Recently, a few studies also analyzed morphological alterations induced by acute stress and corticosterone. Acute corticosterone treatment of rats was shown to induce delayed and time-dependent opposite changes of dendrite morphology in medial prefrontal cortex pyramidal neurons, compared to basolateral amygdala spiny neurons [55]. Similarly, 5 hours of multimodal combined physical/psychological stress was demonstrated to induce a corticotropin-releasing hormone-dependent reduction of spine density in hippocampal area CA3 [54]. In a more recent study, one single session of acute footshock stress was shown to reduce the apical dendritic length of pyramidal neurons in medial prefrontal cortex layers II-III [56]. Intriguingly, this effect was measured as early as one day after stress and lasted for up to 14 days. Furthermore, the effect was partly prevented by chronic treatment with antidepressants before the stress session.

On the other hand, in line with the rapid enhancement of excitatory transmission induced by stress and improvement of working memory performance [44, 45], both acute footshock and acute restraint stress were shown to remarkably increase the number of excitatory axoshaft and axospinous synapses in the medial prefrontal cortex of rats [57] and to induce sprouting of new spines one day after stress [56]. Accordingly, Liston and Gan [58] have shown that acute treatment with corticosterone promotes a dose-dependent increase of spine formation in medial prefrontal cortex pyramidal neurons. Furthermore, acute corticosterone induces rapid GR-dependent spinogenesis in hippocampal slices [59]. These findings suggest that the increased number of synapses induced by acute stress is likely a corticosterone-dependent effect.

2.3. Epigenetic Changes Induced by Stress. The term "epigenetics" refers to mechanisms modulating gene expression independently of changes in nucleotide sequence and includes alterations of DNA methylation, posttranslational modification of histone proteins, and regulation by small noncoding RNAs (essentially, microRNAs, miR) [60, 61]. Compelling evidence showed that behavioral stress induces epigenetic changes in selected brain areas, leading to regulation of gene expression and neuronal function [61–63].

A few studies have assessed changes in DNA methylation (a modification associated with gene silencing) in stress animal models. Chronic social stress in mice induced persistent demethylation at the corticotrophin-releasing factor promoter in the paraventricular nucleus of the hypothalamus, suggesting hyperactivation of the HPA axis [64]. Moreover, in a recent study, changes in the global DNA methylation profile were measured after acute restraint stress in the hippocampus, cerebral cortex, and periaqueductal gray matter, while these alterations were prevented by physical exercise [65].

A growing body of literature reported posttranslational modification of histone proteins after exposure to acute and chronic stress protocols. A genome-wide chromatin immunoprecipitation study reported changes in histone H3 lysine 9 dimethylation levels (inducing repression of gene expression) in the nucleus accumbens of mice susceptible to chronic social defeat stress, and not in resilient animals [66, 67]; importantly, chronic antidepressants reversed these modifications [68]. A recent study also showed that both chronic social defeat in mice and depression in humans reduced the expression of the RAS-related C3 botulinum toxin substrate 1 (Rac1) gene in the nucleus accumbens, through a mechanism involving increased histone H3 lysine 9 dimethylation [69]. On the other hand, permissive histone H3 acetylation is transiently reduced and then persistently increased in the nucleus accumbens of susceptible, but not of resilient, animals subjected to chronic social stress [66]. In the same paper, similar results were obtained in postmortem studies, reporting increased levels of histone H3 acetylation in the nucleus accumbens of depressed patients. However, since local infusion of histone deacetylase inhibitors showed an antidepressant-like effect, the authors hypothesized that the increase in H3 acetylation measured in susceptible animals might mediate long-lasting positive neuronal adaptations to chronic stress.

In the hippocampus, selected and time-dependent changes in histone H3 methylation at lysines 4, 9, and 27 (resp., associated with increased transcription, heterochromatin formation, and transcriptional repression) were demonstrated after acute and repeated restraint stress in rats [70]. A further study from the same group showed that, soon after one single session of restraint stress, repressive histone H3 lysine 9 trimethylation is selectively increased in the hippocampus, especially at transposable element loci [71]. Individual variations of histone H3 acetylation levels were also reported in the hippocampus of rats subjected to repeated social defeat stress [72, 73]. Moreover, it was shown that the acquisition of behavioral immobility response induced by acute forced swim stress was dependent on increased histone H3 phosphoacetylation in the hippocampus and GR-induced activation of the NMDA/extracellular signal-regulated kinases (ERK)/mitogen- and stress-activated kinases (MSK) 1/2 pathway [74].

Repeated social defeat stress was also found to increase histone H3 acetylation in the infralimbic (and not prelimbic) prefrontal cortex [73, 75]. A recent study on postmortem PFC from patients with mood disorders reported increased levels of the presynaptic protein synapsin 2, together with increased

histone H3 lysine 4 trimethylation at its promoter, suggesting epigenetic regulation of synapsin 2 gene expression [76].

Intriguingly, a number of studies reported stress-induced epigenetic regulation of the brain-derived neurotrophic factor (BDNF), a neurotrophin with key roles in neuroplasticity and synaptic function, as well as in the pathophysiology of neuropsychiatric disorders [77, 78]. The expression of BDNF is mediated by the transcription of different mRNAs, driven by dedicated promoters and derived by the splicing of one of multiple 5′ noncoding exons (at least eight in rodents) with the 3′ coding exon [79].

Social defeat stress induced long-lasting downregulation of BDNF transcripts containing exons IV and VI, by increasing dimethylation of histone H3 at specific exon promoters in the mouse hippocampus, and chronic imipramine reversed this downregulation increasing histone acetylation at the same promoters [80]. Similarly, the reduction of total BDNF transcript and mRNAs containing exons I and IV expression, induced by single immobilization stress in the rat hippocampus, was shown to be accompanied by a significant decrease in histone H3 acetylation at respective promoters [78]. In a more recent study, physical exercise was found to counteract the downregulation of selected BDNF transcripts induced by acute restraint stress and to increase the levels of histone H3 acetylation at related promoters [81].

MicroRNAs are small noncoding RNAs regulating gene expression, generally repressing the expression of target mRNAs [82]. In recent years, research studies have been conducted on the involvement of microRNAs in the stress response and onset of neuropsychiatric disorders [83]. It was shown that both acute restraint stress and chronic social defeat in mice markedly upregulated miR-34 levels in amygdala and that miR-34 overexpression in the central amygdala exerted anxiolytic effect [84]. In the same study, *in vitro* experiments showed that miR-34 reduced the activation of the corticotropin-releasing hormone receptor 1, suggesting a role of miR-34 in functional regulation of the stress response. In more recent papers from the same research group, miR-135 in serotonergic neurons was found to have a key role in determining stress resiliency and antidepressant efficacy [85], while the increase of amygdalar miR-19b induced by chronic social defeat stress was suggested to be related to behavioral responses to stress, through mechanisms involving the adrenergic receptor β-1 [86].

3. Perinatal Reprogramming of the Stress Response

The high majority of functional and morphological changes promoted by behavioral stress and corticosteroids were reported in "naïve" young adult animals or mature neuronal cultures. Nevertheless, early-life experiences shape the stress response in adulthood, leading to the reprogramming of coping strategies against environmental challenges and having a strong impact on behavior and susceptibility to neuropsychiatric disorders (see Section 1).

Intriguingly, a few studies on humans aimed at separating the effects of the objective exposure to a stressor and the mother's subjective reaction [87–90]. According to King and Laplante [87], exposure to a natural disaster (Project Ice Storm) occurring during the gestational period allows for a reliable study of the effects of prenatal stress on child health and development [91].

However, the reprogramming effects observed in the offspring likely recapitulate the cumulative experience *in utero* and the quality of the postnatal environment, which is, in turn, mostly associated with the quantity, quality, and reliability of maternal care [92–97]. Thus, we will refer to "perinatal" reprogramming to include events occurring prenatally and/or during the lactation period. Considering that the limitations of retrospective studies constrain the number of epidemiological findings in humans, a large number of data come from evidence in rodents and nonhuman primates [21, 24, 98–104].

3.1. Changes in Excitatory Neurotransmission Induced by Perinatal Stress. Overall, the changes in excitatory transmission and neuronal remodeling, induced by both acute and chronic stress (reviewed in Section 2.1), strongly suggest a key role of the glutamate synapse in the adaptive and maladaptive response to stressful stimuli. However, the study of the effects of exposure to perinatal stress on the activity of glutamatergic neurons is still at its infancy.

Morphological studies have shown that prenatal stress is associated with reduction of dendritic arborization and synaptic loss in prefrontal cortex and hippocampus in adult life, suggesting that stress in gestational period might induce long-lasting impairments of glutamate neuron and transmission [105–108].

A number of studies reported changes in the expression of glutamate receptors and transporters, in adult animals subjected to stress during the perinatal life [109–115]. Maternal separation in rats was found to decrease mRNA expression levels of ionotropic glutamate receptors, together with increasing GLutamate ASpartate Transporter (GLAST) levels, selectively in the hippocampus and not in the prefrontal cortex [109]. It was also demonstrated that maternal separation significantly reduced the expression of type 4 metabotropic glutamate receptor in hippocampus, a change reversed by chronic fluoxetine treatment [110]. Similarly, adult male offspring of pregnant dams subjected to restraint stress during pregnancy display impairment of N-methyl-D-aspartate (NMDA) receptor-mediated long-term potentiation, decreased NMDA receptor subunits [111], and reduced expression of group I/II metabotropic glutamate receptors [112] in the hippocampus. In a more recent study, Adrover and collaborators [113] have shown increased mRNA and protein expression levels of the glial glutamate transporter (GLT-1) in the hippocampus and enhanced glutamate uptake and vesicular glutamate transporter 1 (v-Glut-1) protein levels in the prefrontal cortex of prenatally stressed rats.

Overall, the high majority of studies reported that early stress both decreases the expression of glutamate receptors, suggesting reduced transmission efficacy, and increases glutamate transporters, which may imply an increased rate of glutamate metabolism. Of note, others have found higher levels of ionotropic and metabotropic glutamate receptors [114]

and increased NMDA receptor activation [115]. Others have shown impairment of long-term potentiation and enhancement of long-term depression in young rats subjected to prenatal stress [116]. These abnormalities were correlated with increased pro-brain-derived neurotrophic factor (pro-BDNF), decreased mature BDNF levels, and no changes in NMDA receptor subunits expression [116]. Although changes in the expression of glutamate receptors and transporters are only rough indicators for predicting glutamate release and transmission, these data strongly suggest that perinatal stress exerts a long-term influence on the glutamate system.

It was recently demonstrated that the increase of anxiety-like behavior induced by prenatal stress in rats is causally associated with a reduction of depolarization-evoked presynaptic glutamate release in the ventral hippocampus [117, 118], a brain region encoding memories related to stress and emotions [119]. Interestingly, this effect is blocked by activation of oxytocin receptor [120] (see below for oxytocin and reprogramming). Although the mechanisms by which prenatal stress may cause long-lasting dampening of glutamate neurotransmission in the ventral hippocampus have been poorly clarified, it was hypothesized that prenatal stress, besides enhancing glutamate metabolism, might induce long-lasting dysfunction in the intrinsic machinery controlling exocytotic glutamate release [117].

3.2. Modulation of HPA Axis Reactivity Induced by Perinatal Stress. HPA axis alterations are the characteristic feature of the endophenotypes induced by perinatal stress [21, 121–126].

A pioneering study by Levine showed that maternal separation induced downregulation of the stress response, consistent with weight reduction of adrenal glands [127]. To date, the literature about the long-term effects of perinatal stress on the HPA axis is contradictory, although in many species including mice, rats, guinea pigs, and nonhuman primates, prenatal stress has been shown to increase the overall production of glucocorticoid and/or the feedback regulation [100, 101, 103, 128–130]. For example, peer rearing in monkeys has been shown to exaggerate stress reactivity [122, 123], stereotypies and self-directed behaviors [124], and abnormal brain morphology [125]. Moreover, maternal separated rodents show general upregulation of stress and fear responses [126, 131–133], increased hypothalamic CRF expression, reduced cortical GR expression [134], increased immobility in the forced-swim test [135], and poorer memory performance [134]. Curiously, in rats, 3 hours of daily maternal separation during the first two weeks after birth increases the vulnerability to stress in the adulthood [136, 137], whereas 8 hours of separation decreases the response of the HPA axis [138]. Similarly, prenatally restraint stressed rats display prolonged corticosterone secretion associated with downregulation of GR and MR receptors in the hippocampus [21, 92]. Interestingly, these effects are reversed by prenatal adrenalectomy [93] or postnatal cross-fostering [92].

3.3. Epigenetic Reprogramming of the HPA Axis: Regulation of GR Expression. An ever growing number of studies focused on short- and long-term epigenetic changes induced by stress in early life (recently extensively reviewed in [22, 92, 139–141]). The mechanisms involved in the epigenetic reprogramming are highly complex and strongly depend on the gender of the individual, the type of stressor, and its intensity and duration. Here, we will focus on the epigenetic regulation of GR in the offspring induced by prenatal and postnatal maternal stress.

At the epigenetic level, the GR gene is consistently affected by natural variation of maternal care in rodents (measured as licking/grooming, arched-back and blanket nursing, and nest building) [142, 143]. Indeed, low absolute levels of maternal care selectively modify the DNA methylation status of GR promoter in the hippocampus of the offspring, suggesting reduced expression of the receptor as well as increased HPA reactivity. Conversely, offspring receiving high levels of maternal care exhibit lower level of DNA methylation of the GR promoter and increased histone H3 lysine 9 acetylation (a marker of transcriptional activation).

The GR gene expression and promoter methylation have also been examined in humans following early-life trauma, with similar epigenetic outcomes. McGowan and collaborators [144] found decreased levels of hippocampal GR mRNA and increased cytosine methylation of the GR promoter in subjects with a history of childhood abuse. Similarly, childhood maltreatment has been associated with decreased hippocampal GR expression and increased stress responses in adulthood. Again, such effects are mediated by DNA methylation and hydroxymethylation across GR promoter regions [145]. A compelling study in genocide survivors suggested that the increased DNA methylation at the promoter region of the GR was associated with less intrusive memory of the traumatic event and sex-specific reduced PTSD risk [146]. Together, these findings indicate that the epigenetic regulation of GR expression is a key factor in the reprogramming of the HPA axis induced by early stress.

3.4. Mother-Offspring Interplay: Role of 11β-Hydroxysteroid Dehydrogenases and Oxytocin. The mother/infant interaction is a critical intermediary to study early-life reprogramming. Such interplay is mainly mediated by the placenta, which modulates fetal exposure to maternal factors. As an example, glucocorticoids, despite circulating across the placenta, are significantly lower in the fetus than in the mother. This key tissue-specific barrier control is exerted by the placental 11β-hydroxysteroid dehydrogenase, an enzyme that converts cortisol and corticosterone into inactive cortisone and 11-dehydrocorticosterone (11β-HSD2), and vice versa (11β-HSD1) [147–149]. Recently, it has been shown that 11β-HSD2 undergoes epigenetic regulation in the placenta and fetal brain [150–152]. Curiously, Appleton and collaborators [153] have shown that women experiencing adversity during pregnancy display low levels of 11β-HSD2 methylation. This accounts for increased levels of placental 11β-HSD aimed at protecting the fetus from excessive glucocorticoid exposure. Others have shown that pregnant rats exposed to repeated episodes of restraint stress, a model that recapitulates the main feature of anxiety and depression in the adult offspring, display a reduction of 11β-HSD2 activity in the placenta, thus increasing the amount of nonmetabolized corticosterone

reaching the fetus [55]. Also, high methylation in the promoter region of placental 11β-HSD2 has been associated with low infant birth weight [154]. However, it is unclear whether the modifications of the 11β-HSD2 are exclusively disruptive and/or directly associated with pathological endophenotypes in late life. For example, the downregulation of 11β-HSD2 may provide the fetus with a reliable signal about the maternal stressful environment, thereby predicting the milieu it is likely to cope with after birth.

The maternal HPA axis itself also plays a pivotal role in the mother-offspring interplay. The HPA axis is normally attenuated from midpregnancy to the end of lactation [155–157]; such attenuation is generally associated with maternal behavioral changes including reduced anxiety [158, 159], enhanced maternal behavior [160], and increased aggressiveness [161, 162]. Of note, the central oxytocinergic system exerts this inhibitory effect on the maternal HPA axis [160, 163–167]. Oxytocin is a neurohypophysial peptide, which plays a key role in parturition, lactation, mother/infant interaction, and social behavior [168]. Interestingly, intracerebroventricular administration of oxytocin stimulates maternal behavior in ovariectomized virgin rats [160]. Moreover, enhanced maternal care increases the expression of oxytocin receptor in the central nucleus of the amygdala [169]. Remarkably, in subjects with a history of early-life stress, the inhibitory effect of oxytocin on the HPA axis is diminished or even reversed [120, 170]. Interestingly, impaired social behavior and increased anxiety have been associated with an altered number of oxytocin neurons in the paraventricular and supraoptic nuclei [171] and increased expression of oxytocin receptor in the hippocampus and amygdala [120] of adult prenatally stressed rats. Moreover, it has been shown that the activation of presynaptic oxytocin receptor during the adulthood could both correct the abnormal glucocorticoid feedback of the HPA axis and normalize the expression of GR and MR in the hippocampus in prenatally restraint stressed rats [120]. In humans, it has been shown that intranasal administration of oxytocin dampens the enhanced stress-induced functional connectivity between the amygdala and the hippocampus in subject with a history of early-life stress [172]. Finally, variations in maternal care have been associated with DNA methylation of oxytocin receptor in blood cells both in rodents [173] and in humans [174].

Together, the evidence reviewed in this section emphasizes the molecular and neuroendocrine mechanisms that underlie the critical role of mother-infant interaction in the reprogramming of stress response and vulnerability to neuropsychiatric disorders.

4. Conclusion

The "cumulative stress hypothesis" of neuropsychiatric disorders states that repeated exposure to stressful events is the main environmental factor for pathological onset [1, 8, 32]. Thus, it is conceivable that repeated adverse events, especially when added to perinatal stress, exacerbate psychopathological conditions. On the other hand, according to the "match/mismatch hypothesis," early-life stress might also be somehow protective against stressors in late life, leading to higher achievement of adaptation and survival [7, 9, 175]. Yet, these hypotheses are strictly related and interconnected. Indeed, the deleterious effects of stress rely not only on *when* or *how often* stress occurs, but especially on *how intense* the stress is and *how much* it impacts an individual, depending on one's genetic background [176, 177]. In this context, repeated subjective mild stressors may act improving adaptation to environmental challenges [3, 178], while a single overwhelming adverse event may precipitate neuropsychiatric diseases, as in the case of posttraumatic stress disorder [179, 180].

It is also important to notice that whereas the physiological stress response activates adequate coping strategies, leading to stress resiliency and adaptation in the high majority of subjects, vulnerability toward stress is dependent on individual behavioral, physiological, and genetic factors [4–6]. Thus, individual reprogramming of the stress response induced by early-life stress could be both adaptive and maladaptive. In line with this hypothesis, Macrì and collaborators [181] have suggested that mild neonatal changes may reduce the HPA axis reactivity, leading to resilience, whereas severe neonatal challenges would increase the adult HPA axis reactivity, with the ensuing increased vulnerability to stress-related disorders. Intriguingly, it was recently reported that early-life trauma in humans can also promote early maturation of amygdala-prefrontal cortex connectivity, in line with enhanced emotion regulation and reduced anxious behavior [182].

Integration of perinatal and late-life experiences may induce long-lasting consequences on neuronal excitatory transmission and morphology, especially in corticolimbic areas (Figure 1). If acute stress in adult life was consistently shown to increase glutamate transmission and release, at least in the hippocampus and prefrontal cortex [2, 26–28], chronic stress has the opposite effect, inducing impairments in neuronal transmission and synaptic activity [31] (Figure 1(a)). In turn, the effects of chronic stress are generally associated with behavioral deficits and depressive/anxious-like behavior [32, 61, 183], while the response to acute stress can be both adaptive, with improved behavioral and cognitive functions [44, 45], and maladaptive [7]. However, when the individual is subjected to stress in early life, the stress response in adulthood may be shaped by prior experiences (Figure 1(b)). It was shown that depolarization-dependent release of glutamate in dorsal hippocampus is decreased in animals subjected to chronic stress in the prenatal life [117, 118]; however, little is known on the reprogramming of the stress response at the level of excitatory transmission induced by early-life stress. A very recent cross-sectional observational study examined the effects of early- and late-life trauma in Korean college students, showing a significant correlation between early trauma, stress, and psychological distress [184].

We speculate that the long-lasting attenuation of the stress response induced by early-life stress might also affect the changes in excitatory transmission usually induced by stress in adult life. Thus, hypothetically, both the rise of glutamate transmission induced by acute stress and the attenuation of excitatory currents caused by chronic stress might be

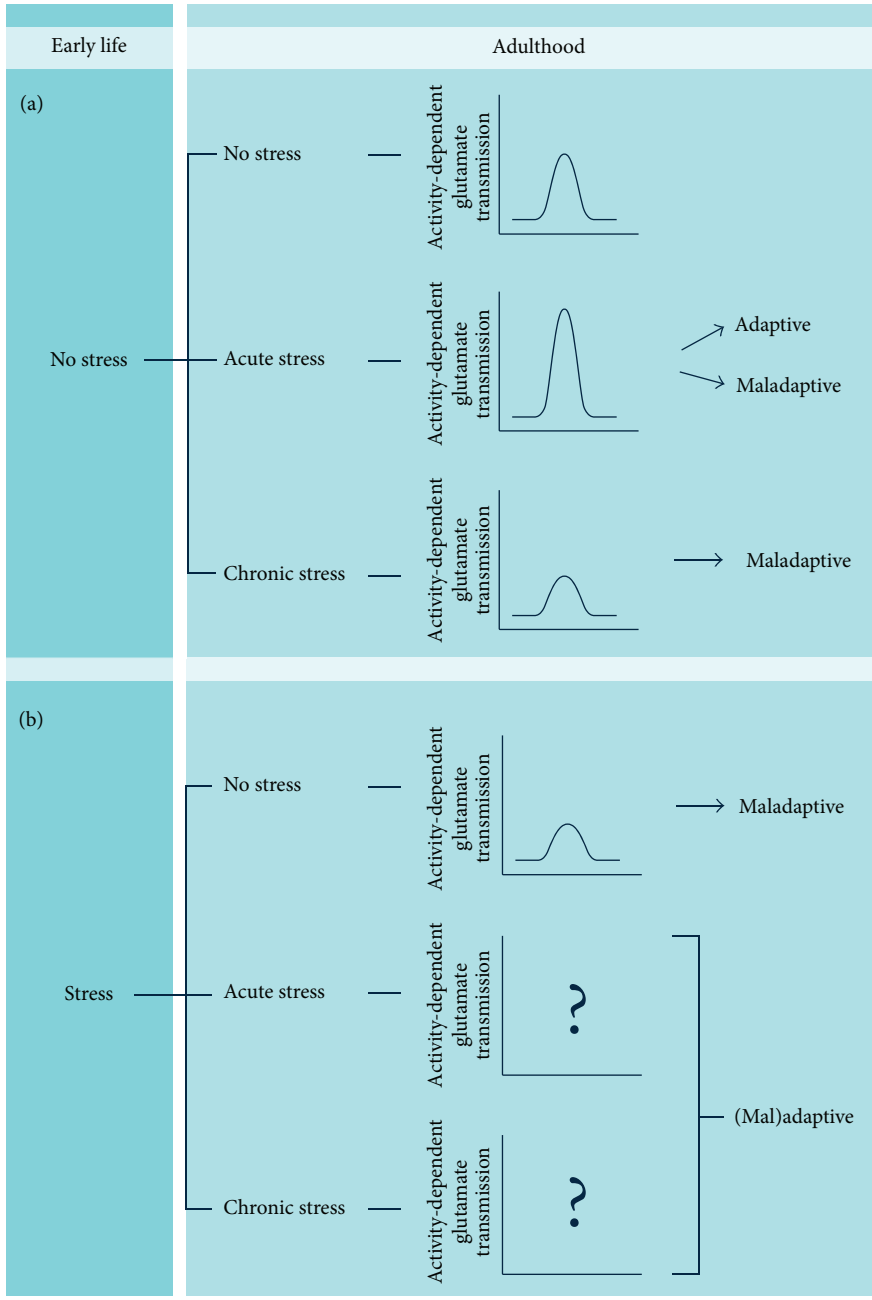


Figure 1: Influence of early-life stress on neuronal excitatory neurotransmission in corticolimbic areas. (a) In subjects with no history of perinatal adverse challenges, acute stress induces an increase in stimulation-evoked glutamate release. This response can be both adaptive and maladaptive. On the other hand, exposure to repeated episodes of stress (chronic stress) induces hypofunction of the glutamatergic synapse with reduced evoked glutamate release, associated with increased vulnerability to stress-related neuropsychiatric disorders. (b) Perinatal stress induces hypofunction of the glutamatergic synapse in adult life, with ensuing reduction in evoked glutamate release. The effects of the association between early- and late-life stress are largely unknown. See text for details.

affected by reprogramming of the stress response induced by early-life stress, thus likely leading to adaptive or maladaptive changes, depending on the intensities of the stressors. Experimental evidence is required to validate or falsify the hypothesis.

Excitotoxicity caused by excessive glutamate release and epigenetic reprogramming are reasonably among the main mechanisms involved in long-lasting neuroplastic alterations induced by stress [1, 185] (Figure 2). Excitoxicity is generally associated with reduced ability to clear the synaptic glutamate, resulting in glutamate spillover and activation of extrasynaptic glutamate receptors [32]. However, it is likely that exposure to high levels of corticosteroids (Figure 2(b)), together with inducing changes in excitatory

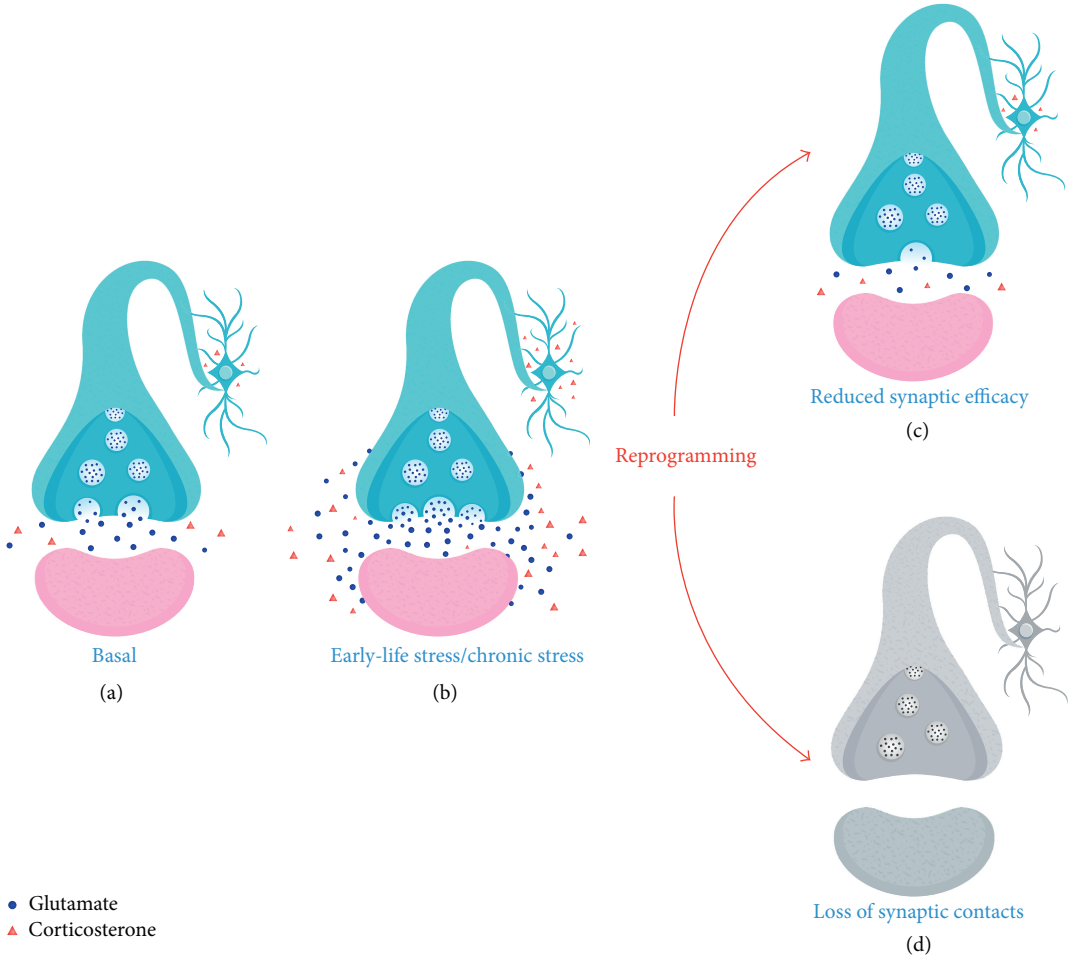


Figure 2: Long-term neuroplastic alterations induced by early-life stress and chronic stress. (a) Basal condition: presynaptic neuron (light blue), postsynaptic neuron (pink). (b) Repeated episodes of stress in early life or in adulthood induce an increase in glucocorticoids associated with a transient increase in glutamate release both in the synaptic cleft and in the extrasynaptic space. Increase in glutamate release may activate reprogramming mechanisms that lead to either reduced synaptic efficacy (c) or loss of synaptic contacts (d).

transmission, activates epigenetic mechanisms, which modulate gene expression and neuronal responsiveness to stress. Of note, mounting evidence suggests that perinatal stress reprogramming of the neuroendocrine stress response and the ensuing behavioral state can cross multiple generations, thus supporting the hypothesis that epigenetic mechanisms underlie the reprogramming of the "stressed synapse" [22, 186]. This in turn leads to functional and structural consequences, in line with reduced synaptic efficacy [31, 117, 118] (Figure 2(c)) and number of synaptic contacts [52, 53, 105–108] (Figure 2(d)).

The mechanisms by which early-life events affect stress resilience via the reprogramming of the stress response and the modulation of excitatory neurotransmission warrant further investigation. In-depth studies of changes in glutamate transmission and dendrite remodeling induced by stress in early and late life will help to elucidate the biological underpinnings of the (mal)adaptive strategies the brain adopts to cope with environmental challenges in one's life.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank Fabio Persico for illustrations and graphical layout and Elisabeth Donnelly for editing and proofreading. Special thanks are due to Professors Bruce S. McEwen and Maurizio Popoli for their valued comments and suggestions.

References

[1] B. S. McEwen, "Allostasis and allostatic load: implications for neuropsychopharmacology," *Neuropsychopharmacology*, vol. 22, no. 2, pp. 108–124, 2000.

[2] B. Myers, J. M. McKlveen, and J. P. Herman, "Glucocorticoid actions on synapses, circuits, and behavior: implications for the

energetics of stress," *Frontiers in Neuroendocrinology*, vol. 35, no. 2, pp. 180–196, 2014.

[3] E. T. Uchoa, G. Aguilera, J. P. Herman, J. L. Fiedler, T. Deak, and M. B. C. de Sousa, "Novel aspects of glucocorticoid actions," *Journal of Neuroendocrinology*, vol. 26, no. 9, pp. 557–572, 2014.

[4] J. Obradović and W. T. Boyce, "Individual differences in behavioral, physiological, and genetic sensitivities to contexts: implications for development and adaptation," *Developmental Neuroscience*, vol. 31, no. 4, pp. 300–308, 2009.

[5] S. J. Russo, J. W. Murrough, M.-H. Han, D. S. Charney, and E. J. Nestler, "Neurobiology of resilience," *Nature Neuroscience*, vol. 15, no. 11, pp. 1475–1484, 2012.

[6] T. B. Franklin, B. J. Saab, and I. M. Mansuy, "Neural mechanisms of stress resilience and vulnerability," *Neuron*, vol. 75, no. 5, pp. 747–761, 2012.

[7] N. P. Daskalakis, R. C. Bagot, K. J. Parker, C. H. Vinkers, and E. R. de Kloet, "The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome," *Psychoneuroendocrinology*, vol. 38, no. 9, pp. 1858–1873, 2013.

[8] B. S. McEwen, "Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators," *European Journal of Pharmacology*, vol. 583, no. 2-3, pp. 174–185, 2008.

[9] M. V. Schmidt, "Animal models for depression and the mismatch hypothesis of disease," *Psychoneuroendocrinology*, vol. 36, no. 3, pp. 330–338, 2011.

[10] S. Santarelli, S. L. Lesuis, X.-D. Wang et al., "Evidence supporting the match/mismatch hypothesis of psychiatric disorders," *European Neuropsychopharmacology*, vol. 24, no. 6, pp. 907–918, 2014.

[11] D. H. Stott, "Follow up study from birth of the effects of prenatal stresses," *Developmental Medicine and Child Neurology*, vol. 15, no. 6, pp. 770–787, 1973.

[12] S. Blomberg, "Influence of maternal distress during pregnancy on postnatal development," *Acta Psychiatrica Scandinavica*, vol. 62, no. 5, pp. 405–417, 1980.

[13] I. L. Ward and J. Weisz, "Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers," *Endocrinology*, vol. 114, no. 5, pp. 1635–1644, 1984.

[14] A. Meijer, "Child psychiatric sequelae of maternal war stress," *Acta Psychiatrica Scandinavica*, vol. 72, no. 6, pp. 505–511, 1985.

[15] C. J. Homer, S. A. A. Beresford, S. A. James, E. Siegel, and S. Wilcox, "Work-related physical exertion and risk of preterm, low birthweight delivery," *Paediatric and Perinatal Epidemiology*, vol. 4, no. 2, pp. 161–174, 1990.

[16] L. B. Holmes, "Looking for long-term effects from prenatal exposures to anticonvulsants," *Teratology*, vol. 64, no. 4, pp. 175–176, 2001.

[17] M. Weinstock, "Effects of maternal stress on development and behaviour in rat offspring," *Stress*, vol. 4, no. 3, pp. 157–167, 2001.

[18] M. Darnaudéry and S. Maccari, "Epigenetic programming of the stress response in male and female rats by prenatal restraint stress," *Brain Research Reviews*, vol. 57, no. 2, pp. 571–585, 2008.

[19] S. J. Lupien, B. S. McEwen, M. R. Gunnar, and C. Heim, "Effects of stress throughout the lifespan on the brain, behaviour and cognition," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 434–445, 2009.

[20] P. J. Brunton and J. A. Russell, "Endocrine induced changes in brain function during pregnancy," *Brain Research*, vol. 1364, pp. 198–215, 2010.

[21] S. Maccari, H. J. Krugers, S. Morley-Fletcher, M. Szyf, and P. J. Brunton, "The consequences of early-life adversity: neurobiological, behavioural and epigenetic adaptations," *Journal of Neuroendocrinology*, vol. 26, no. 10, pp. 707–723, 2014.

[22] T. L. Bale, "Epigenetic and transgenerational reprogramming of brain development," *Nature Reviews Neuroscience*, vol. 16, no. 6, pp. 332–344, 2015.

[23] D. G. Gee and B. J. Casey, "The impact of developmental timing for stress and recovery," *Neurobiology of Stress*, vol. 1, pp. 184–194, 2015.

[24] M. J. Meaney, "Effects of the social environment and early life stress on neurodevelopment, cognition, behaviour and health," *Psychoneuroendocrinology*, vol. 61, p. 11, 2015.

[25] F. L. Groeneweg, H. Karst, E. R. de Kloet, and M. Joëls, "Mineralocorticoid and glucocorticoid receptors at the neuronal membrane, regulators of nongenomic corticosteroid signalling," *Molecular and Cellular Endocrinology*, vol. 350, no. 2, pp. 299–309, 2012.

[26] M. Joëls, N. Pasricha, and H. Karst, "The interplay between rapid and slow corticosteroid actions in brain," *European Journal of Pharmacology*, vol. 719, no. 1–3, pp. 44–52, 2013.

[27] L. Musazzi, G. Treccani, and M. Popoli, "Functional and structural remodeling of glutamate synapses in prefrontal and frontal cortex induced by behavioral stress," *Frontiers in Psychiatry*, vol. 6, article 60, 2015.

[28] W. Timmermans, H. Xiong, C. C. Hoogenraad, and H. J. Krugers, "Stress and excitatory synapses: from health to disease," *Neuroscience*, vol. 248, pp. 626–636, 2013.

[29] L. Groc, D. Choquet, and F. Chaouloff, "The stress hormone corticosterone conditions AMPAR surface trafficking and synaptic potentiation," *Nature Neuroscience*, vol. 11, no. 8, pp. 868–870, 2008.

[30] J. J. Kim and D. M. Diamond, "The stressed hippocampus, synaptic plasticity and lost memories," *Nature Reviews Neuroscience*, vol. 3, no. 6, pp. 453–462, 2002.

[31] M. Joëls, R. A. Sarabdjitsingh, and H. Karst, "Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes," *Pharmacological Reviews*, vol. 64, no. 4, pp. 901–938, 2012.

[32] M. Popoli, Z. Yan, B. S. McEwen, and G. Sanacora, "The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission," *Nature Reviews Neuroscience*, vol. 13, no. 1, pp. 22–37, 2012.

[33] K. Hashimoto, "Emerging role of glutamate in the pathophysiology of major depressive disorder," *Brain Research Reviews*, vol. 61, no. 2, pp. 105–123, 2009.

[34] J. Maguire, "Stress-induced plasticity of GABAergic inhibition," *Frontiers in Cellular Neuroscience*, vol. 8, article 157, 2014.

[35] H. Karst, S. Berger, M. Turiault, F. Tronche, G. Schütz, and M. Joëls, "Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 52, pp. 19204–19207, 2005.

[36] J. E. Olijslagers, E. R. De Kloet, Y. Elgersma, G. M. Van Woerden, M. Joëls, and H. Karst, "Rapid changes in hippocampal CA1 pyramidal cell function via pre- as well as postsynaptic membrane mineralocorticoid receptors," *European Journal of Neuroscience*, vol. 27, no. 10, pp. 2542–2550, 2008.

[37] N. Pasricha, M. Joëls, and H. Karst, "Rapid effects of corticosterone in the mouse dentate gyrus via a nongenomic pathway," *Journal of Neuroendocrinology*, vol. 23, no. 2, pp. 143–147, 2011.

[38] H. Karst and M. Joëls, "Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal cells," *Journal of Neurophysiology*, vol. 94, no. 5, pp. 3479–3486, 2005.

[39] S. Martin, J. M. Henley, D. Holman et al., "Corticosterone alters AMPAR mobility and facilitates bidirectional synaptic plasticity," *PLoS ONE*, vol. 4, no. 3, Article ID e4714, 2009.

[40] C.-C. Chen, C.-H. Yang, C.-C. Huang, and K.-S. Hsu, "Acute stress impairs hippocampal mossy fiber-CA3 long-term potentiation by enhancing cAMP-specific phosphodiesterase 4 activity," *Neuropsychopharmacology*, vol. 35, no. 7, pp. 1605–1617, 2010.

[41] L. Musazzi, M. Milanese, P. Farisello et al., "Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants," *PLoS ONE*, vol. 5, no. 1, Article ID e8566, 2010.

[42] G. Treccani, L. Musazzi, C. Perego et al., "Stress and corticosterone increase the readily releasable pool of glutamate vesicles in synaptic terminals of prefrontal and frontal cortex," *Molecular Psychiatry*, vol. 19, no. 4, pp. 433–443, 2014.

[43] G. Treccani, L. Musazzi, C. Perego et al., "Acute stress rapidly increases the readily releasable pool of glutamate vesicles in prefrontal and frontal cortex through non-genomic action of corticosterone," *Molecular Psychiatry*, vol. 19, no. 4, article 401, 2014.

[44] E. Y. Yuen, W. Liu, I. N. Karatsoreos, J. Feng, B. S. McEwen, and Z. Yan, "Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 33, pp. 14075–14079, 2009.

[45] E. Y. Yuen, W. Liu, I. N. Karatsoreos et al., "Mechanisms for acute stress-induced enhancement of glutamatergic transmission and working memory," *Molecular Psychiatry*, vol. 16, no. 2, pp. 156–170, 2011.

[46] J. B. Lee, J. Wei, W. Liu, J. Cheng, J. Feng, and Z. Yan, "Histone deacetylase 6 gates the synaptic action of acute stress in prefrontal cortex," *Journal of Physiology*, vol. 590, no. 7, pp. 1535–1546, 2012.

[47] R. A. Sarabdjitsingh and M. Joëls, "Rapid corticosteroid actions on synaptic plasticity in the mouse basolateral amygdala: relevance of recent stress history and β-adrenergic signaling," *Neurobiology of Learning and Memory*, vol. 112, pp. 168–175, 2014.

[48] J. Radley, D. Morilak, V. Viau, and S. Campeau, "Chronic stress and brain plasticity: mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders," *Neuroscience & Biobehavioral Reviews*, vol. 58, pp. 79–91, 2015.

[49] C. H. Duman and R. S. Duman, "Spine synapse remodeling in the pathophysiology and treatment of depression," *Neuroscience Letters*, vol. 601, pp. 20–29, 2015.

[50] S. Ghosh, T. R. Laxmi, and S. Chattarji, "Functional connectivity from the amygdala to the hippocampus grows stronger after stress," *Journal of Neuroscience*, vol. 33, no. 17, pp. 7234–7244, 2013.

[51] B. Leuner and T. J. Shors, "Stress, anxiety, and dendritic spines: what are the connections?" *Neuroscience*, vol. 251, pp. 108–119, 2013.

[52] A. F. Arnsten, "Stress weakens prefrontal networks: molecular insults to higher cognition," *Nature Neuroscience*, vol. 18, no. 10, pp. 1376–1385, 2015.

[53] P. Licznerski and R. S. Duman, "Remodeling of axo-spinous synapses in the pathophysiology and treatment of depression," *Neuroscience*, vol. 251, pp. 33–50, 2013.

[54] Y. Chen, C. S. Rex, C. J. Rice et al., "Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 29, pp. 13123–13128, 2010.

[55] H. Kim, J. H. Yi, K. Choi, S. Hong, K. S. Shin, and S. J. Kang, "Regional differences in acute corticosterone-induced dendritic remodeling in the rat brain and their behavioral consequences," *BMC Neuroscience*, vol. 15, article 65, 2014.

[56] N. Nava, G. Treccani, A. Alabsi et al., "Temporal dynamics of acute stress-induced dendritic remodeling in medial prefrontal cortex and the protective effect of desipramine," *Cerebral Cortex*, 2015.

[57] N. Nava, G. Treccani, N. Liebenberg et al., "Chronic desipramine prevents acute stress-induced reorganization of medial prefrontal cortex architecture by blocking glutamate vesicle accumulation and excitatory synapse increase," *International Journal of Neuropsychopharmacology*, vol. 18, no. 3, 2015.

[58] C. Liston and W.-B. Gan, "Glucocorticoids are critical regulators of dendritic spine development and plasticity in vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 38, pp. 16074–16079, 2011.

[59] Y. Komatsuzaki, Y. Hatanaka, G. Murakami et al., "Corticosterone induces rapid spinogenesis via synaptic glucocorticoid receptors and kinase networks in hippocampus," *PLoS ONE*, vol. 7, no. 4, Article ID e34124, 2012.

[60] D. Tardito, A. Mallei, and M. Popoli, "Lost in translation. New unexplored avenues for neuropsychopharmacology: epigenetics and microRNAs," *Expert Opinion on Investigational Drugs*, vol. 22, no. 2, pp. 217–233, 2013.

[61] R. C. Bagot, B. Labonté, C. J. Peña, and E. J. Nestler, "Epigenetic signaling in psychiatric disorders: stress and depression," *Dialogues in Clinical Neuroscience*, vol. 16, no. 3, pp. 281–295, 2014.

[62] M. Fuchikami, S. Yamamoto, S. Morinobu, S. Takei, and S. Yamawaki, "Epigenetic regulation of BDNF gene in response to stress," *Psychiatry Investigation*, vol. 7, no. 4, pp. 251–256, 2010.

[63] B. S. McEwen, C. Nasca, and J. D. Gray, "Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex," *Neuropsychopharmacology*, vol. 41, no. 1, pp. 3–23, 2015.

[64] E. Elliott, G. Ezra-Nevo, L. Regev, A. Neufeld-Cohen, and A. Chen, "Resilience to social stress coincides with functional DNA methylation of the Crf gene in adult mice," *Nature Neuroscience*, vol. 13, no. 11, pp. 1351–1353, 2010.

[65] G. M. Rodrigues Jr., L. V. Toffoli, M. H. Manfredo et al., "Acute stress affects the global DNA methylation profile in rat brain: modulation by physical exercise," *Behavioural Brain Research*, vol. 279, pp. 123–128, 2015.

[66] H. E. Covington III, I. Maze, Q. C. LaPlant et al., "Antidepressant actions of histone deacetylase inhibitors," *Journal of Neuroscience*, vol. 29, no. 37, pp. 11451–11460, 2009.

[67] H. E. Covington, V. F. Vialou, Q. LaPlant, Y. N. Ohnishi, and E. J. Nestler, "Hippocampal-dependent antidepressant-like activity of histone deacetylase inhibition," *Neuroscience Letters*, vol. 493, no. 3, pp. 122–126, 2011.

[68] M. B. Wilkinson, G. Xiao, A. Kumar et al., "Imipramine treatment and resiliency exhibit similar chromatin regulation in the mouse nucleus accumbens in depression models," *Journal of Neuroscience*, vol. 29, no. 24, pp. 7820–7832, 2009.

[69] S. A. Golden, D. J. Christoffel, M. Heshmati et al., "Epigenetic regulation of RAC1 induces synaptic remodeling in stress disorders and depression," *Nature Medicine*, vol. 19, no. 3, pp. 337–344, 2013.

[70] R. G. Hunter, K. J. McCarthy, T. A. Milne, D. W. Pfaff, and B. S. McEwen, "Regulation of hippocampal H3 histone methylation by acute and chronic stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 49, pp. 20912–20917, 2009.

[71] R. G. Hunter, G. Murakami, S. Dewell et al., "Acute stress and hippocampal histone H3 lysine 9 trimethylation, a retrotransposon silencing response," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 43, pp. 17657–17662, 2012.

[72] F. Hollis, F. Duclot, A. Gunjan, and M. Kabbaj, "Individual differences in the effect of social defeat on anhedonia and histone acetylation in the rat hippocampus," *Hormones and Behavior*, vol. 59, no. 3, pp. 331–337, 2011.

[73] C. A. Kenworthy, A. Sengupta, S. M. Luz et al., "Social defeat induces changes in histone acetylation and expression of histone modifying enzymes in the ventral hippocampus, prefrontal cortex, and dorsal raphe nucleus," *Neuroscience*, vol. 264, pp. 88–98, 2014.

[74] Y. Chandramohan, S. K. Droste, J. S. C. Arthur, and J. M. H. M. Reul, "The forced swimming-induced behavioural immobility response involves histone H3 phospho-acetylation and c-Fos induction in dentate gyrus granule neurons via activation of the N-methyl-D-aspartate/extracellular signal-regulated kinase/mitogen- and stress-activated kinase signalling pathway," *European Journal of Neuroscience*, vol. 27, no. 10, pp. 2701–2713, 2008.

[75] M. Hinwood, R. J. Tynan, T. A. Day, and F. R. Walker, "Repeated social defeat selectively increases δfosB expression and histone h3 acetylation in the infralimbic medial prefrontal cortex," *Cerebral Cortex*, vol. 21, no. 2, pp. 262–271, 2011.

[76] C. Cruceanu, M. Alda, C. Nagy, E. Freemantle, G. A. Rouleau, and G. Turecki, "H3K4 tri-methylation in synapsin genes leads to different expression patterns in bipolar disorder and major depression," *International Journal of Neuropsychopharmacology*, vol. 16, no. 2, pp. 289–299, 2013.

[77] V. Vialou, J. Feng, A. J. Robison, and E. J. Nestler, "Epigenetic mechanisms of depression and antidepressant action," *Annual Review of Pharmacology and Toxicology*, vol. 53, pp. 59–87, 2013.

[78] M. Fuchikami, S. Morinobu, A. Kurata, S. Yamamoto, and S. Yamawaki, "Single immobilization stress differentially alters the expression profile of transcripts of the brain-derived neurotrophic factor (BDNF) gene and histone acetylation at its promoters in the rat hippocampus," *International Journal of Neuropsychopharmacology*, vol. 12, no. 1, pp. 73–82, 2009.

[79] T. Aid, A. Kazantseva, M. Piirsoo, K. Palm, and T. Timmusk, "Mouse and rat BDNF gene structure and expression revisited," *Journal of Neuroscience Research*, vol. 85, no. 3, pp. 525–535, 2007.

[80] N. M. Tsankova, O. Berton, W. Renthal, A. Kumar, R. L. Neve, and E. J. Nestler, "Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action," *Nature Neuroscience*, vol. 9, no. 4, pp. 519–525, 2006.

[81] A. Ieraci, A. Mallei, L. Musazzi, and M. Popoli, "Physical exercise and acute restraint stress differentially modulate hippocampal brain-derived neurotrophic factor transcripts and epigenetic mechanisms in mice," *Hippocampus*, vol. 25, no. 11, pp. 1380–1392, 2015.

[82] A. E. Pasquinelli, "MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship," *Nature Reviews Genetics*, vol. 13, no. 4, pp. 271–282, 2012.

[83] O. Issler and A. Chen, "Determining the role of microRNAs in psychiatric disorders," *Nature Reviews Neuroscience*, vol. 16, no. 4, pp. 201–212, 2015.

[84] S. Haramati, I. Navon, O. Issler et al., "MicroRNA as repressors of stress-induced anxiety: the case of amygdalar miR-34," *Journal of Neuroscience*, vol. 31, no. 40, pp. 14191–14203, 2011.

[85] O. Issler, S. Haramati, E. D. Paul et al., "MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity," *Neuron*, vol. 83, no. 2, pp. 344–360, 2014.

[86] N. Volk, E. D. Paul, S. Haramati et al., "MicroRNA-19b associates with Ago2 in the amygdala following chronic stress and regulates the adrenergic receptor beta 1," *The Journal of Neuroscience*, vol. 34, no. 45, pp. 15070–15082, 2014.

[87] S. King and D. P. Laplante, "The effects of prenatal maternal stress on children's cognitive development: project ice storm," *Stress*, vol. 8, no. 1, pp. 35–45, 2005.

[88] L. Cao-Lei, R. Massart, M. J. Suderman et al., "DNA methylation signatures triggered by prenatal maternal stress exposure to a natural disaster: Project ice storm," *PLoS ONE*, vol. 9, no. 9, Article ID e107653, 2014.

[89] L. Cao-Lei, G. Elgbeili, R. Massart, D. P. Laplante, M. Szyf, and S. King, "Pregnant women's cognitive appraisal of a natural disaster affects DNA methylation in their children 13 years later: Project Ice Storm," *Translational Psychiatry*, vol. 2, article e515, 2015.

[90] D. P. Laplante, A. Brunet, and S. King, "The effects of maternal stress and illness during pregnancy on infant temperament: project Ice Storm," *Pediatric Research*, vol. 79, pp. 107–113, 2015.

[91] S. King, K. Dancause, A.-M. Turcotte-Tremblay, F. Veru, and D. P. Laplante, "Using natural disasters to study the effects of prenatal maternal stress on child health and development," *Birth Defects Research Part C—Embryo Today: Reviews*, vol. 96, no. 4, pp. 273–288, 2012.

[92] S. Maccari, P. V. Piazza, M. Kabbaj, A. Barbazanges, H. Simon, and M. Le Moal, "Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress," *The Journal of Neuroscience*, vol. 15, no. 1, part 1, pp. 110–116, 1995.

[93] A. Barbazanges, P. V. Piazza, M. Le Moal, and S. Maccari, "Maternal glucocorticoid secretion mediates long-term effects of prenatal stress," *The Journal of Neuroscience*, vol. 16, no. 12, pp. 3943–3949, 1996.

[94] A. S. Brown and E. S. Susser, "Prenatal nutritional deficiency and risk of adult schizophrenia," *Schizophrenia Bulletin*, vol. 34, no. 6, pp. 1054–1063, 2008.

[95] A. S. Brown, S. Vinogradov, W. S. Kremen et al., "Prenatal exposure to maternal infection and executive dysfunction in adult schizophrenia," *The American Journal of Psychiatry*, vol. 166, no. 6, pp. 683–690, 2009.

[96] A. S. Brown, "Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism," *Developmental Neurobiology*, vol. 72, no. 10, pp. 1272–1276, 2012.

[97] M. J. Meaney, "Epigenetics and the biological definition of gene X environment interactions," *Child Development*, vol. 81, no. 1, pp. 41–79, 2010.

[98] D. M. Lyons, F. L. Martel, S. Levine, N. J. Risch, and A. F. Schatzberg, "Postnatal experiences and genetic effects on squirrel monkey social affinities and emotional distress," *Hormones and Behavior*, vol. 36, no. 3, pp. 266–275, 1999.

[99] M. M. Sánchez, C. O. Ladd, and P. M. Plotsky, "Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models," *Development and Psychopathology*, vol. 13, no. 3, pp. 419–449, 2001.

[100] M. L. Schneider, C. F. Moore, G. W. Kraemer, A. D. Roberts, and O. T. DeJesus, "The impact of prenatal stress, fetal alcohol exposure, or both on development: perspectives from a primate model," *Psychoneuroendocrinology*, vol. 27, no. 1-2, pp. 285–298, 2002.

[101] C. L. Coe, M. Kramer, B. Czéh et al., "Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile Rhesus monkeys," *Biological Psychiatry*, vol. 54, no. 10, pp. 1025–1034, 2003.

[102] B. R. Mueller and T. L. Bale, "Sex-specific programming of offspring emotionality after stress early in pregnancy," *The Journal of Neuroscience*, vol. 28, no. 36, pp. 9055–9065, 2008.

[103] A. Kapoor, A. Kostaki, C. Janus, and S. G. Matthews, "The effects of prenatal stress on learning in adult offspring is dependent on the timing of the stressor," *Behavioural Brain Research*, vol. 197, no. 1, pp. 144–149, 2009.

[104] A. Korosi and T. Z. Baram, "Plasticity of the stress response early in life: mechanisms and significance," *Developmental Psychobiology*, vol. 52, no. 7, pp. 661–670, 2010.

[105] R. I. Martínez-Téllez, E. Hernández-Torres, C. Gamboa, and G. Flores, "Prenatal stress alters spine density and dendritic length of nucleus accumbens and hippocampus neurons in rat offspring," *Synapse*, vol. 63, no. 9, pp. 794–804, 2009.

[106] V. G. Barros, M. Duhalde-Vega, L. Caltana, A. Brusco, and M. C. Antonelli, "Astrocyte-neuron vulnerability to prenatal stress in the adult rat brain," *Journal of Neuroscience Research*, vol. 83, no. 5, pp. 787–800, 2006.

[107] M. S. Murmu, S. Salomon, Y. Biala, M. Weinstock, K. Braun, and J. Bock, "Changes of spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy," *European Journal of Neuroscience*, vol. 24, no. 5, pp. 1477–1487, 2006.

[108] T. Suenaga, M. Yukie, S. Gao, and D. Nakahara, "Sex-specific effects of prenatal stress on neuronal development in the medial prefrontal cortex and the hippocampus," *NeuroReport*, vol. 23, no. 7, pp. 430–435, 2012.

[109] C. Pickering, L. Gustafsson, A. Cebere, I. Nylander, and S. Liljequist, "Repeated maternal separation of male Wistar rats alters glutamate receptor expression in the hippocampus but not the prefrontal cortex," *Brain Research*, vol. 1099, no. 1, pp. 101–108, 2006.

[110] R. M. O'Connor, M. M. Pusceddu, T. G. Dinan, and J. F. Cryan, "Impact of early-life stress, on group III mGlu receptor levels in the rat hippocampus: effects of ketamine, electroconvulsive shock therapy and fluoxetine treatment," *Neuropharmacology*, vol. 66, pp. 236–241, 2013.

[111] G. H. Son, D. Geum, S. Chung et al., "Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity," *The Journal of Neuroscience*, vol. 26, no. 12, pp. 3309–3318, 2006.

[112] A. R. Zuena, J. Mairesse, P. Casolini et al., "Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats," *PLoS ONE*, vol. 3, no. 5, Article ID e2170, 2008.

[113] E. Adrover, M. E. Pallarés, C. J. Baier et al., "Glutamate neurotransmission is affected in prenatally stressed offspring," *Neurochemistry International*, vol. 88, pp. 73–87, 2015.

[114] M. A. Berger, V. G. Barros, M. I. Sarchi, F. I. Tarazi, and M. C. Antonelli, "Long-term effects of prenatal stress on dopamine and glutamate receptors in adult rat brain," *Neurochemical Research*, vol. 27, no. 11, pp. 1525–1533, 2002.

[115] V. G. Barros, M. A. Berger, I. D. Martijena et al., "Early adoption modifies the effects of prenatal stress on dopamine and glutamate receptors in adult rat brain," *Journal of Neuroscience Research*, vol. 76, no. 4, pp. 488–496, 2004.

[116] C.-M. Yeh, C.-C. Huang, and K.-S. Hsu, "Prenatal stress alters hippocampal synaptic plasticity in young rat offspring through preventing the proteolytic conversion of pro-brain-derived neurotrophic factor (BDNF) to mature BDNF," *The Journal of Physiology*, vol. 590, no. 4, pp. 991–1010, 2012.

[117] J. Marrocco, J. Mairesse, R. T. Ngomba et al., "Anxiety-like behavior of prenatally stressed rats is associated with a selective reduction of glutamate release in the ventral hippocampus," *The Journal of Neuroscience*, vol. 32, no. 48, pp. 17143–17154, 2012.

[118] J. Marrocco, M.-L. Reynaert, E. Gatta et al., "The effects of antidepressant treatment in prenatally stressed rats support the glutamatergic hypothesis of stress-related disorders," *The Journal of Neuroscience*, vol. 34, no. 6, pp. 2015–2024, 2014.

[119] M. S. Fanselow and H.-W. Dong, "Are the dorsal and ventral hippocampus functionally distinct structures?" *Neuron*, vol. 65, no. 1, pp. 7–19, 2010.

[120] J. Mairesse, E. Gatta, M. Reynaert et al., "Activation of presynaptic oxytocin receptors enhances glutamate release in the ventral hippocampus of prenatally restraint stressed rats," *Psychoneuroendocrinology*, vol. 62, pp. 36–46, 2015.

[121] L. A. M. Welberg, J. R. Seckl, and M. C. Holmes, "Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: possible implications for behaviour," *Neuroscience*, vol. 104, no. 1, pp. 71–79, 2001.

[122] S. J. Suomi, "Early stress and adult emotional reactivity in rhesus monkeys," *Ciba Foundation Symposium*, vol. 156, pp. 171–188, 1991.

[123] G. Contia, C. Hansman, J. J. Heckman, M. F. X. Novak, A. Ruggiero, and S. J. Suomi, "Primate evidence on the late health effects of early-life adversity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 23, pp. 8866–8871, 2012.

[124] F. Cirulli, N. Francia, I. Branchi et al., "Changes in plasma levels of BDNF and NGF reveal a gender-selective vulnerability to early adversity in rhesus macaques," *Psychoneuroendocrinology*, vol. 34, no. 2, pp. 172–180, 2009.

[125] S. Spinelli, S. Chefer, S. J. Suomi, J. D. Higley, C. S. Barr, and E. Stein, "Early-life stress induces long-term morphologic changes in primate brain," *Archives of General Psychiatry*, vol. 66, no. 6, pp. 658–665, 2009.

[126] R. L. Huot, M. E. Gonzalez, C. O. Ladd, K. V. Thrivikraman, and P. M. Plotsky, "Foster litters prevent hypothalamic-pituitary-adrenal axis sensitization mediated by neonatal maternal separation," *Psychoneuroendocrinology*, vol. 29, no. 2, pp. 279–289, 2004.

[127] S. Levine, "Infantile experience and resistance to physiological stress," *Science*, vol. 126, no. 3270, article 405, 1957.

[128] V. Lemaire, M. Koehl, M. Le Moal, and D. N. Abrous, "Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 20, pp. 11032–11037, 2000.

[129] L. A. M. Welberg and J. R. Seckl, "Prenatal stress, glucocorticoids and the programming of the brain," *Journal of Neuroendocrinology*, vol. 13, no. 2, pp. 113–128, 2001.

[130] P. J. Brunton, "Programming the brain and behaviour by early-life stress: a focus on neuroactive steroids," *Journal of Neuroendocrinology*, vol. 27, no. 6, pp. 468–480, 2015.

[131] S. Macrì and G. Laviola, "Single episode of maternal deprivation and adult depressive profile in mice: interaction with cannabinoid exposure during adolescence," *Behavioural Brain Research*, vol. 154, no. 1, pp. 231–238, 2004.

[132] P. M. Plotsky and M. J. Meaney, "Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats," *Molecular Brain Research*, vol. 18, no. 3, pp. 195–200, 1993.

[133] C. M. McCormick, P. Kehoe, and S. Kovacs, "Corticosterone release in response to repeated, short episodes of neonatal isolation: evidence of sensitization," *International Journal of Developmental Neuroscience*, vol. 16, no. 3-4, pp. 175–185, 1998.

[134] R. L. Huot, P. M. Plotsky, R. H. Lenox, and R. K. McNamara, "Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats," *Brain Research*, vol. 950, no. 1-2, pp. 52–63, 2002.

[135] J.-H. Lee, H. J. Kim, J. G. Kim et al., "Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation," *Neuroscience Research*, vol. 58, no. 1, pp. 32–39, 2007.

[136] W. M. U. Daniels, C. Y. Pietersen, M. E. Carstens, and D. J. Stein, "Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to a subsequent stressor," *Metabolic Brain Disease*, vol. 19, no. 1-2, pp. 3–14, 2004.

[137] S. Uchida, K. Hara, A. Kobayashi et al., "Early life stress enhances behavioral vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents," *The Journal of Neuroscience*, vol. 30, no. 45, pp. 15007–15018, 2010.

[138] L. Enthoven, M. S. Oitzl, N. Koning, M. van der Mark, and E. R. de Kloet, "Hypothalamic-pituitary-adrenal axis activity of newborn mice rapidly desensitizes to repeated maternal absence but becomes highly responsive to novelty," *Endocrinology*, vol. 149, no. 12, pp. 6366–6377, 2008.

[139] M. Kundakovic and F. A. Champagne, "Early-life experience, epigenetics, and the developing brain," *Neuropsychopharmacology*, vol. 40, no. 1, pp. 141–153, 2015.

[140] N. Provençal and E. B. Binder, "The effects of early life stress on the epigenome: from the womb to adulthood and even before," *Experimental Neurology*, vol. 268, pp. 10–20, 2015.

[141] O. Babenko, I. Kovalchuk, and G. A. S. Metz, "Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health," *Neuroscience and Biobehavioral Reviews*, vol. 48, pp. 70–91, 2015.

[142] I. C. G. Weaver, N. Cervoni, F. A. Champagne et al., "Epigenetic programming by maternal behavior," *Nature Neuroscience*, vol. 7, no. 8, pp. 847–854, 2004.

[143] I. C. G. Weaver, "Epigenetic programming by maternal behavior and pharmacological intervention—nature versus nurture: let's call the whole thing off," *Epigenetics*, vol. 2, no. 1, pp. 22–28, 2007.

[144] P. O. McGowan, A. Sasaki, A. C. D'Alessio et al., "Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse," *Nature Neuroscience*, vol. 12, no. 3, pp. 342–348, 2009.

[145] T. Y. Zhang, B. Labonté, X. L. Wen, G. Turecki, and M. J. Meaney, "Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans," *Neuropsychopharmacology*, vol. 38, no. 1, pp. 111–123, 2013.

[146] V. Vukojevic, I.-T. Kolassa, M. Fastenrath et al., "Epigenetic modification of the glucocorticoid receptor gene is linked to traumatic memory and post-traumatic stress disorder risk in genocide survivors," *The Journal of Neuroscience*, vol. 34, no. 31, pp. 10274–10284, 2014.

[147] D. Amelung, H. J. Hubener, L. Roka, and G. Meyerheim, "Conversion of cortisone to compound F," *The Journal of Clinical Endocrinology and Metabolism*, vol. 13, no. 9, pp. 1125–1126, 1953.

[148] J. R. Seckl and K. E. Chapman, "The 11β-hydroxysteroid dehydrogenase system, a determinant of glucocorticoid and mineralocorticoid action: medical and physiological aspects of the 11β-hydroxysteroid dehydrogenase system," *European Journal of Biochemistry*, vol. 249, no. 2, pp. 361–364, 1997.

[149] J. R. Seckl and B. R. Walker, "Minireview: 11β-hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action," *Endocrinology*, vol. 142, no. 4, pp. 1371–1376, 2001.

[150] C. Jensen Peña, C. Monk, and F. A. Champagne, "Epigenetic effects of prenatal stress on 11β-hydroxysteroid dehydrogenase-2 in the placenta and fetal brain," *PLoS ONE*, vol. 7, no. 6, Article ID e39791, 2012.

[151] K. L. Togher, M. M. O'Keeffe, A. S. Khashan, H. Gutierrez, L. C. Kenny, and G. W. O'Keeffe, "Epigenetic regulation of the placental HSD11B2 barrier and its role as a critical regulator of fetal development," *Epigenetics*, vol. 9, no. 6, pp. 816–822, 2014.

[152] B. M. Nugent and T. L. Bale, "The omniscient placenta: metabolic and epigenetic regulation of fetal programming," *Frontiers in Neuroendocrinology*, vol. 39, pp. 28–37, 2015.

[153] A. A. Appleton, D. A. Armstrong, C. Lesseur et al., "Patterning in placental 11-B hydroxysteroid dehydrogenase methylation according to prenatal socioeconomic adversity," *PLoS ONE*, vol. 8, no. 9, Article ID e74691, 2013.

[154] C. J. Marsit, M. A. Maccani, J. F. Padbury, and B. M. Lester, "Placental 11-beta hydroxysteroid dehydrogenase methylation is associated with newborn growth and a measure of neurobehavioral outcome," *PLoS ONE*, vol. 7, no. 3, Article ID e33794, 2012.

[155] J. M. Stern, L. Goldman, and S. Levine, "Pituitary adrenal responsiveness during lactation in rats," *Neuroendocrinology*, vol. 12, no. 3, pp. 179–191, 1973.

[156] I. D. Neumann, H. A. Johnstone, M. Hatzinger et al., "Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes," *The Journal of Physiology*, vol. 508, no. 1, pp. 289–300, 1998.

[157] S. L. Lightman, R. J. Windle, S. A. Wood, Y. M. Kershaw, N. Shanks, and C. D. Ingram, "Peripartum plasticity within the hypothalamo-pituitary-adrenal axis," *Progress in Brain Research*, vol. 133, pp. 111–129, 2001.

[158] R. J. Windle, S. Wood, N. Shanks et al., "Endocrine and behavioural responses to noise stress: comparison of virgin and lactating female rats during non-disrupted maternal activity," *Journal of Neuroendocrinology*, vol. 9, no. 6, pp. 407–414, 1997.

[159] I. D. Neumann, "Brain mechanisms underlying emotional alterations in the peripartum period in rats," *Depression and Anxiety*, vol. 17, no. 3, pp. 111–121, 2003.

[160] C. A. Pedersen, J. A. Ascher, Y. L. Monroe, and A. J. Prange Jr., "Oxytocin induces maternal behavior in virgin female rats," *Science*, vol. 216, no. 4546, pp. 648–649, 1982.

[161] S. C. Gammie, A. Negron, S. M. Newman, and J. S. Rhodes, "Corticotropin-releasing factor inhibits maternal aggression in mice," *Behavioral Neuroscience*, vol. 118, no. 4, pp. 805–814, 2004.

[162] O. J. Bosch and I. D. Neumann, "Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: from central release to sites of action," *Hormones and Behavior*, vol. 61, no. 3, pp. 293–303, 2012.

[163] E. R. de Kloet, D. A. M. Voorhuis, Y. Boschma, and J. Elands, "Estradiol modulates density of putative 'oxytocin receptors' in discrete rat brain regions," *Neuroendocrinology*, vol. 44, no. 4, pp. 415–421, 1986.

[164] T. L. Bale, D. M. Dorsa, and C. A. Johnston, "Oxytocin receptor mRNA expression in the ventromedial hypothalamus during the estrous cycle," *The Journal of Neuroscience*, vol. 15, no. 7 I, pp. 5058–5064, 1995.

[165] L. J. Young, S. Muns, Z. Wang, and T. R. Insel, "Changes in oxytocin receptor mRNA in rat brain during pregnancy and the effects of estrogen and interleukin-6," *Journal of Neuroendocrinology*, vol. 9, no. 11, pp. 859–865, 1997.

[166] D. Huber, P. Veinante, and R. Stoop, "Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala," *Science*, vol. 308, no. 5719, pp. 245–248, 2005.

[167] D. A. Slattery and I. D. Neumann, "No stress please! Mechanisms of stress hyporesponsiveness of the maternal brain," *Journal of Physiology*, vol. 586, no. 2, pp. 377–385, 2008.

[168] O. J. Bosch, S. L. Meddle, D. I. Beiderbeck, A. J. Douglas, and I. D. Neumann, "Brain oxytocin correlates with maternal aggression: Link to anxiety," *The Journal of Neuroscience*, vol. 25, no. 29, pp. 6807–6815, 2005.

[169] D. D. Francis, F. C. Champagne, and M. J. Meaney, "Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat," *Journal of Neuroendocrinology*, vol. 12, no. 12, pp. 1145–1148, 2000.

[170] S. Grimm, K. Pestke, M. Feeser et al., "Early life stress modulates oxytocin effects on limbic system during acute psychosocial stress," *Social Cognitive and Affective Neuroscience*, vol. 9, no. 11, pp. 1828–1835, 2014.

[171] M. A. de Souza, L. A. Centenaro, P. R. Menegotto et al., "Prenatal stress produces social behavior deficits and alters the number of oxytocin and vasopressin neurons in adult rats," *Neurochemical Research*, vol. 38, no. 7, pp. 1479–1489, 2013.

[172] Y. Fan, K. Pestke, M. Feeser et al., "Amygdala-hippocampal connectivity changes during acute psychosocial stress: joint effect of early life stress and oxytocin," *Neuropsychopharmacology*, vol. 40, no. 12, pp. 2736–2744, 2015.

[173] A. K. Beery, L. M. McEwen, J. L. MacIsaac, D. D. Francis, and M. S. Kobor, "Natural variation in maternal care and cross-tissue patterns of oxytocin receptor gene methylation in rats," *Hormones and Behavior*, vol. 77, pp. 42–52, 2016.

[174] E. Unternaehrer, A. H. Meyer, S. C. Burkhardt et al., "Childhood maternal care is associated with DNA methylation of the genes for brain-derived neurotrophic factor (BDNF) and oxytocin receptor (OXTR) in peripheral blood cells in adult men and women," *Stress*, vol. 18, no. 4, pp. 451–461, 2015.

[175] J. Belsky and M. Pluess, "Beyond diathesis stress: differential susceptibility to environmental influences," *Psychological Bulletin*, vol. 135, no. 6, pp. 885–908, 2009.

[176] T. Klengel and E. B. Binder, "Gene × environment interactions in the prediction of response to antidepressant treatment," *International Journal of Neuropsychopharmacology*, vol. 16, no. 3, pp. 701–711, 2013.

[177] C. Finsterwald and C. M. Alberini, "Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: from adaptive responses to psychopathologies," *Neurobiology of Learning and Memory*, vol. 112, pp. 17–29, 2014.

[178] G. Aguilera, "HPA axis responsiveness to stress: implications for healthy aging," *Experimental Gerontology*, vol. 46, no. 2-3, pp. 90–95, 2011.

[179] R. D. Marshall and A. Garakani, "Psychobiology of the acute stress response and its relationship to the psychobiology of post-traumatic stress disorder," *Psychiatric Clinics of North America*, vol. 25, no. 2, pp. 385–395, 2002.

[180] S. Deppermann, H. Storchak, A. J. Fallgatter, and A.-C. Ehlis, "Stress-induced neuroplasticity: (mal)adaptation to adverse life events in patients with PTSD—a critical overview," *Neuroscience*, vol. 283, pp. 166–177, 2014.

[181] S. Macrì, F. Zoratto, and G. Laviola, "Early-stress regulates resilience, vulnerability and experimental validity in laboratory rodents through mother-offspring hormonal transfer," *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 7, pp. 1534–1543, 2011.

[182] D. G. Gee, K. L. Humphreys, J. Flannery et al., "A developmental shift from positive to negative connectivity in human amygdala-prefrontal circuitry," *The Journal of Neuroscience*, vol. 33, no. 10, pp. 4584–4593, 2013.

[183] P. Willner, "Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS," *Neuropsychobiology*, vol. 52, no. 2, pp. 90–110, 2005.

[184] S. Kim, D. Noh, and S. I. Park, "Mediating effect of stress on the association between early trauma and psychological distress in Korean college students: a cross-sectional observational study," *Journal of Psychiatric and Mental Health Nursing*, vol. 22, no. 10, pp. 784–791, 2015.

[185] E. J. Nestler, C. J. Peña, M. Kundakovic, A. Mitchell, and S. Akbarian, "Epigenetic basis of mental illness," *The Neuroscientist*, 2015.

[186] N. J. Grundwald and P. J. Brunton, "Trans-generational effects of prenatal stress on the neuroendocrine stress axis in rats," *Psychoneuroendocrinology*, vol. 61, article 30, 2015.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 5054275, 20 pages
http://dx.doi.org/10.1155/2016/5054275

Research Article

Postischemic Anhedonia Associated with Neurodegenerative Changes in the Hippocampal Dentate Gyrus of Rats

Jiro Kasahara, Hiroto Uchida, Kenta Tezuka, and Nanae Oka

Department of Neurobiology and Therapeutics, Institute of Biomedical Sciences, Graduate School and Faculty of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan

Correspondence should be addressed to Jiro Kasahara; awajiro@tokushima-u.ac.jp

Received 25 September 2015; Revised 10 January 2016; Accepted 17 January 2016

Academic Editor: Laura Musazzi

Poststroke depression is one of the major symptoms observed in the chronic stage of brain stroke such as cerebral ischemia. Its pathophysiological mechanisms, however, are not well understood. Using the transient right middle cerebral artery occlusion- (MCAO-, 90 min) operated rats as an ischemia model in this study, we first observed that aggravation of anhedonia spontaneously occurred especially after 20 weeks of MCAO, and it was prevented by chronic antidepressants treatment (imipramine or fluvoxamine). The anhedonia specifically associated with loss of the granular neurons in the ipsilateral side of hippocampal dentate gyrus and was also prevented by an antidepressant imipramine. Immunohistochemical analysis showed increased apoptosis inside the granular cell layer prior to and associated with the neuronal loss, and imipramine seemed to recover the survival signal rather than suppressing the death signal to prevent neurons from apoptosis. Proliferation and development of the neural stem cells were increased transiently in the subgranular zone of both ipsi- and contralateral hippocampus within one week after MCAO and then decreased and almost ceased after 6 weeks of MCAO, while chronic imipramine treatment prevented them partially. Overall, our study suggests new insights for the mechanistic correlation between poststroke depression and the delayed neurodegenerative changes in the hippocampal dentate gyrus with effective use of antidepressants on them.

1. Introduction

Transient focal cerebral ischemia is the most common type of stroke caused by occlusion of a cerebral artery [1]. It causes both acute and chronic dysfunctions in the central nervous system (CNS) and lowers the quality of life in patients for a long period of lifetime. The middle cerebral artery (MCA) is most frequently infarcted in the cerebral ischemia, and various animal models have been developed including nonhuman primates and rodents [2, 3]. In the models of transient focal cerebral ischemia, neurons in the ischemic core including cerebral cortex and some parts of the striatum were immediately damaged after the ischemia-reperfusion manipulation, sometimes followed by the delayed neuronal death in the areas apart from the ischemic core, including a part of thalamus, substantia nigra, and hippocampus [4–6]. They cause various dysfunctions such as cognitive, mood/emotional, and motor impairments in the chronic stage after stroke.

Among the CNS dysfunctions in the chronic stage of cerebral ischemia, depression is one of the major mood/emotional impairments known as poststroke depression (PSD). It has been generally recognized that PSD occurred in around 40% of the stroke patients [7, 8], although it varies depending on the studies from around 20% [9] to 72% [10]. Because the physical disabilities lowering the activities of daily living are the stressor on the stroke patients, PSD has been believed to occur as the result of psychogenic and systemic responses to the stressed conditions with complicated mechanisms of pathogenesis [11]. Robinson and Price reported their follow-up study of 103 stroke patients with evaluating PSD [12, 13], confirming that the lesion location (frontal area in the left hemisphere and posterior area in the right hemisphere) determined frequency and severity of depression. It was the first study suggesting the PSD pathogenesis as a neurodegenerative lesion in a particular brain area. Now, PSD pathogenesis is considered to be multifactorial with neurodegenerative, psychogenic, and genetic mechanisms.

Animal models of PSD have been reported especially using rodents [14], mostly combining a surgical operation (MCA occlusion) with application of extra stressors such as unpredicted chronic mild stress (UCMS) [15, 16], ovariectomy [17], and spatial restraint stress [18].

Among various symptoms seen in PSD, anhedonia is one of the typical ones: loss of interest or pleasure in almost all the activities and things that one previously liked [19]. Pathogenesis of anhedonia includes brain areas such as orbitofrontal cortex, nuclear accumbens, and ventral pallidum. A recent study reported a positive correlation between the poststischemic anhedonia with salivary cortisol levels and reduction of volume by lesion in parahippocampal/hippocampal area [20].

Hippocampus is one of the vulnerable areas to the ischemic stress, showing delayed neuronal death in CA1 region within a few days to a week after MCA occlusion (MCAO) [6, 21, 22]. Because hippocampus is deeply related to higher brain functions such as cognition, learning, and memory, CA1 degeneration causes functional impairments of them after stroke. Different from CA1, other regions such as CA3 and dentate gyrus (DG) in the hippocampus are considered to be resistant to the ischemic stress [23, 24]. The neurogenesis in subgranular zone (SGZ) of DG as well as cortical subventricular zone (SVZ) produces newly generated neurons even in adulthood and is known to increase neural stem cells (NSCs) proliferation and differentiation into neurons after the transient brain ischemia [25, 26]. Because the rats that received UCMS after MCAO treatment had reduced neurogenesis in DG, it was considered as an adaptive or a compensatory process against the poststroke stressors [15]. Proliferation and differentiation of NSCs in SGZ are controlled by various factors such as stress, mood/emotion, environment, corticosteroids, and antidepressants [27, 28].

Based on these backgrounds, we initially examined whether anhedonia could be spontaneously induced after MCAO in rats while observing them for up to 30 weeks together with the effects of antidepressant imipramine (IMP) or fluvoxamine (FLV) in this study. The reason why we chose anhedonia rather than other depression-related behaviors such as the forced swimming or tail suspension was to minimize the negative effects of the motor impairments directly influenced by MCAO. We also evaluated the long-term neurodegenerative changes of the hippocampal DG, including apoptosis of the granular cells (GC) and the neurogenesis in SGZ, with the effects of IMP.

2. Materials and Methods

2.1. Experimental Animal. Young male SD rats weighing 200–250 g at 6 weeks of age were purchased from Nihon SLC, Co., Shizuoka, Japan. They were housed in a controlled environment (23 ± 1°C, 50 ± 5% humidity) and were allowed food and tap water *ad libitum* and habituated for 2 weeks before the surgical operation. The room lights were on between 8:00 and 20:00 (illuminated with 950 lux). The procedure of the surgical operations for transient MCAO was described in our previous publications [22, 29–31]. In brief, the right middle cerebral artery of the rat was occluded for 90 min by inserting a silicone-coated 4-0 monofilament after the right carotid artery was exposed and separated into the internal and the external ones with careful conservation of the vagus nerve under anesthetization with 2% halothane in a mixture of 30% oxygen and 70% nitrous oxide. One day after the reperfusion of blood flow by withdrawing the filament, animals that showed grade 3 or over of Hunter's neurological score [32] were used for further experiments. To examine the behavioral changes after the surgical operation for 30 weeks, 40 rats were divided into 4 groups: sham, sham + antidepressants, MCAO, and MCAO + antidepressants (N = 10/group, total N = 40). For the immunohistochemical examinations, another 80 rats were divided equally into two groups of sham and MCAO, and 5 rats/group were sacrificed at 1 day (1 d), 3 days (3 d), 1 week (1 w), 2 weeks (2 w), 6 weeks (6 w), 20 weeks (20 w), and 30 weeks (30 w) after the surgical operation. Naïve animal (N = 5) means the rats fixed without any surgical operation at 8 weeks of age.

All handling and procedures of animal experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications number 8023, revised 1978) and approved by the Committee for Animal Experiments of Tokushima University.

2.2. Antidepressant Treatment. From 1 week after MCAO, the antidepressant imipramine hydrochloride or a selective serotonin reuptake inhibitor (SSRI) fluvoxamine maleate (Sigma, St. Louis, MO, USA) was dissolved directly in tap water based on the daily amount of water consumption so that the rats could absorb the drugs with 20 mg/kg/day. The doses were determined based on the previous reports [33–36]. For drug administration, we avoided a direct handling procedure such as intraperitoneal injection to minimize any noxious stressor on rats after MCAO.

2.3. Sucrose Preference Test. This test is to evaluate anhedonia, one of indices of depressiveness [37]. For habituation during one week before MCAO, animals were exposed to water containing 1% sucrose twice for 2 successive days separated by a 2-day interval substituted by normal tap water. The tests were performed at 1 w, 2 w, 6 w, 20 w, and 30 w after MCAO during the dark period (20:00–8:00) with presenting two bottles of water simultaneously (one was normal while the other one contained 1% sucrose), and the percentage of sucrose preference was calculated as (sucrose water intake)/(total water intake).

2.4. Open Field Test. This test was performed to evaluate the spontaneous activity of the rats at 1 w, 2 w, 6 w, 20 w, and 30 w after MCAO. The rats were put in an open field (50 cm × 50 cm, divided into 25 squares of 10 cm × 10 cm by line grids) under the 950-lux illumination for 10 min and were recorded by a video camera set at 60 cm high above the field. The number of stepping instances over the grids was counted as the horizontal activity, and the number of rearing instances was counted as the vertical activity, by an examiner unaware of the grouping details.

2.5. Immunohistochemistry. The procedure of the immunohistochemical examinations was followed essentially by the previous studies in our laboratory [22, 29–31]. Rats were anesthetized with intraperitoneal injection of 50 mg/kg of sodium pentobarbital solution at 1 d, 3 d, 1 w, 2 w, 6 w, 20 w, and 30 w after MCAO, flashed with 1% heparin-containing saline, and fixed with perfusion of 4% paraformaldehyde (Wako, Osaka, Japan) in phosphate buffered saline (PBS, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 10 mM KH_2PO_4). One day after the fixation, brains were excised out and kept in the same fixative solution for one night. The fixed brains were washed with PBS for 1 hr × 3 times and dehydrated in ethanol (70, 80, 95, and 100%) and then embedded in paraffin. The coronal sections of the dorsal hippocampus (between bregmata −2.5 mm and −4.5 mm) with 5 μm thickness were sliced out by a sliding microtome (TU-213, Yamato, Saitama, Japan), and 4 slices/animal (for one primary antibody) were taken out from each of the 0.5 mm ranges and mounted on the slide glasses. For deparaffinization, the slices were heated at 59°C for 1 hr, treated with xylene, ethanol, and dH_2O, and then served for the immunohistochemical examinations. They were washed with PBS for 3 min × 3 times and incubated with the blocking solution containing 10% normal horse serum (Vector Labs, CA, USA) and 0.3% Triton X-100 in PBS for 60 min at room temperature.

For the primary antibodies of immunostaining, anti-neuronal nuclei (NeuN, monoclonal, Chemicon-Millipore, CA, USA, 1 : 200), anti-βIII-tubulin (monoclonal, Abcam, MA, USA, 1 : 400), anti-MAP2 (monoclonal, Abcam, MA, USA, 1 : 1500), anti-Bax (polyclonal, Santa Cruz, CA, USA, 1 : 100), anti-Bcl-2α (monoclonal, Lab Vision, ND, USA, 1 : 50), anti-Nestin (monoclonal, Chemicon, CA, USA, 1 : 500), anti-Ki67 (polyclonal, Abcam, MA, USA, 1 : 400), and anti-doublecortin (DCX, polyclonal, Abcam, MA, USA, 1 : 1000) were used. They were diluted in the blocking solution and incubated with the slices for one night at 4°C. The slices were washed with PBS for 3 min × 3 times before incubating with the secondary antibody. To visualize the bound primary antibodies, a Vectastain elite ABC kit and 3′,3′-diaminobenzidine (DAB) substrate kit (Vector Labs, CA, USA) were used according to the manufacturer's protocol. As negative control sections, each primary antibody or the secondary antibody was omitted. All the slices on the slide glasses were covered by cover glasses with VECTASHIELD mounting medium (Vector Labs, CA, USA). They were observed and recorded with a digital camera-equipped BX51 light microscope (Olympus, Tokyo, Japan) at a magnitude of 12.5x (cresyl violet) or 200x (NeuN, Bax, Bcl-2α, and DCX) or 400x (βIII-tubulin, MAP2, Nestin, and Ki67). To detect anti-cleaved caspase-3 antibody (polyclonal, Bio Vision, CA, USA, 1 : 100), TSA system with FITC kit (GE Health Science, MA, USA) combined with Höchst 33258 (Invitrogen, OR, USA) nuclear staining was used. The images were observed and captured with LSM510 confocal laser scanning microscope system (Carl Zeiss, Heidelberg, Germany) at a magnitude of 200x using ZEN 2009 interface with line mode, 3 μm thickness of pinhole setting, and 4 × averaging with scan speed 4 (2 min, 5 sec).

2.6. Analysis of the Immunoreactivity. The images of DG from 4 slices/animal were captured and analyzed using a computer-associated image analyzer (WinROOF Version 5; Mitani Corporation, Fukui, Japan) by an examiner who was not aware of the grouping details. Images from one slice were collected from both ipsi- and contralateral DG so that 70% or more of the area of GCL could be examined. We defined the granular cell layer (GCL) in each image with 100 μm width and SGZ at the inner edge of GCL with 20 μm width and measured the defined area as mm^2 automatically with the analyzer. The number of cresyl violet-stained or immunopositive cells (NeuN, cleaved caspase-3, Bax, Bcl-2α, Nestin, Ki67, and DCX) or % immunopositive density (βIII-tubulin and MAP2) in each image was measured, summed up for 4 slices/animal, and was calculated as per mm^2 of the measured area in each animal.

2.7. Statistical Analysis. All the data are given as means ± standard error of mean (SEM). The following analysis was used: behavioral examinations, two-way (time, group) analysis of variance (ANOVA) followed by Scheffe *post hoc* test at each time point; immunohistochemical examinations of the time course analysis, two-way (time, group) ANOVA followed by Tukey-Kramer *post hoc* test (Nissl, NeuN, βIII-tubulin, MAP2, Nestin, Ki67, and DCX) or unpaired t-test (cleaved caspase-3, Bax, and Bcl-2α) at each time point; immunohistochemical examinations with IMP at 30 weeks after the surgical operation, one-way (group) ANOVA followed by Tukey-Kramer *post hoc* test; comparisons of two independent groups, unpaired t-test. $P < 0.05$ was regarded as statistically significant. All of the analysis was done using StatView Ver. 5.0 (SAS Institute, USA).

3. Results

3.1. Aggravation of Anhedonia after MCAO. We first examined whether depression-related behavior anhedonia would be spontaneously expressed after MCAO in rats. Normal rats usually show 80–90% of sucrose preference (87.9 ± 2.8% at 30 weeks in sham), as shown in Figures 1(a) and 1(b) (open symbols). MCAO-operated rats (closed symbols) tended to less prefer sucrose containing water compared to sham groups (open symbols) throughout the time course after MCAO. Interestingly, sucrose preference was further decreased significantly at 20 and 30 weeks after MCAO to around 50–60% (56.8 ± 4.5% at 30 weeks in MCAO, $P < 0.01$ versus sham), and it was prevented by chronic administration of IMP (Figure 1(a), 78.9 ± 3.8% at 30 weeks in MCAO + IMP, $P < 0.05$ versus MCAO) or FLV (Figure 1(b), 76.7 ± 2.2% in MCAO + FLV at 30 weeks, $P < 0.05$ versus MCAO) significantly. Daily amount of water consumption was decreased significantly to around 60% in MCAO-operated rats (59.2 ± 3.3 mL in sham versus 33.7 ± 1.8 mL in MCAO at 30 weeks, $P < 0.01$) and was not prevented by chronic IMP treatment (Figure 1(c), 34.4 ± 3.1 mL in MCAO + IMP at 30 weeks, $P > 0.05$ versus MCAO). The body weight of the MCAO-operated rats initially decreased significantly (230.1 ± 3.7 g in sham versus 170.4 ± 5.3 g in MCAO at 3 days, $P < 0.01$) and stayed

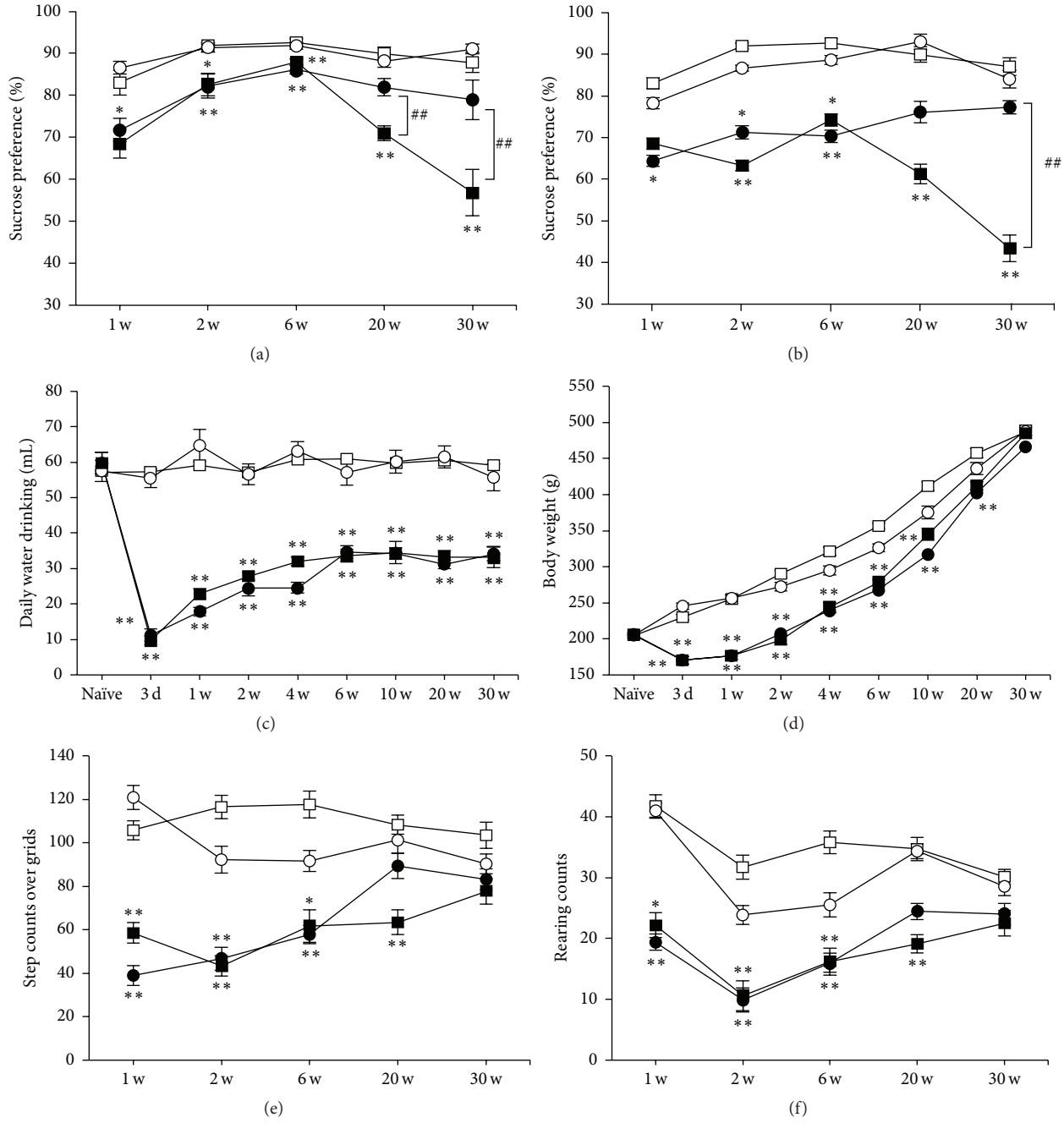


Figure 1: Behavioral analysis of the rats after MCAO. Symbols used are as follows: open square, sham; open circle, sham + antidepressant; closed square, MCAO; closed circle, MCAO + antidepressant; $N = 10$/group, total $N = 40$. For antidepressants treatment, vehicle (open and closed square), 20 mg/kg/day of IMP in (a) and (c) to (f) or FLV in (b) (open and closed circles) was administered for 29 weeks. Statistical significance was evaluated with two-way ANOVA followed by Scheffe *post hoc* test. (a) Sucrose preference test with IMP. $F_{(\text{time})\,4} = 17.4$; $F_{(\text{group})\,3} = 63.9$; $F_{(\text{time}*\text{group})\,12,180} = 9.131$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham; $^{\#\#}P < 0.01$ versus MCAO + IMP. (b) Sucrose preference test with FLV. $F_{(\text{time})\,4} = 2.76$; $F_{(\text{group})\,3} = 39.5$; $F_{(\text{time}*\text{group})\,12,180} = 2.13$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham; $^{\#\#}P < 0.01$ versus MCAO + FLV. (c) Daily amount of water drinking before (Naïve) and after MCAO. $F_{(\text{time})\,8} = 27.2$; $F_{(\text{group})\,3} = 351.1$; $F_{(\text{time}*\text{group})\,24,324} = 9.70$; $^{**}P < 0.01$ versus sham. (d) Changes of body weight before (Naïve) and after MCAO. $F_{(\text{time})\,8} = 1291.6$; $F_{(\text{group})\,3} = 249.5$; $F_{(\text{time}*\text{group})\,24,324} = 11.1$; $^{**}P < 0.01$ versus sham. (e, f) Open field test. (e) Horizontal activity during a period of 10 min. $F_{(\text{time})\,4} = 3.43$; $F_{(\text{group})\,3} = 56.8$; $F_{(\text{time}*\text{group})\,12,180} = 3.45$; $^{**}P < 0.01$ versus sham. (f) Vertical activity characterized by the number of rearing instances during a period of 10 min. $F_{(\text{time})\,4} = 9.79$; $F_{(\text{group})\,3} = 41.0$; $F_{(\text{time}*\text{group})\,12,180} = 1.59$; $^{**}P < 0.01$ versus sham.

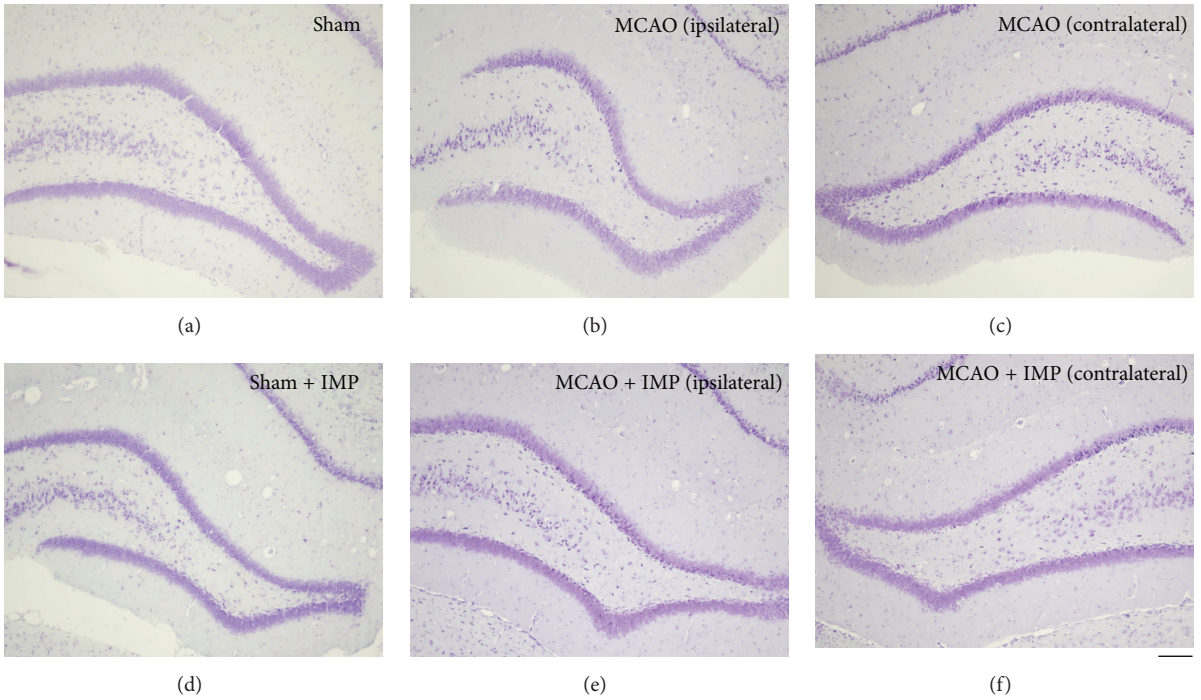


Figure 2: Typical images of the cresyl violet staining in the hippocampal dentate gyrus of rats. Note that the entire shape of GCL in (b) (ipsilateral MCAO) is shrunk compared to the others. Scale bar indicates 200 μm.

lower than sham groups until around 6 weeks after MCAO and then gradually recovered and was almost the same as sham group at 30 weeks (Figure 1(d): 487.4 ± 6.2 g in sham versus 485.2 ± 7.1 g in MCAO at 30 weeks, $P > 0.05$). Chronic IMP treatment did not affect them after MCAO (465.9 ± 7.3 g in MCAO + IMP at 30 weeks, $P > 0.05$ versus MCAO). The rats given FLV also showed similar results to Figures 1(c) and 1(d) (data not shown).

3.2. Spontaneous Activity of the Rats after MCAO. We next examined the spontaneous activity of rats with open field test (Figures 1(e) and 1(f)). It revealed that both horizontal and vertical activities of MCAO-operated rats (closed symbols) were decreased significantly compared to sham groups (open symbols). However, they gradually recovered and reached a similar level to that of sham group at 30 weeks after MCAO (Figures 1(e) and 1(f): horizontal activity, 103.4 ± 4.3 in sham versus 77.6 ± 5.8 in MCAO, $P > 0.05$; vertical activity, 30.1 ± 1.2 in sham versus 23.5 ± 2.2 in MCAO, $P > 0.05$). Chronic IMP treatment did not affect them (Figures 1(e) and 1(f): horizontal activity, 83.2 ± 4.9 in MCAO + IMP, $P > 0.05$ versus MCAO; vertical activity, 24.2 ± 1.8 in MCAO + IMP, $P > 0.05$ versus MCAO). Similar results with FLV-treated rats were observed (data not shown).

3.3. Specific Loss of the Granular Neurons in the Ipsilateral DG after MCAO. In the paraffin sections prepared from the brains at 30 weeks after MCAO, DG of the dorsal hippocampus was observed with cresyl violet staining (Figure 2). We noticed that the shape of GCL was shrunk specifically in the slices prepared from MCAO-operated ipsilateral hippocampus (Figure 2(b)). Time course analysis of the cresyl violet-stained cells revealed the significant loss of the cells in GCL observed at 20 and 30 weeks after MCAO (Figure 3(b): 4699.6 ± 119.2 cells/mm^2 in sham versus 3561.8 ± 177.3 cells/mm^2 in ipsilateral MCAO at 30 weeks, $P < 0.01$), and it was prevented by chronic administration of IMP from 1 week to 30 weeks after MCAO (Figure 3(c): 4531.0±262.5 cells/mm^2 in ipsilateral MCAO + IMP, $P < 0.05$ versus ipsilateral MCAO).

We also quantified the number of the cells in which the condensed cytosol was observed with cresyl violet staining at 30 weeks after MCAO ($F_{5,24} = 1.67$, one-way ANOVA followed by Tukey-Kramer *post hoc* test). Compared with sham (102.7 ± 14.7 cells/mm^2), MCAO tended to increase the condensed cells in both ipsi- and contralateral GCL without statistical significance (147.5 ± 9.6 cells/mm^2 in ipsilateral MCAO and 129.2 ± 16.7 cells/mm^2 in contralateral MCAO, $P > 0.05$ versus sham), and IMP did not reverse them (137.4 ± 14.8 cells/mm^2 in ipsilateral MCAO + IMP and 133.2 ± 21.4 cells/mm^2 in contralateral MCAO + IMP, $P > 0.05$ versus sham).

Because loss of GC in DG after ischemia had never been reported yet, we examined NeuN immunoreactivity as a marker of the matured neurons in GCL (Figure 4). Similar to the result of cresyl violet staining in Figure 3, time course analysis revealed that the number of NeuN immunopositive cells in GCL was decreased at 20 and 30 weeks after MCAO significantly (Figure 4(b): 4401.0 ± 110.4 cells/mm^2 in sham versus 3230.9 ± 235.8 cells/mm^2 in

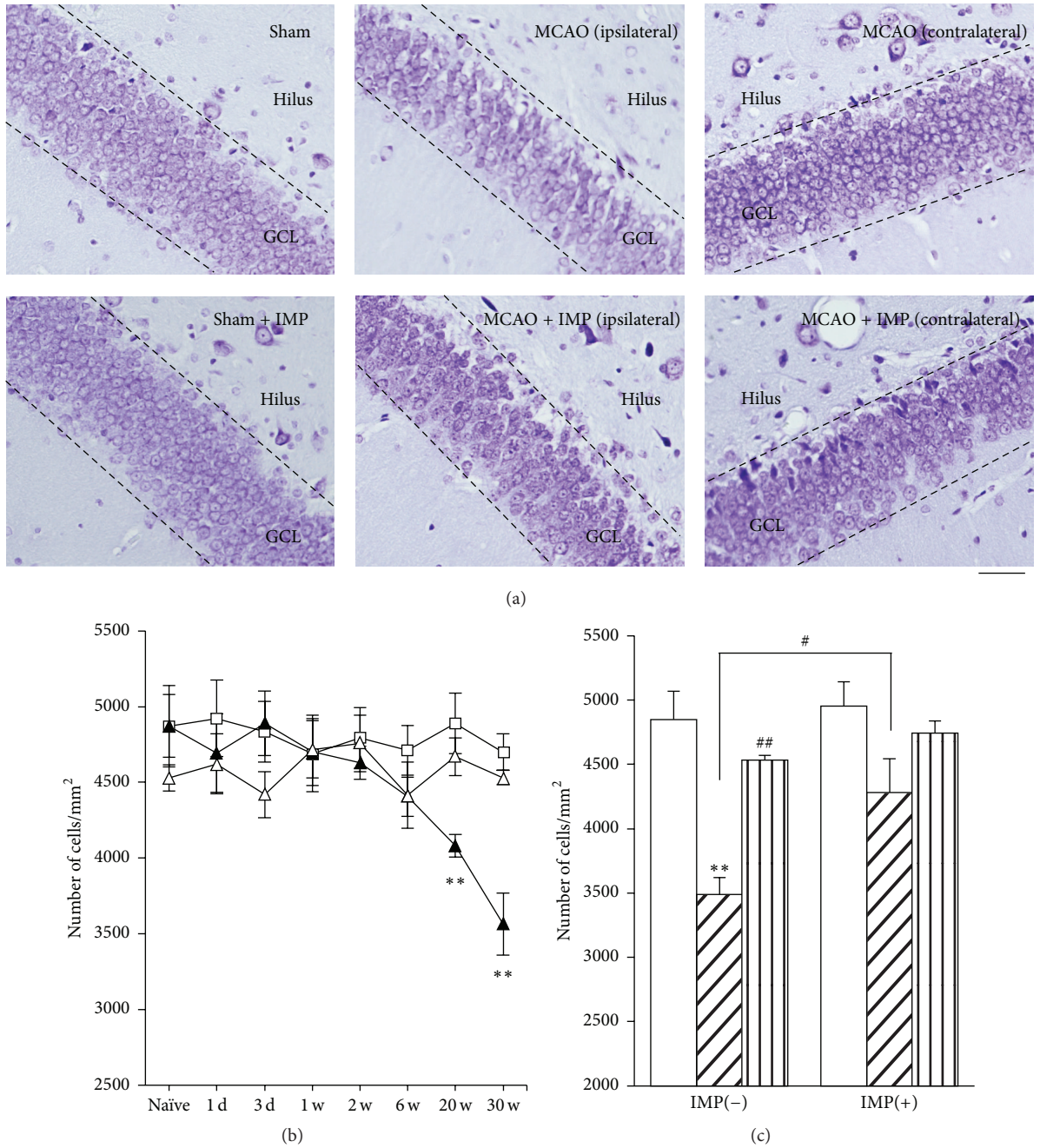


Figure 3: Analysis of the cresyl violet-stained cells in GCL. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 50 μm. (b) Time course changes of Nissl positive cells after MCAO in GCL. Symbols used are as follows: open square, sham; closed triangle, ipsilateral MCAO; open triangle, contralateral MCAO. Statistical significance was evaluated with two-way ANOVA followed by Tukey-Kramer test at each time point ($F_{(\text{time})\,7} = 2.96$; $F_{(\text{group})\,2} = 7.37$; $F_{(\text{time}*\text{group})\,14,96} = 2.42$; $^{**}P < 0.01$ versus sham). (c) Analysis of the effect of IMP on the number of Nissl positive cells at 30 weeks after MCAO. Open columns, sham; hatched columns, ipsilateral MCAO; striped columns, contralateral MCAO. Vehicle [IMP(−)] or 20 mg/kg/day of IMP for 29 weeks [IMP(+)] from 1 week after the surgical operation was administered. Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer test ($F_{5,24} = 8.78$; $^{**}P < 0.01$ versus sham [IMP(−)]; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus ipsilateral MCAO; $N = 5$/group).

ipsilateral MCAO at 30 weeks, $P < 0.01$) and the decrease was prevented by chronic IMP treatment for 29 weeks after MCAO (Figure 4(c): 4169.8 ± 287.5 cells/mm^2 in ipsilateral MCAO + IMP, $P < 0.05$ versus ipsilateral MCAO).

We further examined other antibodies for the neuronal markers βIII-tubulin and MAP2 (Figures 5 and 6) in GCL with measuring % density of the immunoreactive area. Immunoreactivity of βIII-tubulin in ipsilateral GCL started

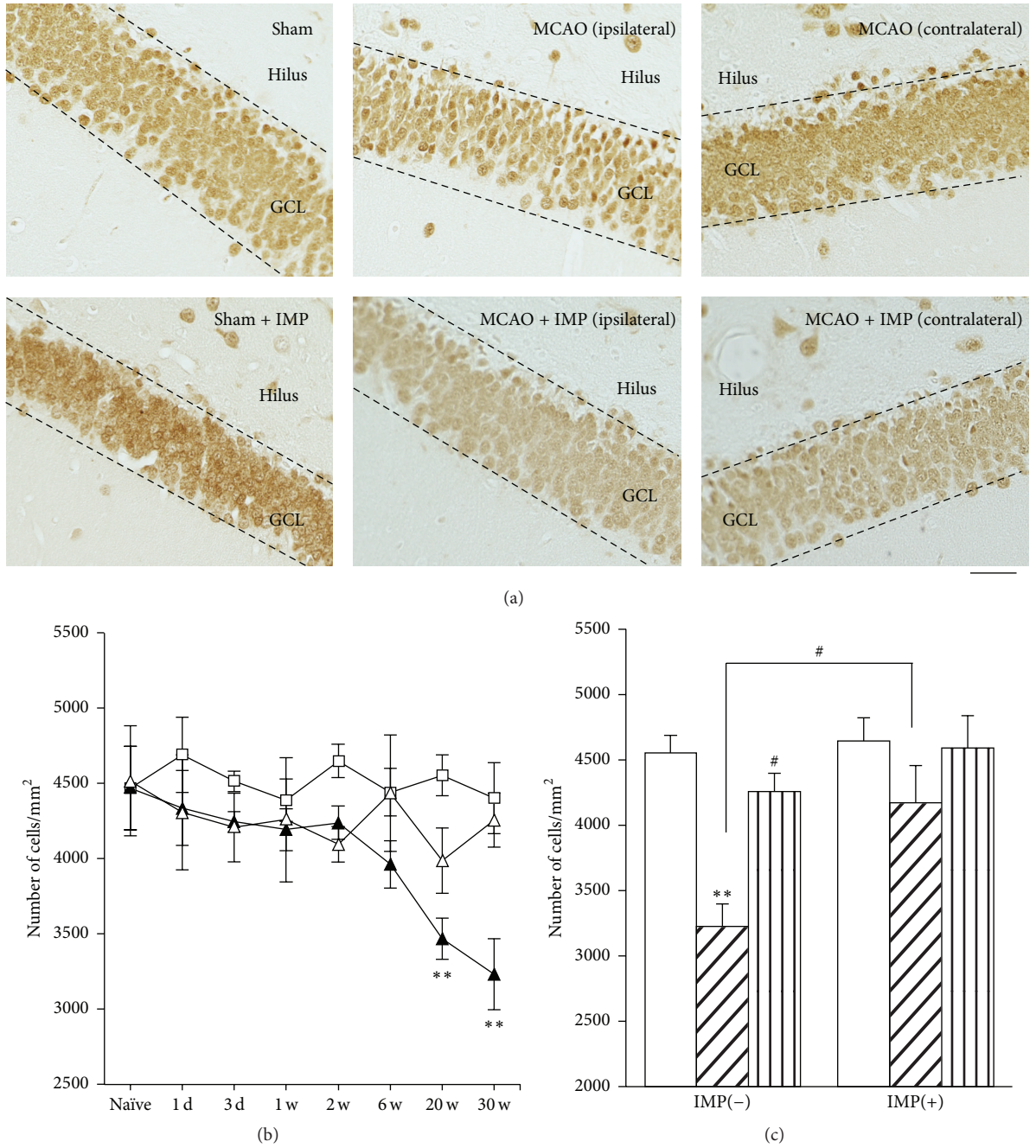


Figure 4: Analysis of NeuN immunopositive cells in GCL. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 50 μm. (b) Time course changes of NeuN immunopositive cells after MCAO in GCL. Symbols used are as follows: open square, sham; closed triangle, ipsilateral MCAO; open triangle, contralateral MCAO. Statistical significance was evaluated with two-way ANOVA followed by Tukey-Kramer test at each time point ($F_{(\text{time})\,7} = 2.78$; $F_{(\text{group})\,2} = 11.0$; $F_{(\text{time}*\text{group})\,14,96} = 1.57$; $^{**}P < 0.01$ versus sham). (c) Analysis of the effect of IMP on the number of NeuN immunopositive cells at 30 weeks after MCAO. Vehicle [IMP(−)] or 20 mg/kg/day of IMP for 29 weeks [IMP(+)] from 1 week after the surgical operation was administered. Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer test ($F_{5,24} = 7.00$; $^{**}P < 0.01$ versus sham [IMP(−)]; $^{\#}P < 0.05$ versus ipsilateral MCAO [IMP(−)]; $N = 5$/group).

to decrease at 6 weeks after MCAO (Figure 5(b): 18.8 ± 0.90% in sham versus 14.6 ± 0.61% in ipsilateral MCAO, $P < 0.05$) and further decreased at 20 and 30 weeks (12.4 ± 0.48%, $P < 0.01$ versus sham), and it was reversed by chronic IMP treatment (15.7 ± 0.72%, $P < 0.01$). Similar results were obtained from the examination of MAP2 immunoreactivity (Figure 6). At 30 weeks after the surgical operation, 19.6 ± 0.50% in sham was decreased to 14.2 ± 0.51% in ipsilateral MCAO ($P < 0.01$), and chronic IMP treatment prevented it (17.0 ± 1.20%, $P > 0.05$ versus sham).

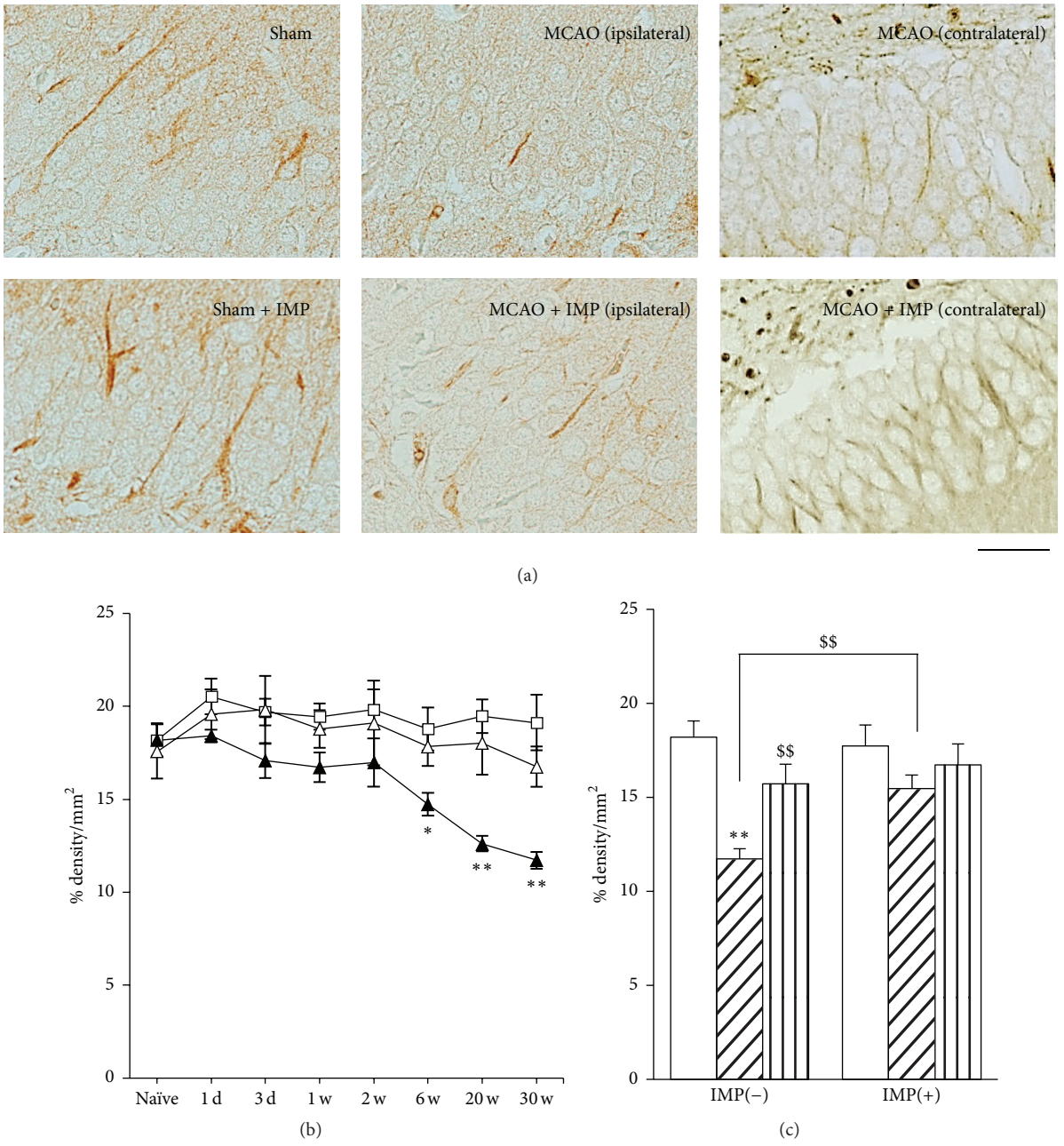


Figure 5: Analysis of βIII-tubulin immunopositive areas in GCL. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 30 μm. (b) Time course changes of βIII-tubulin immunopositive areas after MCAO in GCL. Symbols used are as follows: open square, sham; closed triangle, ipsilateral MCAO; open triangle, contralateral MCAO. Statistical significance was evaluated with two-way ANOVA followed by Tukey-Kramer test at each time point ($F_{(\text{time})\,7} = 3.06$; $F_{(\text{group})\,2} = 19.6$; $F_{(\text{time}*\text{group})\,14,96} = 1.17$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham). (c) Analysis of the effect of IMP on βIII-tubulin immunopositive areas at 30 weeks after MCAO. Vehicle [IMP(−)] or 20 mg/kg/day of IMP for 29 weeks [IMP(+)] from 1 week after the surgical operation was administered. Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer test ($F_{5,24} = 6.05$; $^{**}P < 0.01$ versus sham [IMP(−)]; $N = 5$/group). Unpaired t-test was performed with ipsilateral MCAO [IMP(−)] versus contralateral MCAO [IMP(−)] ($t = 3.41$, $^{\$\$}P < 0.01$) or versus ipsilateral MCAO [IMP(+)] ($t = 4.12$, $^{\$\$}P < 0.01$).

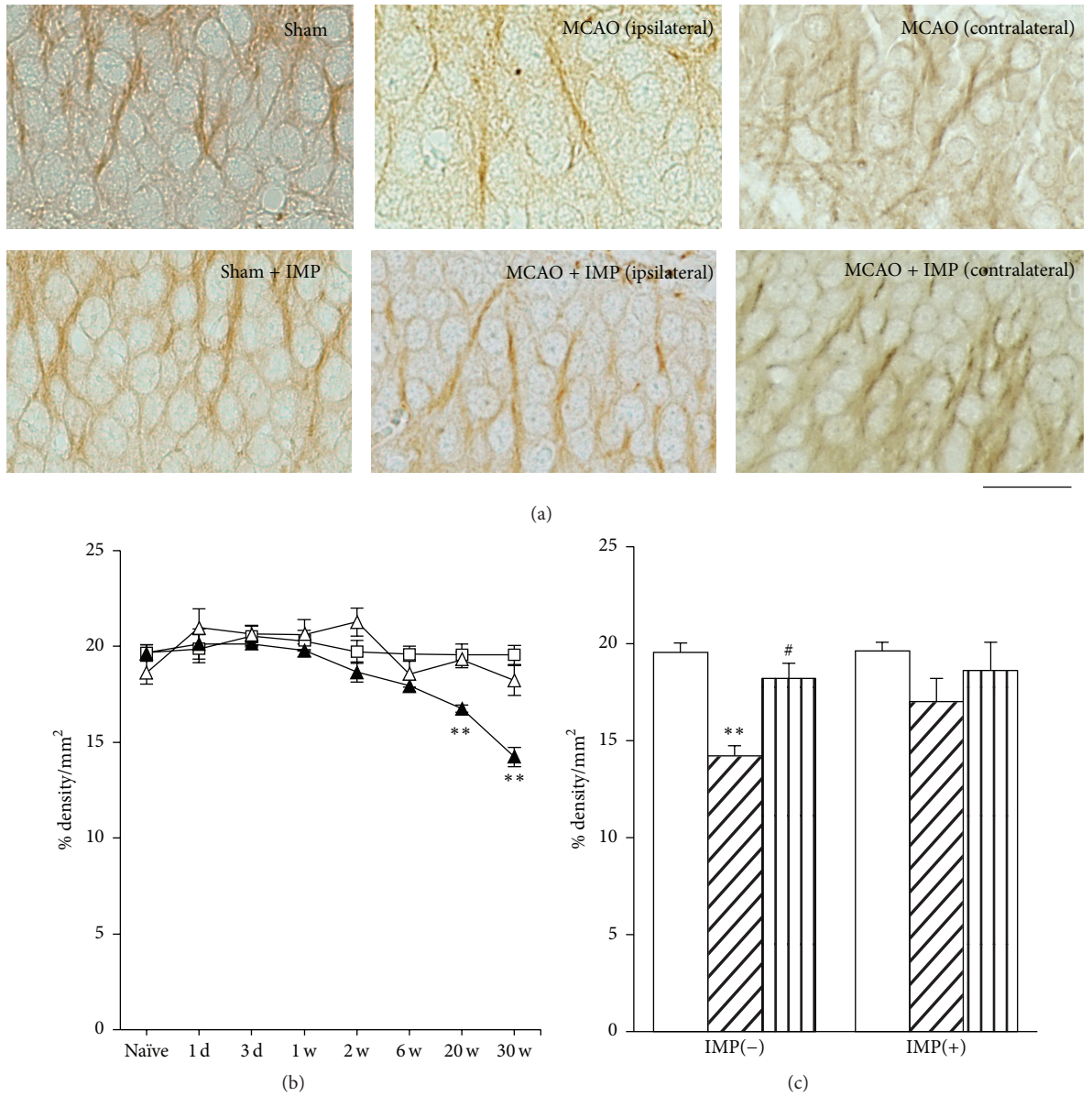


Figure 6: Analysis of MAP2 immunopositive areas in GCL. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 20 μm. (b) Time course changes of MAP2 immunopositive areas after MCAO in GCL. Symbols used are as follows: open square, sham; closed triangle, ipsilateral MCAO; open triangle, contralateral MCAO. Statistical significance was evaluated with two-way ANOVA followed by Tukey-Kramer test at each time point ($F_{(\text{time})\,7} = 11.0$; $F_{(\text{group})\,2} = 16.1$; $F_{(\text{time}*\text{group})\,14,96} = 3.65$; $^{**}P < 0.01$ versus sham). (c) Analysis of the effect of IMP on MAP2 immunopositive areas at 30 weeks after MCAO. Vehicle [IMP(−)] or 20 mg/kg/day of IMP for 29 weeks [IMP(+)] from 1 week after the surgical operation was administered. Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer test ($F_{5,24} = 5.07$; $^{**}P < 0.01$ versus sham [IMP(−)]; $^{\#}P < 0.05$ versus ipsilateral MCAO [IMP(−)]; $N = 5$/group).

All of these data indicated the degenerative loss of GC in the ipsilateral DG at chronic stages after MCAO.

3.4. Increased Apoptosis in GCL after MCAO. In Section 3.3, we showed that delayed neuronal loss occurred in the ipsilateral GCL of the hippocampal DG at chronic stages after MCAO. To examine whether the degenerative loss of GC involved neuronal apoptosis, we next performed immunostaining of cleaved caspase-3, which executes apoptosis. We analyzed the ipsilateral GCL and SGZ separately to distinguish the matured neurons and the other cells including NSCs in DG. In GCL, the number of cleaved caspase-3 immunopositive cells was significantly increased at 2 through 30 weeks of MCAO (Figure 7(b): 17.7 ± 1.44 cells/mm^2 in sham versus 55.1 ± 4.76 cells/mm^2 in MCAO at 30 weeks, $P <$ 0.01) and the decrease was mostly prevented by chronic IMP treatment at 30 weeks after MCAO (Figures 7(a) and 7(d): 19.8 ± 1.01 cells/mm^2 in MCAO + IMP at 30 weeks, $P < 0.01$

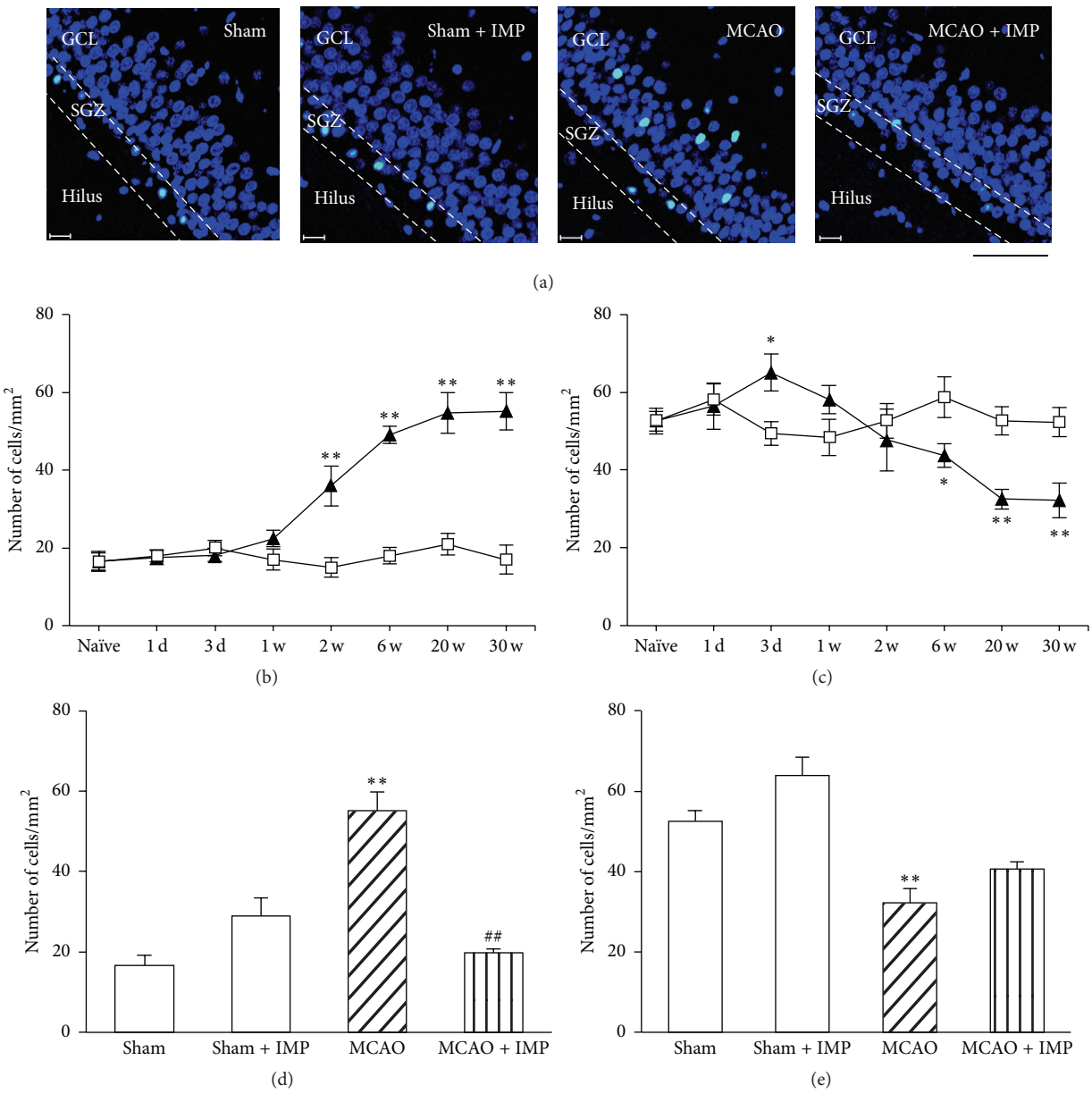


FIGURE 7: Analysis of the number of cleaved caspase-3 immunopositive cells in ipsilateral GCL and SGZ. (a) Typical images of the slices taken at 30 weeks after MCAO. Blue signal is the nuclei stained with Höchst 33258, while green signal of FITC is from cleaved caspase-3 immunopositive cells. Scale bar indicates 50 μm. (b, c) Time course changes of cleaved caspase-3 immunopositive cells after MCAO in GCL (b) and SGZ (c). Symbols used are as follows: open square, sham; closed triangle, MCAO. Statistical significance was evaluated with two-way ANOVA followed by unpaired t-test at each time point (GCL: $F_{(\text{time})\,7} = 21.2$, $F_{(\text{group})\,1} = 129.6$, $F_{(\text{time}*\text{group})\,7,64} = 19.1$, $t_{(2\,\text{w})} = 3.71$, $t_{(6\,\text{w})} = 12.35$, $t_{(20\,\text{w})} = 6.24$, and $t_{(30\,\text{w})} = 7.53$; SGZ: $F_{(\text{time})\,7} = 4.50$, $F_{(\text{group})\,1} = 5.84$, $F_{(\text{time}*\text{group})\,7,64} = 6.06$, $t_{(3\,\text{d})} = 3.29$, $t_{(6\,\text{w})} = -2.79$, $t_{(20\,\text{w})} = -4.86$, and $t_{(30\,\text{w})} = -3.95$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham). (d, e) Analysis of the effect of IMP (20 mg/kg/day for 29 weeks) on the number of cleaved caspase-3 immunopositive cells at 30 weeks after MCAO in GCL (d) and SGZ (e). Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer *post hoc* test ($F_{(\text{GCL})\,3,16} = 24.63$; $F_{(\text{SGZ})\,3,16} = 18.41$; $^{**}P < 0.01$ versus sham [IMP(−)]; $^{\#\#}P < 0.01$ versus MCAO [IMP(−)]; $N = 5$/group).

versus MCAO). In contrast, the number of cleaved caspase-3 immunopositive cells in SGZ was initially increased at 3 days (49.3 ± 3.03 cells/mm^2 in sham versus 65.1 ± 3.71 cells/mm^2 in MCAO, $P < 0.05$) and then decreased at 6 through 30 weeks of MCAO (Figure 7(c): 52.3 ± 3.73 cells/mm^2 in sham versus 32.2±3.48 cells/mm^2 in MCAO at 30 weeks, $P < 0.01$), and chronic IMP treatment tended to prevent it (Figure 7(e): 40.6±1.79 cells/mm^2, $P > 0.05$ versus sham). It is notable that the number of cleaved caspase-3 immunopositive cells in SGZ was much higher than that of GCL in sham groups, whereas

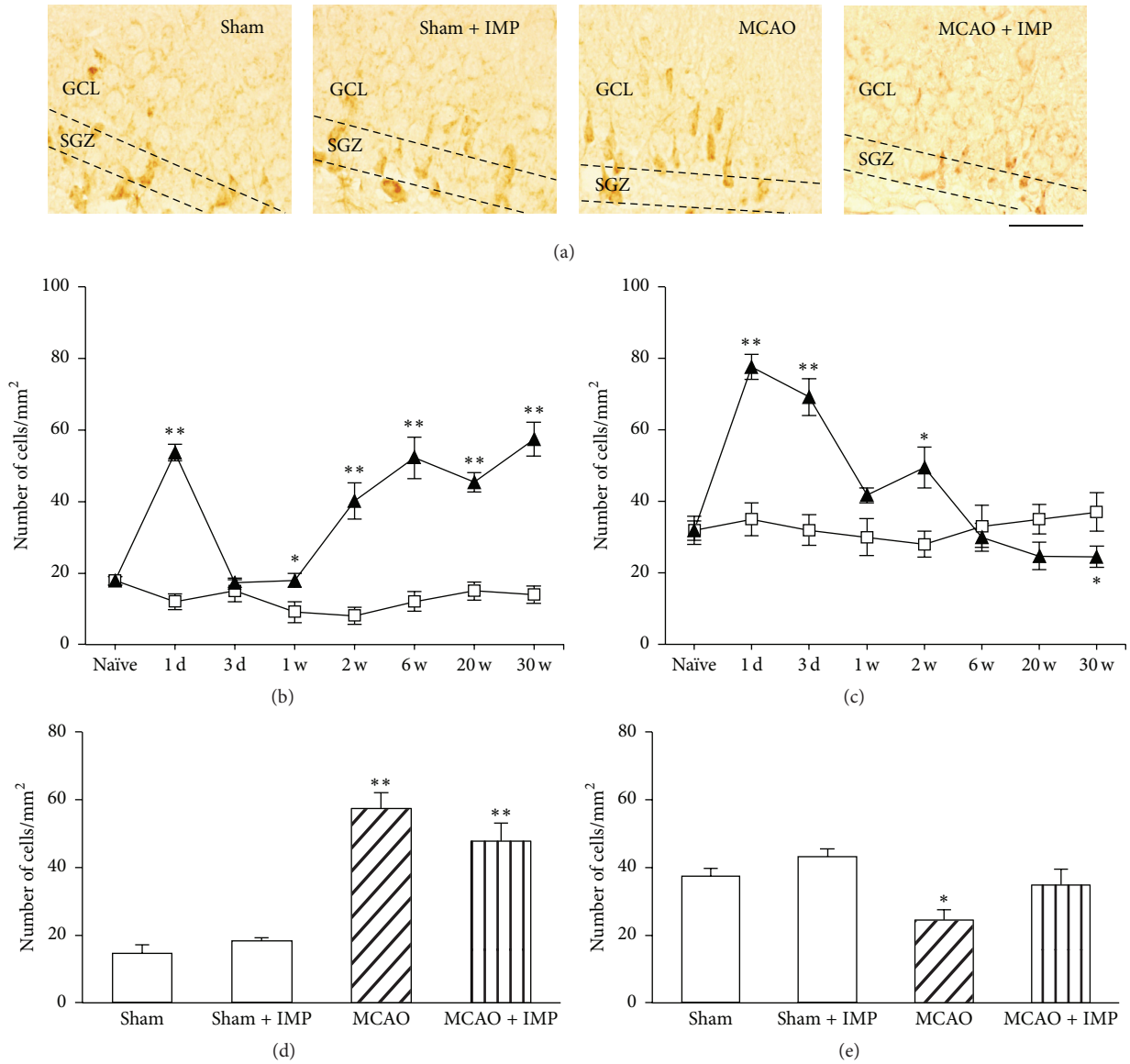


Figure 8: Analysis of the number of Bax immunopositive cells in the ipsilateral GCL and SGZ. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 50 μm. (b, c) Time course changes of Bax immunopositive cells after MCAO in GCL (b) and SGZ (c). Symbols used are as follows: open square, sham; closed triangle, MCAO. Statistical significance was evaluated with two-way ANOVA followed by unpaired t-test at each time point (GCL: $F_{(\text{time})\,7} = 18.1$, $F_{(\text{group})\,1} = 286.4$, $F_{(\text{time}*\text{group})\,7,64} = 19.3$, $t_{(1\,\text{d})} = 12.8$, $t_{(1\,\text{w})} = 2.46$, $t_{(2\,\text{w})} = 5.61$, $t_{(6\,\text{w})} = 6.30$, $t_{(20\,\text{w})} = 11.3$, and $t_{(30\,\text{w})} = 7.95$; SGZ: $F_{(\text{time})\,7} = 11.2$, $F_{(\text{group})\,1} = 27.8$, $F_{(\text{time}*\text{group})\,7,64} = 12.3$, $t_{(1\,\text{d})} = 7.51$, $t_{(3\,\text{d})} = 5.52$, $t_{(2\,\text{w})} = 3.17$, and $t_{(30\,\text{w})} = -2.00$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham). (d, e) Analysis of the effect of IMP (20 mg/kg/day for 29 weeks) on the number of Bax immunopositive cells at 30 weeks after MCAO in GCL (d) and SGZ (e). Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer *post hoc* test ($F_{(\text{GCL})\,3,16} = 31.1$; $F_{(\text{SGZ})\,3,16} = 6.07$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham [IMP(−)]; $N = 5$/group).

it was reversed after 20 and 30 weeks of MCAO, suggesting the increased apoptosis of the matured GC and decreased apoptosis of NSCs in DG after MCAO.

3.5. Analysis of the Death and Survival Signaling in DG after MCAO. Related to the increased apoptosis in the ipsilateral GCL, we characterized the immunoreactivities of Bax as a marker of death signaling and Bcl-2α as a marker of survival signaling in DG using specific antibodies for them (Figures 8 and 9, Supplementary Figures 1 and 2 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/5054275).

In the ipsilateral GCL, Bax immunoreactivity increased transiently at 1 day after MCAO (Figure 8(b): 11.9 ± 2.23 cells/mm^2 in sham versus 53.7 ± 2.38 cells/mm^2 in MCAO, $P < 0.01$), returned to the sham level in 3 days, and then increased again and stayed higher than the sham group significantly at 1 through 30 weeks of MCAO (Figure 8(b): 14.7 ± 2.47 cells/mm^2 in sham versus 57.4 ± 4.78 cells/mm^2 in MCAO at 30 weeks, $P < 0.01$). The increased Bax was not

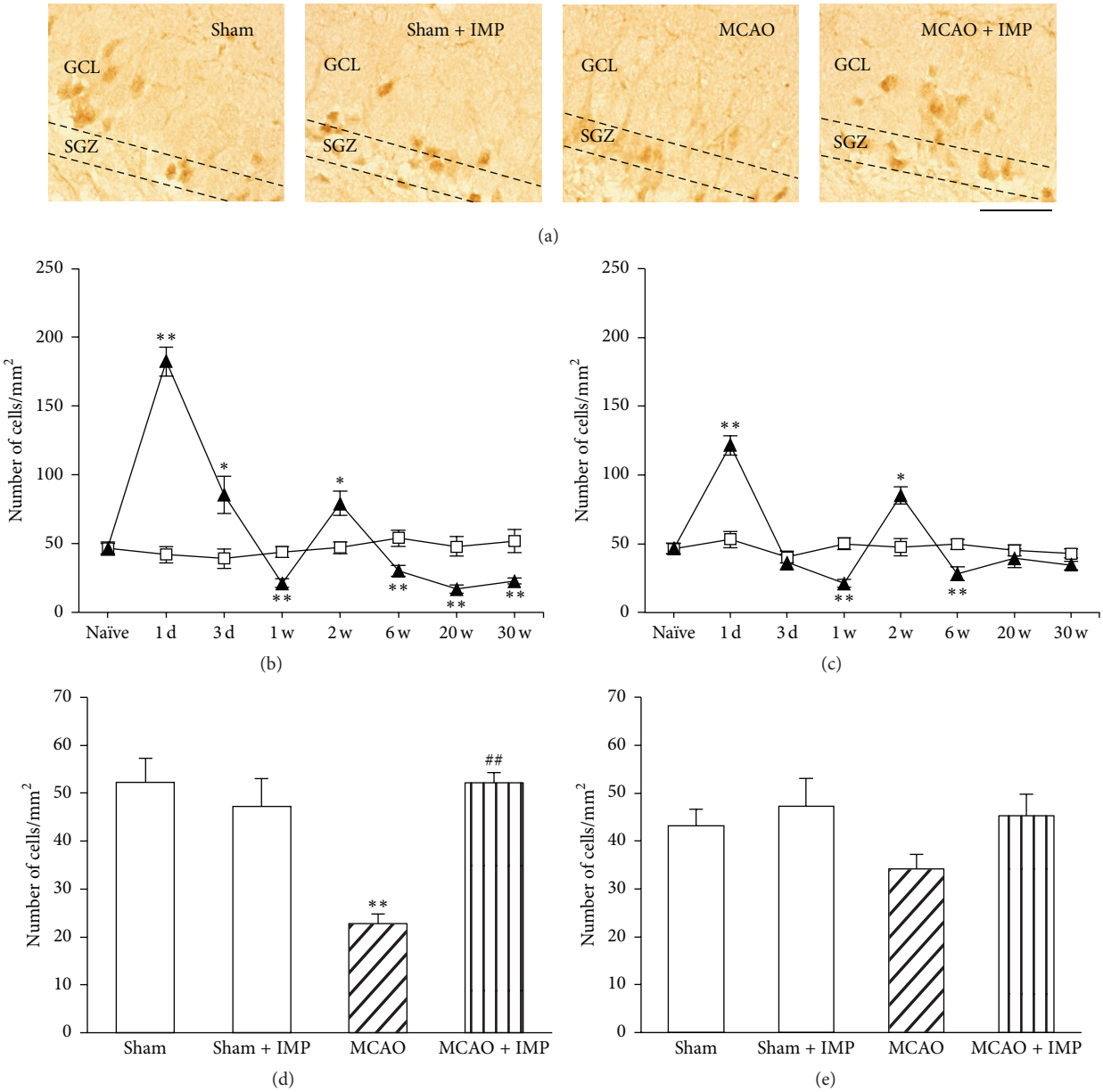


FIGURE 9: Analysis of the number of Bcl-2α immunopositive cells in the ipsilateral GCL and SGZ. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 50 μm. (b, c) Time course changes of Bcl-2α immunopositive cells after MCAO in GCL (b) and SGZ (c). Symbols used are as follows: open square, sham; closed triangle, MCAO. Statistical significance was evaluated with two-way ANOVA followed by unpaired t-test at each time point (GCL: $F_{(\text{time})\,7} = 40.3$, $F_{(\text{group})\,1} = 20.8$, $F_{(\text{time}*\text{group})\,7,64} = 48.7$, $t_{(1\,\text{d})} = 12.4$, $t_{(3\,\text{d})} = 3.15$, $t_{(1\,\text{w})} = -5.54$, $t_{(2\,\text{w})} = 3.31$, $t_{(6\,\text{w})} = -4.16$, $t_{(20\,\text{w})} = -5.73$, and $t_{(30\,\text{w})} = -5.44$; SGZ: $F_{(\text{time})\,7} = 23.1$, $F_{(\text{group})\,1} = 2.36$, $F_{(\text{time}*\text{group})\,7,64} = 18.2$, $t_{(1\,\text{d})} = 7.36$, $t_{(1\,\text{w})} = -5.38$, $t_{(2\,\text{w})} = 2.90$, and $t_{(6\,\text{w})} = -3.64$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham). (d, e) Analysis of the effect of IMP (20 mg/kg/day for 29 weeks) on the number of Bcl-2α immunopositive cells at 30 weeks after MCAO in GCL (d) and SGZ (e). Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer *post hoc* test ($F_{(\text{GCL})\,3,16} = 11.9$; $F_{(\text{SGZ})\,3,16} = 1.84$; $^{**}P < 0.01$ versus sham [IMP(−)]; $^{\#\#}P < 0.01$ versus MCAO; $N = 5$/group).

prevented by chronic IMP treatment at 30 weeks after MCAO (Figure 8(d): 47.8±5.29 cells/mm^2 in MCAO + IMP, $P < 0.01$ versus sham). In the ipsilateral SGZ, Bax immunoreactivity showed a biphasic increase with its initial peak at 1–3 days (Figure 8(c): 77.7 ± 3.52 cells/mm^2 at 1 day, $P < 0.01$) and the second peak at 2 weeks (49.4 ± 5.70 cells/mm^2, $P < 0.05$) and then returned to the sham level and was lower than sham at 30 weeks after MCAO (24.5 ± 2.97 cells/mm^2, $P < 0.05$). Chronic IMP treatment tended to prevent this decrease at 30 weeks (Figure 8(e): 34.8 ± 4.66 cells/mm^2, $P > 0.05$ versus sham). The same analysis was performed in the contralateral DG (Supplementary Figure 1). Different from the ipsilateral

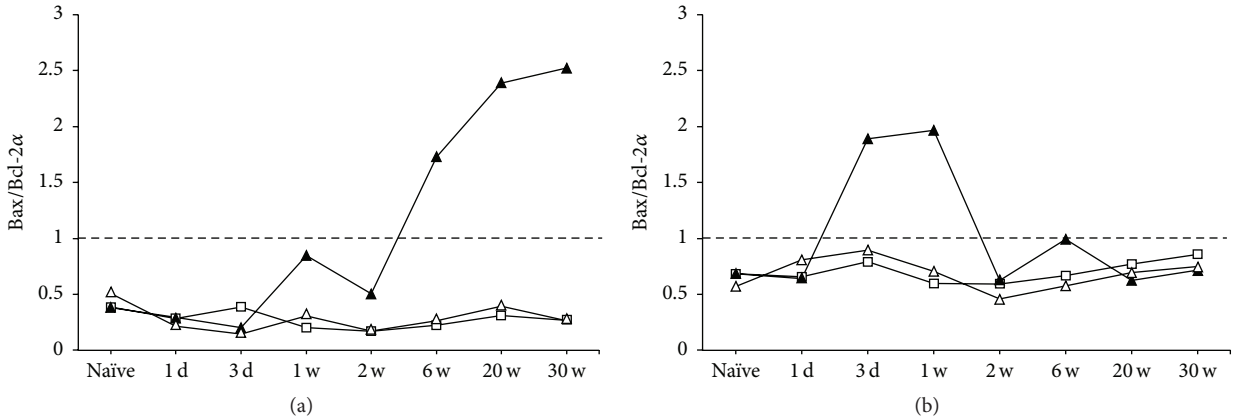


Figure 10: Time course changes of Bax/Bcl-2α index in GCL (a) and SGZ (b). Symbols used are as follows: open square, sham; closed triangles, ipsilateral MCAO; open triangles, contralateral MCAO.

GCL, Bax immunoreactivity in the contralateral GCL only showed the initial increase at 1 day (Supplementary Figure 1B: 16.7 ± 2.98 cells/mm^2 in sham versus 61.9 ± 5.38 cells/mm^2 in MCAO, $P < 0.01$) and returned to the basal level at 3 days through 30 weeks after MCAO, and chronic IMP treatment had no effect on it (Supplementary Figure 1D). The contralateral SGZ showed a similar time course change to the ipsilateral SGZ, although it only had a significant increase at 1 and 3 days after MCAO (Supplementary Figure 1C: 28.5±4.31 cells/mm^2 in sham versus 73.31±3.80 cells/mm^2 in MCAO at 1 day, $P < 0.01$) and returned to the sham level at 1 week through 30 weeks after MCAO. Chronic IMP treatment had no effect on it (Supplementary Figure 1E).

Immunoreactivity of Bcl-2α in the ipsilateral GCL (Figure 9(b): 47.0 ± 1.50 cells/mm^2 in sham throughout the time course) showed a biphasic increase with two peaks at 1 day (182.4 ± 10.7 cells/mm^2, $P < 0.01$) and 2 weeks (79.4 ± 8.99 cells/mm^2, $P < 0.05$) after MCAO. However, it was significantly lower than sham at 1, 6, 20, and 30 weeks (22.8 ± 2.04 cells/mm^2 at 30 weeks, $P < 0.01$), and chronic IMP treatment normalized it at 30 weeks (Figure 9(d): 52.2 ± 2.15 cells/mm^2, $P < 0.05$ versus MCAO). Similar time course change of Bcl-2α to GCL was observed in the ipsilateral SGZ with two peaks at 1 day (121.6 ± 7.11 cells/mm^2, $P < 0.01$) and 2 weeks (85.2 ± 6.38 cells/mm^2, $P < 0.05$) after MCAO (Figure 9(c)). As was in GCL, it was significantly lower than sham at 1 and 6 weeks, although it returned to the sham level at 20 and 30 weeks after MCAO, and there was no difference between sham and MCAO groups with or without IMP (Figure 9(e)). In the contralateral GCL (Supplementary Figure 2B: 45.9 ± 1.68 cells/mm^2 throughout the time course in sham), it also showed a biphasic increase with two peaks at 1 day (268.1 ± 6.60 cells/mm^2, $P < 0.01$) and 2 weeks (139.3 ± 25.2 cells/mm^2, $P < 0.01$). Different from the ipsilateral GCL, it never dropped below the sham level throughout the time course. At 30 weeks after MCAO, there was no significant difference between sham and MCAO with or without chronic IMP treatment (Supplementary Figure 2D). Similar result was obtained from the contralateral SGZ (Supplementary Figures 2C and 2E).

From these results of Bax and Bcl-2α, we calculated Bax/Bcl-2α ratio [38] to see how the death and the survival signaling were changed after MCAO in GCL and in SGZ (Figure 10). In the ipsilateral GCL (Figure 10(a), closed triangle), it exceeded 1.0 at 6 through 30 weeks after MCAO, suggesting that the death signaling was dominant in chronic stages. In contrast, the index in SGZ (Figure 10(b)) increased at early stages after MCAO, exceeded 1.0 at 3 days and 1 week, and returned to the normal level at 2 through 30 weeks after MCAO. In the contralateral GCL and SGZ (open triangle), it kept below 1.0 as was in sham (open square) throughout the time course for 30 weeks after MCAO.

3.6. Analysis of Neural Stem Cell Proliferation and Development in SGZ after MCAO. We further analyzed the proliferation and development of NSCs in SGZ because they are well related to mood, depressiveness, and effects of antidepressants.

In MCAO rats, immunoreactivity of NSCs marker Nestin increased transiently at 3 days and 1 week after MCAO in both the ipsilateral and contralateral SGZ of DG (Figure 11(b) at 3 days: 58.4±4.13 cells/mm^2 in sham; 101.4±7.55 cells/mm^2 in ipsilateral SGZ, $P < 0.01$ versus sham; 88.4 ± 7.18 cells/mm^2 in contralateral SGZ, $P < 0.05$ versus sham) and then decreased and reached almost 50% of sham group (at 30 weeks: 29.9 ± 2.12 cells/mm^2 in ipsilateral MCAO, $P < 0.01$ versus sham; 36.1 ± 3.5 cells/mm^2 in contralateral MCAO, $P < 0.01$ versus sham). Chronic IMP treatment for 29 weeks significantly prevented the decrease of Nestin-positive cells observed in ipsilateral MCAO group at 30 weeks (39.9 ± 1.47 cells/mm^2, $P < 0.05$ versus MCAO), although they were still lower than those of sham group significantly (Figure 11(c)).

We also examined Ki67 immunoreactivity as a proliferating cell marker. The result was similar to those observed in Nestin. Ki67 immunoreactivity increased transiently with

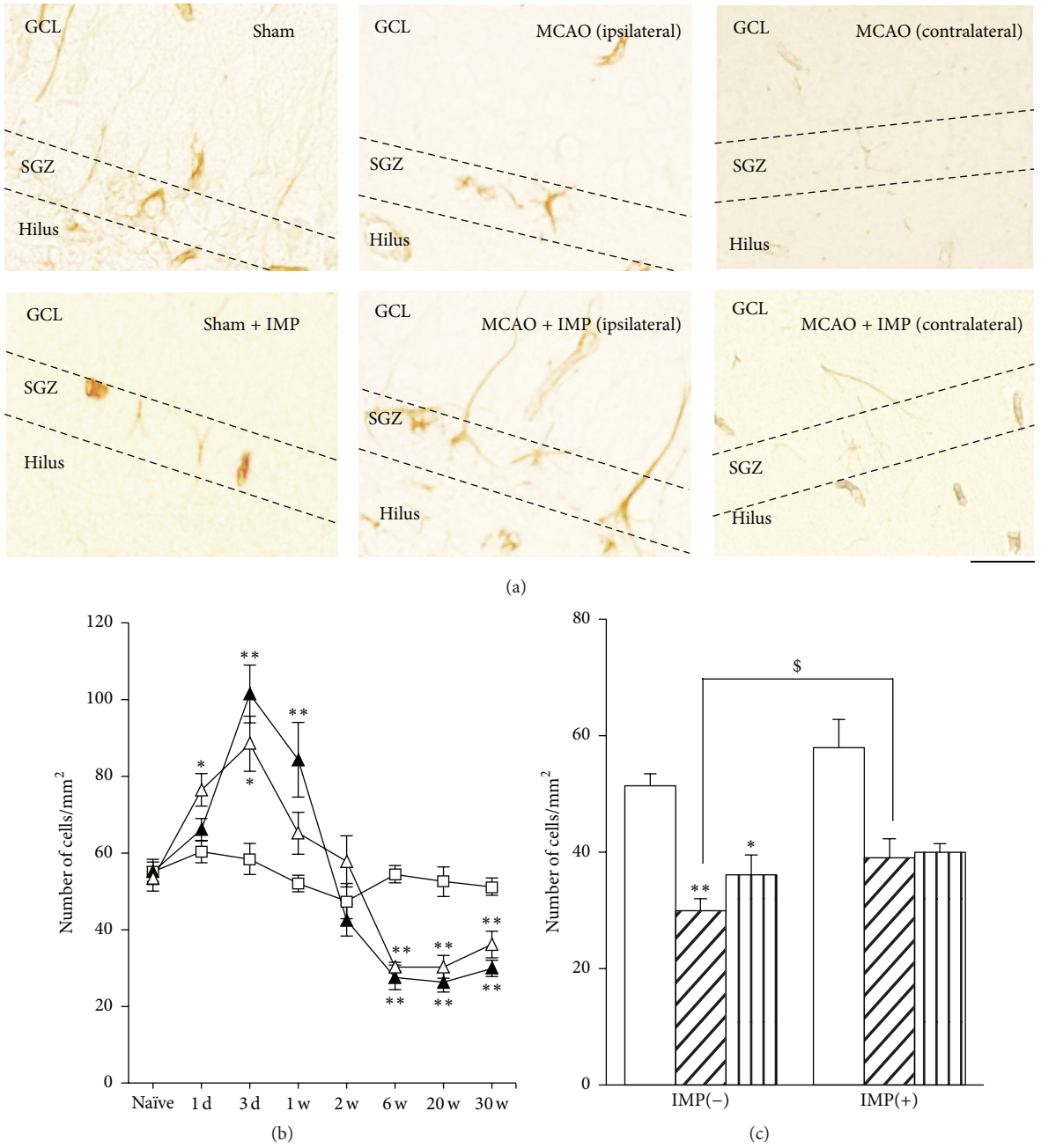


Figure 11: Analysis of Nestin immunopositive cells in GCL. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 20 μm. (b) Time course changes of Nestin immunopositive cells after MCAO in GCL. Symbols used are as follows: open square, sham; closed triangle, ipsilateral MCAO; open triangle, contralateral MCAO. Statistical significance was evaluated with two-way ANOVA followed by Tukey-Kramer test at each time point ($F_{(\text{time})\,7} = 53.9$; $F_{(\text{group})\,2} = 0.097$; $F_{(\text{time}*\text{group})\,14,96} = 12.5$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham). (c) Analysis of the effect of imipramine (IMP) on the number of Nestin immunopositive cells at 30 weeks after MCAO. Vehicle [IMP(−)] or 20 mg/kg/day of IMP for 29 weeks [IMP(+)] from 1 week after the surgical operation was administered. Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer test ($F_{5,24} = 11.36$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham [IMP(−)]; $N = 5$/group). Unpaired t-test was performed with ipsilateral MCAO [IMP(−)] versus [IMP(+)] ($t = -2.40$, $^{\$}P < 0.05$).

its peak at 3 days after MCAO (Figure 12(b) at 3 days: 50.4 ± 6.29 cells/mm^2 in sham; 175.9 ± 12.8 cells/mm^2 in ipsilateral SGZ, $P < 0.01$ versus sham; 90.1 ± 5.12 cells/mm^2 in contralateral SGZ, $P < 0.05$ versus sham) and then decreased and stayed with a few cells per mm^2 at 6 through 30 weeks (Figure 12(b) at 30 weeks: 6.40 ± 0.79 cells/mm^2 in ipsilateral MCAO, $P < 0.01$ versus sham; 8.51 ± 2.55 cells/mm^2 in contralateral MCAO, $P < 0.01$ versus sham). Chronic IMP treatment significantly prevented the decreased Ki67 immunoreactivity at 30 weeks after MCAO in

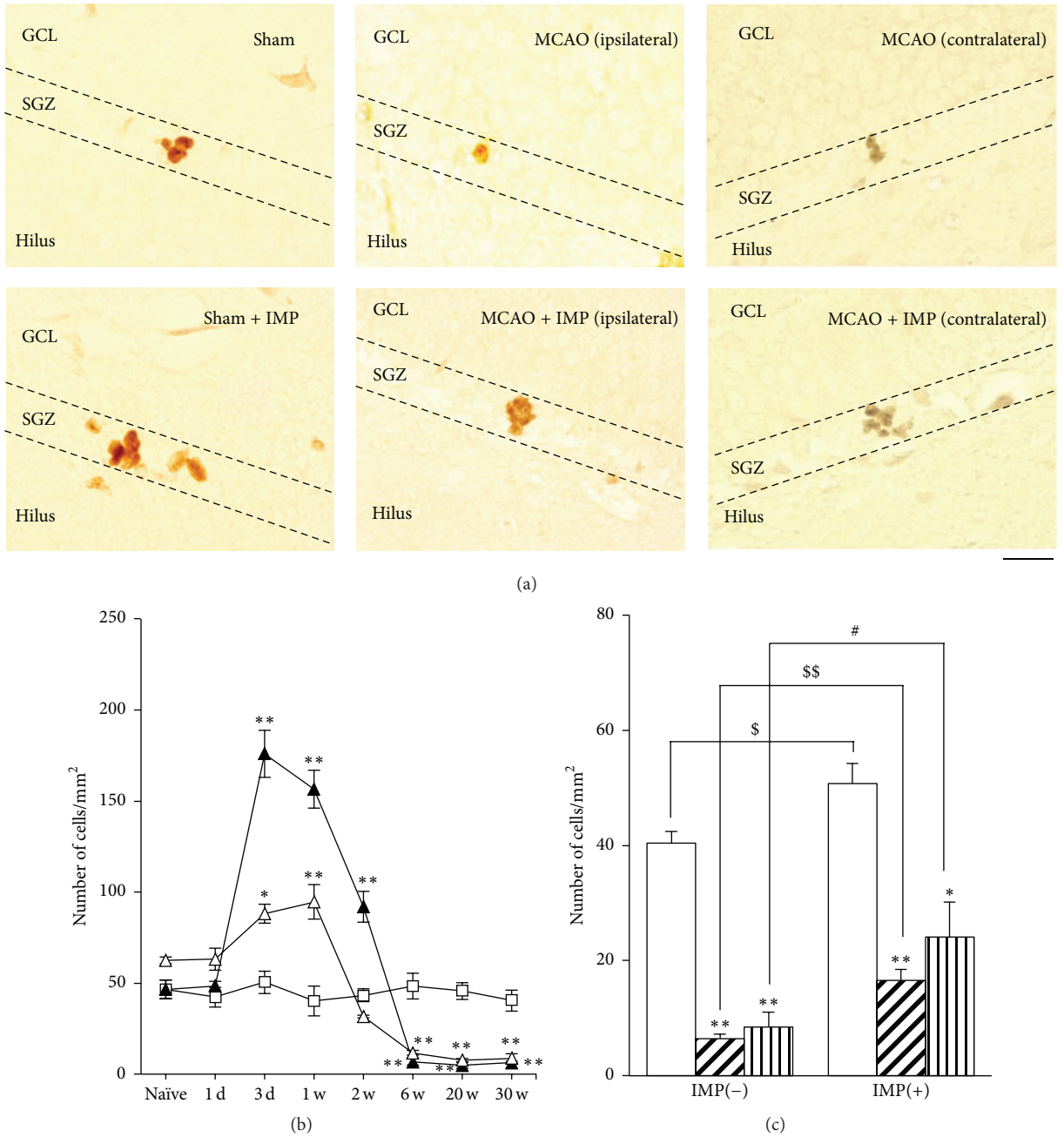


Figure 12: Analysis of Ki67 immunopositive cells in GCL. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 20 μm. (b) Time course changes of Ki67 immunopositive cells after MCAO in GCL. Symbols used are as follows: open square, sham; closed triangle, ipsilateral MCAO; open triangle, contralateral MCAO. Statistical significance was evaluated with two-way ANOVA followed by Tukey-Kramer test at each time point ($F_{(\text{time})\,7} = 101.1$; $F_{(\text{group})\,2} = 38.2$; $F_{(\text{time}*\text{group})\,14,96} = 37.7$; $^{**}P < 0.01$ versus sham). (c) Analysis of the effect of imipramine (IMP) on the number of Ki67 immunopositive cells at 30 weeks after MCAO. Vehicle [IMP(−)] or 20 mg/kg/day of IMP for 29 weeks [IMP(+)] from 1 week after the surgical operation was administered. Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer test ($F_{5,24} = 29.53$; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus sham [IMP(−)]; $^{\#}P < 0.05$ versus contralateral MCAO [IMP(−)]; $N = 5$/group). Unpaired t-test was performed with sham [IMP(−)] versus [IMP(+)] ($t = -2.53$, $^{\$}P < 0.05$) or with ipsilateral MCAO [IMP(−)] versus [IMP(+)] ($t = -4.74$, $^{\$\$}P < 0.01$).

all groups, although they were still around 50% of sham group (Figure 12(c): 16.5 ± 1.98 cells/mm^2 in ipsilateral MCAO + IMP; 24.1 ± 6.05 cells/mm^2 in contralateral MCAO + IMP; $P < 0.05$ versus its corresponding MCAO).

Finally, we examined DCX immunoreactivity as a developmental marker of neurons in GCL. Although we analyzed entire GCL, most of the immunopositive cells are found around SGZ. As was observed in Nestin and Ki67,

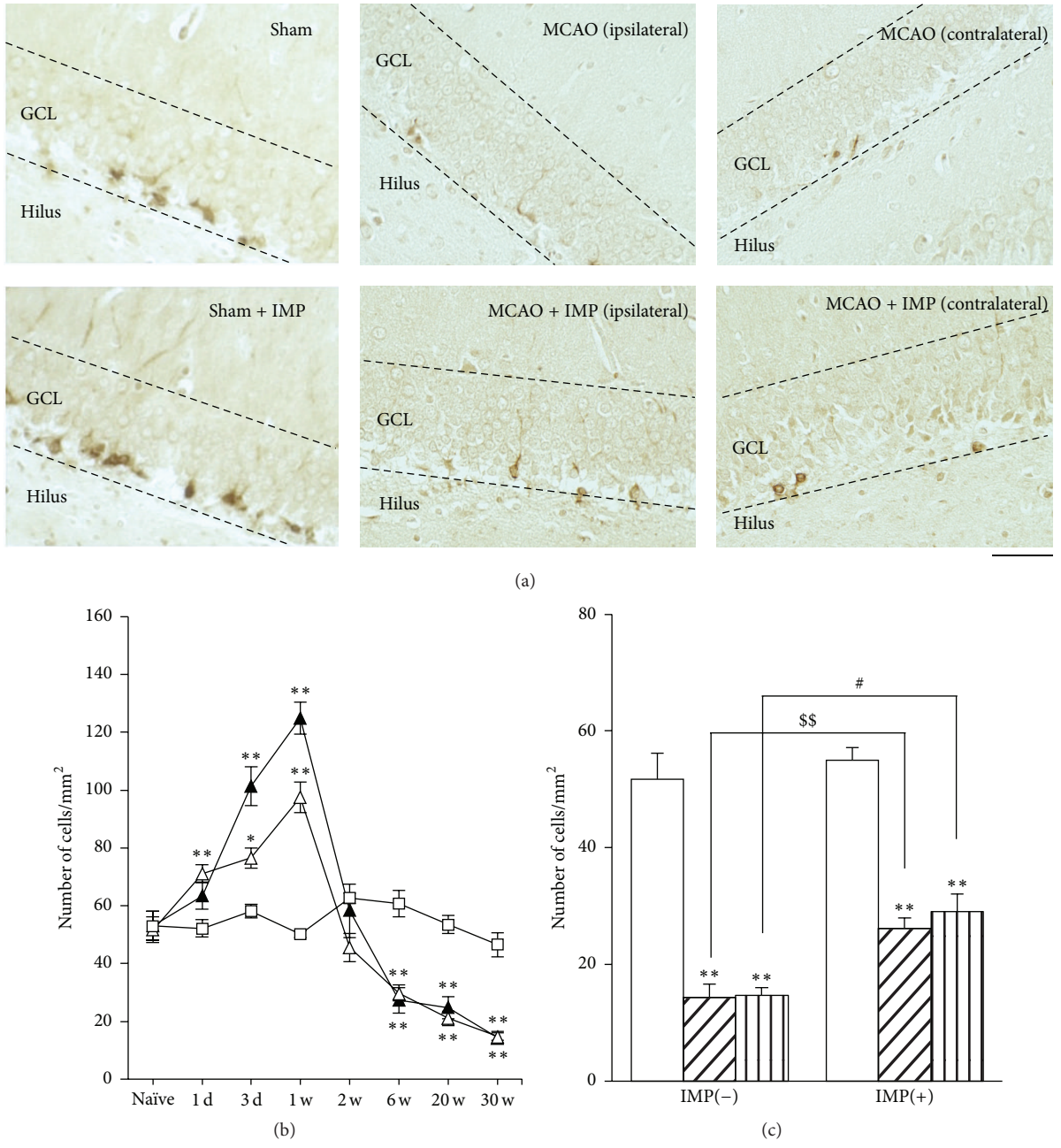


FIGURE 13: Analysis of DCX immunopositive cells in GCL. (a) Typical images of the slices taken at 30 weeks after MCAO. Scale bar indicates 50 μm. (b) Time course changes of DCX immunopositive cells after MCAO in GCL. Symbols used are as follows: open square, sham; closed triangle, ipsilateral MCAO; open triangle, contralateral MCAO. Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer test at each time point ($F_{(\text{time})\,7} = 80.7$; $F_{(\text{group})\,2} = 5.91$; $F_{(\text{time}*\text{group})\,14,96} = 22.7$; $^{**}P < 0.01$ versus sham; $^{\#}P < 0.05$ versus contralateral MCAO [IMP(−)]; $N = 5$/group). (c) Analysis of the effect of imipramine (IMP) on the number of DCX immunopositive cells at 30 weeks after MCAO. Vehicle [IMP(−)] or 20 mg/kg/day of IMP for 29 weeks [IMP(+)] from 1 week after the surgical operation was administered. Statistical significance was evaluated with one-way ANOVA followed by Tukey-Kramer test ($F_{5,24} = 41.60$; $^{**}P < 0.01$ versus sham; $^{\#}P < 0.05$ versus contralateral MCAO [IMP(−)]; $N = 5$/group). Unpaired t-test was performed with ipsilateral MCAO [IMP(−)] versus [IMP(+)] ($t = -4.10$, $^{\$\$}P < 0.01$).

it also increased in early stages with its peak at 1 week (Figure 13(b) at 1 week: 58.2 ± 3.39 cells/mm^2 in sham; 124.9 ± 5.54 cells/mm^2 in ipsilateral SGZ; 97.5 ± 5.36 cells/mm^2 in contralateral SGZ, $P < 0.01$ versus sham) and then decreased and stayed lower than sham significantly at 6 through 30 weeks after MCAO (Figure 13(b) at 30 weeks: 14.4 ± 2.21 cells/mm^2 in ipsilateral MCAO; 14.8 ± 1.30 cells/mm^2 in contralateral MCAO, $P < 0.01$ versus sham). Chronic

IMP treatment significantly prevented the decreased DCX immunoreactivity at 30 weeks in MCAO groups, although they were not normalized as sham group (Figure 13(c): 26.2 ± 1.83 cells/mm^2 in ipsilateral MCAO + IMP; 29.1 ± 3.84 cells/mm^2 in contralateral MCAO + IMP; $P < 0.05$ versus its corresponding MCAO).

4. Discussion

Here we characterized a possible animal model of the postischemic depression after the transient MCAO in rats with a behavioral and an immunohistochemical analysis. Our findings in this study are as follows: (i) we observed spontaneous aggravation of anhedonia in MCAO-operated rats at chronic stages (20 and 30 weeks) after MCAO, which was prevented by chronic IMP or FLV treatment; (ii) this aggravation of anhedonia associated with delayed neuronal death of the granular neurons specifically in the ipsilateral DG, suggesting it would be a part of cellular and molecular pathophysiological mechanisms; (iii) the increased proliferation of NSCs and the following neural development in SGZ at early stages after MCAO were decreased significantly at chronic stages with almost ceased NSCs proliferation; (iv) the neurodegenerative changes in DG were prevented by chronic IMP treatment.

In the following, we discuss these subjects in further detail.

4.1. Postischemic Anhedonia in MCAO Rodents. From many previous studies combining MCAO and additional stressor in rodents [14], it is strongly suggested that the stress vulnerability after stroke is increased significantly. Without adding any contemplated stress, Boyko et al. reported % sucrose preference in permanent MCAO rats (operated on at 20 weeks of age and examined at 3 weeks after MCAO) decreased to around 60%, together with the increased immobility in FST and decreased responses in the shuttle avoidance task [39]. Craft and DeVries reported that the transient ischemia model of mice showed anhedonia with around 50% sucrose preference at 7 days after the surgical operation and was rescued by administration of an antagonist of interleukin-1 (IL-1) receptor [37]. Although both of them did not examine the time course change of anhedonia nor effect of antidepressants on it, these reports with our results in Figures 1(a) and 1(b) showed that PSD in rodents could be induced after MCAO without combining extra stressor stimuli. Sensitivity of the experimental anhedonia to antidepressants should carefully be examined because as shown in Figures 1(a) and 1(b), lessor sucrose preference in MCAO rats was observed throughout the time course from 1 day to 6 weeks after MCAO, and it was insensitive to IMP or FLV. It should also be noted that the decreased amount of daily water consumption in MCAO rats was not rescued by IMP nor by FLV, suggesting that some hypothalamic functions controlling water intake may be affected after MCAO. Because of this reason, we had to increase the concentration of antidepressants in the drinking water of MCAO rats so that they take the same amount of antidepressants as sham rats. To avoid this issue, use of an osmotic minipump, for example, would be examined in further studies.

4.2. Postischemic Neuronal Loss and Apoptosis in GCL. As mentioned in the Introduction, delayed neuronal death of the hippocampal CA1 pyramidal neurons after ischemia has been well characterized, and other regions such as CA3 and DG were thought to be resistant to ischemic stress. In this study, however, we demonstrated delayed neuronal death of the granular neurons in DG at chronic stages after MCAO for the first time, which was consistent with the time course of the aggravation of anhedonia (Figures 1(a), 2–6). Considering the increased immunoreactivities of cleaved caspase-3 and Bax together with decreased Bcl-2α at chronic stages in the ipsilateral GCL (Figures 7–9), it seems that the death signal exceeded the survival signal in GCL after 1 week of MCAO (Figure 10), resulting in the increased apoptotic neuronal death of the matured GC. The increased Bax after 1 week stayed higher than that of sham, whereas Bcl-2α significantly decreased compared to sham in those periods, suggesting that the death signal exceeded the survival signal. Further studies are required to identify what kind of molecular factor triggers this neurodegenerative condition. One possible mechanism is the increase of the inflammatory factors such as tumor-necrosis factor α (TNFα), IL-1β, IL-6, and iNOS from glial cells [22, 40, 41]. In fact, trimethyltin was reported to induce selective loss of granular neurons in DG with activating TNFα-mediated apoptotic signaling [42, 43]. Other possible molecular candidates are the decreased trophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) [44, 45], and vascular endothelial growth factor (VEGF) [46]. BDNF expression in PSD animals has been examined by researchers [14, 18, 39]. Considering the neuronal activity-dependent production of neurotrophic factors [47], it is also possible that the brain areas innervating DG such as the entorhinal cortex might be damaged after MCAO with decreasing the neuronal input to DG; thus it reduced the neuronal activities of DG, resulting in the loss of neurotrophic conditions. Further electrophysiological, neurochemical, and immunohistochemical examinations will explore the detailed pathological mechanisms of the neurodegeneration in the ipsilateral DG after MCAO.

4.3. Postischemic Proliferation of the Neural Stem Cells in SGZ. Increased proliferation of NSCs after ischemic event in SGZ was reported previously [25, 26]. It was also indicated that the proliferated NSCs differentiated into mature neurons and integrated in GCL [48]. In fact, our results of Nestin, Ki67, and DCX immunostaining at early stages after MCAO (Figures 11–13) were consistent with these reports. Although this initial increase is thought to be important to keep the number of mature GC after MCAO for at least 20 weeks, it did not last for 2 weeks and dropped under the sham levels after 6 weeks of MCAO in our observations (Figures 11–13). It seemed that NSCs in SGZ after 6 weeks of MCAO escaped from cell cycle and stayed quiescent. The decreased apoptosis in SGZ after 20 weeks of MCAO also supports this idea (Figures 7 and 8). Different from the loss of GC specifically observed in the ipsilateral GCL, time course changes of NSCs

were observed in both ipsi- and contralateral sides of SGZ, suggesting that they were controlled by systemic regulatory mechanisms such as glucocorticoids released according to the activation of hypothalamic-pituitary-adrenal (HPA) axis [49]. GC with condensed cytosol stained by cresyl violet also tended to increase in both ipsi- and contralateral DG (see Section 3.3, 2nd paragraph). Together with the apoptotic loss of the ipsilateral GC, the suppressed neurogenesis of the bilateral SGZ would also contribute to the aggravation of anhedonia as a result of response to the psychogenic stressor triggered by such motor impairments.

4.4. Effects of Antidepressants after MCAO. Because both IMP and FLV prevented aggravation of anhedonia after 20 weeks of MCAO (Figure 1), we confirmed it was closely related to PSD. FLV is a SSRI, which primarily increases serotonin and dopamine together with stimulating sigma-1 receptor [50]. Recent study of Zhang et al. showed decreased sucrose preference, increased immobility in forced swim and tail suspension tests, and exacerbated neurological functions with reduced body weight by combining MCAO and spatial restraint stress in mice [18]. They also showed the decreased serotonin and dopamine levels in hypothalamus, hippocampus, and cortex in their PSD mice, which were reversed by chronic IMP treatment. Thus, PSD is thought to imply decreased serotonin and dopamine levels in brain with increased cortisol level in serum.

Based on the results of Bax and Bcl-2α analysis shown in Figures 8 and 9, chronic IMP treatment seemed to stimulate the survival signal rather than suppressing the death signal in GCL. Neuroprotective effect of antidepressants was suggested to mediate BDNF-cyclic AMP responsive element-binding (CREB) protein pathway in a previous report [51], and this could be one possible molecular mechanism to keep GC alive by antidepressants after MCAO. Antidepressants including IMP and FLV are also known to stimulate proliferation of NSCs in SGZ, thus promoting the increase of adult neurogenesis [27]. It is understood to be one of the essential mechanisms for the effects of antidepressants [52]. In fact, our results in Figures 11–13 showed that IMP tended to stimulate the proliferation of NSCs, although it did not normalize to the sham level in MCAO groups in our experimental condition. Considering our time course data of both apoptosis and neurogenesis with the effect of antidepressants, favorable timing for the onset of antidepressant treatment on PSD seems to be within 2 weeks after MCAO, although it should be carefully examined with considering the species difference between rodents and humans. Moreover, effects of antidepressants after establishing anhedonia should be examined in our future study, because it will clarify whether antidepressants have therapeutic potency in addition to neuroprotective effects in our PSD model.

5. Conclusions

Spontaneous aggravation of anhedonia was observed at the chronic stage in a rat model of transient cerebral focal ischemia. It associated with the neurodegenerative changes in the hippocampal dentate gyrus: apoptotic neuronal loss of the granular cells and decreased proliferation of the neural stem cells. They are prevented by chronic administration of antidepressants. Our study provides new insights into the pathophysiological mechanisms of poststroke depression and effective use of antidepressants on it.

Abbreviations

ANOVA:	Analysis of variance
BDNF:	Brain-derived neurotrophic factor
CNS:	Central nervous system
CREB protein:	Cyclic AMP responsive element-binding protein
DAB:	3′,3′-Diaminobenzidine
DCX:	Doublecortin
DG:	Dentate gyrus
FLV:	Fluvoxamine
GCL:	Granular cell layer
HPA axis:	Hypothalamus-pituitary-adrenal axis
IL:	Interleukin
IMP:	Imipramine
MCAO:	Middle cerebral artery occlusion
NGF:	Nerve growth factor
NSCs:	Neural stem cells
PSD:	Poststroke depression
SGZ:	Subgranular zone
SSRI:	Selective serotonin reuptake inhibitor
SVZ:	Subventricular zone
TNFα:	Tumor-necrosis factor α
UCMS:	Unpredicted chronic mild stress
VEGF:	Vascular endothelial growth factor.

Competing Interests

All the authors declare that they have no competing interests related to this study.

Authors' Contributions

Jiro Kasahara conceived, designed, and supervised all the experiments, conducted the data analysis, and wrote the paper. Hiroto Uchida and Kenta Tezuka performed the animal experiments, immunohistochemical examinations, and data analysis. Nanae Oka participated in data analysis.

Acknowledgments

Jiro Kasahara would like to thank Dr. Takashi Kitamura in MIT for his valuable comments. This study was supported by an ordinary budget in Tokushima University for research and education in each laboratory.

References

[1] U. Dirnagl, C. Iadecola, and M. A. Moskowitz, "Pathobiology of ischaemic stroke: an integrated view," *Trends in Neurosciences*, vol. 22, no. 9, pp. 391–397, 1999.

[2] W. R. Hudgins and J. H. Garcia, "Transorbital approach to the middle cerebral artery of the squirrel monkey: a technique for

experimental cerebral infarction applicable to ultrastructural studies," *Stroke*, vol. 1, no. 2, pp. 107–111, 1970.

[3] R. G. Robinson, "A model for the study of stroke using the rat: surgical ligation of the middle cerebral artery in the rat," *The American Journal of Pathology*, vol. 104, no. 1, pp. 103–105, 1981.

[4] A. Tamura, Y. Tahira, H. Nagashima et al., "Thalamic atrophy following cerebral infarction in the territory of the middle cerebral artery," *Stroke*, vol. 22, no. 5, pp. 615–618, 1991.

[5] A. Tamura, T. Kirino, K. Sano, K. Takagi, and O. Hidemune, "Atrophy of the ipsilateral substantia nigra following middle cerebral artery occlusion in the rat," *Brain Research*, vol. 510, no. 1, pp. 154–157, 1990.

[6] T. Kirino, "Delayed neuronal death in the gerbil hippocampus following ischemia," *Brain Research*, vol. 239, no. 1, pp. 57–69, 1982.

[7] R. G. Robinson, "Neuropsychiatric consequences of stroke," *Annual Review of Medicine*, vol. 48, pp. 217–229, 1997.

[8] V. Hachinski, "Post-stroke depression, not to be underestimated," *The Lancet*, vol. 353, no. 9166, p. 1728, 1999.

[9] P. W. Burvill, G. A. Johnson, K. D. Jamrozik, C. S. Anderson, E. G. Stewart-Wynne, and T. M. H. Chakera, "Prevalence of depression after stroke: the Perth Community Stroke Study," *British Journal of Psychiatry*, vol. 166, pp. 320–327, 1995.

[10] D. S. P. Schubert, C. Taylor, S. Lee, A. Mentari, and W. Tamaklo, "Detection of depression in the stroke patient," *Psychosomatics*, vol. 33, no. 3, pp. 290–294, 1992.

[11] I. Loubinoux, G. Kronenberg, M. Endres et al., "Post-stroke depression: mechanisms, translation and therapy," *Journal of Cellular and Molecular Medicine*, vol. 16, no. 9, pp. 1961–1969, 2012.

[12] R. G. Robinson and T. R. Price, "Post-stroke depressive disorders: a follow-up study of 103 patients," *Stroke*, vol. 13, no. 5, pp. 635–641, 1982.

[13] R. G. Robinson, L. B. Starr, K. L. Kubos, and T. R. Price, "A two-year longitudinal study of post-stroke mood disorders: findings during the initial evaluation," *Stroke*, vol. 14, no. 5, pp. 736–741, 1983.

[14] G. Kronenberg, K. Gertz, A. Heinz, and M. Endres, "Of mice and men: Modelling post-stroke depression experimentally," *British Journal of Pharmacology*, vol. 171, no. 20, pp. 4673–4689, 2014.

[15] S.-H. Wang, Z.-J. Zhang, Y.-J. Guo, G.-J. Teng, and B.-A. Chen, "Hippocampal neurogenesis and behavioural studies on adult ischemic rat response to chronic mild stress," *Behavioural Brain Research*, vol. 189, no. 1, pp. 9–16, 2008.

[16] C. Wu, J. Zhang, and Y. Chen, "Study on the behavioral changes of a post-stroke depression rat model," *Experimental and Therapeutic Medicine*, vol. 10, no. 1, pp. 159–163, 2015.

[17] Y. Cheng, Q. Su, B. Shao et al., "17β-estradiol attenuates poststroke depression and increases neurogenesis in female ovariectomized rats," *BioMed Research International*, vol. 2013, Article ID 392434, 10 pages, 2013.

[18] G. Zhang, L. Chen, L. Yang et al., "Combined use of spatial restraint stress and middle cerebral artery occlusion is a novel model of post-stroke depression in mice," *Scientific Reports*, vol. 5, p. 16751, 2015.

[19] A. Der-Avakian and A. Markou, "The neurobiology of anhedonia and other reward-related deficits," *Trends in Neurosciences*, vol. 35, no. 1, pp. 68–77, 2012.

[20] L. Terroni, E. Amaro Jr., D. V. Iosifescu et al., "The association of post-stroke anhedonia with salivary cortisol levels and stroke lesion in hippocampal/parahippocampal region," *Neuropsychiatric Disease and Treatment*, vol. 11, pp. 233–242, 2015.

[21] J. Bachevalier and M. Meunier, "Cerebral ischemia: are the memory deficits associated with hippocampal cell loss?" *Hippocampus*, vol. 6, no. 5, pp. 553–560, 1996.

[22] H. Uchida, Y. Fujita, M. Matsueda et al., "Damage to neurons and oligodendrocytes in the hippocampal CA1 sector after transient focal ischemia in rats," *Cellular and Molecular Neurobiology*, vol. 30, no. 7, pp. 1125–1134, 2010.

[23] K. Vass, W. J. Welch, and T. S. Nowak Jr., "Localization of 70-kDa stress protein induction in gerbil brain after ischemia," *Acta Neuropathologica*, vol. 77, no. 2, pp. 128–135, 1988.

[24] M. Zhang, W.-B. Li, Y.-X. Liu et al., "High expression of GLT-1 in hippocampal CA3 and dentate gyrus subfields contributes to their inherent resistance to ischemia in rats," *Neurochemistry International*, vol. 59, no. 7, pp. 1019–1028, 2011.

[25] J. Liu, K. Solway, R. O. Messing, and F. R. Sharp, "Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils," *The Journal of Neuroscience*, vol. 18, no. 19, pp. 7768–7778, 1998.

[26] N. J. Kee, E. Preston, and J. M. Wojtowicz, "Enhanced neurogenesis after transient global ischemia in the dentate gyrus of the rat," *Experimental Brain Research*, vol. 136, no. 1, pp. 313–320, 2001.

[27] G. Kemperman, *Adult Neurogenesis: Stem Cells and Neuronal Development in the Adult Brain*, Oxford University Press, 2005.

[28] B. R. Miller and R. Hen, "The current state of the neurogenic theory of depression and anxiety," *Current Opinion in Neurobiology*, vol. 30, pp. 51–58, 2015.

[29] H. Uchida, H. Yokoyama, H. Kimoto, H. Kato, and T. Araki, "Long-term changes in the ipsilateral substantia nigra after transient focal cerebral ischaemia in rats," *International Journal of Experimental Pathology*, vol. 91, no. 3, pp. 256–266, 2010.

[30] S. Matsuda, M. Umeda, H. Uchida, H. Kato, and T. Araki, "Alterations of oxidative stress markers and apoptosis markers in the striatum after transient focal cerebral ischemia in rats," *Journal of Neural Transmission*, vol. 116, no. 4, pp. 395–404, 2009.

[31] S. Matsuda, M. Umeda, H. Kato, and T. Araki, "Glial damage after transient focal cerebral ischemia in rats," *Journal of Molecular Neuroscience*, vol. 38, no. 2, pp. 220–226, 2009.

[32] A. J. Hunter, J. Hatcher, D. Virley et al., "Functional assessments in mice and rats after focal stroke," *Neuropharmacology*, vol. 39, no. 5, pp. 806–816, 2000.

[33] J. C. Von Frijtag, R. Van den Bos, and B. M. Spruijt, "Imipramine restores the long-term impairment of appetitive behavior in socially stressed rats," *Psychopharmacology*, vol. 162, no. 3, pp. 232–238, 2002.

[34] M. E. Breuer, L. Groenink, R. S. Oosting, H. G. M. Westenberg, and B. Olivier, "Long-term behavioral changes after cessation of chronic antidepressant treatment in olfactory bulbectomized rats," *Biological Psychiatry*, vol. 61, no. 8, pp. 990–995, 2007.

[35] H. Miura, H. Qiao, T. Kitagami, T. Ohta, and N. Ozaki, "Effects of fluvoxamine on levels of dopamine, serotonin, and their metabolites in the hippocampus elicited by isolation housing and novelty stress in adult rats," *International Journal of Neuroscience*, vol. 115, no. 3, pp. 367–378, 2005.

[36] S. Sato, T. Kawamata, T. Kobayashi, and Y. Okada, "Antidepressant fluvoxamine reduces cerebral infarct volume and ameliorates sensorimotor dysfunction in experimental stroke," *Neuroreport*, vol. 25, no. 10, pp. 731–736, 2014.

[37] T. K. S. Craft and A. C. DeVries, "Role of IL-1 in post-stroke depressive-like behavior in mice," *Biological Psychiatry*, vol. 60, no. 8, pp. 812–818, 2006.

[38] Y. T. Mak, W. Y. Chan, W. P. Lam, and D. T. Yew, "Immunohistological evidences of *Ginkgo biloba* extract altering Bax to Bcl-2 expression ratio in the hippocampus and motor cortex of senescence accelerated mice," *Microscopy Research and Technique*, vol. 69, no. 8, pp. 601–605, 2006.

[39] M. Boyko, R. Kutz, B. F. Gruenbaum et al., "The influence of aging on poststroke depression using a rat model via middle cerebral artery occlusion," *Cognitive, Affective & Behavioral Neuroscience*, vol. 13, no. 4, pp. 847–859, 2013.

[40] F. Block, M. Dihné, and M. Loos, "Inflammation in areas of remote changes following focal brain lesion," *Progress in Neurobiology*, vol. 75, no. 5, pp. 342–365, 2005.

[41] M. Kubera, E. Obuchowicz, L. Goehler, J. Brzeszcz, and M. Maes, "In animal models, psychosocial stress-induced (neuro)inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 35, no. 3, pp. 744–759, 2011.

[42] C. A. McPherson, J. Kubik, R. N. Wine, C. L. D'Hellencourt, and G. J. Harry, "Alterations in cyclin A, B, and D1 in mouse dentate gyrus following TMT-induced hippocampal damage," *Neurotoxicity Research*, vol. 5, no. 5, pp. 339–354, 2003.

[43] G. J. Harry, C. Lefebvre D'Hellencourt, C. A. McPherson, J. A. Funk, M. Aoyama, and R. N. Wine, "Tumor necrosis factor p55 and p75 receptors are involved in chemical-induced apoptosis of dentate granule neurons," *Journal of Neurochemistry*, vol. 106, no. 1, pp. 281–298, 2008.

[44] J. D. Gray, T. A. Milner, and B. S. McEwen, "Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors," *Neuroscience*, vol. 239, pp. 214–227, 2013.

[45] C. Jiang and S. R. Salton, "The role of neurotrophins in major depressive disorder," *Translational Neuroscience*, vol. 4, no. 1, pp. 46–58, 2013.

[46] M. J. During and L. Cao, "VEGF, a mediator of the effect of experience on hippocampal neurogenesis," *Current Alzheimer Research*, vol. 3, no. 1, pp. 29–33, 2006.

[47] D. Lindholm, E. Castrén, M. Berzaghi, A. Blöchl, and H. Thoenen, "Activity-dependent and hormonal regulation of neurotrophin mRNA levels in the brain—implications for neuronal plasticity," *Journal of Neurobiology*, vol. 25, no. 11, pp. 1362–1372, 1994.

[48] C. Wang, M. Zhang, C. Sun et al., "Sustained increase in adult neurogenesis in the rat hippocampal dentate gyrus after transient brain ischemia," *Neuroscience Letters*, vol. 488, no. 1, pp. 70–75, 2011.

[49] B. S. McEwen, "Plasticity of the hippocampus: adaptation to chronic stress and allostatic load," *Annals of the New York Academy of Sciences*, vol. 933, pp. 265–277, 2001.

[50] Y. Ago, K. Yano, N. Hiramatsu, K. Takuma, and T. Matsuda, "Fluvoxamine enhances prefrontal dopaminergic neurotransmission in adrenalectomized/castrated mice via both 5-HT reuptake inhibition and $\sigma(1)$ receptor activation," *Psychopharmacology (Berlin)*, vol. 217, no. 3, pp. 377–386, 2011.

[51] M. Nibuya, E. J. Nestler, and R. S. Duman, "Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus," *Journal of Neuroscience*, vol. 16, no. 7, pp. 2365–2372, 1996.

[52] L. Santarelli, M. Saxe, C. Gross et al., "Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants," *Science*, vol. 301, no. 5634, pp. 805–809, 2003.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 5460732, 16 pages
http://dx.doi.org/10.1155/2016/5460732

Review Article

Comorbidity Factors and Brain Mechanisms Linking Chronic Stress and Systemic Illness

Vanja Duric, Sarah Clayton, Mai Lan Leong, and Li-Lian Yuan

Department of Physiology and Pharmacology, Des Moines University, Des Moines, IA 50312, USA

Correspondence should be addressed to Vanja Duric; vanja.duric@dmu.edu

Received 23 July 2015; Revised 11 October 2015; Accepted 25 October 2015

Academic Editor: Laura Musazzi

Neuropsychiatric symptoms and mental illness are commonly present in patients with chronic systemic diseases. Mood disorders, such as depression, are present in up to 50% of these patients, resulting in impaired physical recovery and more intricate treatment regimen. Stress associated with both physical and emotional aspects of systemic illness is thought to elicit detrimental effects to initiate comorbid mental disorders. However, clinical reports also indicate that the relationship between systemic and psychiatric illnesses is bidirectional, further increasing the complexity of the underlying pathophysiological processes. In this review, we discuss the recent evidence linking chronic stress and systemic illness, such as activation of the immune response system and release of common proinflammatory mediators. Altogether, discovery of new targets is needed for development of better treatments for stress-related psychiatric illnesses as well as improvement of mental health aspects of different systemic diseases.

1. Introduction

Stress can be defined as the presence of acute or persistent physiological or psychological threats to the organism that results in significant strain on the body's compensatory systems. Goldstein and McEwen described stress as a condition where a discrepancy exists between the current or anticipated perceptions and expected perceptions of the internal or external environment [1]. Stress is further characterized by the existence of compensatory responses that generally deviate from and extend normal physiological regulation in order to protect the living organism against severe threats and sustain life.

To date, activation of the hypothalamic-pituitary-adrenal (HPA) axis has been widely accepted as one of the central physiological mechanisms involved in stress response. It is primarily dependent on stimulation of corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) release from the hypothalamic paraventricular nucleus (PVN) and the anterior pituitary, respectively, leading to increased production and systemic release of glucocorticoids from adrenal glands. However, high glucocorticoid levels that are associated with repeated or chronic stress can also lead to hyperactivation of the HPA axis due to diminished function of negative feedback mechanisms, especially within the limbic brain areas that regulate mood and emotional responses. Moreover, chronic stress is also accompanied by increased secretion of proinflammatory cytokines (PICs) that can further impair neuronal transmission and plasticity within these brain circuits; *for a more comprehensive review see* [2].

In contrast to coordinated physiological processes that underlie maintenance of steady state or homeostasis, prolonged psychological or traumatic stress can lead to disruption of cellular and systemic equilibriums resulting in dysfunction of both the nervous system and peripheral organ systems. Thus, chronic stress can ultimately lead to alterations and dysfunction of internal systems that control stress responses and consequent development of both neurological and psychosomatic illnesses. This concept is further supported by clinical reports indicating that psychological stress and systemic disorders are both commonly associated with adverse impact on mental health and development of comorbid psychiatric illnesses. Indeed, clinical depression is present in up to 50% of patients with chronic systemic conditions (e.g., pain, stroke, cardiovascular disease, obesity, diabetes, and cancer), much higher than the rate of 5–8%

in the general population [3–5]. However, despite the abundance of empirical evidence and the well-established link between different physical disease states and mental illness, the question remains why so many different systemic disorders are frequently accompanied by deterioration in mental health. Adaptation to stress or allostasis, a well-known phenomenon that requires integration of autonomic, endocrine, and behavioral response systems, most likely plays an important role in such comorbidities; however, the precise underlying physiological and biochemical mechanisms are still poorly understood. Thus, in this review we will discuss the pathophysiological consequences of chronic stress on the brain and central nervous system (CNS) with particular focus on alterations in neural plasticity and function, as well as the correlation between different systemic illnesses and mental disorders such as clinical depression.

2. Effects of Chronic Stress on Neural Mechanisms Involved in Development of Mood Disorders

2.1. Changes in Neuronal Structure and Function. Clinical and basic science research has provided strong evidence that prolonged exposure to psychological stress can lead to the development of behavioral deficits and psychiatric illness, especially depression and anxiety. Stress affects the nervous system and brain as a whole; however, several limbic areas within the mood-regulating neurocircuitry, such as the hippocampus and prefrontal cortex (PFC), seem to be especially vulnerable to chronic stress [6, 7]. The negative effects of stress have been consistently observed in these brain structures, especially morphological and molecular adaptations shown to impair normal neural and glial cell function (Figure 1). For example, examinations of stressed brains in rodent models, as well as imaging studies and post-mortem analysis of depressed human brains, have revealed significant atrophy (e.g., reductions in dendritic arborization and spine density), cell loss, and reduced tissue volume in the hippocampal and PFC regions [8–16]. Decreased density and number of glial cells, both astrocytes and oligodendrocytes, have also been observed in depressed and bipolar brains, within the dorsolateral PFC, orbitofrontal cortex [17, 18], and anterior cingulate cortex [10, 19]. Furthermore, stress was also shown to decrease neurogenesis (i.e., production of new neurons) in the adult hippocampus. Although the exact role of neurogenesis in the etiology and maintenance of depression is still unclear, currently available reports indicate that neurogenesis is increased by antidepressant treatments and may be required for development of antidepressant behavioral responses [20–23]. At the molecular and biochemical levels, stress initially evokes responses such as increased glucocorticoid release and altered neurotransmitter activity, followed by glutamatergic excitotoxicity, decreased neurotrophic support, and diminished synaptic function [24–26]. With chronic exposure to stress, these initial adjustments may progress into cellular destabilization and morphological changes eventually resulting in dysfunction of both individual neurons and entire neural networks encompassing both limbic and cortical brain areas [7].

2.2. Role of Neurotrophic Factors and Proinflammatory Cytokines. Stress-evoked brain alterations mentioned above may represent some of the key neuropathological factors underlying development of behavioral deficits and an overall depressive phenotype; however, the exact molecular adaptations and subsequent cellular remodeling mechanisms are still not well defined. During the last three decades, a number of different modulators and intracellular pathways have been linked to stress responses. A plethora of evidence suggests that adequate brain levels of neurotrophic factor signaling, which is involved in neuronal growth, differentiation, and survival, may have a critical role in both the etiology of mood disorders and treatment response and, thus, have led to formation of the neurotrophic hypothesis of depression [27–29]. For example, decreased expression levels of brain-derived neurotrophic factor (BDNF), a member of nerve growth factor family, and its preferred receptor tyrosine kinase B (TrkB) were found in the hippocampus and PFC of both depressed humans and animals exposed to stress [30–32]. In contrast, chronic antidepressant treatments were shown to increase *in vivo* BDNF levels [33, 34]. Further studies also showed that intrahippocampal administration of BDNF produces antidepressant-like behavioral effects [35], while actions of chemical antidepressants are attenuated in BDNF knockout mice [36]. Moreover, actions of BDNF in this framework also seem to be multifaceted and brain circuitry-dependent, as its increased activity within the mesolimbic dopamine reward pathway (i.e., ventral tegmental area and nucleus accumbens) results in development of depressive-like behaviors [37]. However, when administered by routes that impact multiple brain areas and different neuronal circuits (i.e., intracerebroventricular or systemic), BDNF largely produces antidepressant responses [38, 39]. In addition to BDNF, a number of recent studies using combinations of *in vivo* and *in vitro* approaches have identified several additional key neurotrophic factors that are involved in stress processing and/or antidepressant actions including fibroblast growth factor 2 (FGF2), neuritin, vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), and VGF (nonacronymic); *for a more comprehensive review see* [7, 23].

Activated immune cells, such as macrophages, T-lymphocytes, and CNS's microglia, synthesize and secrete cytokines that function as signaling molecules implicated in regulation of cellular homeostasis and chronic inflammatory processes [40]. The idea that immune system activation may also be associated with stress and depression pathophysiology (Figure 1) is supported by clinical findings showing elevated blood levels of proinflammatory cytokines (PICs), primarily interleukin- (IL-) 1β, IL-6, and tumor necrosis factor alpha (TNFα), in depressed patients [41, 42]. Likewise, robust upregulation of genes coding for different inflammatory cytokines was found in a recent microarray analysis of depressed PFC [43]. Overall, increased activity of PICs is thought to contribute to the CNS effects of stress to induce a depressive phenotype, including symptoms such as anhedonia and sleeping abnormalities [41]. Additional evidence linking the negative effects of stress and PICs comes from recent reports suggesting that stress-induced activation of IL-1β leads to inhibition of neurogenesis in animal models

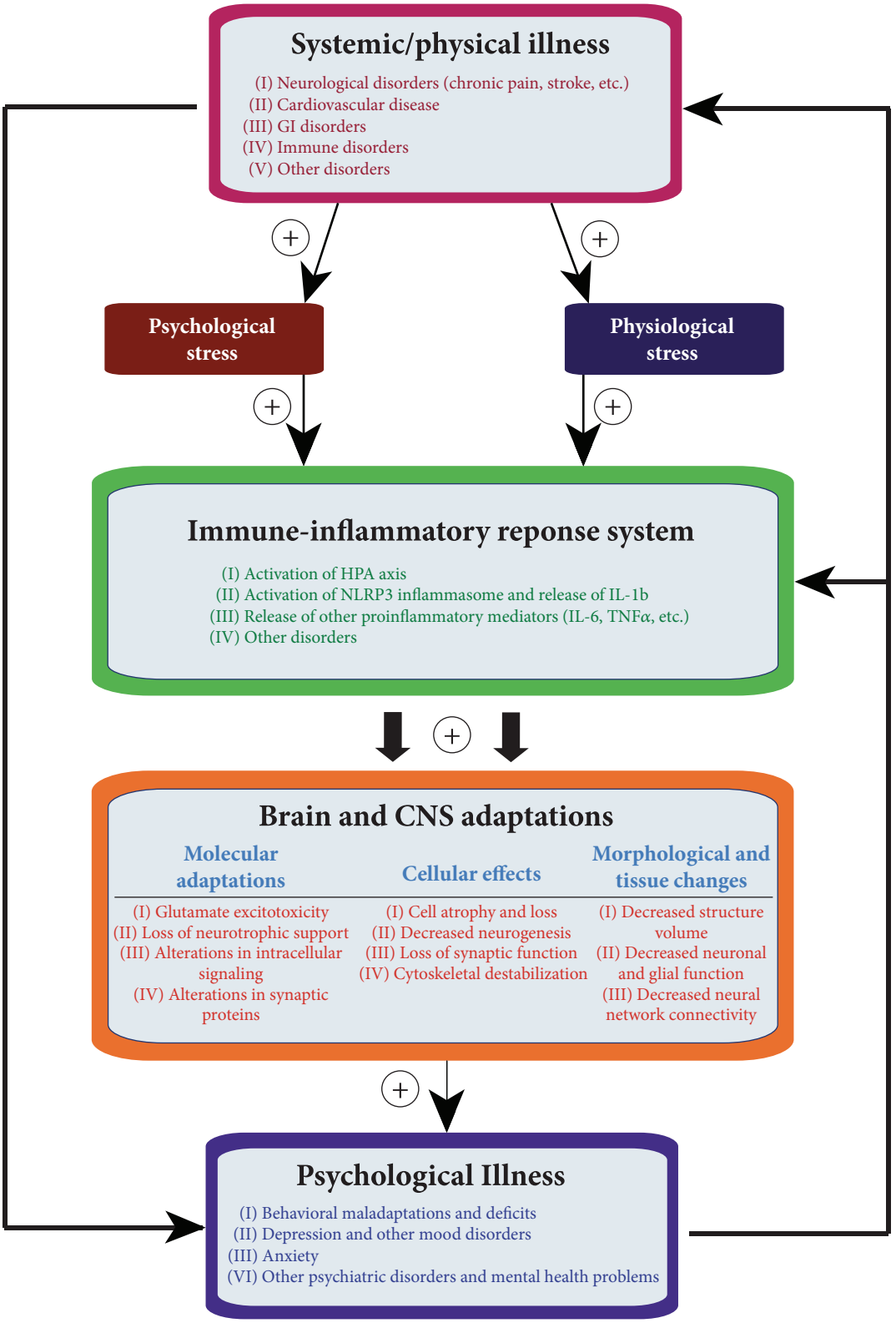


Figure 1: Bidirectional relationship between systemic illness and psychiatric disorders. Physical and/or psychological stress associated with systemic illness can lead to activation of immune response system resulting in increased local and systemic release of proinflammatory cytokines. Increased levels of inflammatory mediators in the CNS are potentially key contributors to the damaging cellular and morphological adaptations that underlie development of comorbid mental illness.

[44–46]. Specifically, administration of IL-1β was shown to suppress cell proliferation within the hippocampal dentate gyrus subregion, while blockade of IL-1β signaling was protective against antineurogenic effects and depressive-like behaviors caused by stress [44].

2.3. Altered Neuronal Plasticity, Synaptic Dysfunction, and Synaptogenesis. Recent studies have revealed that stress and depression are also associated with decreased synaptic function and reduced overall neural connectivity in the PFC and hippocampus, possibly due to loss of neurotrophic signaling and glucocorticoid overactivity in these brain areas [47]. Neural processes involved in synaptic plasticity and synaptogenesis (i.e., production of new neuronal synapses) were shown to be particularly susceptible to stress and may play a critical role in antidepressant effect [48, 49]. This idea is further supported by evidence demonstrating that administration of drugs such as ketamine (a glutamate receptor antagonist) evokes robust increases in synaptogenesis resulting in a rapid antidepressant response, even in treatment-resistant depressed patients [50–52]. Moreover, these rapid actions of ketamine are contingent upon increased BDNF release and activation of mammalian target of rapamycin (mTOR) intracellular pathways, leading to downstream production of synaptic proteins involved in formation of new dendritic spines and synaptogenesis [53, 54]. Thus, enhanced synaptic activity and neural network connectivity, especially within the PFC, are thought to underlie rapid antidepressant behavioral responses and provide new directions for development of improved treatment and diagnosis of depression and other psychiatric illnesses.

3. Comorbidity of Stress-Related Mood Disorders and Systemic Illness

3.1. Neurological Disorders

3.1.1. Chronic Pain. Chronic pain is commonly associated with altered mood. Numerous clinical reports indicate the high prevalence of depression and other psychiatric illnesses among patients with all types of chronic pain conditions [55–59]. It is estimated that comorbid depression can be present in 30–50% of clinical chronic pain patients [60]. Although the physiological mechanisms linking pain and mood disorders are not clear, recent human and animal studies have demonstrated that pain produces morphological, organizational, and functional changes in both cortical and subcortical brain structures [61]. Neuroimaging studies have revealed that direct pain exposure activates sites within the brain network (i.e., "pain matrix") that are responsible for defining cognitive and emotional aspect of pain and are mostly independent of sites involved in the sensory pain processing [62–66]. This component of pain-activated neurologic signature [67] involves primarily limbic neurocircuitry, including brain areas such as the anterior cingulate cortex, insula, PFC, amygdala, and hippocampus, and is thought to also help incorporate aspects such as attention, anticipation, memory, and empathy into the formation and characterization of the overall pain experience and perception [68–71]. However, in chronic pain states, prolonged nociceptive activation of the limbic neurocircuitry may also lead to dysfunction of affective pain processing resulting in development of secondary pathologies including mental illness [72–74]. This idea is further supported by studies showing a high correlation between severity of depression and the duration and severity of pain, number of pain sites, number of pain days, frequency of breakthrough pain, and general pain-related inhibition in daily functioning [56, 59, 75–77]. Likewise, additional factors such as age, gender, level of motor dysfunction, marital status, and other socioeconomic conditions have been shown to affect the incidence and severity of pain-related depression [59, 78, 79]. Other clinical observations further suggest that the relationship between pain and mood disorders is most likely reciprocal, as depressed patients commonly experience increased pain complaints (e.g., headache and stomach pain), and the perception of endogenous pain stimuli is, in part, correlated to modality, severity, and duration of pain conditions [80, 81]. Such findings could be explained by the effects of chronic stress, via elevation of glucocorticoid levels, on the function of corticolimbic and brainstem structures that modulate endogenous pain facilitation [82, 83]. Altogether, these clinical observations suggest potential impairments of the sensory systems (i.e., ascending and descending pain-control pathways) in patients with mood disorders, as well as robust alterations in activity of stress- and mood-regulating brain areas in chronic pain patients.

Pain- and stress-evoked overactivation and dysfunction of the HPA axis may, in part, underlie the physiological and pathological events that link these disease states [84]; however, the exact CNS mechanisms are still unknown. Our previous findings have indicated that persistent pain causes cellular and molecular adjustments within the limbic brain areas similar to those evoked by prolonged stress. Specifically, we found robust decreases in neurotrophic factors signaling (e.g., BDNF) and diminished rate of neurogenesis in the hippocampus of animals exposed to 21 days of peripheral, inflammatory nociception [85–88]. Similar findings were also more recently observed in animal models of neuropathic pain, where pain-evoked decreases in hippocampal neurogenesis and synaptic deficits were linked to anxiety-like behaviors and deficits in learning and memory [89]. At the molecular level, neuropathic pain also evokes increased expression of PICs in the brain, which is thought to contribute to the development of depressive-like behaviors [90, 91]. Likewise, pain-related development of allodynia is correlated with sustained elevation of hippocampal PICs, specifically IL-1β and IL-6 [92]. Furthermore, these initial pain-evoked increases in IL-1β were shown to be dependent on ATP signaling and P2X7 receptor activation, which suggest involvement of microglial cells in supraspinal pain processing [93, 94]. This notion is supported by a recent study showing that neuropathic pain causes increases in activated microglia within the reward neurocircuitry leading to disruption of dopamine-mediated reward behavior [95]. In total, these preclinical reports indicate that neural mechanisms linking pain and mood alterations may be dependent on induction

of neuroinflammation within the brain structures involved in regulation of emotional states and pain perception.

3.1.2. Substance Use Disorders and Alcoholism. Large-scale epidemiological studies have also revealed a high extent of comorbidity between substance abuse and mood disorders [96–98]. There is a near-twofold increase in the lifetime prevalence of depression in individuals with a substance use disorder (SUD), and vice versa, reflecting the reciprocal nature of these disorders.

Several mechanisms have been proposed to underlie the high cooccurrence of SUD and mood disorders [99]. First, mood disorders and SUD may be risk factors for each other due to their negative pathological effects. In the case of alcoholism, which includes both alcohol abuse and dependence, excessive consumption of alcohol can cause negative emotional and social problems. Conversely, those suffering from mood disorders may seek alcohol and/or other drugs of abuse as self-medication to cope with their stress and anxiety symptoms, resulting in a higher risk of developing alcoholism and/or drug addiction. Furthermore, there may exist overlapping neural circuits that are targets of pathological manifestations of both SUD and stress. Recent neural imaging studies have revealed that both patients with substance dependence and mood disorders exhibit increased activation in certain regions of the brain's reward system during performance of emotional and reward-processing task; *for a more comprehensive review see* [100]. Finally, common genetic factors may also contribute to the cooccurrence [101, 102]. This idea is supported by a recent study showing that simultaneous treatment of clinically depressed alcoholic patients with both a selective serotonin reuptake inhibitor (SSRI) and an opioid antagonist (i.e., for alcohol dependence) produces a greater therapeutic effect [103]. Overall, such findings suggest that comorbidity of mood disorders and SUD require development of treatment strategies that address both disorders, preferably through combining psychosocial treatment with pharmacotherapy.

3.1.3. Stroke. Different types of psychiatric disorders are commonly observed following stroke, including depression, anxiety, emotional incontinence, delusions, and hallucinations [104]. Poststroke depression (PSD) is the most frequent neuropsychiatric complication after stroke, affecting approximately 30–50% patients within the first year of recovery [104–106]. PSD not only was shown to impact cognitive function and mental health but also impairs rehabilitation progress and functional motor recovery in stroke patients. PSD is also associated with increased risk of suicide and increased mortality; however, it is frequently undetected and remains untreated [107–110]. The pathophysiology of PSD has been shown to be multifactorial, including aspects of neuroanatomical size, location and number of lesions, stroke subtype and severity, social difficulties, family support, and overall stroke burden [104]. Moreover, emerging evidence suggests that risk factors contributing to the vulnerability to depression involve neuroanatomical alterations (e.g., smaller amygdala), disruption of basal ganglia-prefrontal pathways, and cerebrovascular impairments, such as hypertension, atherosclerosis, and hyperlipidemia [111–114]. In addition to these factors, preexisting depression is another independent risk factor for stroke development and recurrence, indicating a reciprocal association between these disease processes [115, 116]. Overall, the association between stroke and depression is well documented; however, the etiology of PSD is highly complex and remains poorly understood.

3.1.4. Alzheimer's Disease. Several meta-analyses have linked a history of clinical depression or depressive-like symptoms with Alzheimer's Disease (AD) [117–120]. Specifically, late-life depression appears to be a significant risk factor for future development of AD [121–123]. Similarly, patients with a history of depression prior to the diagnosis of AD are more likely to experience new depressive episodes throughout the course of AD [124, 125] and the corresponding depressive symptoms tend to worsen with progression of the disease [126]. Although there is significant clinical evidence supporting this comorbidity between depression and AD, very little is understood about the neural mechanisms linking the two disease states. Recent studies show that rodent models of AD do exhibit depressive-like behavioral deficits such as helplessness, indicated by prolonged immobility in the forced swim test [127]. Furthermore, at the molecular level, decreased BDNF mRNA expression and protein levels were found in the postmortem AD brains, especially within the areas linked to depression pathophysiology such as the hippocampus and frontal cortex [128–131]. These observations of depleted growth factor levels in the AD brains are similar to previous findings in depressed brains and may underlie the progressive cell death and neurodegeneration characteristic to both illnesses. Thus, overall lack of neurotrophic support in key brain areas may represent one common pathogenic mechanism linking AD and depression.

Numerous studies also report altered levels of various cytokines in the peripheral circulation of AD patients [132–134]. More importantly, general patterns of rise in the serum levels of several PICs, including IL-1β, TNFα, and IL-6, observed in AD patients [133, 135] are very similar to increases in circulating levels of proinflammatory cytokines in depressed patients [136, 137]. Interestingly, in patients with comorbid AD and depression, blood PIC levels are even more elevated [138]. Moreover, these peripheral immune studies are seemingly corroborated by genetic studies investigating the involvement of alleles associated with higher production of PICs in a sample of elderly patients with AD and depression [139]. In sum, besides alterations in growth factor activity within the brain, changes in the activation of peripheral immune system may signify another pathogenic mechanism linking clinical features of AD and various mood disorders, including depression.

3.2. Cardiovascular Disease (CVD). Cardiovascular health is influenced by many factors. Traditionally, the major risk factors for cardiovascular disease are high blood pressure, high blood lipids, diabetes, and family history. However, other risk factors have recently emerged to predict cardiovascular health, especially ones that include lifestyle choices (e.g.,

exercise and nutrition). Moreover, emerging research has identified chronic stress as another important risk factor for development of cardiovascular disease. This idea is supported by a number of clinical studies that have established a strong link between prolonged exposures to psychological stress and increased incidences of stroke, myocardial infarction, atherosclerosis, and coronary artery disease [140–145].

The risk for myocardial infarction (MI) can also be significantly increased by previous exposures to psychological stressors, as demonstrated by the *INTERHEART* study [141]. In the Nurse's Health Study 2 respondents suffering posttraumatic stress disorder (PTSD, by the clinical definition) were shown to have a 60% enhanced risk of MI or stroke [145]. Furthermore, this study also found the presence of additional behavioral risk factors (e.g., smoking and sedentary lifestyle) among subjects at highest risk of MI. The relationship between chronic stress and behavioral risk factors has also been shown in other cohorts [146, 147]. For example, chronic stress is associated with poor food choices, leading to obesity and metabolic disorders, which are independent risk factors for CVD. A recent study by Bergmann and colleagues examined the role of chronic stress on the development of the metabolic syndrome, a constellation of disorders including obesity, high blood pressure, hyperglycemia/insulin resistance, and hyperlipidemia [148]. Interestingly, the energy homeostasis was shown to be significantly impaired in stressed animals in comparison to control animals when both were fed a high-fat diet. Stressed animals on high-fat diet also exhibited altered levels of leptin, the "satiety hormone" previously shown to have the capability to change the activity of specific brain regions, including those involved in control of sympathetic outflow and regulation of stress responses [149–151]. Moreover, presence of stress-related hyperglycemia has also been documented and implicated in increased activation of proinflammatory signaling and promotion of oxidant stress [152, 153].

Recent human and animal studies have further demonstrated that stress can also lead to the development of vascular dysfunction resulting in a predisposition to CVD [154–157]. In addition, both acute and chronic stressors have been shown to impair vascular reactivity. Although the exact mechanism and effect seem to be dependent on the type of the stressor and/or the model, there is evidence to suggest that reduced vascular dilation and enhanced constriction may play an important role [156, 157]. In humans, acute psychological stress reduces the magnitude of flow-mediated dilation of the brachial artery, a measure of the reactivity of the peripheral vasculature that is thought to be the result of altered nitric oxide handling [154]. Furthermore, in the Takotsubo syndrome, an acute coronary condition usually resulting from an exposure to a major emotional or physical stressor, a reaction is triggered that mimics coronary artery disease, which is suggested to be a result of increased sympathetic activity leading to impaired coronary vascular reactivity [158]. Animal studies have also shown changes in coronary hemodynamics as a result of psychological stress. For example, studies in dogs have demonstrated that psychological stress can reduce coronary blood flow, resulting in electrocardiographic changes resembling an acute MI, and an enhanced risk for arrhythmias, possibly through alterations in the effective refractory period [159, 160]. Similarly to chronic stress, a number of recent studies have linked depression to peripheral arterial disease [161], coronary artery disease [162], MI/stroke [163, 164], heart failure [165–167], and hypertension [168, 169], suggesting that depression, perhaps as a result of sustained stressors, can also have a major impact on cardiovascular health.

In both clinical and preclinical studies, chronic stress has been shown to increase proinflammatory signaling cascades and reactive oxygen species, enhance vasoactive agents (e.g., endothelin-1 and angiotensin II), and activate both the HPA axis and sympathetic nervous system activity, while reducing the activity of vasodilators (i.e., nitric oxide) and abrogating the activity of the parasympathetic nervous system. Sympathetic activity is also increased with stress, mainly due to enhanced neuronal discharge from the paraventricular nucleus of the hypothalamus and the rostral ventrolateral medulla, leading to overactivation of sympathetic preganglionic neurons [170, 171]. Additionally, neurohumoral factors including angiotensin II are also increased in chronic stress [172, 173]. Angiotensin II is linked to cardiovascular disease through direct vasoconstriction, altered salt and water excretion from the kidney, and cellular remodeling. Moreover, angiotensin II can perpetuate the proinflammatory state through activation of nuclear factor- (NF-) κB, among other transcription factors [174–177].

3.3. Gastrointestinal Disorders. Gastrointestinal (GI) disorders are often associated with stressful symptoms including chronic abdominal pain, severe diarrhea, and changed bowel habits. Some of the major functional/structural GI disorders, such as the inflammatory bowel disease (IBD), are characterized by chronic inflammation of the digestive tract; however, there are no identifiable anatomic or biochemical abnormalities that can be attributed to the symptoms. In addition to the symptoms of physical illness, patients with GI disorders commonly exhibit mental health problems and are often diagnosed with psychiatric illness. Depression and mood disorders are among the most common psychiatric comorbidities with a much higher prevalence than the general population [178]. The Manitoba IBD cohort study reported a lifetime depression rate of 27% in IBD patients compared to 12% in healthy controls [179]. Similarly to other systemic illnesses, the relationship between mood and GI disorders appears to be bidirectional. Patients with depression have been found to have a significantly higher frequency of IBS and at greater risk for subsequent development of GI symptoms [180].

Potential mechanisms linking GI and mood disorders include, but are not limited to, alterations in brain regions involved in emotion regulation, gut-brain axis, HPA axis, and proinflammatory signaling. There is increasing evidence suggesting a bidirectional communication between the gut-residing microbiota/microbiome and the brain [181, 182]. This interaction has been demonstrated in animal models where perception of stress by the brain was shown to reduce the diversity of the microbiota in the gut [183] and, in turn, the microbiome also influences stress responses

and depressive behaviors through modulating brain activity [184]. Recent clinical studies further support this relationship as chronically depressed patients were shown to have increased plasma levels of immunoglobulins (Ig), especially IgA and IgM, indicating enhanced immune responses against lipopolysaccharides (LPS) of different commensal Gram-negative bacteria [185, 186]. This type of systemic activation of IgA/M-mediated inflammatory response system (IRS) suggests that greater permeability of the gut wall (i.e., "leaky gut") and enhanced bacterial translocation may signify additional phenomena that can play important roles in development of both sickness behavior symptoms and pathophysiology of chronic depression [187].

At the molecular level, multiple genetic factors may contribute to the comorbid occurrence of mood and GI disorders, including polymorphisms of BDNF and serotonin transporter genes [188, 189]. It is also worth noting that in female IBS patients enhanced tryptophan degradation has been detected, which results in depletion of tryptophan, the precursor for serotonin synthesis [190]. Thus, it is plausible that hypofunction of serotonin links IBS with depression. Moreover, symptoms associated with physical aspects of GI disorders, such as chronic pain and inflammation, may also serve as risk factors for development of depression. Conversely, mood disorders in turn can adversely affect the course of GI disorders as well as the treatment outcomes. More effective treatments, aimed at breaking this vicious circle, are needed with the intention to mitigate the comorbid mood disorders simultaneously [191]. These treatments, both pharmacotherapy (e.g., antidepressants) and psychotherapy, should demonstrate effectiveness in the context of depression cooccurring with GI symptoms.

3.4. Obesity and Metabolic Disorders. In addition to the above-mentioned disorders, systemic activation of innate immune processes has been linked to a range of different factors that represent potential risks for comorbid depression including psychosocial stressors, daily practices, and lifestyle choices (i.e., lack of exercise and physical activity, sleep deprivation, smoking, poor diet, etc.) as well as pathological states such as obesity and metabolic disorders; *for a more comprehensive review see* [187, 192]. Obesity is usually linked to poor dietary habits and currently represents a significant public health and medical concern in terms of both prevalence and related secondary physical illnesses, respectively [193–195]. Obesity is also associated with cognitive deficits and number of comorbid mood disorders. More specifically, obesity and depression seem to be correlated in a bidirectional manner, as clinical studies have shown that obesity predisposes to the development of clinical depression, while depression can significantly increase the risk of developing obesity [187, 196, 197]. Moreover, obesity is thought to induce a state of systemic low-grade inflammation characterized by increased production of PICs that promote development of other disorders including diabetes, cardiovascular dysfunction, and psychiatric illnesses [198]. Indeed, adipose tissue contributes to development of systemic inflammation through the release of adipokines such as proinflammatory leptin and predominantly anti-inflammatory adiponectin [199]. Thus, controlling the release and activity of these adipokines is essential for maintenance of energy balance and overall homeostasis. Moreover, clinical studies have shown that obesity is associated with a reduction in adiponectin, which potentially underlies the development of type 2 diabetes (T2D), hypertension, and atherosclerosis [200]. The exact actions of these circulating metabolic regulators on CNS and brain function are still being investigated; however, several recent studies have linked alterations in central metabolic mechanisms with systemic dysregulation [201]. Additional factors including dysbiosis and excessive nutrient intake as well as different "disease clusters," such as metabolic syndrome (MetS) and polycystic ovarian syndrome (PCOS), have also been associated with proinflammatory processes leading to obesity, metabolic dysregulation, and impaired innate immunity [202–206]; however, further research is necessary to determine how these processes and pathological states influence chronic psychological dysfunction and psychiatric illnesses.

4. Role of Inflammatory Processes in Linking Stress and Systemic Illness

Based on the clinical and preclinical evidence discussed so far, the elucidation of the precise mechanisms underlying how psychological and physical stressors can influence the CNS and peripheral organ systems as well as the development of systemic diseases remains unsolved. A recently proposed immune-inflammation based hypothesis of depression and related comorbid systemic illnesses may help answer this question [5]. This hypothesis suggests that an inflammasome protein complex and related inflammatory reactions are the main driving force underlying the reciprocal/bidirectional relationship between the psychological stress-related psychiatric illness and comorbid systemic disease (Figure 2).

The idea that psychological and physiological stressors can stimulate inflammatory cytokine activity, leading to neuronal dysfunction and ensuing development of psychiatric illness such as MDD, has been promoted for over 30 years [207, 208]. Although exact physiological and molecular pathways are not fully understood, recent findings have demonstrated/indicated bidirectional/reciprocal communication between neuroendocrine and immune systems [209]. As previously mentioned, several meta-analysis studies have provided strong evidence that depression is accompanied by alterations in immune system function and activation of inflammatory response system (IRS) as increased levels of PICs (i.e., IL-1β, IL-6, and TNFα) were found in the blood of depressed patients [210, 211]. In contrast, serum levels of these cytokines are normalized by treatments with tricyclic antidepressants (TCAs) and SSRIs [212]. Furthermore, increased levels of PICs (i.e., IL-1β and TNFα) were also found in brain tissue after exposure to psychological stressors, an observation consistent with their role as potent activators of the HPA axis, which is commonly dysregulated in mood disorders [213, 214]. For example, approximately two-thirds of patients suffering from depression exhibit elevated cortisol levels [215]. At the cellular level, cytokines are involved in release of different stress hormones implicated in HPA axis

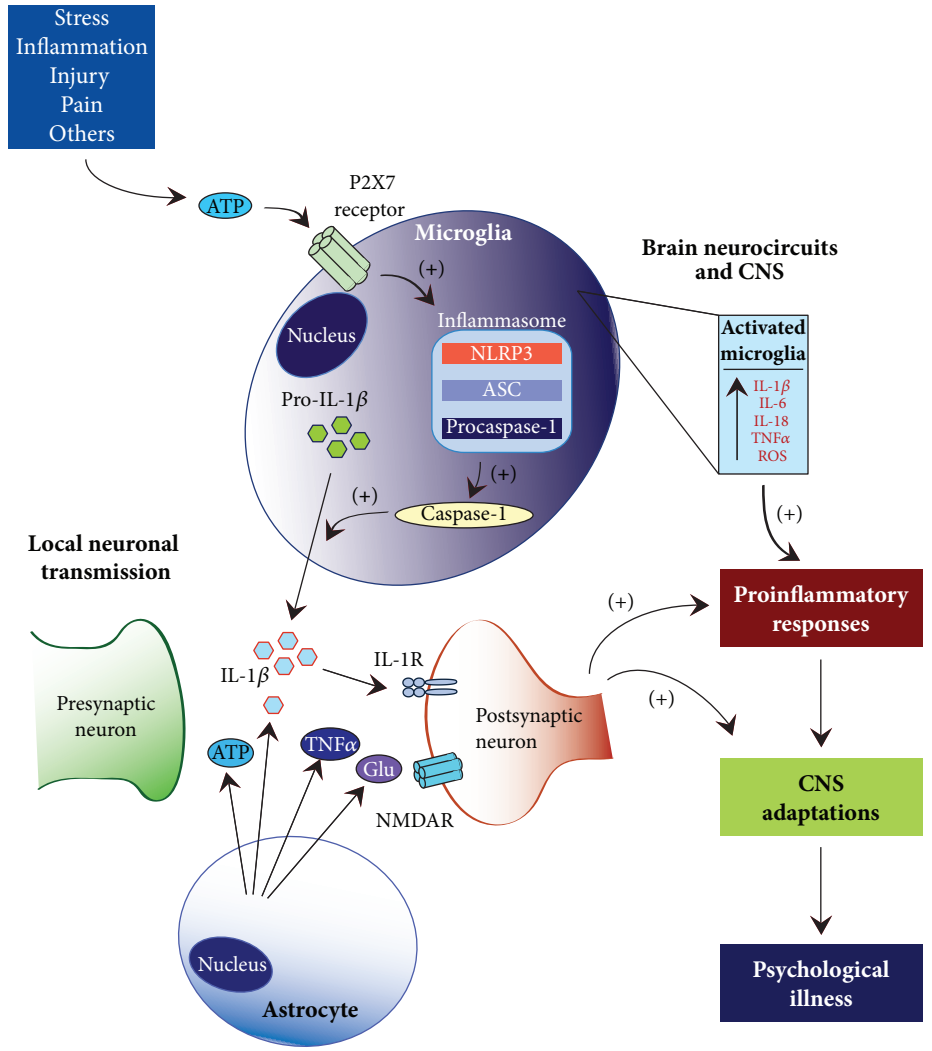


Figure 2: Role of microglia and inflammasome complex in development of inflammatory responses. Systemic disorders and pathological states can induce activation of microglial cells leading to a local release of proinflammatory cytokines (PICs). This process includes stimulation of microglial P2X7 receptors and downstream activation of the NLRP3 inflammasome. Consequent systemic increases in circulating PICs may signify one of the fundamental mechanisms responsible for initiation of molecular and functional alteration within the CNS that underlie development of mental illness. Interleukin (IL), tumor necrosis factor alpha (TNFα), reactive oxygen species (ROS), NOD-like receptor family, pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), *N*-methyl-D-aspartate receptor (NMDAR), and purinergic type 2X7 (P2X7).

activity, primarily the CRH and ACTH [216, 217]. Moreover, enhanced activation of these cytokines has been linked with sickness behaviors in animal models and mood alterations, such as dysphoria and anxiety, that resemble behavioral symptoms of depression in humans [209]. Commonly, the release of these proinflammatory cytokines transpires as a result of activation of the innate immune system defense mechanisms in response to the presence of potentially survival-threatening pathogenic molecules and/or an array of danger signals [5]. In line with this idea, preclinical studies have identified IL-1β as a critical mediator of stress-induced behaviors. Several lines of empirical evidence support this notion: (1) stress was shown to increase hippocampal IL-1β levels, (2) IL-1β robustly suppresses hippocampal cell proliferation, and (3) genetic deletion or pharmacological blockade of IL-1β receptor completely protects against damaging effects of stress and development of depressive-like behaviors [44]. In addition, these IL-1β-related prodepressive effects are thought to occur through activation of the NF-kβ signaling pathway [218].

Recent mechanistic studies have identified toll-like receptors (TLRs) as key contributors to the recognition and initiation of an immune response in the presence of pathogenic molecules leading to increased gene transcription and release of IL-1β, IL-6, and TNFα [219, 220]. Moreover, the induction and final release of mature IL-1β protein also entail cleavage from inactive pro-IL-1β form. This process was shown to be dependent on stimulation of the ATP purinergic type 2X7 (P2X7) receptors and downstream activation of the NLRP3 inflammasome complex that includes caspase-1,

an enzyme responsible for cleavage of pro-IL-1β (Figure 2) [221–224]. In the CNS, the NLRP3 inflammasome is constitutively expressed in microglial cells which are present throughout the brain, especially in the hippocampus, a mood-regulating area susceptible to chronic stress [5]. Finally, the recently proposed idea that activation of NLRP3 inflammasome is the central mechanism linking systemic illness and comorbid mood disorders is further supported by observations that a number of different neurological, cardiovascular, metabolic, and inflammatory diseases have been associated with NLRP3 and elevated IL-1β release; *for a more comprehensive review see* [5]. Altogether, these findings suggest that inflammatory cytokines and NLRP3 inflammasome may act as key neuromodulators in neurochemical and physiological alterations that underlie development and maintenance of behavioral deficits associated with both mood disorders and systemic illnesses.

5. Conclusions

Significant amounts of evidence from human and animal research have demonstrated that psychological and/or physical stress is a powerful driving force behind both the pathophysiology of and comorbidity between psychiatric and systemic disorders. Stress-related detrimental modifications in structure, organization, and function of CNS may, in part, explain the coincidence and comorbidity between these pathological states. This concept is further supported by clinical observations showing that mood disorders, such as depression, are some of the most prevalent and debilitating psychiatric illnesses associated with other systemic disorders. Recent findings point towards the immunoinflammatory system as a key endogenous response mechanism involved in this elusive, bidirectional interaction between stress and illness; however, further studies are needed to reveal the exact pathophysiological mechanism(s) involved. Nonetheless, the question remains whether the mental health (e.g., behavioral/mood deficits) should be addressed as part of the standard treatment of systemic disease. Future studies may link different areas of biomedical sciences and open new research avenues that may ultimately identify novel clinical strategies for improved management of systemic illness by also improving the mental health aspects of these disorders.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work is funded by grants from PhRMA Foundation (Vanja Duric), Iowa Science Foundation (Vanja Duric), and NIH R15MH108043 (Li-Lian Yuan).

References

[1] D. S. Goldstein and B. McEwen, "Allostasis, homeostats, and the nature of stress," *Stress*, vol. 5, no. 1, pp. 55–58, 2002.

[2] A. Gądek-Michalska, J. Tadeusz, P. Rachwalska, and J. Bugajski, "Cytokines, prostaglandins and nitric oxide in the regulation of stress-response systems," *Pharmacological Reports*, vol. 65, no. 6, pp. 1655–1662, 2013.

[3] R. C. Kessler, P. Berglund, O. Demler et al., "The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R)," *Journal of the American Medical Association*, vol. 289, no. 23, pp. 3095–3105, 2003.

[4] D. L. Evans, D. S. Charney, L. Lewis et al., "Mood disorders in the medically ill: scientific review and recommendations," *Biological Psychiatry*, vol. 58, no. 3, pp. 175–189, 2005.

[5] M. Iwata, K. T. Ota, and R. S. Duman, "The inflammasome: pathways linking psychological stress, depression, and systemic illnesses," *Brain, Behavior, and Immunity*, vol. 31, pp. 105–114, 2013.

[6] V. Krishnan and E. J. Nestler, "Linking molecules to mood: new insight into the biology of depression," *The American Journal of Psychiatry*, vol. 167, no. 11, pp. 1305–1320, 2010.

[7] V. Duric and R. S. Duman, "Depression and treatment response: dynamic interplay of signaling pathways and altered neural processes," *Cellular and Molecular Life Sciences*, vol. 70, no. 1, pp. 39–53, 2013.

[8] Y. Watanabe, E. Gould, and B. S. McEwen, "Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons," *Brain Research*, vol. 588, no. 2, pp. 341–345, 1992.

[9] Y. I. Sheline, P. W. Wang, M. H. Gado, J. G. Csernansky, and M. W. Vannier, "Hippocampal atrophy in recurrent major depression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 9, pp. 3908–3913, 1996.

[10] D. Cotter, D. Mackay, S. Landau, R. Kerwin, and I. Everall, "Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder," *Archives of General Psychiatry*, vol. 58, no. 6, pp. 545–553, 2001.

[11] S. Campbell, M. Marriott, C. Nahmias, and G. M. MacQueen, "Lower hippocampal volume in patients suffering from depression: a meta-analysis," *The American Journal of Psychiatry*, vol. 161, no. 4, pp. 598–607, 2004.

[12] C. A. Stockmeier, G. J. Mahajan, L. C. Konick et al., "Cellular changes in the postmortem hippocampus in major depression," *Biological Psychiatry*, vol. 56, no. 9, pp. 640–650, 2004.

[13] P. Videbech and B. Ravnkilde, "Hippocampal volume and depression: a meta-analysis of MRI studies," *The American Journal of Psychiatry*, vol. 161, no. 11, pp. 1957–1966, 2004.

[14] J. J. Radley, A. B. Rocher, M. Miller et al., "Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex," *Cerebral Cortex*, vol. 16, no. 3, pp. 313–320, 2006.

[15] J. Cole, S. G. Costafreda, P. McGuffin, and C. H. Y. Fu, "Hippocampal atrophy in first episode depression: a meta-analysis of magnetic resonance imaging studies," *Journal of Affective Disorders*, vol. 134, no. 1–3, pp. 483–487, 2011.

[16] M. J. Kempton, Z. Salvador, M. R. Munafò et al., "Structural neuroimaging studies in major depressive disorder. Meta-analysis and comparison with bipolar disorder," *Archives of General Psychiatry*, vol. 68, no. 7, pp. 675–690, 2011.

[17] G. Rajkowska, "Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells," *Biological Psychiatry*, vol. 48, no. 8, pp. 766–777, 2000.

[18] D. Cotter, D. Mackay, G. Chana, C. Beasley, S. Landau, and I. P. Everall, "Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder," *Cerebral Cortex*, vol. 12, no. 4, pp. 386–394, 2002.

[19] D. Öngür, W. C. Drevets, and J. L. Price, "Glial reduction in the subgenual prefrontal cortex in mood disorders," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 22, pp. 13290–13295, 1998.

[20] J. E. Malberg, A. J. Eisch, E. J. Nestler, and R. S. Duman, "Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus," *The Journal of Neuroscience*, vol. 20, no. 24, pp. 9104–9110, 2000.

[21] R. S. Duman, J. Malberg, and S. Nakagawa, "Regulation of adult neurogenesis by psychotropic drugs and stress," *Journal of Pharmacology and Experimental Therapeutics*, vol. 299, no. 2, pp. 401–407, 2001.

[22] L. Santarelli, M. Saxe, C. Gross et al., "Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants," *Science*, vol. 301, no. 5634, pp. 805–809, 2003.

[23] H. D. Schmidt and R. S. Duman, "The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior," *Behavioural Pharmacology*, vol. 18, no. 5-6, pp. 391–418, 2007.

[24] M. B. Knable, B. M. Barci, M. J. Webster, J. Meador-Woodruff, and E. F. Torrey, "Molecular abnormalities of the hippocampus in severe psychiatric illness: postmortem findings from the Stanley Neuropathology Consortium," *Molecular Psychiatry*, vol. 9, no. 6, pp. 609–620, 2004.

[25] M. Banasr, J. M. Dwyer, and R. S. Duman, "Cell atrophy and loss in depression: reversal by antidepressant treatment," *Current Opinion in Cell Biology*, vol. 23, no. 6, pp. 730–737, 2011.

[26] R. Bernard, I. A. Kerman, R. C. Thompson et al., "Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression," *Molecular Psychiatry*, vol. 16, no. 6, pp. 634–646, 2011.

[27] R. S. Duman, G. R. Heninger, and E. J. Nestler, "A molecular and cellular theory of depression," *Archives of General Psychiatry*, vol. 54, no. 7, pp. 597–606, 1997.

[28] R. S. Duman, "Role of neurotrophic factors in the etiology and treatment of mood disorders," *NeuroMolecular Medicine*, vol. 5, no. 1, pp. 11–25, 2004.

[29] E. Castrén, V. Võikar, and T. Rantamäki, "Role of neurotrophic factors in depression," *Current Opinion in Pharmacology*, vol. 7, no. 1, pp. 18–21, 2007.

[30] A. M. Rasmusson, L. Shi, and R. Duman, "Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock," *Neuropsychopharmacology*, vol. 27, no. 2, pp. 133–142, 2002.

[31] Y. Dwivedi, H. S. Rizavi, R. R. Conley, R. C. Roberts, C. A. Tamminga, and G. N. Pandey, "Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects," *Archives of General Psychiatry*, vol. 60, no. 8, pp. 804–815, 2003.

[32] Y. Dwivedi, A. C. Mondal, H. S. Rizavi, and R. R. Conley, "Suicide brain is associated with decreased expression of neurotrophins," *Biological Psychiatry*, vol. 58, no. 4, pp. 315–324, 2005.

[33] M. Nibuya, S. Morinobu, and R. S. Duman, "Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments," *The Journal of Neuroscience*, vol. 15, no. 11, pp. 7539–7547, 1995.

[34] A. A. Russo-Neustadt, R. C. Beard, Y. M. Huang, and C. W. Cotman, "Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus," *Neuroscience*, vol. 101, no. 2, pp. 305–312, 2000.

[35] Y. Shirayama, A. C.-H. Chen, S. Nakagawa, D. S. Russell, and R. S. Duman, "Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression," *Journal of Neuroscience*, vol. 22, no. 8, pp. 3251–3261, 2002.

[36] L. M. Monteggia, "Elucidating the role of brain-derived neurotrophic factor in the brain," *The American Journal of Psychiatry*, vol. 164, no. 12, p. 1790, 2007.

[37] A. J. Eisch, C. A. Bolaños, J. de Wit et al., "Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression," *Biological Psychiatry*, vol. 54, no. 10, pp. 994–1005, 2003.

[38] B. A. Hoshaw, J. E. Malberg, and I. Lucki, "Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects," *Brain Research*, vol. 1037, no. 1-2, pp. 204–208, 2005.

[39] H. D. Schmidt and R. S. Duman, "Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models," *Neuropsychopharmacology*, vol. 35, no. 12, pp. 2378–2391, 2010.

[40] H. Anisman, "Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder," *Journal of Psychiatry and Neuroscience*, vol. 34, no. 1, pp. 4–20, 2009.

[41] C. L. Raison, L. Capuron, and A. H. Miller, "Cytokines sing the blues: inflammation and the pathogenesis of depression," *Trends in Immunology*, vol. 27, no. 1, pp. 24–31, 2006.

[42] A. H. Miller, V. Maletic, and C. L. Raison, "Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression," *Biological Psychiatry*, vol. 65, no. 9, pp. 732–741, 2009.

[43] R. C. Shelton, J. Claiborne, M. Sidoryk-Wegrzynowicz et al., "Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression," *Molecular Psychiatry*, vol. 16, no. 7, pp. 751–762, 2011.

[44] J. W. Koo and R. S. Duman, "IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 2, pp. 751–756, 2008.

[45] J. W. Koo and R. S. Duman, "Evidence for IL-1 receptor blockade as a therapeutic strategy for the treatment of depression," *Current Opinion in Investigational Drugs*, vol. 10, no. 7, pp. 664–671, 2009.

[46] J. W. Koo and R. S. Duman, "Interleukin-1 receptor null mutant mice show decreased anxiety-like behavior and enhanced fear memory," *Neuroscience Letters*, vol. 456, no. 1, pp. 39–43, 2009.

[47] R. S. Duman, "Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections," *Dialogues in Clinical Neuroscience*, vol. 16, no. 1, pp. 11–27, 2014.

[48] B. S. McEwen, "Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators," *European Journal of Pharmacology*, vol. 583, no. 2-3, pp. 174–185, 2008.

[49] R. M. Shansky, C. Hamo, P. R. Hof, B. S. McEwen, and J. H. Morrison, "Stress-induced dendritic remodeling in the prefrontal cortex is circuit specific," *Cerebral Cortex*, vol. 19, no. 10, pp. 2479–2484, 2009.

[50] R. M. Berman, A. Cappiello, A. Anand et al., "Antidepressant effects of ketamine in depressed patients," *Biological Psychiatry*, vol. 47, no. 4, pp. 351–354, 2000.

[51] C. A. Zarate Jr., J. B. Singh, P. J. Carlson et al., "A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression," *Archives of General Psychiatry*, vol. 63, no. 8, pp. 856–864, 2006.

[52] R. S. Duman, N. Li, R.-J. Liu, V. Duric, and G. Aghajanian, "Signaling pathways underlying the rapid antidepressant actions of ketamine," *Neuropharmacology*, vol. 62, no. 1, pp. 35–41, 2012.

[53] N. Li, B. Lee, R.-J. Liu et al., "mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists," *Science*, vol. 329, no. 5994, pp. 959–964, 2010.

[54] A. E. Autry, M. Adachi, E. Nosyreva et al., "NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses," *Nature*, vol. 475, no. 7354, pp. 91–95, 2011.

[55] G. E. Ruoff, "Depression in the patient with chronic pain," *Journal of Family Practice*, vol. 43, no. 6, pp. S25–S34, 1996.

[56] D. A. Fishbain, R. Cutler, H. L. Rosomoff, and R. S. Rosomoff, "Chronic pain-associated depression: antecedent or consequence of chronic pain? A review," *Clinical Journal of Pain*, vol. 13, no. 2, pp. 116–137, 1997.

[57] L. Manchikanti, B. Fellows, and V. Singh, "Understanding psychological aspects of chronic pain in interventional pain management," *Pain Physician*, vol. 5, no. 1, pp. 57–82, 2002.

[58] M. J. Bair, R. L. Robinson, W. Katon, and K. Kroenke, "Depression and pain comorbidity: a literature review," *Archives of Internal Medicine*, vol. 163, no. 20, pp. 2433–2445, 2003.

[59] S. R. Currie and J. Wang, "Chronic back pain and major depression in the general Canadian population," *Pain*, vol. 107, no. 1-2, pp. 54–60, 2004.

[60] M. J. L. Sullivan, K. Reesor, S. Mikail, and R. Fisher, "The treatment of depression in chronic low back pain: review and recommendations," *Pain*, vol. 50, no. 1, pp. 5–13, 1992.

[61] M. N. Baliki, T. J. Schnitzer, W. R. Bauer, and A. V. Apkarian, "Brain morphological signatures for chronic pain," *PLoS ONE*, vol. 6, no. 10, Article ID e26010, 2011.

[62] R. Melzack and P. D. Wall, "Pain mechanisms: a new theory," *Science*, vol. 150, no. 3699, pp. 971–979, 1965.

[63] R. Peyron, B. Laurent, and L. García-Larrea, "Functional imaging of brain responses to pain. A review and meta-analysis (2000)," *Neurophysiologie Clinique*, vol. 30, no. 5, pp. 263–288, 2000.

[64] I. Tracey, L. Becerra, I. Chang et al., "Noxious hot and cold stimulation produce common patterns of brain activation in humans: a functional magnetic resonance imaging study," *Neuroscience Letters*, vol. 288, no. 2, pp. 159–162, 2000.

[65] M. J. Farrell, A. R. Laird, and G. F. Egan, "Brain activity associated with painfully hot stimuli applied to the upper limb: a meta-analysis," *Human Brain Mapping*, vol. 25, no. 1, pp. 129–139, 2005.

[66] G. Salimi-Khorshidi, S. M. Smith, J. R. Keltner, T. D. Wager, and T. E. Nichols, "Meta-analysis of neuroimaging data: a comparison of image-based and coordinate-based pooling of studies," *NeuroImage*, vol. 45, no. 3, pp. 810–823, 2009.

[67] T. D. Wager, L. Y. Atlas, M. A. Lindquist, M. Roy, C.-W. Woo, and E. Kross, "An fMRI-based neurologic signature of physical pain," *The New England Journal of Medicine*, vol. 368, no. 15, pp. 1388–1397, 2013.

[68] R. Peyron, L. García-Larrea, M.-C. Grégoire et al., "Haemodynamic brain responses to acute pain in humans: sensory and attentional networks," *Brain*, vol. 122, part 9, pp. 1765–1779, 1999.

[69] S. J. Bantick, R. G. Wise, A. Ploghaus, S. Clare, S. M. Smith, and I. Tracey, "Imaging how attention modulates pain in humans using functional MRI," *Brain*, vol. 125, no. 2, pp. 310–319, 2002.

[70] G. Ji, H. Sun, Y. Fu et al., "Cognitive impairment in pain through amygdala-driven prefrontal cortical deactivation," *Journal of Neuroscience*, vol. 30, no. 15, pp. 5451–5464, 2010.

[71] C. Corradi-Dell'Acqua, C. Hofstetter, and P. Vuilleumier, "Felt and seen pain evoke the same local patterns of cortical activity in insular and cingulate cortex," *Journal of Neuroscience*, vol. 31, no. 49, pp. 17996–18006, 2011.

[72] J. F. Bernard, H. Bester, and J. M. Besson, "Involvement of the spino-parabrachio -amygdaloid and -hypothalamic pathways in the autonomic and affective emotional aspects of pain," *Progress in Brain Research*, vol. 107, pp. 243–255, 1996.

[73] W. D. Willis and K. N. Westlund, "Neuroanatomy of the pain system and of the pathways that modulate pain," *Journal of Clinical Neurophysiology*, vol. 14, no. 1, pp. 2–31, 1997.

[74] J. F. Bernard and R. Bandler, "Parallel circuits for emotional coping behaviour: new pieces in the puzzle," *Journal of Comparative Neurology*, vol. 401, no. 4, pp. 429–436, 1998.

[75] M. Von Korff, S. F. Dworkin, L. Le Resche, and A. Kruger, "An epidemiologic comparison of pain complaints," *Pain*, vol. 32, no. 2, pp. 173–183, 1988.

[76] M. Von Korff and G. Simon, "The relationship between pain and depression," *British Journal of Psychiatry*, vol. 168, no. 30, pp. 101–108, 1996.

[77] A. J. Rush, P. Polatin, and R. J. Gatchel, "Depression and chronic low back pain: establishing priorities in treatment," *Spine*, vol. 25, no. 20, pp. 2566–2571, 2000.

[78] G. Magni, C. Caldieron, S. Rigatti-Luchini, and H. Merskey, "Chronic musculoskeletal pain and depressive symptoms in the general population. An analysis of the 1st National Health and Nutrition Examination Survey data," *Pain*, vol. 43, no. 3, pp. 299–307, 1990.

[79] G. Magni, M. Marchetti, C. Moreschi, H. Merskey, and S. R. Luchini, "Chronic musculoskeletal pain and depressive symptoms in the National Health and Nutrition Examination. I. Epidemiologic follow-up study," *Pain*, vol. 53, no. 2, pp. 163–168, 1993.

[80] S. Lautenbacher, J. Spernal, W. Schreiber, and J.-C. Krieg, "Relationship between clinical pain complaints and pain sensitivity in patients with depression and panic disorder," *Psychosomatic Medicine*, vol. 61, no. 6, pp. 822–827, 1999.

[81] K.-J. Bär, S. Brehm, M. K. Boettger, S. Boettger, G. Wagner, and H. Sauer, "Pain perception in major depression depends on pain modality," *Pain*, vol. 117, no. 1-2, pp. 97–103, 2005.

[82] J. J. Cerqueira, J. M. Pêgo, R. Taipa, J. M. Bessa, O. F. X. Almeida, and N. Sousa, "Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors," *Journal of Neuroscience*, vol. 25, no. 34, pp. 7792–7800, 2005.

[83] R. Mitra and R. M. Sapolsky, "Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 14, pp. 5573–5578, 2008.

[84] G. Blackburn-Munro and R. E. Blackburn-Munro, "Chronic pain, chronic stress and depression: coincidence or consequence?" *Journal of Neuroendocrinology*, vol. 13, no. 12, pp. 1009–1023, 2001.

[85] V. Duric and K. E. McCarson, "Hippocampal neurokinin-1 receptor and brain-derived neurotrophic factor gene expression is decreased in rat models of pain and stress," *Neuroscience*, vol. 133, no. 4, pp. 999–1006, 2005.

[86] V. Duric and K. E. McCarson, "Effects of analgesic or antidepressant drugs on pain- or stress-evoked hippocampal and spinal neurokinin-1 receptor and brain-derived neurotrophic factor gene expression in the rat," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 319, no. 3, pp. 1235–1243, 2006.

[87] V. Duric and K. E. McCarson, "Persistent pain produces stress-like alterations in hippocampal neurogenesis and gene expression," *The Journal of Pain*, vol. 7, no. 8, pp. 544–555, 2006.

[88] V. Duric and K. E. McCarson, "Neurokinin-1 (NK-1) receptor and brain-derived neurotrophic factor (BDNF) gene expression is differentially modulated in the rat spinal dorsal horn and hippocampus during inflammatory pain," *Molecular Pain*, vol. 3, article 32, 2007.

[89] A. A. Mutso, D. Radzicki, M. N. Baliki et al., "Abnormalities in hippocampal functioning with persistent pain," *The Journal of Neuroscience*, vol. 32, no. 17, pp. 5747–5756, 2012.

[90] A. V. Apkarian, S. Lavarello, A. Randolf et al., "Expression of IL-1beta in supraspinal brain regions in rats with neuropathic pain," *Neuroscience Letters*, vol. 407, no. 2, pp. 176–181, 2006.

[91] G. J. Norman, K. Karelina, N. Zhang, J. C. Walton, J. S. Morris, and A. C. Devries, "Stress and IL-1β contribute to the development of depressive-like behavior following peripheral nerve injury," *Molecular Psychiatry*, vol. 15, no. 4, pp. 404–414, 2010.

[92] A. del Rey, H.-J. Yau, A. Randolf et al., "Chronic neuropathic pain-like behavior correlates with IL-1β expression and disrupts cytokine interactions in the hippocampus," *Pain*, vol. 152, no. 12, pp. 2827–2835, 2011.

[93] K. J. Whitehead, C. G. S. Smith, S. A. Delaney et al., "Dynamic regulation of spinal pro-inflammatory cytokine release in the rat in vivo following peripheral nerve injury," *Brain, Behavior, and Immunity*, vol. 24, no. 4, pp. 569–576, 2010.

[94] S. Nadeau, M. Filali, J. Zhang et al., "Functional recovery after peripheral nerve injury is dependent on the pro-inflammatory cytokines IL-1beta and TNF: implications for neuropathic pain," *Journal of Neuroscience*, vol. 31, no. 35, pp. 12533–12542, 2011.

[95] A. M. Taylor, A. Castonguay, A. J. Taylor et al., "Microglia disrupt mesolimbic reward circuitry in chronic pain," *Journal of Neuroscience*, vol. 35, no. 22, pp. 8442–8450, 2015.

[96] D. A. Regier, M. E. Farmer, D. S. Rae et al., "Comorbidity of mental disorders with alcohol and other drug abuse. Results from the epidemiologic catchment area (ECA) study," *The Journal of the American Medical Association*, vol. 264, no. 19, pp. 2511–2518, 1990.

[97] R. C. Kessler, K. A. McGonagle, S. Zhao et al., "Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey," *Archives of General Psychiatry*, vol. 51, no. 1, pp. 8–19, 1994.

[98] R. C. Kessler, R. M. Crum, L. A. Warner, C. B. Nelson, J. Schulenberg, and J. C. Anthony, "Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the national comorbidity survey," *Archives of General Psychiatry*, vol. 54, no. 4, pp. 313–321, 1997.

[99] S. B. Quello, K. T. Brady, and S. C. Sonne, "Mood disorders and substance use disorder: a complex comorbidity," *Science & Practice Perspectives*, vol. 3, no. 1, pp. 13–21, 2005.

[100] E. K. Diekhof, P. Falkai, and O. Gruber, "Functional neuroimaging of reward processing and decision-making: a review of aberrant motivational and affective processing in addiction and mood disorders," *Brain Research Reviews*, vol. 59, no. 1, pp. 164–184, 2008.

[101] C. A. Prescott, S. H. Aggen, and K. S. Kendler, "Sex-specific genetic influences on the comorbidity of alcoholism and major depression in a population-based sample of US twins," *Archives of General Psychiatry*, vol. 57, no. 8, pp. 803–811, 2000.

[102] D. O. Procopio, L. M. Saba, H. Walter et al., "Genetic markers of comorbid depression and alcoholism in women," *Alcoholism: Clinical and Experimental Research*, vol. 37, no. 6, pp. 896–904, 2013.

[103] H. M. Pettinati, D. W. Oslin, K. M. Kampman et al., "A double-blind, placebo-controlled trial combining sertraline and naltrexone for treating co-occurring depression and alcohol dependence," *The American Journal of Psychiatry*, vol. 167, no. 6, pp. 668–675, 2010.

[104] M. Flaster, A. Sharma, and M. Rao, "Poststroke depression: a review emphasizing the role of prophylactic treatment and synergy with treatment for motor recovery," *Topics in Stroke Rehabilitation*, vol. 20, no. 2, pp. 139–150, 2013.

[105] E. M. Whyte and B. H. Mulsant, "Post stroke depression: epidemiology, pathophysiology, and biological treatment," *Biological Psychiatry*, vol. 52, no. 3, pp. 253–264, 2002.

[106] E. M. Whyte, B. H. Mulsant, J. Vanderbilt, H. H. Dodge, and M. Ganguli, "Depression after stroke: a prospective epidemiological study," *Journal of the American Geriatrics Society*, vol. 52, no. 5, pp. 774–778, 2004.

[107] Y. Kishi, R. G. Robinson, and J. T. Kosier, "Suicidal plans in patients with stroke: comparison between acute-onset and delayed-onset suicidal plans," *International Psychogeriatrics*, vol. 8, no. 4, pp. 623–634, 1996.

[108] A. Singh, S. E. Black, N. Herrmann et al., "Functional and neuroanatomic correlations in poststroke depression: the Sunnybrook Stroke study," *Stroke*, vol. 31, no. 3, pp. 637–644, 2000.

[109] R. Gillen, H. Tennen, T. E. McKee, P. Gernert-Dott, and G. Affleck, "Depressive symptoms and history of depression predict rehabilitation efficiency in stroke patients," *Archives of Physical Medicine and Rehabilitation*, vol. 82, no. 12, pp. 1645–1649, 2001.

[110] A. House, P. Knapp, J. Bamford, and A. Vail, "Mortality at 12 and 24 months after stroke may be associated with depressive symptoms at 1 month," *Stroke*, vol. 32, no. 3, pp. 696–701, 2001.

[111] J. M. Lyness, E. D. Caine, D. A. King, Y. Conwell, C. Cox, and P. R. Duberstein, "Cerebrovascular risk factors and depression in older primary care patients: testing a vascular brain disease model of depression," *American Journal of Geriatric Psychiatry*, vol. 7, no. 3, pp. 252–258, 1999.

[112] J. C. de Groot, F.-E. de Leeuw, M. Oudkerk, A. Hofman, J. Jolles, and M. M. B. Breteler, "Cerebral white matter lesions and depressive symptoms in elderly adults," *Archives of General Psychiatry*, vol. 57, no. 11, pp. 1071–1076, 2000.

[113] D. C. Steffens, K. R. R. Krishnan, C. Crump, and G. L. Burke, "Cerebrovascular disease and evolution of depressive symptoms in the cardiovascular health study," *Stroke*, vol. 33, no. 6, pp. 1636–1644, 2002.

[114] P. S. Sachdev, X. Chen, A. Joscelyne, W. Wen, and H. Brodaty, "Amygdala in stroke/transient ischemic attack patients and its relationship to cognitive impairment and psychopathology: the Sydney Stroke Study," *American Journal of Geriatric Psychiatry*, vol. 15, no. 6, pp. 487–496, 2007.

[115] K. J. Salaycik, M. Kelly-Hayes, A. Beiser et al., "Depressive symptoms and risk of stroke: the Framingham Study," *Stroke*, vol. 38, no. 1, pp. 16–21, 2007.

[116] J.-Y. Dong, Y.-H. Zhang, J. Tong, and L.-Q. Qin, "Depression and risk of stroke: a meta-analysis of prospective studies," *Stroke*, vol. 43, no. 1, pp. 32–37, 2012.

[117] G. S. Alexopoulos, R. C. Young, K. W. Lieberman, and C. A. Shamoian, "Platelet MAO activity in geriatric patients with depression and dementia," *The American Journal of Psychiatry*, vol. 144, no. 11, pp. 1480–1483, 1987.

[118] P. Chen, M. Ganguli, B. H. Mulsant, and S. T. DeKosky, "The temporal relationship between depressive symptoms and dementia: a community-based prospective study," *Archives of General Psychiatry*, vol. 56, no. 3, pp. 261–266, 1999.

[119] R. L. Ownby, E. Crocco, A. Acevedo, V. John, and D. Loewenstein, "Depression and risk for Alzheimer disease: systematic review, meta-analysis, and metaregression analysis," *Archives of General Psychiatry*, vol. 63, no. 5, pp. 530–538, 2006.

[120] H. Lenoir, C. Dufouil, S. Auriacombe et al., "Depression history, depressive symptoms, and incident dementia: the 3C study," *Journal of Alzheimer's Disease*, vol. 26, no. 1, pp. 27–38, 2011.

[121] M. A. Butters, J. B. Young, O. Lopez et al., "Pathways linking late-life depression to persistent cognitive impairment and dementia," *Dialogues in Clinical Neuroscience*, vol. 10, no. 3, pp. 345–357, 2008.

[122] B. S. Diniz and A. L. Teixeira, "Brain-derived neurotrophic factor and Alzheimer's disease: physiopathology and beyond," *NeuroMolecular Medicine*, vol. 13, no. 4, pp. 217–222, 2011.

[123] B. S. Diniz, M. A. Butters, S. M. Albert, M. A. Dew, and C. F. Reynolds III, "Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies," *The British Journal of Psychiatry*, vol. 202, no. 5, pp. 329–335, 2013.

[124] G. D. Pearlson, C. A. Ross, W. D. Lohr, B. W. Rovner, G. A. Chase, and M. F. Folstein, "Association between family history of affective disorder and the depressive syndrome of alzheimer's disease," *The American Journal of Psychiatry*, vol. 147, no. 4, pp. 452–456, 1990.

[125] M. E. Strauss and P. K. Ogrocki, "Confirmation of an association between family history of affective disorder and the depressive syndrome in Alzheimer's disease," *The American Journal of Psychiatry*, vol. 153, no. 10, pp. 1340–1342, 1996.

[126] M. S. Mega, J. L. Cummings, T. Fiorello, and J. Gornbein, "The spectrum of behavioral changes in Alzheimer's disease," *Neurology*, vol. 46, no. 1, pp. 130–135, 1996.

[127] M. Filali, R. Lalonde, and S. Rivest, "Cognitive and non-cognitive behaviors in an APPswe/PS1 bigenic model of Alzheimer's disease," *Genes, Brain and Behavior*, vol. 8, no. 2, pp. 143–148, 2009.

[128] H. S. Phillips, J. M. Hains, M. Armanini, G. R. Laramee, S. A. Johnson, and J. W. Winslow, "BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease," *Neuron*, vol. 7, no. 5, pp. 695–702, 1991.

[129] V. Soontornniyomkij, G. Wang, C. A. Pittman, R. L. Hamilton, C. A. Wiley, and C. L. Achim, "Absence of brain-derived neurotrophic factor and trkB receptor immunoreactivity in glia of Alzheimer's disease," *Acta Neuropathologica*, vol. 98, no. 4, pp. 345–348, 1999.

[130] C. Hock, K. Heese, C. Hulette, C. Rosenberg, and U. Otten, "Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas," *Archives of Neurology*, vol. 57, no. 6, pp. 846–851, 2000.

[131] R. M. D. Holsinger, J. Schnarr, P. Henry, V. T. Castelo, and M. Fahnestock, "Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease," *Molecular Brain Research*, vol. 76, no. 2, pp. 347–354, 2000.

[132] X. Antón Alvarez, A. Franco, L. Fernández-Novoa, and R. Cacabelos, "Blood levels of histamine, IL-1β, and TNF-α in patients with mild to moderate Alzheimer disease," *Molecular and Chemical Neuropathology*, vol. 29, no. 2-3, pp. 237–252, 1996.

[133] C. Humpel and T. Hochstrasser, "Cerebrospinal fluid and blood biomarkers in Alzheimer's disease," *World Journal of Psychiatry*, vol. 1, no. 1, pp. 8–18, 2011.

[134] D. Gezen-Ak, E. Dursun, H. Hanağası et al., "BDNF, TNFα, HSP90, CFH, and IL-10 serum levels in patients with early or late onset Alzheimer's disease or mild cognitive impairment," *Journal of Alzheimer's Disease*, vol. 37, no. 1, pp. 185–195, 2013.

[135] W. Swardfager, K. Lanctôt, L. Rothenburg, A. Wong, J. Cappell, and N. Herrmann, "A meta-analysis of cytokines in Alzheimer's disease," *Biological Psychiatry*, vol. 68, no. 10, pp. 930–941, 2010.

[136] B. E. Leonard, "Inflammation, depression and dementia: are they connected?" *Neurochemical Research*, vol. 32, no. 10, pp. 1749–1756, 2007.

[137] R. A. Khairova, R. MacHado-Vieira, J. Du, and H. K. Manji, "A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder," *International Journal of Neuropsychopharmacology*, vol. 12, no. 4, pp. 561–578, 2009.

[138] V. K. Khemka, A. Ganguly, D. Bagchi et al., "Raised serum proinflammatory cytokines in Alzheimer's disease with depression," *Aging and Disease*, vol. 5, no. 3, pp. 170–176, 2014.

[139] H.-J. Kang, J.-M. Kim, S.-W. Kim et al., "Associations of cytokine genes with Alzheimer's disease and depression in an elderly Korean population," *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 88, no. 9, pp. 1002–1007, 2014.

[140] E. C. D. Gullette, J. A. Blumenthal, M. Babyak et al., "Effects of mental stress on myocardial ischemia during daily life," *The Journal of the American Medical Association*, vol. 277, no. 19, pp. 1521–1526, 1997.

[141] A. Rosengren, S. Hawken, S. Ôunpuu et al., "Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study," *The Lancet*, vol. 364, no. 9438, pp. 953–962, 2004.

[142] A. Seldenrijk, N. Vogelzangs, H. P. J. van Hout, H. W. J. van Marwijk, M. Diamant, and B. W. J. H. Penninx, "Depressive and anxiety disorders and risk of subclinical atherosclerosis. Findings from the Netherlands Study of Depression and Anxiety (NESDA)," *Journal of Psychosomatic Research*, vol. 69, no. 2, pp. 203–210, 2010.

[143] H. Karimi-Moonaghi, M. Mojalli, and S. Khosravan, "Psychosocial complications of coronary artery disease," *Iranian Red Crescent Medical Journal*, vol. 16, no. 6, Article ID e18162, 2014.

[144] J. Wei, C. Rooks, R. Ramadan et al., "Meta-analysis of mental stress-induced myocardial ischemia and subsequent cardiac events in patients with coronary artery disease," *The American Journal of Cardiology*, vol. 114, no. 2, pp. 187–192, 2014.

[145] J. A. Sumner, L. D. Kubzansky, M. S. V. Elkind et al., "Trauma exposure and posttraumatic stress disorder symptoms predict onset of cardiovascular events in women," *Circulation*, 2015.

[146] K. E. Joynt, D. J. Whellan, and C. M. O'Connor, "Depression and cardiovascular disease: mechanisms of interaction," *Biological Psychiatry*, vol. 54, no. 3, pp. 248–261, 2003.

[147] K. N. Kershaw, G. A. Brenes, L. E. Charles et al., "Associations of stressful life events and social strain with incident cardiovascular disease in the Women's Health Initiative," *Journal of the American Heart Association*, vol. 3, no. 3, Article ID e000687, 2014.

[148] N. Bergmann, F. Gyntelberg, and J. Faber, "The appraisal of chronic stress and the development of the metabolic syndrome: a systematic review of prospective cohort studies," *Endocrine Connections*, vol. 3, no. 2, pp. R55–R80, 2014.

[149] A. A. da Silva, J. M. do Carmo, and J. E. Hall, "Role of leptin and central nervous system melanocortins in obesity hypertension," *Current Opinion in Nephrology and Hypertension*, vol. 22, no. 2, pp. 135–140, 2013.

[150] A. C. Arnold and D. I. Diz, "Endogenous leptin contributes to baroreflex suppression within the solitary tract nucleus of aged rats," *American Journal of Physiology—Heart and Circulatory Physiology*, vol. 307, no. 11, pp. H1539–H1546, 2014.

[151] M. J. Barnes and D. H. McDougal, "Leptin into the rostral ventral lateral medulla (RVLM) augments renal sympathetic nerve activity and blood pressure," *Frontiers in Neuroscience*, vol. 8, article 232, 2014.

[152] G. G. Gauglitz, D. N. Herndon, and M. G. Jeschke, "Insulin resistance postburn: underlying mechanisms and current therapeutic strategies," *Journal of Burn Care and Research*, vol. 29, no. 5, pp. 683–694, 2008.

[153] E. S. Epel, "Psychological and metabolic stress: a recipe for accelerated cellular aging?" *Hormones*, vol. 8, no. 1, pp. 7–22, 2009.

[154] L. Ghiadoni, A. E. Donald, M. Cropley et al., "Mental stress induces transient endothelial dysfunction in humans," *Circulation*, vol. 102, no. 20, pp. 2473–2478, 2000.

[155] P. H. Black and L. D. Garbutt, "Stress, inflammation and cardiovascular disease," *Journal of Psychosomatic Research*, vol. 52, no. 1, pp. 1–23, 2002.

[156] E. Isingrini, A. Surget, C. Belzung et al., "Altered aortic vascular reactivity in the unpredictable chronic mild stress model of depression in mice: UCMS causes relaxation impairment to ACh," *Physiology & Behavior*, vol. 103, no. 5, pp. 540–546, 2011.

[157] A. Puzserova, J. Torok, R. Sotnikova, A. Zemancikova, and I. Bernatova, "Reactivity of the mesenteric bed arteries of normotensive rats exposed to chronic social stress," *General Physiology and Biophysics*, vol. 31, no. 3, pp. 279–290, 2012.

[158] F. Pelliccia, C. Greco, C. Vitale, G. Rosano, C. Gaudio, and J. C. Kaski, "Takotsubo syndrome (stress cardiomyopathy): an intriguing clinical condition in search of its identity," *The American Journal of Medicine*, vol. 127, no. 8, pp. 699–704, 2014.

[159] R. L. Verrier, E. L. Hagestad, and B. Lown, "Delayed myocardial ischemia induced by anger," *Circulation*, vol. 75, no. 1, pp. 249–254, 1987.

[160] L. Carnevali, M. Trombini, S. Rossi et al., "Structural and electrical myocardial remodeling in a rodent model of depression," *Psychosomatic Medicine*, vol. 75, no. 1, pp. 42–51, 2013.

[161] S. M. Grenon, J. Hiramoto, K. G. Smolderen, E. Vittinghoff, M. A. Whooley, and B. E. Cohen, "Association between depression and peripheral artery disease: insights from the heart and soul study," *Journal of the American Heart Association*, vol. 1, Article ID e002667, 2012.

[162] J. C. Stewart, D. J. Zielke, M. A. W. Hawkins et al., "Depressive symptom clusters and 5-year incidence of coronary artery calcification: the coronary artery risk development in young adults study," *Circulation*, vol. 126, no. 4, pp. 410–417, 2012.

[163] S. Ye, P. Muntner, D. Shimbo et al., "Behavioral mechanisms, elevated depressive symptoms, and the risk for myocardial infarction or death in individuals with coronary heart disease: the REGARDS (Reason for Geographic and Racial Differences in Stroke) study," *Journal of the American College of Cardiology*, vol. 61, no. 6, pp. 622–630, 2013.

[164] C. Alcantara, P. Muntner, D. Edmondson et al., "Perfect storm: concurrent stress and depressive symptoms increase risk of myocardial infarction or death," *Circulation: Cardiovascular Quality and Outcomes*, vol. 8, no. 2, pp. 146–154, 2015.

[165] C. E. Angermann, G. Gelbrich, S. Störk et al., "Rationale and design of a randomised, controlled, multicenter trial investigating the effects of selective serotonin re-uptake inhibition on morbidity, mortality and mood in depressed heart failure patients (MOOD-HF)," *European Journal of Heart Failure*, vol. 9, no. 12, pp. 1212–1222, 2007.

[166] W. Jiang, R. Krishnan, M. Kuchibhatla et al., "Characteristics of depression remission and its relation with cardiovascular outcome among patients with chronic heart failure (from the SADHART-CHF study)," *American Journal of Cardiology*, vol. 107, no. 4, pp. 545–551, 2011.

[167] K. G. Smolderen, K. M. Strait, R. P. Dreyer et al., "Depressive symptoms in younger women and men with acute myocardial infarction: insights from the VIRGO study," *Journal of the American Heart Association*, vol. 4, no. 4, Article ID e001424, 2015.

[168] A. M. Al Madany, F. H. Hassan, A. A. Al-Nabawy, M. E. Ramadan, and A. A. Ismail, "Study of depression among a sample of hypertensive patients," *Journal of the Egyptian Society of Parasitology*, vol. 45, no. 1, pp. 207–216, 2015.

[169] B. E. Cohen, D. Edmondson, and I. M. Kronish, "State of the art review: depression, stress, anxiety, and cardiovascular disease," *American Journal of Hypertension*, vol. 28, no. 11, pp. 1295–1302, 2015.

[170] E. C. Dumont, S. Rafrafi, S. Laforest, and G. Drolet, "Involvement of central angiotensin receptors in stress adaptation," *Neuroscience*, vol. 93, no. 3, pp. 877–884, 1999.

[171] D. L. Daubert, B. M. Looney, R. R. Clifton, J. N. Cho, and D. A. Scheuer, "Elevated corticosterone in the dorsal hindbrain increases plasma norepinephrine and neuropeptide Y, and recruits a vasopressin response to stress," *American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 307, no. 2, pp. R212–R224, 2014.

[172] G. Yang, Z.-X. Xi, Y. Wan, H. Wang, and G. Bi, "Changes in circulating and tissue angiotensin II during acute and chronic stress," *Biological Signals*, vol. 2, no. 3, pp. 166–172, 1993.

[173] M. Groeschel and B. Braam, "Connecting chronic and recurrent stress to vascular dysfunction: no relaxed role for the renin-angiotensin system," *The American Journal of Physiology—Renal Physiology*, vol. 300, no. 1, pp. F1–F10, 2011.

[174] M. Ruiz-Ortega, O. Lorenzo, M. Rupérez, V. Esteban, S. Mezzano, and J. Egido, "Renin-angiotensin system and renal damage: emerging data on angiotensin II as a proinflammatory mediator," *Contributions to Nephrology*, vol. 135, pp. 123–137, 2001.

[175] M. Ruiz-Ortega, M. Ruperez, O. Lorenzo et al., "Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney," *Kidney International, Supplement*, vol. 62, no. 82, pp. S12–S22, 2002.

[176] G. Wolf, U. Wenzel, K. D. Burns, R. C. Harris, R. A. K. Stahl, and F. Thaiss, "Angiotensin II activates nuclear transcription factor-κB through AT1 and AT2 receptors," *Kidney International*, vol. 61, no. 6, pp. 1986–1995, 2002.

[177] V. Esteban, O. Lorenzo, M. Rupérez et al., "Angiotensin II, via AT_1 and AT_2 receptors and NF-κB pathway, regulates the inflammatory response in unilateral ureteral obstruction," *Journal of the American Society of Nephrology*, vol. 15, no. 6, pp. 1514–1529, 2004.

[178] M. Fadgyas-Stanculete, A. Buga, A. Popa-Wagner, and D. L. Dumitrascu, "The relationship between irritable bowel syndrome and psychiatric disorders: from molecular changes to clinical manifestations," *Journal of Molecular Psychiatry*, vol. 2, no. 4, 2014.

[179] J. R. Walker, J. P. Ediger, L. A. Graff et al., "The Manitoba IBD cohort study: a population-based study of the prevalence of lifetime and 12-month anxiety and mood disorders," *The American Journal of Gastroenterology*, vol. 103, no. 8, pp. 1989–1997, 2008.

[180] M. T. Hillilä, J. Hämäläinen, M. E. Heikkinen, and M. A. Färkkilä, "Gastrointestinal complaints among subjects with depressive symptoms in the general population," *Alimentary Pharmacology & Therapeutics*, vol. 28, no. 5, pp. 648–654, 2008.

[181] S. R. Knowles, E. A. Nelson, and E. A. Palombo, "Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: a possible mechanism underlying susceptibility to illness," *Biological Psychology*, vol. 77, no. 2, pp. 132–137, 2008.

[182] J. F. Cryan and T. G. Dinan, "Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour," *Nature Reviews Neuroscience*, vol. 13, no. 10, pp. 701–712, 2012.

[183] A. J. Park, J. Collins, P. A. Blennerhassett et al., "Altered colonic function and microbiota profile in a mouse model of chronic depression," *Neurogastroenterology & Motility*, vol. 25, no. 9, p. 733-e575, 2013.

[184] J. A. Bravo, P. Forsythe, M. V. Chew et al., "Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 38, pp. 16050–16055, 2011.

[185] M. Maes, M. Kubera, and J.-C. Leunis, "The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression," *Neuroendocrinology Letters*, vol. 29, no. 1, pp. 117–124, 2008.

[186] M. Maes, M. Kubera, J.-C. Leunis, and M. Berk, "Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut," *Journal of Affective Disorders*, vol. 141, no. 1, pp. 55–62, 2012.

[187] M. Berk, L. J. Williams, F. N. Jacka et al., "So depression is an inflammatory disease, but where does the inflammation come from?" *BMC Medicine*, vol. 11, no. 1, article 200, 2013.

[188] M. E. Jarrett, R. Kohen, K. C. Cain et al., "Relationship of SERT polymorphisms to depressive and anxiety symptoms in irritable bowel syndrome," *Biological Research for Nursing*, vol. 9, no. 2, pp. 161–169, 2007.

[189] H. Frielingsdorf, K. G. Bath, F. Soliman, J. Difede, B. J. Casey, and F. S. Lee, "Variant brain-derived neurotrophic factor Val66Met endophenotypes: implications for posttraumatic stress disorder," *Annals of the New York Academy of Sciences*, vol. 1208, no. 1, pp. 150–157, 2010.

[190] P. Fitzgerald, M. Cassidy Eugene, G. Clarke et al., "Tryptophan catabolism in females with irritable bowel syndrome: relationship to interferon-gamma, severity of symptoms and psychiatric co-morbidity," *Neurogastroenterology and Motility*, vol. 20, no. 12, pp. 1291–1297, 2008.

[191] M. Tonini and F. Pace, "Drugs acting on serotonin receptors for the treatment of functional GI disorders," *Digestive Diseases*, vol. 24, no. 1-2, pp. 59–69, 2006.

[192] J. Lasselin and L. Capuron, "Chronic low-grade inflammation in metabolic disorders: relevance for behavioral symptoms," *Neuroimmunomodulation*, vol. 21, no. 2-3, pp. 95–101, 2014.

[193] P. G. Kopelman, "Obesity as a medical problem," *Nature*, vol. 404, no. 6778, pp. 635–643, 2000.

[194] S. B. Racette, S. S. Deusinger, and R. H. Deusinger, "Obesity: overview of prevalence, etiology, and treatment," *Physical Therapy*, vol. 83, no. 3, pp. 276–288, 2003.

[195] J. A. Pasco, G. C. Nicholson, S. L. Brennan, and M. A. Kotowicz, "Prevalence of obesity and the relationship between the body mass index and body fat: cross-sectional, population-based data," *PLoS ONE*, vol. 7, Article ID e29580, 2012.

[196] F. S. Luppino, L. M. de Wit, P. F. Bouvy et al., "Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies," *Archives of General Psychiatry*, vol. 67, no. 3, pp. 220–229, 2010.

[197] M. S. Faith, M. Butryn, T. A. Wadden, A. Fabricatore, A. M. Nguyen, and S. B. Heymsfield, "Evidence for prospective associations among depression and obesity in population-based studies," *Obesity Reviews*, vol. 12, no. 501, pp. e438–e453, 2011.

[198] A. Aguilar-Valles, W. Inoue, C. Rummel, and G. N. Luheshi, "Obesity, adipokines and neuroinflammation," *Neuropharmacology*, vol. 96, pp. 124–134, 2015.

[199] H. Münzberg, "Differential leptin access into the brain—a hierarchical organization of hypothalamic leptin target sites?" *Physiology and Behavior*, vol. 94, no. 5, pp. 664–669, 2008.

[200] K. Ohashi, D. Yuasa, R. Shibata, T. Murohara, and N. Ouchi, "Adiponectin as a target in obesity-related inflammatory state," *Endocrine, Metabolic & Immune Disorders Drug Targets*, vol. 15, no. 2, pp. 145–150, 2015.

[201] H. Jin, J. M. Meyer, S. Mudaliar, and D. V. Jeste, "Impact of atypical antipsychotic therapy on leptin, ghrelin, and adiponectin," *Schizophrenia Research*, vol. 100, no. 1–3, pp. 70–85, 2008.

[202] A. Repaci, A. Gambineri, and R. Pasquali, "The role of low-grade inflammation in the polycystic ovary syndrome," *Molecular and Cellular Endocrinology*, vol. 335, no. 1, pp. 30–41, 2011.

[203] C. Jin and R. A. Flavell, "Innate sensors of pathogen and stress: linking inflammation to obesity," *Journal of Allergy and Clinical Immunology*, vol. 132, no. 2, pp. 287–294, 2013.

[204] N. Castanon, G. Luheshi, and S. Layé, "Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity," *Frontiers in Neuroscience*, vol. 9, article 229, 2015.

[205] M. Esfahani, A. Movahedian, M. Baranchi, and M. T. Goodarzi, "Adiponectin: an adipokine with protective features against metabolic syndrome," *Iranian Journal of Basic Medical Sciences*, vol. 18, pp. 430–442, 2015.

[206] A. Iyer, L. Brown, J. P. Whitehead, J. B. Prins, and D. P. Fairlie, "Nutrient and immune sensing are obligate pathways in metabolism, immunity, and disease," *The FASEB Journal*, vol. 29, pp. 3612–3625, 2015.

[207] E. S. Tecoma and L. Y. Huey, "Psychic distress and the immune response," *Life Sciences*, vol. 36, no. 19, pp. 1799–1812, 1985.

[208] S. F. Maier, L. R. Watkins, and M. Fleshner, "Psychoneuroimmunology. The interface between behavior, brain, and immunity," *American Psychologist*, vol. 49, no. 12, pp. 1004–1017, 1994.

[209] O. J. G. Schiepers, M. C. Wichers, and M. Maes, "Cytokines and major depression," *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 29, no. 2, pp. 201–217, 2005.

[210] M. B. Howren, D. M. Lamkin, and J. Suls, "Associations of depression with c-reactive protein, IL-1, and IL-6: a meta-analysis," *Psychosomatic Medicine*, vol. 71, no. 2, pp. 171–186, 2009.

[211] Y. Dowlati, N. Herrmann, W. Swardfager et al., "A meta-analysis of cytokines in major depression," *Biological Psychiatry*, vol. 67, no. 5, pp. 446–457, 2010.

[212] G. Kenis and M. Maes, "Effects of antidepressants on the production of cytokines," *The International Journal of Neuropsychopharmacology*, vol. 5, no. 4, pp. 401–412, 2002.

[213] J. L. M. Madrigal, O. Hurtado, M. A. Moro et al., "The increase in TNF-alpha levels is implicated in NF-kappaB activation and inducible nitric oxide synthase expression in brain cortex after immobilization stress," *Neuropsychopharmacology*, vol. 26, no. 2, pp. 155–163, 2002.

[214] K. A. O'Connor, J. D. Johnson, M. K. Hansen et al., "Peripheral and central proinflammatory cytokine response to a severe acute stressor," *Brain Research*, vol. 991, no. 1-2, pp. 123–132, 2003.

[215] C. Stetler and G. E. Miller, "Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research," *Psychosomatic Medicine*, vol. 73, no. 2, pp. 114–126, 2011.

[216] F. Berkenbosch, J. van Oers, A. del Rey, F. Tilders, and H. Besedovsky, "Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1," *Science*, vol. 238, no. 4826, pp. 524–526, 1987.

[217] R. Sapolsky, C. Rivier, G. Yamamoto, P. Plotsky, and W. Vale, "Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor," *Science*, vol. 238, no. 4826, pp. 522–524, 1987.

[218] J. W. Koo, S. J. Russo, D. Ferguson, E. J. Nestler, and R. S. Duman, "Nuclear factor-kappaB is a critical mediator of stress-impaired neurogenesis and depressive behavior," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 6, pp. 2669–2674, 2010.

[219] J. Tschopp, F. Martinon, and K. Burns, "NALPs: a novel protein family involved in inflammation," *Nature Reviews Molecular Cell Biology*, vol. 4, no. 2, pp. 95–104, 2003.

[220] K. Schroder and J. Tschopp, "The inflammasomes," *Cell*, vol. 140, no. 6, pp. 821–832, 2010.

[221] D. Ferrari, P. Chiozzi, S. Falzoni et al., "Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages," *Journal of Immunology*, vol. 159, no. 3, pp. 1451–1458, 1997.

[222] F. Martinon, K. Burns, and J. Tschopp, "The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β," *Molecular Cell*, vol. 10, no. 2, pp. 417–426, 2002.

[223] D. Ferrari, C. Pizzirani, E. Adinolfi et al., "The P2X7 receptor: a key player in IL-1 processing and release," *Journal of Immunology*, vol. 176, no. 7, pp. 3877–3883, 2006.

[224] C. Bryant and K. A. Fitzgerald, "Molecular mechanisms involved in inflammasome activation," *Trends in Cell Biology*, vol. 19, no. 9, pp. 455–464, 2009.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 7267865, 10 pages
http://dx.doi.org/10.1155/2016/7267865

Research Article

Acute Footshock Stress Induces Time-Dependent Modifications of AMPA/NMDA Protein Expression and AMPA Phosphorylation

Daniela Bonini,[1] Cristina Mora,[1] Paolo Tornese,[2] Nathalie Sala,[2] Alice Filippini,[1] Luca La Via,[1] Marco Milanese,[3] Stefano Calza,[4] Gianbattista Bonanno,[3] Giorgio Racagni,[2] Massimo Gennarelli,[1,5] Maurizio Popoli,[2] Laura Musazzi,[2] and Alessandro Barbon[1]

[1]*Biology and Genetic Division, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy*
[2]*Laboratorio di Neuropsicofarmacologia e Neurogenomica Funzionale, Dipartimento di Scienze Farmacologiche e Biomolecolari and CEND, Università degli Studi di Milano, 20133 Milano, Italy*
[3]*Department of Pharmacy, Unit of Pharmacology and Toxicology and Center of Excellence for Biomedical Research, University of Genoa, 16148 Genoa, Italy*
[4]*Unit of Biostatistics and Biomathematics, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy*
[5]*Genetic Unit, IRCCS Centro San Giovanni di Dio Fatebenefratelli, 25125 Brescia, Italy*

Correspondence should be addressed to Alessandro Barbon; alessandro.barbon@unibs.it

Received 25 September 2015; Revised 21 December 2015; Accepted 10 January 2016

Academic Editor: Zygmunt Galdzicki

Clinical studies on patients with stress-related neuropsychiatric disorders reported functional and morphological changes in brain areas where glutamatergic transmission is predominant, including frontal and prefrontal areas. In line with this evidence, several preclinical works suggest that glutamate receptors are targets of both rapid and long-lasting effects of stress. Here we found that acute footshock- (FS-) stress, although inducing no transcriptional and RNA editing alterations of ionotropic AMPA and NMDA glutamate receptor subunits, rapidly and transiently modulates their protein expression, phosphorylation, and localization at postsynaptic spines in prefrontal and frontal cortex. In total extract, FS-stress increased the phosphorylation levels of GluA1 AMPA subunit at Ser^{845} immediately after stress and of GluA2 Ser^{880} 2 h after start of stress. At postsynaptic spines, stress induced a rapid decrease of GluA2 expression, together with an increase of its phosphorylation at Ser^{880}, suggesting internalization of GluA2 AMPA containing receptors. GluN1 and GluN2A NMDA receptor subunits were found markedly upregulated in postsynaptic spines, 2 h after start of stress. These results suggest selected time-dependent changes in glutamatergic receptor subunits induced by acute stress, which may suggest early and transient enhancement of AMPA-mediated currents, followed by a transient activation of NMDA receptors.

1. Introduction

Stress can be defined as any condition that perturbs the physiological homeostasis [1]. A stressful event rapidly activates both the hypothalamic-pituitary-adrenocortical axis, leading to secretion of glucocorticoids (mainly cortisol in humans, corticosterone in rats), and the autonomic nervous system, which releases catecholamines (noradrenaline, adrenaline). The stress response is physiologically proadaptive, when efficiently turned on and then shut off, but may became maladaptive, particularly in subjects with a genetic background of vulnerability or when the stressful stimulus is chronic or overwhelming [2, 3].

The prefrontal cortex (PFC), a region involved in working memory, decision-making, and behavioral flexibility, as well as in social interaction and emotional processing, is a main target of the stress hormones [4–6]. A large body of literature has consistently shown that the fast response to

stress involves increased attention, vigilance, and improved PFC-mediated cognitive performance, mainly mediated by potentiation of glutamate transmission [7–9]. Indeed, acute stress and glucocorticoids rapidly modulate glutamate release and excitatory synaptic transmission in PFC [8, 10–12]. In particular, it has been shown that acute stress induces a rapid and transient enhancement of N-methyl-D-aspartic acid- (NMDA-) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid- (AMPA-) receptor-mediated currents in PFC in juvenile rats, together with increasing the surface expression of AMPA and NMDA receptor subunits [10, 11].

Taken together, these data strongly suggest that the enrichment, localization, and posttranslational modifications, as well as posttranscriptional and translational regulations of glutamate receptors, may be involved in the neuronal response to behavioral stress.

In this study, we exposed adult male rats to acute footshock- (FS-) stress and investigated time-dependent modifications of AMPA and NMDA receptor subunits mRNA and protein expression, RNA editing, and posttranslational regulation. The analyses have been performed in the prefrontal and frontal cortex (PFC/FC) at different time points (immediately after the 40 min of stress and 2 hours and 24 hours after stress start), to monitor the early and delayed effects of acute stress on the regulatory mechanisms of ionotropic glutamate receptors.

The results provided here indicate that exposure to acute stress causes transient and time-dependent subunit-specific changes in glutamate receptor, in line with previously observed adaptive modifications of excitatory synaptic transmission in the PFC/FC.

2. Materials and Methods

2.1. Footshock Stress Procedure. All experimental procedures involving animals were performed in accordance with the European Community Council Directive 86/609/EEC and were approved by the Italian legislation on animal experimentation (Decreto Ministeriale 116/1992). Experiments were performed with adult male Sprague-Dawley rats (275–300 g). Rats were housed two per cage and maintained on a 12/12 h light/dark schedule (lights on at 7:00 am), in a temperature controlled facility with free access to food and water. The experiments were performed during the light phase (between 9:00 and 12:00 am), at least one week after arrival from the supplier (Charles River, Wilmington, MA, USA). The footshock- (FS-) stress protocol was performed essentially as previously reported (40 min FS-stress: 0.8 mA, 20 min total of actual shock with random intershock length between 2 and 8 sec) [8, 12, 13]. Sham-stressed rats (controls) were kept in the stress apparatus without delivering of shocks. Rats were killed by decapitation at different time points (10 rats/group): immediately after the stress session ($t = 0$) and 2 or 24 h after stress start. The 2 and 24 h groups were left undisturbed in their cages after the 40 min stress session. Sham-groups were prepared at each time point, as specific controls for respective stressed groups.

The whole frontal lobe, referred to as PFC/FC, was quickly dissected on ice and right and left hemiareas were randomly assigned to RNA extraction or postsynaptic spine membranes (triton insoluble fraction; TIF) purification.

Serum corticosterone levels were measured using a commercial kit (Corticosterone ELISA kit, Enzo Life Sciences, Farmingdale, NY, USA).

2.2. RNA Extraction and Retrotranscription. Samples from PFC/FC of each animal were homogenized, and total RNA was extracted using TRIZOL reagent (Life Technologies, Milano, Italy). RNA was recovered by precipitation with isopropyl alcohol, washed with a 75% ethanol solution, and dissolved in RNase-free water. RNA quantification and quality controls were carried out using both spectrophotometric analysis and AGILENT Bioanalyzer 2100 lab-on-a-chip technology (AGILENT Technologies, Santa Clara, CA, USA). Reverse-transcription (RT) was done using Moloney murine leukemia virus-reverse transcriptase (MMLV-RT) (Life Technologies). Briefly, 2.5 μg of total RNA from each sample was mixed with 2.2 μL of 0.2 ng/μL random hexamers, 10 μL of 5x buffer, 10 μL of 2 mM dNTPs, 1 μL of 1 mM DTT, 0.4 μL of 33 U/μL RNaseout, and 2 μL MMLV-RT (200 U/μL) in a final volume of 50 μL. The reaction mix was incubated at 37°C for 2 h and the enzyme was then heat inactivated at 95° for 10 min.

2.3. Quantitative Real-Time PCR and RNA Editing Quantification. RNA expression pattern of the glutamate receptors was analyzed by means of an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). PCR was carried out by using TaqMan Universal PCR Master Mix (Applied Biosystems). 25 ng of sample was used in each real-time PCR reaction (TaqMan Gene Expression Assay id probes: GluA1: Rn00709588_m1; GluA2: Rn00568514_m1; GluN1: RN01436038_m1; GluN2A: Rn00561341_m1; GluN2B: Rn00561352_m1, Applied Biosystems). The expression ratio of target genes in treated sample groups, compared to control group, was calculated using the $\Delta\Delta$Ct method and H2AFZ, GAPDH, and PolII geometric mean as reference (ID H2AFZ TaqMan probe: Rn00821133_g1; ID GAPDH: Rn99999916_m1; ID PolII: Rn00580118_m1). Each individual determination was repeated in triplicate. The quantification for AMPA receptor subunits GluA2 Q/R and GluA2, GluA3, and GluA4 R/G editing levels were measured by sequence analysis as previously described [14, 15].

2.4. Protein Extracts and Western Blotting. PFC/FC were homogenized in 0.32 M ice-cold sucrose containing 1 mM HEPES, 1 mM $MgCl_2$, 1 mM EDTA, 1 mM $NaHCO_3$, and 0.1 mM PMSF, at pH 7.4, 2 mg/mL of protease inhibitor cocktail (Thermo scientific, Rockford, IL, USA), and phosphatases inhibitors (Sigma-Aldrich, Milan, Italy), pH 7.4. 200 μL of homogenate was aliquoted and immediately frozen.

Triton-X-100 insoluble fractions (TIF) were purified as previously reported [12]. The homogenized tissue was centrifuged at 1000 ×g for 10 min. The resulting supernatant (S1) was centrifuged at 3000 ×g for 15 min to obtain a crude

Table 1: Corticosterone serum levels.

	$t = 0$	2 h	24 h
Control	60.32 ± 13.06 ($n = 10$)	11.85 ± 3.82 ($n = 11$)	21.53 ± 6.93 ($n = 9$)
FS-stress	308.30 ± 23.30*** ($n = 10$)	57.20 ± 17.34*** ($n = 11$)	53.63 ± 13.41 ($n = 9$)

Data are expressed as ng/mL and reported as mean ± SE. *** $p < 0.001$.

membrane fraction (P2 fraction). The pellet was resuspended in 1 mM HEPES and centrifuged at 100,000 ×g for 1 h. The pellet (P3) was resuspended in buffer containing 75 mM KCl and 1% Triton-X-100 and centrifuged at 100,000 ×g for 1 h. The supernatant was stored and referred to as Triton-X-100-soluble fraction (TSF) (S4). The final pellet (P4) was homogenized in 20 mM HEPES. Then, an equal volume of glycerol was added, and this fraction, referred to as TIF, was stored at −80°C until processing.

The BCA protein concentration assay (Sigma-Aldrich, St. Louis, MO, USA) was used for protein quantitation. Before electrophoresis, each sample was incubated at 75°C for 10 min. Equal amounts of proteins were applied to precast SDS polyacrylamide gels (4–12% NuPAGEBis-Tris gels; Life Technologies, Milan, Italy), and proteins were electrophoretically transferred to a Hybond-P PVDF Transfer Membrane (GE Healthcare Life Science), for 2 h at a 1 mA/cm^2 of membrane surface. Membranes were blocked for 60 min with 3–5% nonfat dry milk or 5% BSA in TBS-T (Tris-buffered saline with 0.2% Tween-20, Sigma-Aldrich, Milan, Italy). Immunoblotting was carried out overnight at 4°C with specific antibodies against phosphoSer^{831}-GluA1 (1 : 1000, cod. ab109464, Abcam, Cambridge, UK), phosphoSer^{845}-GluA1 (1 : 1000, cod. ab3901, Abcam), and phosphoSer^{880}-GluA2 (1 : 1.000, cod. Ab52180, Abcam). Immunoblotting was also carried out on the same stripped membranes with antibodies against total GluA1 (1 : 200, cod. AGC004, Alomone Labs, Jerusalem, Israel) and GluA2 (1 : 2500, cod. AGC005, Alomone) in blocking buffer. Primary antibodies were used to detect GluN1 (1 : 500, cod. AB9864, Millipore, Billerica, MA, USA), GluN2A (1 : 500, AB1555P, Millipore), and GluN2B (1 : 500, cod. 454582, Calbiochem-Millipore). Mouse monoclonal anti-GAPDH (1 : 40.000, cod. Mab374, Millipore) or rabbit monoclonal anti-β-Actin (1 : 3000, cod.04-1116, Millipore) were used as internal controls. Membranes were washed five times with TBS-Tween-20 0.2% and incubated for 1 h at room temperature with AP-conjugated secondary antibodies (Promega, Milan, Italy). Immunolabeled proteins were detected by incubation with Supersignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA) or CDPStar (Roche Applied Science) detection reagents and then exposed to imaging film. Prestained Novex Sharp Protein Standards (Life Technologies) were used as molecular weight standards loaded on the same gel. The intensity of immunoreactive bands was analyzed with Image-Pro Plus. Data are presented as optical density ratios of the investigated protein band normalized for GAPDH or β-Act bands in the same line and are expressed as percentage of controls. The levels of GluA phosphorylated subunits were normalized to total GluA levels, based on previous reports [16, 17].

2.5. Data Analysis. All the analyses were carried out in individual animals (independent determinations).

Preliminary data inspection showed a fairly constant coefficient of variation among groups, as well as a multiplicative effect on the mean. Therefore, we modeled data using a gamma regression model with log-link (via Generalized Linear Models, GLM [18]), with treatment (stress, control), time, and their interaction as predictors. Where needed, a robust Generalized Linear Model was used to account for potential outliers. Due to some additional heteroskedasticity in corticosterone levels between groups, tests were performed using "sandwich" robust standard error estimates. Data in the text are reported as estimated fold changes (FC) and 95% confidence intervals (CI 95%). The interaction between treatment and time (treatment-×-time) was considered the main effect of interest, as it indicates a differential effect on stressed versus control groups during time. Pairwise contrasts p values between groups were adjusted by Bonferroni Post Hoc Test (reported as p_{adj}). Statistical significance was assumed at $p < 0.05$.

For simplicity, data on graphs are represented as estimated group mean values + standard errors of the means (SEM). Stressed groups are represented as percentage of controls at each time point. Statistical analysis was carried out by using *R* [19].

3. Results

3.1. Corticosterone Levels. To test the efficacy of the stress protocol, we evaluated plasma corticosterone levels in all the animals. As expected, the FS-stress procedure markedly and transiently increased serum corticosterone levels as shown in Table 1.

We observed a significant increase in corticosterone levels in stressed animals sacrificed immediately after the stress session ($t = 0$ FC = 5.11, CI 95% = 2.42–10.77, $p_{adj} < 0.001$), with a relatively slow decrease in the following time points (2 h FC = 4.83, CI 95% = 2.19–10.64, $p_{adj} < 0.001$; 24 h FC = 2.49, CI 95% = 0.84–7.39, $p_{adj} = 0.13$).

3.2. Acute Stress Does Not Induce Any Alteration in Transcriptional Levels and Editing of Ionotropic Glutamate Receptors. mRNA expression analysis of glutamate receptor subunits showed no changes in transcript levels, at the three time points analyzed (see Supplemental Figure 1 in

Supplementary Material available online at http://dx.doi.org/10.1155/2016/7267865). Furthermore, no alterations were observed for Q/R and R/G editing sites of GluA2, GluA3, and GluA4 AMPA subunits (Supplemental Figure 2).

3.3. Modulation of AMPA Receptor Subunits Expression and Phosphorylation Induced by Acute Stress. To assess time-dependent changes induced by acute stress in glutamate receptor subunits expression, Western blot analyses for AMPA and NMDA receptor subunits were performed on PFC/FC total homogenates and purified postsynaptic spine membranes (TIF) of rats subjected to acute FS-stress and sacrificed at the different time points.

In total PFC/FC homogenate, no significant effects of FS-stress were found on the total expression of GluA1 and GluA2 AMPA receptor subunits at different time points (GluA1: interaction term, $p = 0.47$; GluA2: interaction term, $p = 0.94$) (Figures 1(a) and 1(b), resp.), although a trend for increase could be observed for GluA1, 2 h after the stress beginning (FC = 1.21, $p_{adj} = 0.08$).

No significant changes were also observed for GluA1 Ser^{831} phosphorylation in FS-stress animals at different time points (interaction term $p = 0.15$, Figure 1(c)), despite single comparison at 2 hours after stress had a marginally significant effect (FC = 0.78; $p = 0.045$). In contrast, we measured a significant treatment-×-time interaction for GluA1 Ser^{845} phosphorylation ($p = 0.026$, Figure 1(d)). In particular, exclusively immediately after the stress protocol, a marked upregulation of GluA1 Ser^{845} phosphorylation was observed (FC = 1.32, CI 95% = 1.12–1.55, $p_{adj} < 0.001$), with no significant variations at other time points (2 h FC = 1.09, CI 95% = 0.93–1.28, $p_{adj} = 0.49$; 24 h FC = 1.03, CI 95% = 0.87–1.21, $p_{adj} = 0.97$). Moreover, we found a significant treatment-×-time effect for GluA2 Ser^{880} phosphorylation ($p = 0.002$; Figure 1(e)): acute stress caused an increase in GluA2 Ser^{880} phosphorylation 2 h after its start (FC = 1.33, CI 95% = 1.09–1.62, $p_{adj} = 0.0015$), while no significant changes were observed at the other time points (2 h FC = 0.90, $p_{adj} = 0.62$; 24 h FC = 1.00, $p_{adj} = 0.99$).

At postsynaptic membranes, no significant modifications were observed for GluA1 (interaction term, $p = 0.32$; Figure 2(a)), while treatment-×-time interaction was significant for GluA2 subunit ($p = 0.013$; Figure 2(b)), with a significant downregulation immediately after the stress protocol (FC = 0.77, CI 95% = 0.63–0.95, $p_{adj} = 0.01$). No significant modifications were found in GluA1 phosphorylation at Ser^{831} (interaction term, $p = 0.27$; Figure 2(c)) or Ser^{845} (interaction term $p = 0.76$; Figure 2(d)). On the contrary, we observed a significant treatment-×-time interaction for GluA2 at Ser^{880} phosphorylation levels ($p = 0.025$; Figure 2(e)), which were significantly increased immediately after the stress protocol (FC = 1.37, CI 95% = 1.11–1.69, $p_{adj} = 0.0013$) and reduced at following time points (2 h FC = 1.12, CI 95% = 0.91–1.38, $p_{adj} = 0.49$; 24 h FC = 1.02, CI 95% = 0.83–1.26, $p_{adj} = 0.99$).

3.4. Acute Stress Induces Alterations in NMDA Receptor Subunits Expression. In total PFC/FC homogenates, we found no effect of stress on GluN1 subunit expression levels at different time points (interaction term, $p = 0.94$, Figure 3(a)). Instead, with regard to GluN2A, a significant treatment-×-time interaction term was found ($p = 0.022$; Figure 3(b)). Indeed, total GluN2A expression levels were found increased in total homogenates of PFC/FC from FS-stress rats, selectively 2 h after the beginning of stress (FC = 1.41, CI 95% = 1.07–1.86, $p_{adj} = 0.008$), and not at the other time points analyzed ($t = 0$ FC = 1.04, $p_{adj} = 0.99$; 24 h FC = 0.97, $p_{adj} = 0.99$). With regard to GluN2B subunit, a trend for decrease although not statistically significant was observed 2 h after the beginning of stress (FC = 0.85, $p_{adj} = 0.53$; Figure 3(c)).

Given the key role of GluN2A/GluN2B ratio in regulating glutamatergic synapses activity [20], the ratio between the two subunits has also been calculated (Figure 3(d)). We found a significant treatment-×-time interaction effect ($p = 0.003$), with GluN2A/GluN2B ratio significantly higher in PFC/FC total homogenates from stressed rats sacrificed 2 h after the stress beginning (FC = 1.71, CI 95% = 1.22–2.40, $p_{adj} < 0.001$), and no significant changes in GluN2A/GluN2B ratio at other time points ($t = 0$ FC = 1.02, $p_{adj} = 0.99$; 24 h FC = 0.93, $p_{adj} = 0.94$).

At postsynaptic membranes, we observed a significant treatment-×-time interaction (Robust GLM, $p = 0.005$) for GluN1 expression levels (Figure 4(a)), which were significantly increased in FS-stressed rats sacrificed 2 h after the beginning of stress (FC = 1.36, CI 95% = 1.05–1.68, $p_{adj} = 0.0013$). A similar result was found for GluN2A subunit (Figure 4(b)). GluN2A protein expression level showed a significant stress-×-time interaction (Robust GLM, $p = 0.0009$), with a marked increase 2 h after stress beginning (FC = 1.50, CI 95% = 1.16–1.93, $p_{adj} = 0.0005$). On the contrary, no alterations were found either in postsynaptic level of GluN2B (interaction term, $p = 0.85$; Figure 4(c)) or in GluN2A/GluN2B ratio (interaction term, $p = 0.39$; Figure 4(d)).

4. Discussion

We report here that acute footshock- (FS-) stress, although inducing no transcriptional or posttranscriptional alterations of ionotropic AMPA and NMDA glutamate receptor subunits, modulates, in a time- and subunit-dependent way, their protein expression, phosphorylation, and localization at postsynaptic spines in PFC/FC of rats.

In particular, FS-stress rapidly increased phosphorylation of GluA1, selectively at Ser^{845} (not at Ser^{831}), and of GluA2 at Ser^{880} in total homogenate, while reducing GluA2 levels, together with increasing its phosphorylation at Ser^{880}, in postsynaptic spine membranes. Acute stress exerted no effect on GluA1 and GluA2 protein expression levels in total homogenate, as previously reported [10]. All the changes in AMPA receptor subunits expression and phosphorylation levels were selectively measured immediately after the 40 min of stress session (except for increased GluA2 phospho-Ser^{880} levels in total homogenate, which were selectively increased

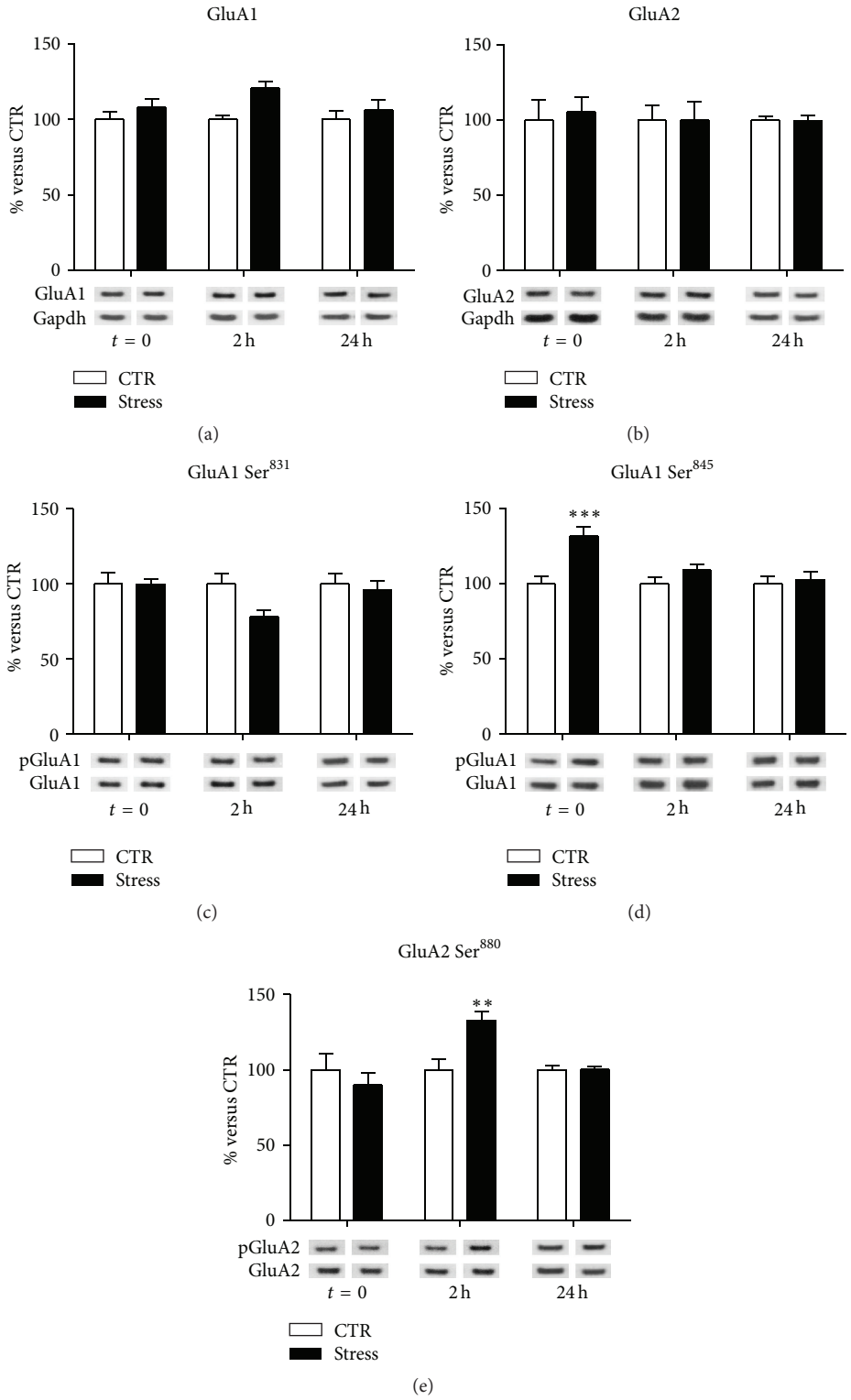


FIGURE 1: Time-dependent changes of protein expression levels of GluA1 (a), GluA2 (b), GluA1 phospho-Ser^{831} (c), GluA1 phospho-Ser^{845} (d), and GluA2 phospho-Ser^{880} (e) in PFC/FC total homogenate of rats subjected to FS-stress and sacrificed immediately after stress and 2 h and 24 h from stress beginning. Data are represented as percentage of controls at each time point, as means ± SEM ($n = 8$). Statistics: Generalized Linear Models (GLM) and Bonferroni Post Hoc Test (see Section 2 for details). $^{**}p < 0.01$; $^{***}p < 0.001$.

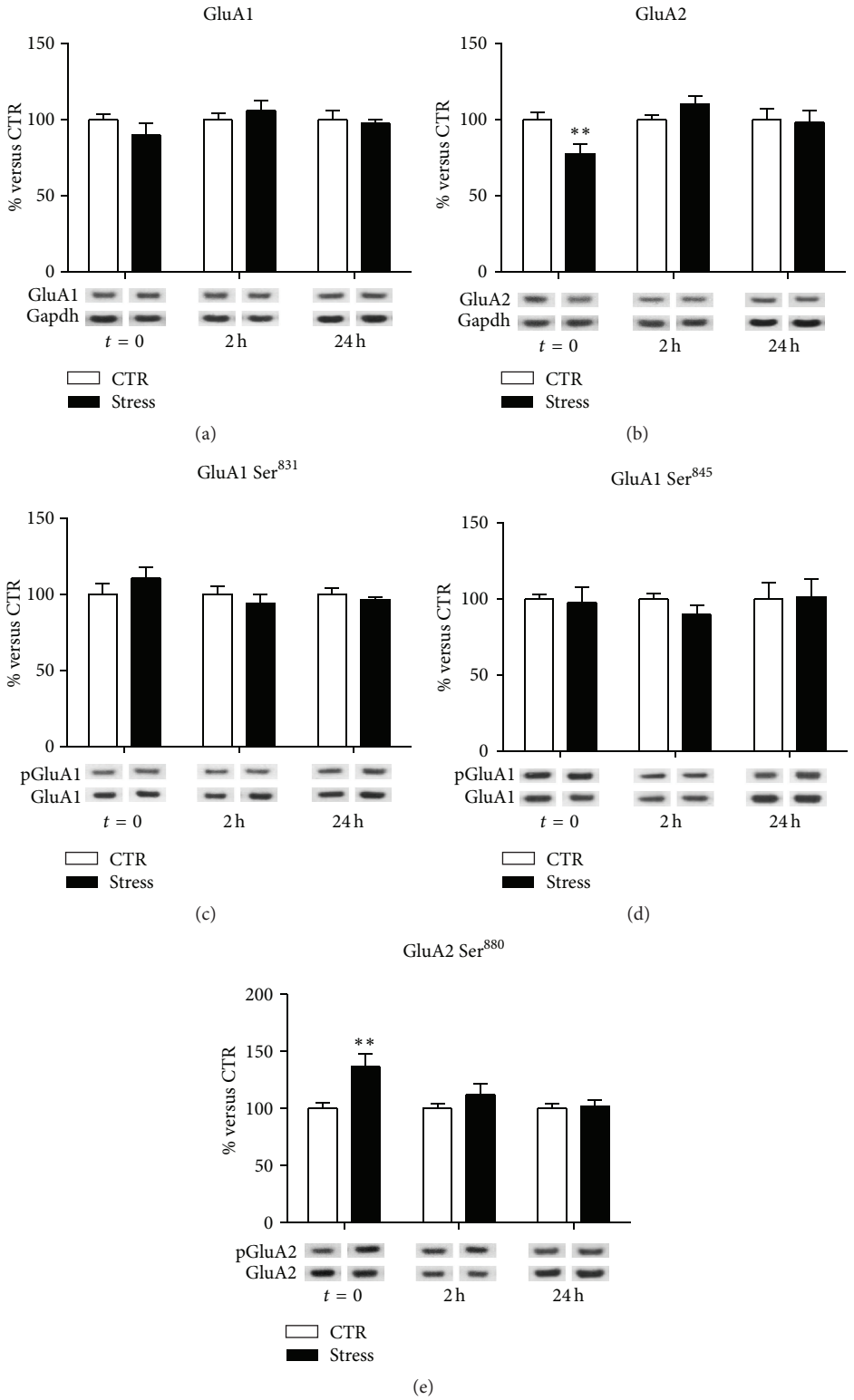


Figure 2: Time-dependent changes of protein expression levels of GluA1 (a), GluA2 (b), GluA1 phospho-Ser^{831} (c), GluA1 phospho-Ser^{845} (d), and GluA2 phospho-Ser^{880} (e) in PFC/FC postsynaptic spine membranes of rats subjected to FS-stress and sacrificed immediately after stress and 2 h and 24 h from stress beginning. Data are represented as percentage of controls at each time point, as means ± SEM ($n = 8$). Statistics: Generalized Linear Models (GLM) and Bonferroni Post Hoc Test (see Section 2 for details). $^{**}p < 0.01$.

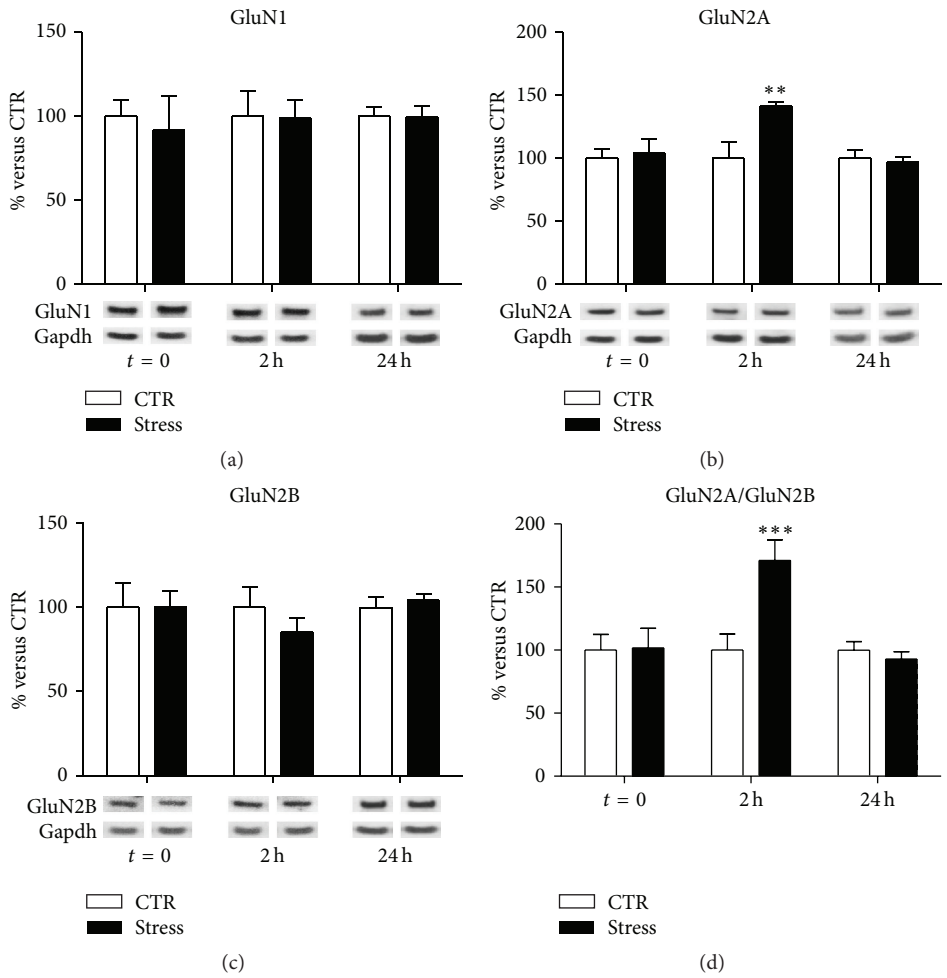


Figure 3: Time-dependent changes of protein expression levels of GluN1 (a), GluN2A (b), GluN2B (c), and GluN2A/GluN2B (d) in PFC/FC total homogenate of rats subjected to FS-stress and sacrificed immediately after stress and 2 h and 24 h from stress beginning. Data are represented as percentage of controls at each time point, as means ± SEM ($n = 8$). Statistics: Generalized Linear Models (GLM) and Bonferroni Post Hoc Test (see Section 2 for details). $^{**}p < 0.01$; $^{***}p < 0.001$.

2 h after stress start), suggesting fast and transient modulation of AMPA receptor subunits at PFC/FC synapses induced by acute stress.

Phosphorylation of GluA1 at Ser^{831} and Ser^{845} has been shown to modulate potentiation of AMPA receptor-mediated synaptic currents and to be involved in both Long Term Potentiation (LTP) and Long Term Depression (LTD) [21]. In particular, phosphorylation at Ser^{845} increases the open channel probability, and the peak amplitude of currents mediated by AMPA receptors [21]. Therefore, the increase of GluA1 phosphorylation at Ser^{845} rapidly induced by FS-stress is in line with increased AMPA receptor currents.

Phosphorylation of GluA2 at Ser^{880} was shown to affect its association with PDZ domain-containing proteins, thereby modifying trafficking and redistribution of the subunit at synaptic sites, facilitating GluA2 internalization [22–25], and subsequent lysosomal degradation [26]. GluA2 is a critical subunit in determining the function of AMPA receptors. Indeed, GluA2-containing AMPA receptors are Ca^{2+}-impermeable and have a relatively low single channel conductance [27], while AMPA receptors lacking GluA2 subunit have a higher Ca^{2+} permeability and conductance [28, 29]. Intriguingly, it was shown that homomeric GluA1 AMPA receptors are delivered to synapses after LTP induction, whereas homomeric GluA2 or GluA3 AMPA receptors are constitutively inserted [30, 31].

Taken together, this body of evidence strongly suggests that acute FS-stress, increasing GluA1 phosphorylation at Ser^{845} and reducing the levels of GluA2-containing AMPA receptors at postsynaptic membranes, may rapidly and transiently activate AMPA receptor-mediated synaptic currents.

We have also found here that acute FS-stress markedly increased GluN2A expression levels and GluN2A/GluN2B ratio in PFC/FC homogenate and GluN1 and GluN2A protein levels in postsynaptic spine membranes, 2 h after the stress session. Notably, no changes were detected in NMDA

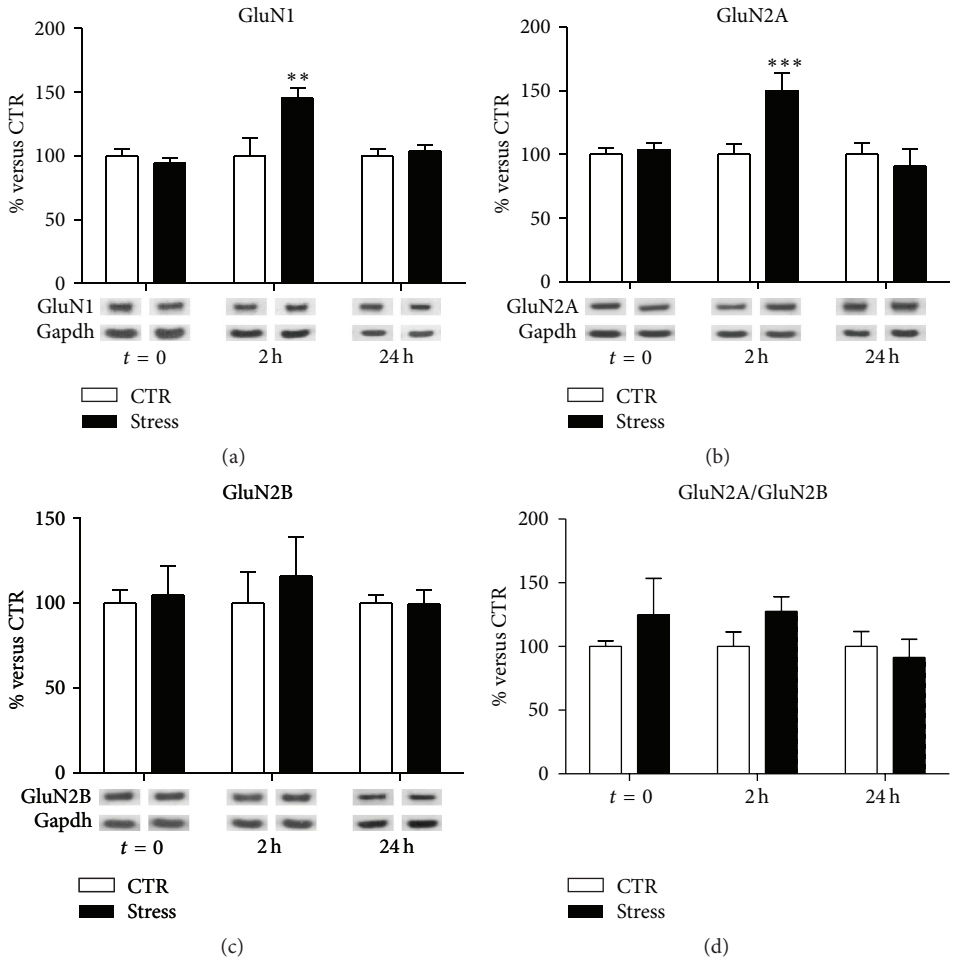


Figure 4: Time-dependent changes of protein expression levels of GluN1 (a), GluN2A (b), GluN2B (c), and GluN2A/GluN2B (d) in PFC/FC postsynaptic spine membranes of rats subjected to FS-stress and sacrificed immediately after stress and 2 h and 24 h from stress beginning. Data are represented as percentage of controls at each time point, as means ± SEM ($n = 8$). Statistics: Generalized Linear Models (GLM) and Bonferroni Post Hoc Test (see Section 2 for details). $^{**}p < 0.01$; $^{***}p < 0.001$.

receptor subunits at other time points, suggesting a time-dependent modulation of GluN2A and GluN2B subunits induced by acute stress.

In the forebrain, NMDA receptors are composed of one GluN1 subunit and one or more GluN2A or GluN2B subunits, and the precise combination of subunits determines the functional properties of the receptor [32]. It is well known that NMDA receptor subunit composition changes during development: while GluN2B is abundant in the early postnatal brain, the level of GluN2A, characterized by faster rising and decay kinetics, increases progressively during development [33]. In the adult brain, GluN2A is enriched at synaptic sites, while GluN2B is mainly extrasynaptic [34], and the GluN2A/GluN2B ratio was shown to be dependent on neuronal activity [35]. In particular, since increased GluN2A/GluN2B ratio is related with increased synaptic stimulation and transmission, its dynamic regulation is a major determinant of synaptic plasticity [36]. In this context, the increase of GluN2A/GluN2B ratio in homogenate, together with enrichment of GluN1- and GluN2A-containing NMDA receptors in postsynaptic spine membranes measured 2 h, but not 24 h, after the start of FS-stress, is in line with a delayed and transient enhancement of NMDA receptor-mediated synaptic currents.

In line with our results, in previous studies it was shown that both acute stress *in vivo* and short-term incubation of PFC neurons with corticosterone *in vitro* increase AMPA and NMDA receptor-mediated synaptic transmission and expression levels at membranes [10, 37]. However, contrary to the fast and transient effect measured after FS-stress, acute forced-swim stress was shown to induce a long-lasting increase (from 1–4 h, to 24 h after stress) of AMPA and NMDA-mediated excitatory postsynaptic currents amplitude and surface expression [10]. The apparent discrepancy with our results may be dependent on a number of factors, including different types of stress used, different age of the rats (juvenile versus adult), time points analyzed, and measurement of glutamate receptor subunits expression in different compartments (total membrane fraction versus postsynaptic spine membranes). Moreover, although the

changes in AMPA and NMDA receptor subunits expression and phosphorylation levels induced by FS-stress were found to be transient, it cannot be excluded that FS-stress may induce long-lasting alterations of synaptic transmission, mediated by other molecular mechanisms. Further studies are required to address this point.

In previous studies, we showed that FS-stress, together with enhancing depolarization-dependent release of endogenous glutamate, increases excitatory postsynaptic currents amplitude (measured immediately after the stress session) [8]. Acute FS-stress also strongly decreases synaptic facilitation and its calcium-dependence, in line with an increase in release probability. The results obtained in the present study strongly suggest that postsynaptic mechanisms may also be involved in the enhancement of glutamate transmission induced by FS-stress in PFC/FC. In addition, we have also shown recently, by using electron microscopy stereology, that the total number of nonperforated and axoshaft excitatory synapses in medial PFC is increased remarkably (over 40%) immediately after acute FS-stress [38], demonstrating that the early functional changes in glutamate transmission are accompanied by large-scale changes in brain architecture at a fast pace.

5. Conclusion

In this study, we reported a time-dependent modulation of both AMPA and NMDA receptor subunits expression and phosphorylation induced by acute FS-stress in PFC/FC.

Although further studies are warranted to dissect the time-dependent functional, molecular, and structural alterations induced by stress in PFC/FC, the present study may further support the evidence of an enhancement of glutamatergic synaptic transmission as early response to acute stress.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Daniela Bonini and Cristina Mora equally contributed to this work. Laura Musazzi and Alessandro Barbon equally contributed to this work.

Acknowledgments

This work was supported by a grant from MIUR (PRIN 2012 prot.2012A9T2S9_004). The authors gratefully acknowledge Dr. Giulia Treccani for technical help.

References

[1] B. S. McEwen, "Allostasis and allostatic load: implications for neuropsychopharmacology," *Neuropsychopharmacology*, vol. 22, no. 2, pp. 108–124, 2000.

[2] I. N. Karatsoreos and B. S. McEwen, "Psychobiological allostasis: resistance, resilience and vulnerability," *Trends in Cognitive Sciences*, vol. 15, no. 12, pp. 576–584, 2011.

[3] N. Sousa and O. F. X. Almeida, "Disconnection and reconnection: the morphological basis of (mal)adaptation to stress," *Trends in Neurosciences*, vol. 35, no. 12, pp. 742–751, 2012.

[4] M. Popoli, Z. Yan, B. S. McEwen, and G. Sanacora, "The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission," *Nature Reviews Neuroscience*, vol. 13, no. 1, pp. 22–37, 2012.

[5] A. F. T. Arnsten, M. J. Wang, and C. D. Paspalas, "Neuromodulation of thought: flexibilities and vulnerabilities in prefrontal cortical network synapses," *Neuron*, vol. 76, no. 1, pp. 223–239, 2012.

[6] B. S. McEwen and J. H. Morrison, "The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course," *Neuron*, vol. 79, no. 1, pp. 16–29, 2013.

[7] B. S. McEwen, C. Nasca, and J. D. Gray, "Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex," *Neuropsychopharmacology*, vol. 41, no. 1, pp. 3–23, 2015.

[8] L. Musazzi, M. Milanese, P. Farisello et al., "Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants," *PLoS ONE*, vol. 5, no. 1, Article ID e8566, 2010.

[9] R. S. Duman, "Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections," *Dialogues in Clinical Neuroscience*, vol. 16, no. 1, pp. 11–27, 2014.

[10] E. Y. Yuen, W. Liu, I. N. Karatsoreos, J. Feng, B. S. McEwen, and Z. Yan, "Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 33, pp. 14075–14079, 2009.

[11] E. Y. Yuen, J. Wei, W. Liu, P. Zhong, X. Li, and Z. Yan, "Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex," *Neuron*, vol. 73, no. 5, pp. 962–977, 2012.

[12] G. Treccani, L. Musazzi, C. Perego et al., "Stress and corticosterone increase the readily releasable pool of glutamate vesicles in synaptic terminals of prefrontal and frontal cortex," *Molecular Psychiatry*, vol. 19, no. 4, pp. 433–443, 2014.

[13] G. Bonanno, R. Giambelli, L. Raiteri et al., "Chronic antidepressants reduce depolarization-evoked glutamate release and protein interactions favoring formation of SNARE complex in hippocampus," *The Journal of Neuroscience*, vol. 25, no. 13, pp. 3270–3279, 2005.

[14] A. Barbon, F. Fumagalli, L. Caracciolo et al., "Acute spinal cord injury persistently reduces R/G RNA editing of AMPA receptors," *Journal of Neurochemistry*, vol. 114, no. 2, pp. 397–407, 2010.

[15] D. Bonini, A. Filippini, L. La Via et al., "Chronic glutamate treatment selectively modulates AMPA RNA editing and ADAR expression and activity in primary cortical neurons," *RNA Biology*, vol. 12, no. 1, pp. 43–53, 2015.

[16] D. Caudal, B. P. Godsil, F. Mailliet, D. Bergerot, and T. M. Jay, "Acute stress induces contrasting changes in AMPA receptor subunit phosphorylation within the prefrontal cortex, amygdala and hippocampus," *PLoS ONE*, vol. 5, no. 12, Article ID e15282, 2010.

[17] P. Svenningsson, H. Bateup, H. Qi et al., "Involvement of AMPA receptor phosphorylation in antidepressant actions with special reference to tianeptine," *European Journal of Neuroscience*, vol. 26, no. 12, pp. 3509–3517, 2007.

[18] P. McCullagh and J. A. Nelder, *Generalized Linear Models*, Chapman & Hall/CRC, 2nd edition, 1989.

[19] R Development Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2011.

[20] T. E. Bartlett and Y. T. Wang, "The intersections of NMDAR-dependent synaptic plasticity and cell survival," *Neuropharmacology*, vol. 74, pp. 59–68, 2013.

[21] J. Q. Wang, A. Arora, L. Yang et al., "Phosphorylation of AMPA receptors: mechanisms and synaptic plasticity," *Molecular Neurobiology*, vol. 32, no. 3, pp. 237–249, 2005.

[22] R. H. Scannevin and R. L. Huganir, "Postsynaptic organization and regulation of excitatory synapses," *Nature Reviews Neuroscience*, vol. 1, no. 2, pp. 133–141, 2000.

[23] S. Matsuda, S. Mikawa, and H. Hirai, "Phosphorylation of serine-880 in GluR2 by protein kinase C prevents its C terminus from binding with glutamate receptor-interacting protein," *Journal of Neurochemistry*, vol. 73, no. 4, pp. 1765–1768, 1999.

[24] W. Lu and K. W. Roche, "Posttranslational regulation of AMPA receptor trafficking and function," *Current Opinion in Neurobiology*, vol. 22, no. 3, pp. 470–479, 2012.

[25] H. J. Chung, J. Xia, R. H. Scannevin, X. Zhang, and R. L. Huganir, "Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins," *Journal of Neuroscience*, vol. 20, no. 19, pp. 7258–7267, 2000.

[26] M. D. Ehlers, "Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting," *Neuron*, vol. 28, no. 2, pp. 511–525, 2000.

[27] J. T. R. Isaac, M. C. Ashby, and C. J. McBain, "The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity," *Neuron*, vol. 54, no. 6, pp. 859–871, 2007.

[28] H. Lomeli, J. Mosbacher, T. Melcher et al., "Control of kinetic properties of AMPA receptor channels by nuclear RNA editing," *Science*, vol. 266, no. 5191, pp. 1709–1713, 1994.

[29] G. T. Swanson, D. Feldmeyer, M. Kaneda, and S. G. Cull-Candy, "Effect of RNA editing and subunit co-assembly single-channel properties of recombinant kainate receptors," *The Journal of Physiology*, vol. 492, part 1, pp. 129–142, 1996.

[30] S.-H. Shi, Y. Hayashi, J. A. Esteban, and R. Malinow, "Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons," *Cell*, vol. 105, no. 3, pp. 331–343, 2001.

[31] S. Bassani, A. Folci, J. Zapata, and M. Passafaro, "AMPAR trafficking in synapse maturation and plasticity," *Cellular and Molecular Life Sciences*, vol. 70, no. 23, pp. 4411–4430, 2013.

[32] A. Sanz-Clemente, R. A. Nicoll, and K. W. Roche, "Diversity in NMDA receptor composition: many regulators, many consequences," *Neuroscientist*, vol. 19, no. 1, pp. 62–75, 2013.

[33] C. Lohmann and H. W. Kessels, "The developmental stages of synaptic plasticity," *Journal of Physiology*, vol. 592, no. 1, pp. 13–31, 2014.

[34] C. Bellone and R. A. Nicoll, "Rapid bidirectional switching of synaptic NMDA receptors," *Neuron*, vol. 55, no. 5, pp. 779–785, 2007.

[35] K. Yashiro and B. D. Philpot, "Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity," *Neuropharmacology*, vol. 55, no. 7, pp. 1081–1094, 2008.

[36] R. C. Malenka and M. F. Bear, "LTP and LTD: an embarrassment of riches," *Neuron*, vol. 44, no. 1, pp. 5–21, 2004.

[37] E. Y. Yuen, W. Liu, I. N. Karatsoreos et al., "Mechanisms for acute stress-induced enhancement of glutamatergic transmission and working memory," *Molecular Psychiatry*, vol. 16, no. 2, pp. 156–170, 2011.

[38] N. Nava, G. Treccani, N. Liebenberg et al., "Chronic desipramine prevents acute stress-induced reorganization of medial prefrontal cortex architecture by blocking glutamate vesicle accumulation and excitatory synapse increase," *International Journal of Neuropsychopharmacology*, vol. 18, no. 3, 2015.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 7539065, 16 pages
http://dx.doi.org/10.1155/2016/7539065

Review Article

Impaired Functional Connectivity in the Prefrontal Cortex: A Mechanism for Chronic Stress-Induced Neuropsychiatric Disorders

Ignacio Negrón-Oyarzo, Francisco Aboitiz, and Pablo Fuentealba

Departamento de Psiquiatría, Facultad de Medicina, Centro Interdisciplinario de Neurociencia, Pontificia Universidad Católica de Chile, Avenida Marcoleta No. 391, 8320000 Santiago, Chile

Correspondence should be addressed to Pablo Fuentealba; pfuentealba@uc.cl

Received 14 August 2015; Revised 25 November 2015; Accepted 16 December 2015

Academic Editor: Laura Musazzi

Chronic stress-related psychiatric diseases, such as major depression, posttraumatic stress disorder, and schizophrenia, are characterized by a maladaptive organization of behavioral responses that strongly affect the well-being of patients. Current evidence suggests that a functional impairment of the prefrontal cortex (PFC) is implicated in the pathophysiology of these diseases. Therefore, chronic stress may impair PFC functions required for the adaptive orchestration of behavioral responses. In the present review, we integrate evidence obtained from cognitive neuroscience with neurophysiological research with animal models, to put forward a hypothesis that addresses stress-induced behavioral dysfunctions observed in stress-related neuropsychiatric disorders. We propose that chronic stress impairs mechanisms involved in neuronal functional connectivity in the PFC that are required for the formation of adaptive representations for the execution of adaptive behavioral responses. These considerations could be particularly relevant for understanding the pathophysiology of chronic stress-related neuropsychiatric disorders.

1. Introduction

The enriched image we have of the world as well as the great number of behavioral options implies the need for appropriate and adaptive accommodation of behavioral responses for our survival. This demand is especially evident in neuropsychiatric disorders like major depressive disorder (MDD), anxiety disorders (including posttraumatic stress disorder, PTSD), and schizophrenia (SZ), in which subjects develop aberrant and maladaptive behavioral responses which negatively affects well-being (reviewed in [1, 2]). For example, MDD is characterized by a bias in explicit memory that favors negative self-related information and increased bias to interpret perceptual stimuli as negative [3, 4]. Similarly, anxiety disorders are characterized by selective attention favoring threatening information and increased retention of aversive memories [3, 5, 6], while SZ patients display a tendency for ambiguous stimuli to be misinterpreted as threatening, together with misinterpretation of the intentions of others [2, 7]. Simultaneously, these disorders are also characterized by impaired behavioral flexibility, that is, the ability to adapt behavioral responses to current environmental demands [8]. This evidence suggests a clear impairment in the adaptive control of behavior.

The adaptive organization of goal-directed behavioral responses is supported by the prefrontal cortex (PFC) [9, 10] and its appropriate connectivity with other brain structures, as the hippocampus (HPC) and the amygdala [11–14]. It is argued that neuropsychiatric disorders involve alterations to the behavioral control system supported by the PFC and its connectivity with other brain systems [2, 15, 16]. In support of this idea, it has been shown that patients suffering from these disorders display alterations of PFC-dependent cognitive functions, like cognitive flexibility [17], working memory [18, 19], and fear extinction [20]. In the same line of evidence, functional alterations of the PFC have been observed in patients suffering from neuropsychiatric disorders [4, 21–24]. Also, these patients display altered functional connectivity in the PFC-HPC and the PFC-amygdala pathway [25–27]. These evidences suggest that such disorders are related

to the maladaptive accommodation of behavioral responses supported by the PFC, and its connection with other structures. On the other hand, chronic stress, that is, an intense and sustained maladaptive response to environmental threats [28, 29], is considered one of the most important risk conditions for the development of neuropsychiatric disorders [30–32]. Therefore, connecting this evidence, it has been suggested that chronic stress affects the accurate functioning of the PFC [33, 34], which is expressed as the maladaptive generation of behavioral responses observed in neuropsychiatric disorders. However, how chronic stress affects the neural representation of behavior in the PFC and how these alterations are expressed as maladaptive behavioral responses remain still elusive.

In the present review we integrate theoretical considerations and empirical evidence, mainly from rodents animal models, to relate the effects of chronic stress on neural functional connectivity related to the organization of behavior mediated by the PFC. In Section 2, we approach theoretical frameworks proposing that the control of behavior is mediated by the formation of cognitive maps in the PFC. In Section 3, we propose these cognitive maps are represented as functional connectivity of neuronal assemblies in the PFC. We then in Section 4 propose synaptic plasticity and oscillatory synchrony as neurophysiological mechanisms involved in the formation of these neural assemblies in the PFC. We also report evidence of the chronic stress-induced impairment of these mechanisms. In Section 5, we approach the long-term storage of neural assemblies representing cognitive maps in the PFC, the neurophysiological mechanisms involved, and in Section 6 the consequences of chronic stress on these mechanisms. In Section 7, we propose a hypothesis for chronic stress-induced psychiatric disorders based on functional connectivity in the PFC. Finally, we discuss future research in this area, in particular concerning the pathophysiology of chronic stress-related neuropsychiatric disorders.

2. Adaptive Control of Behavior by the PFC

2.1. Function of the PFC. The first clues about the role of the PFC came from studies of PFC lesions in humans. Lesions of the PFC have long been known to produce impairment of cognitive functions, such as attention, set-shifting, working memory, planning, temporal integration, decision making, retrieving and manipulating old memories, and inhibitory control (reviewed extensively in [35]). Although rodents do not possess anatomical features of primate PFC, a large body of evidence has shown that lesions to the rodent medial-PFC (mPFC) subserves a range of cognitive and behavioral processes homologous to those mediated by the primate PFC (reviewed in [36], [37]). Lesions to the rodent mPFC impair set-shifting [38], working memory [39], and the recall of extinction of conditioned fear, a type of emotional reversal learning [40]. Thus, rodent models have important properties to the study of behavioral and neural properties related to the control of behavior.

The lesion studies of the PFC in both primates and rodents led to the idea that the principle and more general behavioral impairments associated with PFC lesions are not explained by failure of a particular function but imply a failure to coordinate a set of cognitive processes. A large body of evidence leads to the hypothesis that the main function of the PFC is cognitive control of behavior, that is, the adaptive accommodation of behavioral responses to current perceptual conditions toward the attainment of goals [41], also known as executive control [10]. To accomplish this function, the PFC integrates perceptual information about the current context and execute an appropriate behavioral response a process known as the perception-action cycle [9].

To the execution of the perception-action cycle, both the primate PFC and rodent mPFC are anatomically positioned between the perceptual input and motor and visceral output neural systems [9]. The perceptual information communicates about the internal state (needs), the significance and motivational and emotional information (valence and arousal), contextual information of the stimuli, and previous memories related to similar perceptual information.

Perceptual information about the external and internal environment is transmitted mainly from the orbitofrontal cortex (OFC) and the insula, respectively [42]. The PFC also receives inputs from the thalamus (mediodorsal and reuniens nuclei) [43], which is involved in learning new information, from the hippocampus (HPC), which is involved in spatial and episodic memory and in memory consolidation [44, 45], and from the amygdala, which carries information about the motivational significance of sensory stimuli [46, 47]. The ascending arousal system also projects densely to the PFC [48–51]. Thus, the PFC integrates perceptual, contextual, and motivational information about the environment, together with internal states and needs.

For the execution of the perception-action cycle, the PFC projects to neural systems involved in generating behavioral responses and executing actions [52, 53]. The PFC projects to several outputs, such as the premotor cortex, the hypothalamus (reviewed in [53]), the striatum [54], and the ascending-arousal system [55]. Thus, the PFC projects to motor, neuromodulatory, and visceral brain systems to generate an appropriate response according to internal and external demand in a goal-directed manner.

It is important to take into consideration that, based on anatomical and physiological criteria, both the primate and rodent PFC may be subdivided into different compartments which could have different behavioral roles (reviewed in [9, 52, 53]). Thus, for example, the rodent PFC is subdivided into the dorsal anterior cingulate cortex (Acc), the prelimbic cortex (PL), and infralimbic cortex (IL). Projection of the PL includes the agranular insular cortex, the claustrum, ACC (and extended ventral striatum), basolateral amygdala, the paraventricular, RE and MD of thalamus, VTA/SNc, and raphe nuclei of the midbrain (SLN, DR, and MR). Thus, the connectivity pattern of the dorsal mPFC is consistent with a role in limbic-cognitive functions homologous to the dorsolateral prefrontal cortex of primates. On the other hand, the IL projects mainly to forebrain and brainstem sites controlling autonomic/visceromotor activity, projections that are consistent with a role for IL in the control of visceral/autonomic activity homologous to the orbitomedial prefrontal cortex of primates [52, 53, 56]. Also PL and IL are

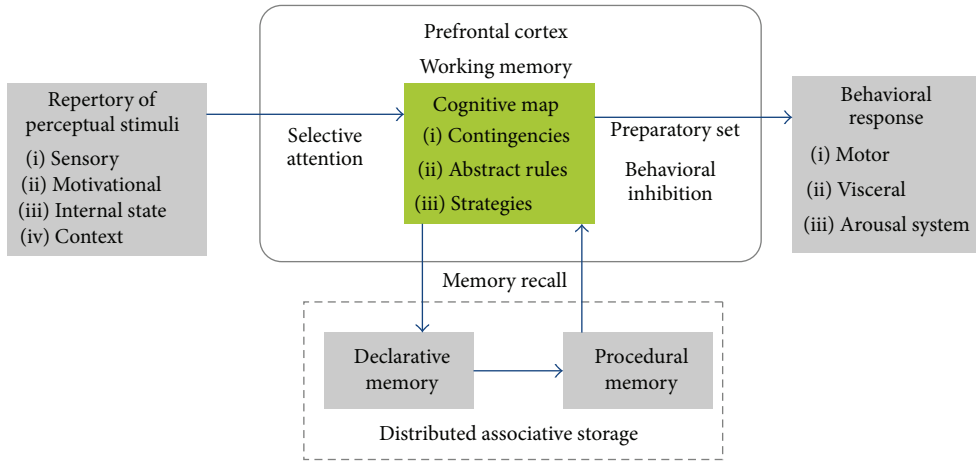


Figure 1: Cognitive map of the perception-action cycle in the PFC. We present a diagram of the execution of cognitive behavioral control by the PFC in the perception-action cycle. Sensory, motivational, contextual, and internal state information is sent to the PFC, where it is integrated through synaptic inputs from other brain systems (OFC, HPC, amygdala, insula, ascending arousal system, etc.). Cognitive function, as selective attention, participates in the integration of perceptual stimuli. Once integrated, a labile cognitive map is formed in the PFC, which represents the coupling of perception and actions as required for adaptive goal-directed behaviors. This link is provided by the projection of the PFC to neural systems involved in executing actions and behavioral responses (premotor cortex, striatum, ascending-arousal system, and hypothalamus). The cognitive map formed in the PFC contains contingencies, abstract rules, and strategies related to the accomplishment of the goal. Simultaneously, through memory recall, this cognitive map retains links to and among distributed associative memories in other brain systems (declarative and procedural memories), allowing for retrieving memories and other representations when needed. Cognitive function as working memory is involved in the "online" attainment of the cognitive map, while preparatory set and inhibitory control participates in the generation of accurate behavioral responses.

strongly interconnected [53], suggesting the integration of both visceral and cognitive elements, which seems to be necessary to the generation of adaptive behavioral responses.

2.2. Formation of the Cognitive Map Related to Control of Behavioral in the PFC. Theoretical work by Fuster [9, 57, 58] proposes that once perceptual information is selected and integrated in the PFC, a cognitive map of the perception-action cycle is formed in this structure that integrates and engages the repertory of perceptions and actions coupled as required in a specific adaptive goal-directed behavioral response [9, 58]. This cognitive map may include simple associations between cues and goals (contingency), and associations between different rules and strategies to accomplish goals (Figure 1).

In a first step, through selective attention, the perceptual information relevant to the goal is focused and selected. During the acquisition of the perception-action cycle, the PFC recalls and engages sparse representations of perception and of actions stored in different brain systems (declarative and procedural memories, Figure 1), allowing for memories and other tasks to be brought "online" when needed, for which executive functions like working memory are implemented. Other executive functions are also required, as a preparatory set for the selection of the action coupled with perceptual information, and inhibitory control to suppress cognitive or emotional contents and operations that may interfere with the goal.

After acquisition, the cognitive map may be labile in time, confirming the short-term nature of the perception-action cycle [56]. If the cognitive map has motivational significance, it is transformed into a static cognitive map in the PFC, corresponding to the remote memory of the perception-action cycle ([59] see below). If current perceptual information is similar to a previously stored cognitive map, it induces reactivation of the map (memory recall), a process that brings "to mind" the previously linked distributed associative representations through working memory as required [56]. Thus, stored cognitive maps are activated whenever the subject faces a similar environmental challenge. However, if perceptual stimuli are novel or ambiguous, the perceptual information is not explicitly linked to a clear action, and the subject must update the response on the basis of constantly changing stimuli [10, 60]. Under these conditions, a stored cognitive map may be updated in agreement with new circumstances, or different internal cognitive maps may compete to generate the correct response [10], a feature that requires behavioral flexibility [8]. Hence, given that "new memories consist invariably of the updating and expansion of old ones which new experience activates by association and recall" [9], the cognitive map is also a dynamic map that is constantly updated in agreement with new perceptual information.

2.3. Chronic Stress Impairs PFC-Dependent Behavioral Tasks. It has been suggested that the maladaptive organization of behavioral responses observed in chronic-stress psychiatric disorders is related to the aberrant cognitive function of the PFC [61]. Chronic stress alters several executive functions of the PFC in humans, such as working memory, selective attention, and behavioral flexibility [62–64]. Similarly,

chronic stress also affects executive functions mediated by the mPFC in rodents, like working memory [65–67], behavioral flexibility [62, 65, 68], recall of the extinction of conditioned fear [69–72], and decision making [73]. Hence, we suggest that these behavioral alterations have repercussions in the formation of cognitive maps for the perception-action cycle in the PFC, resulting in maladaptive behavioral responses, as observed in chronic stress-related psychiatric diseases.

3. Encoding and Representation of Cognitive Maps in the PFC

3.1. Neural Assemblies as Representation in the PFC. How are executive functions encoded in the PFC? A large body of evidence has shown that cognitive events take the form of modulations of the neuronal firing rate in the PFC. An early observation of this phenomenon was the modulation of the firing rate in the primate PFC that correlated to delayed response in working memory tasks [74–76]. Similarly to primates, changes in the firing rate in the rodent mPFC have been associated with different behavioral requirements [77]. Likewise, changes in firing rate in the mPFC have been associated with fear conditioning [78], motivational salience of places [79], control of fear expression [80, 81] strategy switches [82], recognition of safe versus unsafe places [83], object-in-place [84], and appetitive behavior [85].

However, due to variability of firing rates and limited representation capacity [86], the single-neuron spiking model is limited for encoding complex parameters [87]. Hebb [88] hypothesized that a discrete, strongly interconnected group of active neurons, the "neural assembly," codes for distinct cognitive entities. The neural assembly hypothesis is based on the following three principles (reviewed extensively in [89]):

(1) *The neural assembly is made up of a relatively small set of neurons that encode a behavioral parameter.* This principle assumes that a given representation is encoded by the synchronized activation of a population of neurons. The activation of the assembly is manifested by the synchronized firing of neurons, which is supported by synaptic connectivity among neurons that form the assembly. This principle also assumes that any given neuron is a member of different neural assemblies.

(2) *As a behavioral parameter is learned, the neural assembly that represents it is formed.* This principle assumes that as a behavioral parameter is learned, the connectivity of the neurons that represent this behavioral parameter is gradually formed and strengthened through changes in synaptic weight (synaptic plasticity). It also assumes that the neural assembly is formed by the activity-dependent repeated coactivation of a group of neurons during behavior.

(3) *Activation of the neural assembly correlates to behavior.* This assumes that behavioral performance is paralleled with activation of the neural assembly, thus providing a functional meaning to the assembly.

Therefore, a neural assembly that represents a cognitive map in the PFC is composed of functional connectivity among neurons whose coordinated activity reflects learned relationships with cognitive-relevant elements, such as strategies, decisions, and goals [90]. Experimentally, an assembly is a task-related synchronized overlapping firing of multiple neurons, and the task-dependent dynamics of the functional connectivity among multiple neurons [90]. Therefore, in order to detect neural assemblies it is necessary to record a large number of active neurons simultaneously during the execution of behavioral tasks [91]. In recent decades, Hebb's hypothesis of neural assemblies has been empirically supported thanks to advances in multielectrode recording techniques [91, 92] and statistical analysis for signal processing [87]. However, given that multielectrode recording is a highly invasive technique, most recordings of neural assemblies have been performed in freely moving animals, especially rodents. To date, the best-known examples of neural assemblies are "place cells" in the rodent HPC [93, 94] and the "grid cells" in the entorhinal cortex [95], which are considered different levels of internal representations of the location of the animal in space [93, 95].

3.2. Cognitive Functions Are Encoded by Neural Assemblies in the PFC. The emergence of neuronal assemblies related to executive functions like working memory has been well demonstrated in the rodent mPFC [96–98]. The activity of these assemblies related to working memory increases progressively in parallel to behavioral performance [98–100], suggesting that learning is related to the gradual formation of the assembly. In the study of Fujisawa et al. [101] the neural assemblies showed an increased firing specifically at the choice point of the T-maze in a working memory task. Similarly, Benchenane et al. [98] and Fujisawa and Buzsáki [102] showed that the activation of the assembly in the mPFC also occurs at the choice point in the Y-maze after learning, and that activation of assemblies predicts the choice of reward, suggesting that the assembly encodes for strategy representation. Thus, assemblies are well correlated with behavioral outcomes, suggesting that this is a neural representation of cognitive activity implemented by the mPFC. There is also evidence of neural assemblies in the mPFC relative to a set-shifting paradigm, a model of cognitive flexibility [103]. Importantly, these neuronal assemblies switch from encoding a familiar rule to a completely novel rule, and neural assemblies are predictive of behavioral choices even before a trial starts [103]. Altogether, the evidence presented suggests that neural assemblies support cognitive maps of the perception-action cycle, and that these neural assemblies are gradually formed in the mPFC during acquisition of a perception-action cycle.

The rodent mPFC is subdivided into PL and IL, and it has been suggested that both subdivisions have particular behavioral roles [9, 52, 53]. Some studies have not found significant differences in the neural representation of behavioral outcomes between these subdivisions [77–79, 99]. From these studies it could be suggested that there is no important difference between PL and IL in the representation of different aspects of goal-directed behavior. However, some studies

reported important differences in the neural activity in PL and IL referent to behavioral correlates. The most well known differences between these structures are related to the control of the expression of conditioned fear. Milad and Quirk [104] found that neurons in the IL, but not PL, fire to the tone only when rats are recalling extinction of conditioned fear on the following day, which correlated to reduced freezing. On the other hand, Burgos-Robles et al. [80] found that neurons in PL fire correlated with fear expression, and that persistence of PL responses after extinction training was associated with failure to express extinction memory. These results suggest that PL and IL have opposite roles in the control of the expression of fear after extinction (reviewed in [105]). Differences between PL and IL have also been found in appetitive behavior. Burgos-Robles et al. [85] reported that PL neurons exhibited fast and transient responses to reward, whereas IL neurons exhibited delayed and prolonged activity to reward collection. Finally, Rich and Shapiro [82] found that neurons in PL and IL code for strategy switches; however, fire in the PL codes for anticipated adoption of new strategy, whereas IL neurons established new representation only after learning criteria have been established, suggesting that the two regions help to initiate and establish new strategies, respectively. Considering that PL and IL are strongly interconnected [106], altogether these results suggest that both PL and IL could represent different aspects of the same behavioral response.

3.3. Effect of Chronic Stress on Neural Assemblies. It has been suggested that dysfunction in neural assemblies is associated with stress-related disorders like SZ [107]. Indeed, there is evidence of impaired coordinated activation of firing in the mPFC in genetic and developmental models of schizophrenia [108, 109]. However, whether chronic stress impacts on neural assemblies that represent executive functions in the mPFC is unknown to date. Nonetheless, it has been shown that chronic stress disrupts stability of place cells in the HPC [110], impairment that is paralleled with decreased spatial memory. This suggests that chronic stress affects neural assemblies that code for cognitive functions in the PFC, likely altering the neural mechanism for their formation.

4. Mechanism for the Formation of Neural Representation of Cognitive Maps in the PFC

4.1. Role of Synaptic Plasticity in the Formation of Neural Assemblies. Hebb's hypothesis proposes that the formation, storage, and reactivation of neural assemblies depend on the alteration of the efficacy and connectivity of synapses in relevant neural networks [88], a concept known as synaptic plasticity [111]. This hypothesis poses that rapid, long-lasting change in synapse strength between consistently correlated pre- and postsynaptic activity drives strengthening of synaptic transmission, while weakly correlated activity drives weakening of synaptic transmission ("neurons that fire together, wire together"; [88]). Synaptic plasticity induced by neuronal activity, which generates neural network dynamics based on experience and training-induced activity, is especially important for the formation of assemblies with behavioral relevance [89]. Synaptic plasticity is implicated not only in the formation of neural assemblies, but also in their updating and long-term memory [112]. Long-term synaptic plasticity, the effects of which last from hours to days, plays an important role in the long-term storage of memory representations [112]. Experimentally, long-term synaptic plasticity has been assessed through long-term potentiation (LTP) and long-term depression (LTD), a long-lasting increase or decrease, respectively, in synaptic strength induced by a specific neural activity pattern, usually brief and strongly correlated to pre- and postsynaptic activity [113]. Importantly, synaptic plasticity results in structural remodeling of activated synapses, thus modifying structural connectivity among neurons (reviewed extensively by [114]). Thus, through long-term synaptic plasticity, sustained neural activity produces long-lasting synaptic modifications among neurons [115].

4.2. Synaptic Plasticity in the PFC. Short- and long-term synaptic plasticity have been observed in rodent mPFC slices *in vitro* [45, 116–123]. Also synaptic plasticity has been shown *in vivo* between the mPFC and other brain structures, as the HPC and the thalamus [124–128]. However, because of technical considerations, there is no direct evidence of the involvement of synaptic plasticity in the formation of neural assemblies in the mPFC. Some clues support the role of synaptic interactions in the formation of assemblies in the PFC. For example, Fujisawa et al. [101] found that 20% of the neurons of the assembly in the mPFC related to the choice of the animal in the T-maze showed monosynaptic interaction, suggesting that the efficacy of synaptic transmission between neurons varies according to task requirements. Courtin et al. [81] found that 63% of recorded neurons displayed excitatory or inhibitory interaction in the mPFC related to fear expression. These observations suggest that synaptic interaction is a critical process to integrate neurons in neural assemblies in the mPFC in relation to behavioral functions.

4.3. Chronic Stress Affects Synaptic Function in the PFC. It has been proposed that alteration in synaptic plasticity is an important mechanism in the development of stress-related psychiatric disorders [129–131]. Behavioral alterations induced by chronic stress are paralleled with alterations in synaptic transmission in the mPFC [72, 132], particularly in glutamatergic synaptic transmission [132, 133] (reviewed in [134]). The significance of these findings is that synaptic inputs from other brain systems, like the amygdala and HPC, as well as internal network connectivity, are mainly mediated by glutamatergic transmission [45, 46, 119]. It has been shown that glutamatergic synaptic transmission plays a critical role in firing activity in cortical neurons [135]. Indeed, chronic stress, either at the prenatal or at postnatal stage, reduces firing and burst activity of principal neurons in the mPFC *in vivo* [67, 71, 134, 136]. Chronic stress also reduces long-term synaptic plasticity in the mPFC both prefrontal in slices [133, 137] and *in vivo* in the HPC-mPFC axis [68, 138, 139], the mediodorsal thalamus-mPFC pathway [140], and the nucleus accumbens-mPFC neural pathway [141]. These data suggest

that chronic stress impairs activity-dependent changes in synapse strength both in the internal circuitry of the mPFC and with the connectivity with other neural structures. These alterations are accompanied by severe disruption of working memory and cognitive flexibility [68, 140]. Interestingly, in both early life and adulthood, chronic stress blocked LTP of the hippocampal-mPFC pathway, impairment that was associated with an increased fear response after fear extinction [142, 143].

Whether chronic stress has direct repercussions on synaptic plasticity related to the formation of neural assemblies in the mPFC is still unknown. However, as Kim et al. [110] showed, the impaired formation of place cells induced by chronic stress in the HPC is accompanied by a decrease in LTP, suggesting that synaptic plasticity is an important mechanism for the stability of neural assemblies. The same principle can be applied to neural assemblies in the PFC.

4.4. Role of Neural Oscillations in the PFC. Neural oscillations are patterns of rhythmic electrical activity expressed as voltage changes in the extracellular space that arises from complex interactions between neurons and the networks to which they belong [144]. Given that long-term synaptic plasticity requires precise synchronization between pre- and postsynaptic neurons [145], it has been suggested that neural oscillations are responsible for synchronization of synaptic and neural activity involved in the formation and activation of neural assemblies [146–148]. This hypothesis, known as binding-by-synchronization [146], proposes that oscillations provide a "tag" that binds those neurons representing the same perceptual object. This is because oscillatory activity induces changes in the somatic membrane potential that strongly entrain action potentials, thus modulating both spike probability and timing [149, 150]. Thus, oscillatory activity may segregate the activity of neural populations that encode different aspects, or even segregate individual neural assemblies [89].

Oscillatory activity is significant because neuronal events related to cognitive activity are integrated by oscillatory rhythms [151]. For example, theta oscillations (4–10 Hz) arise during "active behaviors" and therefore synchronize specific neuron populations in the mPFC related to the requirements of the task, allowing the activation and emergence of neural assemblies [81, 98, 102, 108, 152–155]. Theta activity synchronizes spikes in the mPFC specifically in moments or places of special significance for the accomplishment of the task [98, 102]. Moreover, theta entrainment of prefrontal spikes, and not the firing rate, predicts successful working memory in rodents [154]. Benchenane et al. [98] showed that the activities of neural assemblies in the mPFC related to correct choice in a working memory task were synchronized to theta rhythm. Similarly, Fujisawa and Buzsáki [102] found that prefrontal goal-predicting neurons were significantly more synchronized by a 4 Hz oscillation than nonpredicting neurons. Moreover, synaptic transmission between pairs of neurons in the mPFC was significantly modulated by a 4 Hz rhythm in the choice point of the maze [102]. On the other hand, gamma oscillations (30–80 Hz), which emerge from local neural interactions, allow precise timing of action potentials by promoting the activation of a population of neurons and facilitating spike-dependent synaptic plasticity [147]. Theta oscillations entrain gamma activity in the mPFC, a modulation that is evident in the choice point in the T-maze and correlates with working memory performance [102, 156]. Therefore, through oscillatory synchrony, theta oscillations collectively coordinate gamma oscillations and neuronal assemblies in a task-relevant manner. Altogether, this evidence suggests that transient and dynamic synchronization of spikes to oscillatory activity in the mPFC is important to coordinate behavioral functions.

4.5. Oscillatory Spectral Coherence Synchronize Active Neurons in the mPFC. As described in Section 2.1, the efficient connectivity between the mPFC and other brain structures has a relevant role in the function of the control of behavior mediated by the mPFC. A special feature of neural oscillations is that they synchronize spikes and synaptic activity between different brain systems. Thus, spectral coherence, a measure of oscillatory synchrony between different brain areas [157], facilitates functional interactions and communication between neuron populations [151, 158], promoting synaptic plasticity for the formation of neural assemblies [159].

Oscillatory synchrony between the mPFC and HPC has been observed in rodents [102, 152, 153, 156, 160–166]. Interestingly, an increase in spectral coherence is observed in the theta range between the mPFC and the HPC during executive functions, such as working memory or inhibitory control [98, 102, 152, 153, 155, 156, 162]. This increase in spectral coherence at the theta frequency emerges in moments and places of special significance for the accomplishment of tasks [98, 102, 108, 153]. The increase in spectral coherence between the mPFC and HPC synchronizes mPFC spikes in a task-dependent manner, promoting the emergence and activation of neural assemblies that predict correct choice in a working memory task [98]. This work has important implications: first, to accomplish a given behavioral task, both the mPFC and HPC synchronize their oscillatory activity in the theta frequency, which is reflected as an increase in spectral coherence; second, an increase in coherence does not occur during the entire behavioral task, but only at relevant points in the maze; and finally, synchronization of oscillatory activity between the mPFC and HPC may provide, through spectral coherence in the theta frequency, the tag for neuronal communication of these structures required for the behavioral task.

4.6. Chronic Stress Affects Oscillatory Activity in the mPFC. Alterations in oscillatory activity have been suggested as a critical component in stress-related mental disorders [167–169]. Genetic and developmental models of SZ show reduced entrainment of prefrontal spikes to spectral coherence, suggesting a critical role of neuronal synchrony in physiopathology of psychiatric disorders [108, 109]. However, few studies have approached the effect of chronic stress on oscillatory activity in the mPFC. It has been shown that chronic stress decreases oscillatory synchrony between the thalamus and mPFC in the delta and theta frequency bands [139, 140]. Chronic stress also reduces spectral coherence between the mPFC and amygdala in the theta frequency band [170].

Oliveira et al. [171] showed that chronic stress-induced variations in HPC-to-mPFC coherence correlated with stress-induced behavioral deficits in a spatial reference memory task. Similarly, chronic stress increases spectral coherence at theta frequencies between the mPFC and HPC [172]. Therefore, we suggest that these chronic stress-induced alterations of oscillations impair synchrony between prefrontal neurons required for the activation of neural assemblies that represent executive functions.

5. Long-Term Storage of Cognitive Maps in the PFC

5.1. Memory Consolidation. Although not considered an executive function, the recall of remote memories has been shown to be linked to the PFC [173, 174]. It has been suggested that remote memory is associated with the long-term storage of cognitive maps of the perception-action cycle in the PFC [9, 56]. This static cognitive map in the PFC may link sparse representations stored in different brain systems, allowing bringing memories and other task-related knowledge "online" when needed and mixing new memories with existing ones [9, 56, 58]. Thus, this static cognitive map could be considered as a "schema" [175]. The transformation of the transient cognitive map of the perception-action cycle into a stable cognitive map occurs by a process known as memory consolidation, in which memories are gradually established from a relatively labile form (short-term or recent memory) to a more permanent state (long-term or remote memory) [176].

Currently, the most accepted model for memory consolidation is the two-stage model [177], which proposes that new information is first acquired and stored transiently during the waking state and then consolidated "off line" during sleep [178–181]. In this model, the interplay between the PFC and the HPC when subject is awake acts as a transient storage structure for recently acquired information [182, 183]. During slow-wave sleep (SWS), the interplay between the PFC and HPC may be required to consolidate the new cognitive maps in the PFC ([178] see below), inducing the increase and strengthening of neural connectivity within prefrontal networks [184]. Thus, the stable cognitive map is formed in the PFC and becomes independent of the HPC [173]. This process also allows the gradual integration of new memories with previously consolidated cognitive maps.

The proposed biological advantage of this two-stage model is that memories are not consolidated until the significance of an experience has been evaluated [185]. This implies that not all labile memories are transformed into remote memories. Indeed, it is well known that emotionally arousing experiences generally create strong, long-lasting memories [185], and that only memories that have motivational (or emotional) significance are consolidated [186, 187]. The amygdala, through the connection to the PFC, provides emotional information (both valence and arousal), whether aversive [188] or appetitive [189]. The activation of the amygdala by emotionally significant events, by tagging such events, facilitates the transmission of information between the HPC and the PFC needed for consolidation of cognitive maps (see below, [187]).

5.2. Oscillatory Interplay between the PFC and HPC Assists in the Long-Term Storage of Neural Assemblies in the mPFC. Neural assemblies that represent rules or strategies in goal-directed tasks are highly stable in the mPFC even for some time after acquisition, suggesting long-term stability of the neural assembly [103, 190]. It has been suggested that neural assemblies that represent cognitive events are consolidated through their reactivation during sleep. The first evidence of this phenomenon was the reactivation of place cells in the HPC during sleep [191]. In the mPFC, neuronal assemblies that are active and have behavioral relevance during wakefulness are replayed during SWS [96–98]. Interestingly, the replay of assemblies in the mPFC during SWS correlates with behavioral performance during wakefulness [96, 97, 103], suggesting that neural processes involved in the stabilization and consolidation of neural representation of cognitive activity carried out by neural assemblies take place during SWS.

It has been suggested that replay is accomplished by oscillatory synchrony between the neocortex and HPC [177]. During SWS, when delta activity is prominent [161, 163], particular patterns of spindle-shaped neural oscillations (periods of 1-2 s of waxing-and-waning patterns at 7–12 Hz, [192]) emerge in the neocortex, including the PFC, while sharp-wave ripples (SWRs, events of high-frequency 100–300 Hz oscillations, [193]) emerge exclusively in the HPC. Both SWR and spindles have been implicated in memory consolidation [192, 193]. During SWS, delta activity synchronizes prefrontal spindles and spikes to hippocampal SWR [160, 161, 163, 164]. Furthermore, putative pyramidal neurons and interneurons are modulated by spindles in the mPFC, showing enhanced responsiveness to hippocampal SPWRs during sleep [194]. This study also showed that gamma activity in the mPFC is modulated by spindles and SWRs, indicating that activity patterns in the mPFC and HPC are strongly synchronized by network oscillatory activity during SWS.

Synchrony between the mPFC and HPC also allows the replay of prefrontal neural assemblies during sleep [96–98]. Neural assemblies formed in the mPFC related to working memory are reactivated during sleep, especially in synchrony with hippocampal SWRs [97, 98]. This suggests that hippocampal SWRs serve to select neocortical neurons to be preferentially activated based on the information placed in hippocampal networks by past experience [160]. Together, these studies suggest that synchrony of discrete oscillatory patterns between the mPFC and HPC during SWS represents the offline transference of information from the HPC to cortical networks, a process that appears critical for the consolidation of memories. Thus, assembly recruitment by oscillatory activity during SWS seems to be critical for long-term memory consolidation of representations supported by these assemblies.

5.3. Possible Role of the Amygdala in the Consolidation of Cognitive Maps in the PFC. The amygdala promotes the transmission of information between the HPC and the neocortex needed for consolidation of memories by tagging events that

are to be consolidated [187]. Interestingly, spectral coherence between the mPFC and the amygdala has been observed at the theta frequency [195, 196]. It has been proposed that oscillations in the amygdala, especially theta activity, are involved in the consolidation of emotionally significant memories [197, 198]. Theta activity is present in the amygdala only during emotional arousal [199], suggesting that this activity could inform the emotional value of current conditions. The amygdala has strong reciprocal connections with the mPFC and the HPC [46, 200] and theta activity between these structures is highly synchronous during emotional arousal [201–203]. Lesting et al. [195] showed that theta synchrony between the mPFC, HPC, and amygdala, as well as synchrony of neural firing, increased during retrieval of conditioned fear and decreased during extinction learning [195, 204]. Hence, it has been proposed that amygdalar theta activity during emotional arousal promotes memory consolidation by facilitating interactions between the mPFC and HPC [197]. Amygdalar theta oscillations could magnify the periods of effective synaptic interactions promoting synaptic plasticity in coactive structures like the mPFC and HPC during memory storage [197]. In support of this idea, an increase in spectral coherence at the theta frequency band during paradoxical sleep after fear learning between the mPFC, HPC, and amygdala has been reported, which correlated with behavioral performance [205]. Interestingly, about 10–20% of mPFC and amygdala neurons were activated and synchronized to theta activity during paradoxical sleep, suggesting the replay of neurons that represent fear learning [205]. Altogether, these results support the hypothesis that the amygdala, through oscillatory synchrony with the mPFC and HPC, provides a mechanism for interareal coordination related to aversive stimuli, providing contextual emotional information for subsequent behavior. Thus, communication through oscillatory synchrony may promote consolidation of emotionally significant memories.

5.4. Effect of Chronic Stress on Memory Consolidation. Some stress-related psychiatric disorders, like MDD and PTSD, are characterized by an aberrant persistence of aversive memories [3, 6]. Recent data indicate that chronic stress enhances retention of aversive long-term memories in rodents [206]. Also, chronic stress during gestation induces persistence of aversive long-term memory in the offspring at adulthood [136, 207]. This persistence of aversive long-term memory is paralleled with a decrease in the firing rate in the mPFC, and a reduced cross-correlation between hippocampal SWR and prefrontal spikes during delta activity [136], suggesting an association of synchrony of activity patterns between the mPFC and the HPC, and the persistence of aversive memories.

It is important to take into consideration that the aberrant persistence of aversive memories observed in stress related disorders is related to aversive memories [3, 6] but not to other types of memories. This suggests that chronic stress induces the selection of the memories to be consolidated, a function that may depend on the amygdala [187]. Other studies have reported that chronic stress generates hyperactivation of the amygdala [208]. For example, chronic stress increases firing in the amygdala [209] and also increases spectral coherence between the HPC and amygdala [172, 210]. Thus, the hyperactivation of the amygdala may strengthen memory consolidation for aversive experiences by increasing the number of neurons that best represent that event.

6. Summary: Effect of Chronic Stress

In the previous sections we integrate evidence from basic and cognitive neuroscience that approach how the mPFC participates in the implementation of the adaptive organization of behavioral responses. We also put evidence with focus on how chronic stress could affect the organization of behavioral responses mediated by the mPFC. This evidence, systematized in Figure 2, can be summarized as follows:

(1) Chronic stress impairs glutamatergic synaptic transmission (Section 4.3), reducing the probability to firing of prefrontal neurons. Chronic stress also impairs long-term synaptic plasticity (Section 4.3), resulting in the impaired ability to modify synaptic connectivity in an activity-dependent manner between prefrontal neurons and between prefrontal neurons with neurons of other brain systems.

(2) Chronic stress impairs also oscillatory coherence between the mPFC and other brain systems (as HPC and thalamus) in behaviorally relevant frequency bands (theta, gamma, and SWR) (Section 4.6). This impairs neural synchrony necessary for synaptic plasticity, impacting the formation of neural assemblies. On the other side, reduced neural synchrony affects the coordinated firing of prefrontal neurons, resulting in a reduced activation of previously formed neural assemblies.

(3) Altogether, these alteration induced by chronic stress may result in the impairment of the formation and consolidation of neural assemblies that represent adaptive cognitive maps in the mPFC, in a similar manner as what occurs with hippocampal place cells ([110]; Section 3.3).

(4) In both humans and rodent models, these neurophysiological alterations induced by chronic stress result in the impairment of the generation of behavioral responses dependent on the prefrontal network, such as working memory, selective attention, behavioral flexibility, recall of the extinction of conditioned fear, and decision making (Section 2.3). These behavioral alterations can be attributed to an inaccurate formation, consolidation, and activation of neural assemblies that represent cognitive maps in the PFC.

7. Hypothesis

The goal of the present review is to propose a framework that unifies cognitive neuroscience and neurophysiological evidence that attempts to explain the impairment of behavioral responses observed in chronic stress-related mental disorders. As a first proposal, our hypothesis is that the

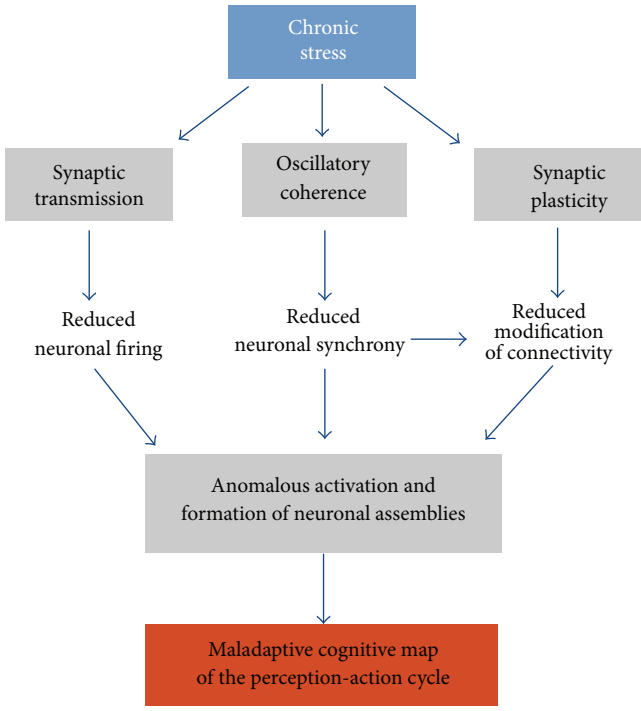


Figure 2: Proposed mechanism by which chronic stress affects the formation of neural assemblies related to cognitive maps in the PFC. In a first step, chronic stress reduces excitatory synaptic transmission in the PFC. This induces a reduction of firing in principal neurons. Chronic stress also reduces activity-dependent synaptic plasticity in the internal circuitry, and between the PFC and other brain systems. Also chronic stress reduces oscillatory coherence in cognitive relevant frequency bands between the PFC and other brain structures, resulting in a decreased synchrony between these and the PFC. The decreased oscillatory coherence together with the decreased synaptic plasticity results in an important reduction of the ability to modify functional connectivity dependent on neural activity in the PFC. Collectively, these impairments induce an aberrant formation and activation of neural assemblies in the PFC, which result in the development of maladaptive cognitive maps that link perceptual information to a repertory of erroneous actions, resulting in the maladaptive behavioral response directed to goals.

behavioral alterations observed in chronic stress-related psychiatric disorders can be categorized into three classes [1]: (i) exacerbated formation and implementation of maladaptive behavioral responses, a feature known as negative-cognitive bias, which manifests itself as the tendency to misinterpret perceptual stimuli as aversive or threatening [61, 211], (ii) exacerbated long-term recall of maladaptive memories, as increased retention and recall of aversive memories [3, 6, 15], and (iii) behavioral perseverance, manifested as the inability to adaptively update behavioral responses to novel environmental conditions [8].

The second proposal states that the above-mentioned cognitive defects observed in psychiatric diseases are the result of the anomalous and maladaptive formation of cognitive maps that represent perception-action cycles in the PFC. And the third proposal is that these anomalous representations are the consequence of the chronic stress-induced impairment of the neural mechanisms involved in the formation and/or consolidation of neural assemblies that represent these cognitive maps in the PFC.

7.1. Exacerbated Formation and Implementation of Maladaptive Behavioral Responses. These impairments result in the development of aberrant cognitive maps that link perceptual information to a repertory of erroneous actions, resulting in the maladaptive behavioral response directed to goals. How are these aberrant cognitive maps formed? In a first step, during the integration of perceptual information by the mPFC, the hyperactivation of the amygdala induced by chronic stress [209] can excessively transmit aversive-related information to the PFC, through a chronic stress-induced enhancement of spectral coherence at the theta band, between the amygdala and the mPFC [172, 210]. This could form an association between "neutral" perceptual environmental information and "aversive" information transmitted by the amygdala, resulting in a labile cognitive map and an incorrect perception-action cycle. This association is encoded by neural assemblies in the PFC that engage sparse representations stored in other neural systems with the aversive information [56]. Simultaneously, chronic stress decreases synaptic transmission and neural firing in the PFC [72, 132, 133, 136, 137], which results in an impairment for the implementation of executive functions, like working memory, selective attention, and inhibitory control [67–69]. The latter is required to inhibit amygdalar function [212], which may favor the acquisition of maladaptive cognitive maps, resulting in the implementation of maladaptive associations between different rules and strategies to accomplish different goals.

7.2. Exacerbated Long-Term Recall of Maladaptive Memories. In a second step, the long-term storage of these maladaptive cognitive maps may also be favored by chronic stress. Again, hyperactivity of the amygdala may favor synchrony at the theta oscillatory band between the PFC and the HPC during sleep, required to consolidate already formed cognitive maps in the PFC by favoring the replay of existing neural assemblies [205]. The chronic stress-induced decrease in PFC firing favors consolidation through an increased correlation between spikes and hippocampal SWR [136], resulting in the replay and stabilization of maladaptive neural assemblies in the PFC [96, 97]. Thus, labile acquired cognitive maps become stable (and maladaptive). This process can also be applied to the consolidation of aversive memories, as observed in PTSD.

In a third step, chronic stress may enhance activation (i.e., recall) of these maladaptive cognitive maps. Upon the presentation of ambiguous perceptual information, chronically stressed subjects activate more consolidated cognitive maps that correspond to previously acquired and consolidated maladaptive maps. This may be the consequence of the activation of neural assemblies that represent previously linked distributed associative events related to the negative-biasing

interpretation of environmental information observed in chronic stress-induced disorders.

7.3. Impairment in Cognitive Flexibility. Chronic stress impairs cognitive flexibility as observed in reversal learning and set-shifting tasks [62, 68], suggesting that chronic stress impairs the ability to update previously formed cognitive maps or the perception-action cycle in the mPFC. Thus, even when environmental conditions change, stereotyped and maladaptive behavioral responses are maintained, as is observed in the impairment of extinction of conditioned fear induced by chronic stress [69, 72]. Importantly, neural assemblies that represent rules in the mPFC are updated with the change of contingency [103], suggesting that the impairment of behavioral flexibility prevents updating neural assemblies. For example, Wilber et al. [71] showed that chronic stress blocks the decrease of the firing rate in the mPFC required for successful recall of extinction of conditioned fear memory [80]. The impairment of synaptic transmission and plasticity in the mPFC induced by chronic stress [68, 132, 133, 137] may in turn impair the accommodation of previously formed neural assemblies, resulting in persistence of maladaptive cognitive maps for the perception-action cycle.

8. Concluding Remarks

In this review, we integrate evidence from cognitive neuroscience and behavioral neurophysiology with current knowledge of chronic stress-induced impairment in behavioral and neuronal function in the PFC to formulate a hypothesis on cognitive dysfunctions observed in stress-related neuropsychiatric disorders. We hypothesize that chronic stress-induced impairment of neural processes required for structuring neuronal assemblies in the PFC plays an important role in the abnormal organization of behavior observed in chronic stress-induced neuropsychiatric disorders. However, our hypothesis, to date, is mainly sustained by indirect evidence like the effect of chronic stress on the mechanisms involved in configuring neuronal assemblies, rather than observation of the effect of chronic stress on neural assemblies *per se*. Further research will complement and support the current hypothesis. It is necessary to record neuronal assemblies in the mPFC while executing cognitive functions in animals subjected to chronic stress. Large-scale neuronal recording methods [213] and high-resolution optical imaging techniques to assess the precise spatiotemporal properties of neuronal assemblies [214], together with manipulation of neuronal assemblies [215, 216], will offer a comprehensive picture leading us to an understanding of the physiopathology of chronic stress-related psychiatric disorders. These experiments offer the opportunity not only to clarify the relationship between chronic stress and neuropsychiatric disorders, but also to associate the modification of neural representations with behavioral performance.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors were supported by Millennium Center for the Neuroscience of Memory, NC10-001-F, from the Ministry of Economy, Development and Tourism, Chile (Pablo Fuentealba and Francisco Aboitiz); FONDECYT Grant no. 1141089 to Pablo Fuentealba; and FONDECYT for the postdoctoral Grant no. 3140370 to Ignacio Negrón-Oyarzo.

References

[1] M. T. Banich, K. L. Mackiewicz, B. E. Depue, A. J. Whitmer, G. A. Miller, and W. Heller, "Cognitive control mechanisms, emotion and memory: a neural perspective with implications for psychopathology," *Neuroscience and Biobehavioral Reviews*, vol. 33, no. 5, pp. 613–630, 2009.

[2] M. L. Phillips, W. C. Drevets, S. L. Rauch, and R. Lane, "Neurobiology of emotion perception I: the neural basis of normal emotion perception," *Biological Psychiatry*, vol. 54, no. 5, pp. 504–514, 2003.

[3] A. Mathews and C. MacLeod, "Cognitive vulnerability to emotional disorders," *Annual Review of Clinical Psychology*, vol. 1, pp. 167–195, 2005.

[4] T. Johnstone, C. M. van Reekum, H. L. Urry, N. H. Kalin, and R. J. Davidson, "Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression," *The Journal of Neuroscience*, vol. 27, no. 33, pp. 8877–8884, 2007.

[5] S. J. Bishop, "Neurocognitive mechanisms of anxiety: an integrative account," *Trends in Cognitive Sciences*, vol. 11, no. 7, pp. 307–316, 2007.

[6] R. G. Parsons and K. J. Ressler, "Implications of memory modulation for post-traumatic stress and fear disorders," *Nature Neuroscience*, vol. 16, no. 2, pp. 146–153, 2013.

[7] D. M. Barch and A. Ceaser, "Cognition in schizophrenia: core psychological and neural mechanisms," *Trends in Cognitive Sciences*, vol. 16, no. 1, pp. 27–34, 2012.

[8] S. B. Floresco, Y. Zhang, and T. Enomoto, "Neural circuits subserving behavioral flexibility and their relevance to schizophrenia," *Behavioural Brain Research*, vol. 204, no. 2, pp. 396–409, 2009.

[9] J. M. Fuster, "The prefrontal cortex—an update: time is of the essence," *Neuron*, vol. 30, no. 2, pp. 319–333, 2001.

[10] E. K. Miller and J. D. Cohen, "An integrative theory of prefrontal cortex function," *Annual Review of Neuroscience*, vol. 24, pp. 167–202, 2001.

[11] S. Laroche, S. Davis, and T. M. Jay, "Plasticity at hippocampal to prefrontal cortex synapses: dual roles in working memory and consolidation," *Hippocampus*, vol. 10, no. 4, pp. 438–446, 2000.

[12] A. R. Preston and H. Eichenbaum, "Interplay of hippocampus and prefrontal cortex in memory," *Current Biology*, vol. 23, no. 17, pp. R764–R773, 2013.

[13] J. Y. Yu and L. M. Frank, "Hippocampal-cortical interaction in decision making," *Neurobiology of Learning and Memory*, vol. 117, pp. 34–41, 2015.

[14] R. Marek, C. Strobel, T. W. Bredy, and P. Sah, "The amygdala and medial prefrontal cortex: partners in the fear circuit," *The Journal of Physiology*, vol. 591, part 10, pp. 2381–2391, 2013.

[15] M. W. Cole, G. Repovš, and A. Anticevic, "The frontoparietal control system: a central role in mental health," *Neuroscientist*, vol. 20, no. 6, pp. 652–664, 2014.

[16] B. P. Godsil, J. P. Kiss, M. Spedding, and T. M. Jay, "The hippocampal-prefrontal pathway: the weak link in psychiatric disorders?" *European Neuropsychopharmacology*, vol. 23, no. 10, pp. 1165–1181, 2013.

[17] M.-P. Austin, P. Mitchell, and G. M. Goodwin, "Cognitive deficits in depression: possible implications for functional neuropathology," *British Journal of Psychiatry*, vol. 178, pp. 200–206, 2001.

[18] S. Park and P. S. Holzman, "Schizophrenics show spatial working memory deficits," *Archives of General Psychiatry*, vol. 49, no. 12, pp. 975–982, 1992.

[19] J. Lee and S. Park, "Working memory impairments in schizophrenia: a meta-analysis," *Journal of Abnormal Psychology*, vol. 114, no. 4, pp. 599–611, 2005.

[20] J. Blechert, T. Michael, N. Vriends, J. Margraf, and F. H. Wilhelm, "Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses," *Behaviour Research and Therapy*, vol. 45, no. 9, pp. 2019–2033, 2007.

[21] W. C. Drevets, J. L. Price, J. R. Simpson Jr. et al., "Subgenual prefrontal cortex abnormalities in mood disorders," *Nature*, vol. 386, no. 6627, pp. 824–827, 1997.

[22] I. Liberzon and B. Martis, "Neuroimaging studies of emotional responses in PTSD," *Annals of the New York Academy of Sciences*, vol. 1071, pp. 87–109, 2006.

[23] I. Liberzon and K. L. Phan, "Brain-imaging studies of posttraumatic stress disorder," *CNS Spectrums*, vol. 8, no. 9, pp. 641–650, 2003.

[24] F. Ferrarelli, M. Massimini, M. J. Peterson et al., "Reduced evoked gamma oscillations in the frontal cortex in schizophrenia patients: a TMS/EEG study," *The American Journal of Psychiatry*, vol. 165, no. 8, pp. 996–1005, 2008.

[25] Y. Yu, H. Shen, L.-L. Zeng, Q. Ma, and D. Hu, "Convergent and divergent functional connectivity patterns in schizophrenia and depression," *PLoS ONE*, vol. 8, no. 7, Article ID e68250, 2013.

[26] L. Genzel, M. Dresler, M. Cornu et al., "Medial prefrontal-hippocampal connectivity and motor memory consolidation in depression and schizophrenia," *Biological Psychiatry*, vol. 77, no. 2, pp. 177–186, 2015.

[27] B. Vai, G. Sferrazza Papa, S. Poletti et al., "Abnormal cortico-limbic connectivity during emotional processing correlates with symptom severity in schizophrenia," *European Psychiatry*, vol. 30, no. 5, pp. 590–597, 2015.

[28] B. S. McEwen, "Physiology and neurobiology of stress and adaptation: central role of the brain," *Physiological Reviews*, vol. 87, no. 3, pp. 873–904, 2007.

[29] S. M. Rodrigues, J. E. LeDoux, and R. M. Sapolsky, "The influence of stress hormones on fear circuitry," *Annual Review of Neuroscience*, vol. 32, pp. 289–313, 2009.

[30] S. N. Brixey, B. J. Gallagher III, J. A. McFalls Jr., and L. F. Parmelee, "Gestational and neonatal factors in the etiology of schizophrenia," *Journal of Clinical Psychology*, vol. 49, no. 3, pp. 447–456, 1993.

[31] C. Hammen, "Stress and depression," *Annual Review of Clinical Psychology*, vol. 1, no. 1, pp. 293–319, 2005.

[32] R. K. Pitman, A. M. Rasmusson, K. C. Koenen et al., "Biological studies of post-traumatic stress disorder," *Nature Reviews Neuroscience*, vol. 13, no. 11, pp. 769–787, 2012.

[33] A. F. T. Arnsten, "Stress signalling pathways that impair prefrontal cortex structure and function," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 410–422, 2009.

[34] R. M. Shansky and J. Lipps, "Stress-induced cognitive dysfunction: hormone-neurotransmitter interactions in the prefrontal cortex," *Frontiers in Human Neuroscience*, vol. 7, article 123, 2013.

[35] J. M. Fuster, *The Prefrontal Cortex*, vol. 1, 2008.

[36] H. B. M. Uylings, H. J. Groenewegen, and B. Kolb, "Do rats have a prefrontal cortex?" *Behavioural Brain Research*, vol. 146, no. 1-2, pp. 3–17, 2003.

[37] Y. Chudasama, "Animal models of prefrontal-executive function," *Behavioral Neuroscience*, vol. 125, no. 3, pp. 327–343, 2011.

[38] K. Schwabe, T. Enkel, S. Klein, M. Schütte, and M. Koch, "Effects of neonatal lesions of the medial prefrontal cortex on adult rat behaviour," *Behavioural Brain Research*, vol. 153, no. 1, pp. 21–34, 2004.

[39] B. Delatour and P. Gisquet-Verrier, "Prelimbic cortex specific lesions disrupt delayed-variable response tasks in the rat," *Behavioral Neuroscience*, vol. 110, no. 6, pp. 1282–1298, 1996.

[40] G. J. Quirk, G. K. Russo, J. L. Barron, and K. Lebron, "The role of ventromedial prefrontal cortex in the recovery of extinguished fear," *Journal of Neuroscience*, vol. 20, no. 16, pp. 6225–6231, 2000.

[41] S. Funahashi, "Neuronal mechanisms of executive control by the prefrontal cortex," *Neuroscience Research*, vol. 39, no. 2, pp. 147–165, 2001.

[42] B. Zingg, H. Hintiryan, L. Gou et al., "Neural networks of the mouse neocortex," *Cell*, vol. 156, no. 5, pp. 1096–1111, 2014.

[43] A. S. Mitchell and S. Chakraborty, "What does the mediodorsal thalamus do?" *Frontiers in Systems Neuroscience*, vol. 7, article 37, 2013.

[44] T. M. Jay and M. P. Witter, "Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of *Phaseolus vulgaris*-leucoagglutinin," *Journal of Comparative Neurology*, vol. 313, no. 4, pp. 574–586, 1991.

[45] M. A. Parent, L. Wang, J. Su, T. Netoff, and L.-L. Yuan, "Identification of the hippocampal input to medial prefrontal cortex in vitro," *Cerebral Cortex*, vol. 20, no. 2, pp. 393–403, 2010.

[46] L. Orozco-Cabal, S. Pollandt, J. Liu, L. Vergara, P. Shinnick-Gallagher, and J. P. Gallagher, "A novel rat medial prefrontal cortical slice preparation to investigate synaptic transmission from amygdala to layer V prelimbic pyramidal neurons," *Journal of Neuroscience Methods*, vol. 151, no. 2, pp. 148–158, 2006.

[47] S. J. Bacon, A. J. N. Headlam, P. L. A. Gabbott, and A. D. Smith, "Amygdala input to medial prefrontal cortex (mPFC) in the rat: a light and electron microscope study," *Brain Research*, vol. 720, no. 1-2, pp. 211–219, 1996.

[48] Z.-W. Zhang, M. W. Burke, N. Calakos, J.-M. Beaulieu, and E. Vaucher, "Confocal analysis of cholinergic and dopaminergic inputs onto pyramidal cells in the prefrontal cortex of rodents," *Frontiers in Neuroanatomy*, vol. 4, article 21, 2010.

[49] E. Sobel and D. Corbett, "Axonal branching of ventral tegmental and raphe projections to the frontal cortex in the rat," *Neuroscience Letters*, vol. 48, no. 2, pp. 121–125, 1984.

[50] E. R. Samuels and E. Szabadi, "Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation," *Current Neuropharmacology*, vol. 6, no. 3, pp. 235–253, 2008.

[51] C. A. Köhler, W. C. da Silva, F. Benetti, and J. S. Bonini, "Histaminergic mechanisms for modulation of memory systems," *Neural Plasticity*, vol. 2011, Article ID 328602, 16 pages, 2011.

[52] P. L. A. Gabbott, T. A. Warner, P. R. L. Jays, P. Salway, and S. J. Busby, "Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers," *Journal of Comparative Neurology*, vol. 492, no. 2, pp. 145–177, 2005.

[53] R. P. Vertes, "Differential projections of the infralimbic and prelimbic cortex in the rat," *Synapse*, vol. 51, no. 1, pp. 32–58, 2004.

[54] H. W. Berendse, Y. Galis-de Graaf, and H. J. Groenewegen, "Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat," *Journal of Comparative Neurology*, vol. 316, no. 3, pp. 314–347, 1992.

[55] L. Gonçalves, M. I. Nogueira, S. J. Shammah-Lagnado, and M. Metzger, "Prefrontal afferents to the dorsal raphe nucleus in the rat," *Brain Research Bulletin*, vol. 78, no. 4-5, pp. 240–247, 2009.

[56] D. R. Euston, A. J. Gruber, and B. L. McNaughton, "The role of medial prefrontal cortex in memory and decision making," *Neuron*, vol. 76, no. 6, pp. 1057–1070, 2012.

[57] J. M. Fuster, "Prefrontal cortex and the bridging of temporal gaps in the perception-action cycle," *Annals of the New York Academy of Sciences*, vol. 608, pp. 318–336, 1990.

[58] J. M. Fuster, "The cognit: a network model of cortical representation," *International Journal of Psychophysiology*, vol. 60, no. 2, pp. 125–132, 2006.

[59] N. Axmacher, C. E. Elger, and J. Fell, "Memory formation by refinement of neural representations: the inhibition hypothesis," *Behavioural Brain Research*, vol. 189, no. 1, pp. 1–8, 2008.

[60] S. Otani, "Memory trace in prefrontal cortex: theory for the cognitive switch," *Biological Reviews of the Cambridge Philosophical Society*, vol. 77, no. 4, pp. 563–577, 2002.

[61] M. L. Phillips, W. C. Drevets, S. L. Rauch, and R. Lane, "Neurobiology of emotion perception II: implications for major psychiatric disorders," *Biological Psychiatry*, vol. 54, no. 5, pp. 515–528, 2003.

[62] C. Liston, M. M. Miller, D. S. Goldwater et al., "Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting," *The Journal of Neuroscience*, vol. 26, no. 30, pp. 7870–7874, 2006.

[63] L. Öhman, S. Nordin, J. Bergdahl, L. S. Birgander, and A. S. Neely, "Cognitive function in outpatients with perceived chronic stress," *Scandinavian Journal of Work, Environment and Health*, vol. 33, no. 3, pp. 223–232, 2007.

[64] G. LaGarde, J. Doyon, and A. Brunet, "Memory and executive dysfunctions associated with acute posttraumatic stress disorder," *Psychiatry Research*, vol. 177, no. 1-2, pp. 144–149, 2010.

[65] A. Del Arco, G. Segovia, P. Garrido, M. de Blas, and F. Mora, "Stress, prefrontal cortex and environmental enrichment: studies on dopamine and acetylcholine release and working memory performance in rats," *Behavioural Brain Research*, vol. 176, no. 2, pp. 267–273, 2007.

[66] B. L. Murphy, A. F. T. Arnsten, J. D. Jentsch, and R. H. Roth, "Dopamine and spatial working memory in rats and monkeys: pharmacological reversal of stress-induced impairment," *Journal of Neuroscience*, vol. 16, no. 23, pp. 7768–7775, 1996.

[67] K. Mizoguchi, M. Yuzurihara, A. Ishige, H. Sasaki, D.-H. Chui, and T. Tabira, "Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction," *Journal of Neuroscience*, vol. 20, no. 4, pp. 1568–1574, 2000.

[68] J. J. Cerqueira, F. Mailliet, O. F. X. Almeida, T. M. Jay, and N. Sousa, "The prefrontal cortex as a key target of the maladaptive response to stress," *The Journal of Neuroscience*, vol. 27, no. 11, pp. 2781–2787, 2007.

[69] A. D. Miracle, M. F. Brace, K. D. Huyck, S. A. Singler, and C. L. Wellman, "Chronic stress impairs recall of extinction of conditioned fear," *Neurobiology of Learning and Memory*, vol. 85, no. 3, pp. 213–218, 2006.

[70] A. Izquierdo, C. L. Wellman, and A. Holmes, "Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice," *The Journal of Neuroscience*, vol. 26, no. 21, pp. 5733–5738, 2006.

[71] A. A. Wilber, A. G. Walker, C. J. Southwood et al., "Chronic stress alters neural activity in medial prefrontal cortex during retrieval of extinction," *Neuroscience*, vol. 174, pp. 115–131, 2011.

[72] I. Negrón-Oyarzo, M. Á. Pérez, G. Terreros, P. Muñoz, and A. Dagnino-Subiabre, "Effects of chronic stress in adolescence on learned fear, anxiety, and synaptic transmission in the rat prelimbic cortex," *Behavioural Brain Research*, vol. 259, pp. 342–353, 2014.

[73] E. Dias-Ferreira, J. C. Sousa, I. Melo et al., "Chronic stress causes frontostriatal reorganization and affects decision-making," *Science*, vol. 325, no. 5940, pp. 621–625, 2009.

[74] J. Fuster and G. Alexander, "Neuron activity related to short-term memory," *Science*, vol. 173, no. 3997, pp. 652–654, 1971.

[75] K. Kubota and H. Niki, "Prefrontal cortical unit activity and delayed alternation performance in monkeys," *Journal of Neurophysiology*, vol. 34, no. 3, pp. 337–347, 1971.

[76] S. Funahashi, C. J. Bruce, and P. S. Goldman-Rakic, "Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex," *Journal of Neurophysiology*, vol. 61, no. 2, pp. 331–349, 1989.

[77] M. W. Jung, Y. Qin, B. L. McNaughton, and C. A. Barnes, "Firing characteristics of deep layer neurons in prefrontal cortex in rats performing spatial working memory tasks," *Cerebral Cortex*, vol. 8, no. 5, pp. 437–450, 1998.

[78] E. H. Baeg, Y. B. Kim, J. Jang, H. T. Kim, I. Mook-Jung, and M. W. Jung, "Fast spiking and regular spiking neural correlates of fear conditioning in the medial prefrontal cortex of the rat," *Cerebral Cortex*, vol. 11, no. 5, pp. 441–451, 2001.

[79] V. Hok, E. Save, P. P. Lenck-Santini, and B. Poucet, "Coding for spatial goals in the prelimbic/infralimbic area of the rat frontal cortex," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 12, pp. 4602–4607, 2005.

[80] A. Burgos-Robles, I. Vidal-Gonzalez, and G. J. Quirk, "Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure," *Journal of Neuroscience*, vol. 29, no. 26, pp. 8474–8482, 2009.

[81] J. Courtin, F. Chaudun, R. R. Rozeske et al., "Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression," *Nature*, vol. 505, no. 7481, pp. 92–96, 2014.

[82] E. L. Rich and M. Shapiro, "Rat prefrontal cortical neurons selectively code strategy switches," *Journal of Neuroscience*, vol. 29, no. 22, pp. 7208–7219, 2009.

[83] A. Adhikari, M. A. Topiwala, and J. A. Gordon, "Single units in the medial prefrontal cortex with anxiety-related firing patterns are preferentially influenced by ventral hippocampal activity," *Neuron*, vol. 71, no. 5, pp. 898–910, 2011.

[84] J. Kim, S. Delcasso, and I. Lee, "Neural correlates of object-in-place learning in hippocampus and prefrontal cortex," *Journal of Neuroscience*, vol. 31, no. 47, pp. 16991–17006, 2011.

[85] A. Burgos-Robles, H. Bravo-Rivera, and G. J. Quirk, "Prelimbic and infralimbic neurons signal distinct aspects of appetitive

instrumental behavior," *PLoS ONE*, vol. 8, no. 2, Article ID e57575, 2013.

[86] C. Von Der Malsburg, "The what and why of binding: the modeler's perspective," *Neuron*, vol. 24, no. 1, pp. 95–104, 1999.

[87] S. A. Deadwyler and R. E. Hampson, "The significance of neural ensemble codes during behavior and cognition," *Annual Review of Neuroscience*, vol. 20, pp. 217–244, 1997.

[88] D. O. Hebb, "The organization of behavior: a neuropsychological theory," *Science Education*, vol. 44, no. 1, p. 335, 1949.

[89] G. Buzsáki, "Neural syntax: cell assemblies, synapsembles, and readers," *Neuron*, vol. 68, no. 3, pp. 362–385, 2010.

[90] Y. Sakurai, "How do cell assemblies encode information in the brain?" *Neuroscience and Biobehavioral Reviews*, vol. 23, no. 6, pp. 785–796, 1999.

[91] G. Buzsáki, "Large-scale recording of neuronal ensembles," *Nature Neuroscience*, vol. 7, no. 5, pp. 446–451, 2004.

[92] M. A. L. Nicolelis, A. A. Ghazanfar, B. M. Faggin, S. Votaw, and L. M. O. Oliveira, "Reconstructing the engram: simultaneous, multisite, many single neuron recordings," *Neuron*, vol. 18, no. 4, pp. 529–537, 1997.

[93] K. D. Harris, J. Csicsvari, H. Hirase, G. Dragoi, and G. Buzsáki, "Organization of cell assemblies in the hippocampus," *Nature*, vol. 424, no. 6948, pp. 552–556, 2003.

[94] K. D. Harris, "Neural signatures of cell assembly organization," *Nature Reviews Neuroscience*, vol. 6, no. 5, pp. 399–407, 2005.

[95] E. I. Moser, Y. Roudi, M. P. Witter, C. Kentros, T. Bonhoeffer, and M.-B. Moser, "Grid cells and cortical representation," *Nature Reviews Neuroscience*, vol. 15, no. 7, pp. 466–481, 2014.

[96] D. R. Euston, M. Tatsuno, and B. L. McNaughton, "Fast-forward playback of recent memory sequences in prefrontal cortex during sleep," *Science*, vol. 318, no. 5853, pp. 1147–1150, 2007.

[97] A. Peyrache, M. Khamassi, K. Benchenane, S. I. Wiener, and F. P. Battaglia, "Replay of rule-learning related neural patterns in the prefrontal cortex during sleep," *Nature Neuroscience*, vol. 12, no. 7, pp. 919–926, 2009.

[98] K. Benchenane, A. Peyrache, M. Khamassi et al., "Coherent theta oscillations and reorganization of spike timing in the hippocampal- prefrontal network upon learning," *Neuron*, vol. 66, no. 6, pp. 921–936, 2010.

[99] E. H. Baeg, Y. B. Kim, K. Huh, I. Mook-Jung, H. T. Kim, and M. W. Jung, "Dynamics of population code for working memory in the prefrontal cortex," *Neuron*, vol. 40, no. 1, pp. 177–188, 2003.

[100] E. H. Baeg, Y. B. Kim, J. Kim, J.-W. Ghim, J. J. Kim, and M. W. Jung, "Learning-induced enduring changes in functional connectivity among prefrontal cortical neurons," *Journal of Neuroscience*, vol. 27, no. 4, pp. 909–918, 2007.

[101] S. Fujisawa, A. Amarasingham, M. T. Harrison, and G. Buzsáki, "Behavior-dependent short-term assembly dynamics in the medial prefrontal cortex," *Nature Neuroscience*, vol. 11, no. 7, pp. 823–833, 2008.

[102] S. Fujisawa and G. Buzsáki, "A 4 Hz oscillation adaptively synchronizes prefrontal, VTA, and hippocampal activities," *Neuron*, vol. 72, no. 1, pp. 153–165, 2011.

[103] D. Durstewitz, N. M. Vittoz, S. B. Floresco, and J. K. Seamans, "Abrupt transitions between prefrontal neural ensemble states accompany behavioral transitions during rule learning," *Neuron*, vol. 66, no. 3, pp. 438–448, 2010.

[104] M. R. Milad and G. J. Quirk, "Neurons in medial prefrontal cortex signal memory for fear extinction," *Nature*, vol. 420, no. 6911, pp. 70–74, 2002.

[105] J. Peters, P. W. Kalivas, and G. J. Quirk, "Extinction circuits for fear and addiction overlap in prefrontal cortex," *Learning and Memory*, vol. 16, no. 5, pp. 279–288, 2009.

[106] R. P. Vertes, "Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat," *Neuroscience*, vol. 142, no. 1, pp. 1–20, 2006.

[107] M. W. Jones, "Errant ensembles: dysfunctional neuronal network dynamics in schizophrenia," *Biochemical Society Transactions*, vol. 38, no. 2, pp. 516–521, 2010.

[108] T. Sigurdsson, K. L. Stark, M. Karayiorgou, J. A. Gogos, and J. A. Gordon, "Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia," *Nature*, vol. 464, no. 7289, pp. 763–767, 2010.

[109] K. G. Phillips, U. Bartsch, A. P. McCarthy et al., "Decoupling of sleep-dependent cortical and hippocampal interactions in a neurodevelopmental model of schizophrenia," *Neuron*, vol. 76, no. 3, pp. 526–533, 2012.

[110] J. J. Kim, H. J. Lee, A. C. Welday et al., "Stress-induced alterations in hippocampal plasticity, place cells, and spatial memory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 46, pp. 18297–18302, 2007.

[111] G. Berlucchi and H. A. Buchtel, "Neuronal plasticity: historical roots and evolution of meaning," *Experimental Brain Research*, vol. 192, no. 3, pp. 307–319, 2009.

[112] S. J. Martin, P. D. Grimwood, and R. G. M. Morris, "Synaptic plasticity and memory: an evaluation of the hypothesis," *Annual Review of Neuroscience*, vol. 23, pp. 649–711, 2000.

[113] R. C. Malenka and M. F. Bear, "LTP and LTD: an embarrassment of riches," *Neuron*, vol. 44, no. 1, pp. 5–21, 2004.

[114] A. Holtmaat and K. Svoboda, "Experience-dependent structural synaptic plasticity in the mammalian brain," *Nature Reviews Neuroscience*, vol. 10, no. 9, pp. 647–658, 2009.

[115] Q. Yuan, J. S. Isaacson, and M. Scanziani, "Linking neuronal ensembles by associative synaptic plasticity," *PLoS ONE*, vol. 6, no. 6, Article ID e20486, 2011.

[116] J. C. Hirsch and F. Crepel, "Use-dependent changes in synaptic efficacy in rat profrontal neurons in vitro," *Journal of Physiology*, vol. 427, pp. 31–49, 1990.

[117] S. Otani, O. Blond, J.-M. Desce, and F. Crépel, "Dopamine facilitates long-term depression of glutamatergic transmission in rat prefrontal cortex," *Neuroscience*, vol. 85, no. 3, pp. 669–676, 1998.

[118] S. H. Morris, S. Knevett, E. G. Lerner, and L. J. Bindman, "Group I mGluR agonist DHPG facilitates the induction of LTP in rat prelimbic cortex in vitro," *Journal of Neurophysiology*, vol. 82, no. 4, pp. 1927–1933, 1999.

[119] C. M. Hempel, K. H. Hartman, X.-J. Wang, G. G. Turrigiano, and S. B. Nelson, "Multiple forms of short-term plasticity at excitatory synapses in rat medial prefrontal cortex," *Journal of Neurophysiology*, vol. 83, no. 5, pp. 3031–3041, 2000.

[120] Y.-Y. Huang, E. Simpson, C. Kellendonk, and E. R. Kandel, "Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 9, pp. 3236–3241, 2004.

[121] Y. Matsuda, A. Marzo, and S. Otani, "The presence of background dopamine signal converts long-term synaptic depression to potentiation in rat prefrontal cortex," *Journal of Neuroscience*, vol. 26, no. 18, pp. 4803–4810, 2006.

[122] B. Kolomiets, A. Marzo, J. Caboche, P. Vanhoutte, and S. Otani, "Background dopamine concentration dependently facilitates long-term potentiation in rat prefrontal cortex through postsynaptic activation of extracellular signal-regulated kinases," *Cerebral Cortex*, vol. 19, no. 11, pp. 2708–2718, 2009.

[123] A. Marzo, J. Bai, J. Caboche, P. Vanhoutte, and S. Otani, "Cellular mechanisms of long-term depression induced by noradrenaline in rat prefrontal neurons," *Neuroscience*, vol. 169, no. 1, pp. 74–86, 2010.

[124] T. M. Jay, F. Burette, and S. Laroche, "NMDA receptor-dependent long-term potentiation in the hippocampal afferent fibre system to the prefrontal cortex in the rat," *European Journal of Neuroscience*, vol. 7, no. 2, pp. 247–250, 1995.

[125] T. M. Jay, H. Gurden, and T. Yamaguchi, "Rapid increase in PKA activity during long-term potentiation in the hippocampal afferent fibre system to the prefrontal cortex in vivo," *European Journal of Neuroscience*, vol. 10, no. 10, pp. 3302–3306, 1998.

[126] Y. Izaki, M. Takita, T. M. Jay, H. Kaneko, S. S. Suzuki, and M. Nomura, "Effect of long-term potentiation induction on gamma-band electroencephalograms in prefrontal cortex following stimulation of rat hippocampus in vivo," *Neuroscience Letters*, vol. 305, no. 1, pp. 57–60, 2001.

[127] M. Takita, Y. Izaki, M. Kuramochi, H. Yokoi, and M. Ohtomi, "Synaptic plasticity dynamics in the hippocampal-prefrontal pathway in vivo," *NeuroReport*, vol. 21, no. 1, pp. 68–72, 2010.

[128] L. S. Bueno-Junior, C. Lopes-Aguiar, R. N. Ruggiero, R. N. Romcy-Pereira, and J. P. Leite, "Muscarinic and nicotinic modulation of thalamo-prefrontal cortex synaptic pasticity in vivo," *PLoS ONE*, vol. 7, no. 10, Article ID e47484, 2012.

[129] F. Calabrese, R. Molteni, G. Racagni, and M. A. Riva, "Neuronal plasticity: a link between stress and mood disorders," *Psychoneuroendocrinology*, vol. 34, no. 1, pp. S208–S216, 2009.

[130] C. Pittenger and R. S. Duman, "Stress, depression, and neuroplasticity: a convergence of mechanisms," *Neuropsychopharmacology*, vol. 33, no. 1, pp. 88–109, 2008.

[131] Y. Goto, C. R. Yang, and S. Otani, "Functional and dysfunctional synaptic plasticity in prefrontal cortex: roles in psychiatric disorders," *Biological Psychiatry*, vol. 67, no. 3, pp. 199–207, 2010.

[132] E. Y. Yuen, J. Wei, W. Liu, P. Zhong, X. Li, and Z. Yan, "Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex," *Neuron*, vol. 73, no. 5, pp. 962–977, 2012.

[133] I. Negrón-Oyarzo, A. Dagnino-Subiabre, and P. Muñoz Carvajal, "Synaptic impairment in layer 1 of the prefrontal cortex induced by repeated stress during adolescence is reversed in adulthood," *Frontiers in Cellular Neuroscience*, vol. 9, no. 442, 2015.

[134] M. Popoli, Z. Yan, B. S. McEwen, and G. Sanacora, "The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission," *Nature Reviews Neuroscience*, vol. 13, no. 1, pp. 22–37, 2012.

[135] D. C. Rotaru, H. Yoshino, D. A. Lewis, G. B. Ermentrout, and G. Gonzalez-Burgos, "Glutamate receptor subtypes mediating synaptic activation of prefrontal cortex neurons: relevance for schizophrenia," *Journal of Neuroscience*, vol. 31, no. 1, pp. 142–156, 2011.

[136] I. Negrón-Oyarzo, D. Neira, N. Espinosa, P. Fuentealba, and F. Aboitiz, "Prenatal stress produces persistence of remote memory and disrupts functional connectivity in the hippocampal-prefrontal cortex axis," *Cerebral Cortex*, vol. 25, no. 9, pp. 3132–3143, 2015.

[137] D. S. Goldwater, C. Pavlides, R. G. Hunter et al., "Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery," *Neuroscience*, vol. 164, no. 2, pp. 798–808, 2009.

[138] C. Rocher, M. Spedding, C. Munoz, and T. M. Jay, "Acute stress-induced changes in hippocampal/prefrontal circuits in rats: effects of antidepressants," *Cerebral Cortex*, vol. 14, no. 2, pp. 224–229, 2004.

[139] C. Zheng, M. Quan, and T. Zhang, "Decreased thalamo-cortical connectivity by alteration of neural information flow in theta oscillation in depression-model rats," *Journal of Computational Neuroscience*, vol. 33, no. 3, pp. 547–558, 2012.

[140] M. Quan, C. Zheng, N. Zhang et al., "Impairments of behavior, information flow between thalamus and cortex, and prefrontal cortical synaptic plasticity in an animal model of depression," *Brain Research Bulletin*, vol. 85, no. 3-4, pp. 109–116, 2011.

[141] H. Abush and I. Akirav, "Cannabinoids ameliorate impairments induced by chronic stress to synaptic plasticity and short-term memory," *Neuropsychopharmacology*, vol. 38, no. 8, pp. 1521–1534, 2013.

[142] R. Garcia, G. Spennato, L. Nilsson-Todd, J.-L. Moreau, and O. Deschaux, "Hippocampal low-frequency stimulation and chronic mild stress similarly disrupt fear extinction memory in rats," *Neurobiology of Learning and Memory*, vol. 89, no. 4, pp. 560–566, 2008.

[143] C. Judo, M. Matsumoto, D. Yamazaki et al., "Early stress exposure impairs synaptic potentiation in the rat medial prefrontal cortex underlying contextual fear extinction," *Neuroscience*, vol. 169, no. 4, pp. 1705–1714, 2010.

[144] G. Buzsáki, C. A. Anastassiou, and C. Koch, "The origin of extracellular fields and currents—EEG, ECoG, LFP and spikes," *Nature Reviews Neuroscience*, vol. 13, no. 6, pp. 407–420, 2012.

[145] W. B. Levy and O. Steward, "Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus," *Neuroscience*, vol. 8, no. 4, pp. 791–797, 1983.

[146] P. Fries, "A mechanism for cognitive dynamics: neuronal communication through neuronal coherence," *Trends in Cognitive Sciences*, vol. 9, no. 10, pp. 474–480, 2005.

[147] P. Fries, "Neuronal gamma-band synchronization as a fundamental process in cortical computation," *Annual Review of Neuroscience*, vol. 32, pp. 209–224, 2009.

[148] N. Axmacher, F. Mormann, G. Fernández, C. E. Elger, and J. Fell, "Memory formation by neuronal synchronization," *Brain Research Reviews*, vol. 52, no. 1, pp. 170–182, 2006.

[149] C. A. Anastassiou, R. Perin, H. Markram, and C. Koch, "Ephaptic coupling of cortical neurons," *Nature Neuroscience*, vol. 14, no. 2, pp. 217–224, 2011.

[150] M. Volgushev, M. Chistiakova, and W. Singer, "Modification of discharge patterns of neocortical neurons by induced oscillations of the membrane potential," *Neuroscience*, vol. 83, no. 1, pp. 15–25, 1998.

[151] F. Varela, J.-P. Lachaux, E. Rodriguez, and J. Martinerie, "The brainweb: phase synchronization and large-scale integration," *Nature Reviews Neuroscience*, vol. 2, no. 4, pp. 229–239, 2001.

[152] M. W. Jones and M. A. Wilson, "Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task," *PLoS Biology*, vol. 3, no. 12, article e402, 2005.

[153] P.-K. O'Neill, J. A. Gordon, and T. Sigurdsson, "Theta oscillations in the medial prefrontal cortex are modulated by spatial working memory and synchronize with the hippocampus through its ventral subregion," *The Journal of Neuroscience*, vol. 33, no. 35, pp. 14211–14224, 2013.

[154] J. M. Hyman, E. A. Zilli, A. M. Paley, and M. E. Hasselmo, "Working memory performance correlates with prefrontal-hippocampal theta interactions but not with prefrontal neuron firing rates," *Frontiers in Integrative Neuroscience*, vol. 4, article 2, 2010.

[155] A. Adhikari, M. A. Topiwala, and J. A. Gordon, "Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety," *Neuron*, vol. 65, no. 2, pp. 257–269, 2010.

[156] A. Sirota, S. Montgomery, S. Fujisawa, Y. Isomura, M. Zugaro, and G. Buzsáki, "Entrainment of neocortical neurons and gamma oscillations by the hippocampal theta rhythm," *Neuron*, vol. 60, no. 4, pp. 683–697, 2008.

[157] R. T. Canolty, K. Ganguly, S. W. Kennerley et al., "Oscillatory phase coupling coordinates anatomically dispersed functional cell assemblies," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 40, pp. 17356–17361, 2010.

[158] M. Siegel, T. H. Donner, and A. K. Engel, "Spectral fingerprints of large-scale neuronal interactions," *Nature Reviews Neuroscience*, vol. 13, no. 2, pp. 121–134, 2012.

[159] J. Fell and N. Axmacher, "The role of phase synchronization in memory processes," *Nature Reviews Neuroscience*, vol. 12, no. 2, pp. 105–118, 2011.

[160] A. G. Siapas and M. A. Wilson, "Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep," *Neuron*, vol. 21, no. 5, pp. 1123–1128, 1998.

[161] A. Sirota, J. Csicsvari, D. Buhl, and G. Buzsáki, "Communication between neocortex and hippocampus during sleep in rodents," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 4, pp. 2065–2069, 2003.

[162] A. G. Siapas, E. V. Lubenov, and M. A. Wilson, "Prefrontal phase locking to hippocampal theta oscillations," *Neuron*, vol. 46, no. 1, pp. 141–151, 2005.

[163] M. Mölle, O. Yeshenko, L. Marshall, S. J. Sara, and J. Born, "Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep," *Journal of Neurophysiology*, vol. 96, no. 1, pp. 62–70, 2006.

[164] C. M. Wierzynski, E. V. Lubenov, M. Gu, and A. G. Siapas, "State-dependent spike-timing relationships between hippocampal and prefrontal circuits during sleep," *Neuron*, vol. 61, no. 4, pp. 587–596, 2009.

[165] K. Benchenane, P. H. Tiesinga, and F. P. Battaglia, "Oscillations in the prefrontal cortex: a gateway to memory and attention," *Current Opinion in Neurobiology*, vol. 21, no. 3, pp. 475–485, 2011.

[166] M. D. Brockmann, B. Pöschel, N. Cichon, and I. L. Hanganu-Opatz, "Coupled oscillations mediate directed interactions between prefrontal cortex and hippocampus of the neonatal rat," *Neuron*, vol. 71, no. 2, pp. 332–347, 2011.

[167] P. J. Uhlhaas and W. Singer, "Abnormal neural oscillations and synchrony in schizophrenia," *Nature Reviews Neuroscience*, vol. 11, no. 2, pp. 100–113, 2010.

[168] G. A. Light, J. L. Hsu, M. H. Hsieh et al., "Gamma band oscillations reveal neural network cortical coherence dysfunction in schizophrenia patients," *Biological Psychiatry*, vol. 60, no. 11, pp. 1231–1240, 2006.

[169] O. L. Smart, V. R. Tiruvadi, and H. S. Mayberg, "Multimodal approaches to define network oscillations in depression," *Biological Psychiatry*, vol. 77, no. 12, pp. 1061–1070, 2015.

[170] Y. A. Lee, P. Poirier, S. Otani, and Y. Goto, "Dorsal-ventral distinction of chronic stress-induced electrophysiological alterations in the rat medial prefrontal cortex," *Neuroscience*, vol. 183, pp. 108–120, 2011.

[171] J. F. Oliveira, N. S. Dias, M. Correia et al., "Chronic stress disrupts neural coherence between cortico-limbic structures," *Frontiers in Neural Circuits*, vol. 7, article 10, 2013.

[172] L. R. Jacinto, J. S. Reis, N. S. Dias, J. J. Cerqueira, J. H. Correia, and N. Sousa, "Stress affects theta activity in limbic networks and impairs novelty-induced exploration and familiarization," *Frontiers in Behavioral Neuroscience*, vol. 7, article 127, Article ID 127, 2013.

[173] P. W. Frankland and B. Bontempi, "The organization of recent and remote memories," *Nature Reviews Neuroscience*, vol. 6, no. 2, pp. 119–130, 2005.

[174] I. L. C. Nieuwenhuis and A. Takashima, "The role of the ventromedial prefrontal cortex in memory consolidation," *Behavioural Brain Research*, vol. 218, no. 2, pp. 325–334, 2011.

[175] V. E. Ghosh and A. Gilboa, "What is a memory schema? A historical perspective on current neuroscience literature," *Neuropsychologia*, vol. 53, no. 1, pp. 104–114, 2014.

[176] L. R. Squire and P. Alvarez, "Retrograde amnesia and memory consolidation: a neurobiological perspective," *Current Opinion in Neurobiology*, vol. 5, no. 2, pp. 169–177, 1995.

[177] G. Buzsáki, "Two-stage model of memory trace formation: a role for 'noisy' brain states," *Neuroscience*, vol. 31, no. 3, pp. 551–570, 1989.

[178] S. Ribeiro and M. A. L. Nicolelis, "Reverberation, storage, and postsynaptic propagation of memories during sleep," *Learning and Memory*, vol. 11, no. 6, pp. 686–696, 2004.

[179] R. Stickgold, "Sleep-dependent memory consolidation," *Nature*, vol. 437, no. 7063, pp. 1272–1278, 2005.

[180] S. Diekelmann and J. Born, "The memory function of sleep," *Nature Reviews Neuroscience*, vol. 11, no. 2, pp. 114–126, 2010.

[181] J. O'Neill, B. Pleydell-Bouverie, D. Dupret, and J. Csicsvari, "Play it again: reactivation of waking experience and memory," *Trends in Neurosciences*, vol. 33, no. 5, pp. 220–229, 2010.

[182] L. R. Squire, P. C. Slater, and P. M. Chace, "Retrograde amnesia: temporal gradient in very long term memory following electroconvulsive therapy," *Science*, vol. 187, no. 4171, pp. 77–79, 1975.

[183] L. E. Jarrard, "What does the hippocampus really do?" *Behavioural Brain Research*, vol. 71, no. 1-2, pp. 1–10, 1995.

[184] P. A. Lewis and S. J. Durrant, "Overlapping memory replay during sleep builds cognitive schemata," *Trends in Cognitive Sciences*, vol. 15, no. 8, pp. 343–351, 2011.

[185] J. L. McGaugh, "Memory consolidation and the amygdala: a systems perspective," *Trends in Neurosciences*, vol. 25, no. 9, pp. 456–461, 2002.

[186] J. L. McGaugh, "Memory—a century of consolidation," *Science*, vol. 287, no. 5451, pp. 248–251, 2000.

[187] E. J. Hermans, F. P. Battaglia, P. Atsak, L. D. De Voogd, G. Fernández, and B. Roozendaal, "How the amygdala affects emotional memory by altering brain network properties," *Neurobiology of Learning and Memory*, vol. 112, pp. 2–16, 2014.

[188] J. L. McGaugh, "The amygdala modulates the consolidation of memories of emotionally arousing experiences," *Annual Review of Neuroscience*, vol. 27, pp. 1–28, 2004.

[189] P. C. Holland and M. Gallagher, "Amygdala-frontal interactions and reward expectancy," *Current Opinion in Neurobiology*, vol. 14, no. 2, pp. 148–155, 2004.

[190] N. J. Powell and A. D. Redish, "Complex neural codes in rat prelimbic cortex are stable across days on a spatial decision task," *Frontiers in Behavioral Neuroscience*, vol. 8, article 120, 2014.

[191] M. A. Wilson and B. L. McNaughton, "Reactivation of hippocampal ensemble memories during sleep," *Science*, vol. 265, no. 5172, pp. 676–679, 1994.

[192] S. M. Fogel and C. T. Smith, "The function of the sleep spindle: a physiological index of intelligence and a mechanism for sleep-dependent memory consolidation," *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 5, pp. 1154–1165, 2011.

[193] G. Girardeau and M. Zugaro, "Hippocampal ripples and memory consolidation," *Current Opinion in Neurobiology*, vol. 21, no. 3, pp. 452–459, 2011.

[194] A. Peyrache, F. P. Battaglia, and A. Destexhe, "Inhibition recruitment in prefrontal cortex during sleep spindles and gating of hippocampal inputs," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 41, pp. 17207–17212, 2011.

[195] J. Lesting, R. T. Narayanan, C. Kluge, S. Sangha, T. Seidenbecher, and H.-C. Pape, "Patterns of coupled theta activity in amygdala-hippocampal-prefrontal cortical circuits during fear extinction," *PLoS ONE*, vol. 6, no. 6, Article ID e21714, 2011.

[196] E. Likhtik, J. M. Stujenske, M. A. Topiwala, A. Z. Harris, and J. A. Gordon, "Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety," *Nature Neuroscience*, vol. 17, no. 1, pp. 106–113, 2014.

[197] D. Paré, D. R. Collins, and J. G. Pelletier, "Amygdala oscillations and the consolidation of emotional memories," *Trends in Cognitive Sciences*, vol. 6, no. 7, pp. 306–314, 2002.

[198] J. G. Pelletier and D. Paré, "Role of amygdala oscillations in the consolidation of emotional memories," *Biological Psychiatry*, vol. 55, no. 6, pp. 559–562, 2004.

[199] D. Paré, H.-C. Pape, and J. Dong, "Bursting and oscillating neurons of the cat basolateral amygdaloid complex in vivo: electrophysiological properties and morphological features," *Journal of Neurophysiology*, vol. 74, no. 3, pp. 1179–1191, 1995.

[200] S. Maren and M. S. Fanselow, "Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo," *Journal of Neuroscience*, vol. 15, no. 11, pp. 7548–7564, 1995.

[201] T. Seidenbecher, T. R. Laxmi, O. Stork, and H.-C. Pape, "Amygdalar and hippocampal theta rhythm synchronization during fear memory retrieval," *Science*, vol. 301, no. 5634, pp. 846–850, 2003.

[202] H.-C. Pape, R. T. Narayanan, J. Smid, O. Stork, and T. Seidenbecher, "Theta activity in neurons and networks of the amygdala related to long-term fear memory," *Hippocampus*, vol. 15, no. 7, pp. 874–880, 2005.

[203] R. T. Narayanan, T. Seidenbecher, C. Kluge, J. Bergado, O. Stork, and H.-C. Pape, "Dissociated theta phase synchronization in amygdalo-hippocampal circuits during various stages of fear memory," *European Journal of Neuroscience*, vol. 25, no. 6, pp. 1823–1831, 2007.

[204] J. Lesting, T. Daldrup, V. Narayanan, C. Himpe, T. Seidenbecher, and H.-C. Pape, "Directional theta coherence in prefrontal cortical to amygdalo-hippocampal pathways signals fear extinction," *PLoS ONE*, vol. 8, no. 10, Article ID e77707, 2013.

[205] D. Popa, S. Duvarci, A. T. Popescu, C. Léna, and D. Paré, "Coherent amygdalocortical theta promotes fear memory consolidation during paradoxical sleep," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 14, pp. 6516–6519, 2010.

[206] A. N. Hoffman, A. Parga, P. R. Paode et al., "Chronic stress enhanced fear memories are associated with increased amygdala zif268 mRNA expression and are resistant to reconsolidation," *Neurobiology of Learning and Memory*, vol. 120, pp. 61–68, 2015.

[207] B. R. Mueller and T. L. Bale, "Early prenatal stress impact on coping strategies and learning performance is sex dependent," *Physiology and Behavior*, vol. 91, no. 1, pp. 55–65, 2007.

[208] B. Roozendaal, B. S. McEwen, and S. Chattarji, "Stress, memory and the amygdala," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 423–433, 2009.

[209] W. Zhang and J. A. Rosenkranz, "Repeated restraint stress increases basolateral amygdala neuronal activity in an age-dependent manner," *Neuroscience*, vol. 226, pp. 459–474, 2012.

[210] S. Ghosh, T. R. Laxmi, and S. Chattarji, "Functional connectivity from the amygdala to the hippocampus grows stronger after stress," *Journal of Neuroscience*, vol. 33, no. 17, pp. 7234–7244, 2013.

[211] L. Clark, S. R. Chamberlain, and B. J. Sahakian, "Neurocognitive mechanisms in depression: implications for treatment," *Annual Review of Neuroscience*, vol. 32, pp. 57–74, 2009.

[212] T. Amano, C. T. Unal, and D. Paré, "Synaptic correlates of fear extinction in the amygdala," *Nature Neuroscience*, vol. 13, no. 4, pp. 489–494, 2010.

[213] D. Khodagholy, J. N. Gelinas, T. Thesen et al., "NeuroGrid: recording action potentials from the surface of the brain," *Nature Neuroscience*, vol. 18, no. 2, pp. 310–315, 2015.

[214] B. F. Grewe and F. Helmchen, "Optical probing of neuronal ensemble activity," *Current Opinion in Neurobiology*, vol. 19, no. 5, pp. 520–529, 2009.

[215] S. Ramirez, X. Liu, C. J. MacDonald et al., "Activating positive memory engrams suppresses depression-like behaviour," *Nature*, vol. 522, no. 7556, pp. 335–339, 2015.

[216] D. Riga, M. R. Matos, A. Glas, A. B. Smit, S. Spijker, and M. C. Van den Oever, "Optogenetic dissection of medial prefrontal cortex circuitry," *Frontiers in Systems Neuroscience*, vol. 8, article 230, 2014.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 3985063, 13 pages
http://dx.doi.org/10.1155/2016/3985063

Review Article

Linking Mitochondria to Synapses: New Insights for Stress-Related Neuropsychiatric Disorders

Freddy Jeanneteau and Margarita Arango-Lievano

Team AVENIR "Stress Hormones and Plasticity", INSERM U1191, CNRS UMR5203, Institut de Génomique Fonctionnelle, 34094 Montpellier, France

Correspondence should be addressed to Freddy Jeanneteau; freddy.jeanneteau@igf.cnrs.fr

Received 25 September 2015; Accepted 9 November 2015

Academic Editor: Jordan Marrocco

The brain evolved cellular mechanisms for adapting synaptic function to energy supply. This is particularly evident when homeostasis is challenged by stress. Signaling loops between the mitochondria and synapses scale neuronal connectivity with bioenergetics capacity. A biphasic "inverted U shape" response to the stress hormone glucocorticoids is demonstrated in mitochondria and at synapses, modulating neural plasticity and physiological responses. Low dose enhances neurotransmission, synaptic growth, mitochondrial functions, learning, and memory whereas chronic, higher doses produce inhibition of these functions. The range of physiological effects by stress and glucocorticoid depends on the dose, duration, and context at exposure. These criteria are met by neuronal activity and the circadian, stress-sensitive and ultradian, stress-insensitive modes of glucocorticoid secretion. A major hallmark of stress-related neuropsychiatric disorders is the disrupted glucocorticoid rhythms and tissue resistance to signaling with the glucocorticoid receptor (GR). GR resistance could result from the loss of context-dependent glucocorticoid signaling mediated by the downregulation of the activity-dependent neurotrophin BDNF. The coincidence of BDNF and GR signaling changes glucocorticoid signaling output with consequences on mitochondrial respiration efficiency, synaptic plasticity, and adaptive trajectories.

1. Introduction

One paramount feature of higher organisms is to learn and adapt to changing environments. It is a matter of survival that requires the brain to convert immediate stimuli into long-lasting changes of neural circuits through alterations of neuronal structure and function [1–3]. Failure to adjust body wide homeostasis in changing environment can cause the organism to follow maladaptive trajectories with presumed pathological consequences [1, 4, 5].

The goal of the present review is to give the cell biologists vision of (mal)adaptive trajectories to stress focusing on the link between the mitochondria and synapses to keep in check "neuronal fitness" in changing environment. If adaptive plasticity necessitates neurons to derail from homeostasis, then disease vulnerability checkpoints could lie in the signaling loops linking mitochondria to synapses. Several studies provide a useful framework for a pathway to neuropsychiatric disorders by the concomitant reorganization of synaptic territories and a dysfunction of mitochondria; but these two responses are often investigated separately [6–10].

Adaptation of metabolism with respect to neural connectivity is presented as a mechanism of neuronal preservation to positive and negative external stimuli. That is, neurons could choose quiescence or growth to fit in their network demands. In particular, the relation between synapses and mitochondria, a pivot of neuronal plasticity, is here discussed, emphasizing on its modulation by the stress hormone, glucocorticoid.

2. Linking Mitochondria to Synapse: A Role in Neuronal Plasticity

Several studies indicate that physical proximity between mitochondria and synapses is regulated by neuronal activity [11, 12]. A significant fraction of neuronal mitochondria

constantly moves along microtubule networks while the remaining pool is captured where metabolic demand rises [13]. This relation is proportionate, at least in the presynaptic terminals. In the dendrites, the distribution of mitochondria is heterogeneous and difficult to link morphologically with one particular synapse given that mitochondria rarely penetrate into postsynaptic dendritic spines [14]. The functional properties of mitochondria differ in axons and dendrites. For example, mitochondria are more motile in the axons as compared with the dendrites, and there is a greater proportion of highly charged, metabolically active mitochondria in dendrites compared to axons [15]. The following subsections describe mitochondrial functions responding to neurotransmission and likely essential for the plasticity of neuronal networks.

2.1. ATP Production. Synaptic activity consumes energy mainly supplied by astrocytes, the biggest reservoir of glycogen in the brain. An elegant study [16] indicated that hippocampal activity induced glycogenolysis, whose end product, lactate, could be used by neurons to produce ATP via glycolytic enzymes that others and we previously found at the synapse [17, 18]. Disruption of lactate transport between astrocytes and neurons impaired the retention of new memories of inhibitory avoidance [16]. Although this study raised the importance of lactate as a signaling molecule or as a source of energy for neurotransmission and behavior, it did not refute the critical role of ATP derived from mitochondria during synaptic plasticity. Neurons rely on mitochondria more than glycolysis to produce ATP [19]. Previous experiments in cultures of hippocampal neurons mostly deprived of astrocytes demonstrated a role for ATP derived from mitochondria in long-term potentiation (LTP) and dendritic spine morphogenesis. For instance, hippocampal neurons, which express dominantly the mitochondrial isoform of the creatine kinase [20], responded to a treatment of exogenous creatine by increasing the number and activity of dendritic mitochondria that coincidently nearly doubled the number of synapses [21].

2.2. Calcium Homeostasis. Mitochondria are more than just portable power stations. Mitochondria buffer calcium in the presynaptic terminals and in dendrites after activation of the NMDA receptors by physically coupling with the smooth endoplasmic reticulum (SER) [22, 23]. The capture of cytoplasmic calcium by mitochondria has a modulatory role for neurotransmission at central synapses [24]. The negatively charged electron gradient across the mitochondrial membrane attracts calcium into the matrix via the low-affinity calcium uniport. This phenomenon is reversible so brain mitochondria can store and release calcium [25]. Low levels of matrix calcium levels can control the activity of the oxidative phosphorylation pathway thus changing the rate of ATP production [26, 27]. In contrast, massive accumulation of calcium within mitochondria is cytotoxic and a typical response of glutamate hyperexcitation that can result from disease conditions like stroke or epilepsy [25]. Interestingly, the expression of a cluster of activity-dependent and calcium-sensitive genes can provide neuroprotection through a common process that renders mitochondria more resistant to hyperexcitability [28].

2.3. Superoxide Production. Mitochondria produce and release reactive oxygen and nitrogen species that are necessary for synaptic plasticity, learning, and memory [29]. But the abundance of superoxides is extremely damaging for proteins, mitochondrial DNA, and lipids. Therefore, the redox function of mitochondria is also critical for neuronal plasticity during ageing and disease conditions [30]. Remarkably, transgenic mice overexpressing the Superoxide Dismutase 2 (SOD2) exhibit lower levels of mitochondrial superoxides accompanied by normal long-term potentiation (LTP), learning, and memory [31]. This study raises the question whether mitochondrial redox functions are more useful in disease conditions than in health. Indeed, mitochondrial redox functions are breached in disease conditions, causing substantial oxidative stress [32].

2.4. Nonapoptotic Caspase Signaling. Mitochondria also activate caspases in dendrites to trigger postsynaptic spine elimination in response to NMDA-dependent long-term depression (LTD) [33]. In particular, the cascade end product caspase-3 is a protease for a broad range of cytoskeletal substrates abundant in dendritic spines (e.g., gelsolin, spectrin, fodrin, and cofilin), in dendritic shafts (e.g., tau), and for glutamatergic/neurotrophic signaling (e.g., calcineurin, AKT) [34]. Consequently, it is hypothesized that mitochondria could participate in LTD by the pruning of synapses. The question remains how synapses to be eliminated are targeted by mitochondrial mechanisms. One possibility is to prevent the spread of caspase signaling to unrelated synapses by the deactivation of the ubiquitin proteasome in hotspots of the dendrites [35]. Alternatively, the expression of caspases is in tight equilibrium with the inhibitors of apoptosis protein (IAP) that suppress generalized caspase activation and neuronal death.

2.5. Mitochondrial Maintenance. The size and distribution of mitochondria vary, spanning more than one postsynaptic dendritic spine for elongated mitochondria, while large clusters of neighboring spines can be deprived of dendritic mitochondria. The distribution of dendritic mitochondria depends on the processes of fission/fusion mainly regulated by GTPases (DRP1, OPA1, MFN1, and MFN2) [21, 36]. The fragmentation of mitochondria could serve as a mechanism to cover the needs of distant clusters of spines. In contrast, the elongation of mitochondria could represent a mechanism to increase the ratio of mitochondria per dendrites independently of mitochondrial biogenesis. In terms of plasticity, trains of electrical stimulation elicit slow and prolonged changes in mitochondrial ultrastructure and membrane potential, modifying the proton gradients across mitochondrial membranes necessary to produce ATP [37]. High tetanic stimulation elicits the fragmentation and translocation of mitochondria into enlarged dendritic protrusions in the stimulated region but not in the distant part

of the same dendrite [21]. Both rapid and slow mechanisms are likely involved in the maintenance of mitochondrial function upon neuronal activation. Dendritic mitochondria could be rapidly transported where ATP consumption and necessity for energy are particularly high, as it takes place at the active presynaptic terminals [38]. Also, the biogenesis of mitochondria is a slow process, proportionate to neuronal differentiation and growth [39].

3. Mitochondria Pay the Price of Synaptic Plasticity

3.1. Mitochondria and Synaptic Scaling. Mitochondrial function is closely related to synaptic function. Synaptic scaling is a form of synaptic plasticity that adjusts the strength of all neuronal synapses to the demand of the network [40]. Popular experimental paradigms for inducing synaptic scaling are through the blockade or enhancement of network activity in cultures [41, 42] or sensory deprivation in animal models [43]. For example, the induction of neuronal activity by electrical stimulation or chemical depolarization in cultures decreased the motility and fusion of mitochondria in dendrites via activity-dependent calcium influx [13, 21]. This phenomenon could reflect the synaptic capture of mitochondria as a function of synaptic activity resulting from the pause of mitochondrial transport machinery at hotspots of intracellular calcium signaling. Notably, the calcium-sensing protein MIRO, which links mitochondria to the kinesins microtubule motor proteins, is essential for mitochondrial arrest upon calcium influx through NMDA receptors [44]. In contrast, global suppression of neuronal activity with TTX increased the motility, fusion, and redistribution of mitochondria along dendrites. Therefore, global changes in synaptic activity and glutamate receptor blockers can modulate mitochondrial depolarization and related functions [45]. Likewise, there is a remarkable parallel between the changes in content of synaptic and mitochondrial proteins in the visual cortex of mice reared in the dark compared to mice living under light/dark conditions [46]. Therefore, mechanisms exist for reciprocal regulation of synapses and mitochondria.

Most brain energy is consumed at synapses to maintain ion gradients and support the signaling responses of neurotransmission. The energetic cost of housekeeping neuronal functions is predicted to be higher in the postsynaptic dendrites than in the presynaptic terminals [47]. There are limits to which mitochondria can support trains of neuronal network activation. One example is the repetitive discharges of excitation at hippocampal neurons during epileptic seizures that produce severe mitochondrial dysfunctions, eventually resulting in neuronal death [48]. Modeling experiments indicate that synaptic depotentiation is likely desirable to support neuronal survival when energetic stores are limited [19]. Therefore, negative feedback mechanisms may have evolved to suppress synaptic potentiation that would drain ATP stores upon high frequency stimulation. One putative target that can sense low metabolic state is the AMP-dependent protein kinase (AMPK), whose activation impedes the transition from the early synaptic potentiation phase to the long-term synaptic potentiation phase [49]. So neurons evolved protective cellular mechanisms for adapting synaptic function with metabolism. By pushing neuronal plasticity beyond the limits of homeostasis, one could learn in the pathways that link mitochondria to synapses how to promote adaptation. The next section will focus on the synaptic and metabolic adaptation to traumatic stress.

3.2. Mitochondria and the Allostatic Load Model. Neuronal networks are capable of adaptive responses because they can reach a novel state of physiological stability each time homeostasis is challenged. This phenomenon termed allostasis requires the setting of new metabolic, housekeeping, and plasticity parameters in accordance with the external demands. It is the nature of the allostatic load that should define the amplitude of adaptation to reach without killing the cells [50]. Therefore, signaling loops between mitochondria and synapses are anticipated to be essential for coping with the allostatic load [51]. The allostatic load model has been extensively studied in the context of stress because of its comorbidity with numerous human neuropsychopathologies and, experimentally, it is admitted that stress is a starting point for the development of chronic disease trajectories. Studies of traumatic stress early in life emphasized the important roles of the age and timing at exposure, the nature, intensity, and duration of the stressor for developing enduring metabolic dysfunction, and neuropsychopathologies in adulthood [52]. Consequently, there is not one but multiple trajectories of adaptation to stress set by the interaction of genetics, lifestyle, and the environment coming in different flavors. Remarkably, twin studies revealed that distinct adaptive trajectories could also evolve from organisms with the same genetic background and history [53–55]. These studies naturally raised the question as to why some individuals are vulnerable to disorders linked to stress (e.g., major depressive disorders, anxiety, and posttraumatic stress disorders) whereas others are not despite similar stressful experiences [56].

Stress, when chronic and uncontrollable, is a major risk factor of neuropsychopathologies. Underlying mechanisms are complex and cannot be ascribed to a single genetic or environmental factor, but they usually result in dysfunctional metabolism and neuronal connectivity of cortical and subcortical micro- and macrocircuits. So, neuropsychiatric disorders induced by stress most likely arise from complex interplay of genetics and environment. The level of stress hormones reflects perceived changes of the external world (e.g., threat, reward, and novelty) by readily diffusing throughout the circulation, virtually accessing all cells of the organism within the same time scale. For this reason, the physiology and adaptive capacity of the hormonal stress response mediated by the hypothalamo-pituitary-adrenal (HPA) axis has been intensively studied [57]. It is admitted that physiological feedforward and feedback neuroendocrine loops control the HPA axis as a function of the frequency and dose of internal and external stimuli [58]. Alterations of these activation and deactivation loops have been reported in patients suffering from stress-related neuropsychiatric disorders and in animal models [59, 60]. One important question remains if the allostatic overload of stress relies on mechanisms of plasticity

that link mitochondrial function to synaptic plasticity. From the characterization of such mechanisms could emerge novel modulators of stress-related neuropathologies.

4. Glucocorticoids Are Fast and Slow Acting Modifiers of Synapses and Mitochondria

Stress and glucocorticoids have potent but complex effects on neurotransmission, learning, and memory. The typical inverted "U" shape response to glucocorticoids and stress depends on the dose, duration, and context at exposure. The convergence of several mechanisms is likely to explain such biphasic responses. First, it could reflect the circadian and ultradian oscillations of glucocorticoids secreted in the bloodstream [57]. Second, it could reflect the interaction of the stress-elicited norepinephrine and glucocorticoids signaling that produces a response distinct from individual pathways during the early learning and memory phases, as a function of stressor intensity and duration [61–63]. Third, it could reflect the dynamics of dendritic spines that can be modified by the changes of glucocorticoids levels in the bloodstream, like the circadian- or stress-mediated changes in oscillatory secretions. Glucocorticoid-induced spine formation is rapid and temporally dissociated from spine survival and elimination, which are slow and required the synthesis of new gene products [64]. Correlation studies suggest that dendritic spine patterning and mnemonic effects on procedural learning afforded by glucocorticoids depend on the dose, duration, and context at exposure [65].

Biphasic modulatory effects of glucocorticoids have been described on mitochondrial function as well [66]. For example, stimulation of primary neurons with corticosterone increased, in time- and dose-dependent manner, mitochondria calcium holding capacity, membrane potential, and redox function. These effects depended also on the context at exposure, producing neurotoxicity or neuroprotection, respectively, with or without a cotreatment of kainic acid [66]. These findings recapitulate that of glucocorticoids on synaptic plasticity, indicating that common or converging pathways control synaptic and mitochondrial functions. There is evidence that such relation exists in vivo as well. Serial section electron microcopy of synapses in the amygdala of animals experiencing fear conditioning revealed that fear learning increased the size of the postsynaptic density, the number of presynaptic docking vesicles, and the number of mitochondria compared to naïve controls and the conditioned inhibition group that learned safety conditioning [67]. This result is in agreement with the glucocorticoid-dependent hypertrophic effects of amygdala neurons in the fear-conditioning paradigm [58]. Other studies indicated that mitochondria ultrastructure and bioenergetics capacity are altered in neurons or brain regions, which demonstrated synaptic pathology (e.g., bipolar disorder, major depressive disorders, schizophrenia, and Alzheimer's disease) [68–71]. In vitro studies indicated that glucocorticoids increased mitochondrial calcium holding capacity, mitochondrial membrane potential, and mitochondrial oxidation rapidly and durably depending on the dose [66]. Glucocorticoid receptors (GR) are required for modulating mitochondria as well as dendritic spine turnover. The former involved the translocation of cytoplasmic GR-BCL2 complexes into the mitochondria [66]; the latter required membrane bound GR presumably localized at the synapse in the motor cortex [64] and previously described in the postsynaptic dendrites of amygdala neurons [72].

The exact roles of GR at the synapse or in mitochondria are uncertain (Figure 1). However, new evidence points toward a role for GR signaling derived from mitochondria and synapses on the dynamics of the actin polymerization between G-actin and F-actin cytoskeleton via the LIMK1-cofilin pathway [64, 73]. The assembly and disassembly of G-actin to F-actin are critical for many cellular processes, including cell motility, migration, dendritic spine morphogenesis, endocytosis, and focal adhesion of mitochondria at pre- and postsynaptic membranes [74]. Interestingly, the broad-spectrum serine/threonine phosphatase calcineurin, essential for functional and structural neuronal plasticity, can be activated on both ends of the mitochondria-synapse pathway. Calcium entry via the NMDA receptors activates calcineurin, and depolarization of mitochondrial membranes results in calcineurin activation, perhaps through the proteolytically activated caspase-3 pathway [75]. Other possible pathways involve the transcriptional regulation of the nuclear and mitochondrial genes bearing GR responsive elements (GRE), demonstrated to participate in the process of oxidative phosphorylation (OXPHOS) in mitochondria [76].

5. Effects of Chronic Stress on Mitochondria and Synapses

Aberrant mitochondrial function and metabolite levels (e.g., ATP) have been documented in patients suffering from stress-related disorders [77, 78], suggesting that people harboring "low power" mitochondria could be more vulnerable to stress. The link between depression and rare polymorphisms in genes encoding for mitochondrial proteins (*TFAM, BCL2, TOMM40, and mitochondrial DNA*) provided valuable mechanistic insights although the sample size is small [77]. The impact of these mutations coincides with the reported increased fragmentation of neuronal mitochondria, the decrease of mitochondrial membrane potential, the increase of ROS production, and the decrease of mitochondrial respiration and calcium buffering capacity after exposition to chronic stress or chronic administration of synthetic glucocorticoids [6]. One consensus is that prolonged glucocorticoid signaling could damage mitochondrial functions whereas acute effects could facilitate mitochondrial functions. Speculatively, the accumulation of damaged mitochondria could erode the bioenergetics capacity of neurons particularly at risk during repetitive and intense challenges. Consistent findings were reported in the brain and peripheral cells of patients with major depressive disorders [79]. Interestingly, the brain regions featuring metabolic deregulation and dysfunctional mitochondria are those also exhibiting changes in neuronal connectivity and neurotransmission at least in animal models [80, 81].

Protective mechanisms driven by the transcription of nuclear genes evolved to limit damage of dangerous challenges. One such putative mechanism of resilience to stress

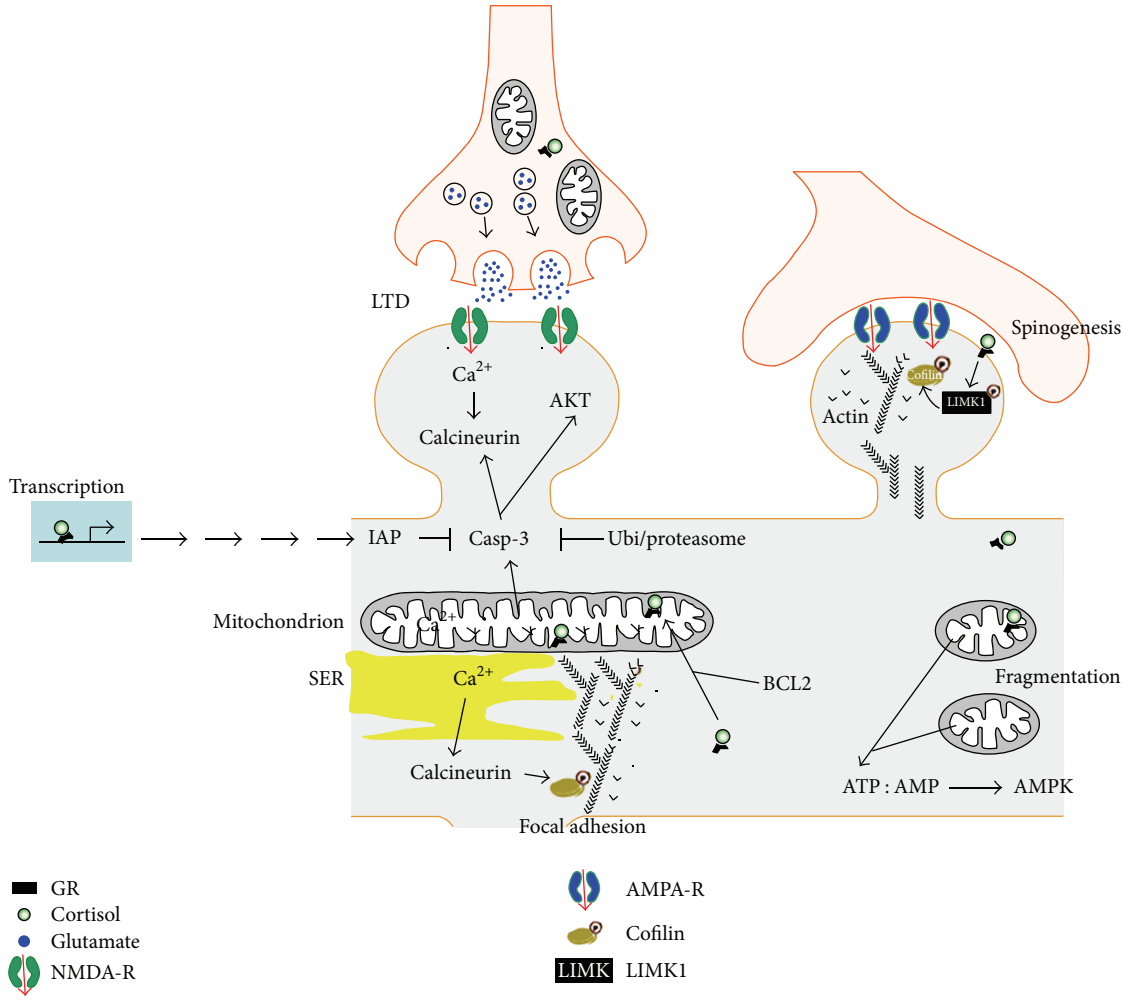


Figure 1: The mitochondria-synapse signaling loop is modulated by glucocorticoids. Acute and moderate glucocorticoid peaks rapidly promote the formation of new dendritic spines via a membrane GR coupled to the activation of the LIMK1-cofilin pathway. In contrast, glucocorticoid-mediated spine elimination is delayed and requires the transcription of new gene products. GR is present at pre- and postsynaptic membranes, in the cytoplasm, the nucleus, and the mitochondria. The exact mechanisms and series of molecular events are unknown. Trains of electrical stimulation impose an intense energy demand that can result in mitochondrial fragmentation if unmet, thereby increasing the ATP : AMP ratio, the activation of the AMP-sensing kinase to signal the local decrease of energy stores. New mitochondria can be captured in a calcium-dependent manner where energy stores are low. The levels of intracellular calcium determine whether or not to activate the calcium-dependent phosphatase calcineurin, which can be disruptive for the focal adhesion of mitochondria by dephosphorylating cofilin, impacting on the polymerization of the acting cytoskeleton tethering membranes to the mitochondria. Additionally, synaptic pruning can result from NMDAR-dependent LTD, calcium-dependent cytochrome c release whose end product is the activated caspase-3. Caspase-3 exerts local nonapoptotic effects via a broad spectrum of synaptic substrates. To this end, caspase-3 activity is retained at hotspots thanks to transcription of inhibitors of apoptosis proteins (IAP), some of which are GR-regulated genes, and by a constitutive active ubiquitin-proteasome degradation system from which caspase-3 can only be protected within the hotspots. Select transcriptional targets of GR have been involved in the regulation of respiration, mitochondrial uncoupling, and elongation, the dynamics of the actin cytoskeleton and synaptic plasticity.

is mitochondrial uncoupling operated by the family of proteins UCP (UCP1–5) [82]. The expression of UCPs, in particular UCP2, increases upon chronic stress resulting in a collapse of mitochondrial membrane polarization, dissipating the proton gradient to produce heat rather than ATP [83]. One advantage is to reduce the production of superoxides, to decrease locally the ATP : AMP ratio, resulting in the activation of the AMPK negative feedback pathway to reduce synaptic potentiation and increase metabolism where needed [84–86]. Another consequence of mitochondrial uncoupling is the elongation of mitochondria, which increases calcium-buffering capacity and counteracts mitophagy thereby enhancing mitochondrial functions not related to OXPHOS. The shape and size of the mitochondria are highly variable depending on the processes of fission and fusion. For example, Mfn1, Mfn2, SOD1, and SOD2 are downregulated by chronic exposure to corticosterone and stress [87]. Remarkably, the knockout of UCP2 exacerbated

the depressive-like phenotypes in a mouse model of chronic inflammation featuring disrupted glucocorticoid levels [88]. Therefore, promoting mitochondrial uncoupling and restoring metabolic functions of mitochondria are potential disease modifying strategies to cope with chronic stress.

6. Effects of Antidepressants on Mitochondria and Synapses

Successful antidepressant therapies eventually increase mitochondrial functions and the number of functional synapses where brain activity is reduced like in the prefrontal cortex [89]. The best-known examples are calorie restriction and voluntary exercise that enhance mitochondrial function with demonstrated antidepressant effects. These studies indicated that mitochondrial dysfunction and cortical atrophy could be reversed by lifestyle modifications [87, 90, 91]. The adaptation capacity of mitochondria and synapses to voluntary exercise was almost null in UCP2 knockouts, suggesting that a mitochondrial mechanism related to UCP2 function is required for appropriate bioenergetics adaptation of neurons to the increased neuronal plasticity induced by voluntary exercise [91].

The effect of tricyclic antidepressants was also evaluated on metabolism and mitochondrial function. In vivo, the incorporation of radiolabelled deoxyglucose is reduced in several brain regions after a single injection of clomipramine in an animal model [92, 93] and in the human brain upon acute treatment with lithium [94]. Specifically, acute treatment of cells in vitro with serotonin reuptake inhibitors (SSRI), noradrenaline serotonin reuptake inhibitors (SNRI), and monoamine oxidases inhibitors (MAOI) deteriorates mitochondrial bioenergetics capacity [77]. Ketamine also impaired mitochondrial bioenergetics capacity in vitro and in vivo [95, 96]. Only one study [97] reported the amelioration of OXPHOS with paroxetine. Although most antidepressants reduced metabolic functions of mitochondria, the redox functions increased in conditions that facilitate synaptic functions [77, 98]. Ketamine, for example, ameliorated the ratio of NADH/NAD+ by increasing SOD activity in the rat brain [96] and in stem cell derived human neurons in culture [99]. Speculatively, deterioration of OXPHOS functions by antidepressants could underlie the many side effects of treatment, whereas beneficial effects could involve the amelioration of mitochondrial redox functions [100].

Strategies to boost mitochondrial bioenergetics and/or redox functions alone or in combination with existing therapies improved symptoms of stress-related disorders in human and animal models. For example, 3 of 4 clinical trials with creatine monohydrate in combination with antidepressant drugs accelerated the efficacy of treatment modalities without improving the maximal therapeutic benefits in an overall small sample size [101–104]. Remarkably, the use of antioxidants (e.g., Zinc, N-acetyl cysteine, vitamin E, and coenzyme Q10) as supplements in the treatment of neuropsychiatric disorders also provided some promising results in major depression and bipolar disorder [105]. Another potentially interesting target of the mitochondria-synapse loop is AMPK because it is activated by intensive voluntary exercise and AMPK triggers the uncoupling mitochondrial response [106]. Remarkably, the antidepressant effects of exercise and ketamine depended on the activation of AMPK at least in the hippocampus [107, 108]. Additionally, activation of AMPK with the small molecule agonist AICAR was sufficient and more efficient than exercise for promoting antidepressant-like effects in diabetic mice [109]. Finally, the brain-derived neurotrophic factor (BDNF) is a necessary target of successful antidepressant therapies, whose signaling modulates mitochondria biogenesis, respiration and redox functions [39, 100, 110, 111], AMPK signaling, synaptogenesis, and neurotransmission [112] as a function of glucocorticoid levels frequently deregulated in patients suffering from stress-related neuropsychiatric disorders [113–118]. This important point raises the possibility that glucocorticoid and BDNF signaling pathways are dependent and both necessary for the efficacy of antidepressant therapies [119, 120].

7. Context-Dependent Glucocorticoid Signaling: BDNF Is Context!

Glucocorticoids effects on mitochondria and synapses depend on the dose, duration, and context at exposure [121]. The effects of dose and duration recapitulated by the acute and chronic stress paradigms were discussed earlier. The context at exposure such as learning at the time of glucocorticoid oscillation peak could produce unique effects, temporally restricted, that are the results of the coupling of neuronal activity with glucocorticoid signaling in select neuronal networks [64, 121]. For example, a single injection of glucocorticoids within minutes of motor skill training enhanced motor learning. In contrast, similar dose administered hours before or after has no effects on behavior, on dendritic spine patterning, or on neurotransmission [64, 122, 123].

Contextual glucocorticoid signaling is determined by (1) *the temporal window*: survival of learning-associated new spines and behavioral performance required that circadian peaks and troughs remain normal during the week after learning to consolidate the acquired memory in the form of clusters of dendritic spines; and by (2) *the spatial window*: plasticity occurred in the amygdala when learning fear conditioning, in the hippocampus CA1 when electrically stimulating the Schaffer collaterals, and in the motor cortex when learning motor skill abilities. If true, glucocorticoid-mediated spine plasticity during motor skill learning should be specific of the motor cortex. Out of the context of motor learning, glucocorticoids enhance the turnover of dendritic spines in all the cortical regions tested (sensory S1, S2, frontal cortex, and motor M1, M2), but these new spines are short-lived and cannot survive at distant time point [65]. Only glucocorticoid signaling within the context of motor learning elicited the survival of clusters of dendritic spines for long periods of time, perhaps to store new information [64]. Such coincidence detection of glucocorticoids signaling with neuronal activity in the cortical circuits involved in learning could be essential for the encoding of the memory trace. It means that glucocorticoid therapies based on chronic nonpulsatile treatment could cause spine patterning defects

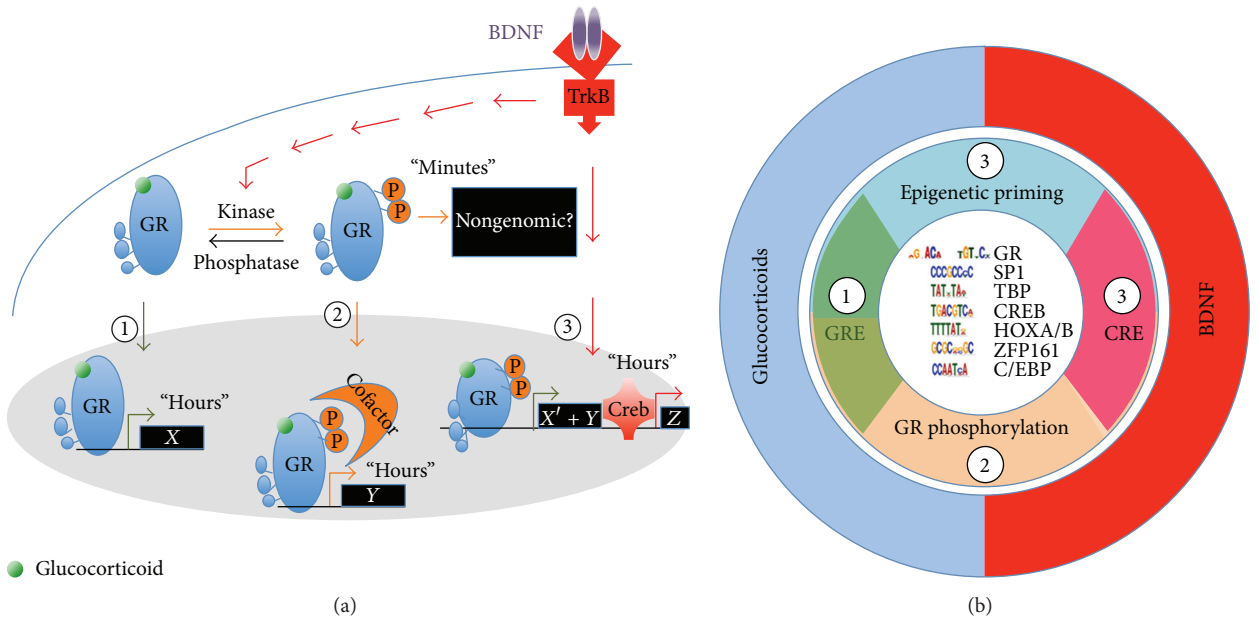


Figure 2: Neurotrophic priming of glucocorticoid signaling. (a) The coincidence of BDNF and glucocorticoid signaling triggers rapid and slow effects different from the sum of genes regulated by individual pathways. *Pathway-1*: glucocorticoids impact the expression of GR-regulated genes. *Pathway-2*: BDNF-induced GR phosphorylation could foster the recruitment of cofactors that change transcriptional output. *Pathway-3*: BDNF/TrkB-responding genes plus epigenetic priming at locus previously unexposed to the activated GR. (b) In the center are listed the most represented genomic DNA ligands bound to GR upon stimulation of cortical neurons with BDNF and dexamethasone. The interplay of BDNF and glucocorticoid signaling uses the mechanisms of epigenetic priming as well as GR phosphorylation to specify the range of targets. Image adapted from [124].

and cognitive disabilities by altering the coincidence [125]. Importantly, the recent discovery that memory does not correlate with the long-term survival of dendritic spines in the hippocampus suggests that cortical regions operate differently [126]. In this case, the role of hippocampal neurogenesis, which is absent from the cortex, is obviously an important aspect for future studies.

What could be the molecular mechanism of coincidence? The convergence of two signaling pathways is anticipated: (1) one known, the glucocorticoid, and (2) one unknown but activity-dependent. If GR signaling is central for glucocorticoid spine plasticity and mitochondrial function, BDNF signaling is a suitable candidate, pivot of activity-dependent synaptic plasticity and mitochondrial function [112, 114, 127, 128]. Other putative candidate modulators of GR signaling have also been described (e.g., norepinephrine, inflammatory cytokines, FGF, and IGF1) [123, 129–132]. BDNF signaling via its receptor TrkB can rewrite GR-mediated gene expression signature [133]. This effect resulted from a composite response of (1) additive effects perhaps due to epigenetic priming and of (2) unique effects at a select cluster of genes that only responded to the coincidence of BDNF and GR signaling (Figure 2).

Mechanistically, BDNF signaling elicits the phosphorylation of GR at serines 134 and 267, which fosters cofactor recruitment like CREB1 to promote a novel gene expression signature [133]. Among target genes, *CRH* expression is decreased in the hypothalamus and increased in the amygdala by GR signaling [58, 134]. In the context of BDNF signaling in the hypothalamus, GR signaling used the CREB coactivator CRTC2 as coincidence detector to activate or suppress CRH expression with physiological consequences on the neuroendocrine responses of stress in mice [135]. In the context of contextual fear learning, glucocorticoid-induced memory consolidation of inhibitory avoidance required coincident BDNF signaling in the hippocampus [136]. The effects of concomitant BDNF and glucocorticoids signaling depended on the dose, duration, and context at exposure [120].

8. Glucocorticoid Resistance in Stress-Related Neuropsychiatric Disorders

Glucocorticoid resistance is a state of reduced tissue responsiveness to glucocorticoids [137, 138] observed in disorders of chronic inflammation, during aging, in neuropsychiatric disorders, and in neurodegenerative diseases. Major hallmarks of these conditions are the elevated levels of circulating cortisol (due to a lack of GR-mediated negative feedback control) and reduced BDNF levels [116, 119, 139, 140]. The question remains whether deficits of BDNF can impair context-dependent GR signaling that may set the stage for developing GR resistance. Transcriptomic analyses of GR-regulated genes in depressed patients compared to healthy controls indicated that the expression of select clusters of genes (e.g., anti-inflammatory) is impaired while others are not affected [141]. Such selectivity at target genes indicates

Table 1: Summary of the cellular and physiological effects of BDNF, glucocorticoids, stress, major depressive disorders, and antidepressant therapies.

	BDNF	Glucocorticoids	Stress	Major depression	Antidepressant therapies
Synaptic structure	Formation, maintenance	Formation, elimination, and maintenance[#¶]	Formation, elimination[#¶]	Elimination[¶]	Formation, maintenance[#¶]
Synaptic function	Potentiation	Potentiation, depression[#¶]	Potentiation, depression[#¶]	Depression[¶]	Potentiation[#¶]
Mitochondria structure	Biogenesis[*]	Augmented/diminished[#*]	Augmented/diminished[#]	Diminished[¶]	Augmented/diminished[#*]
Mitochondria energetics function	Augmented [¶]	Augmented/diminished[#*]	Augmented/diminished[#*]	Diminished[¶]	Augmented/diminished[#*]
Mitochondria redox function	Augmented[¶]	Augmented/diminished[#*]	Augmented/diminished[#]	Diminished[¶]	Augmented[#*]
Learning and memory	Augmented[¶]	Augmented/diminished[#]	Augmented/diminished[#]	Diminished	Augmented[#]
Despair and anxiety	Diminished[¶]	Augmented/diminished[#]	Augmented/diminished[#]	Augmented	Diminished[#]

#: effect depends on the dose, duration, and context at exposure.
¶: effect is specific of the brain region.
∗: effect demonstrated in cultured cells.

that mechanisms of glucocorticoid resistance are complex, resembling a loss of context-dependent GR signaling.

Several mechanisms of GR resistance have been proposed: (i) a decreased expression of GR that has been reported, as much as the increased expression of inactive splice variants [142], (ii) rare mutations in the GR that can cause a generalized glucocorticoid resistance syndrome [143], (iii) impaired epigenetic control of GR-regulated gene expression by HDAC2 [144, 145], and (iv) the transport of GR into mitochondrial matrix which fails in cells resistant to the biological effects of glucocorticoids [146, 147]. Remarkably, glucocorticoids modulate mitochondrial biogenesis and OXPHOS pathway by regulating the transcription of the mitochondrial genome [148, 149]. Amidst the complexity, context-dependent GR signaling in the form of BDNF-priming of GR signaling could unify the neurotrophic hypothesis [150] and the glucocorticoid hypothesis [151] of depression by modulating mitochondrial responses.

9. Conclusions

Glucocorticoid resistance is a common feature of ageing, neurodegenerative diseases, and neuropsychiatric disorders, conditions that can be ameliorated with BDNF mimetic therapies (e.g., electroconvulsive shock, deep brain stimulation, antidepressant drugs, and exercise). Table 1 summarizes the effects of BDNF, glucocorticoids, stress, major depressive disorders, and antidepressant therapies on mitochondrial and synaptic functions. In theory, chronic antidepressant treatment, BDNF signaling, and context-dependent GR signaling should be associated with improved mitochondrial functions and positive neuroplasticity. In reality, antidepressants improve the redox function while deteriorating the bioenergetics capacity, which could explain the mixed benefits of such treatments. On the contrary, inherited and environmental factors that diminished BDNF and GR functions can aggravate the progression of neuropsychiatric disabilities. This is the case of highly penetrant gene mutations in *BDNF* (Val66met, [152, 153]), *TrkB* (Y722C, [154]), *FKBP51* (rs1360780 [155]), *5-HTTLPR* (rs6354 and rs2020936 [156, 157]), and *COMT* (Val158Met [158]). Likewise, chronic stress, disrupted circadian rhythms, chronic neuroinflammation, and chronic high dose glucocorticoid therapies are associated with "low power" mitochondria and negative neuroplasticity.

BDNF and glucocorticoids are essential for cognitive functions and stress coping. Deregulation of their activities is a risk factor for developing psychiatric disorders by impairing synaptic plasticity of brain circuits mediating reward learning, while bolstering circuits mediating aversion learning. Future strategies aiming at boosting mitochondrial functions will identify new targets against GR resistance, perhaps at intersection of BDNF and glucocorticoid signaling pathways in specific circuits.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

The authors are supported by CNRS, INSERM, and the University of Montpellier and they thank the Marie Curie Action, the foundation Jean Valade, and the Fondation de France for sponsoring.

References

[1] E. R. de Kloet, M. Joëls, and F. Holsboer, "Stress and the brain: from adaptation to disease," *Nature Reviews Neuroscience*, vol. 6, no. 6, pp. 463–475, 2005.

[2] B. S. Hall, R. N. Moda, and C. Liston, "Glucocorticoid mechanisms of functional connectivity changes in stress-related neuropsychiatric disorders," *Neurobiology of Stress*, vol. 1, no. 1, pp. 174–183, 2015.

[3] B. S. McEwen and J. H. Morrison, "The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course," *Neuron*, vol. 79, no. 1, pp. 16–29, 2013.

[4] I. N. Karatsoreos and B. S. McEwen, "Psychobiological allostasis: resistance, resilience and vulnerability," *Trends in Cognitive Sciences*, vol. 15, no. 12, pp. 576–584, 2011.

[5] B. S. McEwen and T. Seeman, "Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load," *Annals of the New York Academy of Sciences*, vol. 896, pp. 30–47, 1999.

[6] M. Picard, R.-P. Juster, and B. S. McEwen, "Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids," *Nature Reviews Endocrinology*, vol. 10, no. 5, pp. 303–310, 2014.

[7] C. Liston, M. M. Miller, D. S. Goldwater et al., "Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting," *The Journal of Neuroscience*, vol. 26, no. 30, pp. 7870–7874, 2006.

[8] B. McEwen and J. H. Morrison, "The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course," *Neuron*, vol. 79, no. 1, pp. 16–29, 2013.

[9] C. L. Wellman, "Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration," *Journal of Neurobiology*, vol. 49, no. 3, pp. 245–253, 2001.

[10] M. Popoli, Z. Yan, B. S. McEwen, and G. Sanacora, "The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission," *Nature Reviews Neuroscience*, vol. 13, no. 1, pp. 22–37, 2012.

[11] J. Courchet, T. L. Lewis Jr., S. Lee et al., "Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture," *Cell*, vol. 153, no. 7, pp. 1510–1525, 2013.

[12] Z.-H. Sheng, "Mitochondrial trafficking and anchoring in neurons: new insight and implications," *The Journal of Cell Biology*, vol. 204, no. 7, pp. 1087–1098, 2014.

[13] D. T. W. Chang, A. S. Honick, and I. J. Reynolds, "Mitochondrial trafficking to synapses in cultured primary cortical neurons," *Journal of Neuroscience*, vol. 26, no. 26, pp. 7035–7045, 2006.

[14] N. Kasthuri, K. J. Hayworth, D. R. Berger et al., "Saturated reconstruction of a volume of neocortex," *Cell*, vol. 162, no. 3, pp. 648–661, 2015.

[15] C. C. Overly, H. I. Rieff, and P. J. Hollenbeck, "Organelle motility and metabolism in axons vs dendrites of cultured hippocampal neurons," *Journal of Cell Science*, vol. 109, no. 5, pp. 971–980, 1996.

[16] A. Suzuki, S. A. Stern, O. Bozdagi et al., "Astrocyte-neuron lactate transport is required for long-term memory formation," *Cell*, vol. 144, no. 5, pp. 810–823, 2011.

[17] J. J. Laschet, F. Minier, I. Kurcewicz et al., "Glyceraldehyde-3-phosphate dehydrogenase is a $GABA_A$ receptor kinase linking glycolysis to neuronal inhibition," *Journal of Neuroscience*, vol. 24, no. 35, pp. 7614–7622, 2004.

[18] K. Wu, C. Aoki, A. Elste, A. A. Rogalski-Wilk, and P. Siekevitz, "The synthesis of ATP by glycolytic enzymes in the postsynaptic density and the effect of endogenously generated nitric oxide," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 24, pp. 13273–13278, 1997.

[19] J. J. Harris, R. Jolivet, and D. Attwell, "Synaptic energy use and supply," *Neuron*, vol. 75, no. 5, pp. 762–777, 2012.

[20] M. T. J. Lowe, E. H. Kim, R. L. M. Faull, D. L. Christie, and H. J. Waldvogel, "Dissociated expression of mitochondrial and cytosolic creatine kinases in the human brain: a new perspective on the role of creatine in brain energy metabolism," *Journal of Cerebral Blood Flow and Metabolism*, vol. 33, no. 8, pp. 1295–1306, 2013.

[21] Z. Li, K.-I. Okamoto, Y. Hayashi, and M. Sheng, "The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses," *Cell*, vol. 119, no. 6, pp. 873–887, 2004.

[22] S. S. Kannurpatti, P. G. Joshi, and N. B. Joshi, "Calcium sequestering ability of mitochondria modulates influx of calcium through glutamate receptor channel," *Neurochemical Research*, vol. 25, no. 12, pp. 1527–1536, 2000.

[23] A. Zorzano and M. Claret, "Implications of mitochondrial dynamics on neurodegeneration and on hypothalamic dysfunction," *Frontiers in Aging Neuroscience*, vol. 7, p. 101, 2015.

[24] B. Billups and I. D. Forsythe, "Presynaptic mitochondrial calcium sequestration influences transmission at mammalian central synapses," *Journal of Neuroscience*, vol. 22, no. 14, pp. 5840–5847, 2002.

[25] D. G. Nicholls, "Mitochondrial calcium function and dysfunction in the central nervous system," *Biochimica et Biophysica Acta—Bioenergetics*, vol. 1787, no. 11, pp. 1416–1424, 2009.

[26] J. G. McCormack and R. M. Denton, "The role of mitochondrial Ca^{2+} transport and matrix Ca^{2+} in signal transduction in mammalian tissues," *Biochimica et Biophysica Acta: Bioenergetics*, vol. 1018, no. 2-3, pp. 287–291, 1990.

[27] M. R. Duchen, "Ca^{2+}-dependent changes in the mitochondrial energetics in single dissociated mouse sensory neurons," *Biochemical Journal*, vol. 283, part 1, pp. 41–50, 1992.

[28] S.-J. Zhang, M. Zou, L. Lu et al., "Nuclear calcium signaling controls expression of a large gene pool: identification of a gene program for acquired neuroprotection induced by synaptic activity," *PLoS Genetics*, vol. 5, no. 8, Article ID e1000604, 2009.

[29] C. A. Massaad and E. Klann, "Reactive oxygen species in the regulation of synaptic plasticity and memory," *Antioxidants and Redox Signaling*, vol. 14, no. 10, pp. 2013–2054, 2011.

[30] C. A. Massaad, T. M. Washington, R. G. Pautler, and E. Klann, "Overexpression of SOD-2 reduces hippocampal superoxide and prevents memory deficits in a mouse model of Alzheimer's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 32, pp. 13576–13581, 2009.

[31] D. Hu, P. Cao, E. Thiels et al., "Hippocampal long-term potentiation, memory, and longevity in mice that overexpress mitochondrial superoxide dismutase," *Neurobiology of Learning and Memory*, vol. 87, no. 3, pp. 372–384, 2007.

[32] B. Uttara, A. V. Singh, P. Zamboni, and R. T. Mahajan, "Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options," *Current Neuropharmacology*, vol. 7, no. 1, pp. 65–74, 2009.

[33] Z. Li, J. Jo, J.-M. Jia et al., "Caspase-3 activation via mitochondria is required for long-term depression and AMPA receptor internalization," *Cell*, vol. 141, no. 5, pp. 859–871, 2010.

[34] M. D'Amelio, M. Sheng, and F. Cecconi, "Caspase-3 in the central nervous system: beyond apoptosis," *Trends in Neurosciences*, vol. 35, no. 11, pp. 700–709, 2012.

[35] A. Ertürk, Y. Wang, and M. Sheng, "Local pruning of dendrites and spines by caspase-3-dependent and proteasome-limited mechanisms," *Journal of Neuroscience*, vol. 34, no. 5, pp. 1672–1688, 2014.

[36] M. P. Mattson, M. Gleichmann, and A. Cheng, "Mitochondria in neuroplasticity and neurological disorders," *Neuron*, vol. 60, no. 5, pp. 748–766, 2008.

[37] A. Kaasik, D. Safiulina, A. Zharkovsky, and V. Veksler, "Regulation of mitochondrial matrix volume," *American Journal of Physiology—Cell Physiology*, vol. 292, no. 1, pp. C157–C163, 2007.

[38] J. Y. Sung, O. Engmann, M. A. Teylan, A. C. Nairn, P. Greengard, and Y. Kim, "WAVE1 controls neuronal activity-induced mitochondrial distribution in dendritic spines," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 8, pp. 3112–3116, 2008.

[39] A. Cheng, R. Wan, J.-L. Yang et al., "Involvement of PGC-1alpha in the formation and maintenance of neuronal dendritic spines," *Nature Communications*, vol. 3, article 1250, 2012.

[40] G. G. Turrigiano, "The self-tuning neuron: synaptic scaling of excitatory synapses," *Cell*, vol. 135, no. 3, pp. 422–435, 2008.

[41] T. C. Thiagarajan, M. Lindskog, and R. W. Tsien, "Adaptation to synaptic inactivity in hippocampal neurons," *Neuron*, vol. 47, no. 5, pp. 725–737, 2005.

[42] G. G. Turrigiano, K. R. Leslie, N. S. Desai, L. C. Rutherford, and S. B. Nelson, "Activity-dependent scaling of quantal amplitude in neocortical neurons," *Nature*, vol. 391, no. 6670, pp. 892–896, 1998.

[43] A. Maffei, S. B. Nelson, and G. G. Turrigiano, "Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation," *Nature Neuroscience*, vol. 7, no. 12, pp. 1353–1359, 2004.

[44] X. Wang and T. L. Schwarz, "The mechanism of Ca^{2+}-dependent regulation of kinesin-mediated mitochondrial motility," *Cell*, vol. 136, no. 1, pp. 163–174, 2009.

[45] V. P. Bindokas, C. C. Lee, W. F. Colmers, and R. J. Miller, "Changes in mitochondrial function resulting from synaptic activity in the rat hippocampal slice," *Journal of Neuroscience*, vol. 18, no. 12, pp. 4570–4587, 1998.

[46] M. Dahlhaus, K. W. Li, R. C. van der Schors et al., "The synaptic proteome during development and plasticity of the mouse visual cortex," *Molecular & Cellular Proteomics*, vol. 10, no. 5, Article ID M110.005413, 2011.

[47] D. Attwell and S. B. Laughlin, "An energy budget for signaling in the grey matter of the brain," *Journal of Cerebral Blood Flow and Metabolism*, vol. 21, no. 10, pp. 1133–1145, 2001.

[48] Y.-G. Tang and R. S. Zucker, "Mitochondrial involvement in post-tetanic potentiation of synaptic transmission," *Neuron*, vol. 18, no. 3, pp. 483–491, 1997.

[49] W. B. Potter, K. J. O'Riordan, D. Barnett et al., "Metabolic regulation of neuronal plasticity by the energy sensor AMPK," *PLoS ONE*, vol. 5, no. 2, Article ID e8996, 2010.

[50] B. S. McEwen and J. C. Wingfield, "The concept of allostasis in biology and biomedicine," *Hormones and Behavior*, vol. 43, no. 1, pp. 2–15, 2003.

[51] G. E. Walsberg, "How useful is energy balance as a overall index of stress in animals?" *Hormones and Behavior*, vol. 43, no. 1, pp. 16–17, 2003.

[52] J. Maniam, C. Antoniadis, and M. J. Morris, "Early-life stress, HPA axis adaptation, and mechanisms contributing to later health outcomes," *Frontiers in Endocrinology*, vol. 5, article 73, 2014.

[53] I. Ouellet-Morin, M. Boivin, G. Dionne et al., "Variations in heritability of cortisol reactivity to stress as a function of early familial adversity among 19-month-old twins," *Archives of General Psychiatry*, vol. 65, no. 2, pp. 211–218, 2008.

[54] A. B. Amstadter, J. M. Myers, and K. S. Kendler, "Psychiatric resilience: longitudinal twin study," *British Journal of Psychiatry*, vol. 205, no. 4, pp. 275–280, 2014.

[55] M. W. Logue, A. B. Amstadter, D. G. Baker et al., "The psychiatric genomics consortium posttraumatic stress disorder workgroup: posttraumatic stress disorder enters the age of large-scale genomic collaboration," *Neuropsychopharmacology*, vol. 40, no. 10, pp. 2287–2297, 2015.

[56] R. Yehuda, "Risk and resilience in posttraumatic stress disorder," *Journal of Clinical Psychiatry*, vol. 65, supplement 1, pp. 29–36, 2004.

[57] B. L. Conway-Campbell, J. R. Pooley, G. L. Hager, and S. L. Lightman, "Molecular dynamics of ultradian glucocorticoid receptor action," *Molecular and Cellular Endocrinology*, vol. 348, no. 2, pp. 383–393, 2012.

[58] A. G. Watts, "Glucocorticoid regulation of peptide genes in neuroendocrine CRH neurons: a complexity beyond negative feedback," *Frontiers in Neuroendocrinology*, vol. 26, no. 3-4, pp. 109–130, 2005.

[59] B. S. McEwen, "Physiology and neurobiology of stress and adaptation: central role of the brain," *Physiological Reviews*, vol. 87, no. 3, pp. 873–904, 2007.

[60] S. J. Lupien, B. S. McEwen, M. R. Gunnar, and C. Heim, "Effects of stress throughout the lifespan on the brain, behaviour and cognition," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 434–445, 2009.

[61] B. Roozendaal, E. L. Hahn, S. V. Nathan, D. J.-F. De Quervain, and J. L. McGaugh, "Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala," *Journal of Neuroscience*, vol. 24, no. 37, pp. 8161–8169, 2004.

[62] B. Roozendaal, "Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval," *Neurobiology of Learning and Memory*, vol. 78, no. 3, pp. 578–595, 2002.

[63] H. J. Krugers, H. Karst, and M. Joels, "Interactions between noradrenaline and corticosteroids in the brain: from electrical activity to cognitive performance," *Frontiers in Cellular Neuroscience*, vol. 6, p. 15, 2012.

[64] C. Liston, J. M. Cichon, F. Jeanneteau, Z. Jia, M. V. Chao, and W.-B. Gan, "Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance," *Nature Neuroscience*, vol. 16, no. 6, pp. 698–705, 2013.

[65] C. Liston and W.-B. Gan, "Glucocorticoids are critical regulators of dendritic spine development and plasticity in vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 38, pp. 16074–16079, 2011.

[66] J. Du, Y. Wang, R. Hunter et al., "Dynamic regulation of mitochondrial function by glucocorticoids," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 9, pp. 3543–3548, 2009.

[67] L. E. Ostroff, C. K. Cain, N. Jindal, N. Dar, and J. E. Ledoux, "Stability of presynaptic vesicle pools and changes in synapse

morphology in the amygdala following fear learning in adult rats," *Journal of Comparative Neurology*, vol. 520, no. 2, pp. 295–314, 2012.

[68] G. Morris and M. Berk, "The many roads to mitochondrial dysfunction in neuroimmune and neuropsychiatric disorders," *BMC Medicine*, vol. 13, article 68, 2015.

[69] A. M. Cataldo, D. L. McPhie, N. T. Lange et al., "Abnormalities in mitochondrial structure in cells from patients with bipolar disorder," *The American Journal of Pathology*, vol. 177, no. 2, pp. 575–585, 2010.

[70] H. S. Mayberg, M. Liotti, S. K. Brannan et al., "Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness," *American Journal of Psychiatry*, vol. 156, no. 5, pp. 675–682, 1999.

[71] S. Rigucci, G. Serafini, M. Pompili, G. D. Kotzalidis, and R. Tatarelli, "Anatomical and functional correlates in major depressive disorder: the contribution of neuroimaging studies," *World Journal of Biological Psychiatry*, vol. 11, no. 2, part 2, pp. 165–180, 2010.

[72] L. R. Johnson, C. Farb, J. H. Morrison, B. S. McEwen, and J. E. LeDoux, "Localization of glucocorticoid receptors at postsynaptic membranes in the lateral amygdala," *Neuroscience*, vol. 136, no. 1, pp. 289–299, 2005.

[73] H. Beck, K. Flynn, K. S. Lindenberg et al., "Serum response factor (SRF)-cofilin-actin signaling axis modulates mitochondrial dynamics," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 38, pp. E2523–E2532, 2012.

[74] B. W. Bernstein and J. R. Bamburg, "ADF/Cofilin: a functional node in cell biology," *Trends in Cell Biology*, vol. 20, no. 4, pp. 187–195, 2010.

[75] M. Sheng and A. Ertürk, "Long-term depression: a cell biological view," *Philosophical Transactions of the Royal Society of London B: Biological sciences*, vol. 369, no. 1633, 7 pages, 2014.

[76] A. M. G. Psarra, S. Solakidi, and C. E. Sekeris, "The mitochondrion as a primary site of action of regulatory agents involved in neuroimmunomodulation," *Annals of the New York Academy of Sciences*, vol. 1088, pp. 12–22, 2006.

[77] N. J. Klinedinst and W. T. Regenold, "A mitochondrial bioenergetic basis of depression," *Journal of Bioenergetics and Biomembranes*, vol. 47, no. 1-2, pp. 155–171, 2015.

[78] E. L. Streck, C. L. Gonçalves, C. B. Furlanetto, G. Scaini, F. Dal-Pizzol, and J. Quevedo, "Mitochondria and the central nervous system: searching for a pathophysiological basis of psychiatric disorders," *Revista Brasileira de Psiquiatria*, vol. 36, no. 2, pp. 156–167, 2014.

[79] A. Karabatsiakis, C. Böck, J. Salinas-Manrique et al., "Mitochondrial respiration in peripheral blood mononuclear cells correlates with depressive subsymptoms and severity of major depression," *Translational Psychiatry*, vol. 4, article e397, 2014.

[80] J. L. M. Madrigal, R. Olivenza, M. A. Moro et al., "Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain," *Neuropsychopharmacology*, vol. 24, no. 4, pp. 420–429, 2001.

[81] G. T. Rezin, M. R. Cardoso, C. L. Gonçalves et al., "Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression," *Neurochemistry International*, vol. 53, no. 6–8, pp. 395–400, 2008.

[82] Z. B. Andrews, S. Diano, and T. L. Horvath, "Mitochondrial uncoupling proteins in the CNS: in support of function and survival," *Nature Reviews Neuroscience*, vol. 6, no. 11, pp. 829–840, 2005.

[83] E. Kasahara, A. Sekiyama, M. Hori et al., "Stress-induced glucocorticoid release upregulates uncoupling protein-2 expression and enhances resistance to endotoxin-induced lethality," *Neuroimmunomodulation*, vol. 22, no. 5, pp. 279–292, 2015.

[84] G. R. Steinberg and B. E. Kemp, "AMPK in health and disease," *Physiological Reviews*, vol. 89, no. 3, pp. 1025–1078, 2009.

[85] G. Mairet-Coello, J. Courchet, S. Pieraut, V. Courchet, A. Maximov, and F. Polleux, "The CAMKK2-AMPK kinase pathway mediates the synaptotoxic effects of Abeta oligomers through Tau phosphorylation," *Neuron*, vol. 78, no. 1, pp. 94–108, 2013.

[86] G. Mairet-Coello and F. Polleux, "Involvement of 'stress-response' kinase pathways in Alzheimer's disease progression," *Current Opinion in Neurobiology*, vol. 27, pp. 110–117, 2014.

[87] W. Liu and C. Zhou, "Corticosterone reduces brain mitochondrial function and expression of mitofusin, BDNF in depression-like rodents regardless of exercise preconditioning," *Psychoneuroendocrinology*, vol. 37, no. 7, pp. 1057–1070, 2012.

[88] X.-L. Sun, Y. Liu, T. Dai, J.-H. Ding, and G. Hu, "Uncoupling protein 2 knockout exacerbates depression-like behaviors in mice via enhancing inflammatory response," *Neuroscience*, vol. 192, pp. 507–514, 2011.

[89] A. Moretti, A. Gorini, and R. F. Villa, "Affective disorders, antidepressant drugs and brain metabolism," *Molecular Psychiatry*, vol. 8, no. 9, pp. 773–785, 2003.

[90] T. V. Arumugam, M. Gleichmann, S.-C. Tang, and M. P. Mattson, "Hormesis/preconditioning mechanisms, the nervous system and aging," *Ageing Research Reviews*, vol. 5, no. 2, pp. 165–178, 2006.

[91] M. O. Dietrich, Z. B. Andrews, and T. L. Horvath, "Exercise-induced synaptogenesis in the hippocampus is dependent on UCP2-regulated mitochondrial adaptation," *Journal of Neuroscience*, vol. 28, no. 42, pp. 10766–10771, 2008.

[92] U. Freo, P. Pietrini, M. Dam, G. Pizzolato, and L. Battistin, "The tricyclic antidepressant clomipramine dose-dependently reduces regional cerebral metabolic rates for glucose in awake rats," *Psychopharmacology*, vol. 113, no. 1, pp. 53–59, 1993.

[93] U. Freo, P. Pietrini, G. Pizzolato et al., "Cerebral metabolic responses to clomipramine are greatly reduced following pretreatment with the specific serotonin neurotoxin parachloroamphetamine (PCA). A 2-deoxyglucose study in rats," *Neuropsychopharmacology*, vol. 13, no. 3, pp. 215–222, 1995.

[94] G. M. Goodwin, J. T. O. Cavanagh, M. F. Glabus, R. F. Kehoe, R. E. O'Carroll, and K. P. Ebmeier, "Uptake of 99mTc-exametazime shown by single photon emission computed tomography before and after lithium withdrawal in bipolar patients: associations with mania," *British Journal of Psychiatry*, vol. 170, pp. 426–430, 1997.

[95] K. Weckmann, C. Labermaier, J. M. Asara, M. B. Müller, and C. W. Turck, "Time-dependent metabolomic profiling of Ketamine drug action reveals hippocampal pathway alterations and biomarker candidates," *Translational Psychiatry*, vol. 4, no. 11, article e481, 2014.

[96] C. Venâncio, L. Félix, V. Almeida et al., "Acute ketamine impairs mitochondrial function and promotes superoxide dismutase activity in the rat brain," *Anesthesia and Analgesia*, vol. 120, no. 2, pp. 320–328, 2015.

[97] J. Jeong, M. Park, J. S. Yoon et al., "Requirement of AMPK activation for neuronal metabolic-enhancing effects of antidepressant paroxetine," *NeuroReport*, vol. 26, no. 7, pp. 424–428, 2015.

[98] N. Li, B. Lee, R.-J. Liu et al., "mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists," *Science*, vol. 329, no. 5994, pp. 959–964, 2010.

[99] H. Ito, T. Uchida, and K. Makita, "Ketamine causes mitochondrial dysfunction in human induced pluripotent stem cell-derived neurons," *PLoS ONE*, vol. 10, no. 5, Article ID e0128445, 2015.

[100] S.-Y. Lee, S.-J. Lee, C. Han, A. A. Patkar, P. S. Masand, and C.-U. Pae, "Oxidative/nitrosative stress and antidepressants: targets for novel antidepressants," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 46, pp. 224–235, 2013.

[101] D. G. Kondo, Y.-H. Sung, T. L. Hellem et al., "Open-label adjunctive creatine for female adolescents with SSRI-resistant major depressive disorder: a 31-phosphorus magnetic resonance spectroscopy study," *Journal of Affective Disorders*, vol. 135, no. 1–3, pp. 354–361, 2011.

[102] I. K. Lyoo, S. Yoon, T.-S. Kim et al., "A randomized, double-blind placebo-controlled trial of oral creatine monohydrate augmentation for enhanced response to a selective serotonin reuptake inhibitor in women with major depressive disorder," *American Journal of Psychiatry*, vol. 169, no. 9, pp. 937–945, 2012.

[103] B. Nemets and J. Levine, "A pilot dose-finding clinical trial of creatine monohydrate augmentation to SSRIs/SNRIs/NASA antidepressant treatment in major depression," *International Clinical Psychopharmacology*, vol. 28, no. 3, pp. 127–133, 2013.

[104] S. Roitman, T. Green, Y. Osher, N. Karni, and J. Levine, "Creatine monohydrate in resistant depression: a preliminary study," *Bipolar Disorders*, vol. 9, no. 7, pp. 754–758, 2007.

[105] C. D. Pandya, K. R. Howell, and A. Pillai, "Antioxidants as potential therapeutics for neuropsychiatric disorders," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 46, pp. 214–223, 2013.

[106] D. G. Hardie, "AMP-activated protein kinase as a drug target," *Annual Review of Pharmacology and Toxicology*, vol. 47, pp. 185–210, 2007.

[107] S. X. Xu, Z. Q. Zhou, X. M. Li, M. H. Ji, G. F. Zhang, and J. J. Yang, "The activation of adenosine monophosphate-activated protein kinase in rat hippocampus contributes to the rapid antidepressant effect of ketamine," *Behavioural Brain Research*, vol. 253, pp. 305–309, 2013.

[108] S. Y. Yaua, A. Lia, R. L. C. Hooc et al., "Physical exercise-induced hippocampal neurogenesis and antidepressant effects are mediated by the adipocyte hormone adiponectin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 44, pp. 15810–15815, 2014.

[109] W. Liu, X. Zhai, H. Li, and L. Ji, "Depression-like behaviors in mice subjected to co-treatment of high-fat diet and corticosterone are ameliorated by AICAR and exercise," *Journal of Affective Disorders*, vol. 156, pp. 171–177, 2014.

[110] T. He and Z. S. Katusic, "Brain-derived neurotrophic factor increases expression of MnSOD in human circulating angiogenic cells," *Microvascular Research*, vol. 83, no. 3, pp. 366–371, 2012.

[111] Z. Wu, P. Puigserver, U. Andersson et al., "Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1," *Cell*, vol. 98, no. 1, pp. 115–124, 1999.

[112] F. Jeanneteau, M. V. Chao, and F. Jeanneteau, "Neurotrophins and synaptogenesis," in *Comprehensive Developmental Neuroscience: Cellular Migration and Formation of Neuronal Connections*, J. P. R. Rakic, Ed., pp. 639–659, Elsevier, San Diego, Calif, USA, 2013.

[113] C. D. Wrann, J. White, J. Salogiannnis et al., "Exercise induces hippocampal BDNF through a PGC-1alpha/FNDC5 pathway," *Cell Metabolism*, vol. 18, no. 5, pp. 649–659, 2013.

[114] A. Markham, R. Bains, P. Franklin, and M. Spedding, "Changes in mitochondrial function are pivotal in neurodegenerative and psychiatric disorders: how important is BDNF?" *British Journal of Pharmacology*, vol. 171, no. 8, pp. 2206–2229, 2014.

[115] O. Berton and E. J. Nestler, "New approaches to antidepressant drug discovery: beyond monoamines," *Nature Reviews Neuroscience*, vol. 7, no. 2, pp. 137–151, 2006.

[116] F. Holsboer and M. Ising, "Stress hormone regulation: biological role and translation into therapy," *Annual Review of Psychology*, vol. 61, pp. 81–109, 2010.

[117] A. E. Autry, M. Adachi, E. Nosyreva et al., "NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses," *Nature*, vol. 475, no. 7354, pp. 91–95, 2011.

[118] E. Castrén and T. Rantamäki, "The role of BDNF and its receptors in depression and antidepressant drug action: reactivation of developmental plasticity," *Developmental Neurobiology*, vol. 70, no. 5, pp. 289–297, 2010.

[119] J. D. Gray, T. A. Milner, and B. S. McEwen, "Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors," *Neuroscience*, vol. 239, pp. 214–227, 2013.

[120] F. Jeanneteau and M. V. Chao, "Are BDNF and glucocorticoid activities calibrated?" *Neuroscience*, vol. 239, pp. 173–195, 2013.

[121] M. Arango-Lievano, W. M. Lambert, and F. Jeanneteau, "Molecular biology of glucocorticoid signaling," *Advances in Experimental Medicine and Biology*, vol. 872, pp. 33–57, 2015.

[122] H. Karst and M. Joëls, "Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal cells," *Journal of Neurophysiology*, vol. 94, no. 5, pp. 3479–3486, 2005.

[123] B. Roozendaal, S. Okuda, E. A. Van Der Zee, and J. L. McGaugh, "Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 17, pp. 6741–6746, 2006.

[124] W. M. Lambert, C.-F. Xu, T. A. Neubert et al., "BDNF-signaling rewrites the glucocorticoid transcriptome via glucocorticoid receptor phosphorylation," *Molecular and Cellular Biology*, vol. 33, no. 18, pp. 3700–3714, 2013.

[125] S. J. Lupien, F. Maheu, M. Tu, A. Fiocco, and T. E. Schramek, "The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition," *Brain and Cognition*, vol. 65, no. 3, pp. 209–237, 2007.

[126] A. Attardo, J. E. Fitzgerald, and M. J. Schnitzer, "Impermanence of dendritic spines in live adult CA1 hippocampus," *Nature*, vol. 523, no. 7562, pp. 592–596, 2015.

[127] Y. Lu, K. Christian, and B. Lu, "BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory?" *Neurobiology of Learning and Memory*, vol. 89, no. 3, pp. 312–323, 2008.

[128] M.-M. Poo, "Neurotrophins as synaptic modulators," *Nature Reviews Neuroscience*, vol. 2, no. 1, pp. 24–32, 2001.

[129] P. Schmidt, F. Holsboer, and D. Spengler, "β_2-adrenergic receptors potentiate glucocorticoid receptor transactivation via G protein $\beta\gamma$-subunits and the phosphoinositide 3-kinase pathway," *Molecular Endocrinology*, vol. 15, no. 4, pp. 553–564, 2001.

[130] M. Kaur, J. E. Chivers, M. A. Giembycz, and R. Newton, "Long-acting β2-adrenoceptor agonists synergistically enhance glucocorticoid-dependent transcription in human airway epithelial and smooth muscle cells," *Molecular Pharmacology*, vol. 73, no. 1, pp. 203–214, 2008.

[131] A. H. Miller, C. M. Pariante, and B. D. Pearce, "Effects of cytokines on glucocorticoid receptor expression and function. Glucocorticoid resistance and relevance to depression," *Advances in Experimental Medicine and Biology*, vol. 461, pp. 107–116, 1999.

[132] C. L. Raison, L. Capuron, and A. H. Miller, "Cytokines sing the blues: inflammation and the pathogenesis of depression," *Trends in Immunology*, vol. 27, no. 1, pp. 24–31, 2006.

[133] W. M. Lambert, C.-F. Xu, T. A. Neubert, M. V. Chao, M. J. Garabedian, and F. D. Jeanneteauc, "Brain-derived neurotrophic factor signaling rewrites the glucocorticoid transcriptome via glucocorticoid receptor phosphorylation," *Molecular and Cellular Biology*, vol. 33, no. 18, pp. 3700–3714, 2013.

[134] S. Makino, P. W. Gold, and J. Schulkin, "Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus," *Brain Research*, vol. 640, no. 1-2, pp. 105–112, 1994.

[135] F. D. Jeanneteau, W. M. Lambert, N. Ismaili et al., "BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 4, pp. 1305–1310, 2012.

[136] D. Y. Chen, D. Bambah-Mukku, G. Pollonini, and C. M. Alberini, "Glucocorticoid receptors recruit the CaMKIIα-BDNF-CREB pathways to mediate memory consolidation," *Nature Neuroscience*, vol. 15, no. 12, pp. 1707–1714, 2012.

[137] G. P. Chrousos, S. D. Detera-Wadleigh, and M. Karl, "Syndromes of glucocorticoid resistance," *Annals of Internal Medicine*, vol. 119, no. 11, pp. 1113–1124, 1993.

[138] G. P. Chrousos, A. Vingerhoeds, D. Brandon et al., "Primary cortisol resistance in man. A glucocorticoid receptor-mediated disease," *The Journal of Clinical Investigation*, vol. 69, no. 6, pp. 1261–1269, 1982.

[139] C. M. Pariante, "Risk factors for development of depression and psychosis. Glucocorticoid receptors and pituitary implications for treatment with antidepressant and glucocorticoids," *Annals of the New York Academy of Sciences*, vol. 1179, pp. 144–152, 2009.

[140] K. G. Bath, A. Schilit, and F. S. Lee, "Stress effects on BDNF expression: effects of age, sex, and form of stress," *Neuroscience*, vol. 239, pp. 149–156, 2013.

[141] A. Menke, J. Arloth, B. Pütz et al., "Dexamethasone stimulated gene expression in peripheral blood is a sensitive marker for glucocorticoid receptor resistance in depressed patients," *Neuropsychopharmacology*, vol. 37, no. 6, pp. 1455–1464, 2012.

[142] M. N. Silverman and E. M. Sternberg, "Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction," *Annals of the New York Academy of Sciences*, vol. 1261, no. 1, pp. 55–63, 2012.

[143] E. Charmandari, T. Kino, and G. P. Chrousos, "Primary generalized familial and sporadic glucocorticoid resistance (Chrousos syndrome) and hypersensitivity," *Endocrine Development*, vol. 24, pp. 67–85, 2013.

[144] J. Gräff, D. Rei, J.-S. Guan et al., "An epigenetic blockade of cognitive functions in the neurodegenerating brain," *Nature*, vol. 483, no. 7388, pp. 222–226, 2012.

[145] P. J. Barnes and I. M. Adcock, "Glucocorticoid resistance in inflammatory diseases," *The Lancet*, vol. 373, no. 9678, pp. 1905–1917, 2009.

[146] R. V. Sionov, O. Cohen, S. Kfir, Y. Zilberman, and E. Yefenof, "Role of mitochondrial glucocorticoid receptor in glucocorticoid-induced apoptosis," *Journal of Experimental Medicine*, vol. 203, no. 1, pp. 189–201, 2006.

[147] R. V. Sionov, S. Kfir, E. Zafrir, O. Cohen, Y. Zilberman, and E. Yefenof, "Glucocorticoid-induced apoptosis revisited: a novel role for glucocorticoid receptor translocation to the mitochondria," *Cell Cycle*, vol. 5, no. 10, pp. 1017–1026, 2006.

[148] C. V. Demonacos, N. Karayanni, E. Hatzoglou, C. Tsiriyiotis, D. A. Spandidos, and C. E. Sekeris, "Mitochondrial genes as sites of primary action of steroid hormones," *Steroids*, vol. 61, no. 4, pp. 226–232, 1996.

[149] K. Scheller and C. E. Sekeris, "The effects of steroid hormones on the transcription of genes encoding enzymes of oxidative phosphorylation," *Experimental Physiology*, vol. 88, no. 1, pp. 129–140, 2003.

[150] R. S. Duman and L. M. Monteggia, "A neurotrophic model for stress-related mood disorders," *Biological Psychiatry*, vol. 59, no. 12, pp. 1116–1127, 2006.

[151] F. Holsboer, "The corticosteroid receptor hypothesis of depression," *Neuropsychopharmacology*, vol. 23, no. 5, pp. 477–501, 2000.

[152] M. F. Egan, M. Kojima, J. H. Callicott et al., "The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function," *Cell*, vol. 112, no. 2, pp. 257–269, 2003.

[153] F. Soliman, C. E. Glatt, K. G. Bath et al., "A genetic variant BDNF polymorphism alters extinction learning in both mouse and human," *Science*, vol. 327, no. 5967, pp. 863–866, 2010.

[154] G. S. H. Yeo, C.-C. C. Hung, J. Rochford et al., "A de novo mutation affecting human TrkB associated with severe obesity and developmental delay," *Nature Neuroscience*, vol. 7, no. 11, pp. 1187–1189, 2004.

[155] E. B. Binder, D. Salyakina, P. Lichtner et al., "Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment," *Nature Genetics*, vol. 36, no. 12, pp. 1319–1325, 2004.

[156] N. R. Wray, M. R. James, S. D. Gordon et al., "Accurate, large-scale genotyping of 5HTTLPR and flanking single nucleotide polymorphisms in an association study of depression, anxiety, and personality measures," *Biological Psychiatry*, vol. 66, no. 5, pp. 468–476, 2009.

[157] J. R. Wendland, B. J. Martin, M. R. Kruse, K.-P. Lesch, and D. L. Murphy, "Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531," *Molecular Psychiatry*, vol. 11, no. 3, pp. 224–226, 2006.

[158] R. H. Derijk, "Single nucleotide polymorphisms related to HPA axis reactivity," *Neuroimmunomodulation*, vol. 16, no. 5, pp. 340–352, 2009.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 2540462, 10 pages
http://dx.doi.org/10.1155/2016/2540462

Review Article

Lasting Differential Effects on Plasticity Induced by Prenatal Stress in Dorsal and Ventral Hippocampus

Gayane Grigoryan[1] and Menahem Segal[2]

[1]*Division of Cellular Neurobiology, Zoological Institute, Technische Universität Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany*
[2]*Department of Neurobiology, Weizmann Institute of Science, 76100 Rehovot, Israel*

Correspondence should be addressed to Gayane Grigoryan; g.grigoryan@tu-bs.de

Received 31 August 2015; Revised 18 November 2015; Accepted 20 December 2015

Academic Editor: Jordan Marrocco

Early life adversaries have a profound impact on the developing brain structure and functions that persist long after the original traumatic experience has vanished. One of the extensively studied brain structures in relation to early life stress has been the hippocampus because of its unique association with cognitive processes of the brain. While the entire hippocampus shares the same intrinsic organization, it assumes different functions in its dorsal and ventral sectors (DH and VH, resp.), based on different connectivity with other brain structures. In the present review, we summarize the differences between DH and VH and discuss functional and structural effects of prenatal stress in the two sectors, with the realization that much is yet to be explored in understanding the opposite reactivity of the DH and VH to stressful stimulation.

1. Introduction

Evidence has accumulated in recent years to indicate that early life adversaries have a profound impact on the developing brain structure and functions, long after the original traumatic experience has vanished. One of the extensively studied structures in the brain in relation to early life stress has been the hippocampus. It is a unique structure in that it forms rather late in embryonic life and continues morphogenesis early in postnatal life [1–3]. The hippocampus is a focus of attention because of its unique association with cognitive processes of the brain. However, most studies describe effects of behavioral manipulations on structure/function of the dorsal hippocampus, but there are strong indications that while the entire hippocampus shares the same intrinsic organization, it assumes different functions in its dorsal and ventral sectors (DH and VH, resp.). The two sectors have different connectivity with other brain structures, and they differ in distribution of receptors, which leads to differences in function, different sensitivity, and very often opposite reactions to the same stimulus. In the following review, we will summarize the differences between DH and VH and we will carry on by describing some differential effects of stress in the two sectors, with the realization that much is yet to be explored towards understanding the opposite and long-lasting reactivity of the two sectors to stressful stimulation.

2. Ventral Hippocampus: Is It Different from the Dorsal Hippocampus?

The hippocampus has a curved shape that is conserved across all mammals and is distributed from dorsal (= septal, also called posterior in humans) to ventral (= temporal, anterior in humans) poles. The dorsal and ventral sectors of hippocampus (DH and VH, correspondingly) have different connectivity with cortical and subcortical structures, with the intermediate hippocampus sharing some properties with the DH. VH has more dense connectivity with the amygdala and hypothalamic endocrine and autonomic nuclei than DH [4, 5]. The VH projects preferentially to the medial, intercalated, and basomedial nuclei of amygdala and the amygdala-hippocampal transition area, while the DH distributes its efferents in more lateral regions of the amygdala [6].

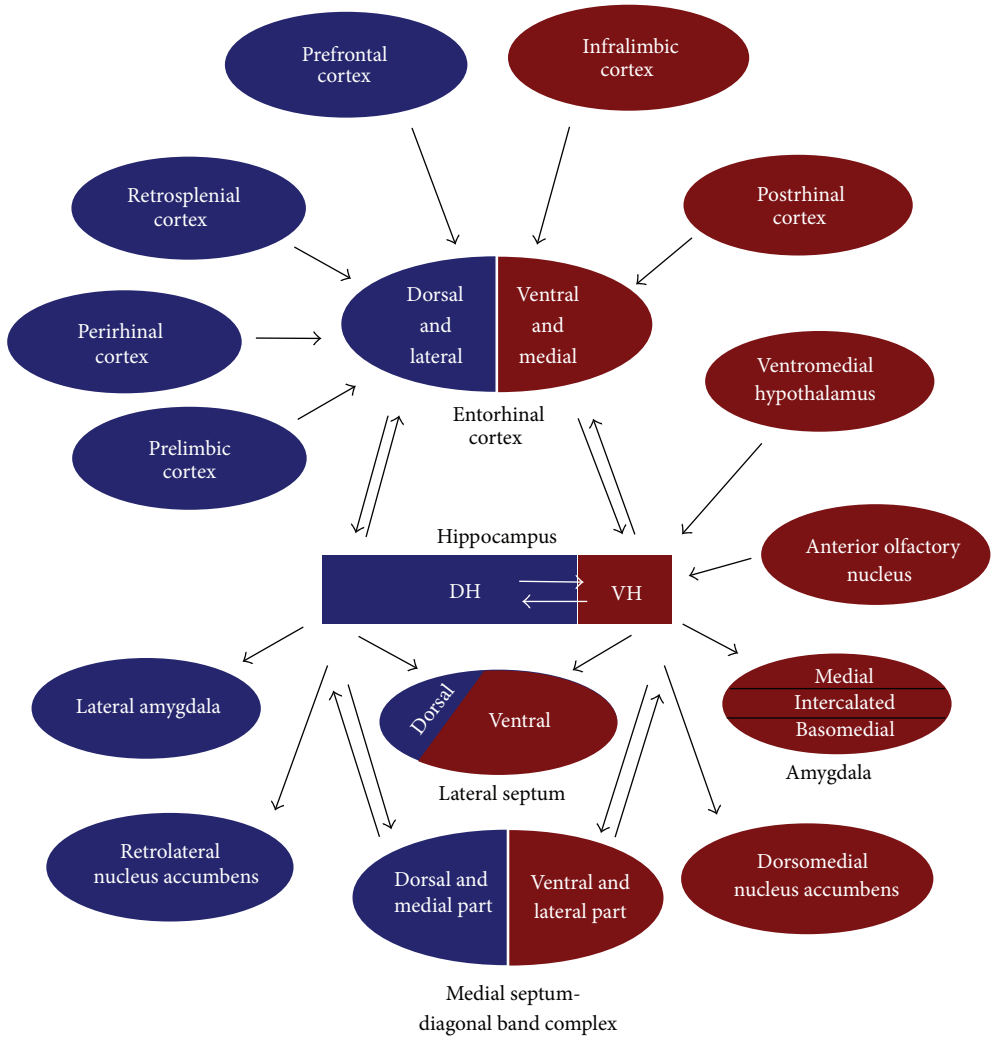


Figure 1: A schematic diagram of the major connections of the dorsal (DH) and ventral (VH) sectors of hippocampus.

The projections from cingulate areas (infralimbic and prelimbic cortices) involved in emotional regulation primarily reach the VH via input to the ventromedial parts of the entorhinal cortex (EC), while the anterior cingulate and retrosplenial cortices involved in spatial processing primarily project to the DH via targeting dorsal and lateral parts of the EC. Projections from the hippocampus to the EC originate in CA1 region and the subiculum and show a topographical organization similar to that of the EC-hippocampus inputs [7, 8]. The projection of the major hippocampal output to the lateral and medial septum (LS and MS, correspondingly) also shows dorsoventral differentiation. Thus, the DH projects to the small dorsal part of LS and dorsal and medial parts of MS, while VH innervates the larger ventral part of LS and lateral and ventral parts of MS [9, 10] (Figure 1).

The special character of the hippocampal connectivity forms a difference in the neurotransmitter composition along the axis of the hippocampus. Thus, cholinergic [11] and dopaminergic [12] innervation is denser in VH. Likewise, the concentrations of norepinephrine [13] and serotonin [14] as well as the density of synaptic terminals containing these transmitters are higher in the VH [15]. This differential distribution of several neuromodulators indicates that the VH is more amenable to neuromodulation than the DH.

Differences in the connectivity of DH and VH determine their functional distinction. Cross-species data show differences in connectivity with cortical and subcortical structures and functional differentiation along the longitudinal axis of hippocampus. This suggests that functional differences along the long axis may exhibit a gradient-like organization [16, 17] but that there are other connections that are restricted to the DH or VH. It has been suggested that the DH plays a crucial role in spatial learning and memory processes and VH is involved in anxiety, fear, defensive behavior, and stress related responses [18–24]. Studies with an animal model of hippocampal damage showed that lesions in DH impair spatial learning on tasks such as the Morris water maze or elevated T maze while lesions in VH disrupt emotional responses without impairments of spatial learning [25–29]. Massive activation of the DH during tasks that require spatial

working memory was demonstrated by cFos staining as well [30]. Interestingly, NR1 N-methyl-D-aspartate (NMDA) receptor subunit deletion from the granule cells of the dentate gyrus (DG) not only impairs short-term spatial memory but also reduces anxiety [18]. The importance of VH in anxiety-related behavior such as anorexia nervosa was also shown [31]. Neonatal excitotoxic lesions of VH in rats result in postpubertal hyperresponsiveness to stress and cognitive abnormalities characteristic to those described in schizophrenia (for review, see [32, 33]). The anterior hippocampus in humans also shows anatomical [34] and functional [35] abnormalities in patients that had suffered from schizophrenia.

Single neurons in the DH and VH vary in their electrophysiological properties. While neurons recorded from CA1 area of DH and VH have similar spike discharge characteristics and could be classified into "complex spike" and "theta" cells, less than one-fourth of cell population in VH have "place" properties, and these have low spatial resolution, while in DH they represent at least half of the cell population with much smaller and better tuned place field size [36].

With respect to evoked field potentials and their plastic properties, in particular, their ability to undergo short- or long-term potentiation or depression (STP, LTP, and LTD, resp., major cellular mechanisms that underlie learning and memory processes [37]), DH and VH exhibit different properties as well. Examination of different forms of synaptic plasticity uncovered an impaired ability of VH to produce STP and LTP [38–41] and weaker synaptic inhibition with lower levels of gamma-aminobutyric acid (GABA) receptor A subunits [42, 43], which makes the VH more vulnerable to epileptic activity [43, 44]. The low ability of VH to express LTP might be due to its biochemical characteristics. Thus, DH and VH are different in the distribution of different subunits of NMDA receptors: the density of both NR2A and NR2B subunits of NMDA receptors is higher in DH than in VH [45, 46]. Moreover, the VH has lower levels of mRNA expression for GluRA, GluRB, and GluRC subunits of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors compared with DH [46].

There is evidence for selective activation of different corticosteroid receptors in DH and VH in response to acute stress exposure. The activation of mineralocorticoid receptors leads to facilitation of LTP by enhancement of voltage-gated Ca^{2+} channels in VH, whereas the suppressed LTP in DH is mediated by activation of a glucocorticoid receptor [38]. Moreover, the VH differs from the DH in its sensitivity to agents that release Ca^{2+} from its internal stores (caffeine/ryanodine). Thus, VH exhibits higher sensitivity to ryanodine than DH which results in a strong response to subthreshold stimulation and is based on a higher level of Ca^{2+}-store-related ryanodine receptors in VH [47, 48].

3. Changes of Synaptic Plasticity in Dorsal versus Ventral Hippocampus

Early life adversaries have an impact on health status and quality of life of individual and society at large; stress during pregnancy also has a profound role in the determination of the destiny of fetus. There are a number of protocols utilized in different laboratories to model prenatal stress (PS) in animals. Simulating PS in laboratory animals and especially interpreting and comparing the results from different groups demand a special care due to many factors related to the nature of the stressor(s) (type, duration, and "severity"), the "time window" during the pregnancy during which stress is experienced, genotype (wild type or genetically manipulated), and species (i.e., mouse, rat, guinea pig, or monkey) of pregnant dams as well as the age and sex of assessed offspring. In the majority of publications, authors do not specify the part of hippocampus that has been studied (DH or VH), but we assume that it is mainly DH.

Severe stressful experience during the last week of pregnancy (immobilization or foot shocks) leads to long-lasting changes of the properties of synaptic plasticity in different brain areas, in particular, in hippocampus of offspring of both genders. Thus, PS favors low-frequency stimulation-induced LTD and inhibits the high-frequency stimulation-induced LTP without affecting basal synaptic transmission in the hippocampus of young (3- or 5-week-old) rats [49–51] as well as in the frontal cortex of adult (3-month-old) animals [52]. Fostering of PS offspring by nonstressed dams to exclude the possible maltreatments of pups by the stressed mother does not abolish the deleterious effects of PS on synaptic plasticity [49, 51]. However, it has been shown that the adoption or postnatal handling can reverse the negative behavioral effects of PS in the adult offspring by altering the activity of the HPA axis and subsequent stress-induced corticosterone release [53, 54].

Yeh and colleagues [51] observed the effects of restraint PS (for 45 minutes three times/day applied at the last week of pregnancy) on synaptic plasticity at young age but the effect disappeared in adult rats (at 8 weeks of age). In contrast, the impairments of hippocampal synaptic plasticity caused by foot shock PS (10 foot shocks/day during the last week of gestation) persist to adulthood (8 weeks of age) in rats as shown by Yang and colleagues [50] but can be cured by an enriched environment treatment at young age. Similar changes of synaptic plasticity after restraint PS during the second week of gestation were seen also in 7-8-week-old male mice [55].

Another approach to PS induction was used by Kinnunen and colleagues [56] as well as by Murmu and colleagues [57]. As shown in [57], the unpredictable stress paradigm consisting of two sessions of three different stressors (restraining in the tube, crowded housing, and forced swim, one on each day during the last week of gestation) is raising blood plasma corticosterone level in pregnant dams and is preventing them from adapting to the stressor. This PS protocol was used in other studies from the same group [58] and was adapted by us [59, 60]. Using this relatively mild stress protocol, Yaka and colleagues [58] were able to show that PS applied during critical period of embryonal development causes deleterious effect on synaptic plasticity of young (4-5-week-old) male offspring expressed as an impaired ability of CA3-CA1 synapses in hippocampus to undergo LTP.

In vivo examination of synapses formed by layer 2 of the entorhinal cortex on the granule cells of the DG (the perforant path) of hippocampus showed that short-lasting mild PS (30 min of restraint, from day 15 to day 17 of gestation) leads to facilitation of potentiation of the perforant path in the adult (at 10 weeks of age) offspring [61].

A recent *in vivo* study employed an amplified broad band traffic noise to induce PS and show that either short- (1 h) or long-term (2 or 4 h) exposure to traffic noise affects basal synaptic transmission and impairs posttetanic and long-term potentiation in hippocampus [62]. Rats that were noise stressed for 1 or 2 hours showed deficit in posttetanic phase of potentiation; however, they expressed similar magnitude of LTP at the end of recording session (~2 hours after tetanus). Rats that were prenatally exposed to 4 hours of noise showed constant decline of EPSP slope, which went under baseline values after 2 hours of recording. This could be interpreted as a PS-induced complete loss of ability to express LTP and an appearance of LTD instead. This observation is actually in line with the findings of Gi and colleagues [55] and Yang and colleagues [49] on facilitation of LTD in prenatally stressed animals. Unfortunately, no information on PS impact on synaptic plasticity in VH was provided in these studies.

PS enhances the responsiveness of organisms to acute stress exposure [49, 50, 63–66] via chronic activation of HPA axis that is confirmed by hypertrophy of the adrenal glands [67], which could also underlie the increased vulnerability to develop affective disorders later in life. It has been shown that PS experienced at the third, but not at the second, week of gestation of Sprague-Dawley rats leads to prolonged elevation of the glucocorticoids level in response to acute stress [68]. The alterations in the reactivity of HPA axis in PS rats are correlated with the functional changes of different types of corticosteroid receptors. Thus, PS results in the downregulation of both high-affinity mineralocorticoid receptors (MRs) and low-affinity glucocorticoid receptors (GRs) in rats' offspring [34, 53, 69–71], affecting the binding capacity of MR only [53].

The mechanisms underlying PS impact on hippocampal synaptic plasticity involve tissue plasminogen activator as well as an imbalance in levels of pro- and mature-BDNF (m-BDNF), most likely due to reduced BDNF gene expression and inhibition of conversion of pro-BDNF to m-BDNF [51, 72, 73]. Interestingly, in mouse model for Alzheimer's disease (APPswe/PS1dE9), PS could cause changes in pro- versus m-BDNF levels in hippocampus of 8-month-old female offspring only [74, 75]. Sierksma and colleagues [74, 75] also used chronic restraint stress comparable with [51] but they applied it during the first week of gestation.

Another mechanism that could be involved in processes of regulating synaptic plasticity includes the changes in the functionality of NMDA receptors and their subunits that are important in the induction of both LTP and LTD [76, 77]. The changes in expression of different subunits of the NMDA receptors in different hippocampal fields after PS exposure were shown in [55, 56, 58, 78, 79]. Unlike Yeh and colleagues [51], these studies found that PS not only reduces the levels of NR1 and NR2B subunits but also impairs synaptic localization of the NMDA receptors (low number of complexes associated with PSD95, a NMDA receptor-anchoring molecule). This suggests that PS induces changes in functional activity and distribution of NMDA receptor subunits between DH and VH resulting in different responsivity to stress of the two sectors of hippocampus [38, 39, 45–47, 80].

PS-induced alterations in neurotransmission could underlie the impaired ability to express LTP. In our own studies, we were able to show that PS affects network properties of hippocampal neurons, by reducing GABA-ergic inhibition [59]. PS-induced epigenetic modification of GABA-ergic interneurons not only in hippocampus but also in frontal cortex of young and adult mice mediated by over-expression of DNA methyltransferase associated with a decrease in reelin and GAD67 expression was shown by Matrisciano and colleagues [81]. The alterations in the main excitatory glutamate neurotransmission that are believed to play a role in the pathophysiology of several neuropsychiatric disorders, including schizophrenia, epilepsy, and anxiety, were shown in a study by Marrocco and colleagues [82]. One of the important aspects of that study is the discrimination between the DH and VH. Interestingly, most of their findings were restricted to the VH. Thus, the restraint of dams during the second half of pregnancy (from day 11 until delivery) caused selectivity to VH long-lasting (tested at 3 months of age) reduction of both glutamate release and synaptic vesicle-associated proteins (such as Rab 3A, Munc-18, synaptobrevin, syntaxin-1, synaptophysin, and synapsin) in PS male offspring. The reduction in the activity of mGlu1/mGlu5 receptors in VH of male but not of female offspring was reported earlier by the same group [73]. Interestingly, the deleterious effects of restraint PS on glutamate release in VH as well as some abnormalities in behavior including increased anxiety-like behavior were successfully ameliorated by antidepressants (fluoxetine and agomelatine) [83].

We also showed that gestational stress in rats selectively modulates noradrenergic (NA) effects in hippocampus of the offspring causing suppression of the ability to convert STP into LTP in the DH and its facilitation in the VH (Figure 2) [60]. An increased plasma noradrenaline level in adult (at 5 months of age) offspring in response to foot shock PS [66] and an impairment in the development of NA neurons in pups from dams exposed to cold stress during the second half of pregnancy were also shown recently [84].

4. Ventral Hippocampus and Anxiety-Like Behavior

PS has a lasting effect on the behavior of animals but the data reported by different research groups is conflicting. For review of human studies, see [85, 86]. In the framework of the current review, we will focus on changes in hippocampus-dependent/related behaviors in rodents. *VH* is believed to be involved in anxiety and fear related behavior [19–21, 24, 25, 87]. One of the widely used behavioral tests to assess anxiety in rodents is the elevated plus maze (EPM) test. It is based on a conflict between the rodent's preference for protected area and its motivation to explore novel environments. The

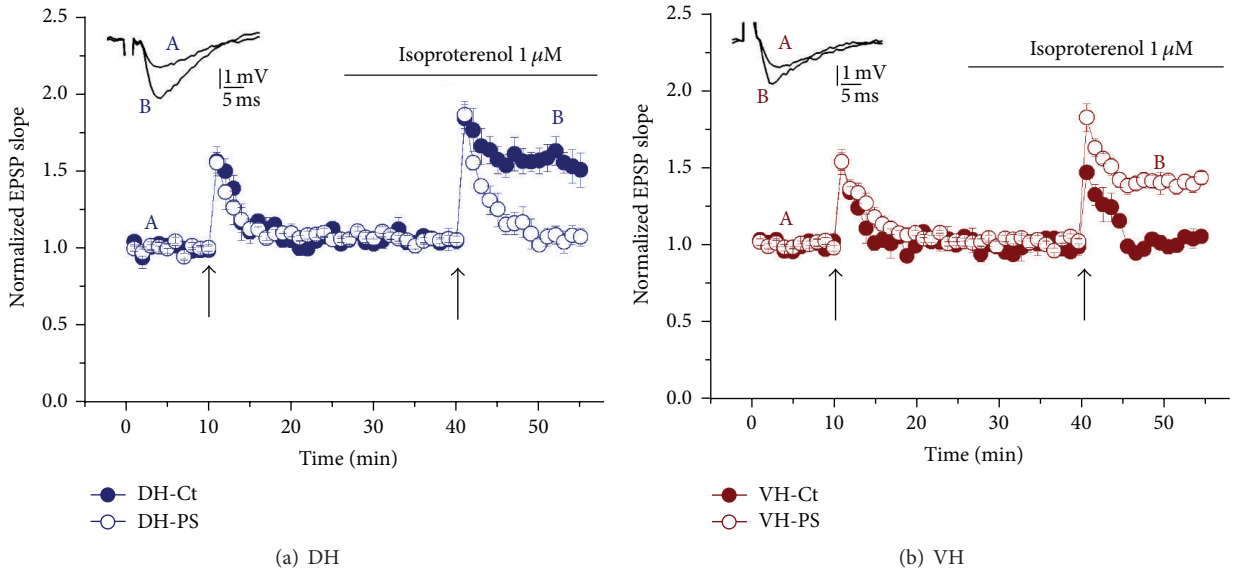


Figure 2: Effect of Isoproterenol, a β-adrenergic agonist, on EPSPs recorded in stratum radiatum of DH (a) and VH (b) hippocampal slices from control and prenatally stressed (PS) rats (at 2-3 weeks of gestation). The arrows denote the points at which short tetanic stimulation (35 stimuli at 100 Hz) was delivered, twice to one pathway. Short tetanic stimulation, which normally produces only short-term potentiation, applied in the presence of Isoproterenol, produced a full-blown LTP in DH slices of control group (full circles, (a)) and in VH slices of PS rats (open circles, (b)), but not in the other conditions tested (*adapted from Grigoryan and Segal [60]*).

avoidance of the open arm by an animal is considered as an anxiety-like behavior [88, 89]. Several studies showed that the exposure to stress during gestation causes changes in emotional status of offspring of both genders. The striking gender-dependent difference in offspring response to PS was exemplified by Zuena and colleagues [73] who showed that males are more prone to developing anxiety-like behavior in the EPM than females that showed reduced anxiety. The increase in anxiety in PS adult offspring (3 months of age) males reported by Zuena and colleagues [73] is consistent with the observations published by the same group [54, 82] as well as with the other studies that utilized traffic noise or varied stressors protocol for prenatal treatments [62, 90, 91]. As to the reduction in anxiety in PS females, it is in disagreement with higher anxiogenic effect of PS in young (5 weeks of age) and young adult (60 days of age) females than in males reported by Salomon and colleagues [90]. In our hands, varying PS during the last week of gestation shifts the emotional balance of young (1 month of age) male offspring into the "less anxious" direction (i.e., PS rats spent more time in the open arm than control rats), and it was correlated with higher motility in the open field and the EPM [59]. Similar effects in young (35 days of age) male offspring as a result of a single but intense PS (120 minutes of maternal immobilization at 16th day of pregnancy) were seen by Cannizzaro and colleagues [92]. Two hours of restraint PS during the second half of pregnancy also leads to more active exploratory behavior in male offspring of the same age [93]. In another study, prenatal restraint stress induced schizophrenic behavior expressed as an increase in locomotion, decreased social interaction, deficit in prepulse inhibition, and contextual fear conditioning was found in adult male rats and male mice [81, 94]. Behavioral profiles indicative of greater emotionality [95] and submissive social rankings [96] were found in the PS offspring of nonhuman primates. In conclusion, the difference in type of maternal stress used as well as the age of the tested animals may lead to the different and sometimes opposite behavioral outcome of PS in the offspring.

5. Prenatal Stress and Spatial Learning

The hippocampus, mainly its dorsal part, is believed to be involved in spatial learning and memory processes in both rats and primates [22]. However, both DH and VH support Morris water maze (MWM) spatial learning task, where animals have to learn to navigate to a hidden platform using distal cues [97, 98]. The impaired performance in spatial learning of PS young and adult rats independently of type of stressor used during the last week of pregnancy was shown in a number of studies [50, 55, 62, 94, 99–101]. Using mild stress protocol, Yaka and colleagues [58] and Yang and colleagues [49] were able to show deleterious effect of PS on performance of 4-5-week-old male offspring in MWM. In contrast, in our studies, PS did not impair the behavior of young male rats in MWM learning task and they were actually improving faster than controls during the acquisition phase [59]. The reasons for this discrepancy could be the difference in experimental setup such as a size of the water pool used for the task as well as the training protocol [97]. The facilitation of learning performance in radial maze of mildly prenatally stressed adult (14-15 weeks of age) offspring was shown in [61]. In another study, spatial learning in MWM was not altered by PS in adult (3 months of age) male

offspring but leads to an improvement of female offspring performance [73]. In contrast, Wu and colleagues [100] showed impaired performance in MWM of female but not of male offspring. The gender-specific effect of PS on learning in rats as shown by longer escape latencies in MWM in adult (4 months old) and old (12 months old) male but not in female offspring, which was correlated with higher basal corticosterone levels and a lower density of hippocampal corticosteroid receptors in females, was reported in [34, 91]. These observations are supported by the findings of long-term maladaptive behavioral stress responsivity found in mice subjected to PS during the first week of gestation [102]. The anxiogenic behavior and the learning deficit in prenatally stressed offspring are completely abolished by adrenalectomy [91], which confirms the importance of elevated maternal corticosterone in developmental origin of brain vulnerability to PS. Interestingly, repetitive restraint stress during the first week of pregnancy as shown in [75] affects long-term memory acquired in object location task in 7-month-old male mice only, while female offspring shows improved spatial memory performance.

The importance of the timing of PS exposure on learning outcome in adult offspring was shown by Kapoor and colleagues [103]. Thus, the male offspring of guinea pigs that were exposed to PS on gestational days 50, 51, and 52 exhibit impaired spatial learning, while the offspring that was stressed during later phase of *in utero* development (days 60, 61, and 62) appears to exhibit enhanced spatial learning [103].

A somewhat unique study demonstrated effects of PS on cognitive functions in lambs. Prenatally stressed lambs were impaired in a maze performance; they were also characterized by increased fear reactions and pessimistic-like judgment in a cognitive bias test [104].

6. Impact of Prenatal Stress on Dorsal and Ventral Hippocampal Morphology

PS influences behavior and memory processes of offspring and it is likely associated with morphological changes in the brain. All studies that have investigated PS-induced changes of brain morphology were focused mainly on DH. To our knowledge, no specific differences of PS effects in DH versus VH have been reported, which leaves an open question of whether PS impacts specifically the morphology of VH.

The age dependence of prenatal restraint stress-induced changes in dendritic morphology of hippocampal pyramidal neurons of areas CA1 and CA3 was shown by Martínez-Téllez and colleagues [93]. They found that CA3 area of the hippocampus is more prone to PS exposure resulting in a decrease in dendritic spine density in prepubertal (at 35 days of age) and adult (at 65 days of age) male offspring, while in the area CA1 the decrease of spine density is characteristic of adult rats only. Interestingly, CA1 pyramidal neurons of hippocampus from prepubertal PS animals were characterized by an increased spine density [93, 105]. In a recent study by Petit and colleagues [106], higher spine density on apical dendrites in the CA1 area of hippocampus of PS lambs immediately after birth was shown [106].

PS during the last week of pregnancy leads to dendritic atrophy expressed as a shortened total length and reduced number of branching points of the apical dendrites of pyramidal neurons of area CA3 of DH also in prepubertal female offspring [78, 105]. In another study that utilized the same stress induction protocol, it was shown that PS does not have an effect either on the total number of neurons or on amount and distribution of both apical and basal dendritic arbors as well as on total spine density of pyramidal neurons of CA1 region of hippocampus in 5-week-old offspring of both genders [51]. Dendritic morphology of CA1 pyramidal cells was not affected in adult (2 months of age) male offspring as well [99].

The decreased synaptic density, length and number of dendritic segments, branching of granule, and CA3 pyramidal hippocampal neurons of young (35-day-old) and adult (2-month-old) male offspring after varied (crowding and daily saline injections during the last week of pregnancy) or restraint PS were demonstrated in other studies as well [99, 107].

PS alters neural and hormonal status also in nonhuman primates as shown in [95]. Thus, 3-year-old male and female offspring of rhesus monkeys that were stressed during early and late periods of their *in utero* development (for 25% of their 24-week gestation, an acoustical startle protocol) were characterized by a 10% reduction in hippocampal volume and inhibition of neurogenesis in the DG associated with increased HPA-axis activity [95]. Prenatal restraint stress as well as varied PS not only induces lifelong reduction of neurogenesis in DG of rat's hippocampus, especially in VH, but also inhibits the facilitation of neurogenesis by learning [67, 108, 109]. However, it was suggested that PS exerts a gender-specific effect on neurogenesis by increasing cell proliferation in the DG of female offspring only [110]. Electron microscopy examination revealed abnormal ultrastructural appearance of hippocampal neurons and myelin sheath in offspring, which was exposed to PS during middle or late stages of embryonal development. In addition, male rats expressed greater impairment than females in these parameters [101]. Moreover, short-lasting mild PS (30 min of restraint, from day 15 to day 17 of gestation) enhances neonatal neurogenesis in hippocampus of 10-week-old male rats, while long-lasting severe PS (240 min of restraint, from day 15 to day 17 of gestation) impairs morphology of hippocampal neurons. Mineralocorticoid and glucocorticoid receptors contribute to PS-induced changes [111].

Morphological analysis of cultured hippocampal cells revealed a reduction in the density of GABA-ergic neurons and the more elaborate dendritic tree of cultured neurons taken from the offspring of PS mothers. However, no difference in dendritic spine density and in the proportion of different spine subtypes was reported [59].

PS causes alterations of neuronal morphology in other young and adult rat brain areas such as nucleus accumbens [93, 112], prefrontal cortex [65, 112], and dorsal anterior cingulate and orbitofrontal cortex [57] as well as corpus callosum of young monkey's brain [113] and prefrontal cortex of brain of newborn lamb [106].

7. Conclusions

There are apparent long-term changes in the brain and specifically in the hippocampus following maternal exposure to stress or to stress hormones. These changes are long lasting, as they are caused by epigenetic regulation of gene expression in the brain, as well as by causing stable morphological change in the young, plastic brain. The outcome of these alterations can lead to neurological and psychiatric disorders at a later age. The possible amelioration of the detrimental effects of the adverse stimulation by activation of brain circuits underlying reward and pleasure is now emerging as a promising avenue of repair.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported by the German-Israeli Project Cooperation (DIP).

References

[1] S. A. Bayer, "Development of the hippocampal region in the rat II. Morphogenesis during embryonic and early postnatal life," *Journal of Comparative Neurology*, vol. 190, no. 1, pp. 115–134, 1980.

[2] D. Rice and S. Barone Jr., "Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models," *Environmental Health Perspectives*, vol. 108, supplement 3, pp. 511–533, 2000.

[3] L. Seress, H. Ábrahám, T. Tornóczky, and G. Kosztolányi, "Cell formation in the human hippocampal formation from mid-gestation to the late postnatal period," *Neuroscience*, vol. 105, no. 4, pp. 831–843, 2001.

[4] P. G. Henke, "Hippocampal pathway to the amygdala and stress ulcer development," *Brain Research Bulletin*, vol. 25, no. 5, pp. 691–695, 1990.

[5] T. van Groen and J. M. Wyss, "Extrinsic projections from area CA_1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections," *Journal of Comparative Neurology*, vol. 302, no. 3, pp. 515–528, 1990.

[6] T. Kishi, T. Tsumori, S. Yokota, and Y. Yasui, "Topographical projection from the hippocampal formation to the amygdala: a combined anterograde and retrograde tracing study in the rat," *Journal of Comparative Neurology*, vol. 496, no. 3, pp. 349–368, 2006.

[7] B. F. Jones and M. P. Witter, "Cingulate cortex projections to the parahippocampal region and hippocampal formation in the rat," *Hippocampus*, vol. 17, no. 10, pp. 957–976, 2007.

[8] M. P. Witter, "Organization of the entorhinal-hippocampal system: a review of current anatomical data," *Hippocampus*, vol. 3, pp. 33–44, 1993.

[9] R. P. A. Gaykema, J. van der Kuil, L. B. Hersh, and P. G. M. Luiten, "Patterns of direct projections from the hippocampus to the medial septum-diagonal band complex: Anterograde tracing with *Phaseolus vulgaris* leucoagglutinin combined with immunohistochemistry of choline acetyltransferase," *Neuroscience*, vol. 43, no. 2-3, pp. 349–360, 1991.

[10] P. Y. Risold and L. W. Swanson, "Structural evidence for functional domains in the rat hippocampus," *Science*, vol. 272, no. 5267, pp. 1484–1486, 1996.

[11] D. G. Amaral and J. Kurz, "An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat," *Journal of Comparative Neurology*, vol. 240, no. 1, pp. 37–59, 1985.

[12] C. Verney, M. Baulac, B. Berger, C. Alvarez, A. Vigny, and K. B. Helle, "Morphological evidence for a dopaminergic terminal field in the hippocampal formation of young and adult rat," *Neuroscience*, vol. 14, no. 4, pp. 1039–1052, 1985.

[13] J. H. Haring and J. N. Davis, "Differential distribution of locus coeruleus projections to the hippocampal formation: anatomical and biochemical evidence," *Brain Research*, vol. 325, no. 1-2, pp. 366–369, 1985.

[14] C. Köhler, V. Chan-Palay, and H. Steinbusch, "The distribution and orientation of serotonin fibers in the entorhinal and other retrohippocampal areas—an immunohistochemical study with anti-serotonin antibodies in the rat's brain," *Anatomy and Embryology*, vol. 161, no. 3, pp. 237–264, 1981.

[15] F. H. Gage and R. G. Thompson, "Differential distribution of norepinephrine and serotonin along the dorsal-ventral axis of the hippocampal formation," *Brain Research Bulletin*, vol. 5, no. 6, pp. 771–773, 1980.

[16] M. Colombo, T. Fernandez, K. Nakamura, and C. G. Gross, "Functional differentiation along the anterior-posterior axis of the hippocampus in monkeys," *Journal of Neurophysiology*, vol. 80, no. 2, pp. 1002–1005, 1998.

[17] B. A. Strange, M. P. Witter, E. S. Lein, and E. I. Moser, "Functional organization of the hippocampal longitudinal axis," *Nature Reviews: Neuroscience*, vol. 15, no. 10, pp. 655–669, 2014.

[18] C. Barkus, S. B. McHugh, R. Sprengel, P. H. Seeburg, J. N. P. Rawlins, and D. M. Bannerman, "Hippocampal NMDA receptors and anxiety: at the interface between cognition and emotion," *European Journal of Pharmacology*, vol. 626, no. 1, pp. 49–56, 2010.

[19] J. Jin and S. Maren, "Fear renewal preferentially activates ventral hippocampal neurons projecting to both amygdala and prefrontal cortex in rats," *Scientific Reports*, vol. 5, Article ID 8388, 2015.

[20] M. A. Kheirbek, L. J. Drew, N. S. Burghardt et al., "Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus," *Neuron*, vol. 77, no. 5, pp. 955–968, 2013.

[21] M.-B. Moser and E. I. Moser, "Functional differentiation in the hippocampus," *Hippocampus*, vol. 8, no. 6, pp. 608–619, 1998.

[22] M.-B. Moser, E. I. Moser, E. Forrest, P. Andersen, and R. G. M. Morris, "Spatial learning with a minislab in the dorsal hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 21, pp. 9697–9701, 1995.

[23] H. B. Nguyen, R. C. Bagot, J. Diorio, T. P. Wong, and M. J. Meaney, "Maternal care differentially affects neuronal excitability and synaptic plasticity in the dorsal and ventral hippocampus," *Neuropsychopharmacology*, vol. 40, no. 7, pp. 1590–1599, 2015.

[24] M. E. Wang, N. P. Fraize, L. Yin et al., "Differential roles of the dorsal and ventral hippocampus in predator odor contextual fear conditioning," *Hippocampus*, vol. 23, no. 6, pp. 451–466, 2013.

[25] D. M. Bannerman, J. N. P. Rawlins, S. B. McHugh et al., "Regional dissociations within the hippocampus—memory and anxiety," *Neuroscience and Biobehavioral Reviews*, vol. 28, no. 3, pp. 273–283, 2004.

[26] M. S. Fanselow and H.-W. Dong, "Are the dorsal and ventral hippocampus functionally distinct structures?" *Neuron*, vol. 65, no. 1, pp. 7–19, 2010.

[27] K. G. Kjelstrup, F. A. Tuvnes, H.-A. Steffenach, R. Murison, E. I. Moser, and M.-B. Moser, "Reduced fear expression after lesions of the ventral hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 16, pp. 10825–10830, 2002.

[28] S. B. McHugh, R. M. J. Deacon, J. N. P. Rawlins, and D. M. Bannerman, "Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety," *Behavioral Neuroscience*, vol. 118, no. 1, pp. 63–78, 2004.

[29] R. G. M. Morris, P. Garrud, J. N. P. Rawlins, and J. O'Keefe, "Place navigation impaired in rats with hippocampal lesions," *Nature*, vol. 297, no. 5868, pp. 681–683, 1982.

[30] S. D. Vann, M. W. Brown, J. T. Erichsen, and J. P. Aggleton, "Fos imaging reveals differential patterns of hippocampal and parahippocampal subfield activation in rats in response to different spatial memory tests," *Journal of Neuroscience*, vol. 20, no. 7, pp. 2711–2718, 2000.

[31] T. G. Chowdhury, N. C. Barbarich-Marsteller, T. E. Chan, and C. Aoki, "Activity-based anorexia has differential effects on apical dendritic branching in dorsal and ventral hippocampal CA1," *Brain Structure and Function*, vol. 219, no. 6, pp. 1935–1945, 2014.

[32] B. K. Lipska and D. R. Weinberger, "To model a psychiatric disorder in animals: schizophrenia as a reality test," *Neuropsychopharmacology*, vol. 23, no. 3, pp. 223–239, 2000.

[33] E. R. Marcotte, D. M. Pearson, and L. K. Srivastava, "Animal models of schizophrenia: a critical review," *Journal of Psychiatry and Neuroscience*, vol. 26, no. 5, pp. 395–410, 2001.

[34] T. F. Szuran, V. Pliska, J. Pokorny, and H. Welzl, "Prenatal stress in rats: effects on plasma corticosterone, hippocampal glucocorticoid receptors, and maze performance," *Physiology and Behavior*, vol. 71, no. 3-4, pp. 353–362, 2000.

[35] T. Pirnia, R. P. Woods, L. S. Hamilton et al., "Hippocampal dysfunction during declarative memory encoding in schizophrenia and effects of genetic liability," *Schizophrenia Research*, vol. 161, no. 2-3, pp. 357–366, 2015.

[36] M. W. Jung, S. I. Wiener, and B. L. McNaughton, "Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat," *The Journal of Neuroscience*, vol. 14, no. 12, pp. 7347–7356, 1994.

[37] T. V. P. Bliss and G. L. Collingridge, "A synaptic model of memory: long-term potentiation in the hippocampus," *Nature*, vol. 361, no. 6407, pp. 31–39, 1993.

[38] N. Maggio and M. Segal, "Striking variations in corticosteroid modulation of long-term potentiation along the septotemporal axis of the hippocampus," *The Journal of Neuroscience*, vol. 27, no. 21, pp. 5757–5765, 2007.

[39] N. Maggio and M. Segal, "Differential modulation of long-term depression by acute stress in the rat dorsal and ventral hippocampus," *Journal of Neuroscience*, vol. 29, no. 27, pp. 8633–8638, 2009.

[40] C. Papatheodoropoulos and G. Kostopoulos, "Dorsal-ventral differentiation of short-term synaptic plasticity in rat CA1 hippocampal region," *Neuroscience Letters*, vol. 286, no. 1, pp. 57–60, 2000.

[41] C. Papatheodoropoulos and G. Kostopoulos, "Decreased ability of rat temporal hippocampal CA1 region to produce long-term potentiation," *Neuroscience Letters*, vol. 279, no. 3, pp. 177–180, 2000.

[42] C. Papatheodoropoulos, E. Asprodini, I. Nikita, C. Koutsona, and G. Kostopoulos, "Weaker synaptic inhibition in CA1 region of ventral compared to dorsal rat hippocampal slices," *Brain Research*, vol. 948, no. 1-2, pp. 117–121, 2002.

[43] E. Sotiriou, C. Papatheodoropoulos, and F. Angelatou, "Differential expression of γ-aminobutyric acid—a receptor subunits in rat dorsal and ventral hippocampus," *Journal of Neuroscience Research*, vol. 82, no. 5, pp. 690–700, 2005.

[44] E. Isaeva, A. Romanov, G. L. Holmes, and D. Isaev, "Status epilepticus results in region-specific alterations in seizure susceptibility along the hippocampal longitudinal axis," *Epilepsy Research*, vol. 110, pp. 166–170, 2015.

[45] N. Maggio, E. Shavit Stein, and M. Segal, "Ischemic LTP: NMDA-dependency and dorso/ventral distribution within the hippocampus," *Hippocampus*, vol. 25, no. 11, pp. 1465–1471, 2015.

[46] C. Pandis, E. Sotiriou, E. Kouvaras, E. Asprodini, C. Papatheodoropoulos, and F. Angelatou, "Differential expression of NMDA and AMPA receptor subunits in rat dorsal and ventral hippocampus," *Neuroscience*, vol. 140, no. 1, pp. 163–175, 2006.

[47] G. Grigoryan, Z. Ardi, A. Albrecht, G. Richter-Levin, and M. Segal, "Juvenile stress alters LTP in ventral hippocampal slices: involvement of noradrenergic mechanisms," *Behavioural Brain Research*, vol. 278, pp. 559–562, 2015.

[48] G. Grigoryan, E. Korkotian, and M. Segal, "Selective facilitation of LTP in the ventral hippocampus by calcium stores," *Hippocampus*, vol. 22, no. 7, pp. 1635–1644, 2012.

[49] J. Yang, H. Han, J. Cao, L. Li, and L. Xu, "Prenatal stress modifies hippocampal synaptic plasticity and spatial learning in young rat offspring," *Hippocampus*, vol. 16, no. 5, pp. 431–436, 2006.

[50] J. Yang, C. Hou, N. Ma et al., "Enriched environment treatment restores impaired hippocampal synaptic plasticity and cognitive deficits induced by prenatal chronic stress," *Neurobiology of Learning and Memory*, vol. 87, no. 2, pp. 257–263, 2007.

[51] C.-M. Yeh, C.-C. Huang, and K.-S. Hsu, "Prenatal stress alters hippocampal synaptic plasticity in young rat offspring through preventing the proteolytic conversion of pro-brain-derived neurotrophic factor (BDNF) to mature BDNF," *The Journal of Physiology*, vol. 590, no. 4, pp. 991–1010, 2012.

[52] J. Sowa, B. Bobula, K. Glombik et al., "Prenatal stress enhances excitatory synaptic transmission and impairs long-term potentiation in the frontal cortex of adult offspring rats," *PLoS ONE*, vol. 10, no. 3, Article ID e0119407, 2015.

[53] S. Maccari, P. V. Piazza, M. Kabbaj, A. Barbazanges, H. Simon, and M. Le Moal, "Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress," *The Journal of Neuroscience*, vol. 15, no. 1 I, pp. 110–116, 1995.

[54] M. Vallée, W. Mayo, F. Dellu, M. L. Moal, H. Simon, and S. Maccari, "Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion," *The Journal of Neuroscience*, vol. 17, no. 7, pp. 2626–2636, 1997.

[55] H. S. Gi, D. Geum, S. Chung et al., "Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity," *Journal of Neuroscience*, vol. 26, no. 12, pp. 3309–3318, 2006.

[56] A. K. Kinnunen, J. I. Koenig, and G. Bilbe, "Repeated variable prenatal stress alters pre- and postsynaptic gene expression in

the rat frontal pole," *Journal of Neurochemistry*, vol. 86, no. 3, pp. 736–748, 2003.

[57] M. S. Murmu, S. Salomon, Y. Biala, M. Weinstock, K. Braun, and J. Bock, "Changes of spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy," *European Journal of Neuroscience*, vol. 24, no. 5, pp. 1477–1487, 2006.

[58] R. Yaka, S. Salomon, H. Matzner, and M. Weinstock, "Effect of varied gestational stress on acquisition of spatial memory, hippocampal LTP and synaptic proteins in juvenile male rats," *Behavioural Brain Research*, vol. 179, no. 1, pp. 126–132, 2007.

[59] G. Grigoryan and M. Segal, "Prenatal stress affects network properties of rat hippocampal neurons," *Biological Psychiatry*, vol. 73, no. 11, pp. 1095–1102, 2013.

[60] G. Grigoryan and M. Segal, "Prenatal stress alters noradrenergic modulation of LTP in hippocampal slices," *Journal of Neurophysiology*, vol. 110, no. 2, pp. 279–285, 2013.

[61] T. Fujioka, A. Fujioka, N. Tan et al., "Mild prenatal stress enhances learning performance in the non-adopted rat offspring," *Neuroscience*, vol. 103, no. 2, pp. 301–307, 2001.

[62] M. Barzegar, F. S. Sajjadi, S. A. Talaei, G. Hamidi, and M. Salami, "Prenatal exposure to noise stress: anxiety, impaired spatial memory, and deteriorated hippocampal plasticity in postnatal life," *Hippocampus*, vol. 25, no. 2, pp. 187–196, 2015.

[63] A. Barbazanges, P. V. Piazza, M. Le Moal, and S. Maccari, "Maternal glucocorticoid secretion mediates long-term effects of prenatal stress," *Journal of Neuroscience*, vol. 16, no. 12, pp. 3943–3949, 1996.

[64] H. Louvart, S. Maccari, and M. Darnaudéry, "Prenatal stress affects behavioral reactivity to an intense stress in adult female rats," *Brain Research*, vol. 1031, no. 1, pp. 67–73, 2005.

[65] K. A. Michelsen, D. L. A. van den Hove, C. Schmitz, O. Segers, J. Prickaerts, and H. W. M. Steinbusch, "Prenatal stress and subsequent exposure to chronic mild stress influence dendritic spine density and morphology in the rat medial prefrontal cortex," *BMC Neuroscience*, vol. 8, article 107, 2007.

[66] M. Weinstock, T. Poltyrev, D. Schorer-Apelbaum, D. Men, and R. McCarty, "Effect of prenatal stress on plasma corticosterone and catecholamines in response to footshock in rats," *Physiology and Behavior*, vol. 64, no. 4, pp. 439–444, 1998.

[67] V. Lemaire, M. Koehl, M. Le Moal, and D. N. Abrous, "Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 20, pp. 11032–11037, 2000.

[68] J. I. Koenig, G. I. Elmer, P. D. Shepard et al., "Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia," *Behavioural Brain Research*, vol. 156, no. 2, pp. 251–261, 2005.

[69] S. Chung, H. S. Gi, H. P. Sung et al., "Differential adaptive responses to chronic stress of maternally stressed male mice offspring," *Endocrinology*, vol. 146, no. 7, pp. 3202–3210, 2005.

[70] C. Henry, M. Kabbaj, H. Simon, M. Le Moal, and S. Maccari, "Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats," *Journal of Neuroendocrinology*, vol. 6, no. 3, pp. 341–345, 1994.

[71] S. Maccari, M. Darnaudery, S. Morley-Fletcher, A. R. Zuena, C. Cinque, and O. Van Reeth, "Prenatal stress and long-term consequences: implications of glucocorticoid hormones," *Neuroscience and Biobehavioral Reviews*, vol. 27, no. 1-2, pp. 119–127, 2003.

[72] A. Berry, P. Panetta, A. Luoni et al., "Decreased Bdnf expression and reduced social behavior in periadolescent rats following prenatal stress," *Developmental Psychobiology*, vol. 57, no. 3, pp. 365–373, 2015.

[73] A. R. Zuena, J. Mairesse, P. Casolini et al., "Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats," *PLoS ONE*, vol. 3, no. 5, Article ID e2170, 2008.

[74] A. S. R. Sierksma, T. Vanmierlo, J. De Vry et al., "Effects of prenatal stress exposure on soluble Aβ and brain-derived neurotrophic factor signaling in male and female APPswe/PS1dE9 mice," *Neurochemistry International*, vol. 61, no. 5, pp. 697–701, 2012.

[75] A. S. R. Sierksma, J. Prickaerts, L. Chouliaras et al., "Behavioral and neurobiological effects of prenatal stress exposure in male and female APPswe/PS1dE9 mice," *Neurobiology of Aging*, vol. 34, no. 1, pp. 319–337, 2013.

[76] R. Li, F.-S. Huang, A.-K. Abbas, and H. Wigström, "Role of NMDA receptor subtypes in different forms of NMDA-dependent synaptic plasticity," *BMC Neuroscience*, vol. 8, article 55, 2007.

[77] R. C. Malenka and M. F. Bear, "LTP and LTD: an embarrassment of riches," *Neuron*, vol. 44, no. 1, pp. 5–21, 2004.

[78] N. Jia, K. Yang, Q. Sun et al., "Prenatal stress causes dendritic atrophy of pyramidal neurons in hippocampal CA3 region by glutamate in offspring rats," *Developmental Neurobiology*, vol. 70, no. 2, pp. 114–125, 2010.

[79] D. Zhao, D. Liu, X. Chen et al., "Prenatal stress disturbs hippocampal KIF17 and NR2B in spatial cognition in male offspring," *Journal of Neuroscience Research*, vol. 91, no. 4, pp. 535–544, 2013.

[80] V. Pinto, J. C. Costa, P. Morgado et al., "Differential impact of chronic stress along the hippocampal dorsal-ventral axis," *Brain Structure and Function*, vol. 220, no. 2, pp. 1205–1212, 2015.

[81] F. Matrisciano, P. Tueting, I. Dalal et al., "Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice," *Neuropharmacology*, vol. 68, pp. 184–194, 2013.

[82] J. Marrocco, J. Mairesse, R. T. Ngomba et al., "Anxiety-like behavior of prenatally stressed rats is associated with a selective reduction of glutamate release in the ventral hippocampus," *The Journal of Neuroscience*, vol. 32, no. 48, pp. 17143–17154, 2012.

[83] J. Marrocco, M.-L. Reynaert, E. Gatta et al., "The effects of antidepressant treatment in prenatally stressed rats support the glutamatergic hypothesis of stress-related disorders," *Journal of Neuroscience*, vol. 34, no. 6, pp. 2015–2024, 2014.

[84] K. Ikemoto, T. Uwano, A. Nishimura, K. Nishi, T. Ono, and H. Nishijo, "Prenatal maternal stress due to repeated exposure to a cold environment affects development of catecholamine neurons in rat offsprings: an immunohistochemical study," *Journal of Neurology & Neurophysiology*, vol. 6, no. 1, pp. 271–273, 2015.

[85] J. Bock, T. Wainstock, K. Braun, and M. Segal, "Stress in utero: prenatal programming of brain plasticity and cognition," *Biological Psychiatry*, vol. 78, no. 5, pp. 315–326, 2015.

[86] M. Weinstock, "The long-term behavioural consequences of prenatal stress," *Neuroscience and Biobehavioral Reviews*, vol. 32, no. 6, pp. 1073–1086, 2008.

[87] L. J. Bertoglio, S. R. L. Joca, and F. S. Guimarães, "Further evidence that anxiety and memory are regionally dissociated within the hippocampus," *Behavioural Brain Research*, vol. 175, no. 1, pp. 183–188, 2006.

[88] S. Pellow, P. Chopin, S. E. File, and M. Briley, "Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat," *Journal of Neuroscience Methods*, vol. 14, no. 3, pp. 149–167, 1985.

[89] A. A. Walf and C. A. Frye, "The use of the elevated plus maze as an assay of anxiety-related behavior in rodents," *Nature Protocols*, vol. 2, no. 2, pp. 322–328, 2007.

[90] S. Salomon, C. Bejar, D. Schorer-Apelbaum, and M. Weinstock, "Corticosterone mediates some but not other behavioural changes induced by prenatal stress in rats," *Journal of Neuroendocrinology*, vol. 23, no. 2, pp. 118–128, 2011.

[91] G. Zagron and M. Weinstock, "Maternal adrenal hormone secretion mediates behavioural alterations induced by prenatal stress in male and female rats," *Behavioural Brain Research*, vol. 175, no. 2, pp. 323–328, 2006.

[92] C. Cannizzaro, F. Plescia, M. Martire et al., "Single, intense prenatal stress decreases emotionality and enhances learning performance in the adolescent rat offspring: interaction with a brief, daily maternal separation," *Behavioural Brain Research*, vol. 169, no. 1, pp. 128–136, 2006.

[93] R. I. Martínez-Téllez, E. Hernández-Torres, C. Gamboa, and G. Flores, "Prenatal stress alters spine density and dendritic length of nucleus accumbens and hippocampus neurons in rat offspring," *Synapse*, vol. 63, no. 9, pp. 794–804, 2009.

[94] J. A. Markham, A. R. Taylor, S. B. Taylor, D. B. Bell, and J. I. Koenig, "Characterization of the cognitive impairments induced by prenatal exposure to stress in the rat," *Frontiers in Behavioral Neuroscience*, vol. 4, article 173, 2010.

[95] C. L. Coe, M. Kramer, B. Czéh et al., "Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys," *Biological Psychiatry*, vol. 54, no. 10, pp. 1025–1034, 2003.

[96] C. L. Coe and G. R. Lubach, "Prenatal origins of individual variation in behavior and immunity," *Neuroscience and Biobehavioral Reviews*, vol. 29, no. 1, pp. 39–49, 2005.

[97] L. de Hoz, J. Knox, and R. G. M. Morris, "Longitudinal axis of the hippocampus: both septal and temporal poles of the hippocampus support water maze spatial learning depending on the training protocol," *Hippocampus*, vol. 13, no. 5, pp. 587–603, 2003.

[98] R. Morris, "Developments of a water-maze procedure for studying spatial learning in the rat," *Journal of Neuroscience Methods*, vol. 11, no. 1, pp. 47–60, 1984.

[99] M. Hosseini-Sharifabad and H. Hadinedoushan, "Prenatal stress induces learning deficits and is associated with a decrease in granules and CA3 cell dendritic tree size in rat hippocampus," *Anatomical Science International*, vol. 82, no. 4, pp. 211–217, 2007.

[100] J. Wu, T.-B. Song, Y.-J. Li, K.-S. He, L. Ge, and L.-R. Wang, "Prenatal restraint stress impairs learning and memory and hippocampal PKCbeta1 expression and translocation in offspring rats," *Brain Research*, vol. 1141, no. 1, pp. 205–213, 2007.

[101] J. Xu, B. Yang, C. Yan et al., "Effects of duration and timing of prenatal stress on hippocampal myelination and synaptophysin expression," *Brain Research*, vol. 1527, pp. 57–66, 2013.

[102] B. R. Mueller and T. L. Bale, "Sex-specific programming of offspring emotionality after stress early in pregnancy," *The Journal of Neuroscience*, vol. 28, no. 36, pp. 9055–9065, 2008.

[103] A. Kapoor, A. Kostaki, C. Janus, and S. G. Matthews, "The effects of prenatal stress on learning in adult offspring is dependent on the timing of the stressor," *Behavioural Brain Research*, vol. 197, no. 1, pp. 144–149, 2009.

[104] M. Coulon, R. Nowak, S. Andanson, B. Petit, F. Lévy, and A. Boissy, "Effects of prenatal stress and emotional reactivity of the mother on emotional and cognitive abilities in lambs," *Developmental Psychobiology*, vol. 57, no. 5, pp. 626–636, 2015.

[105] J. Bock, M. S. Murmu, Y. Biala, M. Weinstock, and K. Braun, "Prenatal stress and neonatal handling induce sex-specific changes in dendritic complexity and dendritic spine density in hippocampal subregions of prepubertal rats," *Neuroscience*, vol. 193, pp. 34–43, 2011.

[106] B. Petit, A. Boissy, A. Zanella et al., "Stress during pregnancy alters dendritic spine density and gene expression in the brain of new-born lambs," *Behavioural Brain Research*, vol. 291, pp. 155–163, 2015.

[107] A. Hayashi, M. Nagaoka, K. Yamada, Y. Ichitani, Y. Miake, and N. Okado, "Maternal stress induces synaptic loss and developmental disabilities of offspring," *International Journal of Developmental Neuroscience*, vol. 16, no. 3-4, pp. 209–216, 1998.

[108] C. D. Mandyam, E. F. Crawford, A. J. Eisen, C. L. Rivier, and H. N. Richardson, "Stress experienced in utero reduces sexual dichotomies in neurogenesis, microenvironment, and cell death in the adult rat hippocampus," *Developmental Neurobiology*, vol. 68, no. 5, pp. 575–589, 2008.

[109] S. Morley-Fletcher, J. Mairesse, A. Soumier et al., "Chronic agomelatine treatment corrects behavioral, cellular, and biochemical abnormalities induced by prenatal stress in rats," *Psychopharmacology*, vol. 217, no. 3, pp. 301–313, 2011.

[110] H. Sun, Q. Su, H. Zhang et al., "Alterations of proliferation and differentiation of hippocampal cells in prenatally stressed rats," *Brain and Development*, vol. 37, no. 6, pp. 563–571, 2015.

[111] A. Fujioka, T. Fujioka, Y. Ishida, T. Maekawa, and S. Nakamura, "Differential effects of prenatal stress on the morphological maturation of hippocampal neurons," *Neuroscience*, vol. 141, no. 2, pp. 907–915, 2006.

[112] A. Muhammad, C. Carroll, and B. Kolb, "Stress during development alters dendritic morphology in the nucleus accumbens and prefrontal cortex," *Neuroscience*, vol. 216, pp. 103–109, 2012.

[113] C. L. Coe, G. R. Lulbach, and M. L. Schneider, "Prenatal disturbance alters the size of the corpus callosum in young monkeys," *Developmental Psychobiology*, vol. 41, no. 2, pp. 178–185, 2002.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 6212983, 13 pages
http://dx.doi.org/10.1155/2016/6212983

Research Article

Social Isolation Stress Induces Anxious-Depressive-Like Behavior and Alterations of Neuroplasticity-Related Genes in Adult Male Mice

Alessandro Ieraci, Alessandra Mallei, and Maurizio Popoli

Laboratorio di Neuropsicofarmacologia e Neurogenomica Funzionale, Dipartimento di Scienze Farmacologiche e Biomolecolari e Centro di Eccellenza sulle Malattie Neurodegenerative, Università di Milano, Milano, Italy

Correspondence should be addressed to Alessandro Ieraci; alessandro.ieraci@unimi.it

Received 4 September 2015; Revised 4 November 2015; Accepted 11 November 2015

Academic Editor: Jordan Marrocco

Stress is a major risk factor in the onset of several neuropsychiatric disorders including anxiety and depression. Although several studies have shown that social isolation stress during postweaning period induces behavioral and brain molecular changes, the effects of social isolation on behavior during adulthood have been less characterized. Aim of this work was to investigate the relationship between the behavioral alterations and brain molecular changes induced by chronic social isolation stress in adult male mice. Plasma corticosterone levels and adrenal glands weight were also analyzed. Socially isolated (SI) mice showed higher locomotor activity, spent less time in the open field center, and displayed higher immobility time in the tail suspension test compared to group-housed (GH) mice. SI mice exhibited reduced plasma corticosterone levels and reduced difference between right and left adrenal glands. SI showed lower mRNA levels of the BDNF-7 splice variant, c-Fos, Arc, and Egr-1 in both hippocampus and prefrontal cortex compared to GH mice. Finally, SI mice exhibited selectively reduced mGluR1 and mGluR2 levels in the prefrontal cortex. Altogether, these results suggest that anxious- and depressive-like behavior induced by social isolation stress correlates with reduction of several neuroplasticity-related genes in the hippocampus and prefrontal cortex of adult male mice.

1. Introduction

Chronic stress is recognized to be a major risk factor for several psychiatric disorders, including anxiety and depression [1–3]. In the presence of an environmental stressor, the body responds with major modifications of the normal homeostasis, including alterations of the hypothalamic-pituitary-adrenal (HPA) axis, the major neuroendocrine system activated by stressful experiences [4, 5]. Activation of the HPA induced by stress culminates with the release of corticosterone (CORT) from the adrenal glands. These processes are finely controlled by neuronal activity of the hippocampus (HPC) and prefrontal cortex (PFC), which exert a negative feedback on the HPA axis activation. Interestingly, it has been reported that both hyper- and hypoactivation of the HPA axis are associated with increased susceptibility to developing psychiatric disorders [6, 7].

In order to better understand the etiology of depression and anxiety and to develop new treatments, valid and reliable animal models are needed [8]. Several chronic stress methods, either physical or psychosocial, have been described to promote modifications in behavior, brain structure, and neuroendocrine system in rodents [8–12]. Because psychiatric disorders in humans have been linked prevalently with social stress and/or reduced social interaction rather than physical stress [13–16], there is an increasing interest in developing chronic social stress paradigms for modeling psychiatric disorders in animal models. Different models of chronic social stress are able to induce various modifications in the behavior, brain function, and neuroendocrine system [17–20]. While it has been extensively reported that social isolation (SI) rearing induces long-lasting effects on behavior and brain structure in rodents, the behavioral consequences of SI in adult animals, especially in mice, have been less

investigated [21–27]. Moreover, the molecular mechanisms underlying these modifications are still not fully understood.

Chronic stress-induced behavioral modifications have been correlated with changes of neuroplasticity-related genes in different brain areas, including neurotrophic factors, metabotropic glutamate receptors, and immediate early genes [11, 28–31]. In particular, Brain-Derived Neurotrophic Factor (BDNF), a brain plasticity marker, has been extensively investigated and associated with stress response, depression, and anxiety [31–36]. The BDNF gene is a complex gene, formed by at least eight $5'$ noncoding exons, each of which can be alternatively spliced to a common $3'$ protein-coding exon, to generate different transcripts [37, 38]. The complex structure of the BDNF gene allows the distinct BDNF splice variants to be differentially expressed in subcellular localizations, in specific brain regions and in response to distinct stimuli [35, 36, 38–41]. However, it is not known whether and how the BDNF splice variants are differentially regulated in the HPC and PFC of socially isolated mice.

In this study, we assessed the effect of SI stress on anxious- and depressive-like behaviors in adult male mice. Moreover, we measured the plasma CORT levels and the adrenal gland weight in socially isolated and grouped mice. Finally, we investigated whether the expression of total BDNF, BDNF splice variants, and other neuroplasticity-related genes (mGluR1, mGluR2, mGluR5, c-Fos, Arc, and Egr-1) were altered in the HPC and PFC of socially isolated compared to grouped adult male mice.

2. Materials and Methods

2.1. Animals. Male C57BL/6J mice that are 8 weeks old were purchased from Charles River (Calco, Italy) and allowed to acclimatize 1 week before being randomly divided in socially isolated (SI) and group-housed (GH) groups. Mice were maintained in a standard 12 h light/dark cycle, temperature controlled room (21 ± 1°C), with access to food and water *ad libitum*.

All animal procedures were conducted according to current regulations for animal experimentation in Italy and the European Union and were approved by the Italian Ministry of Health. To reduce the number of animals used, in agreement with the 3R guidelines, we performed behavioral and molecular analysis in the same cohort of mice. All experimental procedures involving animals were performed in accordance with the European Community Council Directive 86/609/EEC and were approved by Italian legislation on animal experimentation (Decreto Legislativo 116/1992).

2.2. Experimental Procedure. Nine-week-old male mice were individually housed (socially isolated: SI) or group-housed (4 mice per cage; group-housed: GH) in standard mouse cages. Mice were tested in the open field test (OFT) on day 28 and in the tail suspension test (TST) on day 31 (Figure 1(a)). Mice were sacrificed on day 33 and blood, adrenal glands, HPC, and PFC were harvested (Figure 1(a)).

2.3. Open Field Test. OFT was conducted on day 28 as described in Ieraci and Herrera [42] with some modifications. Briefly, mice were placed in the center of a 40 × 40 cm square arena divided into central (20 cm × 20 cm) and peripheral areas, with 35 cm high walls, for 10 min. The test was conducted in a dimly lit room. The total distance traveled and the time spent in the central and peripheral areas were analyzed by a video-tracking system (Any-Maze purchased by Ugo Basile, Varese, Italy).

2.4. Tail Suspension Test. TST was performed on day 31 as described by Steru et al. [43]. Briefly, mice were individually suspended by an adhesive tape, placed about 1 cm from the tip of the tail, on a horizontal bar 50 cm above from the bench top within a visually isolated area. A 6 min test session was videotaped and an experimenter, blinded to the experimental groups, recorded the total immobility time (defined as passive hanging without any movements except respiration).

2.5. Adrenal Weight. To assess whether chronic social isolation stress affects adrenal glands weight, SI and GH mice were sacrificed on day 33 and adrenal glands were removed and pruned from fat and weighed. The left and right adrenals were weighted separately for each animal. Values are reported as the adrenal/total body weight ratio (mg/g).

2.6. Corticosterone Assay. For CORT levels measure, trunk blood was collected on ice-cooled microcentrifuge tube containing EDTA 0.5 M pH 8.00, and plasma was separated by centrifugation and stored at −80°C. CORT levels were measured using the corticosterone ELISA Kit (Enzo Life Sciences, Florence, Italy) according to the manufacturer's instructions.

2.7. RNA Isolation and Reverse Transcription. Total RNA from HPC and PFC was extracted using the Direct-zol RNA MiniPrep (Zymo Research, purchased by Euroclone, Milan, Italy) according to manufacturer's instructions and then quantified by absorption at $A_{260\,\mathrm{nm}}$ measured by UV spectrophotometry (NanoVue, GE Healthcare Europe GmbH, Milan, Italy). cDNA was synthesized from 1 μg of DNase-treated total RNA using the iScript kit (Biorad, Milan, Italy) according to manufacturer's instructions.

2.8. Quantitative Real-Time PCR. qPCR analysis of mRNA expression levels was performed on a 7900HT Fast PCR System (Applied Biosystems, Monza, Italy) and iTaq Universal SYBR Green supermix (Biorad), as previously described [35]. Primers used are listed in Table 1 and were either *de novo* designed or previously published elsewhere [35, 44]. PCR cycling conditions were 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. Relative expression of mRNA for the target genes was performed by the comparative C_T ($\Delta\Delta C_T$) method using β-actin and GAPDH as control reference genes. The relative mRNA levels were expressed as fold change. Analysis of melting curve verified the specificity of the PCR products.

2.9. Western Blot. Western blot was performed as previously described [45]; briefly HPC and PFC were lysed in ice-cold RIPA buffer (0.15 mM NaCl, 0.05 mM Tris_HCl, pH

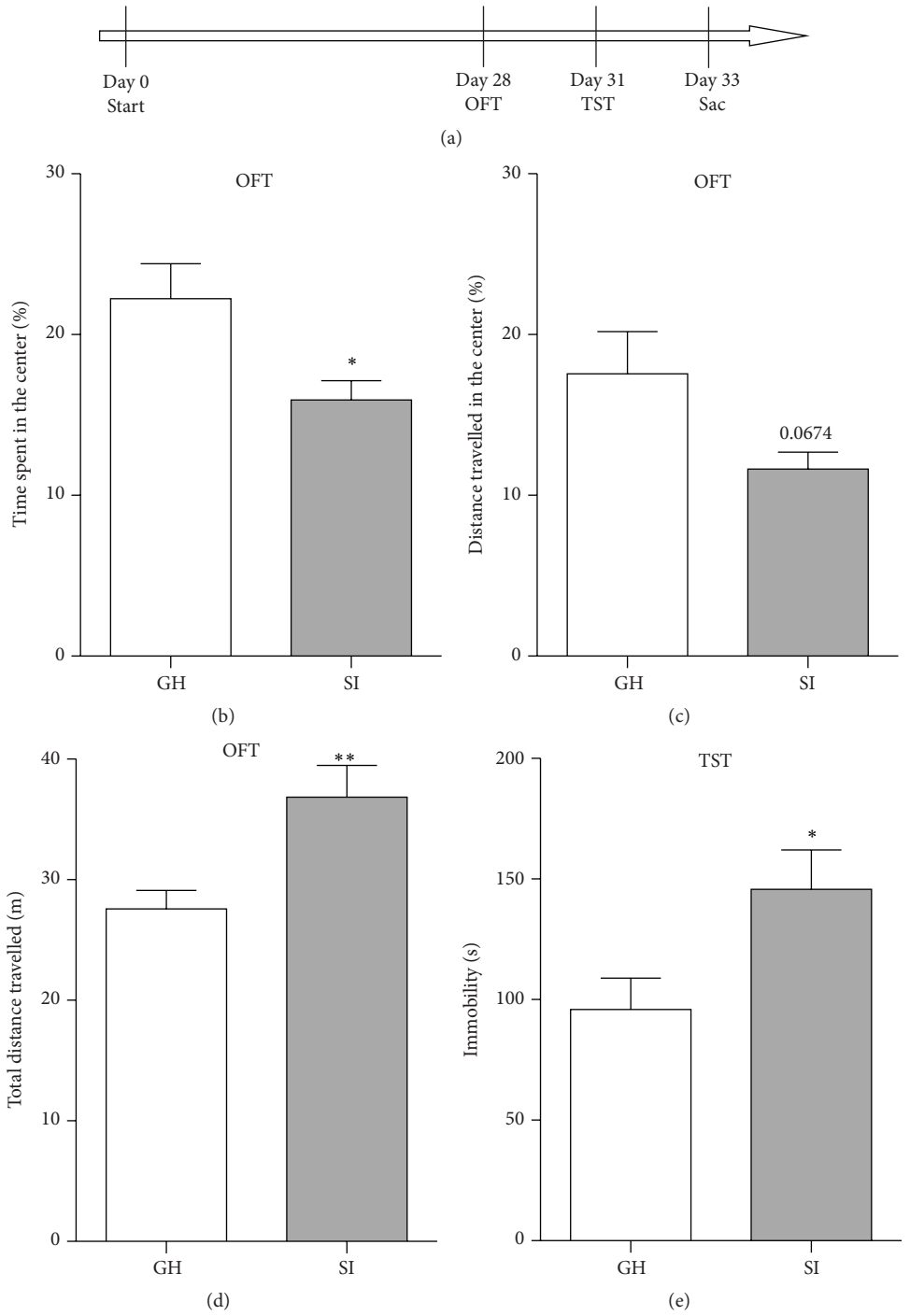


Figure 1: (a) Experimental schedule. Nine-week-old male mice were housed in groups (GH) or socially isolated (SI) throughout the experimental period. The open field test (OFT) and the tail suspension test (TST) were conducted on days 28 and 31, respectively. Animals were sacrificed on day 33. (b–e) Chronic adult social isolation stress induced hyperactivity and anxiety- and depressive-like behaviors in male mice. In the OFT, SI mice showed reduced percentage of time spent in the center (b), increased total distance travelled (d), and only a tendency to decreased percentage of distance travelled in the center (c). In the TST, SI mice showed increased total immobility time (e). Data are presented as mean ± SEM (n = 7-8 per group). $^{*}p < 0.05$; $^{**}p < 0.01$.

Table 1: List and sequence of primers used in this study.

Gene	Forward	Reverse
Real-time PCR		
BDNF-1[a]	CCTGCATCTGTTGGGGAGAC	CGCCTTCATGCAACCGAAGTAT
BDNF-2[a]	ACCTTTTCCTCCTCCTGCG	TGGATGAAGTACTACCACCTCGG
BDNF-3[a]	TGAGACTGCGCTCCACTCCC	CGCCTTCATGCAACCGAAGTAT
BDNF-4[a]	CAGAGCAGCTGCCTTGATGTTT	CGCCTTCATGCAACCGAAGTAT
BDNF-6[a]	ACAATGTGACTCCACTGCCGG	CGCCTTCATGCAACCGAAGTAT
BDNF-7[a]	ACTTACAGGTCCAAGGTCAACG	GGACAGAGGGTCGGATACAG
BDNF-8[a]	ATGACTGTGCATCCCAGGAGAAA	CGCCTTCATGCAACCGAAGTAT
BDNF[a]	TCGTTCCTTTCGAGTTAGCC	TTGGTAAACGGCACAAAAC
mGluR1	CACAGCCCTTGCCAAAGAGAATGAG	CACTCCACTCGAGGTTAACGGA
mGluR2	CACCACCTGTATCATCTGGCTG	GAGCACCACAGAGCCACTGA
mGluR5	AGACGACCTGGCCAAACAAA	CTACTGCTCATGAAAGCCCACA
c-Fos[b]	CTGCAGCCAAGTGCCGGAATC	GGCAATCTCAGTCTGCAACGC
Arc	AGCCCAAACTCAAGCGCTTT	GAAGGCTCAGCTGCCTGCCTC
Egr-1[b]	CCTTCAATCCTCAAGGGGAGC	AACCGAGTCGTTTGGCTGGGA
β-Actin[a]	GCCAGAGCAGTAATCTCCTTCT	AGTGTGACGTTGACATCCGTA
GAPDH[a]	CGTGCCGCCTGGAGAAACC	TGGAAGAGTGGGAGTTGCTGTTG

[a]Ieraci et al., 2015 Hippocampus [35]; [b]Rusconi et al., 2015 Cereb. Cortex [44].

7.2, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) with Protease Inhibitor Cocktail (Sigma-Aldrich, Milan, Italy). Protein concentration was determined by the Quantum Bicinchoninic Protein Assay (Euroclone) and 15 μg of proteins was separated on SDS-PAGE gels and transferred to a polyvinylidene difluoride membrane (GE Healthcare, Amersham, Milan, Italy), followed by blocking in 5% milk dissolved in Tris Buffer Saline-Tween 20 (TBST). Membranes were incubated with anti-BDNF (1 : 2,000, rabbit polyclonal, Alomone, Jerusalem, Israel) or anti-β-actin (1 : 20,000, mouse monoclonal, Sigma) antibodies. After washing with TBST, filters were incubated with peroxidase-conjugated secondary anti-rabbit (1 : 3,000, Sigma-Aldrich) or with the fluorescent IRDye secondary anti-mouse antibody (LI-COR, purchased from Carlo Erba Reagents, Milan, Italy). Peroxidase immunoreactivity bands were revealed by chemiluminescence using ECL detection system (Biorad). Chemiluminescence and fluorescence membrane signals were scanned and quantified in an Odyssey LI-COR scanner (Carlo Erba Reagents).

2.10. Statistical Analysis. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA). Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were made using unpaired t-test or two-way analysis of variance (ANOVA), and LSD procedure was used for multiple comparison analysis. Pearson's r correlations were performed to assess the correlation between CORT levels, gene expression levels, and behavioral analyses (Tables 2–7).

3. Results

3.1. Social Isolation Stress Induces Behavioral Impairments in Adult Male Mice. To assess whether social isolation induces modifications of emotional behavior in adult mice, 9-week-old male mice were randomly housed in group (GH) or socially isolated (SI) for 4 weeks and then tested in the OFT and in the TST to measure the anxious- and depressive-like behaviors, respectively (Figure 1(a)). SI mice spent significantly less time in the center of the arena ($p = 0.029$) (Figure 1(b)) and showed a tendency to a reduced distance travelled in the center ($p = 0.067$) (Figure 1(c)), indicating anxious-like behavior. Interestingly, SI mice showed an increased level of total distance travelled in the OFT as compared to the GH mice ($p = 0.007$) suggesting a hyperactivity phenotype in SI mice (Figure 1(d)). Moreover, in the TST, the SI exhibited a significantly higher period of immobility time compared to the GH mice ($p = 0.03$), indicating a depressive-like behavior in SI mice (Figure 1(e)).

Table 2: Pearson's correlation between corticosterone levels and behavioral analysis.

	HPC	
	r	p
% time spent in the center	0.1178	0.6759
% distance in the center	−0.07975	0.7776
Total distance travelled	−0.4050	0.1343
Tail suspension test	**−0.6367**	**0.0107**

3.2. Corticosterone Plasma Levels Are Decreased in Socially Isolated Adult Male Mice. To study whether the behavioral changes induced by social isolation are associated with alterations of the HPA axis function, we measured the plasma CORT levels and the adrenal glands weight, as an index of terminal phase of the HPA activation, in the SI and GH mice. Interestingly, the levels of plasma CORT were markedly

Table 3: Pearson's correlation between corticosterone and gene expression levels.

	HPC		PFC	
	r	*p*	*r*	*p*
Total BDNF	0.1281	0.6490	−0.1253	0.6438
BDNF-1	0.01566	0.9558	−0.04050	0.8816
BDNF-2	0.003361	0.9905	0.2474	0.3556
BDNF-3	−0.4312	0.1085	0.2589	0.3330
BDNF-4	−0.06950	0.8056	−0.1629	0.5467
BDNF-6	0.1259	0.6549	0.1538	0.5695
BDNF-7	**0.5552**	**0.0317**	**0.4933**	**0.0522**
BDNF-8	−0.09168	0.7452	0.2692	0.3133
mGluR1	0.07396	0.7934	0.3207	0.2439
mGluR2	0.01753	0.9506	0.3320	0.2267
mGluR5	0.2400	0.3890	−0.4608	0.0839
c-Fos	0.3787	0.1639	0.4177	0.1214
Arc	0.4144	0.1246	0.3985	0.1412
Egr-1	0.3721	0.1720	0.4619	0.0830

Table 4: Pearson's correlation between the percentage time spent in the open field center and gene expression levels.

	HPC		PFC	
	r	*p*	*r*	*p*
Total BDNF	**0.5459**	**0.0353**	−0.01596	0.9550
BDNF-1	−0.06687	0.8128	−0.3120	0.2576
BDNF-2	0.3161	0.2510	0.5469	0.0349
BDNF-3	−0.1448	0.6066	0.08998	0.7498
BDNF-4	0.07657	0.7862	−0.1619	0.5643
BDNF-6	0.4217	0.1175	0.1034	0.7138
BDNF-7	0.3897	0.1510	**0.5886**	**0.0210**
BDNF-8	−0.4328	0.1071	−0.02065	0.9418
mGluR1	0.08381	0.7665	**0.6689**	**0.0064**
mGluR2	0.3911	0.1495	0.4856	0.0665
mGluR5	0.2127	0.4465	0.3362	0.2205
c-Fos	**0.6627**	**0.0071**	**0.5797**	**0.0235**
Arc	**0.5360**	**0.0395**	**0.5826**	**0.0227**
Egr-1	0.3196	0.2456	0.5121	0.0510

Table 5: Pearson's correlation between the percentage distance travelled in the open field center and gene expression levels.

	HPC		PFC	
	r	*p*	*r*	*p*
Total BDNF	0.2967	0.2829	−0.2008	0.4731
BDNF-1	−0.1696	0.5456	−0.4613	0.0835
BDNF-2	0.005426	0.9847	0.1432	0.6106
BDNF-3	−0.05189	0.8543	0.09637	0.7326
BDNF-4	0.02956	0.9167	−0.3317	0.2271
BDNF-6	0.2578	0.3536	−0.08952	0.7510
BDNF-7	0.1517	0.5895	0.3941	0.1461
BDNF-8	−0.2511	0.3668	0.01725	0.9514
mGluR1	0.1989	0.4772	**0.5179**	**0.0480**
mGluR2	0.3364	0.2202	0.4008	0.1388
mGluR5	0.2203	0.4302	0.4895	0.0640
c-Fos	0.3250	0.2373	0.3312	0.2278
Arc	0.2202	0.4303	0.2744	0.3222
Egr-1	0.1294	0.6458	0.2010	0.4727

Table 6: Pearson's correlation between the total distance travelled in the open field and gene expression levels.

	HPC		PFC	
	r	*p*	*r*	*p*
Total BDNF	−0.01723	0.9514	0.2982	0.2804
BDNF-1	0.4873	0.0654	0.3025	0.2731
BDNF-2	−0.06728	0.8117	0.1369	0.6266
BDNF-3	0.5139	0.0500	0.3978	0.1420
BDNF-4	0.3485	0.2030	0.1766	0.5289
BDNF-6	0.1649	0.5571	0.2295	0.4107
BDNF-7	−0.3760	0.1672	**−0.5107**	**0.0517**
BDNF-8	0.1553	0.5806	0.09135	0.7461
mGluR1	−0.1733	0.5367	−0.4017	0.1378
mGluR2	0.1448	0.6066	−0.3355	0.2215
mGluR5	0.03919	0.8897	−0.02266	0.9361
c-Fos	−0.4212	0.1180	−0.3366	0.2200
Arc	−0.3860	0.1553	−0.2858	0.3017
Egr-1	−0.1496	0.5947	−0.4556	0.0879

reduced in the SI mice compared to GH mice ($p = 0.002$) (Figure 2(a)). Moreover, a negative Pearson's correlation was specifically detected between the immobility time measured in the TST and the plasma CORT levels ($r = -0.6367$; $p = 0.0107$; Table 2). A two-way ANOVA showed a significant overall effect for the size of the adrenal glands ($F_{(1,26)} = 5.704$; $p = 0.0245$), with the left adrenal gland heavier than the right one (Figure 2(b)), consistent with the data reported in literature [46, 47]. A further Fisher's LSD *post hoc* analysis revealed a significant difference between the right and left adrenal gland for the GH mice ($p = 0.03$), but not for the SI mice ($p = 0.19$) (Figure 2(b)). These differences were not due to changes in body weight ($p = 0.45$) (Figure 2(c)).

3.3. Neuroplasticity-Related Genes Are Reduced in the HPC and PFC of Socially Isolated Adult Male Mice. To investigate the possible molecular mechanisms underlying the social isolation stress-induced behavioral changes, we evaluated the mRNA levels of different genes involved in neuronal plasticity in the HPC and PFC of SI and GH mice. Firstly, we measured the mRNA levels of total BDNF, which is known to modulate stress-induced behavioral changes [34], and BDNF splice variants 1, 2, 3, 4, 6, 7, and 8. We found that BDNF-7 transcript was significantly and selectively downregulated in both the HPC and the PFC of SI mice, compared to GH mice (HPC: $p = 0.028$; PFC: $p = 0.01$) (Figure 3), while no significant differences were revealed for total BDNF and all the other BDNF transcripts analyzed (Figure 3). Protein levels analysis by Western blot revealed only a trend for reduction of pro-BDNF in the HPC ($p = 0.082$; Figure 3(c)) and mature BDNF in the PFC ($p = 0.085$; Figure 3(d)).

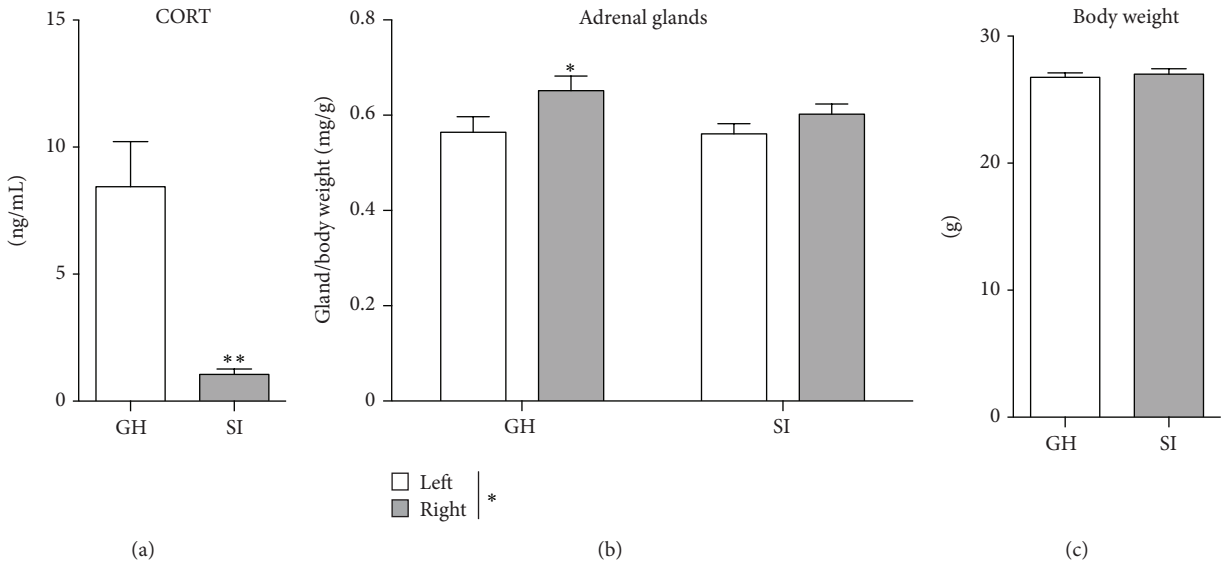


Figure 2: Chronic social isolation stress in adulthood reduces plasma corticosterone levels. (a) Corticosterone levels were reduced in the plasma of socially isolated adult male mice. (b) Socially isolated mice showed reduced differences between the left and right adrenal/body weight ratio. (c) There were no differences in the total body weight between group-housed (GH) and socially isolated (SI) mice. Data are presented mean ± SEM (n = 7-8 per group). $^{*}p < 0.05$; $^{**}p < 0.01$.

Table 7: Pearson's correlation between the immobility time measured in the tail suspension test and gene expression levels.

	HPC		PFC	
	r	p	r	p
Total BDNF	−0.2868	0.3000	−0.3003	0.2768
BDNF-1	0.1625	0.5628	−0.2971	0.2822
BDNF-2	0.1307	0.6425	−0.1610	0.5665
BDNF-3	**0.6580**	**0.0077**	−0.3290	0.2311
BDNF-4	0.2176	0.4359	−0.2689	0.3325
BDNF-6	−0.1178	0.6759	−0.3714	0.1729
BDNF-7	−0.1460	0.6036	−0.3611	0.1860
BDNF-8	0.1389	0.6215	0.1938	0.4888
mGluR1	0.1182	0.6748	−0.1393	0.6205
mGluR2	0.00844	0.9762	−0.05227	0.8532
mGluR5	0.1913	0.4947	0.2808	0.3107
c-Fos	−0.2720	0.3267	−0.2640	0.3417
Arc	−0.3338	0.2241	−0.2845	0.3040
Egr-1	−0.09785	0.7286	−0.1392	0.6208

Interestingly, we have observed a significant positive correlation between the plasma CORT levels and the BDNF-7 mRNA levels measured in the HPC, with only a trend in the PFC (HPC: $r = 0.5552$; $p = 0.0317$; PFC: $r = 0.4933$; $p = 0.0522$; Table 3). Total BDNF mRNA levels in the HPC and BDNF-7 mRNA levels in the PFC positively correlate with the percentage of time spent in the OF center (total BDNF: $r = 0.5459$; $p = 0.0353$; BDNF-7: $r = 0.5886$; $p = 0.021$; Table 4). Moreover, we found a trend for correlation between the cortical BDNF-7 levels and the total distance travelled in the OF ($r = -0.5107$; $p = 0.0517$; Table 6).

Several lines of evidence suggest that metabotropic glutamate receptors are involved in stress-related disorders and in the mechanisms of action of antidepressants [48–50]. Thus, we sought to investigate whether behavioral modifications induced by SI stress correlated with changes in the expression of mGluR1, mGluR2, and mGluR5 in the HPC and PFC. Interestingly, we found that mGluR1 and mGluR2 mRNA levels were significantly reduced in the PFC of SI mice compared to GH mice (mGluR1: $p = 0.012$; mGluR2: $p = 0.038$; Figure 4(b)), while no differences were revealed for mGluR5 mRNA levels in the PFC and for all three mRNA levels in the HPC between SI and GH mice (Figures 4(a)-4(b)). However, only mGluR1 in the PFC showed a positive correlation with the percentage of time spent in the OF center ($r = 0.5886$; $p = 0.021$; Table 4) and the distance travelled in the OF center ($r = 0.5886$; $p = 0.021$; Table 5).

Immediate early genes (IEGs) are genes rapidly expressed upon different stimuli and play important roles in synaptic plasticity [51]. However, constitutive changes in IEGs expression have been reported also after chronic stress and antidepressant treatments [52–54]. Therefore, to investigate whether SI induced alterations in the IEGs expression, we evaluated the mRNA levels of c-Fos, Arc, and Egr-1 in the HPC and PFC of SI and GH mice. We found that mRNA levels of c-Fos, Arc, and Egr-1 were decreased in both HPC and PFC of SI mice compared to GH mice (HPC: c-Fos: $p = 0.0092$; Arc: $p = 0.016$; Egr-1: $p = 0.027$; PFC: c-Fos: $p = 0.0099$; Arc: $p = 0.029$; Egr-1: $p = 0.69$) (Figures 4(c)-4(d)). A significant positive correlation was found for c-Fos and Arc mRNA levels in the HPC and PFC and the percentage of time spent in the OF center (HPC: c-Fos: $r = 0.6627$; $p = 0.0071$; Arc: $r = 0.536$; $p = 0.0395$; PFC: c-Fos: $r = 0.5797$; $p = 0.035$; Arc: $r = 0.5826$; $p = 0.0227$; Table 4).

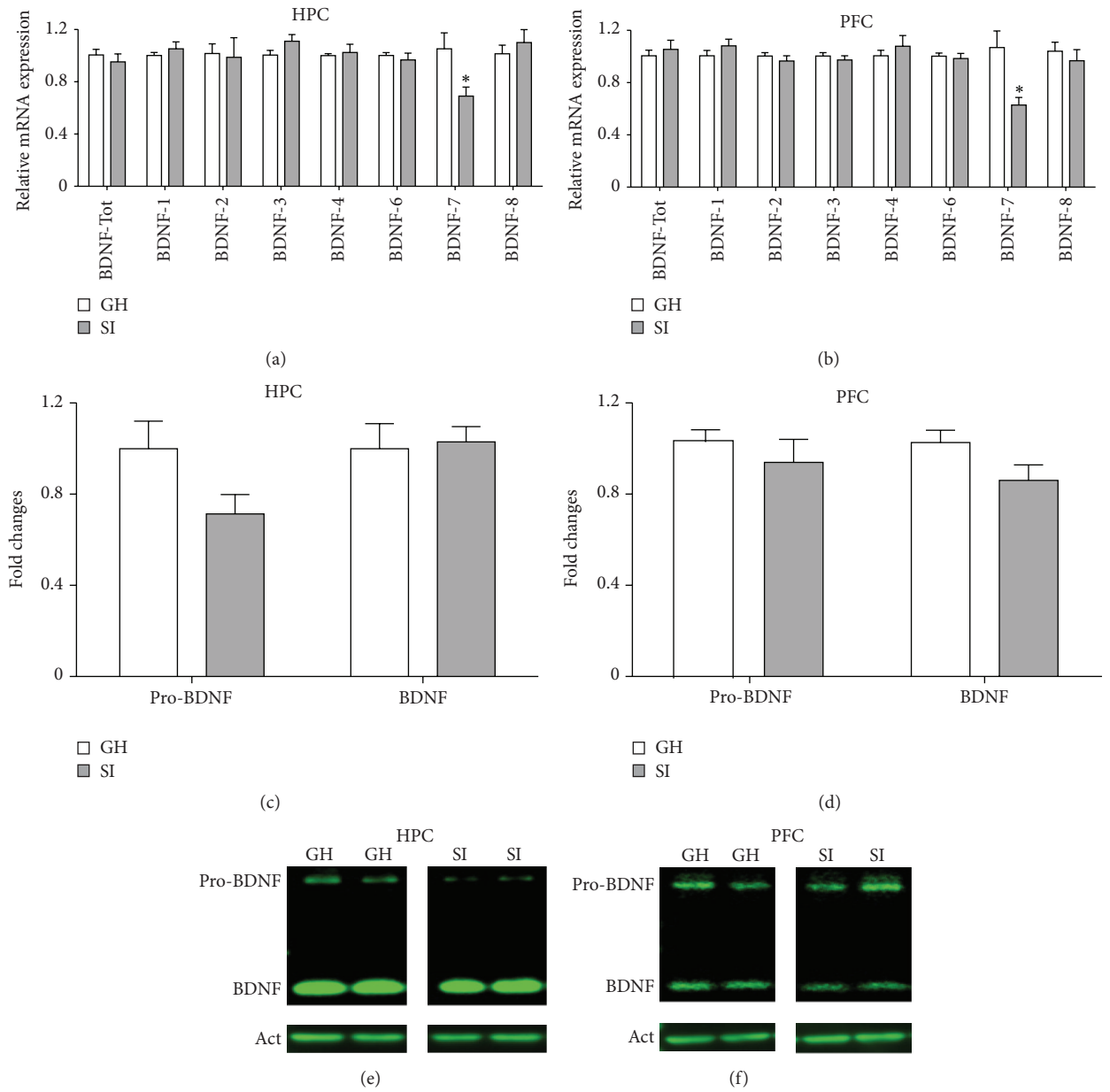


Figure 3: Social isolation stress in adulthood reduces BDNF-7 splice variants in the hippocampus (HPC) and prefrontal cortex (PFC). (a-b) Adult socially isolated mice showed decreased levels of BDNF-7 mRNA transcript in the HPC (a) and PFC (b). On the contrary, total BDNF and BDNF splice variants 1, 2, 3, 4, 6, and 8 were not modulated by adult SI stress. Data are presented as Mean ± SEM (n = 7-8 per group). $^{*}p < 0.05$. (c–f) Pro-BDNF and mature BDNF protein levels in the HPC (c, e) and prefrontal cortex (d, f). Densitometric quantifications were obtained as ratio of Pro-BDNF/β-actin and BDNF/β-actin. Data are presented as mean ± SEM (n = 6 per group). (e-f) Representative Western blot pictures from Pro-BDNF, BDNF, and β-actin.

4. Discussion

In the present study, we have found that SI adult male mice spend less time in the center and are hyperactive in the OFT. Additionally, SI mice showed higher immobility time in the tail suspension test compared to GH mice. Although several studies have reported that SI rearing induces hyperactivity and anxious- and depressive-like behavior in rodents, only a few studies have addressed the consequences of SI in adult rodents, especially in mice [21, 23, 55–59]. Our findings suggest that social deprivation may be deleterious not only during childhood and adolescent period but also during adulthood in male mice. Moreover, our results are consistent with previous data showing that stress induces hyperactivity in rodents in response to exposure to novel environments, such as an open field [52, 60–63].

The reduced plasma CORT levels measured in SI adult mice are paralleled by the decreased difference in weight between the left and right adrenal glands, suggesting that this hypofunction may be due to morphological changes in the

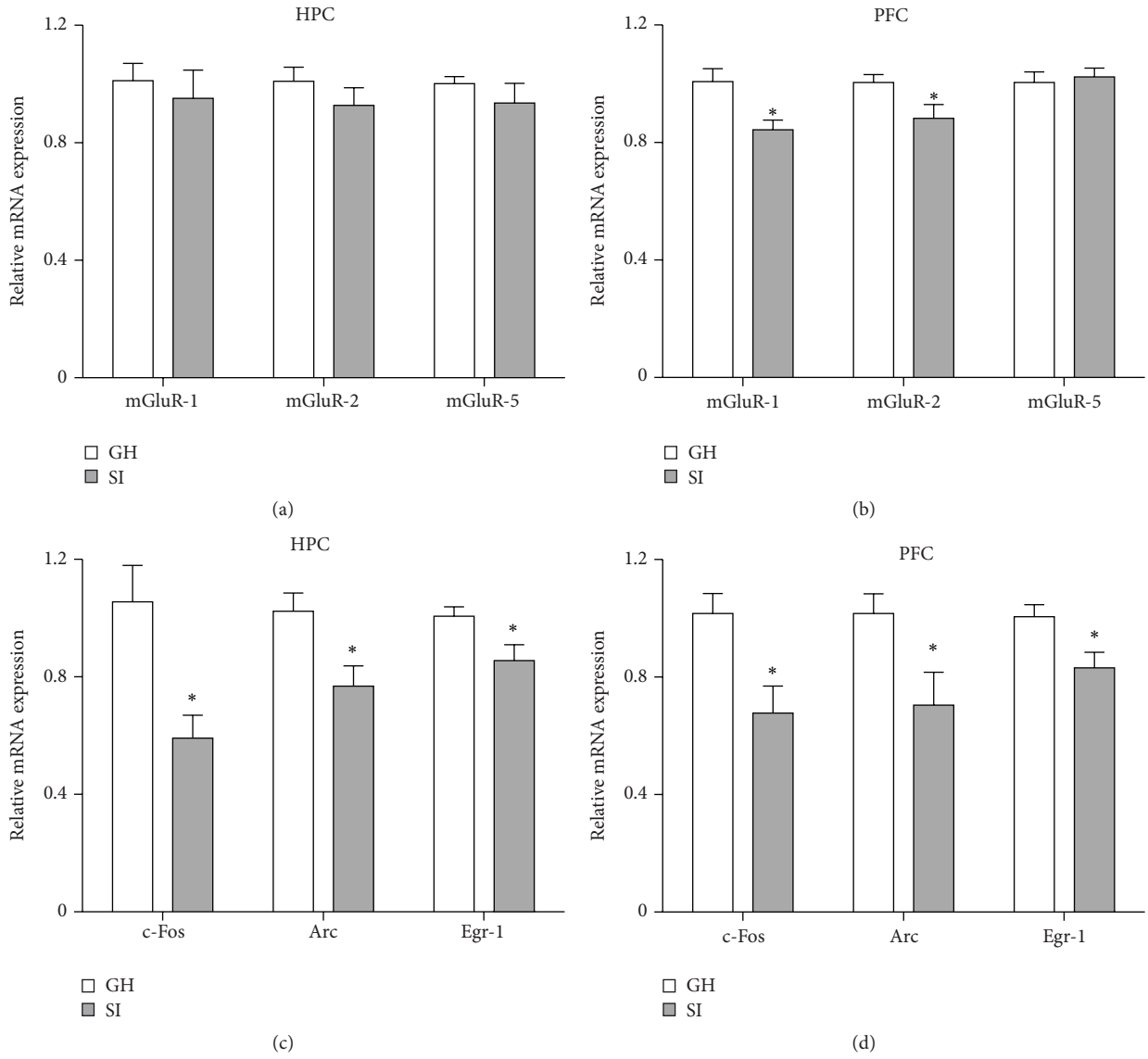


Figure 4: Social isolation stress in adulthood decreases expression of neuroplasticity-related gene in the hippocampus (HPC) and prefrontal cortex (PFC). (a, b) In socially isolated (SI) adult male mice, mGluR1 and mGluR2 mRNA levels were reduced in the PFC (b). No differences were revealed for mGluR5 in the PFC and for mGluR1, mGluR2, and mGluR5 in the HPC (a-b). SI mice showed reduced mRNA levels of c-Fos, Arc, and Egr-1 in the HPC and PFC (c-d). Data are presented as mean ± SEM (n = 7-8 per group). $^{*}p < 0.05$.

HPA axis. Although stress is usually correlated with HPA hyperactivity, our results are in line with previous reports showing reduced levels of CORT in SI adult male rat and female mice [55, 56]. Moreover, lower adrenal activity in response to chronic stress was also reported in social defeat animal models [64]. Interestingly, reduced basal levels of CORT have been reported in patients with posttraumatic stress disorder (PTSD), even decades after the traumatic events [65–67]. Clinical studies have also reported lower CORT levels in some patients at risk for PTSD and in healthy subjects living under constantly stressful environments [68, 69]. Intriguingly, it has been reported that rats with reduced basal levels of CORT display increased anxious-like behavior and low extinctions rates of conditioned fear, behavioral disturbances that model some aspect of PTSD [70]. Delayed and incomplete contextual fear extinction has been also described in SI adult mice [23]. Altogether these lines of evidence suggest that social isolation stress in adult mice could be a suitable model to study the behavioral and molecular alterations related to PTSD [23]. However, further studies will be necessary to fully characterize this model.

The results showing that SI stress in adult male mice downregulated the expression of IEGs are consistent with previous reports showing that social isolation rearing reduced IEGs in rats [52, 71, 72]. IEGs are defined by their capability to be quickly transcribed without new protein synthesis, activated by transcription factors that are regulated by phosphorylation. We measured the levels of gene expression 2

days after the TST to limit the possibility that behavioral tests affected the gene expression. Therefore, the downregulation of IEGs observed here likely reflects an adaptation to the chronic SI stress used, rather than the behavioral manipulation effects. However, we cannot exclude that the downregulation observed is due to the sum of social isolation and behavioral test manipulations.

We found that SI stress decreased Arc expression in both of the HPC and PFC. It has been previously reported that Arc mRNA is delivered to dendrites, where it is specifically accumulated at recently activated synapses and locally translated into protein [73]. Arc has a critical role in neuroplasticity and behavioral processes [74]. Our finding that reduced Arc expression correlated with increased anxiety- and depression-like phenotypes in SI adult mice is consistent with previous data showing that Arc expression is higher in the low-anxious Sprague-Dawley rats compared to the highly anxious hooded PVG strain and that chronic antidepressant treatment increased Arc expression [54, 75]. Altogether, these data suggest that Arc may have a direct role in the synaptic plasticity responses after activation and in behavioral processes, coupling neuronal activity with structural remodeling and functional changes [76, 77]. Interestingly, Egr-1 is a transcriptional factor directly controlling the expression of Arc that has also been associated with some form of synaptic plasticity and may be required for the development of late long-term potentiation and behavioral responses [78–80]. The present results, showing that both Egr-1 and Arc are decreased in HPC and PFC, may suggest that Arc is downregulated as a consequence of stress-induced reduction of Egr-1 expression.

Remarkably, we found that BDNF-7 transcript was selectively reduced in both HPC and PFC of SI mice, while the levels of total BDNF and all other exons were not changed. As a rule, BDNF-7 mRNA levels are lower compared to the most abundant BDNF transcripts in the brain (1, 2, 4, and 6); therefore the reduction of BDNF-7 levels observed in SI mice is probably not enough to significantly reduce the total expression of BDNF (which includes all transcripts). However, the BDNF-7 transcript is more efficiently translated to protein, compared to more highly expressed BDNF transcripts 1, 4, and 6 [81] and is one of the few BDNF transcripts to be present in the dendrites in basal conditions [82]. It has been reported that synaptic plasticity controls local dendritic BDNF translation and that alterations in this pathway may compromise the long-lasting spine plasticity and lead to behavioral dysfunctions [83]. Taken together, these lines of evidence strongly suggest that BDNF-7 may be rapidly translated in the dendritic compartment upon synaptic activation and that reduction of BDNF-7 levels induced by SI stress may contribute to the behavioral impairments observed in SI adult mice.

Our results showing that SI stress decreased the levels of mGluR1 and mGluR2 in the PFC are in line with previous data reporting a significant attenuation in mGluR function in the PFC of SI reared rats [84]. Remarkably, it has been also reported that chronic unpredictable stress (CUS) reduces mGluR2 expression and that upregulation of mGluR2 reverses the anxious- and depressive-like behaviors caused by CUS [48, 50]. Furthermore, a possible role of mGluR2 in controlling hyperactivity has been described as well. Systemic mGluR2/3 agonist administration reverses the SI rearing-induced hyperactivity [85]. Moreover, injecting a mGluR2/3 agonist locally into the PFC blocks the amphetamine-induced hyperactivity [86]. Altogether, these data indicate that downregulation of mGluR2 levels in the PFC of SI mice may contribute to the behavioral impairments observed. Although previous studies have reported behavioral and synaptic plasticity deficits in the mGluR1 null mice [87, 88] and mGluR1/5 agonist reduced the anxiety-like behaviors of DBA/2 mice [89], the possible role of mGluR1 in the PFC has not been specifically investigated yet. Further studies will be necessary to assess whether the expression of mGluR1 in the PFC contributes to the behavioral changes induced by SI.

We specifically found a reduction of mGluR1 and mGluR2 in the PFC but not in the HPC. However, since we have analyzed gene expression in the entire HPC, without subdivision of the different regions (dorsal and ventral hippocampus, dentate gyrus, CA1, and CA3), it is possible that variations in specific regions may be attenuated from the expression in the other regions and therefore not detected by our analyses. Further studies will be necessary to uncover whether changes of the mGluR1 and mGluR2 expression in specific regions of the HPC are actually induced by social isolation. Nevertheless, differential modulation of gene expression in different brain areas is quite common and it has been described for several genes and environmental manipulations.

A major limitation of the present study is the descriptive nature of our analysis. Although we have found significant correlations between behavioral impairments, CORT plasma levels, and gene expression levels in the HPC and PFC, these findings are not sufficient to clearly establish a direct causal effect between these molecular changes and the behavioral alterations. Further studies using both molecular and pharmacological approaches, to specifically modulate the expression of these genes in HPC and PFC, will be necessary. Another limitation of the study is the lack on of time-dependent effects of SI. We have found behavioral and molecular changes after 4 weeks of social isolation stress. It would be interesting in future studies to investigate the shortest period of SI that is able to induce behavioral and molecular changes in adult mice and whether a longer period of isolation is able to worsen the phenotype that we have reported here. Moreover, while we cannot rule out that the molecular changes observed in SI mice are a consequence of the combination of the social isolation paradigm and the behavioral tests, our results show that neuroplasticity-related genes are differentially modulated in GH and SI mice.

Overall, in the present study we showed that SI stress in the adult male mice resulted in anxiety- and depression-like phenotypes. Additionally, we showed that behavioral modifications correlated with decrease of plasma CORT levels and downregulation of neuroplasticity-related genes in HPC and PFC. In particular, the selective reduction of the BDNF-7 transcript in both brain areas of SI mice for the first time links this dendrite-resident BDNF isoform with synaptic plasticity and the behavioral consequences of SI.

Further work to explore the role of this BDNF transcript is warranted.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by a grant from MIUR (PRIN 2010 prot. 2010N8PBAA_004) to Maurizio Popoli and is part of the collaborative consortium "Italian Network on BDNF" (*InBDNF*).

References

[1] E. R. de Kloet, M. Joëls, and F. Holsboer, "Stress and the brain: from adaptation to disease," *Nature Reviews Neuroscience*, vol. 6, no. 6, pp. 463–475, 2005.

[2] B. Leuner and T. J. Shors, "Stress, anxiety, and dendritic spines: what are the connections?" *Neuroscience*, vol. 251, pp. 108–119, 2013.

[3] M. Popoli, Z. Yan, B. S. McEwen, and G. Sanacora, "The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission," *Nature Reviews Neuroscience*, vol. 13, no. 1, pp. 22–37, 2012.

[4] P. W. Gold, "The organization of the stress system and its dysregulation in depressive illness," *Molecular Psychiatry*, vol. 20, no. 1, pp. 32–47, 2015.

[5] C. Tsigos and G. P. Chrousos, "Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress," *Journal of Psychosomatic Research*, vol. 53, no. 4, pp. 865–871, 2002.

[6] C. M. Pariante and S. L. Lightman, "The HPA axis in major depression: classical theories and new developments," *Trends in Neurosciences*, vol. 31, no. 9, pp. 464–468, 2008.

[7] A. Shea, C. Walsh, H. MacMillan, and M. Steiner, "Child maltreatment and HPA axis dysregulation: relationship to major depressive disorder and post traumatic stress disorder in females," *Psychoneuroendocrinology*, vol. 30, no. 2, pp. 162–178, 2005.

[8] V. Krishnan and E. J. Nestler, "Animal models of depression: molecular perspectives," *Current Topics in Behavioral Neurosciences*, vol. 7, no. 1, pp. 121–147, 2011.

[9] I. Toth and I. D. Neumann, "Animal models of social avoidance and social fear," *Cell and Tissue Research*, vol. 354, no. 1, pp. 107–118, 2013.

[10] S. Chiba, T. Numakawa, M. Ninomiya, M. C. Richards, C. Wakabayashi, and H. Kunugi, "Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 39, no. 1, pp. 112–119, 2012.

[11] B. S. McEwen, C. Nasca, and J. D. Gray, "Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex," *Neuropsychopharmacology*, 2015.

[12] J. F. Cryan and D. A. Slattery, "Animal models of mood disorders: recent developments," *Current Opinion in Psychiatry*, vol. 20, no. 1, pp. 1–7, 2007.

[13] A. M. Wood, C. J. Boyce, S. C. Moore, and G. D. A. Brown, "An evolutionary based social rank explanation of why low income predicts mental distress: a 17 year cohort study of 30,000 people," *Journal of Affective Disorders*, vol. 136, no. 3, pp. 882–888, 2012.

[14] F. Lederbogen, P. Kirsch, L. Haddad et al., "City living and urban upbringing affect neural social stress processing in humans," *Nature*, vol. 474, no. 7352, pp. 498–501, 2011.

[15] K. Björkqvist, "Social defeat as a stressor in humans," *Physiology and Behavior*, vol. 73, no. 3, pp. 435–442, 2001.

[16] L. M. Heinrich and E. Gullone, "The clinical significance of loneliness: a literature review," *Clinical Psychology Review*, vol. 26, no. 6, pp. 695–718, 2006.

[17] O. Berton, H. E. Covington III, K. Ebner et al., "Induction of deltaFosB in the periaqueductal gray by stress promotes active coping responses," *Neuron*, vol. 55, no. 2, pp. 289–300, 2007.

[18] H. Dadomo, V. Sanghez, L. Di Cristo et al., "Vulnerability to chronic subordination stress-induced depression-like disorders in adult 129SvEv male mice," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 35, no. 6, pp. 1461–1471, 2011.

[19] D. A. Slattery, N. Uschold, M. Magoni et al., "Behavioural consequences of two chronic psychosocial stress paradigms: anxiety without depression," *Psychoneuroendocrinology*, vol. 37, no. 5, pp. 702–714, 2012.

[20] F. Chaouloff, "Social stress models in depression research: what do they tell us?" *Cell and Tissue Research*, vol. 354, no. 1, pp. 179–190, 2013.

[21] A. Berry, V. Bellisario, S. Capoccia et al., "Social deprivation stress is a triggering factor for the emergence of anxiety- and depression-like behaviours and leads to reduced brain BDNF levels in C57BL/6J mice," *Psychoneuroendocrinology*, vol. 37, no. 6, pp. 762–772, 2012.

[22] A. Djordjevic, J. Djordjevic, I. Elaković, M. Adzic, G. Matić, and M. B. Radojcic, "Fluoxetine affects hippocampal plasticity, apoptosis and depressive-like behavior of chronically isolated rats," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 36, no. 1, pp. 92–100, 2012.

[23] F. Pibiri, M. Nelson, A. Guidotti, E. Costa, and G. Pinna, "Decreased corticolimbic allopregnanolone expression during social isolation enhances contextual fear: a model relevant for posttraumatic stress disorder," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 14, pp. 5567–5572, 2008.

[24] H. Koike, D. Ibi, H. Mizoguchi et al., "Behavioral abnormality and pharmacologic response in social isolation-reared mice," *Behavioural Brain Research*, vol. 202, no. 1, pp. 114–121, 2009.

[25] K. C. F. Fone and M. V. Porkess, "Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders," *Neuroscience and Biobehavioral Reviews*, vol. 32, no. 6, pp. 1087–1102, 2008.

[26] D. L. Wallace, M.-H. Han, D. L. Graham et al., "CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits," *Nature Neuroscience*, vol. 12, no. 2, pp. 200–209, 2009.

[27] A. Thorsell, C. J. Slawecki, A. El Khoury, A. A. Mathe, and C. L. Ehlers, "The effects of social isolation on neuropeptide Y levels, exploratory and anxiety-related behaviors in rats," *Pharmacology Biochemistry and Behavior*, vol. 83, no. 1, pp. 28–34, 2006.

[28] S. Morley-Fletcher, J. Mairesse, A. Soumier et al., "Chronic agomelatine treatment corrects behavioral, cellular, and biochemical abnormalities induced by prenatal stress in rats," *Psychopharmacology*, vol. 217, no. 3, pp. 301–313, 2011.

[29] J. D. Gray, T. A. Milner, and B. S. McEwen, "Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors," *Neuroscience*, vol. 239, pp. 214–227, 2013.

[30] N. Elizalde, P. M. Pastor, Á. L. Garcia-García et al., "Regulation of markers of synaptic function in mouse models of depression: chronic mild stress and decreased expression of VGLUT1," *Journal of Neurochemistry*, vol. 114, no. 5, pp. 1302–1314, 2010.

[31] H. D. Schmidt and R. S. Duman, "Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models," *Neuropsychopharmacology*, vol. 35, no. 12, pp. 2378–2391, 2010.

[32] K. G. Bath, A. Schilit, and F. S. Lee, "Stress effects on BDNF expression: effects of age, sex, and form of stress," *Neuroscience*, vol. 239, pp. 149–156, 2013.

[33] I. Ninan, "Synaptic regulation of affective behaviors; Role of BDNF," *Neuropharmacology C*, vol. 76, pp. 684–695, 2014.

[34] R. S. Duman and L. M. Monteggia, "A neurotrophic model for stress-related mood disorders," *Biological Psychiatry*, vol. 59, no. 12, pp. 1116–1127, 2006.

[35] A. Ieraci, A. Mallei, L. Musazzi, and M. Popoli, "Physical exercise and acute restraint stress differentially modulate hippocampal brain-derived neurotrophic factor transcripts and epigenetic mechanisms in mice," *Hippocampus*, vol. 25, no. 11, pp. 1380–1392, 2015.

[36] A. Mallei, G. Baj, A. Ieraci et al., "Expression and dendritic trafficking of BDNF-6 splice variant are impaired in knock-in mice carrying human BDNF Val66Met polymorphism," *International Journal of Neuropsychopharmacology*, 2015.

[37] T. Aid, A. Kazantseva, M. Piirsoo, K. Palm, and T. Timmusk, "Mouse and rat BDNF gene structure and expression revisited," *Journal of Neuroscience Research*, vol. 85, no. 3, pp. 525–535, 2007.

[38] M. R. Lyons and A. E. West, "Mechanisms of specificity in neuronal activity-regulated gene transcription," *Progress in Neurobiology*, vol. 94, no. 3, pp. 259–295, 2011.

[39] G. Baj, V. D'Alessandro, L. Musazzi et al., "Physical exercise and antidepressants enhance BDNF targeting in hippocampal CA3 dendrites: further evidence of a spatial code for BDNF splice variants," *Neuropsychopharmacology*, vol. 37, no. 7, pp. 1600–1611, 2012.

[40] L. Musazzi, J. M. Rimland, A. Ieraci, G. Racagni, E. Domenici, and M. Popoli, "Pharmacological characterization of BDNF promoters I, II and IV reveals that serotonin and norepinephrine input is sufficient for transcription activation," *International Journal of Neuropsychopharmacology*, vol. 17, no. 5, pp. 779–791, 2014.

[41] M. S. Zajac, T. Y. C. Pang, N. Wong et al., "Wheel running and environmental enrichment differentially modify exon-specific BDNF expression in the hippocampus of wild-type and pre-motor symptomatic male and female Huntington's disease mice," *Hippocampus*, vol. 20, no. 5, pp. 621–636, 2010.

[42] A. Ieraci and D. G. Herrera, "Nicotinamide protects against ethanol-induced apoptotic neurodegeneration in the developing mouse brain," *PLoS Medicine*, vol. 3, no. 4, pp. 547–557, 2006.

[43] L. Steru, R. Chermat, B. Thierry, and P. Simon, "The tail suspension test: a new method for screening antidepressants in mice," *Psychopharmacology*, vol. 85, no. 3, pp. 367–370, 1985.

[44] F. Rusconi, L. Paganini, D. Braida et al., "LSD1 neurospecific alternative splicing controls neuronal excitability in mouse models of epilepsy," *Cerebral Cortex*, vol. 25, no. 9, pp. 2729–2740, 2015.

[45] A. Ieraci, P. E. Forni, and C. Ponzetto, "Viable hypomorphic signaling mutant of the met receptor reveals a role for hepatocyte growth factor in postnatal cerebellar development," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 23, pp. 15200–15205, 2002.

[46] S. K. Droste, A. Gesing, S. Ulbricht, M. B. Müller, A. C. E. Linthorst, and J. M. H. M. Reul, "Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis," *Endocrinology*, vol. 144, no. 7, pp. 3012–3023, 2003.

[47] I. Gerendai and B. Halász, "Neuroendocrine asymmetry," *Frontiers in Neuroendocrinology*, vol. 18, no. 3, pp. 354–381, 1997.

[48] B. Cuccurazzu, V. Bortolotto, M. M. Valente et al., "Upregulation of mGlu2 receptors via NF-κB p65 acetylation is involved in the proneurogenic and antidepressant effects of acetyl-L-carnitine," *Neuropsychopharmacology*, vol. 38, no. 11, pp. 2220–2230, 2013.

[49] B. H. Harvey and M. Shahid, "Metabotropic and ionotropic glutamate receptors as neurobiological targets in anxiety and stress-related disorders: Focus on pharmacology and preclinical translational models," *Pharmacology Biochemistry and Behavior*, vol. 100, no. 4, pp. 775–800, 2012.

[50] C. Nasca, B. Bigio, D. Zelli, F. Nicoletti, and B. S. McEwen, "Mind the gap: glucocorticoids modulate hippocampal glutamate tone underlying individual differences in stress susceptibility," *Molecular Psychiatry*, vol. 20, no. 6, pp. 755–763, 2015.

[51] S. Kubik, T. Miyashita, and J. F. Guzowski, "Using immediate-early genes to map hippocampal subregional functions," *Learning & Memory*, vol. 14, no. 11, pp. 758–770, 2007.

[52] J. B. Levine, R. M. Youngs, M. L. MacDonald et al., "Isolation rearing and hyperlocomotion are associated with reduced immediate early gene expression levels in the medial prefrontal cortex," *Neuroscience*, vol. 145, no. 1, pp. 42–55, 2007.

[53] R. Okada, K. Matsumoto, R. Tsushima, H. Fujiwara, and K. Tsuneyama, "Social isolation stress-induced fear memory deficit is mediated by down-regulated neuro-signaling system and Egr-1 expression in the brain," *Neurochemical Research*, vol. 39, no. 5, pp. 875–882, 2014.

[54] Q. Pei, T. S. C. Zetterström, M. Sprakes, R. Tordera, and T. Sharp, "Antidepressant drug treatment induces Arc gene expression in the rat brain," *Neuroscience*, vol. 121, no. 4, pp. 975–982, 2003.

[55] A. Djordjevic, M. Adzic, J. Djordjevic, and M. B. Radojcic, "Stress type dependence of expression and cytoplasmic-nuclear partitioning of glucocorticoid receptor, Hsp90 and Hsp70 in Wistar rat brain," *Neuropsychobiology*, vol. 59, no. 4, pp. 213–221, 2009.

[56] A. L. Martin and R. E. Brown, "The lonely mouse: verification of a separation-induced model of depression in female mice," *Behavioural Brain Research*, vol. 207, no. 1, pp. 196–207, 2010.

[57] A. Bartolomucci, P. Palanza, P. Sacerdote et al., "Individual housing induces altered immuno-endocrine responses to psychological stress in male mice," *Psychoneuroendocrinology*, vol. 28, no. 4, pp. 540–558, 2003.

[58] X. Liu, R. Wu, F. Tai et al., "Effects of group housing on stress induced emotional and neuroendocrine alterations," *Brain Research*, vol. 1502, pp. 71–80, 2013.

[59] B. M. M. Monteiro, F. A. Moreira, A. R. Massensini, M. F. D. Moraes, and G. S. Pereira, "Enriched environment increases

neurogenesis and improves social memory persistence in socially isolated adult mice," *Hippocampus*, vol. 24, no. 2, pp. 239–248, 2014.

[60] H. Ito, M. Nagano, H. Suzuki, and T. Murakoshi, "Chronic stress enhances synaptic plasticity due to disinhibition in the anterior cingulate cortex and induces hyper-locomotion in mice," *Neuropharmacology*, vol. 58, no. 4-5, pp. 746–757, 2010.

[61] M. Kabbaj, D. P. Devine, V. R. Savage, and H. Akil, "Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules," *The Journal of Neuroscience*, vol. 20, no. 18, pp. 6983–6988, 2000.

[62] V. Võikar, A. Polus, E. Vasar, and H. Rauvala, "Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences," *Genes, Brain and Behavior*, vol. 4, no. 4, pp. 240–252, 2005.

[63] A. Zimprich, L. Garrett, J. M. Deussing et al., "A robust and reliable non-invasive test for stress responsivity in mice," *Frontiers in Behavioral Neuroscience*, vol. 8, article 125, 2014.

[64] G. Beitia, L. Garmendia, A. Azpiroz, O. Vegas, P. F. Brain, and A. Arregi, "Time-dependent behavioral, neurochemical, and immune consequences of repeated experiences of social defeat stress in male mice and the ameliorative effects of fluoxetine," *Brain, Behavior, and Immunity*, vol. 19, no. 6, pp. 530–539, 2005.

[65] L. M. Bierer, L. Tischler, E. Labinsky, S. Cahill, E. Foa, and R. Yehuda, "Clinical correlates of 24-h cortisol and norepinephrine excretion among subjects seeking treatment following the World Trade Center attacks on 9/11," *Annals of the New York Academy of Sciences*, vol. 1071, pp. 514–520, 2006.

[66] D. Hauer, F. Weis, T. Krauseneck, M. Vogeser, G. Schelling, and B. Roozendaal, "Traumatic memories, post-traumatic stress disorder and serum cortisol levels in long-term survivors of the acute respiratory distress syndrome," *Brain Research*, vol. 1293, pp. 114–120, 2009.

[67] T. C. Neylan, A. Brunet, N. Pole et al., "PTSD symptoms predict waking salivary cortisol levels in police officers," *Psychoneuroendocrinology*, vol. 30, no. 4, pp. 373–381, 2005.

[68] D. L. Delahanty, A. J. Raimonde, and E. Spoonster, "Initial posttraumatic urinary cortisol levels predict subsequent PTSD symptoms in motor vehicle accident victims," *Biological Psychiatry*, vol. 48, no. 9, pp. 940–947, 2000.

[69] C. Heim, U. Ehlert, and D. H. Hellhammer, "The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders," *Psychoneuroendocrinology*, vol. 25, no. 1, pp. 1–35, 2000.

[70] R. Reznikov, M. Diwan, J. N. Nobrega, and C. Hamani, "Towards a better preclinical model of PTSD: characterizing animals with weak extinction, maladaptive stress responses and low plasma corticosterone," *Journal of Psychiatric Research*, vol. 61, pp. 158–165, 2015.

[71] K. Matsumoto, K. Ono, H. Ouchi, R. Tsushima, and Y. Murakami, "Social isolation stress down-regulates cortical early growth response 1 (Egr-1) expression in mice," *Neuroscience Research*, vol. 73, no. 3, pp. 257–262, 2012.

[72] M. G. Pisu, R. Dore, M. C. Mostallino et al., "Down-regulation of hippocampal BDNF and Arc associated with improvement in aversive spatial memory performance in socially isolated rats," *Behavioural Brain Research*, vol. 222, no. 1, pp. 73–80, 2011.

[73] O. Steward, S. Farris, P. S. Pirbhoy, J. Darnell, and S. J. Van Driesche, "Localization and local translation of Arc/Arg3.1 mRNA at synapses: some observations and paradoxes," *Frontiers in Molecular Neuroscience*, vol. 7, article 101, 2015.

[74] E. Korb and S. Finkbeiner, "Arc in synaptic plasticity: from gene to behavior," *Trends in Neurosciences*, vol. 34, no. 11, pp. 591–598, 2011.

[75] H. Wang, Y. Z. Zhu, P. T.-H. Wong et al., "cDNA microarray analysis of gene expression in anxious PVG and SD rats after cat-freezing test," *Experimental Brain Research*, vol. 149, no. 4, pp. 413–421, 2003.

[76] C. R. Bramham, P. F. Worley, M. J. Moore, and J. F. Guzowski, "The immediate early gene Arc/Arg3.1: regulation, mechanisms, and function," *The Journal of Neuroscience*, vol. 28, no. 46, pp. 11760–11767, 2008.

[77] O. Steward and P. Worley, "Local synthesis of proteins at synaptic sites on dendrites: role in synaptic plasticity and memory consolidation?" *Neurobiology of Learning and Memory*, vol. 78, no. 3, pp. 508–527, 2002.

[78] J. F. Guzowski, B. Setlow, E. K. Wagner, and J. L. McGaugh, "Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268," *The Journal of Neuroscience*, vol. 21, no. 14, pp. 5089–5098, 2001.

[79] M. W. Jones, M. L. Errington, P. J. French et al., "A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories," *Nature Neuroscience*, vol. 4, no. 3, pp. 289–296, 2001.

[80] L. Li, J. Carter, X. Gao, J. Whitehead, and W. G. Tourtellotte, "The neuroplasticity-associated arc gene is a direct transcriptional target of early growth response (Egr) transcription factors," *Molecular and Cellular Biology*, vol. 25, no. 23, pp. 10286–10300, 2005.

[81] V. Vaghi, A. Polacchini, G. Baj, V. L. M. Pinheiro, A. Vicario, and E. Tongiorgi, "Pharmacological profile of brain-derived neurotrophic factor (BDNF) splice variant translation using a novel drug screening assay: a 'quantitative code'," *The Journal of Biological Chemistry*, vol. 289, no. 40, pp. 27702–27713, 2014.

[82] G. Baj, D. Del Turco, J. Schlaudraff, L. Torelli, T. Deller, and E. Tongiorgi, "Regulation of the spatial code for BDNF mRNA isoforms in the rat hippocampus following pilocarpine-treatment: A systematic analysis using laser microdissection and quantitative real-time PCR," *Hippocampus*, vol. 23, no. 5, pp. 413–423, 2013.

[83] C. Verpelli, G. Piccoli, A. Zanchi et al., "Synaptic activity controls dendritic spine morphology by modulating eEF2-dependent BDNF synthesis," *Journal of Neuroscience*, vol. 30, no. 17, pp. 5830–5842, 2010.

[84] R. I. Melendez, M. L. Gregory, M. T. Bardo, and P. W. Kalivas, "Impoverished rearing environment alters metabotropic glutamate receptor expression and function in the prefrontal cortex," *Neuropsychopharmacology*, vol. 29, no. 11, pp. 1980–1987, 2004.

[85] Y. Ago, R. Araki, K. Yano et al., "The selective metabotropic glutamate 2/3 receptor agonist MGS0028 reverses isolation rearing-induced abnormal behaviors in mice," *Journal of Pharmacological Sciences*, vol. 118, no. 2, pp. 295–298, 2012.

[86] Y. Ago, R. Araki, K. Yano et al., "Activation of metabotropic glutamate 2/3 receptors attenuates methamphetamine-induced hyperlocomotion and increase in prefrontal serotonergic neurotransmission," *Psychopharmacology*, vol. 217, no. 3, pp. 443–452, 2011.

[87] F. Conquet, Z. I. Bashir, C. H. Davies et al., "Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1," *Nature*, vol. 372, no. 6503, pp. 237–243, 1994.

[88] C. Gil-Sanz, J. M. Delgado-García, A. Fairén, and A. Gruart, "Involvement of the mGluR1 receptor in hippocampal synaptic

plasticity and associative learning in behaving mice," *Cerebral Cortex*, vol. 18, no. 7, pp. 1653–1663, 2008.

[89] F. Zhang, B. Liu, Z. Lei, and J.-H. Wang, "MGluR1,5 activation improves network asynchrony and GABAergic synapse attenuation in the amygdala: implication for anxiety-like behavior in DBA/2 mice," *Molecular Brain*, vol. 5, article 20, 2012.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 6503162, 13 pages
http://dx.doi.org/10.1155/2016/6503162

Review Article

Coping with the Forced Swim Stressor: Towards Understanding an Adaptive Mechanism

E. R. de Kloet[1,2] and M. L. Molendijk[3,4]

[1] *Division of Medical Pharmacology and Leiden Academic Center for Drug Research, Leiden University, Einsteinweg 55, 2333 CC Leiden, Netherlands*
[2] *Division of Endocrinology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, Netherlands*
[3] *Institute of Psychology, Leiden University, Wassenaarseweg 52, 2333 AK Leiden, Netherlands*
[4] *Leiden Institute for Brain and Cognition, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, Netherlands*

Correspondence should be addressed to E. R. de Kloet; erdekloet@gmail.com

Received 15 September 2015; Accepted 19 October 2015

Academic Editor: Jordan Marrocco

In the forced swim test (FST) rodents progressively show increased episodes of immobility if immersed in a beaker with water from where escape is not possible. In this test, a compound qualifies as a potential antidepressant if it prevents or delays the transition to this passive (energy conserving) behavioural style. In the past decade however the switch from active to passive "coping" was used increasingly to describe the phenotype of an animal that has been exposed to a stressful history and/or genetic modification. A PubMed analysis revealed that in a rapidly increasing number of papers (currently more than 2,000) stress-related immobility in the FST is labeled as a depression-like phenotype. In this contribution we will examine the different phases of information processing during coping with the forced swim stressor. For this purpose we focus on the action of corticosterone that is mediated by the closely related mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in the limbic brain. The evidence available suggests a model in which we propose that the limbic MR-mediated response selection operates in complementary fashion with dopaminergic accumbens/prefrontal executive functions to regulate the transition between active and passive coping styles. Upon rescue from the beaker the preferred, mostly passive, coping style is stored in the memory via a GR-dependent action in the hippocampal dentate gyrus. It is concluded that the rodent's behavioural response to a forced swim stressor does not reflect depression. Rather the forced swim experience provides a unique paradigm to investigate the mechanistic underpinning of stress coping and adaptation.

1. Introduction

Validated animal models and tests are crucial for understanding the pathogenesis and treatment of mood and anxiety disorders [1]. This contribution is about the exposure of rodents to a forced swim stressor, which was originally designed by Porsolt et al. [2–4] to assess the antidepressant potential of drugs. The so-called forced swim test (FST, see Box 1) is based on the observation that when rats or mice are immersed in a beaker of water from where escape is not possible, they display a progressive increase in the frequency and duration of episodes of immobile floating after initial attempts to escape by swimming, struggling, climbing, or diving. In a retest the animals show the acquired immobility response almost immediately; the total time spent immobile and/or the duration of time until the transition from active to passive behaviour are the read-out parameters of this test. In mice a single session is often applied, which obviously excludes the retention of acquired immobility used in the test-retest design as criterion.

Porsolt's design of the FST was extremely productive for drug screening. The test appeared highly reproducible among different labs, lasted only 2 days, and was applicable for high-throughput [5–7]. However, an unfortunate aspect is the anthropomorphic interpretation of the rodent's progressive immobility during the FST as "lowered mood" or "despair" ... and "giving up hope to escape," which is highlighted as a depression-like phenotype [2–4, 6]. With the advent of

The forced swim test (FST) is a behavioral paradigm that has been developed to screen the potential anti-depressant properties of compounds.
In the original version of the test, developed by Porsolt and his colleagues [2], a rodent is placed in a beaker (width: >20 cm; depth ~15–18 cm) filled with water of 24 ± 2 degrees °C. The rodent is let to swim for 15 minutes. Escape from the beaker is not possible. After the session, the animal is removed from the water, dried, and placed back in the home cage. Twenty-four hours after the initial swim experience, the rodent again is placed in the beaker. During this second swim experience, that usually lasts 5 minutes, most animals start showing passive behavior soon; they stop swimming and show little if any attempts to climb the wall of the cylinder or to dive. When this occurs, the animal is said to be *immobile* or that it *floats*. The time from placement in the cylinder to immobility/floating, often also expressed as the latency to immobility or the percentage of time that the animal stays immobile, is regarded as the main outcome measure of the FST experiment.
Over the years, the original version of the FST has undergone some modifications. One major modification is that many studies choose to use a beaker with a depth of 30 cm (instead of only ~15–18 cm). The prime reason for this is that the rodent is not able to remain stable, without swimming, through tail contact with the bottom of the beaker. A second modification on the classical FST is the use of the test to measure immobility/floating in a single session, thus without the 15 minutes pretest. It has been suggested that the pretest is necessary in order to reliably and more quickly detect the immobile posture of the rodent during the 5-minute test session 24 hours after the 15-minute test session. However, a single swim session may be sufficient to induce stable immobile behavior, in particular for mice. Hence, some studies apply only a single swim session to discern immobility.
For more information on the protocols according to which the FST is used, we refer to Porsolt et al. [2, 3] and Slattery and Cryan [5].

Box 1: The forced swim test.

mouse mutants carrying genetic modifications the FST was adopted as a rapid "animal depression" test. Hence, a dramatic increase occurred in the number of papers reporting in rodents the depressogenic effect of genes (see Figure 1), often in a context of early life adversity as well as later life acute or chronic exposure to stressors of all kind. In 1985 one paper per month was published that reported the results from the FST, today this number amounts to one per day [8]. For discussions of the rodent's forced swim performance as a measure for depression we refer to a series of excellent articles elsewhere [5–7]. For a critical evaluation of animal models for depression, see Nestler and Hyman [1].

In a recent commentary in *Psychoneuroendocrinology*, we presented an analysis of current interpretations of FST behavior [8]. The data for this analysis consisted of random samples of the 4,300 PUBMED listed papers in which the use of the FST was described. We found that the papers in which the FST was used to identify a depression-like phenotype amounted to around 2,020. Rapidly declining over the years (now in total 1,980 papers) was Porsolt's original FST application of the test for identification of a compound's antidepressant potential. In about 820 papers the "depressogenic" effect of stress was studied. We further estimated that in 320 studies the FST was used for phenotyping genetic mouse mutants. Finally, in about 300 papers, and rapidly declining over the years (see Figure 1), the progressive immobility was interpreted as a learning process. These 300 studies demonstrated that the outcome of the FST in the test-retest paradigm could be altered by interfering with acquisition, consolidation, and retention of the immobility response [9, 10].

In this contribution to the *Many Faces of Stress* issue, the progressive immobility that is acquired during the FST is presented as a passive behavioural style of the rodent to cope with the situation that escape from the beaker is not possible.

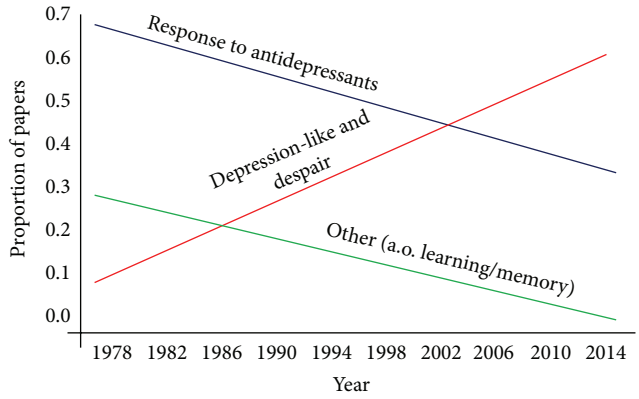


Figure 1: The figure shows the interpretation of the outcomes of the FST as a function of year of publication. The slopes that are plotted indicate that over the years more studies choose to label the outcomes from the FST as "depression-like behaviour" (the red line) and fewer as "antidepressant" properties of drugs (the blue line) or behavior such as "learned immobility" (the green line). For more information on these data we refer to Molendijk and de Kloet [8].

In the first section of this paper we will sketch the transition of active (swimming) to passive (immobility) behaviour as an adaptive learning process that contributes to survival by conserving energy, likely evolving from millions of years of evolution. We will highlight recent reports on the transition from active to passive behaviour and how this adaptive response is stored in memory. It appears that glucocorticoids as well as antidepressants are capable to affect the ability of rodents to consolidate the learned immobility response [9, 11, 12].

In the second section we summarize the corticosteroid receptor balance concept of health and disease [13] and

discuss its implications for immobility learning and memory storage. This includes the selection of the appropriate coping style involving cognitive flexibility and executive dopaminergic functions [14–16], pharmacological experiments to identify the brain sites of mnemonic action of the corticosteroids [17, 18], and a possible epigenetic mechanism as discovered by Reul [19]. We also will discuss the effect of a chronic stress history on the rodent's performance in the FST. We conclude, in the third section, with the notion that synthesis is possible according to the knowledge gained on corticosteroid action in limbic brain and dopaminergic executive functions. Hence, the rodent's response to an acute forced swim stressor provides an excellent opportunity to investigate the mechanism underlying stress coping and adaptation that contributes to survival.

2. Forced Swim

2.1. The Forced Swim Stressor. It is of interest to read the original articles of Porsolt et al. [2] and of Hawkins et al. [4], and the subsequent discussion between the authors of these papers. Hawkins et al. [4] agreed with using the FST as innovative antidepressant screening tool but dismissed Porsolt's notion that immobility in the FST represents despair. Hawkins et al. [4] noted by carefully monitoring the switches between swimming, headshaking, struggling, diving, climbing, and floating that the progressive immobility at the end of the 15 min initial test is an adaptive response *"without the energy expenditure required in swimming."* At retest, 24 hours later, a similar level of immobility is immediately resumed. Moreover, the rats appeared at retest less emotional, observed as a lower amount of emotional defecation, which according to Hawkins supports the idea that "*... having been rescued on day 1, the rats were less fearful on day 2.*" In reply Porsolt maintains the position that the immobility response in the FST measures lowered mood and despair and acknowledges that the procedure is "*not a model for depression in the rat*" but reflects "*... some aspects of depressed mood.*" In addition, Porsolt mentioned that also electroshocks, the treatment of choice when more conventional methods fail in alleviating the symptoms of depression, readily led to decreased immobility scores in the FST mimicking the effects that are observed after administration of pharmacological antidepressants.

In 2009, Castagné and colleagues stated that "*... the FST is not a model for depression because the dependent variable is the response to the acute forced swim stressor rather than the phenotype of the animal*" [20]. Since there is no sign or symptom of depression modeled in the FST it lacks *face validity*. Also construct *validity* is absent since the pathogenesis of depression is a slow process that is often, but not always, precipitated by the inability to cope with the stress of life [21]. Indeed in many experiments animals are subjected to a chronic stress paradigm with or without a genetic mutation and then the FST is used incorrectly to model depression. The FST shows *predictive validity* where it concerns the testing of antidepressant potential of compounds. This validity criterion is without evidence of the mechanism *how* antidepressants affect the switch from active to passive behaviour. Rather, the antidepressants that disrupt immobility in the FST acutely take several weeks before they are clinically effective in a depressed patient [22] suggesting that also this predictive validity of antidepressant action in the FST gives little insight into any pathogenic mechanism. Besides, antidepressants affect multiple functional domains beyond mood, including memory and appetite [22] that potentially could have an effect on FST performance.

There are many *false positives*. For instance drugs like amphetamine, which is not an antidepressant, enhance locomotor activity and prevent the switch to immobility [3, 4]. Also the GABA-A agonist muscimol, barbiturates, benzodiazepines, and anticholinergic agents have been coined as false positives (see De Pablo et al. [23] for an overview). Rats engaged in physical exercise, in humans regarded as being "antidepressant" [24], show increased immobility and secrete increased amounts of corticosterone but also are more resilient [19]. Physical exercise thus could be regarded as a *false negative* in this context. Widely described antidepressant agents of the SSRI type [22] likewise could be regarded as a false negative [25]. Furthermore, animals that are familiar to the test are more immobile [26] just as animals that are exposed to water of 19°C in the initial test which became more immobile if the water at retest was 25°C rather than the original 19°C [19]. Finally, in a brief report on 2 experiments O'Neill and Valentino [27] ruled out that the extent of escapability from the beaker of water reflected a measure of despair: the immobility response during retest was identical irrespective of the presence of an escape option. They also demonstrated that the FST is not a learned helplessness model.

2.2. Active and Passive Coping. As students of the FST, more than 20 years ago, we have performed experiments to examine the role of stress hormones in the acquisition and retention of the rodent's response to the acute swim stressor. We observed the switches between the different behaviours towards longer periods of immobility and that acquired immobility was retained at the 24 hr retest. The retention of acquired immobility may last as long as 4 weeks [28]. In our line of reasoning the switch to immobility behaviour "*... is a successful passive behavioural strategy.*" [29], which appeared affected by antisense manipulation of the glucocorticoid receptor (GR) in the hippocampus if performed at least six hours before the initial test.

Immobility in the FST was also interpreted as passive coping [30]. Since coping has a positive connotation "*... dealing effectively with something difficult*" (Oxford Dictionary; http://www.oxforddictionaries.com, accessed September 5th, 2015) this qualification is somewhat at variance with labeling the passive coping style as a symptom of depression [30, 31]. Cabib and colleagues [10, 18] formulated after a series of elegant experiments using stress-susceptible DBA mice in a test-retest design the hypothesis that "*immobility is the result of extinction-like inhibitory learning involving all available escape responses due to the inescapable/unavoidable nature of the FST*

experience." Other qualifications are "... *that immobility is beneficial in preventing the rats from sinking*": *rodents that float longer probably live longer* [32]. Although some of these explanations suffer from anthropomorphism (e.g., despair, depression-like), the switch between the different behavioural (coping) responses towards increased immobility shows what actually is observed when an animal deals with the forced swim stressor.

Fascinating novel technology currently allows real-time measurement of the transition between active and passive behavioural states with simultaneous *in vivo* electrophysiological recordings. Using these techniques, striking correlates were found between the activity of specific medio prefrontocortical (mPFC) and mesolimbic dopaminergic circuits and the transition between active and passive behavioural states at the 24 h FST retest [15, 30, 31]. These transitions were interpreted as representing elements of neuronal encoding and a subsequent decision-making process that is reflected in the behaviours observed in the FST. Moreover, using optogenetic activation of specific mPFC and midbrain dopaminergic subcircuits (the former projecting to the dorsal raphe nuclei) the behavioural transitions towards immobility were induced suggesting a causal relationship [30].

Tye et al. [31] tested selective ventral tegmental area (VTA) A9 dopaminergic neurons after viral transfection with an enhanced halorhodopsin that shows upon stimulation hyperpolarization and thus dopaminergic inactivation. They found upon AVT inhibition increased immobility in the FST, while locomotor responses were not affected. Moreover, causal relationships of specific circuit activations during the 24 hr retest occurred in parallel with other putative "depression" tests such as the FST, tail suspension, and sucrose preference test [31]. Interestingly, the same authors demonstrated that acquired immobility, enhanced by a history of chronic stress exposure, could be reversed within seconds by light stimulation of the same dopaminergic neurons transduced with channelrhodopsin-2 [31] to achieve the desired neuronal activation. However, opposite results were reported by Chaudhury et al. [33]; see for discussion of these studies Lammel et al. [15]. Noteworthy is that some of the brain circuits linked to passive-active transitions were also identified (i.e., the nucleus accumbens and medial frontal cortex) as targets for the immediate antidepressant effects of deep brain stimulation [34].

Accordingly, these data obtained by optogenetic manipulation of the VTA dopaminergic neurons provide strong evidence for a causal relationship with forced swim performance. The VTA dopaminergic circuit and its mesocortical and mesolimbic branches have however complex afferent and efferent pathways that operate in multiple feedback loops [35]. Cabib and Puglisi-Allegra [16] have built a compelling case that enhanced tonic mesoaccumbens dopamine activity supports the expression of active stress-induced coping styles, while inhibition of dopamine release is required for passive coping (immobility) in the FST. The latter passive behaviour occurs when a stressful condition is appraised as inescapable and/or uncontrollable.

The pioneering research by the Grace group (see for an overview [14]) focused in particular on the balance in afferent pathways from the ventral hippocampus and basolateral amygdala which was found to regulate a spontaneous single-spike firing pattern of the VTA dopamine neurons. This "tonic" pacemaker is driven by the excitatory outflow of the ventral subiculum hippocampus via the nucleus accumbens, ventral pallidum pathway (see [14]). In agreement with Cabib and Puglisi-Allegra [16] and Tye et al., [31] also Grace [36] noted that uncontrollability of the stressor suppressed the mesoaccumbens pathway, while promoting the expression of a passive coping response.

2.3. Consolidation of Acquired Immobility. Jefferys et al. [12] and Veldhuis et al. [11] reported that rats, adrenalectomised 1 week before the initial test, showed levels of immobility that were similar to controls. However, at retest the immobility response was not retained. The naturally occurring glucocorticoid corticosterone and the synthetic glucocorticoids dexamethasone and RU2362 given subcutaneously 15–60 min after the initial test reinstated retention, while mineralocorticoids and progesterone had no effect (see Figure 2). As expected the antiglucocorticoid RU486 given prior to the initial test interfered with the glucocorticoid-induced retention of acquired immobility. Interestingly, removal of the adrenal medulla, secreting adrenaline and opioids, only transiently interfered with retention, which could be restored by administering synthetic enkephalin analogs [37]. Subsequent experiments showed that also thyroid hormone and glucose [38, 39] are effective, suggesting interplay between endocrine and metabolic factors during retention of immobility. It is likely that the action of these factors in promoting immobility also would promote conservation of the energy needed to prolong survival, which is actually one of the lessons for sailors in the *essentials of sea survival* [40].

De Pablo et al. [9] reported a number of well-controlled experiments clearly demonstrating that antidepressants interfere with the consolidation process in the FST (see Table 1). Using an automatic recording procedure to assess mobility, they demonstrated that during the forced swim experience the amount of immobility increased with repeated experience. Exposing the rats to a cylinder with increased water depth led to decreased immobility. This is counterintuitive because more "despair" would have been expected. Finally, antidepressants given after the initial test interfered with consolidation of the acquired immobility response as measured from the rat's performance at retest 24 hr later. Since the protein synthesis inhibitor anisomycin had similar effects as the antidepressants it is evident that effects measured in the FST retest monitor memory storage of the behavioural response acquired at the initial test. The paper by De Pablo et al. [9] is a "must read" for everyone who uses the test-retest design of the FST.

2.4. Conclusion. Exposure to the forced swim stressor induces a profound response of the sympathetic nervous system, the HPA axis, and also of a variety of neurotransmitter circuits (e.g., dopamine, serotonin, GABA) in the brain [31, 41–43]. In particular the VTA-A9 dopaminergic circuitry has received much attention because of its role in

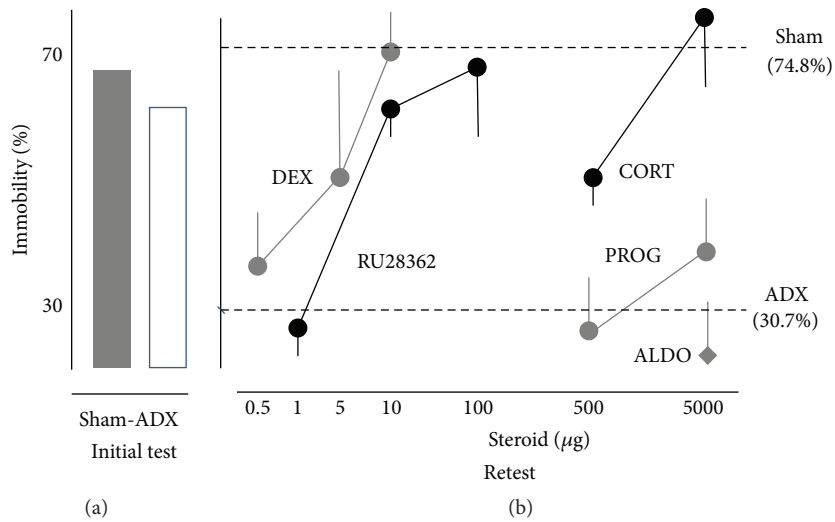


Figure 2: (a) Left panel shows % immobility during the last 5 min of the 15 min initial test of SHAM and adrenalectomised (ADX) animals. (b) Right panel shows the effects of various steroids, given 15 min after the initial swimming exposure, on retention of acquired immobility of ADX rats during the 5 minutes retest period. Data are expressed as mean ± the standard error of the mean as % immobility time. Dashed lines represent the % immobility of SHAM (74,8%) and ADX (30,7%) rats. Fifty ADX and 33 sham rats were used in these experiments. Six animals were used per different dosages of dexamethasone, cortisol, RU38362, and progesterone. Post hoc comparisons following a significant ANOVA revealed that the groups treated with DEX (5 and 10 μg), RU28362 (10 and 100 μg), and cortisol (500 and 5000 μg) differed significantly from ADX but not from sham rats. For more information on these data we refer to [11].

Table 1: The effect of a single dose of 25 mg/kg of imipramine administered one hr before or 15 min after training on day 1 on rat mobility in the forced swim test.

Injection time	N	No. of impulses, day 1	% no. of impulses, day 2
Saline	7	409.85 ± 48.79	42.63 ± 11.50
15 min after	7	420.14 ± 45.93	94.39 ± 8.38*
1 hour before	6	421.66 ± 39.75	93.06 ± 16.44*

*Statistical significance (at $P < .01$) with respect to the saline group.
Adapted and reprinted from De Pablo et al. [9] with permission from Elsevier B.V.

mediating the stress response and in the pathogenesis of stress-related depression and psychosis. Indeed, the mesoaccumbens dopaminergic circuit is important for coping with the forced swim stressor. In the forced swim test transitions proceed progressively from active to passive coping styles culminating in prolonged periods of immobility. These transitions serve as measure in the mouse single trial FST as well as in the retest. They can be evoked by optogenetic stimulation of the VTA dopaminergic neurons. Dopaminergic activity is under control of afferent inputs from limbic areas, that is, amygdala and hippocampus. All these limbic-forebrain areas are targets for corticosteroid hormones released under stress.

3. Corticosteroid Action and Stress

3.1. Corticosteroid Receptors and Action. The naturally occurring glucocorticoids, cortisol in man and corticosterone in man and rodent, are collectively abbreviated as CORT here. CORT regulates energy metabolism and controls the stress response. The hormones, secreted by the adrenals as end product of the HPA axis, coordinate in rhythmic fashion the needs in circadian regulations from food intake to allocation of energy resources. CORT also mediates coping with stress in a manner that the hormones prevent the initial reactions to a stressor from overshooting [44, 45]. These actions exerted by CORT are mediated by mineralocorticoid receptors (NR3C2, MR) and glucocorticoid receptors (NR3C1, GR) [46–49]. MR and GR regulate gene transcription as nuclear receptors and occur also as membrane variants that are engaged in rapid nongenomic membrane actions [50–52].

The MR and GR have different characteristics [48, 53–56]. First, MR expression is abundant in limbic structures, notably the hippocampus, amygdala, lateral septum, and regions of the prefrontal cortex where it is colocalized with the ubiquitously expressed GR. Second, nuclear MR has a tenfold higher affinity for CORT than GR and is therefore always substantially occupied, while GR only becomes occupied after stress and at the ultradian or circadian peaks in circulating CORT. Third, MR in most brain regions is nonselective: CORT and aldosterone have high affinity and also deoxycorticosterone and progesterone bind, the latter as a competitive antagonist [57].

The actions mediated by MR and GR are complementary: in some cells and circuits opposing and elsewhere synergizing [59–61]. On the cellular level MR maintains and enhances the excitatory tone [62, 63]. Activation of the MR membrane variant (which has a lower affinity to CORT than the nuclear MR) by stress stimulates the release of glutamate, which

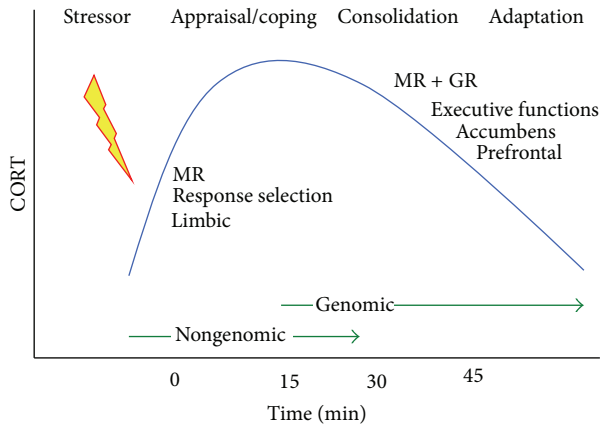


Figure 3: Corticosterone action during processing of stressful information. Increasing corticosterone concentration induced by a stressor initially activates MR modulating appraisal processes and immediate coping and then progressively activates also nuclear GR to reallocate energy to circuits underlying consolidation and retention of the experience in the memory [58]. For this purpose MR and GR mediate in complementary fashion the action of corticosterone in hippocampus and amygdala from decision-making and cognitive flexibility to executive functions in prefrontal brain regions, as is mediated by the mesolimbic dopaminergic system innervating the nucleus accumbens. Adapted from de Kloet et al. [13]. MR is mineralocorticoid receptors; GR is glucocorticoid receptors.

subsequently downregulates the presynaptic Glu2/3 receptors [50, 60, 64, 65]. With rising steroid concentrations CORT suppresses, via the GR, the excitability which is transiently raised by excitatory stimuli [66]. In nongenomic fashion GR promotes the postsynaptic release of endocannabinoids, which inhibit transmitter release presynaptically [67].

On the behavioural level MR and GR mediate distinct functions in the processing of stressful information (see Figure 3). The MR mediates a tonic action on the activity of the HPA axis [68] and is important during the onset of the stress reaction because it regulates anticipation, appraisal, response selection, and thus decision-making processes in coping with novel stressful situations. These are all functions linked to the limbic network [13, 69]. When the stress response develops and CORT concentrations rise, the GR becomes progressively occupied which allocates additional energy resources towards the more executive frontocortical functions [58]. Primarily the action of CORT, mediated by the GR, is aimed to promote behavioural adaptation which terminates the stress reaction. At the same time the outcome of the coping process is stored in memory for future use [70, 71]. When the stress reaction subsides the ultradian rhythm resumes allowing to maintain a state of stress responsiveness.

The GR and MR thus have complementary functions in the processing of stressful information. This has led to the formulation of the Corticosteroid Receptor (CoRe) Balance hypothesis which states that "*upon imbalance of MR:GR-regulated limbic-cortical signaling pathways, the initiation and/or management of the neuroendocrine stress response becomes compromised. At a certain threshold this may lead to a condition of HPA-axis dysregulation and impaired behavioural adaptation, which can enhance susceptibility to stress-related neurodegeneration and mental disorders.*" [13, 54, 55, 68, 69].

3.2. CORT Receptors and FST. Three types of experiments that link CORT receptors to the typical FST behavior will be discussed here. First, it appears that the forced swim stressor itself affects the expression of the CORT receptors differentially. It was shown that the acute stressor induced the expression of MR in the hippocampus as early as 8 hr postinjection and the effect appeared maximal at 24 hr after exposure to the forced swim stressor, at the immunoreactive protein level as well as with radioligand binding [72, 73]. This MR induction depended on CRF, since exogenous CRF induced and CRF antagonist blocked the stress-induced increase in MR. Finally, the CRF-induced MR synthesis appeared functional since in prior forced swim exposed rats antimineralocorticoids were much more effective in disinhibiting the stress-induced HPA-axis activity [72]. Over a period of several weeks the hippocampal MR is profoundly downregulated after exposure to chronic stress, however [74, 75]. This downregulation of MR was prominent in socially defeated mice that showed increased passive coping [76].

Second, GR activation by dexamethasone administration in the low μg range to adrenalectomised animals immediately after the initial 15 min forced swim exposure reinstated dose-dependently the deficit in retention of acquired immobility during the 5 min retest 24 hr later. This effect of dexamethasone in the ADX rats can be prevented by prior subcutaneous administration of the RU486, or other GR antagonist(s) in doses of 1 and 10 mg/kg [17, 77]. Intracerebroventricular adminstration of the GR antagonist to intact rats immediate before the initial test attenuated at retest the retention of acquired immobility in a 100 000 lower dose than needed after systemic administration. In a separate experiment one week later the same low ng dose of RU486 icv increased secretion of CORT [17]. Thus, functional GR is neccesary for retention of acquired immobility.

Figure 4 (adapted from [17]) shows that only 1 ng of RU486 administered in the dentate gyrus is sufficient to impair consolidation of the immobility response. Similar injections in the nucleus parafascicularis and paraventricular nucleus were ineffective, but the GR blockade in the paravenricular nucleus triggered a profound CORT response. Promegestone did not interfere with the RU486 action ruling out a role for the antiprogestin properties of the antagonist. The selective mineralocorticoid antagonist RU28318 was not active excluding, as expected, a role of MR in retention of the passive coping style. The exclusion of MR in memory consolidation is further reinforced by the observation that replacement of the ADX rats with a high dose of CORT occupying both receptor types reinstated the memory deficit of the ADX rats, while a lower dose, mainly occupying MR, did not.

If GR was blocked with RU486 given systemically at 6 hr (but not at 1 hr) prior to the initial test the percentage of immobility was decreased during the initial 15 min test. This decrease was already present in the first 5 min episode and persisted in the retest 24 hr later [29]. A similar result

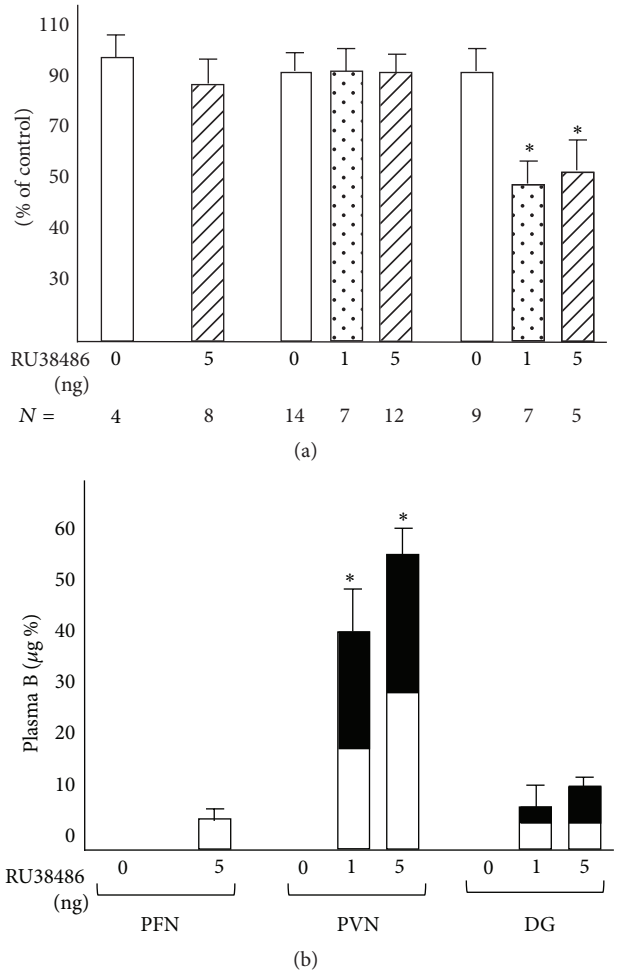


Figure 4: (a) Effect of local intracerebral injection of RU38486 at 5 min prior to initial test on retention of acquired immobility (top) and (b) plasma CORT level (bottom). Behavioural data are expressed as percentage (mean ± the standard error of the mean) of the value observed in rats injected with vehicle. Endocrine data are expressed as micrograms CORT per 100 mL plasma. Open areas represent control injections and closed areas the CORT levels after administration of RU38486. The number of animals is indicated in the figure. The figure comes from de Kloet et al. [17]. $^{*}P < .01$ as compared to controls. PFN is thalamic parafascular nucleus; PVN is hypothalamic paraventricular nucleus; DG is dentate gyrus. Adapted and reprinted with permission from Karger.

was obtained if the synthesis of GR in the dentate gyrus was inhibited by bilateral infusion of 18-mer antisense phosphorothioate oligodeoxynucleotide targeted to GRmRNA 6 hours prior to testing [29]. Likewise daily treatment *plus* a 1 hr pretreatment with GR antagonists also suppressed immobility at pretest, but this design did not include a retest [78, 79]. Reduced immobility was also observed at the initial and retest after metapyrone, which blocks the synthesis of adrenal CORT [80]. Interestingly, these experiments involving blockade of GR or reduction of adrenal output leave MR available for CORT action, supporting indirectly a role of MR in coping with the forced swim stressor.

3.3. MR and GR Function in Acquisition, Consolidation and Retention of Immobility. Colelli et al. [18] demonstrated different levels of immobility learning in DBA/2J and C57Bl/6J mice. In the first experiment both strains showed that the immobility scores in the 10 min initial test were retained in the 5 min retest 24 hr later. Immobility scores in the C57 mice were much higher than in the DBA. In the second experiment it was shown that the immobility performance of the DBA mice correlated, 50 min after initial test, with enhanced expression of cFos in the dorsal striatum, while in the dorsal hippocampus the immediate early gene altered in parallel with C57 immobility. This enhanced activity in the hippocampus aligns with the greater context and spatial memory performance of the C57 that coincides with more CORT output than observed in the DBA strain [81].

Hippocampal MR has a crucial role in the switch from spatial declarative learning towards caudate stimulus response (habit) learning [82]. In the circular hole board test, naive male mice locate with a hippocampal-associated spatial strategy an exit hole at a fixed location flagged by a proximal stimulus. However, if exposed to a stressful context, close to 50% of the mice switched to habit learning associated with hypertrophy of the caudate and atrophy of the hippocampus under chronic stress conditions [83]. Pretreatment with an MR antagonist did prevent the switch towards the stimulus-response strategy [84]. These findings are consistent with evidence that during stress a MR-dependent increase in amygdala connectivity underlies the shift from hippocampal spatial learning to striatal stimulus response or habit learning [82]. With regard to coping with the forced swim stressor, MR antagonists administered prior to the initial test are predicted, therefore, to affect immobility learning in the FST. Indeed, two studies showed that administration of the MR antagonist spironolactone in rats and mice ([85, 86], resp.) reduced the amount of immobility of the animals.

In a series of studies Reul and his colleagues developed the concept that CORT secreted during the initial acute swim stress experience triggers in the dentate gyrus a signaling pathway that activates an epigenetic process underlying increased consolidation and retention of newly acquired stressful information [28]. This mechanism concerned convergence of stress-induced NMDA and GR signaling pathways causing in a distinct and sparse neuroanatomical pattern of dentate gyrus neurons histone modifications, chromatine remodelling, and immediate early gene activation [87–89]. Genetic deletion of specific components (i.e., MSK1/2) in this pathway appeared to prevent the retention of acquired immobility. The significance of this newly identified pathway has been expanded to the role of epigenetics in Morris maze learning, while revealing new interesting twists in their significance for memory consolidation [90].

3.4. The Effects of Chronic Stress. Processing of the forced swim stressor has both physical and psychological components [41]. Physical stressors such as pain, cold, heat, and water immersion each have their inputs to directly stimulate the common final pathway to activation of the sympathetic

nervous system and the HPA axis. Psychological or psychogenic stressors are processed in higher brain regions, potentially using multiple circuits [91, 92] illustrating *the Many Faces of Stress*. However, severe acute stressors can have long-term consequences as well and are of obvious significance as triggers to precipitate an altered phenotype. The acute forced swim stressor has been used for this purpose alone or in combination with another acute single restraint stress exposure [74, 93]. Such animal models for chronic stress exposure also are based on various protocols, for instance exposure of the animals repeatedly during several days to unpredictable stressors, repeated exposure to the same stressor, or daily social defeat, sometimes with a history of early life adversity [94, 95].

The A9 mesolimbic-cortical dopaminergic circuitry is highly responsive to acute and chronic stressors. The responsiveness of this circuitry depends on reciprocal hippocampal ventral subiculum excitatory and amygdala inhibitory inputs [14] including a feedback loop to the A9, but also to the habenular nucleus, dorsal raphe nucleus, basal amygdala, and ventral hippocampus [15]. The circuit has an important function in social and goal-directed behaviour, motivation, pleasure, and reward and is richly endowed with GR. Rodents exposed to repeated social defeat by aggression of a dominant animal develop enduring social aversion and increased anxiety as most prominent behavioural adaptations caused by a CORT-enhanced positive dopaminergic feedback loop. Antiglucocorticoid or GR deletion selectively from the dopaminoceptive neurons reinstated social behaviour linking stress resiliency with dopaminergic tone [96, 97]. As mentioned above, in this circuit correlations were found between circuit activity and the passive-active behavioural transitions during forced swim exposure, which could also be induced optogenetically [15, 30, 31].

Chronic restraint stress induces rapid changes in histone regulation in the hippocampus [98]. Chronically stressed animals likewise show profound changes in neuroendocrine regulations due to an altered phenotype of the CRH neurons expressing much more vasopressin as cosecretagogue [99]. Such chronically stressed animals also display dramatic chromatin reorganizations in CORT brain targets. This altered reorganization becomes apparent only after challenging the stressed individual with an additional acute forced swim stressor. Mice with a stress history exposed to forced swim for 15 min showed much more responsive genes 1 hr later in the hippocampus, and these are particularly genes involved in chromatin modification, epigenetics, and the cytokine/NFκB pathway. The change in some of these genes (e.g., BDNF and GR) persisted for several weeks [94]. Besides, these genes are related to cognitive processes [100] presumably underlying immobility learning in the FST [9, 10].

Interestingly, similar cytokine/NFκB genomic changes were observed after repeated social defeat [101]. The network also showed overlap with the genomic response to CORT applied to rats with a restraint stress history, in this case restricted to the dentate gyrus only [102]. Moreover, in the controls 26 different CORT responsive gene ontology (GO) terms were enriched, whereas this number was only 6 in the stressed group. One highly responsive gene network revealed by this procedure is the mammalian target of rapamycin (mTOR) signalling pathway, which is critical for different forms of synaptic plasticity [103] that may underlie the processes of learning and memory [104].

3.5. Conclusion. GR is expressed abundantly in the ascending A9 mesolimbic and cortical dopaminergic projection innervating frontocortical and nucleus accumbens target regions, while the limbic structures, notably the hippocampal CA1 and CA2 neurons as well as the dentate gyrus neurons, are richly endowed with both MR and GR. The receptors seem to be involved in acquisition and retention of the immobility response. For *acquisition*, pharmacological blockade of GR by systemic RU486 administration and locally by GR knockdown in the dentate gyrus 6 hr *prio*r to the initial test *decreased* the amount of passive behaviour, which was retained in the retest 24 hr later. This effect apparently overrides the small increase in immobility noted after local knockdown of GR in the infralimbic frontocortical dopaminergic target [105]. The *consolidation* and *retention* of the passive behavioural response are promoted after GR activation in the dentate gyrus by an epigenetic mechanism that involves a GR-glutamatergic pathway. However, any blockade of the GR in the limbic regions will result in more dominance of CORT actions via the MR, which could participate in the appraisal, response selection, and immediate coping ability [18]. Such a role of MR has been firmly established in other behavioural paradigms [84, 106].

4. Perspectives

An inevitable consequence of *the Many Faces of Stress* is the discussion centered around the seminal question: "*What is stress?*" For this reason one of the stress pioneers, Levine [107–109], turned to use an operational definition: "*Stress is defined as a composite multidimensional construct in which three components interact: (i) the input, when a stimulus, the stressor, is perceived and appraised, (ii) the processing of stressful information, and (iii) the output, or stress response. The three components interact via complex self-regulating feedback loops with the goal to restore homeostasis through behavioral and physiological adaptations.*"

What happens during processing of the forced swim stressor in the black box of the rodent's brain? The studies by Cabib and coworkers, that are summarized in Cabib and Puglisi-Allegra [16] and Campus et al. [10], point to a genetically determined switch between hippocampus and striatal circuits as a determinant in the choice of behavioural style to cope with the forced swim. Such an MR-induced switch previously was observed in other behavioural paradigms as well [82, 110, 115]. This finding calls for a role of CORT acting via MR during stress, which has been shown crucial for appraisal, immediate coping, response selection, and behavioural flexibility. MR was discovered in electrophysiological studies to mediate fast and rapidly reversible membrane actions of CORT in hippocampus and amygdala [50, 51]. Stress levels of the hormone enhance the frequency of miniature excitatory postsynaptic potentials (mEPSP) in hippocampal CA1

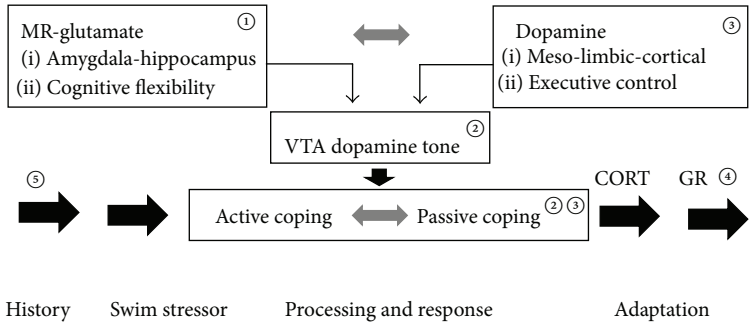


Figure 5: Hypothesis: processing of the forced swim stressor. ① The stressor is perceived and appraised and the appropriate coping style is selected depending on flexibility of amygdala-hippocampus-striatal connectivity, which is controlled by CORT via limbic MR. ② This action of CORT mediated by MR modulates the hippocampal excitatory outflow through enhanced glutamate transmission driving the spontaneous activity of the VTA-A9 neurons and active coping. ③ When time elapses energy is allocated to more executive functions governed by mesocortical and prefrontal circuitry attenuating mesoaccumbens dopamine activity causing a switch from active to passive coping with the inescapable forced swim stressor. ④ The coping response is stored in memory for future use by a mechanism activated by stress-induced levels of CORT acting through the GR in the hippocampal dentate gyrus. ⑤ The information processing during the forced swim is affected by stress history as can be deducted from altered genomic expression in the hippocampus. This hypothesis is based on the following references: [14–19, 28, 30, 31, 36, 50, 51, 58, 64, 82, 84, 94, 102, 110–114]. For more information we refer to the main text of this paper. CORT is corticosterone; MR is mineralocorticoid receptors; GR is glucocorticoid receptors; VTA is ventral tegmental area, A9 dopaminergic neurons.

pyramidal neurons, indicating enhancement of glutamate excitatory outflow from the hippocampus [60, 64, 65, 112].

Grace [36] highlights an important role of the afferent circuits in processing of contextual and emotion-loaded information that operate reciprocally from the hippocampal ventral subiculum and the basolateral amygdala in regulating A9 dopaminergic activity [14]. This afferent excitatory control would be driving the behavioural expression of accumbens dopamine release as can be demonstrated by coping with the forced swim stressor. The optogenetic experiments by Tye et al. [31] indeed show an executive role for the mesolimbic dopaminergic system in the transitions between active and passive coping, while Warden et al. [30] and Cabib and Puglisi-Allegra [16] showed evidence for implication of the mPFC circuitry. Then, after the initial test the preferred passive coping style is stored in memory for future use by a GR dependent process in the hippocampal dentate gyrus [17, 19]. We refer to Figure 5 for some of the elements that may be involved in processing the forced swim stressor in the brain.

How hippocampal MR participates through enhanced excitatory transmission in the transition between active and passive behaviour needs to be investigated. That the subsequent rise in CORT after forced swim activates GR to promote consolidation and retention of the coping style in the memory is firmly established. For this purpose an epigenetic mechanism underlying consolidation of the acquired immobility has been identified in the hippocampal dentate gyrus [19]. Furthermore, a history of chronic stress downregulates in particular hippocampal MR [76, 93], introducing a bias, which is reflected by altering the genomic response in hippocampus and dentate gyrus to forced swim [94]. Part of this response is mimicked by CORT action with consequences in the dentate gyrus neurogenic niche [102, 113].

Floating has been a criterion in the past to judge the witchcraft outcome of forced swim [114], but today it is in use to label a rodent as being depressed. In fact, the number of research papers that intentionally used the FST to assess a depression-like phenotype has shown a dramatic increase in recent years, now amounting to almost one paper per day [8]. Hence, this anthropomorphic interpretation of coping with the forced swim stressor is remarkable, since alternatively the forced swim experience provides a unique challenge to investigate how information processing occurs to achieve stress adaptation.

The use of acquired immobility to diagnose depression in a rat should not be encouraged. What we do encourage is to use the forced swim stressor in research on the mechanism of coping and adaptation that counts to understand an evolutionary-conserved energy-sparing survival mechanism of passive coping with an apparent inescapable/uncontrollable situation.

Conflict of Interests

E. R. de Kloet is on the scientific advisory Board of Dynacorts Therapeutics and Pharmaseed Ltd. and owns stock of Corcept Therapeutics. M. L. Molendijk reports having no potential conflict of interests.

Acknowledgments

The support to E. R. de Kloet by the Royal Netherlands Academy of Arts and Sciences and of EU-Aldosterone and Mineralocorticoid Receptor COST action (ADMIRE) BM1301 is gratefully acknowledged. M. L. Molendijk is supported by Leiden University, The Netherlands.

References

[1] E. J. Nestler and S. E. Hyman, "Animal models of neuropsychiatric disorders," *Nature Neuroscience*, vol. 13, no. 10, pp. 1161–1169, 2010.

[2] R. D. Porsolt, M. Le Pichon, and M. Jalfre, "Depression: a new animal model sensitive to antidepressant treatments," *Nature*, vol. 266, no. 5604, pp. 730–732, 1977.

[3] R. D. Porsolt, A. Bertin, and M. Jalfre, "'Behavioural despair' in rats and mice: strain differences and the effects of imipramine," *European Journal of Pharmacology*, vol. 51, no. 3, pp. 291–294, 1978.

[4] J. Hawkins, R. A. Hicks, N. Phillips, and J. D. Moore, "Swimming rats and human depression," *Nature*, vol. 274, no. 5670, pp. 512–513, 1978.

[5] D. A. Slattery and J. F. Cryan, "Using the rat forced swim test to assess antidepressant-like activity in rodents," *Nature Protocols*, vol. 7, no. 6, pp. 1009–1014, 2012.

[6] J. F. Cryan and C. Mombereau, "In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice," *Molecular Psychiatry*, vol. 9, no. 4, pp. 326–357, 2004.

[7] D. A. Slattery and J. F. Cryan, "The ups and downs of modelling mood disorders in rodents," *ILAR Journal*, vol. 55, no. 2, Article ID ilu026, pp. 297–309, 2014.

[8] M. L. Molendijk and E. R. de Kloet, "Immobility in the Forced Swim Test is adaptive and does not reflect depression," *Psychoneuroendocrinology*, vol. 62, pp. 389–301, 2015.

[9] J. M. De Pablo, A. Parra, S. Segovia, and A. Guillamón, "Learned immobility explains the behavior of rats in the forced swimming test," *Physiology & Behavior*, vol. 46, no. 2, pp. 229–237, 1989.

[10] P. Campus, V. Colelli, C. Orsini, D. Sarra, and S. Cabib, "Evidence for the involvement of extinction-associated inhibitory learning in the forced swimming test," *Behavioural Brain Research*, vol. 278, pp. 348–355, 2015.

[11] H. D. Veldhuis, C. C. M. M. de Korte, and E. R. de Kloet, "Glucocorticoids facilitate the retention of acquired immobility during forced swimming," *European Journal of Pharmacology*, vol. 115, no. 2-3, pp. 211–217, 1985.

[12] D. Jefferys, D. Copolov, D. Irby, and J. Funder, "Behavioural effect of adrenalectomy: reversal by glucocorticoids or [D-ALA2, MET5]enkephalinamide," *European Journal of Pharmacology*, vol. 92, no. 1-2, pp. 99–103, 1983.

[13] E. R. de Kloet, M. Joëls, and F. Holsboer, "Stress and the brain: from adaptation to disease," *Nature Reviews Neuroscience*, vol. 6, no. 6, pp. 463–475, 2005.

[14] P. Belujon and A. A. Grace, "Regulation of dopamine system responsivity and its adaptive and pathological response to stress," *Proceedings of the Royal Society of London B: Biological Sciences*, vol. 282, no. 1805, Article ID 20142516, 2015.

[15] S. Lammel, K. M. Tye, and M. R. Warden, "Progress in understanding mood disorders: optogenetic dissection of neural circuits," *Genes, Brain and Behavior*, vol. 13, no. 1, pp. 38–51, 2014.

[16] S. Cabib and S. Puglisi-Allegra, "The mesoaccumbens dopamine in coping with stress," *Neuroscience and Biobehavioral Reviews*, vol. 36, no. 1, pp. 79–89, 2012.

[17] E. R. de Kloet, S. De Kock, V. Schild, and H. D. Veldhuis, "Antiglucocorticoid RU 38486 attenuates retention of a behaviour and disinhibits the hypothalamic-pituitary adrenal axis at different brain sites," *Neuroendocrinology*, vol. 47, no. 2, pp. 109–115, 1988.

[18] V. Colelli, P. Campus, D. Conversi, C. Orsini, and S. Cabib, "Either the dorsal hippocampus or the dorsolateral striatum is selectively involved in consolidation of forced swim-induced immobility depending on genetic background," *Neurobiology of Learning and Memory*, vol. 111, pp. 49–55, 2014.

[19] J. M. H. M. Reul, "Making memories of stressful events: a journey along epigenetic, gene transcription, and signaling pathways," *Frontiers in Psychiatry*, vol. 5, article 5, 11 pages, 2014.

[20] V. Castagné, P. Moser, and R. D. Porsolt, "Behavioral assessment of antidepressant activity in rodents," in *Methods of Behavior Analysis in Neuroscience*, K. R. Bailey and J. N. Crawley, Eds., CRC Press, Boca Raton, Fla, USA, 2009.

[21] P. Spinhoven, B. M. Elzinga, J. G. F. M. Hovens et al., "The specificity of childhood adversities and negative life events across the life span to anxiety and depressive disorders," *Journal of Affective Disorders*, vol. 126, no. 1-2, pp. 103–112, 2010.

[22] J. J. Mann, "The medical management of depression," *The New England Journal of Medicine*, vol. 353, no. 17, pp. 1819–1834, 2005.

[23] J. M. De Pablo, J. Ortiz-Caro, F. Sanchez-Santed, and A. Guillamón, "Effects of diazepam, pentobarbital, scopolamine and the timing of saline injection on learned immobility in rats," *Physiology & Behavior*, vol. 50, no. 5, pp. 895–899, 1991.

[24] J. Krogh, M. Nordentoft, J. A. C. Sterne, and D. A. Lawlor, "The effect of exercise in clinically depressed adults: systematic review and meta-analysis of randomized controlled trials," *Journal of Clinical Psychiatry*, vol. 72, no. 4, pp. 529–538, 2011.

[25] R. D. Porsolt, "Behavioral despair: past and future," in *New Directions in Affective Disorders*, pp. 17–20, Springer, New York, NY, USA, 1989.

[26] F. Borsini, G. Volterra, and A. Meli, "Does the behavioral 'despair' test measure 'despair'?" *Physiology and Behavior*, vol. 38, no. 3, pp. 385–386, 1986.

[27] K. A. O'Neill and D. Valentino, "Escapability and generalization: effect on 'behavioral despair'," *European Journal of Pharmacology*, vol. 78, no. 3, pp. 379–380, 1982.

[28] M. Gutièrrez-Mecinas, A. F. Trollope, A. Collins et al., "Long-lasting behavioral responses to stress involve a direct interaction of glucocorticoid receptors with ERK1/2-MSK1-Elk-1 signaling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 33, pp. 13806–13811, 2011.

[29] S. M. Korte, S. F. De Boer, E. R. de Kloet, and B. Bohus, "Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus-maze," *Psychoneuroendocrinology*, vol. 20, no. 4, pp. 385–394, 1995.

[30] M. R. Warden, A. Selimbeyoglu, J. J. Mirzabekov et al., "A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge," *Nature*, vol. 492, no. 7429, pp. 428–432, 2012.

[31] K. M. Tye, J. J. Mirzabekov, M. R. Warden et al., "Dopamine neurons modulate neural encoding and expression of depression-related behaviour," *Nature*, vol. 493, no. 7433, pp. 537–541, 2013.

[32] H. Nishimura, A. Tsuda, M. Oguchi, Y. Ida, and M. Tanaka, "Is immobility of rats in the forced swim test 'behavioral despair?'," *Physiology and Behavior*, vol. 42, no. 1, pp. 93–95, 1988.

[33] D. Chaudhury, J. J. Walsh, A. K. Friedman et al., "Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons," *Nature*, vol. 493, no. 7433, pp. 532–536, 2013.

[34] H. S. Mayberg, A. M. Lozano, V. Voon et al., "Deep brain stimulation for treatment-resistant depression," *Neuron*, vol. 45, no. 5, pp. 651–660, 2005.

[35] S. F. de Kloet, H. D. Mansvelder, and T. J. de Vries, "Cholinergic modulation of dopamine pathways through nicotinic acetylcholine receptors," *Biochemical Pharmacology*, vol. 97, no. 4, pp. 425–438, 2015.

[36] A. A. Grace, "Dopamine system dysregulation by the hippocampus: implications for the pathophysiology and treatment of schizophrenia," *Neuropharmacology*, vol. 62, no. 3, pp. 1342–1348, 2012.

[37] D. Jefferys and J. W. Funder, "Glucocorticoids, adrenal medullary opioids, and the retention of a behavioral response after stress," *Endocrinology*, vol. 121, no. 3, pp. 1006–1009, 1987.

[38] D. Jefferys and J. W. Funder, "Thyroid hormones and the acquisition and retention of behavioural responses," *Endocrinology*, vol. 125, no. 2, pp. 1103–1105, 1989.

[39] D. Jefferys and J. W. Funder, "The forced swimming test: effects of glucose administration on the response to food deprivation and adrenalectomy," *European Journal of Pharmacology*, vol. 205, no. 3, pp. 267–269, 1991.

[40] F. Golden and M. Tipton, *Essentials of Sea Survival*, Human Kinetics, 2002.

[41] A. C. E. Linthorst, C. Flachskamm, and J. M. H. M. Reul, "Water temperature determines neurochemical and behavioural responses to forced swim stress: an in vivo microdialysis and biotelemetry study in rats," *Stress*, vol. 11, no. 2, pp. 88–100, 2008.

[42] S. K. Droste, L. de Groote, H. C. Atkinson, S. L. Lightman, J. M. H. M. Reul, and A. C. E. Linthorst, "Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response to forced swim stress," *Endocrinology*, vol. 149, no. 7, pp. 3244–3253, 2008.

[43] L. de Groote and A. C. E. Linthorst, "Exposure to novelty and forced swimming evoke stressor-dependent changes in extracellular GABA in the rat hippocampus," *Neuroscience*, vol. 148, no. 3, pp. 794–805, 2007.

[44] A. Munck, P. M. Guyre, and N. J. Holbrook, "Physiological functions of glucocorticoids in stress and their relation to pharmacological actions," *Endocrine Reviews*, vol. 5, no. 1, pp. 25–44, 1984.

[45] R. M. Sapolsky, L. M. Romero, and A. U. Munck, "How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions," *Endocrine Reviews*, vol. 21, no. 1, pp. 55–89, 2000.

[46] B. S. McEwen, J. M. Weiss, and L. S. Schwartz, "Selective retention of corticosterone by limbic structures in rat brain," *Nature*, vol. 220, no. 5170, pp. 911–912, 1968.

[47] R. de Kloet, G. Wallach, and B. S. McEwen, "Differences in corticosterone and dexamethasone binding to rat brain and pituitary," *Endocrinology*, vol. 96, no. 3, pp. 598–609, 1975.

[48] J. M. H. M. Reul and E. R. de Kloet, "Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation," *Endocrinology*, vol. 117, no. 6, pp. 2505–2511, 1985.

[49] J. L. Arriza, C. Weinberger, G. Cerelli et al., "Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor," *Science*, vol. 237, no. 4812, pp. 268–275, 1987.

[50] H. Karst, S. Berger, M. Turiault, F. Tronche, G. Schütz, and M. Joëls, "Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 52, pp. 19204–19207, 2005.

[51] H. Karst, S. Berger, G. Erdmann, G. Schütz, and M. Joëls, "Metaplasticity of amygdalar responses to the stress hormone corticosterone," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 32, pp. 14449–14454, 2010.

[52] F. L. Groeneweg, H. Karst, E. R. de Kloet, and M. Joëls, "Rapid non-genomic effects of corticosteroids and their role in the central stress response," *Journal of Endocrinology*, vol. 209, no. 2, pp. 153–167, 2011.

[53] J. M. H. M. Reul, F. R. van den Bosch, and E. R. de Kloet, "Relative occupation of type-I and type-II corticosteroid receptors in rat brain following stress and dexamethasone treatment: functional implications," *Journal of Endocrinology*, vol. 115, no. 3, pp. 459–467, 1987.

[54] E. R. de Kloet, "Brain corticosteroid receptor balance and homeostatic control," *Frontiers in Neuroendocrinology*, vol. 12, no. 2, pp. 95–164, 1991.

[55] E. R. de Kloet, E. Vreugdenhil, M. S. Oitzl, and M. Joëls, "Brain corticosteroid receptor balance in health and disease," *Endocrine Reviews*, vol. 19, no. 3, pp. 269–301, 1998.

[56] R. A. Sarabdjitsingh, O. C. Meijer, M. J. M. Schaaf, and E. R. de Kloet, "Subregion-specific differences in translocation patterns of mineralocorticoid and glucocorticoid receptors in rat hippocampus," *Brain Research*, vol. 1249, pp. 43–53, 2009.

[57] M. P. Carey, C. H. Deterd, J. de Koning, F. Helmerhorst, and E. R. de Kloet, "The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat," *Journal of Endocrinology*, vol. 144, no. 2, pp. 311–321, 1995.

[58] E. J. Hermans, M. J. A. G. Henckens, M. Joëls, and G. Fernández, "Dynamic adaptation of large-scale brain networks in response to acute stressors," *Trends in Neurosciences*, vol. 37, no. 6, pp. 304–314, 2014.

[59] M. Joëls, "Corticosteroid effects in the brain: U-shape it," *Trends in Pharmacological Sciences*, vol. 27, no. 5, pp. 244–250, 2006.

[60] M. Joëls, R. A. Sarabdjitsingh, and H. Karst, "Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes," *Pharmacological Reviews*, vol. 64, no. 4, pp. 901–938, 2012.

[61] B. S. McEwen, N. P. Bowles, J. D. Gray et al., "Mechanisms of stress in the brain," *Nature Neuroscience*, vol. 18, no. 10, pp. 1353–1363, 2015.

[62] M. Joels and E. R. de Kloet, "Effects of glucocorticoids and norepinephrine on the excitability in the hippocampus," *Science*, vol. 245, no. 4925, pp. 1502–1505, 1989.

[63] M. Joëls and E. R. de Kloet, "Mineralocorticoid receptor-mediated changes in membrane properties of rat CA1 pyramidal neurons in vitro," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 12, pp. 4495–4498, 1990.

[64] C. Nasca, B. Bigio, D. Zelli, F. Nicoletti, and B. S. McEwen, "Mind the gap: glucocorticoids modulate hippocampal glutamate tone underlying individual differences in stress susceptibility," *Molecular Psychiatry*, vol. 20, no. 6, pp. 755–763, 2015.

[65] M. Joëls, H. Karst, R. DeRijk, and E. R. de Kloet, "The coming out of the brain mineralocorticoid receptor," *Trends in Neurosciences*, vol. 31, no. 1, pp. 1–7, 2008.

[66] M. Joëls and E. R. de Kloet, "Corticosteroid actions on amino acid-mediated transmission in rat CA1 hippocampal cells," *The Journal of Neuroscience*, vol. 13, no. 9, pp. 4082–4090, 1993.

[67] M. N. Hill, S. Patel, P. Campolongo, J. G. Tasker, C. T. Wotjak, and J. S. Bains, "Functional interactions between stress and the

endocannabinoid system: from synaptic signaling to behavioral output," *The Journal of Neuroscience*, vol. 30, no. 45, pp. 14980–14986, 2010.

[68] E. R. de Kloet and J. M. H. M. Reul, "Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems," *Psychoneuroendocrinology*, vol. 12, no. 2, pp. 83–105, 1987.

[69] E. R. de Kloet, "From receptor balance to rational glucocorticoid therapy," *Endocrinology*, vol. 155, no. 8, pp. 2754–2769, 2014.

[70] M. S. Oitzl and E. R. de Kloet, "Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning," *Behavioral Neuroscience*, vol. 106, no. 1, pp. 62–71, 1992.

[71] M. S. Oitzl, D. L. Champagne, R. van der Veen, and E. R. de Kloet, "Brain development under stress: hypotheses of glucocorticoid actions revisited," *Neuroscience & Biobehavioral Reviews*, vol. 34, no. 6, pp. 853–866, 2010.

[72] A. Gesing, A. Bilang-Bleuel, S. K. Droste, A. C. E. Linthorst, F. Holsboer, and J. M. H. M. Reul, "Psychological stress increases hippocampal mineralocorticoid receptor levels: involvement of corticotropin-releasing hormone," *The Journal of Neuroscience*, vol. 21, no. 13, pp. 4822–4829, 2001.

[73] A. H. Veenema, O. C. Meijer, E. R. de Kloet, J. M. Koolhaas, and B. Bohus, "Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression," *Hormones and Behavior*, vol. 43, no. 1, pp. 197–204, 2003.

[74] F. Han, J. Ding, and Y. Shi, "Expression of amygdala mineralocorticoid receptor and glucocorticoid receptor in the single-prolonged stress rats," *BMC Neuroscience*, vol. 15, article 77, 2014.

[75] M. A. Orlovsky, V. E. Dosenko, F. Spiga, G. G. Skibo, and S. L. Lightman, "Hippocampus remodeling by chronic stress accompanied by GR, proteasome and caspase-3 overexpression," *Brain Research*, vol. 1593, pp. 83–94, 2014.

[76] A. H. Veenema, O. C. Meijer, E. R. de Kloet, and J. M. Koolhaas, "Genetic selection for coping style predicts stressor susceptibility," *Journal of Neuroendocrinology*, vol. 15, no. 3, pp. 256–267, 2003.

[77] C. G. Bachmann, A. Bilang-Bleuel, S. De Carli, A. C. E. Linthorst, and J. M. H. M. Reul, "The selective glucocorticoid receptor antagonist ORG 34116 decreases immobility time in the forced swim test and affects cAMP-responsive element-binding protein phosphorylation in rat brain," *Neuroendocrinology*, vol. 81, no. 2, pp. 129–136, 2005.

[78] A. C. Wulsin, J. P. Herman, and M. B. Solomon, "Mifepristone decreases depression-like behavior and modulates neuroendocrine and central hypothalamic-pituitary-adrenocortical axis responsiveness to stress," *Psychoneuroendocrinology*, vol. 35, no. 7, pp. 1100–1112, 2010.

[79] M. B. Solomon, A. C. Wulsin, T. Rice et al., "The selective glucocorticoid receptor antagonist CORT 108297 decreases neuroendocrine stress responses and immobility in the forced swim test," *Hormones and Behavior*, vol. 65, no. 4, pp. 363–371, 2014.

[80] M. Báez and M. Volosin, "Corticosterone influences forced swim-induced immobility," *Pharmacology, Biochemistry and Behavior*, vol. 49, no. 3, pp. 729–736, 1994.

[81] S. Cabib, S. Algeri, C. Perego, and S. Puglisi-Allegra, "Behavioral and biochemical changes monitored in two inbred strains of mice during exploration of an unfamiliar environment," *Physiology and Behavior*, vol. 47, no. 4, pp. 749–753, 1990.

[82] L. Schwabe, M. Tegenthoff, O. Höffken, and O. T. Wolf, "Mineralocorticoid receptor blockade prevents stress-induced modulation of multiple memory systems in the human brain," *Biological Psychiatry*, vol. 74, no. 11, pp. 801–808, 2013.

[83] E. Dias-Ferreira, J. C. Sousa, I. Melo et al., "Chronic stress causes frontostriatal reorganization and affects decision-making," *Science*, vol. 325, no. 5940, pp. 621–625, 2009.

[84] L. Schwabe, H. Schächinger, E. R. de Kloet, and M. S. Oitzl, "Corticosteroids operate as a switch between memory systems," *Journal of Cognitive Neuroscience*, vol. 22, no. 7, pp. 1362–1372, 2010.

[85] C. R. Mostalac-Preciado, P. de Gortari, and C. López-Rubalcava, "Antidepressant-like effects of mineralocorticoid but not glucocorticoid antagonists in the lateral septum: interactions with the serotonergic system," *Behavioural Brain Research*, vol. 223, no. 1, pp. 88–98, 2011.

[86] T.-C. Wu, H.-T. Chen, H.-Y. Chang et al., "Mineralocorticoid receptor antagonist spironolactone prevents chronic corticosterone induced depression-like behavior," *Psychoneuroendocrinology*, vol. 38, no. 6, pp. 871–883, 2013.

[87] A. Bilang-Bleuel, S. Ulbricht, Y. Chandramohan, S. De Carli, S. K. Droste, and J. M. H. M. Reul, "Psychological stress increases histone H3 phosphorylation in adult dentate gyrus granule neurons: involvement in a glucocorticoid receptor-dependent behavioural response," *European Journal of Neuroscience*, vol. 22, no. 7, pp. 1691–1700, 2005.

[88] Y. Chandramohan, S. K. Droste, and J. M. H. M. Reul, "Novelty stress induces phospho-acetylation of histone H3 in rat dentate gyrus granule neurons through coincident signalling via the *N*-methyl-D-aspartate receptor and the glucocorticoid receptor: relevance for c-fos induction," *Journal of Neurochemistry*, vol. 101, no. 3, pp. 815–828, 2007.

[89] Y. Chandramohan, S. K. Droste, J. S. C. Arthur, and J. M. H. M. Reul, "The forced swimming-induced behavioural immobility response involves histone H3 phospho-acetylation and c-Fos induction in dentate gyrus granule neurons via activation of the *N*-methyl-D-aspartate/extracellular signal-regulated kinase/mitogen- and stress-activated kinase signalling pathway," *European Journal of Neuroscience*, vol. 27, no. 10, pp. 2701–2713, 2008.

[90] S. D. Carter, K. R. Mifsud, and J. M. Reul, "Distinct epigenetic and gene expression changes in rat hippocampal neurons after Morris water maze training," *Frontiers in Behavioral Neuroscience*, vol. 9, article 156, 2015.

[91] H. F. Figueiredo, B. L. Bodie, M. Tauchi, C. M. Dolgas, and J. P. Herman, "Stress integration after acute and chronic predator stress: differential activation of central stress circuitry and sensitization of the hypothalamo-pituitary-adrenocortical axis," *Endocrinology*, vol. 144, no. 12, pp. 5249–5258, 2003.

[92] J. P. Herman, H. Figueiredo, N. K. Mueller et al., "Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo–pituitary–adrenocortical responsiveness," *Frontiers in Neuroendocrinology*, vol. 24, no. 3, pp. 151–180, 2003.

[93] D. Zhe, H. Fang, and S. Yuxiu, "Expressions of hippocampal mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) in the single-prolonged stress-rats," *Acta Histochemica et Cytochemica*, vol. 41, no. 4, pp. 89–95, 2008.

[94] J. D. Gray, T. G. Rubin, R. G. Hunter, and B. S. McEwen, "Hippocampal gene expression changes underlying stress sensitization and recovery," *Molecular Psychiatry*, vol. 19, no. 11, pp. 1171–1178, 2014.

[95] N. P. Daskalakis, R. Yehuda, and D. M. Diamond, "Animal models in translational studies of PTSD," *Psychoneuroendocrinology*, vol. 38, no. 9, pp. 1895–1911, 2013.

[96] J. Barik, F. Marti, C. Morel et al., "Chronic stress triggers social aversion via glucocorticoid receptor in dopaminoceptive neurons," *Science*, vol. 339, no. 6117, pp. 332–335, 2013.

[97] M. Niwa, H. Jaaro-Peled, S. Tankou et al., "Adolescent stress-induced epigenetic control of dopaminergic neurons via glucocorticoids," *Science*, vol. 339, no. 6117, pp. 335–339, 2013.

[98] R. G. Hunter, K. J. McCarthy, T. A. Milne, D. W. Pfaff, and B. S. McEwen, "Regulation of hippocampal H3 histone methylation by acute and chronic stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 49, pp. 20912–20917, 2009.

[99] J. P. Herman, "Neural control of chronic stress adaptation," *Frontiers in Behavioral Neuroscience*, vol. 7, article 61, 2013.

[100] C. Finsterwald and C. M. Alberini, "Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: from adaptive responses to psychopathologies," *Neurobiology of Learning and Memory*, vol. 112, pp. 17–29, 2014.

[101] D. E. M. Feldker, M. C. Morsink, A. H. Veenema et al., "The effect of chronic exposure to highly aggressive mice on hippocampal gene expression of non-aggressive subordinates," *Brain Research*, vol. 1089, no. 1, pp. 10–20, 2006.

[102] N. A. Datson, J. M. E. van den Oever, O. B. Korobko, A. M. Magarinos, E. R. de Kloet, and B. S. McEwen, "Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus," *Endocrinology*, vol. 154, no. 9, pp. 3261–3272, 2013.

[103] J. A. E. Polman, R. G. Hunter, N. Speksnijder et al., "Glucocorticoids modulate the mtor pathway in the hippocampus: differential effects depending on stress history," *Endocrinology*, vol. 153, no. 9, pp. 4317–4327, 2012.

[104] G. Neves, S. F. Cooke, and T. V. P. Bliss, "Synaptic plasticity, memory and the hippocampus: a neural network approach to causality," *Nature Reviews Neuroscience*, vol. 9, no. 1, pp. 65–75, 2008.

[105] M. B. Solomon, A. R. Furay, K. Jones et al., "Deletion of forebrain glucocorticoid receptors impairs neuroendocrine stress responses and induces depression-like behavior in males but not females," *Neuroscience*, vol. 203, pp. 135–143, 2012.

[106] A. P. Harris, M. C. Holmes, E. R. de Kloet, K. E. Chapman, and J. R. Seckl, "Mineralocorticoid and glucocorticoid receptor balance in control of HPA axis and behaviour," *Psychoneuroendocrinology*, vol. 38, no. 5, pp. 648–658, 2013.

[107] S. Levine, "Stress and behavior," *Scientific American*, vol. 224, no. 1, pp. 26–31, 1971.

[108] S. Levine, "A definition of stress?" in *Animal Stress*, pp. 51–69, Springer, New York, NY, USA, 1985.

[109] S. Levine, "Stress: an historical perspective," in *Techniques in the Behavioral and Neural Sciences*, vol. 15, chapter 1.1, pp. 3–23, Elsevier, 2005.

[110] J. P. ter Horst, M. van der Mark, J. Kentrop et al., "Deletion of the forebrain mineralocorticoid receptor impairs social discrimination and decision-making in male, but not in female mice," *Frontiers in Behavioral Neuroscience*, vol. 8, article 26, 2014.

[111] M. Joëls and T. Z. Baram, "The neuro-symphony of stress," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 459–466, 2009.

[112] M. Popoli, Z. Yan, B. S. McEwen, and G. Sanacora, "The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission," *Nature Reviews Neuroscience*, vol. 13, no. 1, pp. 22–37, 2012.

[113] C. P. Fitzsimons, L. W. A. van Hooijdonk, M. Schouten et al., "Knockdown of the glucocorticoid receptor alters functional integration of newborn neurons in the adult hippocampus and impairs fear-motivated behavior," *Molecular Psychiatry*, vol. 18, no. 9, pp. 993–1005, 2013.

[114] G. F. Jensen, *The Path of the Devil: Early Modern Witch Hunts*, Rowman & Littlefield, Lanham, Md, USA, 2007.

[115] S. Vogel, F. Klumpers, H. J. Krugers et al., "Blocking the mineralocorticoid receptor in humans prevents the stress-induced enhancement of centromedial amygdala connectivity with the dorsal striatum," *Neuropsychopharmacology*, vol. 40, no. 4, pp. 947–956, 2015.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 4928081, 6 pages
http://dx.doi.org/10.1155/2016/4928081

Review Article

Seeding Stress Resilience through Inoculation

Archana Ashokan, Meenalochani Sivasubramanian, and Rupshi Mitra

School of Biological Sciences, Nanyang Technological University, Singapore 637551

Correspondence should be addressed to Rupshi Mitra; rmitra@ntu.edu.sg

Received 27 June 2015; Revised 11 October 2015; Accepted 4 November 2015

Academic Editor: Laura Musazzi

Stress is a generalized set of physiological and psychological responses observed when an organism is placed under challenging circumstances. The stress response allows organisms to reattain the equilibrium in face of perturbations. Unfortunately, chronic and/or traumatic exposure to stress frequently overwhelms coping ability of an individual. This is manifested as symptoms affecting emotions and cognition in stress-related mental disorders. Thus environmental interventions that promote resilience in face of stress have much clinical relevance. Focus of the bulk of relevant neurobiological research at present remains on negative aspects of health and psychological outcomes of stress exposure. Yet exposure to the stress itself can promote resilience to subsequent stressful episodes later in the life. This is especially true if the prior stress occurs early in life, is mild in its magnitude, and is controllable by the individual. This articulation has been referred to as "stress inoculation," reminiscent of resilience to the pathology generated through vaccination by attenuated pathogen itself. Using experimental evidence from animal models, this review explores relationship between nature of the "inoculum" stress and subsequent psychological resilience.

1. Stress and Stress Inoculation

Stress is a nonspecific response of the body to any demand placed by external environment or internal metabolic milieu [1]. The stress response itself cannot be eradicated or evaded for very long because these challenges are inevitable aspects of life. The concept of stress was first introduced in the work of Selye [1], who observed that individual animals exhibited a "general adaptation syndrome" when confronted with a variety of perturbations. He argued for a common bodily mechanism that was invoked during these challenging episodes and termed it "stress," further remarking that the stress "suffers from the mixed blessing of being too well known and too little understood" [1]. In due course of time psychological dimensions were included in the repertoire of stress syndrome, including emotional and cognitive facets. In recent decades, relationship between stress and predisposition to mental disorders has gained mounting importance [2]. This renewed focus is borne out by reclassification of stress and trauma related disorders as an identifiable mental disorder in recent diagnostic manual [3]. The clinical interest is paralleled by increasing knowledge about neural and endocrine underpinnings of this process. In this regard, feedback loops between adrenal hormones and discrete brain regions have been extensively described and analysed (succinctly reviewed in [4–7]).

Despite its negative connotations, stress has an adaptive value in that it promotes homeostasis. Exposure to stressful events that are not devastating, yet challenging enough to provoke emotional instigation and cognitive processing, might nurture successful coping with subsequent stressors. Thus exposure to prolonged unpredictable and uncontrollable stress induces long-term neurological impairment, but exposure to moderately stressful and controllable events seems to increase efficacy of regulating future stress response. This phenomenon has been referred to as "stress inoculation" [8]. Rhetorically, stress inoculation is reminiscent of protection from a pathology afforded by prior inoculation with attenuated pathological agent. Just as vaccination by a dead or weakened pathogen enables the body to mount a long-lasting immune response, exposure to moderate amount of stress enables organisms to effectively cope with future stressors. Although this hypothesis has great potential, it remains relatively understudied at present.

The effects of stress on health outcomes often vary in a nonlinear manner with severity of the stress, roughly

corresponding to an inverted U shaped reaction norm [9–11]. When starting from a low baseline, moderate amount of stressful challenge enhances both short-term and long-term health outcomes. With successive increase in severity, stress leads to diminished health leading to an inverted U relationship between stress and health parameters. The idea of inverted U shaped reaction norm was first formalized to explain relationship between arousal levels and strength of discrimination based learning [12]. The concept has been subsequently used to empirically explain variation in behaviour with respect to different kinds and strengths of stressor. A few studies have also demonstrated this relationship within a single experimental design. For example, rats swimming in colder water experience greater secretion of stress hormones (16°C > 19°C > 25°C) when undergoing training for spatial learning in radial arm water maze. Animals trained at moderate stress levels of 19°C perform fewer errors compared to those trained at either 16°C or 25°C [13]. Similarly direction of the corticosterone influence on hippocampal primed burst potentiation is dependent upon the concentration of this stress hormone [14]. Briefly, primed burst potentiation utilizes electrical stimulation that mimics pattern of endogenous activity of hippocampal neurons, leading to long-lasting increase in synaptic strength [15]. Low to medium corticosterone levels are positively correlated with primed burst potentiation, representing ascending part of the inverse U. At higher concentration, corticosterone levels become negatively correlated with electrophysiological potentiation, reflecting descending part of the curve.

Various factors influence the shape and inflexion point of such inverted U curve. These include age, sex, and predictability of the stress [16–19]. Very importantly, these factors also include degree, nature, and developmental timing of the historical stress exposure [20]. These factors are capable of shifting the curve from its general course towards either stress pathologies or stress resilience [9]. The essence of stress inoculation thus lies in the idea that prior stress exposure can induce resilience to later stress, if the prior stress is of optimal degree and provided at crucial stage of life.

2. State of Current Animal Models

Animals models are primarily used for stress research due to the methodological and ethical limitations involved in human studies [21]. A good animal model of stress inoculation will ideally comprise face validity, construct validity, and predictive validity (e.g., [22, 23]). In other words, an animal model of stress inoculation must contain elements that are analogous to human stress inoculation. It must comprise measurable endpoint that accurately reflects the unmeasurable theoretical construct of stress inoculation. Additionally, animal model in question must be able to prospectively predict strength of the inoculation. Beyond these, the animal models should be consistent and reproducible and have internal controls to measure influence of confound like locomotion or nutrition. Unfortunately, a consensus about the animal model that meets these criteria for stress inoculation remains elusive at present. A variety of inoculum stressors and correspondingly varied subsequent stressors to test the inoculation have been used. Further work is required to refine animal models with respect to both external and internal validity.

Within the limits of current animal models, researches on rodents and primates support the stress inoculation hypothesis and provide insight into its neurobiological mechanisms. Broadly, early life stress inoculation triggers broad developmental cascades that increase adaptation. For example, studies on male and female squirrel monkeys show that a brief intermittent maternal separation during early childhood enhances long-lasting and trait-like transformation in the multiple domains of adaptive functioning [24]. Young male and female monkeys presented with a moderate stressor in the form of periodic short maternal separation from postnatal week 17 to postnatal week 27 experienced acute distress during the separation periods manifested by agitation and temporary elevation in the stress hormone levels [25]. However later in life, at nine months of age, the same set of monkeys demonstrated lower anxiety and decreased stress hormone levels when compared to the control animals. Further these inoculated monkeys showed higher cognitive control when accessed at 1.5 years of age, higher curiosity when accessed at 2.5 years, and larger prefrontal cortex volume at 3.3 years of age, compared to the age matched noninoculated controls [25, 26]. These results suggest that engagement in new situations that require challenging but not overwhelmingly stressful experiences results in enduring effects that stimulate adaptation in cognitive, motivational, and socioemotional aspect of behaviour in primates.

Early developmental stages of an individual asymmetrically contribute to the shaping of resilience in later life. Several studies have demonstrated entrainment of adult behaviour as a consequence of stress during early development. For example, squirrels change their growth trajectories based on in utero exposure to stress [27], and early childhood stress results in earlier menarche in human females [28] and aversive conditioning during infancy blunts the strength of further conditioning in adulthood [29]. Congruently, majority of studies pertaining to stress inoculation provide initial stress in early life [30–34]. For instance, maternal separation in infant rodents since birth (continuously for 2 weeks, 3 hours per day) gives rise to hyperactive stress responses in the form of heightened stress hormone release [34]. However, intermittent brief separation of these pups from the mother results in adaptive endocrine responses characterizing resilient features [32]. Similarly in primates, when four-month-old squirrel monkeys are exposed to intermittent levels of the same form of maternal separation (ten sessions per week), it leads to emotionally stable responses under stressful situations and lowered release of stress hormones accompanied by more exploration of novel settings [24]. This presents an exemplary case of stress inoculation which is dependent on the developmental stage of an individual.

In should be noted that resilience in models involving maternal separation could result from behavioural change in either mother or the offspring. In other words, it is possible that early life stress promotes resilience because the separation changes maternal behaviour towards pups rather than stress inoculation of the pups themselves. For example,

brief intermittent exposure to foot shock during infancy augments resilience. This is due to the increased maternal stimulation received after the rat pups are returned to their nest. This increased maternal stimulation has been shown to enhance stress regulation in pups that endure into their adulthood [30]. In contrast to rats, similar effects have not been observed in primates. For example, differences in the maternal behaviour did not correspond with differences in the development of arousal regulation in young monkeys [33]. Thus both the locus of initial behavioural change and the outcome can be idiosyncratically specific to the species being studied. This creates further challenge to create an animal model for studying stress inoculation in humans.

While early life has a long-lasting influence on the stress inoculation, several papers have also reported protective effects of preceding stress in adulthood. Thus stress inoculation in male mice by exposure to mild stress (noncontact interaction via resident intrusion) in adulthood leads to more emotionally stable response, lowered depressive like symptoms, and enhanced exploratory behaviour [35]. The same study also showed reduced secretion of stress hormones in response to repeated restraint. Similarly, exposure to three or more mild restraints before inescapable shock or three sessions of inescapable tail-shocks with intervening rest days attenuates development of learned helplessness in male rats [36]. In adult female squirrel monkeys, intermittent separation from group and introduction of novel group partners create a stress inoculation against future stressor [37]. This manifests as reduction in anhedonia and reduced activation of stress hormone axis when inoculated animals are exposed to a subsequent social separation. This generality of inoculation models across developmental stages, if reinforced by further studies, creates greater opportunity for use of this paradigm in the adulthood.

Gender presents an important consideration when interpreting effects of stress inoculation. Sexually dimorphic gonadal hormones robustly interact with brain and behaviour, including the stress response (reviewed in [38–41]). For example, major depression and anxiety disorder are more prevalent in women of reproductive age than corresponding male population [42]. In rats, chronic stress causes lesser anxiogenesis in females compared to males [43–46]. Biological substrates of gender dimorphism pertaining to stress remain understudied. Similarly, reasons for discordant direction of stress effects on humans and rodents are unclear at present.

3. Environmental Manipulation: A Potential Regulator for Hypothalamus-Pituitary-Adrenal Axis Tone

The degree of control that an animal has on a specific stressor plays a key role in defining whether the event will lead to ensuing vulnerability or resilience to the stress. Animals administered with unavoidable and unpredictable shock tend to develop exaggerated fear response, heightened anxiety, and deficits in active coping when faced with subsequent stressor [47], a phenomenon often referred to as learned helplessness [48]. However, animals that are given shock and are concomitantly given the ability to avoid them by modifying their behaviour do not develop learned helplessness [49, 50]. Similar effects have been observed in humans, whereby individuals previously inoculated by a controllable stress acquire resilience to a broader range of other subsequent stressors [51].

In terms of the endocrine activation, stress inoculation results in lower responsiveness and earlier termination of stress hormone secretion, while severe stress results in the opposite effect. For example, repeated maternal separation of rat pups results in lower expression of glucocorticoid receptors (GRs) in the hippocampus when these pups reach adulthood [52]. The hippocampal glucocorticoid receptors bind to circulating corticosterone (CORT), a stress hormone secreted by adrenal glands. The occupancy of these GRs then sends a negative feedback to the hypothalamus-pituitary-adrenal axis, thus terminating further stress hormone release. A reduction in glucocorticoid receptors leads to reduced efficacy of this negative feedback which then blunts the ability to terminate ongoing stress response. This example demonstrates that early environment can have long-lasting implications for future stress response.

CORT, the primary ligand for GR, is often measured to reflect ongoing stress response [53, 54]. Amount of circulating CORT in rodents (cortisol in primates) exhibits robust sensitivity to the environment [55]. For example, introduction of novelty in the environment causes increase in CORT, leading to an emotional arousal in the individual [56]. Modulations in the environment on an intermittent basis might lead to increase or decrease of CORT and when this is done in moderation, it can cause a shift in the threshold of the HPA activity for an individual [55, 57]. The resultant effect would reflect the shift in the inverted U shaped curve of stress response which corresponds to stress inoculation [9]. In brief, stress inoculation can shift dose response curve between future stress and performance leftward (greater performance at lower levels of future stress) and/or rightward (greater tolerance to higher level of stress) [9].

Thus both stress response and CORT are exquisitely responsive to the degree and nature of environmental changes. Interestingly, CORT itself causes differential neural plasticity in different brain regions. For example, chronic stress which raises the circulating CORT leads to neuronal atrophy in the hippocampus but hypertrophy in the basolateral amygdala (BLA) [58–60]. These contrasting effects are suggested to cause reduced memory performance due to chronic stress mediated by its hippocampal effects and increased anxiety mediated by its amygdalar effects. The dorsal region of the hippocampus is necessary for spatial learning and is directly linked to stress-induced memory deficits [61, 62]. Likewise, intra-BLA experimental manipulation suggests necessity and sufficiency of BLA changes for stress-induced anxiogenesis. For example, decreasing excitability of BLA neurons by overexpression of SK2 K^+ channels simultaneously reduces stress-induced stress hormone secretion, anxiety, and BLA hypertrophy [63]. Similarly, rerouting of stress hormone signalling away from glucocorticoid receptors within BLA also reduces anxiety [64–66].

Several studies described above have used CORT levels as a proxy for HPA tone and implicitly as a proxy for stress responsiveness. Yet the relationship between CORT levels and HPA responsiveness is not linear. For example, effects of CORT depend on expression of GR and relative expression of GR/MR, in addition to specific brain regions expressing these receptors. It is noteworthy that an experimental change in ratio between MR and GR expression in the hippocampus can drastically change memory [67, 68]. Similar manipulation in the amygdala reduces anxiety and future endogenous CORT release [64]. This suggests that effects of CORT on brain and behaviour are dependent on expression level of receptors and type of the central receptors (GR and MR) available. The importance of the central receptors is further supported by the observations that stress in rodents and monkeys can downregulate hippocampal GRs and thus is secondarily leading to loss of negative feedback of CORT secretion [69, 70]. In this context, it is interesting that hippocampus and amygdala exhibit differential expression of GR and MR [52, 71].

Cognitive decline associated with hippocampus has been extensively studied in respect to effects of stress (reviewed in [60, 72, 73]). However BLA, which is critical for generation and maintenance of fear and anxiety [74], has been relatively understudied in this regard [75, 76]. Future studies to bridge this gap will hopefully bring more clarity to biological mechanisms of stress inoculation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors were supported by Ministry of Education, Singapore. The authors thank Dr. Ajai Vyas for assistance in editing the final draft.

References

[1] H. Selye, "The evolution of the stress concept: the originator of the concept traces its development from the discovery in 1936 of the alarm reaction to modern therapeutic applications of syntoxic and catatoxic hormones," *American Scientist*, vol. 61, no. 6, pp. 692–699, 1973.

[2] I. N. Karatsoreos and B. S. McEwen, "Psychobiological allostasis: resistance, resilience and vulnerability," *Trends in Cognitive Sciences*, vol. 15, no. 12, pp. 576–584, 2011.

[3] A. P. Association, *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*, ManMag, 2003.

[4] R. G. Hunter and B. S. McEwen, "Stress and anxiety across the lifespan: structural plasticity and epigenetic regulation," *Epigenomics*, vol. 5, no. 2, pp. 177–194, 2013.

[5] B. S. McEwen, "Brain on stress: how the social environment gets under the skin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, supplement 2, pp. 17180–17185, 2012.

[6] B. S. McEwen and L. Getz, "Lifetime experiences, the brain and personalized medicine: an integrative perspective," *Metabolism*, vol. 62, supplement 1, pp. S20–S26, 2013.

[7] M. Popoli, Z. Yan, B. S. McEwen, and G. Sanacora, "The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission," *Nature Reviews Neuroscience*, vol. 13, no. 1, pp. 22–37, 2012.

[8] D. Meichenbaum and R. Cameron, "Stress inoculation training," in *Stress Reduction and Prevention*, D. Meichenbaum and M. Jaremko, Eds., pp. 115–154, Springer, New York, NY, USA, 1989.

[9] S. J. Russo, J. W. Murrough, M.-H. Han, D. S. Charney, and E. J. Nestler, "Neurobiology of resilience," *Nature Neuroscience*, vol. 15, no. 11, pp. 1475–1484, 2012.

[10] R. M. Sapolsky, "Stress and the brain: individual variability and the inverted-U," *Nature Neuroscience*, vol. 18, no. 10, pp. 1344–1346, 2015.

[11] M. Mendl, "Performing under pressure: stress and cognitive function," *Applied Animal Behaviour Science*, vol. 65, no. 3, pp. 221–244, 1999.

[12] R. M. Yerkes and J. D. Dodson, "The relation of strength of stimulus to rapidity of habit-formation," *Journal of Comparative Neurology and Psychology*, vol. 18, no. 5, pp. 459–482, 1908.

[13] B. Salehi, M. I. Cordero, and C. Sandi, "Learning under stress: the inverted-U-shape function revisited," *Learning and Memory*, vol. 17, no. 10, pp. 522–530, 2010.

[14] D. M. Diamond, M. C. Bennett, M. Fleshner, and G. M. Rose, "Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation," *Hippocampus*, vol. 2, no. 4, pp. 421–430, 1992.

[15] G. Rose, D. M. Diamond, K. Pang, and T. V. Dunwiddie, "Primed burst potentiation: lasting synaptic plasticity invoked by physiologically patterned stimulation," in *Synaptic Plasticity in the Hippocampus*, pp. 96–98, Springer, Berlin, Germany, 1988.

[16] S. J. Lupien, B. S. McEwen, M. R. Gunnar, and C. Heim, "Effects of stress throughout the lifespan on the brain, behaviour and cognition," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 434–445, 2009.

[17] V. Luine, "Sex differences in chronic stress effects on memory in rats," *Stress*, vol. 5, no. 3, pp. 205–216, 2002.

[18] A. Q. Martins, D. McIntyre, and C. Ring, "Aversive event unpredictability causes stress-induced hypoalgesia," *Psychophysiology*, vol. 52, no. 8, pp. 1066–1070, 2015.

[19] S. L. Andersen, "Exposure to early adversity: points of cross-species translation that can lead to improved understanding of depression," *Development and Psychopathology*, vol. 27, no. 2, pp. 477–491, 2015.

[20] D. M. Lyons, K. J. Parker, and A. F. Schatzberg, "Animal models of early life stress: implications for understanding resilience," *Developmental Psychobiology*, vol. 52, no. 7, pp. 616–624, 2010.

[21] A. C. Campos, M. V. Fogaça, D. C. Aguiar, and F. S. Guimarães, "Animal models of anxiety disorders and stress," *Revista Brasileira de Psiquiatria*, vol. 35, supplement 2, pp. S101–S111, 2013.

[22] C. Belzung and M. Lemoine, "Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression," *Biology of Mood & Anxiety Disorders*, vol. 1, no. 1, p. 9, 2011.

[23] A. A. Walf and C. A. Frye, "The use of the elevated plus maze as an assay of anxiety-related behavior in rodents," *Nature Protocols*, vol. 2, no. 2, pp. 322–328, 2007.

[24] K. J. Parker, C. L. Buckmaster, A. F. Schatzberg, and D. M. Lyons, "Prospective investigation of stress inoculation in young monkeys," *Archives of General Psychiatry*, vol. 61, no. 9, pp. 933–941, 2004.

[25] K. J. Parker, C. L. Buckmaster, K. R. Justus, A. F. Schatzberg, and D. M. Lyons, "Mild early life stress enhances prefrontal-dependent response inhibition in monkeys," *Biological Psychiatry*, vol. 57, no. 8, pp. 848–855, 2005.

[26] D. M. Lyons and K. J. Parker, "Stress inoculation-induced indications of resilience in monkeys," *Journal of Traumatic Stress*, vol. 20, no. 4, pp. 423–433, 2007.

[27] B. Dantzer, A. E. M. Newman, R. Boonstra et al., "Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal," *Science*, vol. 340, no. 6137, pp. 1215–1217, 2013.

[28] J. S. Chisholm, J. A. Quinlivan, R. W. Petersen, and D. A. Coall, "Early stress predicts age at menarche and first birth, adult attachment, and expected lifespan," *Human Nature*, vol. 16, no. 3, pp. 233–265, 2005.

[29] Y. Sevelinges, S. Moriceau, P. Holman et al., "Enduring effects of infant memories: infant odor-shock conditioning attenuates amygdala activity and adult fear conditioning," *Biological Psychiatry*, vol. 62, no. 10, pp. 1070–1079, 2007.

[30] N. M. Cameron, F. A. Champagne, C. Parent, E. W. Fish, K. Ozaki-Kuroda, and M. J. Meaney, "The programming of individual differences in defensive responses and reproductive strategies in the rat through variations in maternal care," *Neuroscience and Biobehavioral Reviews*, vol. 29, no. 4-5, pp. 843–865, 2005.

[31] C. L. Coe, J. C. Glass, S. G. Wiener, and S. Levine, "Behavioral, but not physiological, adaptation to repeated separation in mother and infant primates," *Psychoneuroendocrinology*, vol. 8, no. 4, pp. 401–409, 1983.

[32] V. H. Denenberg, "Commentary: is maternal stimulation the mediator of the handling effect in infancy?" *Developmental Psychobiology*, vol. 34, no. 1, pp. 1–3, 1999.

[33] T. C. Jordan, M. B. Hennessy, C. A. Gonzalez, and S. Levine, "Social and environmental factors influencing mother-infant separation-reunion in squirrel monkeys," *Physiology and Behavior*, vol. 34, no. 4, pp. 489–493, 1985.

[34] P. M. Plotsky and M. J. Meaney, "Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats," *Molecular Brain Research*, vol. 18, no. 3, pp. 195–200, 1993.

[35] J. Brockhurst, C. Cheleuitte-Nieves, C. L. Buckmaster, A. F. Schatzberg, and D. M. Lyons, "Stress inoculation modeled in mice," *Translational Psychiatry*, vol. 5, no. 3, article e537, 2015.

[36] T. N. Plumb, P. K. Cullen, and T. R. Minor, "Parameters of hormetic stress and resilience to trauma in rats," *Stress*, vol. 18, no. 1, pp. 88–95, 2015.

[37] A. G. Lee, C. L. Buckmaster, E. Yi, A. F. Schatzberg, and D. M. Lyons, "Coping and glucocorticoid receptor regulation by stress inoculation," *Psychoneuroendocrinology*, vol. 49, no. 1, pp. 272–279, 2014.

[38] K. S. Curtis and E. G. Krause, "Sex differences in physiology and behavior: focus on central actions of ovarian hormones," *Physiology and Behavior*, vol. 97, no. 2, pp. 141–142, 2009.

[39] E. Kajantie and D. I. W. Phillips, "The effects of sex and hormonal status on the physiological response to acute psychosocial stress," *Psychoneuroendocrinology*, vol. 31, no. 2, pp. 151–178, 2006.

[40] K. N. Shepard, V. Michopoulos, D. J. Toufexis, and M. E. Wilson, "Genetic, epigenetic and environmental impact on sex differences in social behavior," *Physiology & Behavior*, vol. 97, no. 2, pp. 157–170, 2009.

[41] G. J. Ter Horst, R. Wichmann, M. Gerrits, C. Westenbroek, and Y. Lin, "Sex differences in stress responses: focus on ovarian hormones," *Physiology and Behavior*, vol. 97, no. 2, pp. 239–249, 2009.

[42] R. C. Kessler, K. A. McGonagle, M. Swartz, D. G. Blazer, and C. B. Nelson, "Sex and depression in the National Comorbidity Survey. I: lifetime prevalence, chronicity and recurrence," *Journal of Affective Disorders*, vol. 29, no. 2-3, pp. 85–96, 1993.

[43] A. Vyas and S. Chattarji, "Modulation of different states of anxiety-like behavior by chronic stress," *Behavioral Neuroscience*, vol. 118, no. 6, pp. 1450–1454, 2004.

[44] R. Mitra, A. Vyas, G. Chatterjee, and S. Chattarji, "Chronic-stress induced modulation of different states of anxiety-like behavior in female rats," *Neuroscience Letters*, vol. 383, no. 3, pp. 278–283, 2005.

[45] C. Westenbroek, J. A. Den Boer, and G. J. Ter Horst, "Gender-specific effects of social housing on chronic stress-induced limbic FOS expression," *Neuroscience*, vol. 121, no. 1, pp. 189–199, 2003.

[46] C. Westenbroek, T. A. B. Snijders, J. A. Den Boer, M. Gerrits, D. S. Fokkema, and G. J. Ter Horst, "Pair-housing of male and female rats during chronic stress exposure results in gender-specific behavioral responses," *Hormones and Behavior*, vol. 47, no. 5, pp. 620–628, 2005.

[47] V. Krishnan, M.-H. Han, D. L. Graham et al., "Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions," *Cell*, vol. 131, no. 2, pp. 391–404, 2007.

[48] M. Fleshner, S. F. Maier, D. M. Lyons, and M. A. Raskind, "The neurobiology of the stress-resistant brain," *Stress*, vol. 14, no. 5, pp. 498–502, 2011.

[49] B. N. Greenwood and M. Fleshner, "Exercise, learned helplessness, and the stress-resistant brain," *NeuroMolecular Medicine*, vol. 10, no. 2, pp. 81–98, 2008.

[50] O. Berton, H. E. Covington III, K. Ebner et al., "Induction of ΔFosB in the periaqueductal gray by stress promotes active coping responses," *Neuron*, vol. 55, no. 2, pp. 289–300, 2007.

[51] D. L. Kerr, D. G. McLaren, R. M. Mathy, and J. B. Nitschke, "Controllability modulates the anticipatory response in the human ventromedial prefrontal cortex," *Frontiers in Psychology*, vol. 3, article 557, 2012.

[52] M. Joëls, R. A. Sarabdjitsingh, and H. Karst, "Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes," *Pharmacological Reviews*, vol. 64, no. 4, pp. 901–938, 2012.

[53] S. M. Smith and W. W. Vale, "The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress," *Dialogues in Clinical Neuroscience*, vol. 8, no. 4, pp. 383–395, 2006.

[54] J. P. Herman and W. E. Cullinan, "Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis," *Trends in Neurosciences*, vol. 20, no. 2, pp. 78–84, 1997.

[55] E. J. Crofton, Y. Zhang, and T. A. Green, "Inoculation stress hypothesis of environmental enrichment," *Neuroscience and Biobehavioral Reviews*, vol. 49, pp. 19–31, 2015.

[56] S. Okuda, B. Roozendaal, and J. L. McGaugh, "Glucocorticoid effects on object recognition memory require training-associated emotional arousal," *Proceedings of the National*

Academy of Sciences of the United States of America, vol. 101, no. 3, pp. 853–858, 2004.

[57] B. S. McEwen and P. J. Gianaros, "Stress- and allostasis-induced brain plasticity," *Annual Review of Medicine*, vol. 62, pp. 431–445, 2011.

[58] R. Mitra, S. Jadhav, B. S. McEwen, A. Vyas, and S. Chattarji, "Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 26, pp. 9371–9376, 2005.

[59] A. Vyas, R. Mitra, B. S. Shankaranarayana Rao, and S. Chattarji, "Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons," *Journal of Neuroscience*, vol. 22, no. 15, pp. 6810–6818, 2002.

[60] B. S. McEwen, "Stress and hippocampal plasticity," *Annual Review of Neuroscience*, vol. 22, pp. 105–122, 1999.

[61] E. Moser, M.-B. Moser, and P. Andersen, "Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions," *The Journal of Neuroscience*, vol. 13, no. 9, pp. 3916–3925, 1993.

[62] G. E. Handelmann and D. S. Olton, "Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: impairment and recovery with preoperative training," *Brain Research*, vol. 217, no. 1, pp. 41–58, 1981.

[63] R. Mitra, D. Ferguson, and R. M. Sapolsky, "SK2 potassium channel overexpression in basolateral amygdala reduces anxiety, stress-induced corticosterone secretion and dendritic arborization," *Molecular Psychiatry*, vol. 14, no. 9, pp. 847–855, 2009.

[64] R. Mitra, D. Ferguson, and R. M. Sapolsky, "Mineralocorticoid receptor overexpression in basolateral amygdala reduces corticosterone secretion and anxiety," *Biological Psychiatry*, vol. 66, no. 7, pp. 686–690, 2009.

[65] R. Mitra and R. M. Sapolsky, "Expression of chimeric estrogen-glucocorticoid-receptor in the amygdala reduces anxiety," *Brain Research*, vol. 1342, pp. 33–38, 2010.

[66] R. Mitra and R. M. Sapolsky, "Gene therapy in rodent amygdala against fear disorders," *Expert Opinion on Biological Therapy*, vol. 10, no. 9, pp. 1289–1303, 2010.

[67] D. Ferguson and R. Sapolsky, "Overexpression of mineralocorticoid and transdominant glucocorticoid receptor blocks the impairing effects of glucocorticoids on memory," *Hippocampus*, vol. 18, no. 11, pp. 1103–1111, 2008.

[68] D. Ferguson and R. Sapolsky, "Mineralocorticoid receptor overexpression differentially modulates specific phases of spatial and nonspatial memory," *The Journal of Neuroscience*, vol. 27, no. 30, pp. 8046–8052, 2007.

[69] S. M. Brooke, A. M. De Haas-Johnson, J. R. Kaplan, S. B. Manuck, and R. M. Sapolsky, "Dexamethasone resistance among nonhuman primates associated with a selective decrease of glucocorticoid receptors in the hippocampus and a history of social instability," *Neuroendocrinology*, vol. 60, no. 2, pp. 134–140, 1994.

[70] K. Mizoguchi, A. Ishige, M. Aburada, and T. Tabira, "Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus," *Neuroscience*, vol. 119, no. 3, pp. 887–897, 2003.

[71] J. M. H. M. Reul and E. R. de Kloet, "Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation," *Endocrinology*, vol. 117, no. 6, pp. 2505–2511, 1985.

[72] B. S. McEwen, "Plasticity of the hippocampus: adaptation to chronic stress and allostatic load," *Annals of the New York Academy of Sciences*, vol. 933, pp. 265–277, 2001.

[73] B. S. McEwen, "Sex, stress and the hippocampus: allostasis, allostatic load and the aging process," *Neurobiology of Aging*, vol. 23, no. 5, pp. 921–939, 2002.

[74] M. Davis, D. Rainnie, and M. Cassell, "Neurotransmission in the rat amygdala related to fear and anxiety," *Trends in Neurosciences*, vol. 17, no. 5, pp. 208–214, 1994.

[75] L. M. Boyle, "A neuroplasticity hypothesis of chronic stress in the basolateral amygdala," *Yale Journal of Biology and Medicine*, vol. 86, no. 2, pp. 117–125, 2013.

[76] B. S. McEwen, C. Nasca, and J. D. Gray, "Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex," *Neuropsychopharmacology*, 2015.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 2592319, 11 pages
http://dx.doi.org/10.1155/2016/2592319

Research Article

Chronic Mild Stress Modulates Activity-Dependent Transcription of BDNF in Rat Hippocampal Slices

Raffaella Molteni, Andrea C. Rossetti, Elisa Savino, Giorgio Racagni, and Francesca Calabrese

Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Via Balzaretti 9, 20133 Milano, Italy

Correspondence should be addressed to Francesca Calabrese; francesca.calabrese@unimi.it

Received 27 May 2015; Revised 22 July 2015; Accepted 11 August 2015

Academic Editor: Jordan Marrocco

Although activity-dependent transcription represents a crucial mechanism for long-lasting experience-dependent changes in the hippocampus, limited data exist on its contribution to pathological conditions. We aim to investigate the influence of chronic stress on the activity-dependent transcription of brain-derived neurotrophic factor (BDNF). The *ex vivo* methodology of acute stimulation of hippocampal slices obtained from rats exposed to chronic mild stress (CMS) was used to evaluate whether the adverse experience may alter activity-dependent BDNF gene expression. CMS reduces BDNF expression and that acute depolarization significantly upregulates total BDNF mRNA levels only in control animals, showing that CMS exposure may alter BDNF transcription under basal conditions and during neuronal activation. Moreover, while the basal effect of CMS on total BDNF reflects parallel modulations of all the transcripts examined, isoform-specific changes were found after depolarization. This different effect was also observed in the activation of intracellular signaling pathways related to the neurotrophin. In conclusion, our study discloses a functional alteration of BDNF transcription as a consequence of stress. Being the activity-regulated transcription a critical process in synaptic and neuronal plasticity, the different regulation of individual BDNF promoters may contribute to long-lasting changes, which are fundamental for the vulnerability of the hippocampus to stress-related diseases.

1. Introduction

One of the most remarkable features of the hippocampus is its ability to shape its functions and adapt to environmental changes through different mechanisms allowing neurons to adjust their properties according to their activity. These characteristics are crucial not only because of the role of this brain region in synaptic plasticity in the context of learning and memory [1], but also considering that a deficit in this skill might result in pathologic conditions. For example, given the high sensitivity to stress of the hippocampus [2–4], different studies have shown an association between hippocampal dysfunctions and stress-related diseases, such as major depression or posttraumatic stress disorders [5, 6]. Activity-regulated transcription plays a crucial role in hippocampal function [7] and may be altered under pathologic conditions [8]. A key gene for these mechanisms encodes the neurotrophin brain-derived neurotrophic factor (BDNF) that, in addition to supporting neuronal survival during CNS development [9, 10], represents an important mediator of neuronal plasticity in adulthood [11, 12]. Activity-dependent regulation of BDNF occurs through complex transcriptional mechanisms, with at least eight distinct promoters that drive the transcription of distinct mRNAs, each containing an alternative 5^{I} exon spliced to a common 3^{I} coding exon [13–15]. Although *Bdnf* promoters are differently responsive to neuronal activation [16–18], limited data are available to explain how pathological conditions may affect activity-dependent BDNF transcription. Therefore, the aim of our study was to investigate the activity-dependent transcription of the neurotrophin in the hippocampus of rats exposed to chronic stress. In depth, we used the *ex vivo* methodology of the acute stimulation of hippocampal slices obtained from rats exposed to a chronic unpredictable stress (CMS)

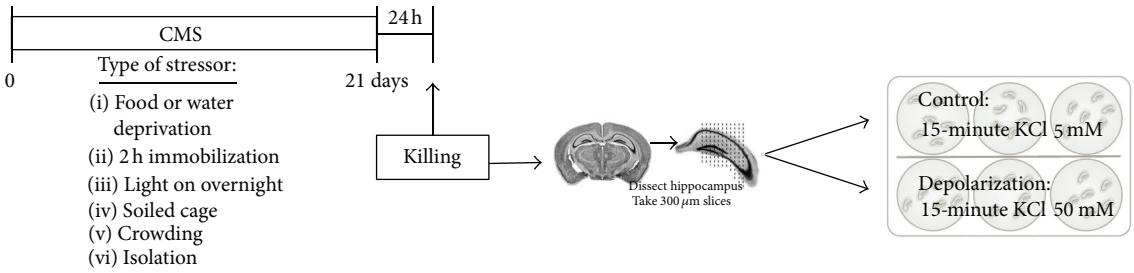


Figure 1: Experimental paradigm. Adult male Sprague Dawley rats were subjected to the stress procedure for 3 weeks and then sacrificed by decapitation after 24 hours from the last stressor. The brains were rapidly removed for hippocampal slices preparation. The slices, after a period of adaptation in oxygenated Krebs buffer, were incubated for 15 minutes in presence or absence of KCl 50 mM to test the effects of *ex vivo* depolarization. After the incubation period, hippocampal slices were collected, frozen on dry ice, and stored at −80°C until the molecular analyses.

paradigm to evaluate the different responsiveness to depolarization in terms of BDNF expression and signaling. This experimental approach replicates many aspects of the *in vivo* context as slices largely preserve the tissue architecture of the brain region they originated from and maintain neuronal activities with intact functional local synaptic circuitry. Hence, they are ideal platforms for dissection of molecular pathways underlying neuronal dysfunction.

2. Methods

General reagents were purchased from Sigma-Aldrich (Milan, Italy), and molecular biology reagents were obtained from Applied Biosystems Italia (Monza, Italy), Bio-Rad Laboratories S.r.l. Italia (Segrate, Italy), Santa Cruz Biotechnology (Santa Cruz, CA, USA), and Cell Signaling Technology (Danvers, MA, USA).

2.1. Animals. Adult male Sprague-Dawley rats (Charles River, Calco, Italy) weighing 300–350 g were used throughout the experiments. Rats were housed in groups of 3 per cage under standard conditions (12 h light/dark cycle with food and water *ad libitum*) and were exposed to daily handling for 2 weeks before any treatment. All animal handling and experimental procedures were approved by the University of Milan Institutional Animal Care and Use Committee and adhered to the Italian legislation on animal experimentation (Decreto Legislativo 116/92), the EU (*EU Directive 2010/63/EU*), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Chronic Mild Stress Paradigm. For chronic mild stress, animals were randomly divided into stressed and no-stressed groups ($n = 6$ in each experimental group). Sham (no-stressed) animals were kept undisturbed in their home cages during the entire experiment except for handling manipulation every 2 days during weighing, while stressed (CMS) rats were instead exposed for 3 weeks to a variable sequence of mild, unpredictable stressors, whose application started at different times every day to minimize habits and therefore predictability. The stressors used were the following: 24 h food deprivation, isolation overnight, 2 h restraint, 24 h empty water bottle, soiled cage overnight, light on overnight, and light on and overcrowding overnight. The impact of CMS was demonstrated by a significant loss of body-weight gain, paralleled by reduced food and water consumption, as well as by reduced preference for sucrose solution with respect to control animals [19]. Twenty-four hours after having been subjected to the last stressor, animals were sacrificed by decapitation and the brains rapidly removed for the hippocampal slices preparation (Figure 1).

2.3. Preparation of Hippocampal Slices. Hippocampal slices were prepared (Figure 1) as described by Gardoni and colleagues [20]. Briefly, removed brains were immediately placed into chilled (4°C) oxygenated Krebs buffer and after removing the meninges, hippocampi were rapidly dissected and quickly sliced with a McIlwain tissue chopper. The slices (300 μm) were then placed for 30 minutes in custom-made chambers continuously equilibrated with O_2 95%–CO_2 5% (v/v) oxygenated Krebs buffer. To induce activity-dependent transcription, potassium chloride depolarization was used. After the equilibration period, slices prepared by CTRL and STRESS rats were incubated for 15 min in presence or absence of KCl 50 mM before being collected, frozen on dry ice, and stored at −80°C until the molecular analyses. The n for the two different experimental conditions was 6 for acute depolarization treatment and 3 for the physiological situation (Krebs buffer).

2.4. RNA Preparation and Quantification of BDNF mRNA Expression by Real-Time RT Quantitative PCR. In order to measure BDNF mRNA levels, total RNA was isolated from hippocampal slices by single step guanidinium isothiocyanate/phenol extraction using PureZol RNA isolation reagent (Bio-Rad Laboratories S.r.l. Italia) according to the manufacturer's instructions and quantified by spectrophotometric analysis. The samples were then processed for real-time polymerase chain reaction (PCR) to assess BDNF mRNA levels as previously reported [21]. Briefly, a 2 μg aliquot of each sample was treated with DNase to avoid DNA contamination and subsequently reverse transcribed using a High-Capacity cDNA Archive commercial kit (Applied Biosystems Italia, Monza, MI, Italy). The real-time PCR reaction was performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems Italia, Monza, MI,

Italy) with the TaqMan Gene Expression Master Mix (Applied Biosystems Italia, Monza, MI, Italy) and the following TaqMan Gene Expression Assay purchased from Applied Biosystems:

Total *Bdnf*: ID Rn02531967_s1; *Bdnf* transcript IV: ID Rn01484927_m1; *Bdnf* transcript VI: ID Rn01484928_m1; *Bdnf* transcript IXa forward primer: TGGTGTCCCCAAGAAAGTAA and reverse primer: CACGTGCTCAAAAGTGTCAG.

After an initial step at 50°C for 2 min and at 95°C for 10 min, 40 cycles of PCR were performed. Each PCR cycle consisted of heating the samples at 95°C for 15 s to enable the melting process and then for 1 min at 60°C for the annealing and extension reaction. Each sample was assayed in duplicate using two independent retrotranscription products. A comparative cycle threshold (Ct) method was used to determine the relative target gene expression. Data have been expressed as percentage calculated from the expression of the target genes normalized on rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression as control gene (ID GAPDH TaqMan probe: Rn99999916_s1).

2.5. Preparation of Protein Extracts. Hippocampal slices were homogenized in a glass-glass potter in cold 0.32 M sucrose buffer pH 7.4 containing 1 mM HEPES, 0.1 mM EGTA, and 0.1 mM PMSF, in presence of commercial cocktails of protease (cod. 11697498001, Roche, Monza, Italy) and phosphatase (cod. P5726, Sigma-Aldrich) inhibitors. The total homogenate (H) was clarified at 1000 g for 10 min obtaining a pellet (P1) corresponding to the nuclear fraction, which was resuspended in a buffer (20 mM HEPES, 0.1 mM DTT, and 0.1 mM EGTA) supplemented with protease and phosphatase inhibitors. Total protein content was measured according to the Bradford Protein Assay procedure (Bio-Rad, Milan, Italy), using bovine serum albumin as calibration standard.

2.6. Western Blot Analysis. By Western blot analysis, protein extracts were used to assess the phosphorylated and the total levels of several components of BDNF-related signaling pathways in the homogenate (ERK1/2, AKT, and GSK-3β) and of the transcription factor CREB in the nuclear fraction.

The same amounts of total protein for all the samples (10 μg for ERK1/2, AKT, and GSK-3β; 20 μg for CREB) were run on an SDS-8% polyacrylamide gel under reducing conditions and then electrophoretically transferred onto nitrocellulose membranes (Bio-Rad, Milan, Italy). The blots were blocked with 10% nonfat dry milk and then incubated with the primary antibodies, following the manufacturer's instructions, as summarized in Table 1. Membranes were then incubated for 1 h at room temperature with the appropriate secondary antibody (see Table 1); immunocomplexes were visualized by chemiluminescence, using the ECL Western blotting kit (Amersham Life Sciences, Milan, Italy), according to the manufacturer's instructions.

Results were standardized to β-actin as control protein, which was detected by evaluating the band density at 43 kDa after probing the membranes with a polyclonal antibody (Sigma, dilution 1 : 10000) followed by a 1 : 10000 dilution of peroxidase-conjugated anti-mouse IgG (Sigma). Quantification of the immunoblots was performed using Quantity One software (Bio-Rad).

Table 1: Antibodies conditions used in the Western blot analyses.

Gene	Primary antibody	Secondary antibody
Phospho-ERK1/2 Y204/Y187 (42–44 kDa)	1 : 1000 (Cell Signaling; **#4370**) 4°C, O/N	Anti-mouse, 1 : 2000, RT, 1 h
ERK1/2 (42–44 kDa)	1 : 5000 (Sigma; **M3807**), RT, 2 h	Anti-rabbit, 1 : 5000, RT, 1 h
Phospho-CREB S133 (43 kDa)	1 : 1000 (Cell Signaling; #**4276**), 4°C, O/N	Anti-rabbit, 1 : 2000, RT, 1 h
CREB (43 kDa)	1 : 1000 (Cell Signaling; #**9197**), 4°C, O/N	Anti-rabbit, 1 : 2000, RT, 1 h
Phospho-AKT S473 (60 kDa)	1 : 1000 (Cell Signaling; #**4060**), 4°C, O/N	Anti-rabbit, 1 : 2000, RT, 1 h
AKT (60 kDa)	1 : 1000 (Cell Signaling; #**9272**), RT, 2 h	Anti-rabbit, 1 : 1000, RT, 1 h
Phospho-GSK3β S9 (46 kDa)	1 : 1000 (Cell Signaling; #**9336**), 4°C, O/N	Anti-rabbit, 1 : 5000, RT, 1 h
GSK3β (46 kDa)	1 : 2500 (BD Transduction; **610201**) RT, 2 h	Anti-mouse, 1 : 5000, RT, 1 h
β-ACTIN	1 : 10000 (Sigma; **A5441**), RT, 1 h	Anti-mouse, 1 : 10000, RT, 1 h

2.7. Statistical Analyses. Behavioral data were analyzed with Student's t-test (weight gain, panel A) and with the one-way analysis of variance (ANOVA) (weight and cage food consumption, panels B and C). Molecular data were analyzed with two-way ANOVA, with stress (No Stress versus Stress) and depolarization (KCl 5 mM versus KCl 50 mM) as independent factors and mRNA or protein levels as dependent variables. When needed, further differences were analyzed by Single Contrast post hoc test (SCPHT). Significance was assumed for $P < 0.05$. For graphic clarity, data are presented as means percent ± standard error (SEM) of control group, namely, hippocampal slices obtained from no-stressed rats and incubated with KCl 5 mM (the same concentration in Krebs buffer).

3. Results

3.1. Effects of CMS on Body Weight. We first established the effectiveness of the adverse manipulation by measuring body weight. As shown in Figure 2(a), animals exposed to 3 weeks of CMS showed significantly less weight gain when compared with control animals ($P < 0.001$, Student's t-test) starting

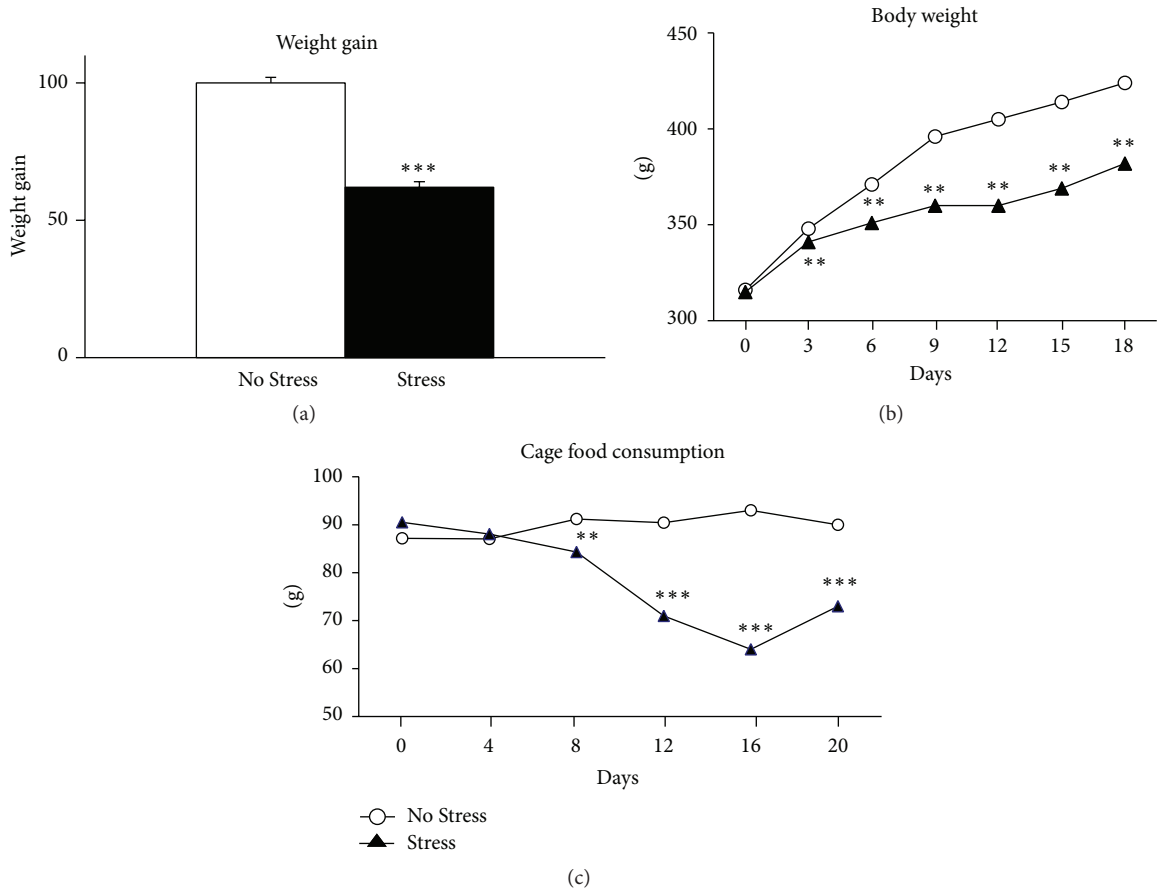


Figure 2: Effects of CMS on weight gain and food consuming behavior. Animals subjected to the stress procedure showed a decrease in body weight, presented as a direct comparison with the control group at 21 days (a) and as a time course during the stress period (b). (c) Showing the profile of food consumption during the 21 days of CMS procedure. $^{**}P < 0.01$, $^{***}P < 0.001$ versus No Stress animals. Student's t-test and one-way ANOVA.

from the third day of stress (Figure 2(b), one-way ANOVA), an effect that may be due also to the reduction of food consumption (Figure 2(c), one-way ANOVA).

Moreover, we previously showed that the exposure to 3 weeks of CMS induced a significantly reduced preference for sucrose solution [19]. These changes are clear indicators of the efficacy of the stressful manipulation.

3.2. Analysis of Bdnf Gene Expression. As a first step, we evaluated the effect of chronic stress on total *Bdnf* (exon IX) gene expression and the CMS paradigm was found to significantly modulate the neurotrophin ($F_{1,18} = 32.240$, $P < 0.001$; ANOVA). In deep, as shown in Figure 3(a), total *Bdnf* mRNA levels were reduced in hippocampal slices prepared from stressed rats (−39% versus No Stress/KCl 5 mM, $P < 0.001$; SCPHT). When hippocampal slices were exposed to depolarizing concentration of KCl, total *Bdnf* mRNAs were significantly modulated ($F_{1,18} = 7.888$, $P < 0.01$). Indeed, BDNF expression increased in slices obtained from unstressed rats (+51% versus No Stress/KCl 5 mM, $P = 0.01$; SCPHT), whereas no changes were found in stressed rats (+9% versus Stress/KCl 5 mM, $P > 0.05$; SCPHT). In order to gain further insight into the different responsiveness to KCl, the expression profile of some neurotrophin transcripts, namely, exons IV, VI, and IXa, was investigated. Similar to what was observed for total *Bdnf*, chronic stress significantly reduced the expression of all these isoforms (Figure 3(b), isoform IV: −40%, $F_{1,15} = 80.819$, $P < 0.001$; Figure 3(c), isoform VI: −31%, $F_{1,15} = 12.719$, $P < 0.01$; Figure 3(d), isoform XIa: −42%, $F_{1,15} = 29.455$, $P < 0.001$). Their gene expression was also affected by depolarization (isoform IV: $F_{1,15} = 15.548$, $P < 0.01$; isoform VI: $F_{1,15} = 16.542$, $P < 0.01$; isoform XIa: $F_{1,15} = 21.278$, $P < 0.001$), but with different effect. Incubation with 50 mM KCl significantly increased isoform IV mRNA levels in control rats (Figure 3(b) +44% versus No Stress/KCl 5 mM, $P < 0.001$; SCPHT) but not in stressed animals (+7% versus Stress/KCl 5 mM). Conversely, under depolarizing conditions the expression of exon VI was upregulated in stressed rats (Figure 3(c) +33% versus Stress/KCl 5 mM, $P < 0.01$; SCPHT) but not in the control group (+4% versus No Stress/KCl 5 mM). Lastly, isoform IXa mRNA levels were increased after depolarization in both unstressed (+36% versus Stress/KCl 5 mM, $P < 0.01$) and stressed animals (+36% versus Stress/KCl 5 mM, $P < 0.05$), as shown in Figure 3(d).

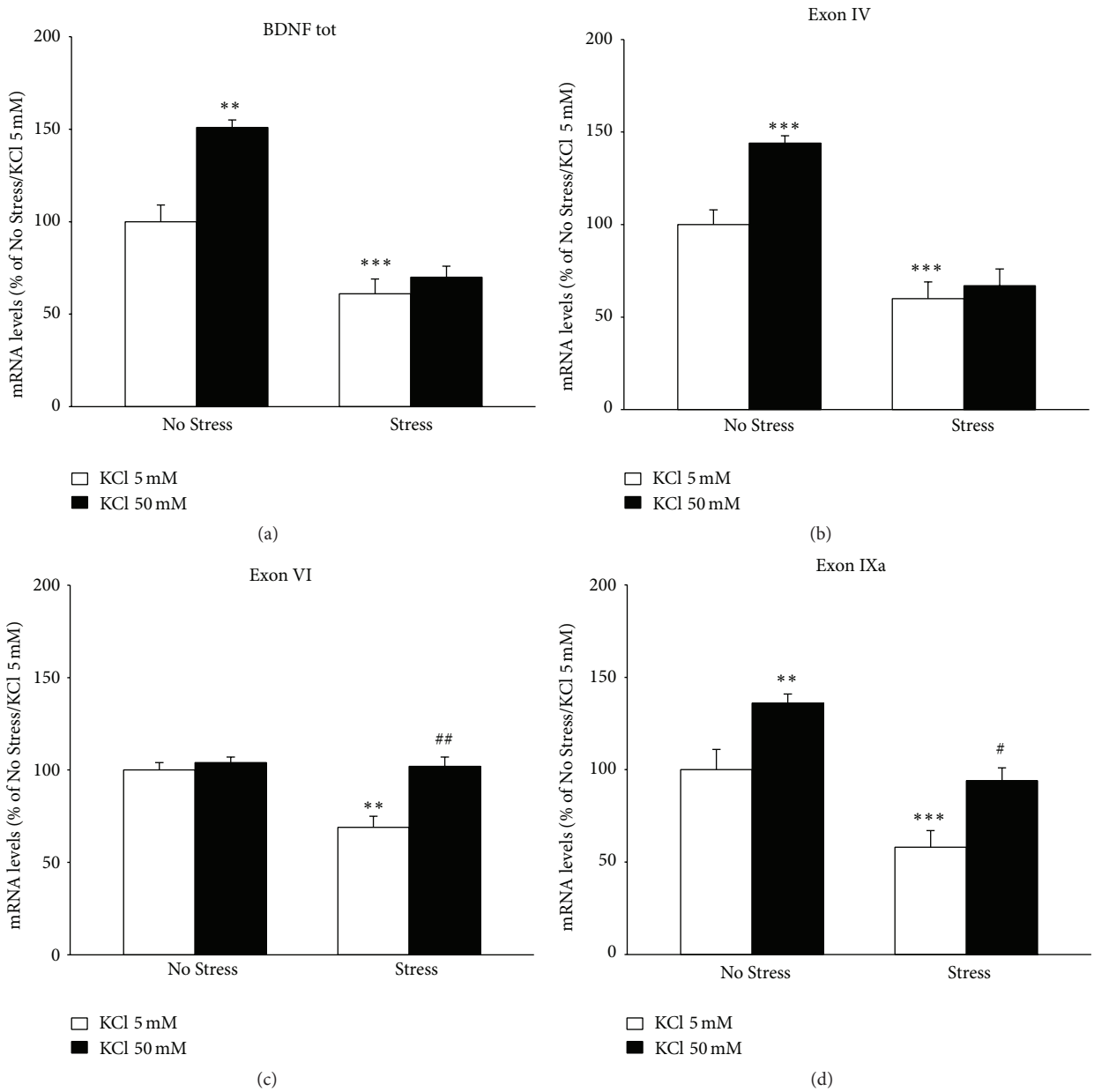


Figure 3: Analyses of BDNF gene expression. The mRNA levels of the total form of BDNF (a), BDNF isoform IV (b), isoform VI (c), and isoform IXa were measured by qRT-PCR in hippocampal slices obtained from unstressed (No Stress) or chronically stressed (Stress) rats exposed to KCl-induced depolarization (KCl 50 mM). The data, shown as a percentage referring to control group (No Stress/KCl 5 nM), are the mean ± SEM of independent determinations. $^{**}P < 0.01$, $^{***}P < 0.001$ versus No Stress animals/KCl 5 nM; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus Stress/KCl 5 nM. Two-way ANOVA with SCPHT.

3.3. Analysis of BDNF Mediated Signaling. Afterwards, we examined whether the different activity-dependent transcription of *Bdnf* seen in CMS rats was paralleled by changes in signaling pathways related to the neurotrophin. We analyzed the expression and the activation (phosphorylated form) of ERK1/ERK2 (Tyr^{204}/Tyr^{187}), Creb (Ser^{133}), and AKT (Ser^{473}) and its downstream target GSK-3β (Ser^{9}) in protein extracts obtained from hippocampal slices, under basal conditions or following KCl-induced depolarization (Figure 4). Although total levels of these signaling proteins were not modulated by CMS or by acute depolarization, we found that the phosphorylation of ERK1 and ERK2 (Figures 5(a) and 5(c)) was significantly affected by stress (pERK1: $F_{1,11} = 41.084$, $P < 0.001$; pERK2: $F_{1,12} = 7.457$, $P < 0.05$; ANOVA) and depolarization (pERK1: $F_{1,11} = 6.432$, $P < 0.05$; pERK2: $F_{1,11} = 1.041$, $P < 0.05$; ANOVA). In depth, pERK1 levels (Figure 5(a)) were significantly reduced in hippocampal slices obtained from stressed animals (pERK1 −42% versus No Stress/KCl 5 mM, $P < 0.001$). Moreover, KCl-induced depolarization increased the phosphorylated forms of both

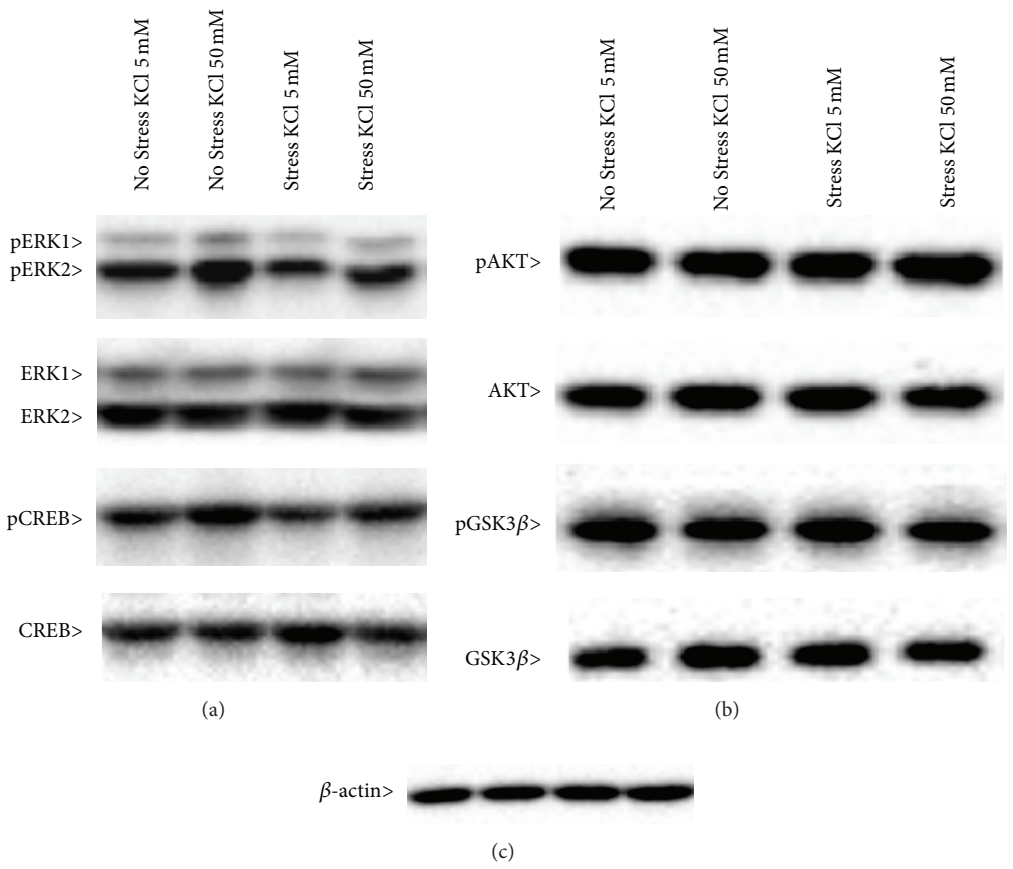


Figure 4: Representative Western blot analyses of the phosphorylated forms of ERK1, ERK2, and CREB (a) and of AKT and GSK3β (b) and their total forms (a and b). β-actin was used as internal standard (c). Experimental conditions are described in Methods.

proteins in hippocampal slices obtained from unstressed rats (Figures 5(a) and 5(c)) (pERK1 +30% versus No Stress/KCl 5 mM, $P < 0.05$; pERK2 +32% versus No Stress/KCl 5 mM, $P < 0.05$; SCPHT), but not in slices obtained from animals exposed to CMS.

Moreover, we investigated the expression levels and the activation (Ser^{133} phosphorylation) (Figures 5(e) and 5(f)) of the transcription factor CREB, which is a crucial downstream element in BDNF-related signaling and a positive regulator of neurotrophin transcription [7]. We observed that both CMS and the acute depolarization displayed significant main effects on pCREB ($F_{1,10} = 58.179$, $P < 0.001$ and $F_{1,10} = 58.179$, $P < 0.05$, resp.). Similar to what was observed for total BDNF and isoform IV expression, pCREB levels were reduced in slices obtained from stressed rats (−32% versus No Stress/KCl 5 mM, $P < 0.001$, SCPHT), whereas they were increased in response to depolarization only in control animals (+25% versus No Stress/KCl 5 mM, $P < 0.05$; Figure 5(e)). Any effect on the levels of the total form of CREB (Figure 5(f)) was found.

Conversely, neither chronic stress nor the acute depolarization was able to modulate the phosphorylation and the total levels of AKT or GSK-3β in the hippocampal slices (Figure 6).

4. Discussion

The results of our experiments disclose a novel and functional level of regulation of BDNF transcription by chronic stress. Indeed our data demonstrate not only that CMS paradigm affects basal BDNF expression but also that it has functional consequences on its activity-dependent regulation.

Different studies have examined the regulation of *Bdnf* under chronic stress, a condition that may reproduce key features of depression [22]. The interpretation of these data is not univocal, since, sometimes, opposite results have emerged based on differences in the experimental paradigm, including timing, length, and type of stressors used [23, 24]. For example, it has been showed [24] that 3 weeks of stress induced a significant increase of the protein levels of BDNF in hippocampus. Even if this effect might seem to be in contrast with our results, several reasons could explain this discrepancy, such as the different stability of the mRNA compared to protein. Another possibility could be that the stress exposure may induce an increase of the translation rate leading to a decrease of mRNA and a concomitant upregulation of the protein levels. Anyway, as explained below, also in the paper of Naert and colleagues [24] the prolonged stress altered the response to a subsequent acute challenge [24] that induced a decrease of BDNF protein levels

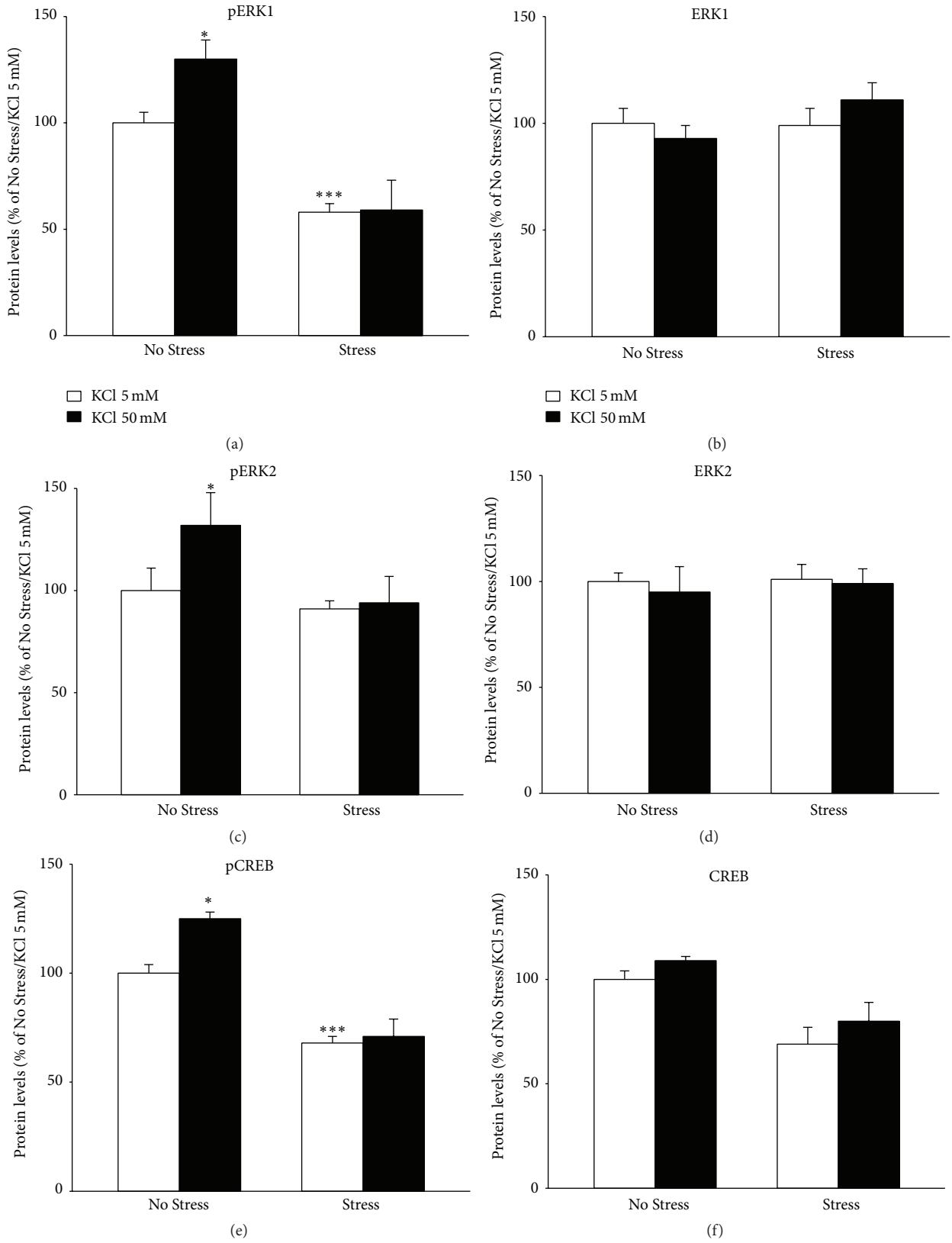


Figure 5: Protein analyses of BDNF mediated signaling: ERK1/2 kinases and CREB. The protein levels of the phosphorylated forms of ERK1 (a), ERK2 (c), and CREB (e) and their total forms (b, d, and f, resp.) were measured by Western blot analyses on protein extracts obtained from hippocampal slices obtained from unstressed (No Stress) or chronically stressed (Stress) rats exposed to KCl-induced depolarization (KCl 50 mM). The data, shown as a percentage referring to control group (No Stress/KCl 5 nM), are the mean ± SEM of independent determinations. $^{*}P < 0.05$, $^{***}P < 0.001$ versus No Stress animals/KCl 5 nM. Two-way ANOVA with SCPHT.

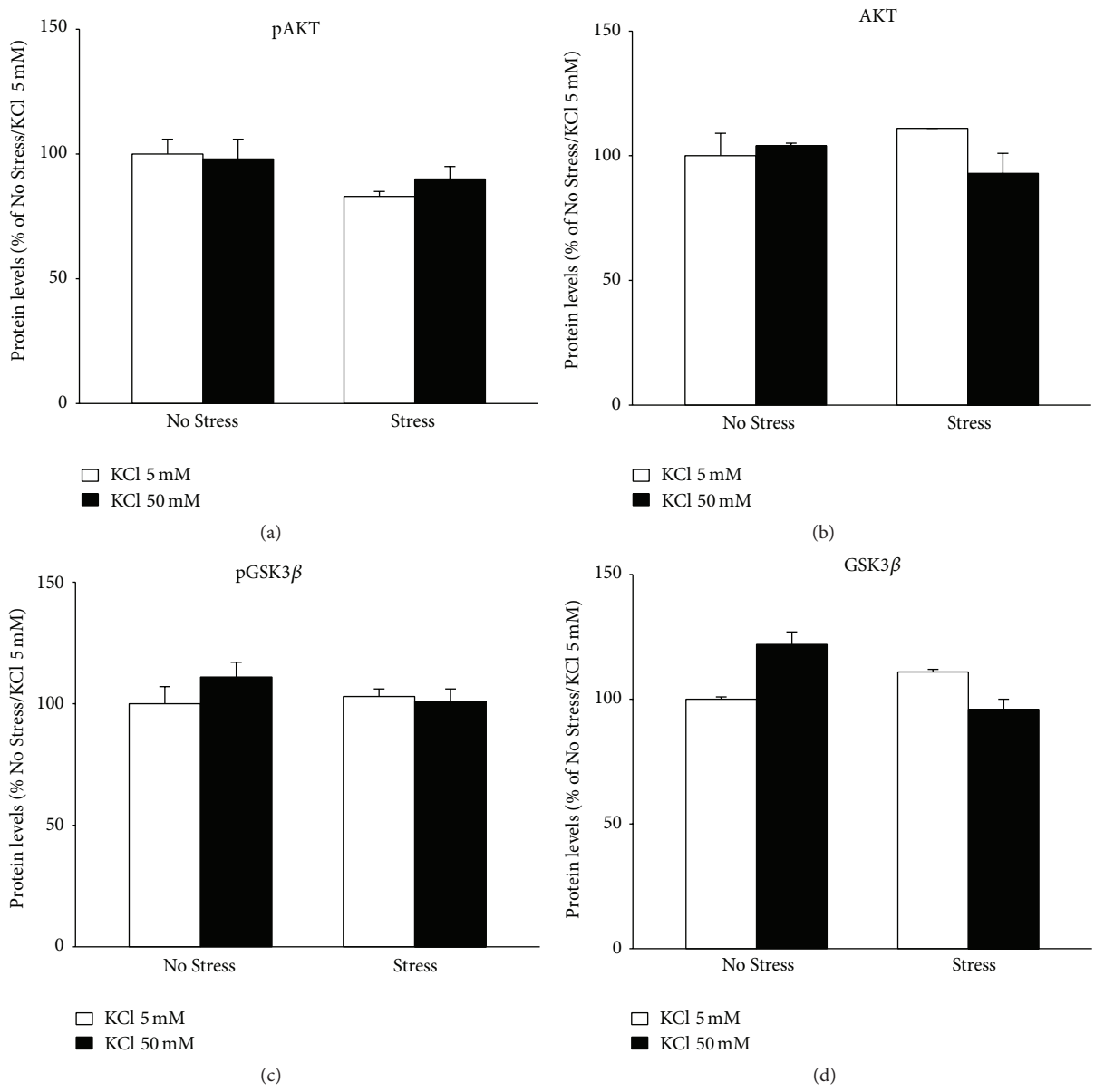


Figure 6: Protein analyses of BDNF mediated signaling: AKT and GSK3β. The protein levels of the phosphorylated forms of AKT (a) and GSK3β (c) and their total forms (b and d, resp.) were measured by Western blot analyses on protein extracts obtained from hippocampal slices obtained from unstressed (No Stress) or chronically stressed (Stress) rats exposed to KCl-induced depolarization (KCl 50 mM). The data, shown as a percentage referring to control group (No Stress/KCl 5 nM), are the mean ± SEM of independent determinations. Two-way ANOVA.

(while, according to our data, an increase was observed in the control rats).

Our results are in line with the "classic" view, according to which CMS may lead to functional impairment through a decreased expression of neurotrophic molecules, such as BDNF [25].

However, our study provides evidence for a novel degree of regulation, demonstrating that activity-dependent modulation of the neurotrophin is impaired in the hippocampus of CMS rats. Since activity-dependent transcription represents a plastic mechanism for sustaining specific neurotrophin functions such as cognition, learning, and memory [26], the impairment of such mechanism in CMS rats may contribute to reduced plasticity and diminished ability to cope with under challenging conditions. Such defect is primarily sustained by changes in the modulation of exon IV, the major activity-dependent transcript in the hippocampus [15] whose deficits have been associated with a depressive phenotype [27, 28]. Indeed, while basal effect of CMS on total *Bdnf* reflects parallel modulation of all the isoforms examined, their analysis, following neuronal activation, provides further insight into mechanisms that may be affected by CMS. Only the expression profile of isoform IV completely reflects the modulation of total *Bdnf*. The increased mRNA

levels of this transcript after depolarization are in line with the well-characterized Ca^{++}-dependent modulation of its promoter [16, 29, 30] and might represent a "positive" response to enhance specific functions. In line with this hypothesis, results obtained in our laboratory have shown that activity-dependent transcription of the neurotrophin is facilitated by chronic treatment with antidepressants [21], and isoform IV specifically participates in the restorative properties of antidepressant in a genetic model of anxiety and depression [31]. Among the calcium-responsive elements mapped in *Bdnf* promoter IV, the cAMP/Ca^{++}-response element (CaRE3/CRE) appears particularly important for the depolarization-induced transcription [17, 32, 33]. Our data on pCREB support this mechanism and clearly show that the activity of the transcription factor may be compromised by chronic stress.

Chronic stress also leads to a significant impairment of the MAPK pathway activation that represents a crucial point of convergence between different extracellular signals. This effect may result from reduced activity-dependent release of BDNF as well as from depolarization-induced changes of neurotransmitters release. Notably, the activation profile of ERK1/ERK2 in our paradigm paralleled the modulation of total *Bdnf* and of isoform IV, thus suggesting that the changes of these kinases may contribute to the alterations found in activity-dependent *Bdnf* transcription. Conversely, any effects on the activation and on the total levels of GSK and AKT were observed. These results seem to be in contrast with other studies [34–36] showing that the stress exposure influences the function of these pathways, but direct comparison between those results and ours is not recommended because of the different experimental conditions used.

Differently to isoform IV, isoform VI is modulated in an opposite manner, with its transcription being upregulated in CMS hippocampal slices exposed to depolarization. This suggests that the systems responsible for isoform VI activity-dependent transcription become more active even though CMS *per se* reduces its mRNA levels. Glucocorticoid hormones, which have an inhibitory control on exon VI transcription [37, 38], may eventually contribute to CMS-induced reduction. Conversely, since different intracellular systems participate in activity-dependent transcription of exon VI [39], it can be inferred that the enhanced levels of its mRNA levels in stimulated slices from CMS rats might result from the contribution and cooperation of multiple pathways differently modulated by stress and depolarization. A different influence of CMS on *Bdnf* activity-dependent transcription was observed for isoform IXa, whose mRNA levels were upregulated by depolarization in both unstressed and stress rats. Given the current lack of information on the regulation of this transcript, we cannot speculate on the mechanisms sustaining the observed effect but only highlight that BDNF transcripts may undergo different stress activity-dependent changes, which may hold implications for the diverse functions that are controlled by the neurotrophin.

To sum up, by using the *ex vivo* methodology of acute stimulation of hippocampal slices, we demonstrated that the activity-dependent modulation of BDNF expression is significantly affected by CMS exposure, thus disclosing a novel functional level of regulation of the neurotrophin by chronic stress. Given the importance of neuronal activity-regulated transcription as a critical process in synaptic and neuronal plasticity, the ability of adverse events to differently modify its control on individual BDNF promoters might be a finely regulated and flexible mechanism that contributes to long-lasting, experience-dependent changes in the hippocampus. Alternatively, the different regulation of BDNF promoters in our paradigm could result in altered translation, trafficking, and activation of signal transduction pathways that may eventually underline divergent consequences for hippocampal structure and function. Further investigations of these mechanisms may provide useful information on upstream or downstream molecular processes that, by contributing to stress-related disorders, may be a potential target for pharmacological intervention.

Abbreviations

BDNF: Brain-derived neurotrophic factor
CREB: cAMP-response element binding protein
CMS: Chronic mild stress
ERK1/2: Extracellular signal-regulated kinases 1/2
GAPDH: Glyceraldehyde 3-phosphate dehydrogenase
GSK-3β: Glycogen synthase kinase 3β.

Conflict of Interests

The author G. Racagni has received compensation as speaker/consultant for Servier, Janssen, and Otsuka. The other authors declare no financial interest or potential conflict of interests.

Authors' Contribution

The authors Molteni and Calabrese conceived and designed the experiments; the authors Savino and Rossetti performed the experiments and analyzed the data; the authors Molteni, Racagni, and Calabrese wrote or contributed to the writing of the paper. All the authors have read and approved the final paper.

Acknowledgment

Special thanks are due to Professor Fabrizio Gardoni for his help in setting the acute stimulation of hippocampal slices.

References

[1] B. C. Dickerson and H. Eichenbaum, "The episodic memory system: neurocircuitry and disorders," *Neuropsychopharmacology*, vol. 35, no. 1, pp. 86–104, 2010.

[2] B. S. McEwen, "Plasticity of the hippocampus: adaptation to chronic stress and allostatic load," *Annals of the New York Academy of Sciences*, vol. 933, pp. 265–277, 2001.

[3] E. R. De Kloet, M. Joëls, and F. Holsboer, "Stress and the brain: from adaptation to disease," *Nature Reviews Neuroscience*, vol. 6, no. 6, pp. 463–475, 2005.

[4] B. S. McEwen, "Physiology and neurobiology of stress and adaptation: central role of the brain," *Physiological Reviews*, vol. 87, no. 3, pp. 873–904, 2007.

[5] G. MacQueen and T. Frodl, "The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research," *Molecular Psychiatry*, vol. 16, no. 3, pp. 252–264, 2011.

[6] K. Wingenfeld and O. T. Wolf, "Stress, memory, and the hippocampus," *Frontiers of Neurology and Neuroscience*, vol. 34, pp. 109–120, 2014.

[7] A. E. West, W. G. Chen, M. B. Dalva et al., "Calcium regulation of neuronal gene expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 20, pp. 11024–11031, 2001.

[8] N. V. Malykhin and N. J. Coupland, "Hippocampal neuroplasticity in major depressive disorder," *Neuroscience*, 2015.

[9] E. J. Huang and L. F. Reichardt, "Neurotrophins: roles in neuronal development and function," *Annual Review of Neuroscience*, vol. 24, pp. 677–736, 2001.

[10] J. Soulé, E. Messaoudi, and C. R. Bramham, "Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain," *Biochemical Society Transactions*, vol. 34, no. 4, pp. 600–604, 2006.

[11] B. Lu, P. T. Pang, and N. H. Woo, "The yin and yang of neurotrophin action," *Nature Reviews Neuroscience*, vol. 6, no. 8, pp. 603–614, 2005.

[12] L. M. Monteggia, "Elucidating the role of brain-derived neurotrophic factor in the brain," *American Journal of Psychiatry*, vol. 164, no. 12, p. 1790, 2007.

[13] T. Aid, A. Kazantseva, M. Piirsoo, K. Palm, and T. Timmusk, "Mouse and rat BDNF gene structure and expression revisited," *Journal of Neuroscience Research*, vol. 85, no. 3, pp. 525–535, 2007.

[14] P. Pruunsild, A. Kazantseval, T. Aid, K. Palm, and T. Timmusk, "Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters," *Genomics*, vol. 90, no. 3, pp. 397–406, 2007.

[15] P. Pruunsild, M. Sepp, E. Orav, I. Koppel, and T. Timmusk, "Identification of cis-elements and transcription factors regulating neuronal activity-dependent transcription of human BDNF gene," *Journal of Neuroscience*, vol. 31, no. 9, pp. 3295–3308, 2011.

[16] A. Tabuchi, R. Nakaoka, K. Amano et al., "Differential activation of brain-derived neurotrophic factor gene promoters I and III by Ca^{2+} signals evoked via L-type voltage-dependent and *N*-methyl-D-aspartate receptor Ca^{2+} channels," *The Journal of Biological Chemistry*, vol. 275, no. 23, pp. 17269–17275, 2000.

[17] X. Tao, S. Finkbeiner, D. B. Arnold, A. J. Shaywitz, and M. E. Greenberg, "Ca^{2+} influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism," *Neuron*, vol. 20, no. 4, pp. 709–726, 1998.

[18] H. Park and M.-M. Poo, "Neurotrophin regulation of neural circuit development and function," *Nature Reviews Neuroscience*, vol. 14, no. 1, pp. 7–23, 2013.

[19] G. Guidotti, F. Calabrese, C. Anacker, G. Racagni, C. M. Pariante, and M. A. Riva, "Glucocorticoid receptor and fkbp5 expression is altered following exposure to chronic stress: modulation by antidepressant treatment," *Neuropsychopharmacology*, vol. 38, no. 4, pp. 616–627, 2013.

[20] F. Gardoni, L. H. Schrama, A. Kamal, W. H. Gispen, F. Cattabeni, and M. Di Luca, "Hippocampal synaptic plasticity involves competition between Ca^{2+}/calmodulin-dependent protein kinase II and postsynaptic density 95 for binding to the NR2A subunit of the NMDA receptor," *The Journal of Neuroscience*, vol. 21, pp. 1501–1509, 2001.

[21] R. Molteni, F. Calabrese, A. Cattaneo et al., "Acute stress responsiveness of the neurotrophin bdnf in the rat hippocampus is modulated by chronic treatment with the antidepressant duloxetine," *Neuropsychopharmacology*, vol. 34, no. 6, pp. 1523–1532, 2009.

[22] R. S. Duman and L. M. Monteggia, "A neurotrophic model for stress-related mood disorders," *Biological Psychiatry*, vol. 59, no. 12, pp. 1116–1127, 2006.

[23] F. Calabrese, R. Molteni, G. Racagni, and M. A. Riva, "Neuronal plasticity: a link between stress and mood disorders," *Psychoneuroendocrinology*, vol. 34, no. 1, pp. S208–S216, 2009.

[24] G. Naert, G. Ixart, T. Maurice, L. Tapia-Arancibia, and L. Givalois, "Brain-derived neurotrophic factor and hypothalamic-pituitary-adrenal axis adaptation processes in a depressive-like state induced by chronic restraint stress," *Molecular and Cellular Neuroscience*, vol. 46, no. 1, pp. 55–66, 2011.

[25] F. Calabrese, R. Molteni, and M. A. Riva, "Antistress properties of antidepressant drugs and their clinical implications," *Pharmacology and Therapeutics*, vol. 132, no. 1, pp. 39–56, 2011.

[26] S. W. Flavell and M. E. Greenberg, "Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system," *Annual Review of Neuroscience*, vol. 31, pp. 563–590, 2008.

[27] R. Molteni, A. Cattaneo, F. Calabrese et al., "Reduced function of the serotonin transporter is associated with decreased expression of BDNF in rodents as well as in humans," *Neurobiology of Disease*, vol. 37, no. 3, pp. 747–755, 2010.

[28] K. Sakata, L. Jin, and S. Jha, "Lack of promoter IV-driven BDNF transcription results in depression-like behavior," *Genes, Brain and Behavior*, vol. 9, no. 7, pp. 712–721, 2010.

[29] X. Tao, A. E. West, W. G. Chen, G. Corfas, and M. E. Greenberg, "A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF," *Neuron*, vol. 33, no. 3, pp. 383–395, 2002.

[30] E. J. Hong, A. E. McCord, and M. E. Greenberg, "A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition," *Neuron*, vol. 60, no. 4, pp. 610–624, 2008.

[31] F. Calabrese, R. Molteni, A. Cattaneo et al., "Long-term duloxetine treatment normalizes altered brain-derived neurotrophic factor expression in serotonin transporter knockout rats through the modulation of specific neurotrophin isoforms," *Molecular Pharmacology*, vol. 77, no. 5, pp. 846–853, 2010.

[32] P. B. Shieh, S.-C. Hu, K. Bobb, T. Timmusk, and A. Ghosh, "Identification of a signaling pathway involved in calcium regulation of BDNF expression," *Neuron*, vol. 20, no. 4, pp. 727–740, 1998.

[33] C.-H. Hang, G. Chen, J.-X. Shi, X. Zhang, and J.-S. Li, "Cortical expression of nuclear factor κB after human brain contusion," *Brain Research*, vol. 1109, no. 1, pp. 14–21, 2006.

[34] K. Zhang, X. Song, Y. Xu et al., "Continuous GSK-3beta overexpression in the hippocampal dentate gyrus induces prodepressant-like effects and increases sensitivity to chronic mild stress in mice," *Journal of Affective Disorders*, vol. 146, no. 1, pp. 45–52, 2013.

[35] H. Qi, F. Mailliet, M. Spedding et al., "Antidepressants reverse the attenuation of the neurotrophic MEK/MAPK cascade in frontal cortex by elevated platform stress; reversal of effects on

LTP is associated with GluA1 phosphorylation," *Neuropharmacology*, vol. 56, no. 1, pp. 37–46, 2009.

[36] Z. H. Fang, C. H. Lee, M. K. Seo et al., "Effect of treadmill exercise on the BDNF-mediated pathway in the hippocampus of stressed rats," *Neuroscience Research*, vol. 76, no. 4, pp. 187–194, 2013.

[37] M. J. M. Schaaf, E. R. De Kloet, and E. Vreugdenhil, "Corticosterone effects on BDNF expression in the hippocampus. Implications for memory formation," *Stress*, vol. 3, no. 3, pp. 201–208, 2000.

[38] A. C. Hansson, W. H. Sommer, M. Metsis, I. Strömberg, L. F. Agnati, and K. Fuxe, "Corticosterone actions on the hippocampal brain-derived neurotrophic factor expression are mediated by exon IV promoter," *Journal of Neuroendocrinology*, vol. 18, no. 2, pp. 104–114, 2006.

[39] Y. Takeuchi, E. Miyamoto, and K. Fukunaga, "Analysis on the promoter region of exon IV brain-derived neurotrophic factor in NG108-15 cells," *Journal of Neurochemistry*, vol. 83, no. 1, pp. 67–79, 2002.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 8574830, 9 pages
http://dx.doi.org/10.1155/2016/8574830

Research Article

Nutritional Omega-3 Deficiency Alters Glucocorticoid Receptor-Signaling Pathway and Neuronal Morphology in Regionally Distinct Brain Structures Associated with Emotional Deficits

Thomas Larrieu,[1,2,3] Muna L. Hilal,[4,5] Véronique De Smedt-Peyrusse,[1,2] Nathalie Sans,[4,5] and Sophie Layé[1,2]

[1]*INRA, Nutrition et Neurobiologie Intégrée, UMR 1286, 33076 Bordeaux, France*
[2]*Univ. Bordeaux, Nutrition et Neurobiologie Intégrée, UMR 1286, 33076 Bordeaux, France*
[3]*Brain Mind Institute, School of Life Sciences, École Polytechnique Federale de Lausanne (EPFL), 1050 Lausanne, Switzerland*
[4]*INSERM, Neurocentre Magendie, UMR 862, 33077 Bordeaux, France*
[5]*Univ. Bordeaux, Neurocentre Magendie, UMR 862, 33077 Bordeaux, France*

Correspondence should be addressed to Thomas Larrieu; thomas.larrieu@epfl.ch and Sophie Layé; sophie.laye@bordeaux.inra.fr

Received 25 June 2015; Revised 7 October 2015; Accepted 19 October 2015

Academic Editor: Laura Musazzi

Extensive evidence suggests that long term dietary n-3 polyunsaturated fatty acids (PUFAs) deficiency results in altered emotional behaviour. We have recently demonstrated that n-3 PUFAs deficiency induces emotional alterations through abnormal corticosterone secretion which leads to altered dendritic arborisation in the prefrontal cortex (PFC). Here we show that hypothalamic-pituitary-adrenal (HPA) axis feedback inhibition was not compromised in n-3 deficient mice. Rather, glucocorticoid receptor (GR) signaling pathway was inactivated in the PFC but not in the hippocampus of n-3 deficient mice. Consequently, only dendritic arborisation in PFC was affected by dietary n-3 PUFAs deficiency. In addition, occlusion experiment with GR blockade altered GR signaling in the PFC of control mice, with no further alterations in n-3 deficient mice. In conclusion, n-3 PUFAs deficiency compromised PFC, leading to dendritic atrophy, but did not change hippocampal GR function and dendritic arborisation. We argue that this GR sensitivity contributes to n-3 PUFAs deficiency-related emotional behaviour deficits.

1. Introduction

Early life stress, including malnutrition, results in altered synaptic and behavioural functions in adult life [1–3]. Among the myriad of components of food, polyunsaturated fatty acids (PUFAs) have received substantial consideration as being relevant to many diseases, including anxiety and depression [4–6]. A compelling body of evidence reveals that anxiety and depressive disorders are linked to dietary lipids, especially the n-3 PUFAs [1, 7–12]. Dysfunction of the hypothalamic-pituitary adrenal (HPA) axis including glucocorticoid receptor (GR) signaling pathway remains one of the cardinal features of depression and anxiety [13–17]. Although several mechanisms underlying the effects of dietary n-3 PUFAs deficiency on emotional behaviour have been described (see [18] for review), those specifically related to HPA axis function remain poorly understood. Using an animal model of maternal dietary n-3 PUFAs deficiency, we recently found that mice that were fed a deficient diet in n-3 PUFAs were under a chronic stress state reflected by behavioural and neuronal changes that resemble those of mice exposed to social defeat stress. These effects were mediated by HPA axis hyperactivity and were reversed by n-3 PUFAs supplementation [19]. Despite the clear importance of dietary n-3 PUFAs in maintaining HPA axis function and preventing emotional impairment, mechanisms by which n-3

PUFAs deficiency induces HPA axis hyperactivity remain largely unexplored. Here, we confirmed and followed up on our initial observations by determining of the processes by which nutritional n-3 PUFAs deficiency induces HPA axis hyperactivity. To do so, we aimed at investigating the effects of maternal dietary n-3 PUFAs deficiency on GR-mediated HPA axis feedback inhibition along with GR signaling pathway and neuronal arborisation in prefrontal and hippocampal brain structures. Here we found that GR signaling pathway was inactivated in the prefrontal cortex (PFC) but not in the hippocampus of omega-3 deficient mice. Consequently, only dendritic arborisation in PFC was affected by dietary n-3 PUFAs deficiency. In addition, occlusion experiment with GR blockade altered GR signaling in the PFC of control mice as well as anxiety and social behaviour with no further alterations in n-3 deficient mice. We argue that this GR sensitivity contributes to n-3 PUFAs deficiency-related HPA axis deregulation.

2. Methods

2.1. Animals. All experiments were performed according to criteria of the European Communities Council Directive (50120103-A). Behavioural and biochemical experiments were performed on C57Bl6/J mice obtained from Charles River (L'Arbresle, France). Mice were maintained under standard housing conditions on corn cob litter in a temperature-controlled ($23 \pm 1^{\circ}$C) and humidity-controlled (40%) animal room with a 12 h light/dark cycle (7:00–19:00), with ad libitum access to food and water. CD1 mice used as the social target were obtained from Charles River. All tests were conducted during the light period. C57BL6/J male mice were housed individually and were 3-4 months old when the behavioural analysis and biochemical analysis were conducted.

2.2. Diets. C57Bl6/J mice were given water and isocaloric experimental diets ad libitum (pellets prepared by UPAE-INRA, Jouy-en-Josas, France, replaced daily) as previously described [1, 20, 21]. After mating, C57BL6/J females were fed throughout gestation and lactation with a diet containing 6% of rapeseed oil (rich in α-linolenic acid, 18:3n-3; the control diet) or 6% fat in the form of sunflower oil (rich in linoleic acid, 18:2n-6; the n-3 deficient diet). After weaning, male offspring were fed with the same diet as their dam until the end of the experiments.

2.3. Surgery and Mifepristone Treatment. For pellets implantation, the skin was lifted on the back side of the C57BL6/J male mice and an incision was made. A pocket was formed with a pair of forceps about 2 cm beyond the incision site. Finally, mifepristone or placebo pellets were implanted into the pocket with forceps. Mifepristone pellets released continuously 20 mg/kg/day for 21 days (Innovative Research of America (IRA)). Innocuousness of the compound at this dose and time has been revealed by measuring body weight change and locomotor activity (data not shown). After 21 days of treatment, open-field and social interaction tests were performed as described below. (see Figure 3(a) for the timeline).

2.4. Behavioural Testing. Social interaction measurement was performed as previously published [1, 20]. Mice were transferred to a new cage (40×40 cm). A social interaction session comprised 5 minutes without target followed by 5-minute exposure of an unfamiliar adult CD1 male enclosed in a wire mesh placed in the corner of the field. Number of active investigatory behaviours (mainly sniffing the anogenital region, mouth, ears, trunk, and tail) was manually counted by an experimenter blind to the conditions.

Open-field test was performed as previously published [19, 20]. The apparatus consisted of a Plexiglas open-field (40×40 cm) with 16 cm high walls. Lighting consisted of four fluorescent bulbs at a height of 2 m above the floor of an open-field apparatus placed on each corner of the experiment room (light intensity of 35 Lux). The floor was cleaned between each trial to avoid olfactory clues. Each mouse was transferred to an open field facing a corner and was allowed to freely explore for 10 minutes an open field. A video tracking system (Smart, Panlab, Spain) recorded the exact track of each mouse as well as total distance travelled (cm) and the percentage of time spent in the centre.

2.5. Plasmatic Corticosterone Analysis. Trunk blood collection in ethylenediaminetetraacetic acid-lined tubes (EDTA) was performed during diurnal rise period, previously determined to occur 60 minutes before lights went off. Corticosterone was measured with an in-house RIA in the plasma as previously described [19]. Briefly, after steroid extraction with absolute ethanol, total corticosterone was measured by competition between cold corticosterone (B) and 3H-B (B^*) by a specific anti-corticosterone antibody provided by Dr. H. Vaudry (University of Rouen, France).

2.6. Morphological Analysis. Brains were quickly removed, washed in PBS, and processed for staining of individual neurons following the manufacturer's instructions for the rapid Golgi kit (FD Neurotech). Golgi stained brain slices of 100 μm containing the PFC were used for morphological analysis. Pyramidal neurons of dorsal CA1 region of hippocampus (from −1.34 to −2.30 mm anterior to bregma) and PFC II/III layers were chosen for morphological analysis in our study. The dorsal PFC can be divided into dorsolateral PFC including frontal association (FrA) cortex (from 2.58 to 3.08 mm anterior to bregma) and dorsomedial PFC including prelimbic (PL) cortex (from 1.5 to 2.3 mm anterior to bregma). Pyramidal neurons within this region are defined as having a cell body which is immediately lateral to layer I, which is relatively absent of cells. These neurons are also defined by the presence of a basilar dendritic tree and a clearly defined single apical dendrite that projects toward the pial surface. For analysis, cells that met the following criteria were chosen: (1) relative isolation of the cell body from neighbouring impregnated neurons; (2) cell bodies existing between 150 and 250 μm from the pial surface to prevent artefacts due to unrepresentative sampling from neurons of varying distance from the midline; and (3) the presence of intact primary, secondary, and tertiary dendrites. As our previous study, which has examined the effects of nutritional omega-3 deficiency and stress on this neuronal class within

the mPFC, has found selective effects on the apical dendrites, as opposed to the basal dendrites, analysis was restricted to the apical dendritic tree. Three to 5 neurons per mouse and per region were reconstructed by a trained experimenter blind to the conditions using a Zeiss microscope Axio Imager 2 (×100) and analysed using the Neurolucida software. Sholl analysis was performed, in which the centre of the soma was used as a reference point and dendritic length was quantified both as a total measure per cell and as a function of radial distance from the soma in 10 μm increments.

2.7. Western Blot Analysis. One day after the last behavioural test, mice used for the occlusion experiment were sacrificed and the brain was quickly removed from the skull and rinsed in cold Milli-Q water to remove any surface blood. Prefrontal cortex was collected with a blade and forceps after removing the olfactory bulbs. Right after rolling out the cortex from both hemispheres, hippocampus was dissected from the cortex using forceps. Collected tissues were flash frozen in dry ice and stored at −80°C until analysis. Western blot measurement was performed as previously described [19, 20]. PFC and hippocampus were homogenised in lysis buffer (TRIS 20 mM pH 7.5, antiprotease cocktail, 5 mM MgCl2, 1 mM DTT, 0.5 M EDTA, 1 mM NaOV, and 1 mM NaF). After centrifugation, protein concentration was determined using a BCA assay kit (Uptima, Montluçon, France). Equal amounts of proteins (50 μg) were loaded onto SDS-PAGE gel (10%) and transferred onto PVDF membrane (Millipore, Billerica, MA, USA). Membranes were incubated overnight (4°C) with anti-GR (M-20) (1 : 5000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-FKBP51 (1 : 500, Santa Cruz Biotechnology), anti-E6AP (H-182) (1 : 500, Santa Cruz Biotechnology), and anti-actin (1 : 2500, Sigma, Saint-Louis, MS, USA) antibodies. After washing, membranes were incubated 1 h with rabbit peroxidase-conjugated secondary antibody (1 : 5000, Jackson ImmunoResearch laboratories, West Grove). Between each revelation, membranes were incubated 15 minutes in stripping buffer (Re-Blot Plus, Millipore) to remove the previous antibody. Staining was revealed with ECL-Plus Western blotting system (Perkin Elmer, Forest City, CA). Chemiluminescence was captured and quantified using GeneTools software (Syngene).

2.8. Dexamethasone Suppression Test. The mice received a single intraperitoneal injection of dexamethasone dissolved in saline (0.9% NaCl, 0.1 mg per kg; 0.1 mL per 10 g of mouse; Sigma-Aldrich, France) or 0.9% NaCl in control condition 6 hours before decapitation and determination of total corticosterone levels in plasma as previously described [22].

2.9. Statistical Analyses. All values are given as mean ± SEM. Results obtained in mifepristone and dexamethasone experiments were all analysed by a two-way analysis of variance (ANOVA), with treatment and diet as fixed factors. Analyses were followed by Bonferroni post hoc test when appropriate. Results obtained in morphological experiments were all analysed by an unpaired t-test. All statistical tests were performed with GraphPad Prism (GraphPad software) using a critical probability of $p < 0.05$. Statistical analyses performed for each experiment are summarised in each legend of figures with the chosen statistical test, n and p values, degree of freedom, and F/t values.

3. Results

We investigated plasma corticosterone levels in undisturbed control diet and n-3 deficient mice to confirm our previous data [19]. Figure 1 shows that dietary n-3 PUFAs deficiency induced a significant increase in total plasma corticosterone levels compared with control diet in basal conditions. In order to understand how dietary n-3 PUFAs deficiency leads to corticosterone hypersecretion, we then tested whether HPA axis feedback regulation was compromised in n-3 deficient mice. Some depressed patients and mice that display depression-related behaviours show impaired suppression of endogenous glucocorticoids by the synthetic glucocorticoid dexamethasone [23]. For this purpose, we used this clinically established neuroendocrinological test, the dexamethasone suppression test [22]. We found that dexamethasone at the dose of 0.1 mg/kg effectively suppressed the corticosterone release in control diet mice (Figure 2). Surprisingly, we found that, six hours after dexamethasone treatment, n-3 deficient mice also exhibited a decrease in total corticosterone levels in plasma as compared to their respective saline group. This result suggests that HPA axis feedback induced by dexamethasone is intact in mice fed with a deficient diet in n-3 PUFAs.

In patients with major depression, diminished GR expression or function has been postulated as causative factor for their increased HPA axis activity. We then asked whether altered GR signaling pathway in distinct brain structures positioned to inhibit the glucocorticoids stress response could partially explain HPA axis hyperactivity observed in n-3 deficient mice. Indeed, GR-mediated signaling in PFC and hippocampus is a pivotal component that contributes to the stress response, particularly in the context of HPA axis feedback desensitisation. In order to evaluate GR signaling in those structures, we used a Western blot method and analysed GR protein as well as FK506 binding protein 51 (FKBP51) protein expression which contains a glucocorticoid response element (GRE) and is modulated by glucocorticoids [24]. We found that expressions of GR and GR-responsive gene FKBP51 were downregulated in PFC of n-3 deficient mice as compared to control diet mice (Figures 3(b) and 3(c)). This result suggests that GR signaling pathway is inactivated in the PFC of n-3 deficient mice. If this hypothesis is true, the effect of chronic GR inactivation with the GR antagonist mifepristone on GR signaling in the PFC should not be detected in n-3 deficient mice. Consistent with this scenario, we found that mifepristone in n-3 deficient mice did not induce any further alterations on both GR (Figure 3(b)) and FKBP51 (Figure 3(c)) expressions in the PFC. Also, GR occlusion experiment revealed that GR signaling impairment found in the PFC of n-3 deficient mice was similar to that observed in control diet mice treated with mifepristone. Although FKBP51 expression was significantly higher in the hippocampus of n-3 deficient mice as compared to control diet mice (Figure 3(f)), no change in GR expression was

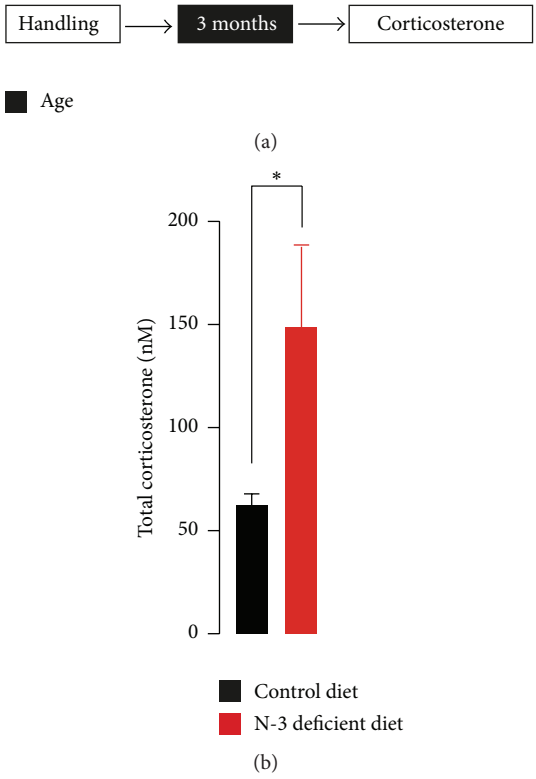


Figure 1: Effects of n-3 deficient diet on total plasma corticosterone levels. N-3 deficient mice showed total corticosterone elevation as compared to control diet mice in steady-state condition ($t_7 = 2.472$, $^*p < 0.05$, unpaired t-test, $n = 4$-5 per group). Data are displayed as mean ± SEM.

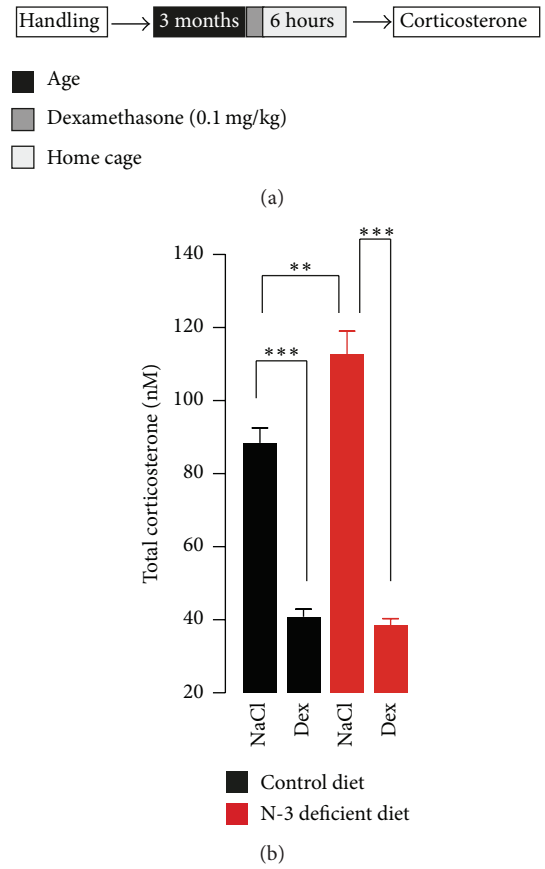


Figure 2: Corticosterone levels after dexamethasone application. (a) Experimental timeline. The mice received a single intraperitoneal injection of dexamethasone dissolved in saline or NaCl 6 hours before decapitation and determination of total corticosterone levels in plasma. (b) A significant interaction between diet and treatment was revealed on corticosterone suppression (interaction: $F_{1,22} = 12.58$, $p < 0.01$, two-way ANOVA, Bonferroni's test; $n = 6$-7 per group). After administration of dexamethasone both control diet and n-3 deficient mice showed decreased corticosterone levels in plasma. Data are displayed as mean ± SEM.

found in the hippocampus (Figure 3(e)). In addition, we found that expression of GR in the hippocampus was reduced after 21 days of mifepristone treatment whatever the diet group (Figure 3(e)), revealing intact GR response to mifepristone in the hippocampus of n-3 deficient mice. Finally, we found that expression of the transcriptional coactivator of GRs E6-associated protein (E6AP) was unaffected by the diet or the treatment in the PFC of n-3 deficient mice as compared to the control diet mice (Figure 3(d)). On the contrary, the hippocampus of n-3 deficient mice showed a significant increase of the expression of E6AP whatever the treatment as compared to the control diet mice (Figure 3(g)).

At the emotional level, exploratory behaviours with emotional load (open-field and social interaction) are impaired in mice with defective HPA axis function [25–27]. We found that n-3 deficient mice did not engage new mice socially and exhibited anxiety-like behaviour in an open-field test, consistent with previous reports from our group [1, 19, 20]. This was revealed by a reduction in the number of active explorations of a new unfamiliar mouse (Figure 3(h)) and in the time spent exploring the centre of an open field (Figure 3(i)) compared to control diet mice. Interestingly, n-3 deficient mice displayed a behavioural impairment comparable with control diet mice treated with the GR antagonist mifepristone. Increased anxiety-related and social avoidance behaviour induced by mifepristone treatment was not linked to impaired motor locomotion. We found no difference regarding the total distance travelled whatever the group (data not shown). Altogether, these findings suggest that altered GR signaling pathway in PFC is closely associated with social and anxiety-related behaviours deficits induced by nutritional n-3 PUFAs deficiency.

Glucocorticoid receptor signaling has a crucial role in shaping neuronal morphology [28]. We have previously shown that nutritional n-3 PUFAs deficiency results in neuronal atrophy in the PFC [19]. Here, we aimed at investigating neuronal morphology in an additional structure, the hippocampus, in which no change was observed in GR protein levels in n-3 deficient mice. 3D morphometric analysis of Golgi-impregnated neurons revealed that exposure to nutritional n-3 PUFAs deficiency induced detrimental effects on the PFC pyramidal neurons. This was shown by

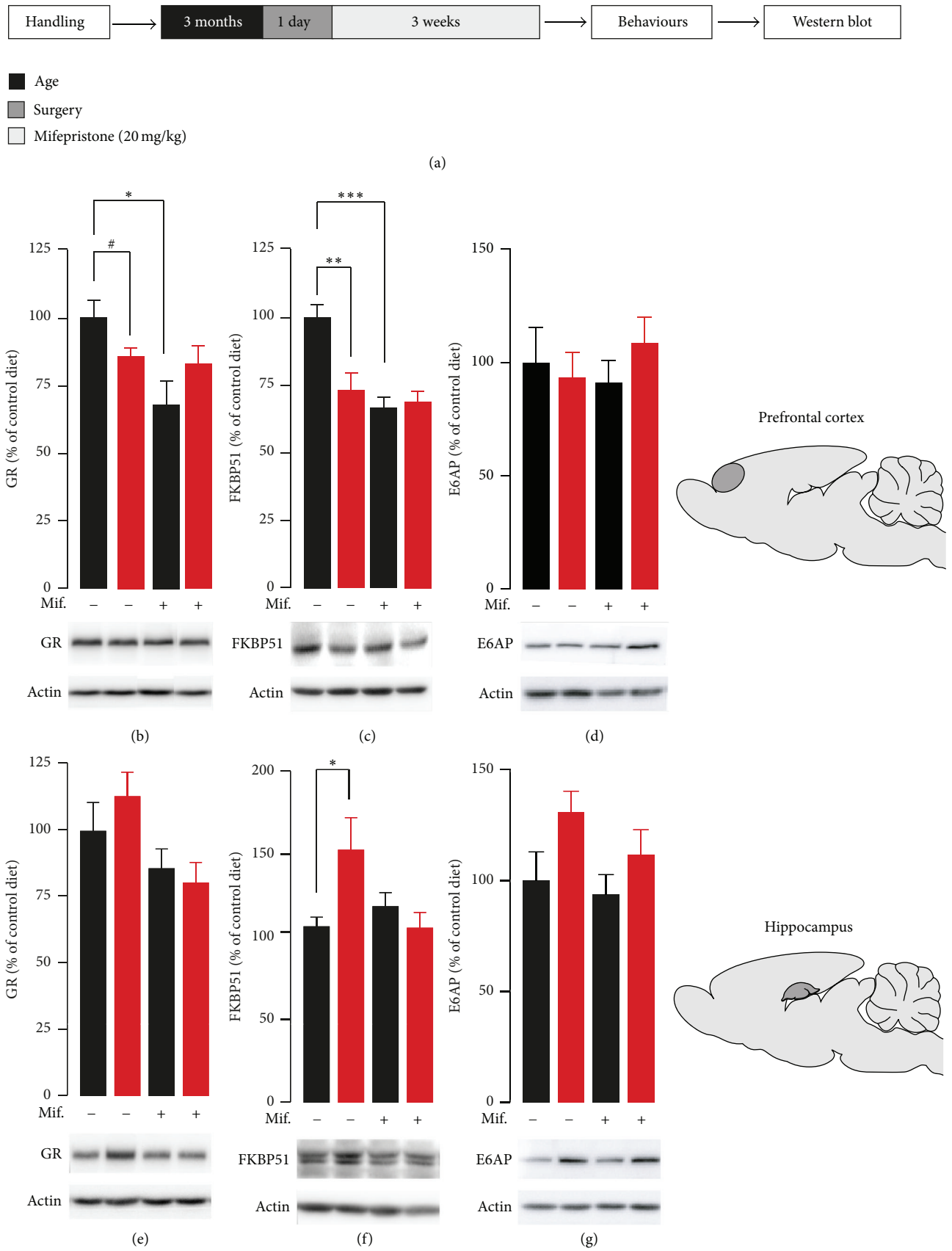


Figure 3: Continued.

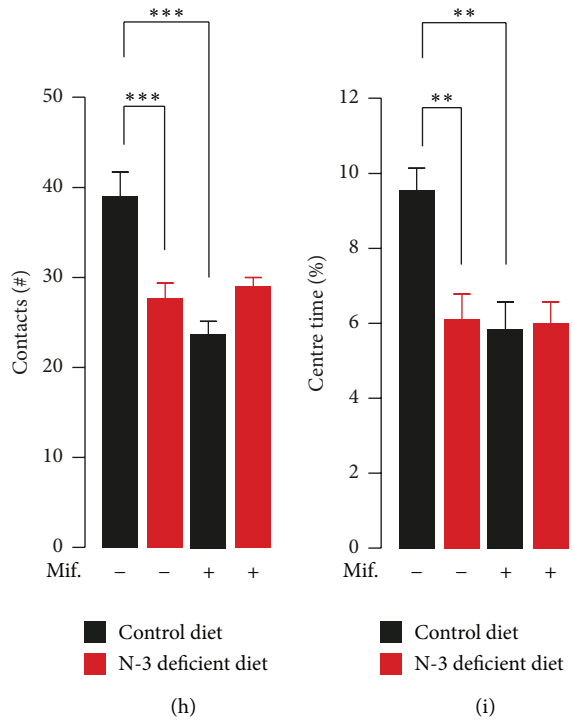


Figure 3: Effects of n-3 deficient diet on GR signaling pathway and emotional behaviours. (a) Experimental timeline. Mifepristone pellets released continuously 20 mg/kg/day for 21 days. After 21 days of treatment, open-field and social interaction tests were performed. One day after the last behavioural test, mice were sacrificed for Western blot analysis. (b, c) A significant interaction between diet and treatment was revealed on both (b) GR (interaction: $F_{1,14} = 5.540$, $p < 0.05$, two-way ANOVA, Bonferroni's test; $n = 4$-5 per group) and (c) FKBP51 expression in the PFC (interaction: $F_{1,17} = 7.584$, $p < 0.05$, two-way ANOVA, Bonferroni's test; $n = 4$-5 per group). (d) No change was revealed in the expression of E6AP in the PFC of mice (interaction: $F_{1,21} = 0.9281$, $p = 0.3463$, diet effect: $F_{1,21} = 0.1845$, $p = 0.6719$, treatment effect: $F_{1,21} = 0.07035$, $p = 0.7934$, two-way ANOVA; $n = 6$-7 per group). (e) Mifepristone effect on GR occurred in the HC regardless of the diet condition (treatment effect: $F_{1,17} = 5.991$, $p < 0.05$, two-way ANOVA; $n = 4$-5 per group). (f) A significant interaction between diet and treatment was revealed on FKBP51 expression in the hippocampus (interaction: $F_{1,16} = 4.631$, $p < 0.05$, two-way ANOVA, Bonferroni's test; $n = 4$ per group). (g) The expression of E6AP in the HC of n-3 deficient mice was reduced as compared to control diet mice whatever the treatment (interaction: $F_{1,20} = 0.3197$, $p = 0.5781$, diet effect: $F_{1,20} = 4.571$, $p = 0.0451$, treatment effect: $F_{1,20} = 1.250$, $p = 0.2769$, two-way ANOVA; $n = 5$–7 per group). A significant interaction between diet and treatment was revealed on both (h) social interaction (interaction: $F_{1,31} = 16.27$, $p < 0.001$, two-way ANOVA, Bonferroni's test; $n = 8$–10 per group) and (i) open-field test (interaction: $F_{1,38} = 7.309$, $p < 0.05$, two-way ANOVA, Bonferroni's test; $n = 10$-11 per group). Data are displayed as mean ± SEM. $^{\#}p < 0.09$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

a decrease in the total apical dendritic material in both dlPFC and dmPFC (Figures 4(a) and 4(b)). Furthermore, Sholl analysis was employed to determine the region of the apical dendritic tree at which the reduction in dendritic material was seen [29, 30]. This analysis revealed that, relative to control diet mice, dietary n-3 PUFAs deficiency reduced dendritic material in dlPFC and dmPFC at segments which were 80–120 μm and 80–150 μm from the soma, respectively. Interestingly, no change in total apical dendritic material was found in the dorsal CA1 of hippocampal pyramidal neurons of n-3 deficient mice as compared to control diet mice (Figure 4(c)). No significant changes were observed in Sholl analysis for apical dendritic arborisation in the dorsal CA1 between control diet and n-3 deficient mice in total length. Collectively, these data suggest region-specific alteration of PFC pyramidal neurons morphology and function.

4. Discussion

The brain is highly enriched in long-chain polyunsaturated fatty acids including arachidonic acid (AA) and docosahexaenoic acid (DHA) that regulate both the structure and the function of neurons [18]. Hence, it is not surprising that modification of brain PUFAs contents might be involved in the genesis of neuropsychopathologies. Nutritional n-3 PUFAs deficiency observed in Western countries has been associated with many diseases including anxiety and depression but the underlying pathophysiological mechanisms remain poorly understood. Here, we report that mice fed with a deficient diet in n-3 PUFAs demonstrated the ability to inhibit HPA axis after dexamethasone treatment which seems to be inconsistent with a depression-like phenotype. This suggests functional negative feedback which may be partially attributable to normal GR expression observed in

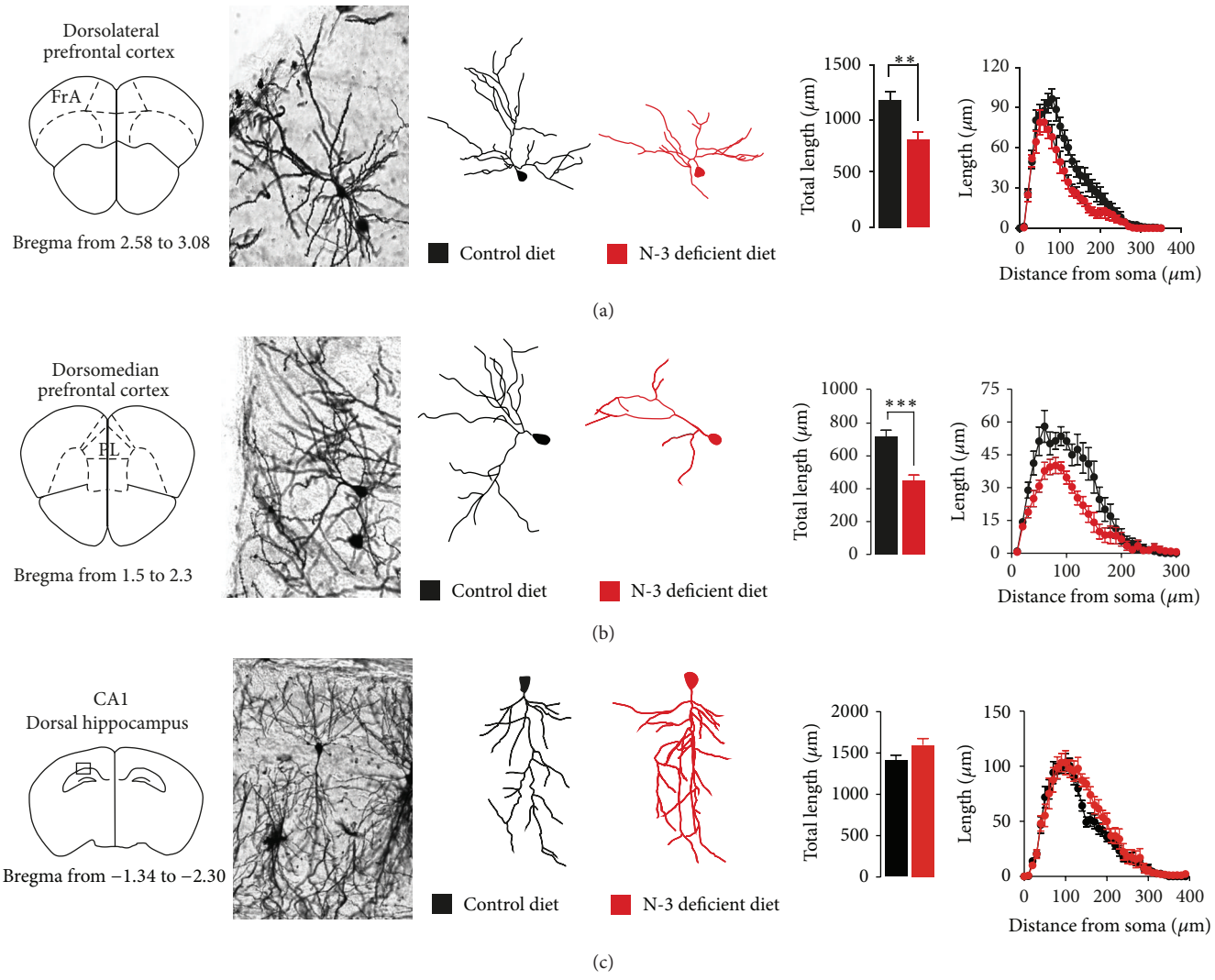


Figure 4: Effect of maternal n-3 deficient diet on dendritic arborisation in the PFC and hippocampus. (a, b, c left) dlPFC, dmPFC, and dorsal CA1 regions are represented. N-3 deficient diet induced shrinking of apical dendritic arborisation on pyramidal neurons of dlPFC ($t_{28} = 3.323$, $^{**}p < 0.01$, unpaired t-test, $n = 15$ neurons per group) and of dmPFC ($t_{34} = 4.125$, $^{***}p < 0.001$, unpaired t-test, $n = 16$ neurons per group). Dietary n-3 PUFAs deficient diet did not alter dendritic arborisation in the dorsal CA1 of the hippocampus ($t_{25} = 1.791$, $p > 0.05$, unpaired t-test, $n = 14$ neurons per group). Data are displayed as mean ± SEM.

the hippocampus of n-3 deficient mice. Indeed, the hippocampus is known to be strongly involved in GR-mediated negative feedback to the paraventricular nucleus of the hypothalamus [31]. To support this idea, we found that mifepristone reduced GR expression in the hippocampus regardless of the diet condition suggesting intact GR functionality in the hippocampus of n-3 deficient mice. In addition, acting as an inhibitor of GR activity, FKBP51 determines GR binding affinity to glucocorticoids. Its expression is also activated by glucocorticoids and is involved in an intracellular ultrashort negative feedback loop for GR activity (see [32] for review). The present profile of high basal corticosterone levels and the overexpression of FKBP51 in the HC of n-3 deficient mice both support the hypothesis of an enhanced inhibition of corticosterone binding to the GR. Furthermore, the overexpression of the transcriptional coactivator of GR E6AP observed in the HC of n-3 deficient mice may explain the fact that no change in GR expression was found. Taken together, these findings suggest a possible compensatory phenomenon involving FKBP51 and E6AP that preserves GR integrity within the HC of n-3 deficient mice. In contrast to the HC, occlusion experiment with mifepristone altered GR signaling in the PFC of control mice, with no further alterations in n-3 deficient mice. Thus, nutritional n-3 PUFAs deficiency alone is able to occlude GR signaling, reflected by a downregulation of GR and GR-responsive gene FKBP51 expressions in the PFC. GR downregulation is unlikely due to transcriptional alterations as no change was found in the expression of the transcriptional coactivator E6AP in the PFC of control diet and n-3 deficient mice.

Interestingly, the disruption of GR-signaling observed in n-3 deficient mice was associated with social deficit and anxiety-like behaviour. Our data agree with previous findings showing that transgenic mice with decreased GR expression in the brain (50%) or GR coactivator Ube3a knockout mice display anxiety- and depression-like behaviours with a reduced expression of FKBP51 in the cortex [22, 25]. In addition, a recent study in a clinical cohort has demonstrated a link between FKBP51 and stress-related psychiatric disorders [33].

Finally, our data revealed distinct regional alterations of neuronal morphology after exposure to dietary n-3 PUFAs deficiency. Nutritional n-3 PUFAs deficiency results in a decrease in the total apical length in the PFC, while no change was observed in the dorsal CA1 region of the hippocampus. In addition, Sholl analysis revealed that this atrophy in dendritic arborisation was driven by a loss of dendritic material restricted to proximal/middle regions of the apical dendritic arborisation (i.e., regions that were short of 180 μm from the cell body). Proximal/middle apical dendrites project radially to local pyramidal cells and interneurons and are the primary target of intracortical projections, while distal apical dendrites in layer II/III pyramidal PFC neurons receive projection from both hippocampal areas CA1 and CA3. Thus, the atrophy of proximal/middle dendrites of layer II-III pyramidal neurons observed within the PFC might be a result of loss of intracortical input with intact input from hippocampal pyramidal neurons. At the behavioural level, both chronic inactivation of GR and nutritional n-3 PUFAs deficiency produced exploratory behaviour impairments as assessed by the open-field and the social interaction tests. As no change in GR expression and dendritic arborisation was found in hippocampal brain structure, the present study strongly suggests that GR sensitivity is closely associated with neuronal atrophy in the PFC of n-3 deficient mice that could be involved in social interaction deficits and increased anxiety-related behaviour. In conclusion, we here provide strong validity of the maternal n-3 PUFAs deficient diet as one of the many faces of stress that deeply affects GR-dependent HPA axis function and neuronal morphology plasticity in brain areas associated with emotional behaviour. This study reinforces the idea of the usefulness of the dietary n-3 PUFAs as an interesting tool for the design and testing of new nonpharmacological strategies in the treatment of neuropsychiatric disorders such as mood-related behaviours [19] and fragile X syndrome [34].

Limitations

It is important to note some limitations of this work. First, corticosterone levels were analysed only at one time point. However, we are aware that it is necessary to take into account the strong circadian secretion rhythms involving glucocorticoids. Second, corticosterone experiments provide us with information regarding the total corticosterone secretion, including the free and protein-bound forms. In future studies, the measurement of both forms of corticosterone would help identify specific alteration induced by dietary n-3 PUFAs deficiency.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This research was financially supported by INRA, FRM, ANR MoodFood, and the Region Aquitaine. Thomas Larrieu was the recipient of a postdoctoral fellowship from the "Société Française de Nutrition" and the Labex BRAIN. The authors thank C. Tridon, M. Cadet, and C. Testa of the animal facility and Dr N. Abrous and M. Koehl for the Neurolucida software. The authors also thank the "Biochemistry Platform" of the Neurocampus funded by the Labex BRAIN.

References

[1] M. Lafourcade, T. Larrieu, S. Mato et al., "Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions," *Nature Neuroscience*, vol. 14, no. 3, pp. 345–350, 2011.

[2] S. Maccari and S. Morley-Fletcher, "Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations," *Psychoneuroendocrinology*, vol. 32, supplement 1, pp. S10–S15, 2007.

[3] D. Cao, K. Kevala, J. Kim et al., "Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function," *Journal of Neurochemistry*, vol. 111, no. 2, pp. 510–521, 2009.

[4] J. R. Hibbeln, "Fish consumption and major depression," *The Lancet*, vol. 351, no. 9110, p. 1213, 1998.

[5] A. P. Simopoulos, "The importance of the ratio of omega-6/omega-3 essential fatty acids," *Biomedicine & Pharmacotherapy*, vol. 56, no. 8, pp. 365–379, 2002.

[6] A. P. Simopoulos, "Evolutionary aspects of the dietary omega-6:omega-3 fatty acid ratio: medical implications," *World Review of Nutrition and Dietetics*, vol. 100, pp. 1–21, 2009.

[7] P. B. Adams, S. Lawson, A. Sanigorski, and A. J. Sinclair, "Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression," *Lipids*, vol. 31, no. 1, pp. S157–S161, 1996.

[8] P. Green, H. Hermesh, A. Monselise, S. Marom, G. Presburger, and A. Weizman, "Red cell membrane omega-3 fatty acids are decreased in nondepressed patients with social anxiety disorder," *European Neuropsychopharmacology*, vol. 16, no. 2, pp. 107–113, 2006.

[9] R. K. McNamara and Y. Liu, "Reduced expression of fatty acid biosynthesis genes in the prefrontal cortex of patients with major depressive disorder," *Journal of Affective Disorders*, vol. 129, no. 1–3, pp. 359–363, 2011.

[10] C. O. Bondi, A. Y. Taha, J. L. Tock et al., "Adolescent behavior and dopamine availability are uniquely sensitive to dietary omega-3 fatty acid deficiency," *Biological Psychiatry*, vol. 75, no. 1, pp. 38–46, 2014.

[11] T. Takeuchi, M. Iwanaga, and E. Harada, "Possible regulatory mechanism of DHA-induced anti-stress reaction in rats," *Brain Research*, vol. 964, no. 1, pp. 136–143, 2003.

[12] J. C. Jr. DeMar, K. Ma, J. M. Bell, M. Igarashi, D. Greenstein, and S. I. Rapoport, "One generation of n-3 polyunsaturated fatty acid deprivation increases depression and aggression test scores

in rats," *The Journal of Lipid Research*, vol. 47, no. 1, pp. 172–180, 2006.

[13] G. P. Chrousos, "Stress and disorders of the stress system," *Nature Reviews Endocrinology*, vol. 5, no. 7, pp. 374–381, 2009.

[14] E. R. de Kloet, "From receptor balance to rational glucocorticoid therapy," *Endocrinology*, vol. 155, no. 8, pp. 2754–2769, 2014.

[15] C. B. Nemeroff, E. Widerlov, G. Bissette et al., "Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients," *Science*, vol. 226, no. 4680, pp. 1342–1344, 1984.

[16] M. Niwa, H. Jaaro-Peled, S. Tankou et al., "Adolescent stress-induced epigenetic control of dopaminergic neurons via glucocorticoids," *Science*, vol. 339, no. 6117, pp. 335–339, 2013.

[17] F. Holsboer, "The corticosteroid receptor hypothesis of depression," *Neuropsychopharmacology*, vol. 23, no. 5, pp. 477–501, 2000.

[18] R. P. Bazinet and S. Layé, "Polyunsaturated fatty acids and their metabolites in brain function and disease," *Nature Reviews Neuroscience*, vol. 15, pp. 771–785, 2014.

[19] T. Larrieu, L. M. Hilal, C. Fourrier et al., "Nutritional omega-3 modulates neuronal morphology in the prefrontal cortex along with depression-related behaviour through corticosterone secretion," *Translational Psychiatry*, vol. 4, article e437, 2014.

[20] T. Larrieu, C. Madore, C. Joffre, and S. Layé, "Nutritional n-3 polyunsaturated fatty acids deficiency alters cannabinoid receptor signaling pathway in the brain and associated anxiety-like behavior in mice," *Journal of Physiology and Biochemistry*, vol. 68, no. 4, pp. 671–681, 2012.

[21] R. Mingam, A. Moranis, R.-M. Bluthé et al., "Uncoupling of interleukin-6 from its signalling pathway by dietary n-3-polyunsaturated fatty acid deprivation alters sickness behaviour in mice," *European Journal of Neuroscience*, vol. 28, no. 9, pp. 1877–1886, 2008.

[22] S. Ridder, S. Chourbaji, R. Hellweg et al., "Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions," *The Journal of Neuroscience*, vol. 25, no. 26, pp. 6243–6250, 2005.

[23] C. Anacker, P. A. Zunszain, L. A. Carvalho, and C. M. Pariante, "The glucocorticoid receptor: pivot of depression and of antidepressant treatment?" *Psychoneuroendocrinology*, vol. 36, no. 3, pp. 415–425, 2011.

[24] R. S. Lee, K. L. K. Tamashiro, X. Yang et al., "A measure of glucocorticoid load provided by DNA methylation of Fkbp5 in mice," *Psychopharmacology*, vol. 218, no. 1, pp. 303–312, 2011.

[25] S. K. Godavarthi, P. Dey, M. Maheshwari, and N. R. Jana, "Defective glucocorticoid hormone receptor signaling leads to increased stress and anxiety in a mouse model of Angelman syndrome," *Human Molecular Genetics*, vol. 21, no. 8, pp. 1824–1834, 2012.

[26] J. Barik, F. Marti, C. Morel et al., "Chronic stress triggers social aversion via glucocorticoid receptor in dopaminoceptive neurons," *Science*, vol. 339, no. 6117, pp. 332–335, 2013.

[27] M. L. Lehmann, R. A. Brachman, K. Martinowich, R. J. Schloesser, and M. Herkenham, "Glucocorticoids orchestrate divergent effects on mood through adult neurogenesis," *The Journal of Neuroscience*, vol. 33, no. 7, pp. 2961–2972, 2013.

[28] D. Suri and V. A. Vaidya, "Glucocorticoid regulation of brain-derived neurotrophic factor: relevance to hippocampal structural and functional plasticity," *Neuroscience*, vol. 239, pp. 196–213, 2013.

[29] M. N. Hill, C. J. Hillard, and B. S. McEwen, "Alterations in corticolimbic dendritic morphology and emotional behavior in cannabinoid CB_1 receptor-deficient mice parallel the effects of chronic stress," *Cerebral Cortex*, vol. 21, no. 9, pp. 2056–2064, 2011.

[30] M. N. Hill, S. A. Kumar, S. B. Filipski et al., "Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure," *Molecular Psychiatry*, vol. 18, no. 10, pp. 1125–1135, 2013.

[31] J. P. Herman, M. M. Ostrander, N. K. Mueller, and H. Figueiredo, "Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 29, no. 8, pp. 1201–1213, 2005.

[32] E. B. Binder, "The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders," *Psychoneuroendocrinology*, vol. 34, no. 1, pp. S186–S195, 2009.

[33] N. Fani, D. Gutman, E. B. Tone et al., "*FKBP5* and attention bias for threat: associations with hippocampal function and shape," *JAMA Psychiatry*, vol. 70, no. 4, pp. 392–400, 2013.

[34] S. Pietropaolo, M. G. Goubran, C. Joffre et al., "Dietary supplementation of omega-3 fatty acids rescues fragile X phenotypes in Fmr1-Ko mice," *Psychoneuroendocrinology*, vol. 49, no. 1, pp. 119–129, 2014.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 7383724, 18 pages
http://dx.doi.org/10.1155/2016/7383724

Research Article

Altered ERK1/2 Signaling in the Brain of Learned Helpless Rats: Relevance in Vulnerability to Developing Stress-Induced Depression

Yogesh Dwivedi and Hui Zhang

Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Correspondence should be addressed to Yogesh Dwivedi; ydwivedi@uab.edu

Received 13 August 2015; Revised 8 October 2015; Accepted 20 October 2015

Academic Editor: Laura Musazzi

Extracellular signal-regulated kinase 1/2- (ERK1/2-) mediated cellular signaling plays a major role in synaptic and structural plasticity. Although ERK1/2 signaling has been shown to be involved in stress and depression, whether vulnerability to develop depression is associated with abnormalities in ERK1/2 signaling is not clearly known. The present study examined ERK1/2 signaling in frontal cortex and hippocampus of rats that showed vulnerability (learned helplessness, (LH)) or resiliency (non-learned helplessness, (non-LH)) to developing stress-induced depression. In frontal cortex and hippocampus of LH rats, we found that mRNA and protein expressions of ERK1 and ERK2 were significantly reduced, which was associated with their reduced activation and phosphorylation in cytosolic and nuclear fractions, where ERK1 and ERK2 target their substrates. In addition, ERK1/2-mediated catalytic activities and phosphorylation of downstream substrates RSK1 (cytosolic and nuclear) and MSK1 (nuclear) were also lower in the frontal cortex and hippocampus of LH rats without any change in their mRNA or protein expression. None of these changes were evident in non-LH rats. Our study indicates that ERK1/2 signaling is differentially regulated in LH and non-LH rats and suggests that abnormalities in ERK1/2 signaling may be crucial in the vulnerability to developing depression.

1. Introduction

Depression is a debilitating psychiatric illness with a lifetime prevalence rate of about 5–20% [1–3]. A large number of depressed patients do not respond to antidepressants and a majority of them show resistance to treatment [4, 5]. This could partially be due to a lack of understanding of the molecular mechanisms associated with the etiology and pathogenesis of depression.

In recent years, the hypothesis that depression is associated with altered gene-environment interaction and impaired synaptic and structural plasticity has gained significant attention [6–9]. Extracellular signal-regulated kinases 1/2 (ERK1/2) signaling, which belongs to a large family of mitogen-activated protein kinase signaling cascades, has consistently been shown to have a major impact on both synaptic plasticity and structural plasticity. This is evident from studies showing their role in long-term potentiation, long-term depression, and the regulation of neuronal survival via neurotrophic/growth factors [10–12]. In this signaling pathway, ERK1 and ERK2 are the two major components. Both ERK1 and ERK2 are activated by upstream mitogen-activated protein kinase kinases MEK1 and MEK2 via phosphorylation at threonine and tyrosine residues within their activation loop [13]. This phosphorylation facilitates transduction of extracellular signals from cell surface receptors to the nucleus because phosphorylated ERK1 and ERK2 are translocated from cytosol to nucleus where they further phosphorylate target proteins and inhibit or activate transcription of a large number of genes [14]. Activated ERK1 and ERK2 can also affect the functions of various proteins within the cytosol. Interestingly, because of a high homology

in their amino acid sequences, ERK1 and ERK2 share several common substrates [15, 16] that regulate neuronal excitability, histone modifications, synaptogenesis, and cell cycle [17–23], which thus participate in behavioral and cognitive processes [24–26]. ERK1/2 signaling is terminated via dephosphorylation by "dual function" MAP kinase phosphatases [27, 28].

Because ERK1 and ERK2 regulate synaptic plasticity and structural plasticity, in recent years, several studies have focused their possible role in stress-related disorders such as depression. We were the first to demonstrate that ERK1/2 signaling was hypoactive in the frontal cortical (Brodmann areas 8, 9, and 10) and hippocampal brain areas of depressed patients [29–31]. Recently, an integrated transcriptome analysis derived from rat and human prefrontal cortex has identified ERK1/2 as one of the leading signaling kinases to be highly associated with depression [32]. At the behavioral level, ERK1 ablation in mice causes hyperactivity and resistance to behavioral despair [33, 34] and treatment of rats with MEK inhibitor induces mood disorder-related behavioral deficits [35]. On the other hand, peripheral injection of MEK inhibitor eliminates the response to antidepressants in behavioral despair [35]. Since adaptive/maladaptive response to stress is crucial in inducing depression, it is interesting to examine whether vulnerability or resiliency to developing depression is associated with differential regulation of ERK1/2 signaling.

To do so, in the present study, we used an animal model of depression that can distinguish vulnerability or susceptibility to developing stress-induced depression. This model is based on proactive interference with the acquisition of escape or avoidance response when animals are subjected to unpredicted and uncontrollable stress [36]. In this model, which is termed as learned helplessness (LH) model of depression, rodents show emotional, cognitive, and motivational deficits. On the other hand, the non-learned helpless animals (non-LH, resilient), although given the same uncontrollable and unpredictable stress, fail to show such responses. This provides an opportunity to distinguish the neurobiological factors associated with resiliency versus vulnerability to developing depression. In our earlier studies, we had modified the stress paradigm in such a way that it significantly prolonged the duration of depressive behavior from 24 hours to 14 days [37–39]. This is quite advantageous in examining the factors associated with chronic depression. Using this animal model, we explored whether ERK1/2 signaling plays a role in developing depressive behavior. For this, in the frontal cortex and hippocampus of LH, non-LH, and tested control (TC) rats, we determined the activation and expression of ERK1 and ERK2 at both transcriptional and translational levels. The activation of ERK1 and ERK2 was determined in cytosolic and nuclear fractions by examining expression levels of phosphorylated ERK1 and ERK2 and their mediated phosphorylation of substrate Elk1. In addition, we examined functional significance of altered ERK1 and ERK2 by determining activation and expression of their downstream common substrates RSK (90 kDa S6 kinase) and MSK (mitogen and stress-activated kinase).

2. Materials and Methods

Male Sprague-Dawley rats (Holtzman strain) weighing between 325 and 370 g were obtained from Harlan Sprague-Dawley Laboratories, USA. Rats were placed at 21 ± 1°C temperature and 55 ± 5% humidity. Initially, during acclimatization, rats were placed randomly (3/cage); however, after initial behavioral testing, they were grouped according to their behavioral phenotype. The light and dark cycle was 12 hours. Rats were given ad libitum food and water. The rats were acclimatized for two weeks prior to the start of the shock paradigm. All the behavioral experiments were performed between 8 and 10 am. The protocol to induce learned helpless behavior was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. All the experiments were done in 6 LH, 6 non-LH, and 6 TC rats.

2.1. Induction of Learned Helpless Behavior. The protocol for the induction of learned helpless behavior has been described in great detail in our earlier publications [37–39]. Briefly, rats were subjected to 100 random inescapable tail shocks (IS) at the intensity of 1.0 mAmp for 5 seconds. The average interval between two shocks was 60 seconds. Escape latency was determined 24 hours later. These rats were given additional IS on day 7 and tested for escape latency on day 8 and again on day 14. Another group of rats were tested for escape latency without giving any shock. These rats were termed as tested control (TC). The escape latency was tested using two different trials: FR1 and FR2. In FR1 (5 trials), rats were given foot shock at the intensity of 0.6 mAmp at variable time intervals. The rats had to escape the foot shock by moving from one chamber to another. In FR2 (25 trials), the rats had to cross from one chamber to the other and had to come back to the original chamber to terminate the shock. The shocks were terminated automatically after 30 sec. Escape latencies were automatically recorded through computer generated programs (Med Associates, USA). All the rats were sacrificed 24 hours after the last escape latency test. Rats were decapitated and blood was collected for plasma corticosterone levels (Abcam, USA). Various brain areas were dissected immediately and kept at −80°C for analyses. Based on escape latency in FR2 trial, rats were divided into two groups: learned helpless (LH, showing escape latency ≥20 seconds) and non-learned helpless (non-LH, showing escape latency <20 seconds). Generally, the rats who showed LH behavior in the FR2 trial (day 2) remained LH throughout the experimental duration (day 14). We found almost equal distribution of rats among LH and non-LH groups.

2.2. Isolation of Cytosolic and Membrane Fractions. These fractions were isolated at 4°C as previously described [37]. Briefly, frontal cortex and hippocampus were homogenized in 10 mM HEPES buffer (pH 7.4), containing various protease and phosphatase inhibitors (NaF [50 mM], phenylmethylsulfonyl fluoride [1 mM], ethylene glycol tetraacetic acid, EGTA [1 mM], sodium orthovanadate [2 mM], ethylenediaminetetraacetic acid, EDTA [1 mM], sodium pyrophosphate [10 mM], para-nitrophenylphosphate [4 Mm], leupeptin

[10 μg/mL], pepstatin A [10 μg/mL], aprotinin [4 μg/mL], and NP-40 [0.5%]). The homogenate was centrifuged at 12,000 ×g for 1 hour. The supernatant was again centrifuged at 100,000 ×g for 1 hour. The pellet was the membrane fraction, whereas the supernatant was used as cytosolic fraction. The pellet containing the membrane fraction was suspended in Tris-HCl buffer (50 mM, pH 7.5) containing various protease and phosphatase inhibitors as described above. The protein contents were measured by Lowry et al. [40].

2.3. Isolation of Nuclear Fraction. Frontal cortex and hippocampus were homogenized in a 10 mM HEPES buffer (pH 7.4) containing various protease and phosphatase inhibitors as detailed above and spun at 100,000 ×g for 30 min. The pellet was again homogenized in 20 mM HEPES buffer (pH 7.4) containing glycerol (50%), NaCl (84 mM), $MgCl_2$ (1.5 mM), EDTA (0.4 mM), and various protease inhibitors, and it was kept at 4°C for 15 min while shaking. The suspension was centrifuged at 20,000 ×g for 15 min. As described earlier [37], the purity of these fractions was confirmed using antibodies against histone H2B (nuclear) and PKA RII subunit (cytosolic) (data not shown).

2.4. ERK1 and ERK2 Assay. The determination of catalytic activities of ERK1 and ERK2 was performed by the procedure described earlier [29]. The active ERK1 and ERK2 were immunoprecipitated using p-ERK1/2 antibody (Santa Cruz Biotechnology, USA). The immunoprecipitation technique was followed as discussed in our earlier publication [41]. Protein A Sepharose beads were used to pull down active ERK1 and ERK2 followed by centrifugation (2,500 rpm for 30 min) and the suspension was washed twice with 20 mM Tris lysis buffer (pH 7.5) containing various phosphatase and protease inhibitors and twice with 25 mM Tris (pH 7.5) kinase buffer containing β-glycerophosphate (5 mM), 2 mM dithiothreitol (2 mM), sodium orthovanadate (0.1 mM), and $MgCl_2$ (10 mM). The kinase reaction was initiated with suspending the pellet in kinase buffer containing ATP (200 μM) and Elk1 fusion protein (2 μg; GST fused to Elk1 codons 307–428). The reaction was carried out for 30 minutes at 30°C and terminated by adding Laemmli buffer. Samples were run for gel electrophoresis followed by transfer to nitrocellulose membrane and incubation with primary (p-Elk1; Santa Cruz Biotechnology, USA) and HRP-conjugated anti-rabbit secondary antibody. Each membrane was stripped and reprobed with β-actin primary antibody (Sigma Chemical Co., USA) and anti-mouse secondary antibody. The optical density of each band was calculated using software provided by Loats Image Analysis System, USA. A ratio of the optical densities of Elk1 and corresponding β-actin band was determined.

2.5. mRNA Expression of ERK1, ERK2, RSK1, and MSK1 by Quantitative Real-Time PCR. RNA from each sample was isolated using TRIzol (Life Technologies, USA), and purity ratios (260/280 nm and 260/230 nm) were assessed by NanoDrop (Thermo Scientific). TaqMan primers and probe sets (Life Technologies, USA) were used for qRT-PCR. The methods for qRT-PCR were followed as described in the manufacturer's protocol and discussed in our earlier publication [39]. β-actin was used as endogenous control (normalizer). Fold changes were calculated using $2^{-\Delta\Delta Ct}$ method [39].

2.6. Immunolabeling of ERK1 and ERK2 and total and ERK1/2-Mediated Phosphorylation of RSK1 and MSK1. Expression levels of total ERK1 and ERK2 were determined in total tissue lysates, whereas total and phosphorylated RSK1 were determined in both the cytosolic and nuclear fractions by Western blot [29, 30]. The levels of total and p-MSK1 were determined in the nuclear fraction. For ERK1/2-mediated phosphorylation of RSK1 and MSK1, tissue samples were immunoprecipitated with p-ERK1/2 antibody as discussed above. Samples containing 25 μg of protein were subjected to gel electrophoresis followed by transfer to nitrocellulose membrane. Following were the primary antibodies used: ERK1, ERK2 (Santa Cruz Biotechnology, USA), RSK1 (Abcam, USA), MSK1 (Santa Cruz Biotechnology, USA), p-ERK1/ERK2 (Santa Cruz Biotechnology, USA), p-RSK1 (phospho-Ser380; Abcam, USA), and p-MSK1 (phospho-Ser360; Abcam, USA). The dilution for each antibody was as follows: ERK1 (1 : 1000), ERK2 (1 : 1000), RSK1 (1 : 1500), MSK1 (1 : 1000), p-ERK1/ERK2 (1 : 1000), p-RSK1 (1 : 1500), or p-MSK1 (1 : 1000). The nitrocellulose membranes were stripped using a buffer (Chemicon International, USA) and exposed with β-actin antibody. The bands on the autoradiograms were determined and ratio of the optical density of the protein of interest to the corresponding β-actin band was calculated. The results are given as percent of the control.

2.7. Immunoprecipitation and Assay of RSK1 and MSK1 Catalytic Activities. Tissues lysates (containing 100 μg protein) were immunoprecipitated using antibodies for MSK1 or RSK1 as described above. Their catalytic activities were assayed essentially by the procedure described by Sapkota et al. [42]. The immunoprecipitates derived from protein A Sepharose were washed with 50 mM Tris-HCl buffer (pH 7.5) containing EGTA (0.1 mM), 2-mercaptoethanol (0.1% v/v), PKA inhibitor (2.5 μM; TTYADFIASGRTGRRNAIHD), and magnesium acetate (10 mM). The reaction was initiated by adding [γ-^{32}P]ATP (~1000 cpm/pmol) and Crosstide (GRPRTSSFAEG, 30 μM; Enzo Life Sciences, USA). The assay was terminated after 15 min. One milliunit of activity denotes the amount of enzyme that catalyzes the phosphorylation of 1 pmol Crosstide per minute. The results are provided as percent of control.

2.8. Statistical Analysis. Statistical Package for the Social Sciences (SPSS) was used for all the data analysis. The data are represented as mean ± standard deviation (SD). TC, LH, and non-LH groups were compared using one-way ANOVA. Post hoc comparisons were calculated by Tukey's method of multiple comparisons. All the 3 groups were compared among each other: LH group was compared with TC and non-LH groups; non-LH group was compared with LH and TC groups; TC group was compared with LH and non-LH groups. Significance level was set at $P < 0.05$. Correlation

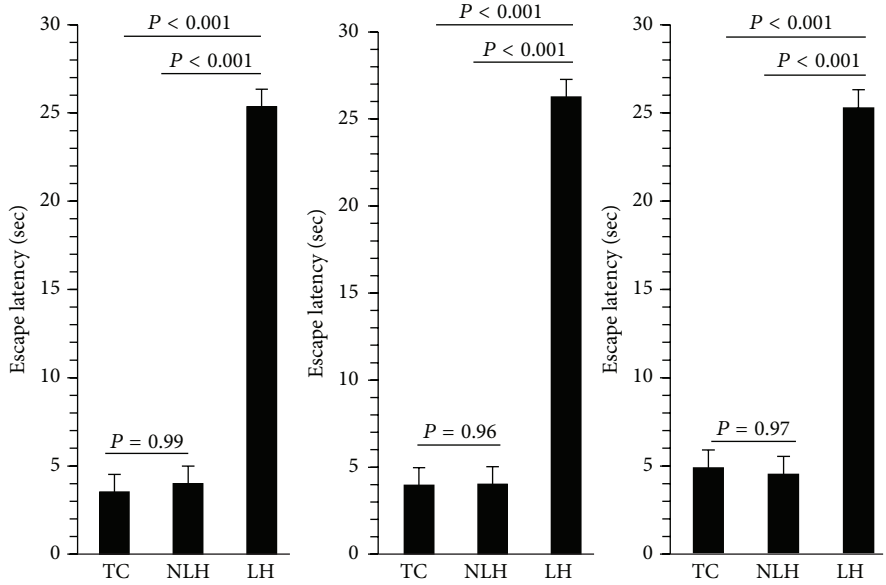


Figure 1: Escape latencies in LH, non-LH, and TC rats measured on days 2, 8, and 14. Data are the mean ± SD from 6 rats in each group. Overall group differences were as follows: day 2: $F = 2, 15$, df = 179, and $P < 0.001$; day 8: $F = 2, 15$, df = 134, and $P < 0.001$; and day 14: $F = 2, 15$, df = 132, and $P < 0.001$. Individual group analysis revealed that, at all these time intervals, LH group was significantly different from NLH or TC groups ($P < 0.001$). There was no significant difference between NLH and TC groups.

analyses were performed using Pearson product moment. Statistical significance levels (overall and individual) are provided in each figure legend.

3. Results

3.1. Escape Latencies. As mentioned above, we determined escape latencies on days 2, 8, and 14. The escape latencies in LH rats were significantly greater than non-LH and TC rats at all these 3 time points. There were no significant differences between NLH and TC groups (Figure 1).

3.2. Serum Corticosterone Level. As indicated in Figure 2, the plasma levels of CORT were not significantly different between non-LH, LH, and TC rats.

3.3. Immunolabeling of ERK1 and ERK2. Representative Western blots for ERK1 and ERK2 in frontal cortex and hippocampus of various groups of rats are given in Figures 3(a) and 3(c), respectively. It was found that the expressions of ERK1 and ERK2 were significantly decreased in frontal cortex (Figure 3(b)) and hippocampus of LH rats (Figure 3(d)) when compared with TC and non-LH rats. On the other hand, the levels of ERK1 and ERK2 were similar in TC and non-LH rats in both these brain areas.

3.4. mRNA Expression of ERK1 and ERK2. The gene expression of these two kinases was determined in the same samples in which their protein levels were examined. As with protein levels, mRNA expressions of ERK1 and ERK2 were significantly decreased in both frontal cortex (Figure 4(a)) and hippocampus (Figure 4(b)) of LH rats, whereas no significant differences were found between non-LH and TC rats.

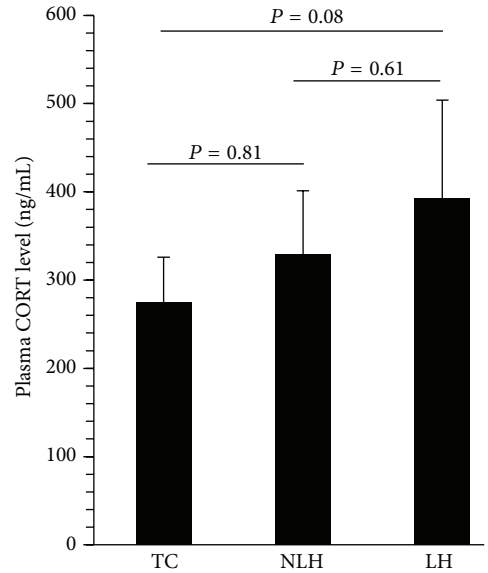


Figure 2: Plasma corticosterone (CORT) levels in TC, non-learned helpless (non-LH), and learned helpless (LH) rats. Data are the mean ± SD from 6 animals per group. The overall group differences were as follows: df = 2, 15, $F = 3.07$, and $P = 0.29$.

3.5. Catalytic Activities of ERK1 and ERK2. In previous studies, we have characterized ERK1 and ERK2 catalytic activities in cytosol and membrane fractions obtained from

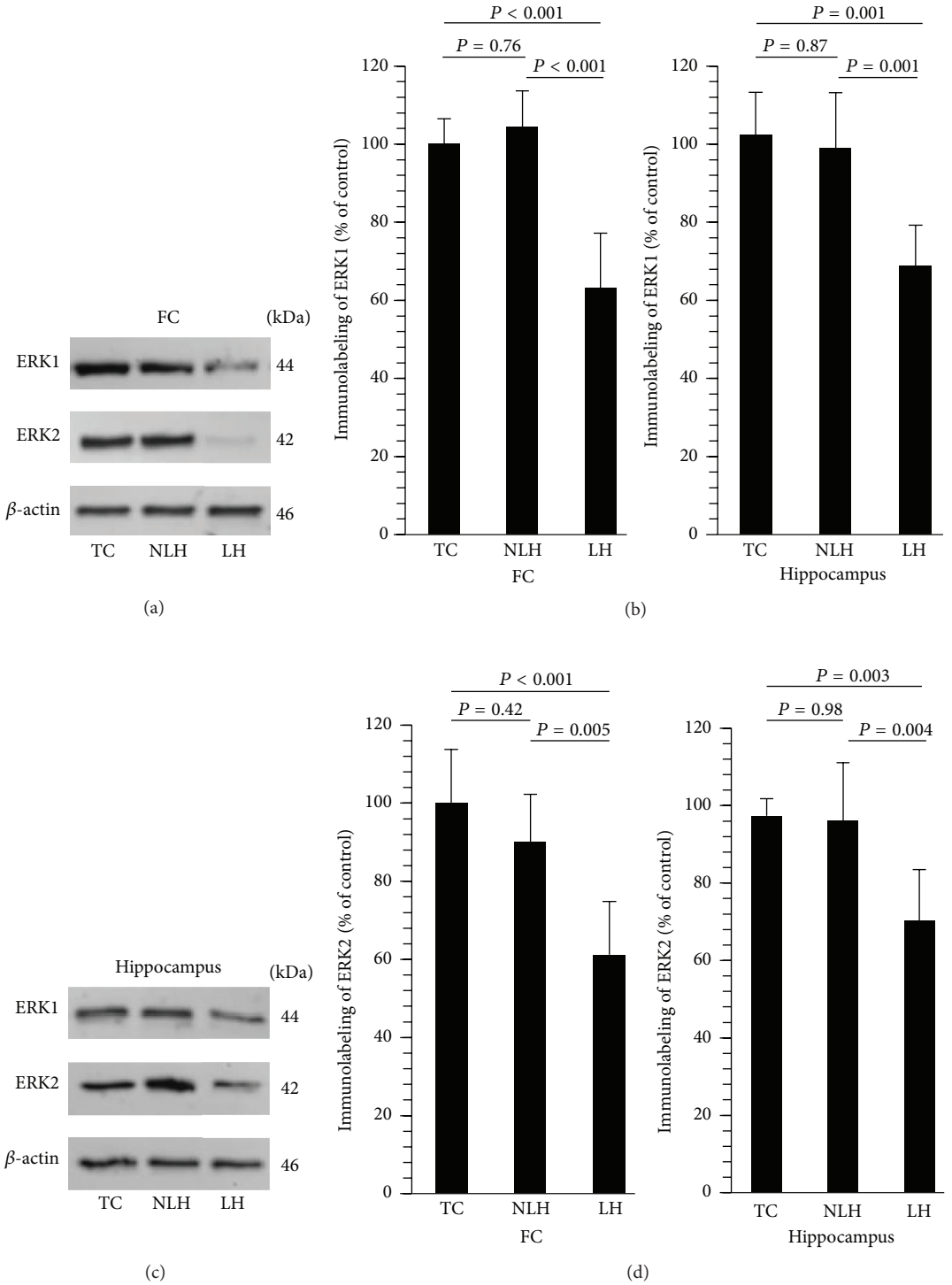
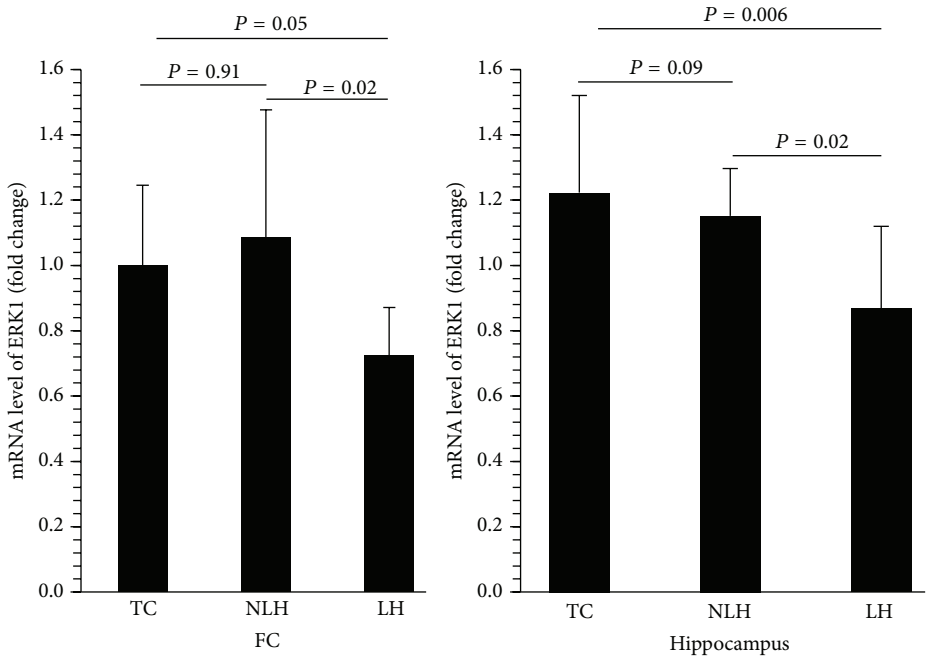
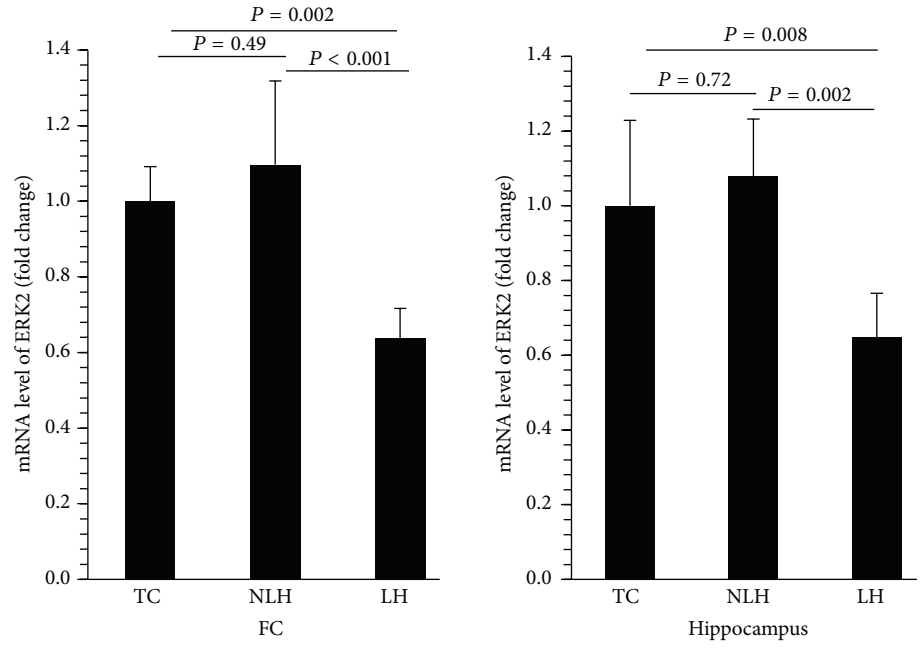


Figure 3: Western blots showing immunolabeling of ERK1 and ERK2 in frontal cortex (FC) (a) and hippocampus (b) of tested control (TC), non-NLH (NLH), and learned helpless (LH) rats. β-actin was used as endogenous control and a ratio of optical density of ERK1 and ERK2 to the optical density of the corresponding β-actin was calculated. (b) Mean ± SD of protein expression levels of ERK1 and ERK2 in FC (c) and hippocampus (d) of TC, non-LH, and LH rats ($n = 6$/group). Overall group differences in the 3 groups are as follows. ERK1: FC, df = 2, 15, $F = 28.10$, and $P < 0.001$; hippocampus, df = 2, 15, $F = 14.35$, and $P < 0.001$. ERK2: FC, df = 2, 15, $F = 14.09$, and $P < 0.001$; hippocampus, df = 2, 15, $F = 10.16$, and $P < 0.002$.



(a)



(b)

Figure 4: mRNA levels of ERK1 and ERK2 in frontal cortex (FC) (a) and hippocampus (b) of TC, non-LH, and LH rats. Data are the mean ± SD from 6 animals in each group. Overall group differences in the 3 groups are as follows. ERK1: FC, df = 2, 15, $F = 5.17$, and $P = 0.02$; hippocampus, df = 2, 15, $F = 7.92$, and $P = 0.004$. ERK2: FC, df = 2, 15, $F = 16.53$, and $P < 0.001$; hippocampus, df = 2, 15, $F = 10.51$, and $P = 0.001$.

human postmortem samples and found that catalytic activities of these kinases reside in the cytosol fraction; in the membrane fraction, their activities were either not detectable or marginally detectable. Similar reports have been shown in previous studies by other investigators [43, 44]. To ensure that similar phenomenon occurs in rat brain, we characterized their activities in the cytosolic and membrane fractions and found similar results (data not shown). Therefore, in subsequent experiments, we determined ERK1 and ERK2 activities in the cytosol fraction. Because there is a translocation of ERK1 and ERK2 from cytosol to nucleus upon phosphorylation, we examined the

catalytic activities of ERK1 and ERK2 also in the nuclear fraction.

Figures 5(a) and 5(c) show representative Western blots depicting ERK1/2 activity (p-Elk1 as a measure of activated ERK1/2) in cytosolic and nuclear fractions of frontal cortex and hippocampus obtained from various groups of rats (TC, non-LH, and LH). The kinase activities of ERK1 and ERK2 were significantly down in cytosolic and nuclear fractions of LH rats both in frontal cortex (Figure 5(b)) and hippocampus (Figure 5(d)). ERK1/2 activity was not altered in non-LH rats compared with TC rats in either cytosol fraction or nuclear fraction.

3.6. Phosphorylation of ERK1 and ERK2. To confirm whether the activity of ERK1 and ERK2 was associated with their altered phosphorylation forms, we measured the levels of p-ERK1/2 in the same tissue lysates of frontal cortex and hippocampus in which we determined ERK1 and ERK2 catalytic activities (Figures 6(a) and 6(c)). As with catalytic activity, phosphorylation of both ERK1 and ERK2 was significantly lower in LH rats (frontal cortex: Figure 6(b); hippocampus: Figure 6(d)). This phosphorylation was not altered in non-LH rats compared with TC rats in either brain area.

3.7. Correlation between Protein Expression Levels of ERK1 and ERK2 and Their Catalytic Activities. In the LH group, the catalytic activity of ERK1/2 was significantly correlated with protein expression of ERK1 in frontal cortex (cytosol: $r = 0.71$ and $P = 0.001$; nuclear: $r = 0.85$ and $P < 0.001$) and hippocampus (cytosol: $r = 0.61$ and $P = 0.007$; nuclear: $r = 0.79$ and $P < 0.001$). Similarly, catalytic activity of ERK1/2 was significantly correlated with protein levels of ERK2 in frontal cortex (cytosol: $r = 0.82$ and $P < 0.001$; nuclear: $r = 0.70$ and $P = 0.001$) and hippocampus (cytosol: $r = 0.79$ and $P < 0.001$; nuclear: $r = 0.83$ and $P < 0.001$) of LH rats.

3.8. ERK1/2-Mediated Phosphorylation of RSK1 and MSK1. We examined ERK1/2-mediated phosphorylation of RSK1 in both cytosolic and nuclear fractions. ERK1/2-mediated phosphorylation of MSK1 was determined only in the nuclear fraction. Immunolabeling of p-RSK1 in the nuclear and cytosolic fractions of frontal cortex and hippocampus is shown in Figures 7(a) and 7(b), respectively. The levels of p-RSK1 were significantly lower in both cytosolic and nuclear fractions of frontal cortex and hippocampus obtained from LH rats (Figure 7(c)). Non-LH group did not show these changes in either frontal cortex or hippocampus compared with TC group. The Western blots of p-MSK1 in the nuclear fraction of frontal cortex and hippocampus are depicted in Figure 7(d) and are depicted as bar diagram in Figure 7(e). The level of MSK1 was significantly reduced in frontal cortex and hippocampus of LH rats without any change in non-LH rats.

3.9. Catalytic Activities of RSK1 and MSK1. ERK1/2-mediated catalytic activities of RSK1 and MSK1 were determined in the tissue lysates of frontal cortex and hippocampus. Both RSK1 and MSK1 catalytic activities were significantly decreased in brain areas of LH rats (Figures 8(a) and 8(b)). These activities were unaltered in non-LH rats compared with TC rats.

3.10. mRNA and Protein Levels of RSK1 and MSK1. mRNA expressions of RSK1 or MSK1 were determined in the total fraction of frontal cortex and hippocampus. We did not find any significant change in the expression of RSK1 or MSK1 in the frontal cortex or hippocampus of any of the groups studied (Figures 9(a) and 9(b)).

Protein levels of RSK1 were determined in cytosolic and nuclear fractions and those of MSK1 were determined in nuclear fraction of frontal cortex and hippocampus of TC, non-LH, and LH rats. Western blots of RSK1 in cytosolic and nuclear fractions of frontal cortex and hippocampus are shown in Figures 10(a) and 10(b), respectively. As with mRNA expression levels, no significant changes were found in the expression of RSK1 in cytosolic fraction or nuclear fraction of frontal cortex (Figure 10(c)) or hippocampus (Figure 10(d)) of LH rats. Similarly, the levels of MSK1 in nuclear fraction were unaltered in LH rats (Figures 10(e) and 10(f)).

3.11. Correlation between p-RSK1 and p-MSK1 with p-ERK1/2 Levels. The levels of p-RSK1 were significantly correlated with levels of p-ERK1/2 in frontal cortex (cytosolic: $r = 0.54$ and $P = 0.02$; nuclear: $r = 0.69$ and $P = 0.002$) and hippocampus (cytosolic: $r = 0.76$ and $P < 0.001$; nuclear: $r = 0.87$ and $P < 0.002$) of LH rats. Similarly, p-MSK1 and p-ERK1/2 were significantly correlated in the nuclear fraction of frontal cortex ($r = 0.82$; $P < 0.001$) and hippocampus ($r = 0.77$; $P < 0.001$) of LH rats.

4. Discussion

In the present study, we found that there is hypoactivation of ERK1/2 signaling in the brain of LH rats. This is based on several observations made in frontal cortex and hippocampus of LH rats compared with non-LH rats. For example, in LH rats, (1) catalytic activity of ERK1/2, measured as ERK1/2-mediated phosphorylation of Elk1, was significantly reduced; (2) phosphorylation and therefore the activation of ERK1 and ERK2 was significantly downregulated; (3) mRNA and protein expression of ERK1 and ERK2, measured independently, were significantly lower; (4) ERK1/2-mediated phosphorylation of downstream substrates RSK1 and MSK1 was significantly reduced; and (5) ERK1/2-mediated catalytic activities of both RSK1 and MSK1 were significantly lower. None of these changes were apparent in non-LH rats. It is pertinent to mention that our findings of reduced ERK1/2 signaling in the brain of LH rats represent abnormalities associated with prolonged depression as repeated stress paradigm used in the current study generated behavioral deficits that persisted for 14 days. In our earlier studies, we had found that single inescapable shock paradigm was sufficient to develop LH behavior; however, these behavioral deficits persisted only for 24 hours and were reversed thereafter [37, 38, 45]. In the future, it will be interesting to examine whether there is a differential regulation of ERK1/2 signaling in acute versus chronic depression as we have earlier reported in case of

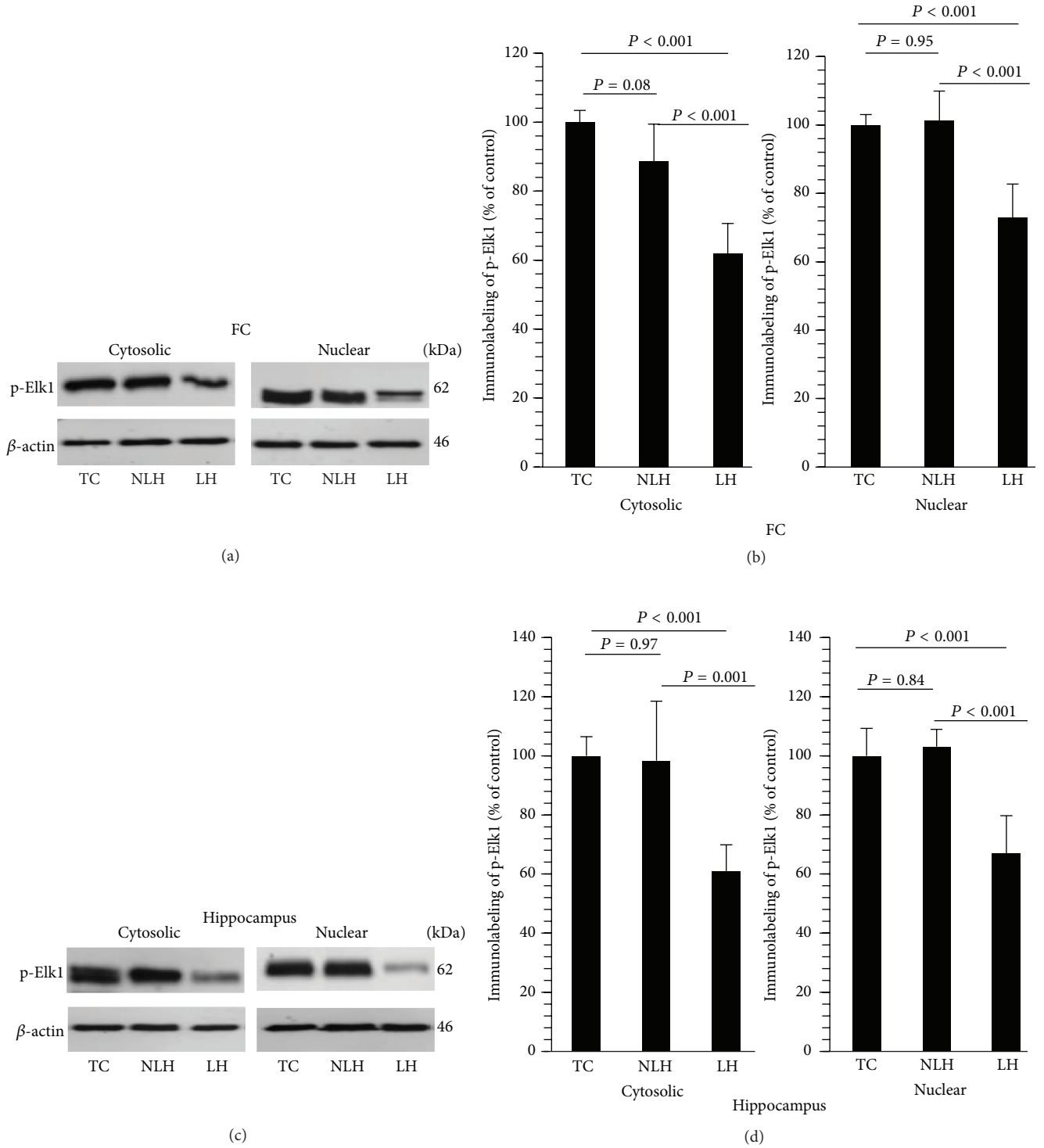


Figure 5: Catalytic activities of ERK1/2 (represented as ERK1/2-mediated phosphorylation of Elk1) in cytosolic and nuclear fractions of FC (a) and hippocampus (c) obtained from TC (tested controls), non-learned helpless (non-LH), and learned helpless (LH) rats. β-actin was used as normalizer. Mean ± SD of Elk1 phosphorylation in cytosolic and nuclear fractions of FC (b) and hippocampus (d) of TC, non-LH, and LH rats ($n = 6$/group). Overall group differences among TC, non-LH, and LH rats are as follows. FC: cytosolic, df = 2, 15, $F = 33.71$, and $P < 0.001$; nuclear, df = 2, 15, $F = 25.93$, and $P < 0.001$. Hippocampus: cytosolic, df = 2, 15, $F = 16.63$, and $P < 0.001$; nuclear, df = 2, 15, $F = 25.57$, and $P < 0.001$.

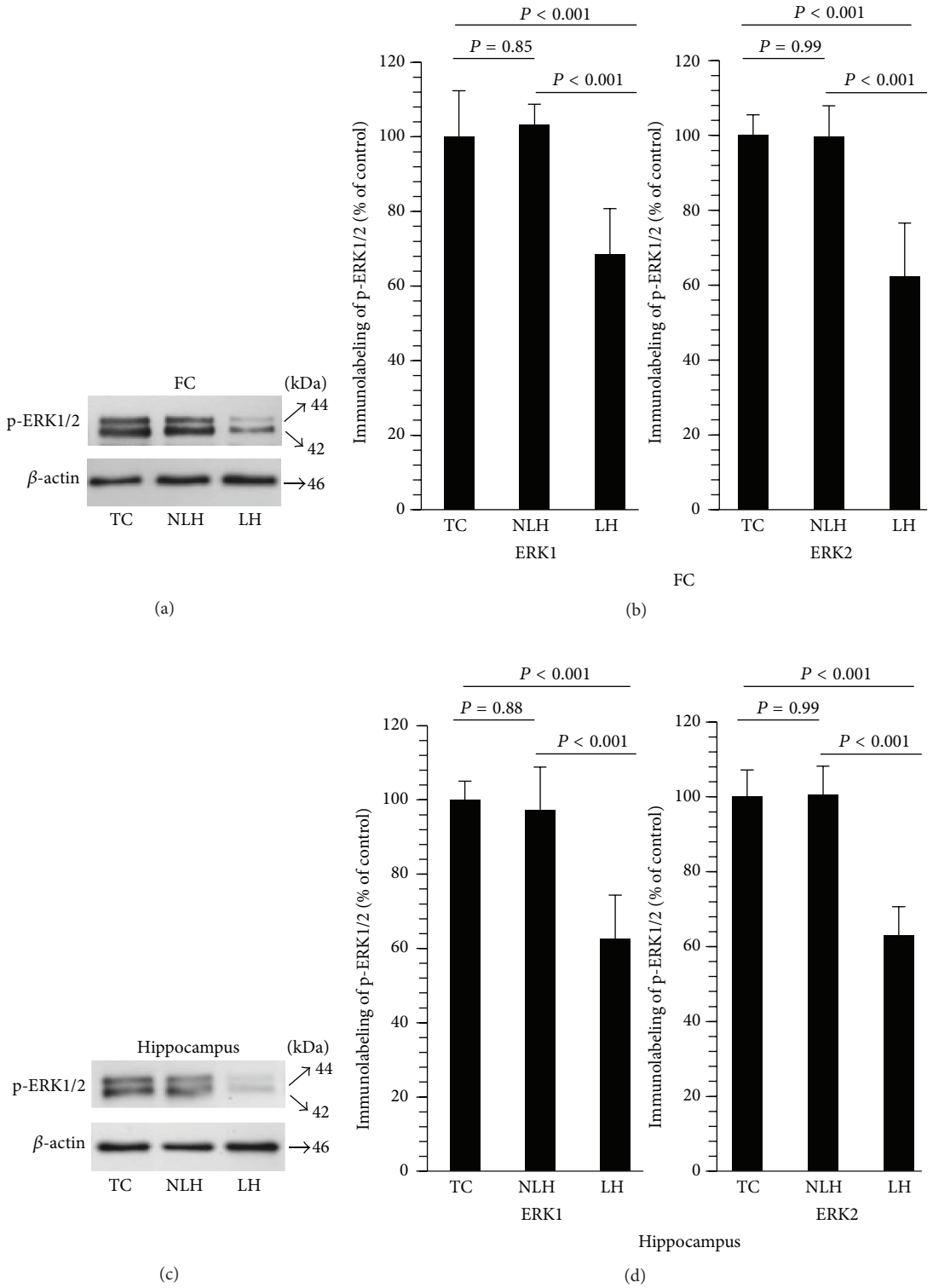


FIGURE 6: Immunolabeling of p-ERK1/2 in FC (a) and hippocampus (c) obtained from tested control (TC), non-learned helpless (non-LH), and learned helpless (LH) rats. β-actin was used as endogenous control. Mean ± SD of ERK1 and ERK2 phosphorylation in FC and hippocampus of TC, non-LH, and LH rats ($n = 6$/group) is depicted in (b) and (d), respectively. Overall group differences in the 3 groups are as follows. FC: ERK1, df = 2, 15, $F = 19.93$, and $P < 0.001$; ERK2, df = 2, 15, $F = 28.04$, and $P < 0.001$. Hippocampus: ERK1, df = 2, 15, $F = 26.12$, and $P < 0.001$; ERK2, df = 2, 15, $F = 48.89$, and $P < 0.001$.

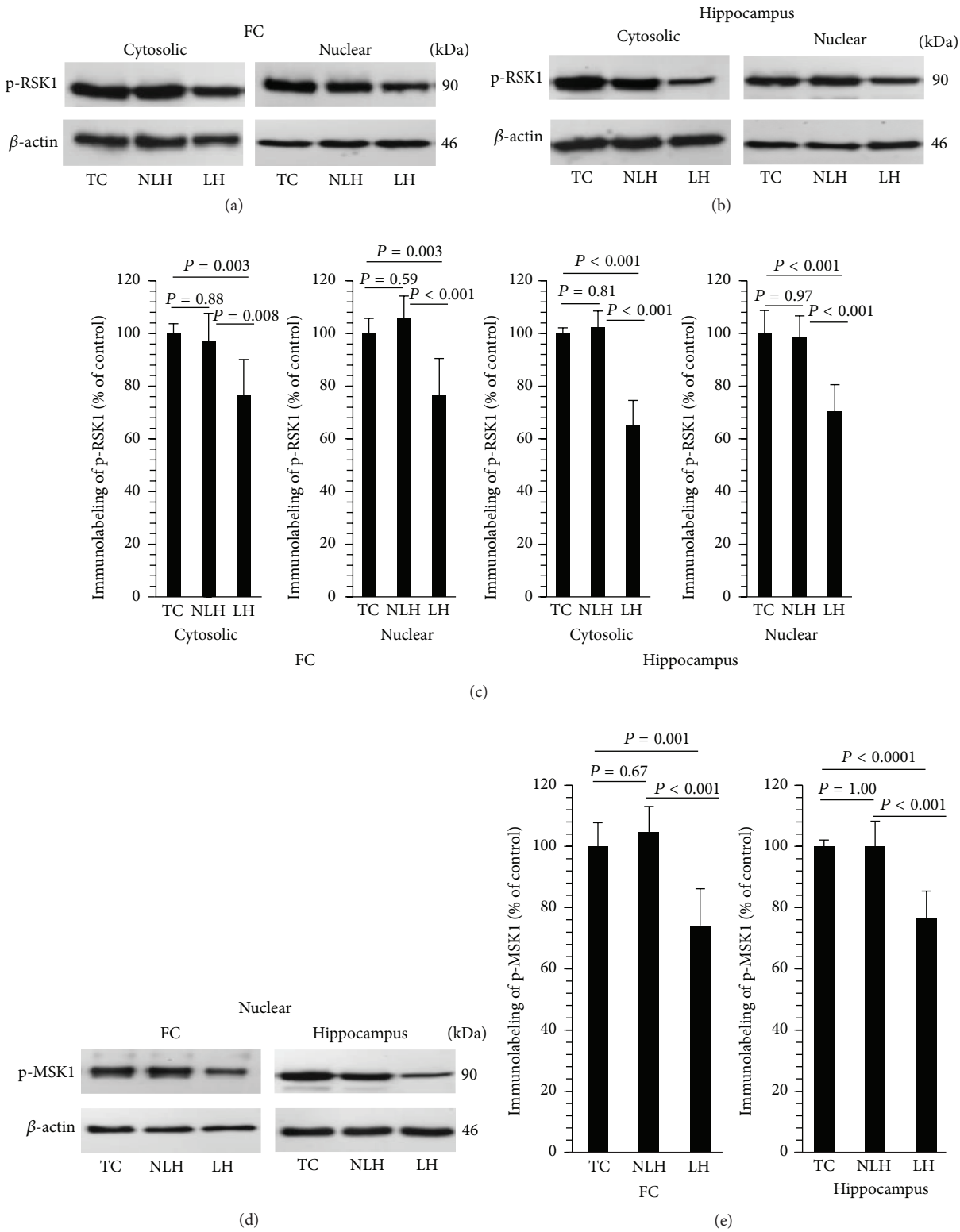


Figure 7: Representative Western blots showing ERK1/2-mediated phosphorylation of RSK1 in cytosolic and nuclear fractions of frontal cortex (FC) (a) and hippocampus (b) and MSK1 in nuclear fraction of FC and hippocampus (d) obtained from tested control (TC), non-learned helpless (non-LH), and learned helpless (LH) rats. β-actin was used as endogenous control. Differences in phosphorylation of RSK1 and MSK1 in FC and hippocampus between TC, non-LH, and LH rats are shown in (c) and (e), respectively. Data are the mean ± SD from 6 rats in each group. Overall group differences for RSK1 among TC, non-LH, and LH rats are as follows. FC: cytosolic, df = 2, 15, $F = 9.62$, and $P = 0.002$; nuclear, df = 2, 15, $F = 14.42$, and $P < 0.001$. Hippocampus: cytosolic, df = 2, 15, $F = 60.13$, and $P < 0.001$; nuclear, df = 2, 15, $F = 20.86$, and $P < 0.001$. Overall group differences for MSK1 in nuclear fraction among TC, non-LH, and LH rats are as follows. FC: df = 2, 15, $F = 17.73$, and $P < 0.001$; hippocampus: df = 2, 15, $F = 21.70$, and $P < 0.001$.

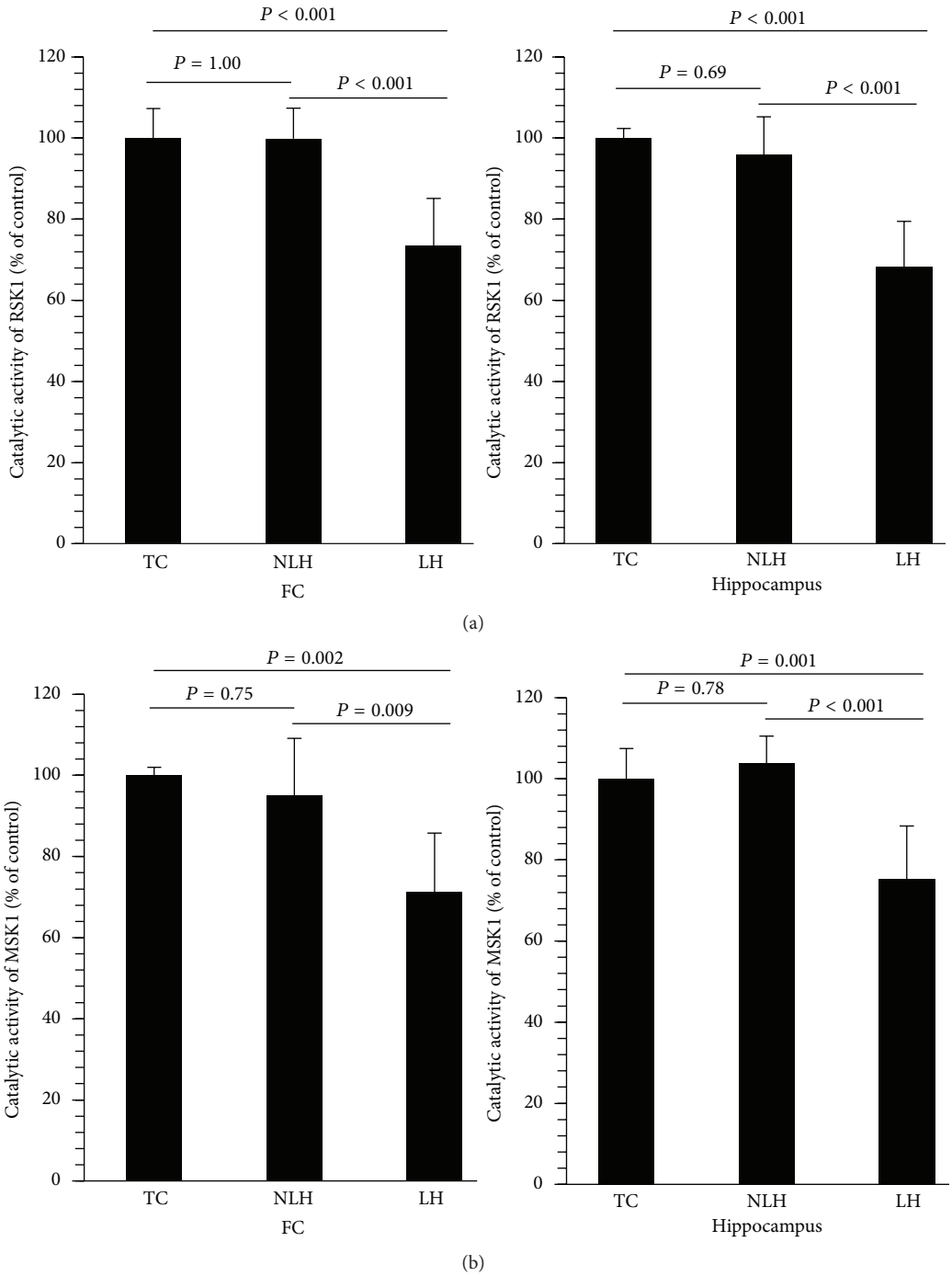


Figure 8: Catalytic activates of RSK1 and MSK1 in frontal cortex (FC) (a) and hippocampus (b) of tested control (TC), non-learned helpless (non-LH), and learned helpless (LH) rats. Overall group differences are as follows. RSK1: FC, df = 2, 15, $F = 17.21$, and $P < 0.001$; hippocampus, df = 2, 15, $F = 24.38$, and $P < 0.001$. MSK1: FC, df = 2, 15, $F = 10.21$, and $P < 0.002$; hippocampus, df = 2, 15, $F = 15.75$, and $P = 0.12$.

serotonergic receptors and associated signaling in LH rats [37, 38, 45], which may reflect adaptive versus maladaptive response.

Translocation of activated ERK1/2 is a crucial phenomenon in targeting substrates within the nucleus. Under resting conditions, ERK1 and ERK2 are primarily localized in cytosol [46]; however, once these kinases are phosphorylated by upstream MEK1 and MEK2, both ERK1 and ERK2 translocate to the nucleus [15]. Initially, we examined levels of activated (phosphorylated form) ERK1/2 in total tissue lysates and found that their activities were significantly reduced in LH rats without any change in non-LH rats.

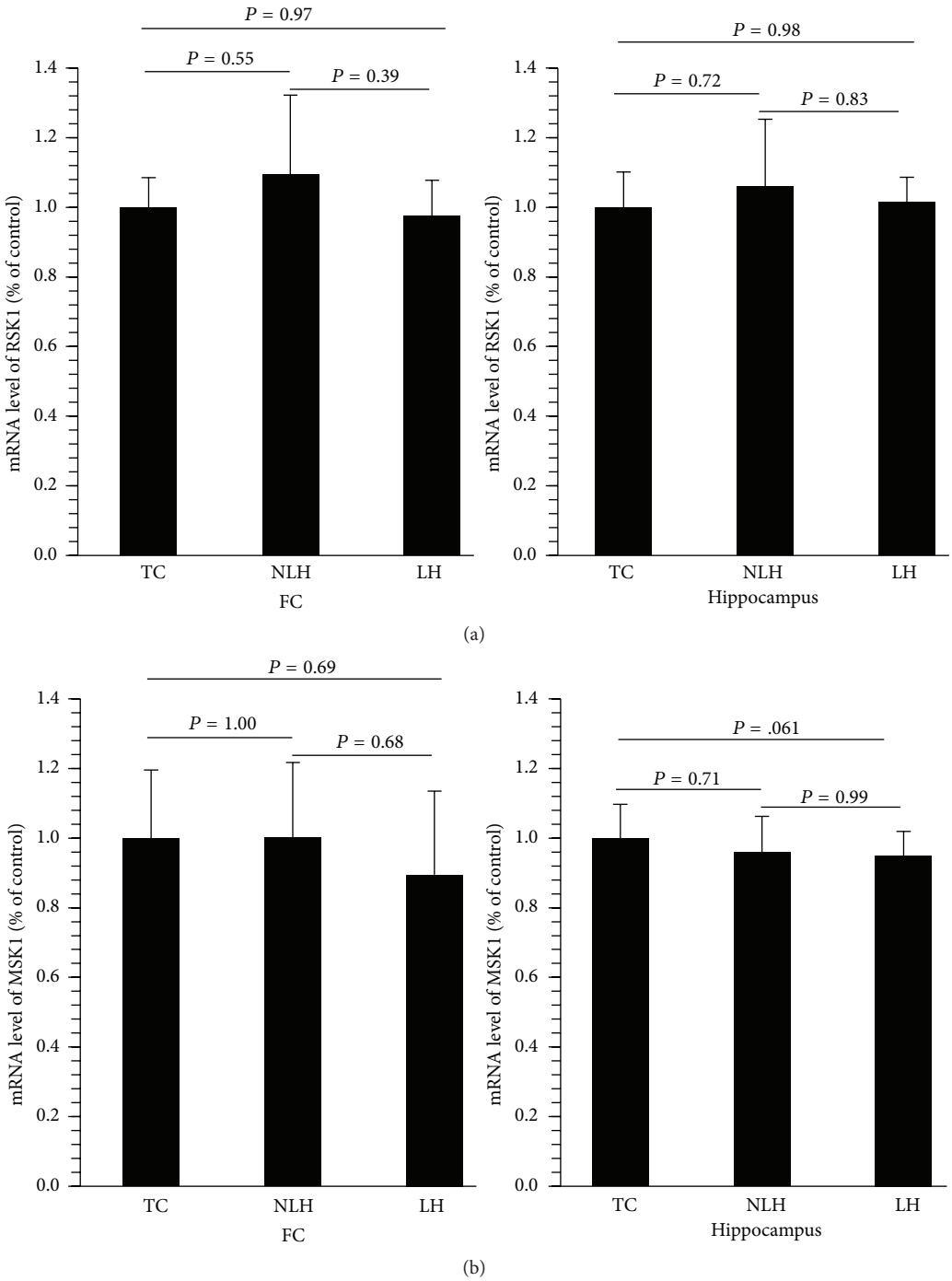


Figure 9: mRNA levels of RSK1 and MSK2 in frontal cortex (FC) (a) and hippocampus (b) of tested controls (TC), non-learned helpless (non-LH), and learned helpless (LH) rats. Data are the mean ± SD from 6 rats in each group. Overall group differences in the 3 groups are as follows. FC: df = 2, 15, $F = 1.00$, and $P = 0.39$; hippocampus: df = 2, 15, $F = 0.33$, and $P = 0.72$.

To further examine whether reduced phosphorylation of ERK1 and ERK2 was associated with alterations in their catalytic activities, we determined ERK1/2-mediated phosphorylation of Elk1 in cytosolic and nuclear fractions of frontal cortex and hippocampus. Interestingly, we found that the levels of p-Elk1 were lower in both these fractions, suggesting that the decrease in ERK1/2 activity is a generalized effect and may not be associated with translocation. This could be attributed to reduced expression of ERK1 and ERK2 as we have found in the brain of LH rats. ERK1 and ERK2 show a very close homology in amino acid sequences such that about 84% of amino acid residues are identical between these two kinases [43]. Also, the activation kinetics and substrate specificity for these two kinases are quite similar [47]. However,

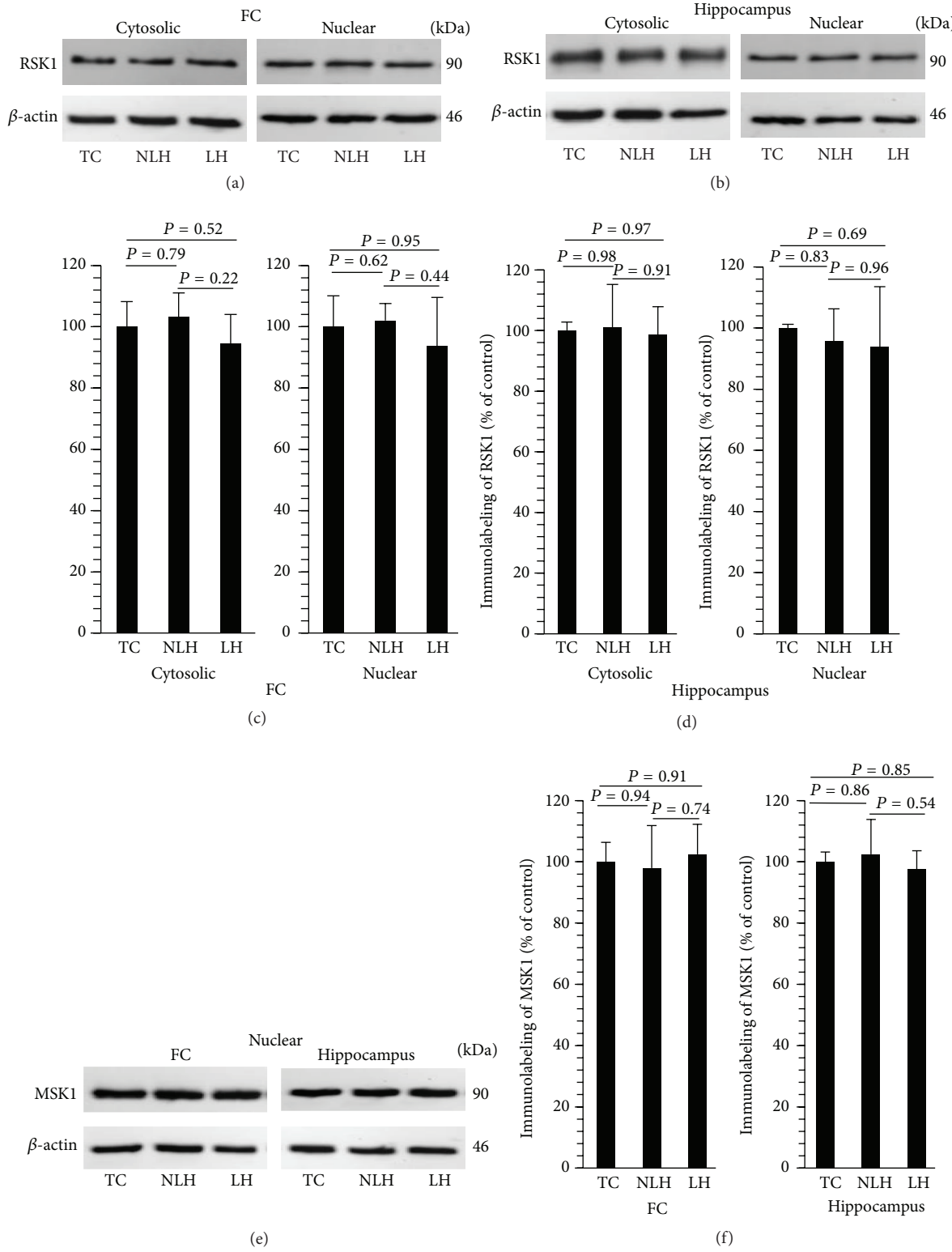


Figure 10: Representative immunoblots of total RSK1 in cytosolic and nuclear fractions of frontal cortex (FC) (a) and hippocampus (b) and MSK1 in nuclear fractions of FC and hippocampus (d) obtained from tested control (TC), non-learned helpless (non-LH), and learned helpless (LH) rats. β-actin was used as endogenous control. Differences in expression of RSK1 and MSK1 in FC and hippocampus between TC, non-LH, and LH rats are shown in (c) and (e), respectively. Data are the mean ± SD from 6 rats in each group. Overall group differences for RSK1 among TC, non-LH, and LH rats are as follows. FC, cytosolic: df = 2, 15, $F = 1.58$, and $P = 0.23$; nuclear: df = 2, 15, $F = 0.85$, and $P = 0.44$. Hippocampus, cytosolic: df = 2, 15, $F = 0.08$, and $P = 0.92$; nuclear: df = 2, 15, $F = 0.36$, and $P = 0.70$. Overall group differences for MSK1 in nuclear fraction among TC, non-LH, and LH rats are as follows. FC: df = 2, 15, $F = 0.27$, and $P = 0.76$; hippocampus: df = 2, 15, $F = 0.58$, and $P = 0.57$. No significant differences were found in expression of RSK1 or MSK1 in any brain area.

several studies point to subtle differences between these two kinases. For example, ERK1 knock-out mice are viable, but these mice show increased synaptic plasticity in the striatum brain area [48]. In contrast, ERK2 knock-out mice do not survive, suggesting that ERK2 deficiency is not compensated with ERK1 [49]. Also, at the cellular level, these two kinases regulate cell cycle in a different manner. Whereas ERK1 acts at G_2/M level, ERK2 regulates G_1 phase of the cell cycle [50]. In addition, *in situ* hybridization studies suggest that whereas ERK2 mRNA is expressed throughout the brain, ERK1 mRNA is confined to cortex, olfactory bulb, regions of hippocampus, amygdala, hypothalamus, and cerebellum. ERK1 mRNA is almost absent in the CA-1 area, whereas ERK2 is present in all neurons of the hippocampus [51, 52]. These studies suggest possible brain region-specific functions mediated by these two ERKs. In light of these observations, we examined the expression of both ERK1 and ERK2 in frontal cortex and hippocampus. We found that mRNA and protein levels of these two kinases were decreased in LH rats and that the degree of change was almost the same in these two brain areas. We also found significant correlations between ERK1 and ERK2 protein and mRNA levels with catalytic activity of ERK1/2. This indicates that lower activation of ERK1 and ERK2 could possibly be associated with less expression of these two isoforms of ERK.

A wide range of functions of ERK1 and ERK2 is mediated through phosphorylation of substrates. Among them, phosphorylation and activation of MAP kinase-activated kinases represent a crucial amplification step in the ERK1/2 cascade. Of these, RSKs and MSKs are directly regulated by ERK1/2. Four different isoforms of RSKs have been identified (RSKs 1–4) with close homology among themselves (~80%). As with ERK1 and ERK2, under resting conditions, RSKs reside in cytoplasm and translocate to the nucleus upon phosphorylation [53]. RSKs are highly expressed in the brain [54, 55] and participate in the regulation of cell cycle and in the proliferation and survival of neurons. The most important substrates of RSKs are serum response factor [56], CREB [57–59], and chromatin-associated histone H3 [60]. RSKs also interact with Ets transcription factors [61], which are required for activation of TIF-1A, a transcription initiation factor involved in the transcription of RNA polymerase I and synthesis of rRNAs. Activation of ERK1 and ERK2 promotes interaction between RSK and CBP, which along with p300 form nucleosome structure and participate in transcriptional activation [62]. RSKs also participate in the survival of neurons by phosphorylating and therefore deactivating a proapoptotic protein Bad [61]. In addition, death associated protein kinase, another apoptotic regulatory protein, is phosphorylated and deactivated upon activation of RSKs [63]. The other crucial substrates of ERK1/2 are MSKs. MSKs exist in two isoforms, MSK1 and MSK2, with ~75% amino acid homology [64]. Both MSK isoforms are highly expressed in the brain; however, at cellular level, unlike RSKs, MSKs are present in the nucleus. Upon activation, MSKs regulate gene transcription by phosphorylating transcription factors ATF-1 and CREB and increase the transcript stability by phosphorylating nuclear proteins [64–67]. In addition, MSKs phosphorylate proapoptotic Bad [68], Akt [69], and translational machinery component 4EBP1 [70]. To examine if reduced expression and activation of ERK1 and ERK2 lead to altered activation of RSKs and MSKs, we determined ERK1/2-mediated catalytic activities of RSK1 and MSK1 as well as ERK1/2-mediated phosphorylation of RSK1 and MSK1. We found that, in LH rats, catalytic activities of both RSK1 and MSK1 were reduced in frontal cortex and hippocampus. ERK1/2-mediated phosphorylation of RSK1 and MSK1 was also reduced in these brain areas of LH rats. None of these changes were apparent in non-LH rats. We examined the expression of RSK1 and MSK1 but did not find any significant change in their mRNA or protein levels. These results suggest that it is not the expression but the reduced activation of upstream ERK1 and ERK2 which contributes to the decreased phosphorylation and therefore activation of RSK1 and MSK1 in LH rats.

In our earlier postmortem brain studies, we have shown that expression and activation of ERK1 and ERK2 are reduced in various cortical and hippocampal brain areas of depressed patients but not in cerebellum [29]. We have also found that upstream kinases MEK1 and MEK2 were less active in these brain areas [30]. The present study confirms our human postmortem brain findings that indeed reduced ERK1/2 signaling is associated with depression. Similar to our human brain study, our preliminary study in LH, NLH, and TC rats demonstrates that ERK1/2 activation was affected in cerebellum (data not shown), suggesting brain region-specific changes in ERK1/2 in LH rats. Interestingly, several studies demonstrate that inhibition of ERK1/2 signaling causes impaired learning behavior in mice which is improved with increased ERK activity [71–73]. This appears to be relevant to the development of LH phenotype as these rats show learning deficit which could be associated with reduced ERK1/2 activation. On the other hand, ERK1/2 signaling remained unaltered in non-LH rats even though these rats were given the same stress paradigm as LH rats. This again shows that the development of resiliency towards depression could be dependent upon the status of ERK activation. In the future, it will be interesting to test whether overexpression of ERK1 or ERK2 in frontal cortex or hippocampus of LH rats can lead to non-LH behavior and whether reduced ERK1/2 activation in these brain areas of non-LH rats can induce LH phenotype. An earlier study has shown that systemic injection of MEK inhibitor resulted in reduced ERK phosphorylation and subsequent depressive-like behavior in rats [35]. This inhibition also blocked the effects of antidepressants in various behavioral tests [35]. This suggests that not only can ERK1/2 inhibition cause depression but reduced ERK1/2 can also block the effectiveness of antidepressants. This is further supported by observations that electroconvulsive shock induces activation and tyrosine phosphorylation of ERK1/2 in the rat hippocampus [74] and that fluoxetine reverses depressive-like behavior in rats by increasing ERK/CREB signaling [75].

The reason behind reduced expression and activation of ERK1 and ERK2 in LH rats is presently unclear; however, the possibility of the role of upstream regulators in altering ERK1/2 signaling cannot be ruled out. As is well known, ERK1 and ERK2 are activated by several G protein coupled

receptors, receptors for tyrosine and nontyrosine kinases, and various effector molecules such as protein kinase A and protein kinase C either directly or via upstream kinases such as MAPK kinase kinases or Raf [11–13, 76]. Interestingly, neurotransmitter receptors such as $5HT_{1A}$ and $5HT_{2A}$ and α_2 and β_2 adrenergic receptors have been shown to be altered in the brain of depressed patients as well as in LH rats [38, 77, 78]. In addition, we have reported less activation of PKC and PKA not only in the brain of depressed patients [79, 80] but also in the frontal cortex and hippocampus of LH rats [37].

The consequence of reduced ERK1/2 signaling at functional levels in the brain of LH rats remains to be explored; however, as mentioned above, by phosphorylating several substrates either directly or indirectly, ERK1/2 can regulate transcription factors, stimulus-induced expression of immediately early genes, histone modifications, and translational machinery [81–83], which can lead to altered synaptic plasticity and physiological responses. In addition, since ERK1/2 is the major signaling pathway for BDNF-mediated response [84, 85] in promoting neuronal survival, proliferation, and differentiation [57], any abnormality in this signaling may also cause altered structural plasticity. It is pertinent to mention that neuronal atrophy of the prefrontal cortex and hippocampus has been reported in depressed patients [86–89], which could be associated with reduced BDNF levels and its mediated ERK1/2 activation [90]. Interestingly, it has been shown that lower ERK activity and reduced gray matter volume in depressed patients are related to depression associated risk allele Ser704Cyst [91] and that haplotypes and gene-gene interaction in the Ras/Raf/MAPK/RSK signaling pathway are involved in antidepressant remission in depressed population [92]. ERK1/2 pathway has been shown to exert its effects, in part, by regulating the synthesis of miRNA via increasing the stability of proteins belonging to the Argonaute complex, including dicer and the human immunodeficiency virus transactivation response RNA-binding protein (TRBP), which participate in the silencing of gene expression [93]. We recently reported that there is an adaptive miRNA response to inescapable shocks in non-LH rats, which was blunted in LH rats [39]. It will be interesting to examine whether differential activation of ERK1/2 has any impact on miRNAs response in LH or non-LH rats and in the development of these phenotypes.

In conclusion, we found differential responsiveness of ERK1/2 signaling in the brain of LH and non-LH rats. Whereas LH rats showed diminished activation and expression of ERK1 and ERK2 in frontal cortex and hippocampus, there was a muted response in non-LH rats. This was also evident at functional level where ERK1/2-mediated activation of RSK and MSK was lower in LH rats, without any change in non-LH rats. Our present and previous human postmortem brain studies [29–31] not only suggest that alterations in ERK1/2 may be important in the pathophysiology of depression but also raise the interesting possibility that ERK1/2 may be involved in generating vulnerability to depression phenotype. Follow-up studies will be needed to further investigate whether manipulation of ERK1/2 in these brain areas can induce or reverse LH phenotype.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The study was supported by grants from the National Institute of Mental Health (R01MH082802, R01MH100616, and R01MH101980) to Dr. Yogesh Dwivedi.

References

[1] C. J. L. Murray and A. D. Lopez, "Evidence-based health policy—lessons from the global burden of disease study," *Science*, vol. 274, no. 5288, pp. 740–743, 1996.

[2] R. C. Kessler, P. Berglund, O. Demler et al., "The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R)," *The Journal of the American Medical Association*, vol. 289, no. 23, pp. 3095–3105, 2003.

[3] K. Demyttenaere, R. Bruffaerts, J. Posada-Villa et al., "Prevalence, severity, and unmet need for treatment of mental disorders in the World Health Organization World Mental Health Surveys," *The Journal of the American Medical Association*, vol. 291, no. 21, pp. 2581–2590, 2004.

[4] M.-L. Wong and J. Licinio, "From monoamines to genomic targets: a paradigm shift for drug discovery in depression," *Nature Reviews Drug Discovery*, vol. 3, no. 2, pp. 136–151, 2004.

[5] A. A. Nierenberg, M. Fava, M. H. Trivedi et al., "A comparison of lithium and T_3 augmentation following two failed medication treatments for depression: a STAR∗D report," *The American Journal of Psychiatry*, vol. 163, no. 9, pp. 1519–1530, 2006.

[6] R. S. Duman, J. Malberg, S. Nakagawa, and C. D'Sa, "Neuronal plasticity and survival in mood disorders," *Biological Psychiatry*, vol. 48, no. 8, pp. 732–739, 2000.

[7] R. S. Duman, "Pathophysiology of depression: the concept of synaptic plasticity," *European Psychiatry*, vol. 17, no. 3, pp. 306–310, 2002.

[8] P. Fossati, A. Radtchenko, and P. Boyer, "Neuroplasticity: from MRI to depressive symptoms," *European Neuropsychopharmacology*, vol. 14, no. 5, pp. S503–S510, 2004.

[9] H. K. Manji, J. A. Quiroz, J. Sporn et al., "Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression," *Biological Psychiatry*, vol. 53, no. 8, pp. 707–742, 2003.

[10] M. G. Giovannini, "The role of the extracellular signal-regulated kinase pathway in memory encoding," *Reviews in the Neurosciences*, vol. 17, no. 6, pp. 619–634, 2006.

[11] K. Fukunaga and E. Miyamoto, "Role of MAP kinase in neurons," *Molecular Neurobiology*, vol. 16, no. 1, pp. 79–95, 1998.

[12] T. Numakawa, S. Suzuki, E. Kumamaru, N. Adachi, M. Richards, and H. Kunugi, "BDNF function and intracellular signaling in neurons," *Histology and Histopathology*, vol. 25, no. 2, pp. 237–258, 2010.

[13] M. Raman, W. Chen, and M. H. Cobb, "Differential regulation and properties of MAPKs," *Oncogene*, vol. 26, no. 22, pp. 3100–3112, 2007.

[14] L.-L. Lin, M. Wartmann, A. Y. Lin, J. L. Knopf, A. Seth, and R. J. Davis, "$cPLA_2$ is phosphorylated and activated by MAP kinase," *Cell*, vol. 72, no. 2, pp. 269–278, 1993.

[15] R.-H. Chen, C. Sarnecki, and J. Blenis, "Nuclear localization and regulation of erk-and rsk-encoded protein kinases," *Molecular and Cellular Biology*, vol. 12, no. 3, pp. 915–927, 1992.

[16] R. Marais, J. Wynne, and R. Treisman, "The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain," *Cell*, vol. 73, no. 2, pp. 381–393, 1993.

[17] L. F. Reichardt, "Neurotrophin-regulated signalling pathways," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 361, no. 1473, pp. 1545–1564, 2006.

[18] K. Ohira and M. Hayashi, "A new aspect of the TrkB signaling pathway in neural plasticity," *Current Neuropharmacology*, vol. 7, no. 4, pp. 276–285, 2009.

[19] S. D. Skaper, "The biology of neurotrophins, signalling pathways, and functional peptide mimetics of neurotrophins and their receptors," *CNS & Neurological Disorders—Drug Targets*, vol. 7, no. 1, pp. 46–62, 2008.

[20] J. J. Day and J. D. Sweatt, "Epigenetic mechanisms in cognition," *Neuron*, vol. 70, no. 5, pp. 813–829, 2011.

[21] A. Ciccarelli and M. Giustetto, "Role of ERK signaling in activity-dependent modifications of histone proteins," *Neuropharmacology*, vol. 80, pp. 34–44, 2014.

[22] K. Kolkova, V. Novitskaya, N. Pedersen, V. Berezin, and E. Bock, "Neural cell adhesion molecule-stimulated neurite outgrowth depends on activation of protein kinase C and the Ras-mitogen-activated protein kinase pathway," *Journal of Neuroscience*, vol. 20, no. 6, pp. 2238–2246, 2000.

[23] S.-H. Yang, A. D. Sharrocks, and A. J. Whitmarsh, "MAP kinase signalling cascades and transcriptional regulation," *Gene*, vol. 513, no. 1, pp. 1–13, 2013.

[24] E. J. Weeber and J. D. Sweatt, "Molecular neurobiology of human cognition," *Neuron*, vol. 33, no. 6, pp. 845–848, 2002.

[25] I. S. Samuels, S. C. Saitta, and G. E. Landreth, "MAP'ing CNS development and cognition: an ERKsome process," *Neuron*, vol. 61, no. 2, pp. 160–167, 2009.

[26] R. L. Stornetta and J. J. Zhu, "Ras and Rap signaling in synaptic plasticity and mental disorders," *Neuroscientist*, vol. 17, no. 1, pp. 54–78, 2011.

[27] D. R. Alessi, N. Gomez, G. Moorhead, T. Lewis, S. M. Keyse, and P. Cohen, "Inactivation of p42 MAP kinase by protein phosphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines," *Current Biology*, vol. 5, no. 3, pp. 283–295, 1995.

[28] K. Gopalbhai and S. Meloche, "Repression of mitogen-activated protein kinases ERK1/ERK2 activity by a protein tyrosine phosphatase in rat fibroblasts transformed by upstream oncoproteins," *Journal of Cellular Physiology*, vol. 174, no. 1, pp. 35–47, 1998.

[29] Y. Dwivedi, H. S. Rizavi, R. C. Roberts, R. C. Conley, C. A. Tamminga, and G. N. Pandey, "Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects," *Journal of Neurochemistry*, vol. 77, no. 3, pp. 916–928, 2001.

[30] Y. Dwivedi, H. S. Rizavi, R. R. Conley, and G. N. Pandey, "ERK MAP kinase signaling in post-mortem brain of suicide subjects: differential regulation of upstream Raf kinases Raf-1 and B-Raf," *Molecular Psychiatry*, vol. 11, no. 1, pp. 86–98, 2006.

[31] Y. Dwivedi, H. S. Rizavi, H. Zhang, R. C. Roberts, R. R. Conley, and G. N. Pandey, "Aberrant extracellular signal-regulated kinase (ERK)1/2 signalling in suicide brain: role of ERK kinase 1 (MEK1)," *International Journal of Neuropsychopharmacology*, vol. 12, no. 10, pp. 1337–1354, 2009.

[32] K. Malki, O. Pain, M. G. Tosto, E. Du Rietz, L. Carboni, and L. C. Schalkwyk, "Identification of genes and gene pathways associated with major depressive disorder by integrative brain analysis of rat and human prefrontal cortex transcriptomes," *Translational Psychiatry*, vol. 5, no. 3, article e519, 2015.

[33] H. Einat, P. Yuan, T. D. Gould et al., "The role of the extracellular signal-regulated kinase signaling pathway in mood modulation," *Journal of Neuroscience*, vol. 23, no. 19, pp. 7311–7316, 2003.

[34] S. R. Engel, T. K. Creson, Y. Hao et al., "The extracellular signal-regulated kinase pathway contributes to the control of behavioral excitement," *Molecular Psychiatry*, vol. 14, no. 4, pp. 448–461, 2009.

[35] C. H. Duman, L. Schlesinger, M. Kodama, D. S. Russell, and R. S. Duman, "A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment," *Biological Psychiatry*, vol. 61, no. 5, pp. 661–670, 2007.

[36] M. E. P. Seligman and G. Beagley, "Learned helplessness in the rat," *Journal of Comparative and Physiological Psychology*, vol. 88, no. 2, pp. 534–541, 1975.

[37] Y. Dwivedi, A. C. Mondal, P. K. Shukla, H. S. Rizavi, and J. Lyons, "Altered protein kinase A in brain of learned helpless rats: effects of acute and repeated stress," *Biological Psychiatry*, vol. 56, no. 1, pp. 30–40, 2004.

[38] Y. Dwivedi, A. C. Mondal, G. V. Payappagoudar, and H. S. Rizavi, "Differential regulation of serotonin (5HT)2A receptor mRNA and protein levels after single and repeated stress in rat brain: role in learned helplessness behavior," *Neuropharmacology*, vol. 48, no. 2, pp. 204–214, 2005.

[39] N. R. Smalheiser, G. Lugli, H. S. Rizavi et al., "MicroRNA expression in rat brain exposed to repeated inescapable shock: differential alterations in learned helplessness vs. non-learned helplessness," *International Journal of Neuropsychopharmacology*, vol. 14, no. 10, pp. 1315–1325, 2011.

[40] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with Folin phenol reagent," *The Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[41] Y. Dwivedi, A. C. Mondal, H. S. Rizavi, and R. R. Conley, "Suicide brain is associated with decreased expression of neurotrophins," *Biological Psychiatry*, vol. 58, no. 4, pp. 315–324, 2005.

[42] G. P. Sapkota, A. Kieloch, J. M. Lizcano et al., "Phosphorylation of the protein kinase mutated in Peutz-Jeghers cancer syndrome, LKB1/STK11, at Ser^{431} by $p90^{RSK}$ and cAMP-dependent protein kinase, but not its farnesylation at Cys^{433}, is essential for LKB1 to suppress cell growth," *The Journal of Biological Chemistry*, vol. 276, no. 22, pp. 19469–19482, 2001.

[43] T. G. Boulton and M. H. Cobb, "Identification of multiple extracellular signal-regulated kinases (ERKs) with antipeptide antibodies," *Cell Regulation*, vol. 2, no. 5, pp. 357–371, 1991.

[44] J. Ortiz, H. W. Harris, X. Guitart, R. Z. Terwilliger, J. W. Haycock, and E. J. Nestler, "Extracellular signal-regulated protein kinases (ERKs) and ERK kinase (MEK) in brain: regional distribution and regulation by chronic morphine," *The Journal of Neuroscience*, vol. 15, no. 2, pp. 1285–1297, 1995.

[45] Y. Dwivedi, A. C. Mondal, H. S. Rizavi, P. K. Shukla, and G. N. Pandey, "Single and repeated stress-induced modulation of phospholipase C catalytic activity and expression: role in LH behavior," *Neuropsychopharmacology*, vol. 30, no. 3, pp. 473–483, 2005.

[46] A. A. Reszka, R. Seger, C. D. Diltz, E. G. Krebs, and E. H. Fischer, "Association of mitogen-activated protein kinase

with the microtubule cytoskeleton," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 19, pp. 8881–8885, 1995.

[47] Y. D. Shaul and R. Seger, "The MEK/ERK cascade: from signaling specificity to diverse functions," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1773, no. 8, pp. 1213–1226, 2007.

[48] C. Mazzucchelli, C. Vantaggiato, A. Ciamei et al., "Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory," *Neuron*, vol. 34, no. 5, pp. 807–820, 2002.

[49] M. K. Saba-El-Leil, F. D. J. Vella, B. Vernay et al., "An essential function of the mitogen-activated protein kinase Erk2 in mouse trophoblast development," *EMBO Reports*, vol. 4, no. 10, pp. 964–968, 2003.

[50] X. Liu, S. Yan, T. Zhou, Y. Terada, and R. L. Erikson, "The MAP kinase pathway is required for entry into mitosis and cell survival," *Oncogene*, vol. 23, no. 3, pp. 763–776, 2004.

[51] K. L. Thomas and S. P. Hunt, "The regional distribution of extracellularly regulated kinase-1 and -2 messenger RNA in the adult rat central nervous system," *Neuroscience*, vol. 56, no. 3, pp. 741–757, 1993.

[52] R. S. Fiore, V. E. Bayer, S. L. Pelech, J. Posada, J. A. Cooper, and J. M. Baraban, "p42 mitogen-activated protein kinase in brain: prominent localization in neuronal cell bodies and dendrites," *Neuroscience*, vol. 55, no. 2, pp. 463–472, 1993.

[53] Y. Zhao, C. Bjørbæk, S. Weremowicz, C. C. Morton, and D. E. Moller, "RSK3 encodes a novel pp90rsk isoform with a unique N-terminal sequence: growth factor-stimulated kinase function and nuclear translocation," *Molecular and Cellular Biology*, vol. 15, no. 8, pp. 4353–4363, 1995.

[54] H. G. Yntema, B. van den Helm, J. Kissing et al., "A novel ribosomal S6-kinase (RSK4; RPS6KA6) is commonly deleted in patients with complex X-linked mental retardation," *Genomics*, vol. 62, no. 3, pp. 332–343, 1999.

[55] M. Zeniou, T. Ding, E. Trivier, and A. Hanauer, "Expression analysis of RSK gene family members: the RSK2 gene, mutated in Coffin-Lowry syndrome, is prominently expressed in brain structures essential for cognitive function and learning," *Human Molecular Genetics*, vol. 11, no. 23, pp. 2929–2940, 2002.

[56] J. C. Brüning, J. A. Gillette, Y. Zhao et al., "Ribosomal subunit kinase-2 is required for growth factor-stimulated transcription of the c-Fos gene," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 6, pp. 2462–2467, 2000.

[57] A. Bonni, A. Brunet, A. E. West, S. R. Datta, M. A. Takasu, and M. E. Greenberg, "Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms," *Science*, vol. 286, no. 5443, pp. 1358–1362, 1999.

[58] D. D. Ginty, A. Bonni, and M. E. Greenberg, "Nerve growth factor activates a Ras-dependent protein kinase that stimulates c-fos transcription via phosphorylation of CREB," *Cell*, vol. 77, no. 5, pp. 713–725, 1994.

[59] J. Xing, D. D. Ginty, and M. E. Greenberg, "Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase," *Science*, vol. 273, no. 5277, pp. 959–963, 1996.

[60] I. S. Strelkov and J. R. Davie, "Ser-10 phosphorylation of histone H3 and immediate early gene expression in oncogene-transformed mouse fibroblasts," *Cancer Research*, vol. 62, no. 1, pp. 75–78, 2002.

[61] J. Wu and R. Janknecht, "Regulation of the ETS transcription factor ER81 by the 90-kDa ribosomal S6 kinase 1 and protein kinase A," *The Journal of Biological Chemistry*, vol. 277, no. 45, pp. 42669–42679, 2002.

[62] T. Nakajima, A. Fukamizu, J. Takahashi et al., "The signal-dependent coactivator CBP is a nuclear target for $pp90_{RSK}$," *Cell*, vol. 86, no. 3, pp. 465–474, 1996.

[63] R. Anjum, P. P. Roux, B. A. Ballif, S. P. Gygi, and J. Blenis, "The tumor suppressor DAP kinase is a target of RSK-mediated survival signaling," *Current Biology*, vol. 15, no. 19, pp. 1762–1767, 2005.

[64] M. Deak, A. D. Clifton, J. M. Lucocq, and D. R. Alessi, "Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB," *The EMBO Journal*, vol. 17, no. 15, pp. 4426–4441, 1998.

[65] J. S. C. Arthur and P. Cohen, "MSK1 is required for CREB phosphorylation in response to mitogens in mouse embryonic stem cells," *FEBS Letters*, vol. 482, no. 1-2, pp. 44–48, 2000.

[66] G. R. Wiggin, A. Soloaga, J. M. Foster, V. Murray-Tait, P. Cohen, and J. S. C. Arthur, "MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts," *Molecular and Cellular Biology*, vol. 22, no. 8, pp. 2871–2881, 2002.

[67] S. Schuck, A. Soloaga, G. Schratt, J. S. C. Arthur, and A. Nordheim, "The kinase MSK1 is required for induction of c-fos by lysophosphatidic acid in mouse embryonic stem cells," *BMC Molecular Biology*, vol. 4, article 6, 2003.

[68] Q.-B. She, M. Wei-Ya, S. Zhong, and Z. Dong, "Activation of JNK1, RSK2, and MSK1 is involved in serine 112 phosphorylation of bad by ultraviolet B radiation," *The Journal of Biological Chemistry*, vol. 277, no. 27, pp. 24039–24048, 2002.

[69] M. Nomura, A. Kaji, W.-Y. Ma et al., "Mitogen- and stress-activated protein kinase 1 mediates activation of Akt by ultraviolet B irradiation," *The Journal of Biological Chemistry*, vol. 276, no. 27, pp. 25558–25567, 2001.

[70] G. Liu, Y. Zhang, A. M. Bode, W.-Y. Ma, and Z. Dong, "Phosphorylation of 4E-BP1 is mediated by the p38/MSK1 pathway in response to UVB irradiation," *The Journal of Biological Chemistry*, vol. 277, no. 11, pp. 8810–8816, 2002.

[71] C. M. Atkins, J. C. Selcher, J. J. Petraitis, J. M. Trzaskos, and J. D. Sweatt, "The MAPK cascade is required for mammalian associative learning," *Nature Neuroscience*, vol. 1, no. 7, pp. 602–609, 1998.

[72] G. E. Schafe, C. M. Atkins, M. W. Swank, E. P. Bauer, J. D. Sweatt, and J. E. LeDoux, "Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning," *Journal of Neuroscience*, vol. 20, no. 21, pp. 8177–8187, 2000.

[73] J. P. Adams and J. D. Sweatt, "Molecular psychology: roles for the ERK MAP kinase cascade in memory," *Annual Review of Pharmacology and Toxicology*, vol. 42, pp. 135–163, 2002.

[74] D. E. Berman, S. Hazvi, K. Rosenblum, R. Seger, and Y. Dudai, "Specific and differential activation of mitogen-activated protein kinase cascades by unfamiliar taste in the insular cortex of the behaving rat," *Journal of Neuroscience*, vol. 18, no. 23, pp. 10037–10044, 1998.

[75] X. Qi, W. Lin, J. Li et al., "Fluoxetine increases the activity of the ERK-CREB signal system and alleviates the depressive-like behavior in rats exposed to chronic forced swim stress," *Neurobiology of Disease*, vol. 31, no. 2, pp. 278–285, 2008.

[76] E. D. Roberson, J. D. English, J. P. Adams, J. C. Selcher, C. Kondratick, and J. D. Sweatt, "The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus," *Journal of Neuroscience*, vol. 19, no. 11, pp. 4337–4348, 1999.

[77] J. J. Mann, "The serotonergic system in mood disorders and suicidal behaviour," *Philosophical Transactions of the Royal Society of London Series B: Biological sciences*, vol. 368, no. 1615, Article ID 20120537, 2013.

[78] G. Flügge, M. Van Kampen, and M. J. Mijnster, "Perturbations in brain monoamine systems during stress," *Cell and Tissue Research*, vol. 315, no. 1, pp. 1–14, 2004.

[79] G. N. Pandey, Y. Dwivedi, S. C. Pandey, R. R. Conley, R. C. Roberts, and C. A. Tamminga, "Protein kinase C in the postmortem brain of teenage suicide victims," *Neuroscience Letters*, vol. 228, no. 2, pp. 111–114, 1997.

[80] G. N. Pandey, Y. Dwivedi, S. C. Pandey et al., "Low phosphoinositide-specific phospholipase C activity and expression of phospholipase C β_1 protein in the prefrontal cortex of teenage suicide subjects," *The American Journal of Psychiatry*, vol. 156, no. 12, pp. 1895–1901, 1999.

[81] Y. Chandramohan, S. K. Droste, J. S. C. Arthur, and J. M. H. M. Reul, "The forced swimming-induced behavioural immobility response involves histone H3 phospho-acetylation and c-Fos induction in dentate gyrus granule neurons via activation of the N-methyl-D-aspartate/extracellular signal-regulated kinase/mitogen- and stress-activated kinase signalling pathway," *European Journal of Neuroscience*, vol. 27, no. 10, pp. 2701–2713, 2008.

[82] M. L. Laorden, C. Núñez, P. Almela, and M. V. Milanés, "Morphine withdrawal-induced c-fos expression in the hypothalamic paraventricular nucleus is dependent on the activation of catecholaminergic neurones," *Journal of Neurochemistry*, vol. 83, no. 1, pp. 132–140, 2002.

[83] E. Valjent, C. Pagès, D. Hervé, J.-A. Girault, and J. Caboche, "Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain," *European Journal of Neuroscience*, vol. 19, no. 7, pp. 1826–1836, 2004.

[84] J. E. Cavanaugh, J. Ham, M. Hetman, S. Poser, C. Yan, and Z. Xia, "Differential regulation of mitogen-activated protein kinases ERK1/2 and ERK5 by neurotrophins, neuronal activity, and cAMP in neurons," *The Journal of Neuroscience*, vol. 21, no. 2, pp. 434–443, 2001.

[85] R. A. Segal and M. E. Greenberg, "Intracellular signaling pathways activated by neurotrophic factors," *Annual Review of Neuroscience*, vol. 19, pp. 463–489, 1996.

[86] Y. I. Sheline, P. W. Wang, M. H. Gado, J. G. Csernansky, and M. W. Vannier, "Hippocampal atrophy in recurrent major depression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 9, pp. 3908–3913, 1996.

[87] D. Öngür, W. C. Drevets, and J. L. Price, "Glial reduction in the subgenual prefrontal cortex in mood disorders," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 22, pp. 13290–13295, 1998.

[88] G. Rajkowska, "Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells," *Biological Psychiatry*, vol. 48, no. 8, pp. 766–777, 2000.

[89] G. Rajkowska, J. J. Miguel-Hidalgo, J. Wei et al., "Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression," *Biological Psychiatry*, vol. 45, no. 9, pp. 1085–1098, 1999.

[90] Y. Dwivedi, "Brain-derived neurotrophic factor: role in depression and suicide," *Neuropsychiatric Disease and Treatment*, vol. 5, no. 1, pp. 433–449, 2009.

[91] R. Hashimoto, T. Numakawa, T. Ohnishi et al., "Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling," *Human Molecular Genetics*, vol. 15, no. 20, pp. 3024–3033, 2006.

[92] C.-J. Wang, Z.-J. Zhang, Z. Xu et al., "Kinase gene haplotypes and gene-gene interactions in the Ras-Raf-MAPK signaling pathway: association with antidepressant remission," *International Clinical Psychopharmacology*, vol. 28, no. 5, pp. 245–254, 2013.

[93] Z. Paroo, X. Ye, S. Chen, and Q. Liu, "Phosphorylation of the human microRNA-generating complex mediates MAPK/Erk signaling," *Cell*, vol. 139, no. 1, pp. 112–122, 2009.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 3136743, 12 pages
http://dx.doi.org/10.1155/2016/3136743

Research Article

Salivary Cortisol Levels and Depressive Symptomatology in Consumers and Nonconsumers of Self-Help Books: A Pilot Study

Catherine Raymond,[1,2] Marie-France Marin,[1,2] Anne Hand,[1] Shireen Sindi,[1,3] Robert-Paul Juster,[1,3] and Sonia J. Lupien[1,4]

[1]*Centre for Studies on Human Stress, Research Centre, Institut Universitaire en Santé Mentale de Montréal, Montreal, Canada H1N 3M5*
[2]*Department of Neuroscience, University of Montreal, Montreal, Canada H3C 3J7*
[3]*Integrated Program in Neuroscience, McGill University, Montreal, Canada H3A 3R1*
[4]*Department of Psychiatry, Faculty of Medicine, University of Montreal, Montreal, Canada H3T 1J4*

Correspondence should be addressed to Sonia J. Lupien; sonia.lupien@umontreal.ca

Received 22 June 2015; Revised 26 August 2015; Accepted 29 September 2015

Academic Editor: Jordan Marrocco

The self-help industry generates billions of dollars yearly in North America. Despite the popularity of this movement, there has been surprisingly little research assessing the characteristics of self-help books consumers, and whether this consumption is associated with physiological and/or psychological markers of stress. The goal of this pilot study was to perform the first psychoneuroendocrine analysis of consumers of self-help books in comparison to nonconsumers. We tested diurnal and reactive salivary cortisol levels, personality, and depressive symptoms in 32 consumers and nonconsumers of self-help books. In an explorative secondary analysis, we also split consumers of self-help books as a function of their preference for problem-focused *versus* growth-oriented self-help books. The results showed that while consumers of growth-oriented self-help books presented increased cortisol reactivity to a psychosocial stressor compared to other groups, consumers of problem-focused self-help books presented higher depressive symptomatology. The results of this pilot study show that consumers with preference for either problem-focused or growth-oriented self-help books present different physiological and psychological markers of stress when compared to nonconsumers of self-help books. This preliminary study underlines the need for additional research on this issue in order to determine the impact the self-help book industry may have on consumers' stress.

1. Introduction

The World Health Organization predicts that by the year 2020, depression will be the first cause of invalidity in the world followed by cardiovascular disease [1]. Although various psychological and pharmacological treatments exist for the treatment of depression (for a review, see [2]), difficult access to psychotherapy due to monetary or transportation issues and/or low acceptance of antidepressant treatments has led to the development of other forms of treatments [3]. In recent years, there has been a rise in the use of self-help treatments that provide users with information on how to self-identify their problems and propose methods to overcome them [3].

Self-help exists in a variety of mediums. The most frequent form of delivery includes books (bibliotherapy) and use of the internet (internet-based therapy; for a review, see [3, 4]). Moreover, self-help treatment can be guided or unguided. Guided self-help treatment implies that some form of support from a therapist is delivered to the patient, either through self-help booklets developed by health professionals or scientists, or via support provided directly by a therapist in addition to utilization of the self-help material [5]. In contrast, unguided self-help represents the use of "self-help books" available in bookstores with no additional support from a health professional [4, 6]. Unguided self-help books represent books written by recognized or unrecognized specialists in the field

that provides guidance on how to live a better life, be happy, and so forth [4, 7].

Two dimensions of self-help are generally proposed in unguided self-help books [4, 6], that is, problem-focused or growth-oriented [4]. Problem-focused self-help books represent books that extensively discuss the nature of problems one can encounter and how to recognize and circumvent them [4] (this category of self-help books has also been named "*victimization* books" [6]). In contrast, growth-oriented books present inspirational messages about life and happiness and propose various methods of coping and development of new skills [4] (this category of self-help books has also been named "*empowerment* books" [6]).

Meta-analyses have shown that guided self-help interventions for depression are more effective than absence of treatment, and guided self-help interventions present similar efficacy to psychotherapies and/or antidepressants [2, 8, 9]. Moreover, guided self-help interventions are now recommended by the National Institute for Health and Clinical Excellence [10]. Although guided self-help interventions presented in books or via the internet have been extensively studied [2, 11, 12], unguided self-help books have received very little attention. Some studies suggest that reading problem-focused self-help books can have positive effects in the treatment of some problematics such as marital conflict [13] and general emotional disorders [14], and others suggest that unguided self-help books could be used to prevent the incidence of depression in high risk groups [15]. However, at this point, there is a lot of cynicism about the potentially positive effects of unguided self-help books, with some authors claiming that self-help books are fraudulent [16], and others suggesting that buying self-help books may be part of a "false hope syndrome" [17]. For many authors, the major limitation of unguided self-help books is their "one-size fits all" approach in which advice is given without taking into account the personality and/or diagnosis and/or personal circumstances of the reader [16–18].

This later point brings attention to the lack of information that exists on the type of readership of unguided self-help books. The few studies that were performed to date showed that consumers of self-help books come from all levels of educational backgrounds, socioeconomic status, and positions, although women tend to consume more self-help books than men [13]. Notwithstanding, the literature is inconsistent in describing whether consumers of self-help books differ from nonconsumers in terms of personality [19]. One study showed that consumers of self-help books present higher neuroticism than nonconsumers [20], a second study did not find such a difference [4, 21], and a third reported that reading self-help books is associated with an increase in self-actualization [13].

Although these data are interesting, they do not inform us about the characteristics of self-help book readership. Indeed, studies assessing why certain people are attracted to self-help books propose that many adults are active consumers of self-help books as a way of self-diagnosing and/or "treating" their own psychological distress, and that this would mainly result from the stigma surrounding depression in adults [22, 23]. In this sense, the active proliferation of the self-help book industry would mainly reflect the underlying depressive symptomatology of individuals, and this industry would be highly successful because individuals need some sort of autotreatment to alleviate their depressive mood and/or disorder. If this is the case, one could predict that active consumers of self-help books might present increased stress physiology and increased depressive symptomatology when compared to nonconsumers of self-help books. The goal of this study was to test this hypothesis.

Impairment in the regulation of the hypothalamic-pituitary-adrenal (HPA) system has been reported in acute and/or chronic episodes of depression [24, 25]. The impaired negative feedback of the HPA system ultimately leads to hypersecretion of CRF, shifting the activity of the HPA axis to greater production of glucocorticoids (cortisol in humans; for a review, see [26]). In this first pilot study, we assessed whether consumers of unguided self-help books present differences in diurnal levels of cortisol, stress reactive cortisol levels, depressive symptoms, and personality traits in comparison to nonconsumers. Personality and depressive symptomatology are important factors to measure in consumers of self-help books as they could potentially be important predictors of increased stress reactivity and/or depressive symptomatology. In line with the goals of pilot studies (for a review on pilot studies, see [27]), we performed this first small scale preliminary study in order to evaluate the feasibility of studying self-help book consumers and potential adverse events related to these types of studies. Most importantly, to guide future research, we aimed to generate effect sizes for our dependent variables (cortisol levels, depressive symptomatology, and personality factors) in order to determine the appropriate sample size needed for a larger experimental study on this issue that has received no empirical evidence.

2. Materials and Methods

2.1. Recruitment and Group Classification. The definition of "self-help books" that we used in this research project is the definition given by the neuropsychologist Paul Pearsall who defines self-help books as "*Books that give advice on how to change your life, attain happiness, find true love, lose weight, and more*" [7]. We defined "consumers of self-help books" as individuals who have bought or browsed a minimum of four self-help books in the previous year. We felt that including only individuals who have bought (and not "browsed") four self-help books might bias the sample toward people from higher socioeconomic status, which could then have a significant impact on the results. Questions about the number and types of books bought and/or browsed by the participants were asked during a recruitment phone interview. Participants defined as "consumers of self-help books" were asked to provide the names of these books during the phone interview in order to ascertain whether they fell into our category of consumers of self-help books.

Online recruitment was performed using advertisements posted on general or university websites. Since the purpose of the study was to compare two different populations (self-help books' consumers and nonconsumers), two different types of

advertisement were used. Nonconsumers were recruited via an advertisement featuring a study on personality traits and stress, without any mention on self-help books consumption. This procedure was used to ensure that the nonconsumer group was not composed of people "against" this type of literature but only "not attracted" to it. These potential nonconsumer participants were then screened on the phone and additional questions were asked to validate that they had never read or browsed that kind of self-help literature and that they were not attracted to it. Only those individuals who did not read self-help books and were not attracted to them were retained in the nonconsumer group.

Self-help book consumers were recruited via an advertisement stating that we were looking for adults who were active consumers of self-help books for a study on personality and stress. During their visit to the lab, participants from that group were evaluated on their preference for problem-focused *versus* growth-oriented self-help books using a classification task that we developed. In this task, we presented the consumer group with 10 books and, after giving them 10 minutes to browse the various books, we asked them to sort out the five books that they would buy given the opportunity. Five of the ten books proposed a growth-oriented approach (e.g., "*The Power of Positive Thinking*"), while five of them proposed a problem-focused approach (e.g., "*How Can I Forgive You?: The Courage to Forgive, the Freedom Not To*"). The 5 books in each category are presented as follows.

Books Used to Assess Preference for Growth-Oriented (Books #1 to #5) versus Problem-Focused (Books #6 to Book #10) Self-Help Books. Growth-Oriented Self-Help Books are the following:

(1) "*The Power of Positive Thinking*" by Norman Vincent Peale, 1952.

(2) "*How to Stop Worrying and Start Living*" by Dale Carnegie, 1990.

(3) "*You're Stronger than You Think*" by Peter Ubel, 2006.

(4) "*You Can Be Happy No Matter What*" by Richard Carlson, 2006.

(5) "*Choices That Change Lives: 15 Ways to Find More Purpose, Meaning, and Joy*" by Hal Urban, 2006.

Problem-Focused Self-Help Books are as follows:

(6) "*Why Is It Always About You*?: *Saving Yourself from the Narcissists in Your Life*" by Sandy Hotchkiss, 2003.

(7) "*I'm Ok, You're My Parents*" by Dale Atkins, 2004.

(8) "*Shame and Guilt*" by Jane Middelton-Moz, 1990.

(9) "*Self Nurture: Learning to Care for Yourself As Effectively As You Care for Everyone Else*" by Alice D. Domar and Henry Dreher, 2001.

(10) "*How Can I Forgive You?: The Courage to Forgive, the Freedom Not To*" by Janis A. Spring, 2005.

A ratio of growth-oriented/problem-focused preference was calculated by adding the number of books from each pole that fell within the category of "books to buy" by the participants. For example, if a participant stated that they would buy three growth-oriented books and two problem-focused books, this participant received a ratio of 3/2 = 1.5. With this ratio, the larger the number, the greater the attraction to growth-oriented books and vice versa for problem-focused books. Participants displaying a ratio of 4 and above were classified in the growth-oriented group as scores lower than 4 were closer to chance level for preference assessment. When presented with books, participants were not aware that the goal of this task was to determine their attractiveness to growth-oriented *versus* problem-focused books. The reason for this is that it can be predicted that most people would choose not to select problem-focused books if told about the two poles (growth-oriented *versus* problem-focused), given the negative social value that may be attached to problem-focused self-help books.

2.2. Participants. Participants from both groups were screened over the phone prior to recruitment in order to make sure that they fulfilled our inclusion criteria. Exclusionary criteria included presence or history of neurological or psychiatric conditions, diabetes, respiratory disease, asthma, infectious illness, thyroid or adrenal dysfunctions, obesity (body massive index > 30), any glucocorticoid or cardiovascular altering medications (e.g., antidepressants, diuretics, antiasthmatics, and b-blockers), and excessive use of drugs or alcohol. Smoking was an exclusion criterion due to its known effect on HPA axis regulation [19].

Thirty-two healthy men and women aged between 18 and 65 ($M = 36.03 \pm 16.09$) participated in this study. Eighteen self-help consumers (75% female) and 14 nonconsumers (75% female) were recruited. The average age of the consumers was 38.33 years old (±3.5) and 33.07 years old (±4.72) for the nonconsumers. Within the group of self-help books consumers, 11 individuals were classified as having a preference for problem-focused books (hereon referred to as the "problem-focused group") and 7 were classified as having a preference for growth-oriented books (hereon referred to as the "growth-oriented group"). Three women were menopausal (one in each condition) and all others were tested in the follicular phase of their menstrual cycle. Women on hormonal therapy were not included in this study. All participants provided written informed consent and were compensated for their participation in the study.

This study was approved by the Research Ethics Board of the Mental Health University Institute respecting the Canadian Tri-Council's Policy statement for the ethical conduct for experimentation using humans, guided by the World Medical Association's Declaration of Helsinki.

2.3. Questionnaires

2.3.1. Personality Traits. We measured personality traits in order to determine whether preference for problem-focused or growth-oriented books would be associated with personality traits that could predict cortisol levels. Personality traits were measured using the NEO Five Factor Inventory (NEO-FFI). This 60-item personality inventory was developed as a short form of the NEO-PI [28]. The subscales

include "neuroticism," "extraversion," "openness to new experiences," "agreeableness," and "conscientiousness". Participants are asked to respond on a Likert-scale with the extent to which they agree with each item ("strongly agree" to "strongly disagree"). The mean coefficient alpha for the revised inventory scale was 0.77.

2.3.2. Locus of Control. Since low sense of control is linked to the cortisol stress response [29], locus of control was measured in order to explain any potential physiological stress response differences between groups. We administered the Belief in Competence and Control Questionnaire (BCC). Using a six-point Likert scale ("not at all true" to "very true"), the BCC yields four scales including "self-concept of own competence," "control expectancy: internality," "control expectancy: externality," and "control expectancy: chance control" [30]. The mean alpha for this questionnaire is 0.82 for young students and 0.83 for the elderly.

2.3.3. Self-Esteem. Self-esteem was measured using the 10-item Rosenberg Self-Esteem Scale (RES [31]), which is a unidimensional scale that measures personal worth, self-confidence, self-respect, and self-depreciation. Participants are asked to respond on a four-point scale with the degree to which they agree with each item ("strongly agree" to "strongly disagree"). The scale shows good reliability ($\alpha = 0.80$) and is a valid test of global self-worth.

2.3.4. Depressive Symptomatology. Self-reported depressive symptoms were assessed using the 21-item Beck Depression Inventory II (BDI II [32]), which is a unidimensional scale that assesses diverse psychological and physiological symptoms related to depression on a four-point scale. The BDI's total score ranges from 0 to 63, displaying a continuum of depression related symptoms. The scale has been found to show good reliability (0.92). Total sum scores were used in the present analysis.

2.4. Diurnal Cortisol Secretion. All participants were provided with a saliva kit to bring home. They were asked to provide samples on two different days, separated by 3 days, with the first day of sampling starting 3 days after their visit to our laboratory. Saliva was collected using passive drool at the time of awakening and 30 minutes after awakening in order to calculate the "Cortisol Awakening Response" (CAR [33]). It has been reported that during the first hour after awakening, cortisol levels show an acute increase [33]. Cortisol determination during this time of day appears to represent a response of the HPA axis to an endogenous stimulation and is a reliable indicator of diurnal HPA activity [34]. Participants were also asked to provide three additional samples at 14:00 and 16:00 and before bedtime. These sampling times have been shown in previous studies to be reliable markers of the diurnal cycle of cortisol secretion [35, 36]. As the nonadherence to saliva sampling in ambulatory settings has been shown to exert a significant impact on the resulting cortisol profile [37], a "daily sampling questionnaire" was also completed. Individuals were asked to record the exact time of each saliva sample to assess participants' compliance.

2.5. Stress Reactivity. Participants were exposed to the Trier Social Stress Test (TSST [38]). The TSST is an established and highly effective psychosocial stress paradigm used to provoke activation of the HPA axis. The version of the TSST that we used in the current study was somewhat different from the original version as we used a "Panel-out" (judges behind a false mirror) instead of a "Panel-in" (judges in the same room as the participants) condition. The reason why we made the decision to use the Panel-out condition in the current study is that the research assistants who acted as judges in our experiment were younger than the participants. We have shown in previous studies that environmental factors such as age of research assistants can lead to a spurious stress response in some individuals [39, 40], and, consequently, we wanted to limit contact between our participants and the judges. The Panel-out version of the TSST was used in many of our studies. While one study reported no significant differences between the Panel-in and the Panel-out conditions in men [41], another study has reported higher cortisol reactivity in the Panel-in compared to the Panel-out condition in women [42]. Recently, our laboratory found no significant differences in terms of cortisol reactivity between the Panel-in and Panel-out condition when comparing 140 men, women in the luteal phase of their menstrual cycle, and women taking oral contraceptives. Therefore both conditions induce a stress response (article in preparation).

In summary, the TSST involves an anticipation phase (10 minutes) and a test phase that comprises 10 minutes of public speaking. The test phase is divided into a mock job interview (5 minutes) followed by mental arithmetic (5 minutes). Throughout their performance, participants face a one-way mirror and a camera. Behind this mirror, two confederates act as judges and pretend to be experts in behavioral analysis while observing the participants and communicating with them via an intercommunication system. Participants underwent the TSST in the afternoon between 13:30 and 16:30. A total of eight saliva samples for cortisol determination were obtained at −20 min and −10 min (baseline), immediately before the TSST as well as +10, +20, +30, +40, and +50 min after the TSST began.

2.6. Procedure. During recruitment, potential participants were told on the phone that the study consisted of one testing day, lasting two hours, and two days of saliva sampling at home were required following the testing session. All participants were tested at the Douglas Institute Research Center.

For the laboratory visit, participants were tested in the afternoon in order to obtain adequate cortisol reactivity to the psychosocial stressor and to control for possible differential effects of the circadian cortisol patterns. Upon arrival at the laboratory, participants were asked to read and sign an informed consent form. Thereafter, they were asked to answer the psychological questionnaires, which took approximately 15 minutes. Participants provided saliva samples by filling a small plastic vial with 1 mL of pure saliva (i.e., passive drool). Participants were instructed about the TSST and prepared their mock job interview speech during a 10-minute anticipation phase. Participants then had to do the verbal (5

minutes) and mental arithmetic (5 minutes) tasks. After the recovery period, they were debriefed with regard to the goal of the public speaking task. Participants were debriefed about the general hypothesis of the study when they brought the home saliva kit back to the lab.

2.7. Salivary Cortisol Assays. Salivary samples were maintained at −20°C until time of cortisol concentration determination. Salivary cortisol concentrations were determined in Dr. Dominique Walker's laboratory at the Douglas Institute Research Center by radioimmunoassay using a kit from DSL (Diagnostic System Laboratories, Inc., Texas, USA). Total binding and nonspecific binding typically range between 47–63% and 0.5–1.5%, respectively. The intra-assay and interassay coefficient of variation for these studies are 4.6% and 5%, respectively. The limit of detection of the assay is 0.01 dl, and all samples were assayed in duplicates.

2.8. Statistical Analysis. All the analyses were done in two separate sets. The first set of analyses was done with Group (2 levels: consumer *versus* nonconsumer) as the independent variable to test whether as a group, consumers of self-help books present different psychoneuroendocrine profiles when compared to nonconsumers of self-help books. In the second set of analyses, consumers of self-help books were split as a function of their preference for growth-oriented or problem-focused books and compared with the nonconsumer group, using Group (3 levels: growth-oriented, problem-focused, and nonconsumer) as the independent variable.

For each analysis, personality traits (as measured by the five NEO subscales "neuroticism," "extroversion," "openness," "agreeableness," and "conscientiousness"), locus of control, self-esteem, and depressive symptoms were included in univariate ANOVAs. For cortisol, both diurnal and reactive cortisol values followed a normal distribution and, for this reason, raw data of cortisol were used for all analyses. For each salivary cortisol analysis, sex and body mass index (BMI) were entered as covariates as these are factors associated with cortisol production [43]. Greenhouse-Geisser values were used when the assumption of sphericity was violated. Diurnal cortisol secretion was calculated using the mean concentration of cortisol for each sample on both days of saliva sampling, resulting in five cortisol means. In order to determine whether self-help book use was related to diurnal cortisol secretion, we calculated the CAR as well as using the trapezoidal method to calculate area under the curve with respect to ground (AUCg; basal cortisol). In order to determine whether self-help book use was related to reactive cortisol secretion, we calculated the area under the curve relative to increase (AUCi; reactive cortisol) [44]. These analyses were made in order to determine whether there were significant group differences in terms of basal and reactive cortisol levels between groups. To ascertain the participant's compliance regarding the diurnal saliva sampling, time when saliva samples were taken was computed into a mean in each group and ANOVAs were used to calculate whether there were significant group differences.

Finally, we calculated the effects size for the comparison between consumers *versus* nonconsumers and the comparison between preference for growth-oriented or problem-focused books in order to determine (1) the statistical power of the significant differences observed and (2) the appropriate sample size for a larger full scale study.

3. Results

3.1. Assessment of Feasibility and Adverse Events from This Pilot Study. In terms of feasibility, we found it quite easy to recruit consumers of self-help books as no differences were observed in terms of time and cost of recruitment of this population compared to other populations we have tested in the past. Recruitment of nonconsumers was more time consuming because we had to validate *a posteriori* the nonconsumption of self-help books in the individuals calling us to participate in the research but, overall, the burden was not high on recruitment. No adverse events were reported during recruitment and testing, although the research assistants working on this project reported that the testing of consumers of self-help books took generally longer than testing of nonconsumers because consumers were generally more verbal and interacted more with the assistants during testing.

3.2. Preliminary Analyses. Figure 1 shows that participants displayed a normal diurnal cortisol rhythm as well as an increase in cortisol in response to the TSST. Preliminary analysis also revealed that groups did not differ in terms of time of saliva sampling (all P values > 0.763) and that groups did not differ in terms of age, BMI, years of education, or sex of the participants (all P values > 0.165). Also, no group differences were observed for personality traits (all P values > 0.112), locus of control (all P values > 0.162), and self-esteem (all P values > 0.295) when we contrasted the consumers to the nonconsumers, and when we split the consumers into those individuals with a preference for growth-oriented or problem-focused books.

3.3. Consumers versus Nonconsumers of Self-Help Books. We first contrasted consumers and nonconsumers on basal/reactive cortisol levels and depressive symptomatology. We found no differences between consumers and nonconsumers on diurnal cortisol levels AUCg ($F(1, 30) = 0.080$, $P = 0.780$; see Figure 2(a)), CAR ($F(1, 30) = 0{,}31$, $P = 0.862$; see Figure 2(b)), and reactive cortisol AUCi ($F(1, 30) = 2.172$, $P = 0.151$; see Figure 2(c)). For depressive symptomatology, the analysis showed a significant between-group effect ($F(1, 31) = 6{,}186$, $P = 0.019$), with the consumer group displaying a higher depressive mean score (7,28 ± 1,01 *versus* 4,14 ± 0,57) when compared to nonconsumers (see Figure 2(d)).

3.4. Preference for Growth-Oriented or Problem-Focused Books. In a second set of analyses splitting the consumer group into those individuals with a preference for growth-oriented or problem-focused books, we found no group differences in AUCg diurnal cortisol levels ($F(2, 29) = 0.789$, $P = 0.464$; see Figure 3(a)) or CAR ($F(2, 29) = 0.015$,

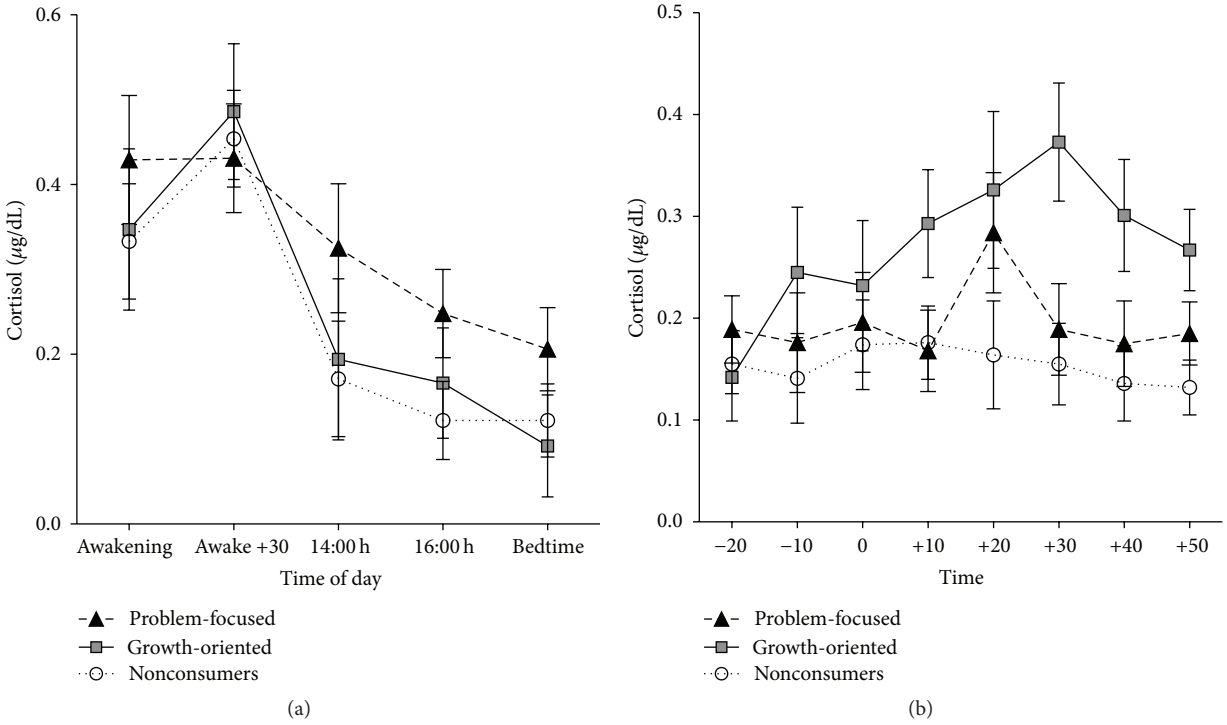


Figure 1: Repeated-measures of (a) diurnal cortisol and (b) reactive cortisol as a functioning of groups based on preference for problem-focused or growth-oriented self-help books. These graphs are used strictly to represent the mean (standard error bars) cortisol concentrations and to show the magnitude of the cortisol response to the TSST in each of the groups tested. As such, they have no relation to the statistical model employed that otherwise used the composite measure of area under the curve for cortisol levels (basal, reactive, and CAR).

$P = 0.985$; see Figure 3(b)). We did, however, find a significant group difference in reactive cortisol levels AUCi [$F(2, 29) = 4.079$, $P = 0.028$]. *Post hoc* analyses showed that the growth-oriented group presented a significantly greater AUCi when compared to the nonconsumer group ($P = 0.040$; see Figure 3(c)). No differences were found between the problem-focused group and nonconsumer group ($P = 1.00$) or between the problem-focused group and the growth-oriented group ($P = 0.10$).

3.5. Supplementary Analyses. Strikingly, when one looks at cortisol levels in response to the TSST in the group of nonconsumers (see Figures 1 and 3(d)), one can see that the cortisol response appears to be quite low compared to that of consumers of self-help books. This could represent either a hyporesponse to the TSST in the nonconsumers of self-help books, or a hyperresponse to the TSST in the consumers of growth-oriented self-help books (see Figure 1).

In order to contextualize the cortisol response to the TSST in the group of nonconsumers, we extracted compiled databases on reactive cortisol in response to TSST (we have more than a thousand participants tested with the same protocol on the TSST in our databases). We extracted data for sex- and age-matched controls and compared their response to the TSST to that of the nonconsumers. The results are presented in Figure 4. We found no significant differences between the cortisol levels in response to the TSST among participants from our previous studies when compared to nonconsumers of self-help books. This suggests that the group of nonconsumers presents a typical cortisol response to the TSST but that the effect seems blunted given the hyperreactivity observed in the group of consumers of growth-oriented self-help books.

3.6. Depressive Symptomatology. When we compared groups on depressive symptomatology, we found a group difference in depressive scores [$F(2, 29) = 5.876$, $P = 0.008$]. Post hoc analyses showed that the problem-focused group presented a significantly higher score on the BDI than the nonconsumer group ($P = 0.006$; see Figure 3(d)). No differences were found between the growth-oriented group and nonconsumer group ($P = 0.795$) or between the problem-focused group and the growth-oriented group ($P = 0.095$).

3.7. Calculation of Effect Size. Cohen's f^2 effect sizes [45] for group differences on depressive symptomatology were large for both the comparison between consumers and nonconsumers of self-help books ($f^2 = 0.454$) and between growth-oriented and problem-focused groups when compared to nonconsumers ($f^2 = 0.63$). We found a similar large effect size for the group difference on reactive cortisol levels when comparing the growth-oriented and problem-focused groups to the nonconsumer group ($f^2 = 0.507$).

Table 1 presents the effect size for all the comparisons performed in the present study. We also calculated the number of participants that would be needed in a future larger scale

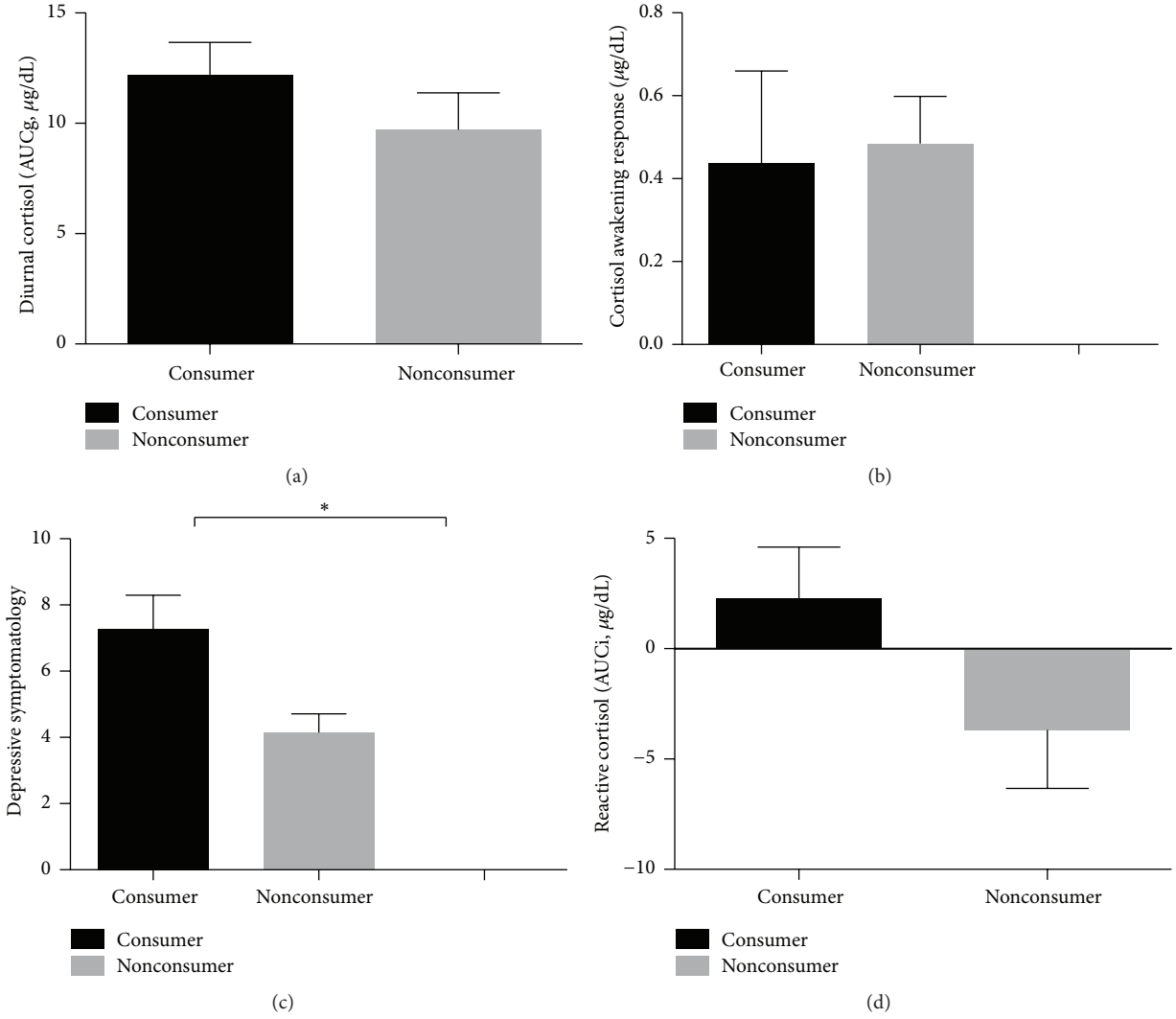


Figure 2: (a) Diurnal salivary cortisol levels (AUCg) as a function of consumer group. (b) Cortisol awakening response as a function of group. (c) Reactive salivary cortisol levels (AUCi) in response to the Trier Social Stress Test as a function of group. (d) Depressive symptomatology as a function of group. The asterisk (∗) means $P < 0.05$. For all figures, the error bars represent the standard error of the mean adjusted for sex and body mass index.

Table 1: Cohen's f^2 effect sizes for the comparisons of basal/reactive cortisol and depressive symptoms between consumers and nonconsumers of self-help books and between consumers of problem-focused *versus* growth-oriented self-help books when compared to nonconsumers.

	AUC basal cortisol levels	AUC reactive cortisol levels	Depressive symptoms
Consumers *versus* nonconsumers	Cohen's $f = 0.0821$ $N > 1000$	Cohen's $f = 0.269$ $N = 112$	Cohen's $f = 0.454$ $N = 42$
Problem-focused *versus* growth-oriented *versus* nonconsumers	Cohen's $f = 0.2418$ $N = 168$	Cohen's $f = 0.507$ $N = 40$	Cohen's $f = 0.633$ $N = 30$

Cohen's f^2 represents one of several effect size measures that is generally used in the context of a F-test for ANOVA. Cohen gives the following guidelines for the psychological and/or social sciences for Cohen's f^2 values: small effect size = 0.10; medium effect size = 0.25; large effect size = 0.40.

study in order to have sufficient statistical power to find group differences on the variables tested. This analysis showed that between 150 and 1000 participants would be needed to find any significant differences in basal cortisol levels as a function of self-help book consumption. By contrast, a much smaller sample size would be needed for reactive cortisol levels ($N = 40$) and depressive symptoms ($N = 30$), based on the medium/large effect sizes found in this small pilot study.

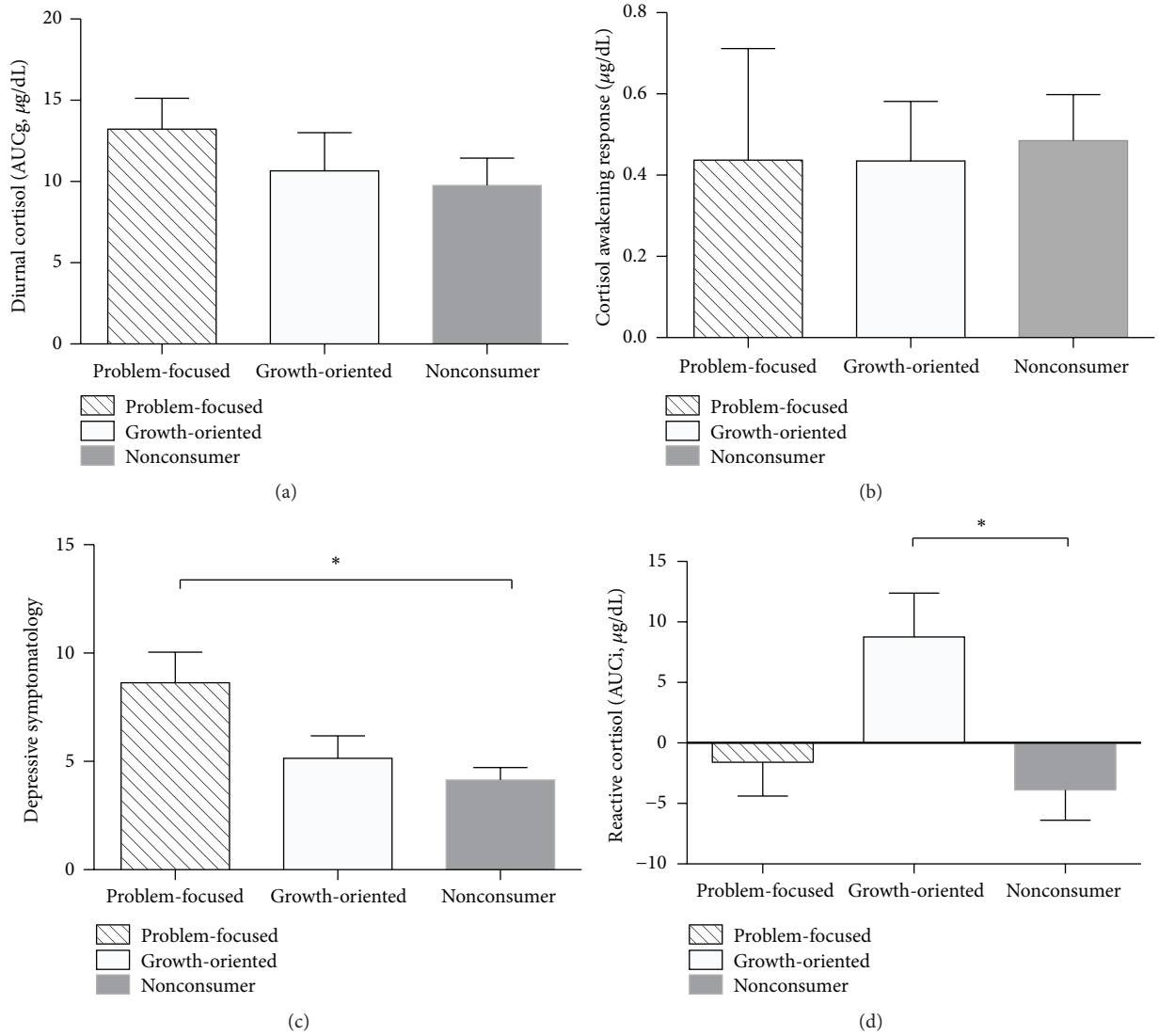


Figure 3: (a) Diurnal salivary cortisol levels (AUCg) as a function of group. (b) Cortisol awakening response as a function of group. (c) Reactive salivary cortisol levels (AUCi) in response to the Trier Social Stress Test as a function of group. (d) Depressive symptomatology as a function of group. The asterisk (∗) means $P < 0.05$. For each figure, the error bars represent the standard error of the mean adjusted for age and body mass index.

4. Discussion

The first goal of this pilot study was to determine whether consumers and nonconsumers of self-help books differ in physiological and/or psychological markers of stress. We found no differences in basal and reactive cortisol levels but reported that consumers of self-help books present increased depressive symptomatology when compared to nonconsumers of self-help books. Although this difference was obtained with a small sample size, the effect size of the difference was large ($f^2 = 0.454$). This first result confirms previous suggestions stating that individuals may buy self-help books in order to self-diagnose and/or treat their psychological distress.

The second goal of this pilot study was to assess whether the *type* of self-help books one has a preference for is a better marker of physiological and psychological markers of stress than general interest in self-help books as a whole. First, we found that consumers of problem-focused self-help books presented significantly more depressive symptoms than consumers of growth-oriented self-help books. Hereto, the effect size obtained was large ($f^2 = 0.633$). This later result shows that the group differences observed between consumers and nonconsumers of self-help books on depressive symptoms is mainly driven by consumers of problem-focused self-help books.

The increased depressive symptoms found in consumers of problem-focused self-help books converge with the literature on depressive symptomatology suggesting that these symptoms are associated with higher self-victimization [46]. Future studies on self-help books consumers should therefore measure self-victimization in order to verify if it mediates

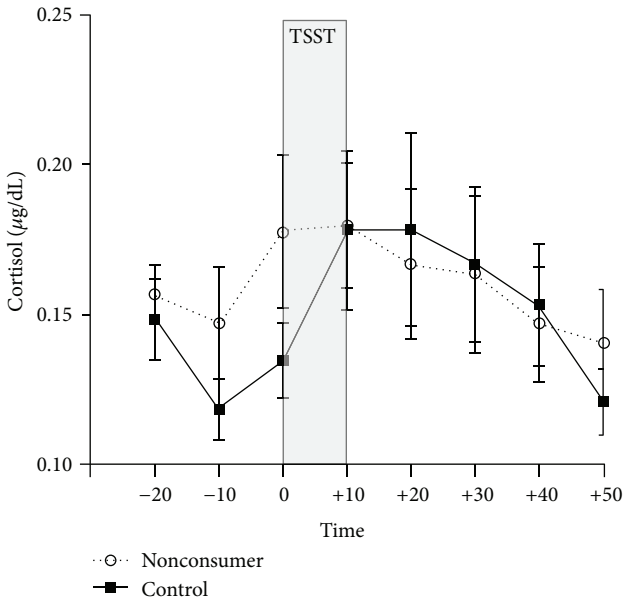


Figure 4: Comparison of reactive salivary cortisol levels in response to the Trier Social Stress Test in nonconsumers of self-help books and a control group of 14 age- and sex-matched individuals extracted from our database. The error bars represent the standard error of the mean.

the association between preference for problem-focused self-help books and depressive symptomatology. While we cannot ascertain that consumers of this literature chose to read these kinds of books because they show higher depressive symptoms, it is possible that using this literature leads to higher depressive symptomatology. Since our cross-sectional design does not allow us to determine the directionality of the association found, a longitudinal study would be necessary to test this. Given the large effect size obtained for this group difference in depressive symptomatology, sample sizes in the range of 20 to 30 participants per group would provide sufficient statistical power to confirm group differences.

In future studies of these populations, it could be interesting to assess potential cognitive behavioral tendencies that have been linked to depression. For example, rumination [46], guilt [47], mind wandering [48], and worries [39, 49, 50] are behavioral tendencies among individuals with depressive symptomatology that may be more prominent among consumers of problem-focused books. Indeed, these cognitions and/or behaviors have been shown to be linked to both depressive symptomatology and stress physiology and could act as mediators in the association between problem-focused self-help books consumption and presence of higher depressive symptomatology. Measuring them in future studies could therefore strengthen our understanding of the psychoneuroendocrine profile of consumers of problem-focused self-help books.

The groups did not differ on diurnal cortisol levels, when consumers were compared to nonconsumers and when the consumer group was split as a function of preference for problem-focused or growth-oriented self-help books. Also, the effect sizes for these differences were very low and we calculated that sample sizes between 150 and >1000 individuals would be necessary to find any statistical differences in diurnal cortisol levels between groups. It is important to note that diurnal cortisol rhythm has been shown to be very stable in healthy populations and that most differences observed in basal cortisol secretion are observed in clinical populations [34, 51]. Therefore, the fact that we recruited healthy consumers of self-help books and that we excluded participants presenting psychopathologies might explain why we were not able to detect any differences in terms of diurnal cortisol levels. Therefore, in future studies, it would be interesting to compare the diurnal cortisol profile of clinically depressed individuals who consume self-help books and clinically depressed nonconsumers if one is interested in measuring diurnal cortisol levels as a function of consumption of self-help books.

When we compared groups on reactive cortisol levels, we found that consumers of growth-oriented self-help books are significantly more reactive to a laboratory psychosocial stressor when compared to consumers of problem-focused self-help books or nonconsumers of self-help books and the effect size was large for this group difference ($f = 0.507$). This is an important finding as we had previously found no significant difference between consumers and nonconsumers of self-help books on reactive cortisol levels. This result suggests that it is the preference for a particular type of self-help books (here, growth-oriented self-help books) that is associated with increased production of cortisol in response to a psychosocial stressor and not general attraction toward self-help books more generally.

Interestingly, no group differences were found in the questionnaire testing locus of control. This suggests that the increased stress reactivity to the TSST that we observed in consumers of growth-oriented self-help books cannot be explained by one's belief that one has control over the situation, as suggested by this type of self-help books. One mechanism that could explain this higher reactivity might be some other personality trait inherent to people who are attracted by this type of literature. Even though we measured basic personality traits using the NEO-FFI and did not find any differences for five factors measured, it is still possible that some other personality traits that elude measurement with the NEO-FFI could explain the greater cortisol reactivity reported in individual having a preference for growth-oriented self-help books.

On the other hand, we do know that HPA axis reactivity to stressors plays a critical role in providing energy resources to face the environment and is therefore both adaptive and necessary [52]. Therefore, another possible mechanism that could explain the higher stress reactivity observed in individuals having a preference for growth-oriented self-help books is that coping mechanisms taught in this literature allow these consumers to react in a more effective way to their environment as required by the situation. This suggestion goes along with studies performed in depressed patients [53] and normal individuals [54] showing that greater use of escape-avoidance coping (unhealthy coping mechanism) is associated with less cortisol reactivity.

4.1. Limitations. The present pilot study is characterized by a number of limitations, including a small sample size, a cross-sectional protocol, and an underrepresentation of men. Although we made sure that our groups were equivalent in a number of factors that are known to have effects on the physiological stress response (such as sex, sex hormones, socioeconomic status, age, and BMI), it is still possible that some of the negative findings reported here are due to a Type II error due to small sample size. Additionally, while the current pilot study relied on the use of a "daily sampling questionnaire" in order to assess participant's compliance when collecting diurnal cortisol saliva samples, this method has been shown to be less reliable than the use of electronic devices [37]. However, a recent study suggests that multiday sampling somewhat tempers this effect in comparison to only one day of sampling [42, 55]. Future studies on consumers of self-help books should consider using electronic devices in the assessment of diurnal cortisol as this method was shown to be more reliable [29].

Furthermore, even though locus of control did not explain the intergroup differences in terms of stress reactivity and depressive symptoms, other factors such as coping strategies that have not been measured in the present study may have predictive value for cortisol secretion in consumers of problem-focused *versus* growth-oriented self-help books. Future studies assessing psychological and/or physiological markers in consumers of self-help books should therefore consider measuring coping strategies, which may explain some of the observed associations between variables. Also, as mentioned earlier, the cross-sectional design prevents us from determining any directionality between variables and, consequently, a longitudinal design measuring stress hormones before and after utilization of self-help books could help disentangle the cause-effects relationship of the self-help book industry on physiological and psychological markers of stress. Finally, given the differences in psychological and biological markers of stress observed in consumers of problem-focused *versus* growth-oriented self-help books, it would be important in future studies to determine whether one group of consumers benefits more from a particular type of unguided self-help literature when compared to the other group.

5. Conclusion

Although we found no general difference in cortisol levels when comparing consumers and nonconsumers of self-help books, we found that consumers of growth-oriented self-help books are more stress reactive when facing a social evaluative threat, while consumers of problem-focused self-help books show higher depressive symptomatology when compared to nonconsumers of self-help books. Our results therefore suggest that preference for a particular *genre* of self-help book (problem-focused *versus* growth-oriented) may be associated with increased stress and/or mental burden in consumers of self-help books. Every year, the self-help industry generates billions of dollars in the US and Canada making it one of the most lucrative businesses in North America. Clinicians are now using guided bibliotherapy to help patients deal with various life conditions and we know that unguided self-help books differ greatly in terms of quality of valid scientific information provided. It is predicted that the self-help book industry will only grow in future years. Consequently, it is essential to understand the impact of different types of self-help books on individuals' physical and mental health.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This pilot study was funded by an internal grant from the Centre for Studies on Human Stress.

References

[1] World Health Organization, *The World Health Organization Report 2001—Mental Health: New Understanding*, WHO, 2001.

[2] C. Velehorschi, P. Bleau, M. Vermani, M. Furtado, and L. J. Klassen, "Understanding the role of adjunctive nonpharmacological therapies in management of the multiple pathways to depression," *Psychiatry Research*, vol. 220, supplement 1, pp. S34–S44, 2014.

[3] K. Hanson, T. L. Webb, P. Sheeran, and G. Turpin, "Attitudes and preferences towards self-help treatments for depression in comparison to psychotherapy and antidepressant medication," *Behavioural and Cognitive Psychotherapy*, 2015.

[4] A. Bergsma, "Do self-help books help?" *Journal of Happiness Studies*, vol. 9, no. 3, pp. 341–360, 2008.

[5] M. Lucock, R. Barber, A. Jones, and J. Lovell, "Service users' views of self-help strategies and research in the UK," *Journal of Mental Health*, vol. 16, no. 6, pp. 795–805, 2007.

[6] S. Salerno, *SHAM: How the Self-Help Movement Made America Helpless*, Crown Publishers, New York, NY, USA, 2005.

[7] P. Pearsall, *The Last Self-Help Book You'll Ever Need*, Books B, New York, NY, USA, 2005.

[8] P. Cuijpers, "Bibliotherapy in unipolar depression: a meta-analysis," *Journal of Behavior Therapy and Experimental Psychiatry*, vol. 28, no. 2, pp. 139–147, 1997.

[9] P. Cuijpers, "The future of psychotherapy research: stop the waste and focus on issues that matter," *Epidemiology and Psychiatric Sciences*, 4 pages, 2015.

[10] NICE, *Depression in Adults: The Treatment and Management of Depression in Adults*, CG 90, NICE, London, UK, 2009.

[11] V. Kaldo, J. Ramnerö, and S. Jernelöv, "Involving clients in treatment methods: a neglected interaction in the therapeutic relationship," *Journal of Consulting and Clinical Psychology*, 2015.

[12] S. El Alaoui, E. Hedman, V. Kaldo et al., "Effectiveness of internet-based cognitive–behavior therapy for social anxiety disorder in clinical psychiatry," *Journal of Consulting and Clinical Psychology*, vol. 83, no. 5, pp. 902–914, 2015.

[13] D. M. Wilson and T. F. Cash, "Who reads self-help books? Development and validation of the self-help reading attitudes survey," *Personality and Individual Differences*, vol. 29, no. 1, pp. 119–129, 2000.

[14] P. C. A. M. den Boer, D. Wiersma, and R. J. Van den Bosch, "Why is self-help neglected in the treatment of emotional disorders? A meta-analysis," *Psychological Medicine*, vol. 34, no. 6, pp. 959–971, 2004.

[15] G. R. W. M. Willemse, F. Smit, P. Cuijpers, and B. G. Tiemens, "Minimal-contact psychotherapy for sub-threshold depression in primary care: randomised trial," *British Journal of Psychiatry*, vol. 185, pp. 416–421, 2004.

[16] W. Styron, *Darkness Visible*, Random House, New York, NY, USA, 1990.

[17] J. Polivy and C. Peter Herman, "If at first you don't succeed: false hopes of self-change," *American Psychologist*, vol. 57, no. 9, pp. 677–689, 2002.

[18] J. K. Norem and E. C. Chang, "The positive psychology of negative thinking," *Journal of Clinical Psychology*, vol. 58, no. 9, pp. 993–1001, 2002.

[19] B. Ehrenreich, *Bright-Sided: How the Relentless Promotion of Positive Thinking Has Undermined America*, MacMillan, 2009.

[20] Z. Saper and J. Forest, "Personality variables and interest in self-help books," *Psychological Reports*, vol. 60, no. 2, pp. 563–566, 1987.

[21] J. J. Forest, "Exploring more on the effects of psychological self-help paperbacks," *Psychological Reports*, vol. 63, no. 3, pp. 891–894, 1988.

[22] A. H. Crisp, M. G. Gelder, S. Rix, H. I. Meltzer, and O. J. Rowlands, "Stigmatisation of people with mental illnesses," *British Journal of Psychiatry*, vol. 177, pp. 4–7, 2000.

[23] J. C. Phelan, E. J. Bromet, and B. G. Link, "Psychiatric illness and family stigma," *Schizophrenia Bulletin*, vol. 24, no. 1, pp. 115–126, 1998.

[24] S. Horstmann, T. Dose, S. Lucae et al., "Suppressive effect of mirtazapine on the HPA system in acutely depressed women seems to be transient and not related to antidepressant action," *Psychoneuroendocrinology*, vol. 34, no. 2, pp. 238–248, 2009.

[25] G. E. Miller, E. Chen, and E. S. Zhou, "If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans," *Psychological Bulletin*, vol. 133, no. 1, pp. 25–45, 2007.

[26] S. J. Lupien, B. S. McEwen, M. R. Gunnar, and C. Heim, "Effects of stress throughout the lifespan on the brain, behaviour and cognition," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 434–445, 2009.

[27] S. B. Hulley, S. R. Cummings, W. S. Browner, D. G. Grady, and T. B. Newman, *Designing Clinical Research*, Wilkins LWA, 2013.

[28] R. R. McCrae and P. T. Costa Jr., "Validation of the five-factor model of personality across instruments and observers," *Journal of Personality and Social Psychology*, vol. 52, no. 1, pp. 81–90, 1987.

[29] J. W. Mason, "A review of psychoendocrine research on the pituitary-adrenal cortical system," *Psychosomatic Medicine*, vol. 30, no. 5, pp. 576–607, 1968.

[30] G. Krampen, *Fragebogen zu Kompetenz- und Kontrollüberzeugungen*, Hogrefe, Göttingen, Germany, 1991.

[31] M. Rosenberg, *Society and the Adolescent Self-Image*, Princeton University Press, Princeton, NJ, USA, 1965.

[32] A. T. Beck, R. A. Steer, and M. G. Carbin, "Psychometric properties of the beck depression inventory: twenty-five years of evaluation," *Clinical Psychology Review*, vol. 8, no. 1, pp. 77–100, 1988.

[33] J. C. Pruessner, D. H. Hellhammer, and C. Kirschbaum, "Burnout, perceived stress, and cortisol responses to awakening," *Psychosomatic Medicine*, vol. 61, no. 2, pp. 197–204, 1999.

[34] Y. Chida and A. Steptoe, "Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis," *Biological Psychology*, vol. 80, no. 3, pp. 265–278, 2009.

[35] S. J. Lupien, A. Fiocco, N. Wan et al., "Stress hormones and human memory function across the lifespan," *Psychoneuroendocrinology*, vol. 30, no. 3, pp. 225–242, 2005.

[36] S. J. Lupien, A. R. Lecours, I. Lussier, G. Schwartz, N. P. V. Nair, and M. J. Meaney, "Basal cortisol levels and cognitive deficits in human aging," *Journal of Neuroscience*, vol. 14, no. 5, pp. 2893–2903, 1994.

[37] B. M. Kudielka, J. E. Broderick, and C. Kirschbaum, "Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects," *Psychosomatic Medicine*, vol. 65, no. 2, pp. 313–319, 2003.

[38] C. Kirschbaum, K.-M. Pirke, and D. H. Hellhammer, "The 'Trier social stress test'—a tool for investigating psychobiological stress responses in a laboratory setting," *Neuropsychobiology*, vol. 28, no. 1-2, pp. 76–81, 1993.

[39] S. Sindi, A. J. Fiocco, R.-P. Juster, C. Lord, J. Pruessner, and S. J. Lupien, "Now you see it, now you don't: testing environments modulate the association between hippocampal volume and cortisol levels in young and older adults," *Hippocampus*, vol. 24, no. 12, pp. 1623–1632, 2014.

[40] S. Sindi, A. J. Fiocco, R.-P. Juster, J. Pruessner, and S. J. Lupien, "When we test, do we stress? Impact of the testing environment on cortisol secretion and memory performance in older adults," *Psychoneuroendocrinology*, vol. 38, no. 8, pp. 1388–1396, 2013.

[41] J. Andrews, M. Wadiwalla, R. P. Juster, C. Lord, S. J. Lupien, and J. C. Pruessner, "Effects of manipulating the amount of social-evaluative threat on the cortisol stress response in young healthy men," *Behavioral Neuroscience*, vol. 121, no. 5, pp. 871–876, 2007.

[42] M. Wadiwalla, J. Andrews, B. Lai, C. Buss, S. J. Lupien, and J. C. Pruessner, "Effects of manipulating the amount of social-evaluative threat on the cortisol stress response in young healthy women," *Stress*, vol. 13, no. 3, pp. 214–220, 2010.

[43] L. Trépanier, R.-P. Juster, M.-F. Marin et al., "Early menarche predicts increased depressive symptoms and cortisol levels in Quebec girls ages 11 to 13," *Development and Psychopathology*, vol. 25, no. 4, pp. 1017–1027, 2013.

[44] J. C. Pruessner, C. Kirschbaum, G. Meinlschmid, and D. H. Hellhammer, "Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change," *Psychoneuroendocrinology*, vol. 28, no. 7, pp. 916–931, 2003.

[45] J. Cohen, *Statistical Power Analysis for the Behavioral Sciences*, Edition S, Lawrence Erlbaum Associates, Hillsdale, NJ, USA, 1988.

[46] K. Takano and Y. Tanno, "Self-rumination, self-reflection, and depression: self-rumination counteracts the adaptive effect of self-reflection," *Behaviour Research and Therapy*, vol. 47, no. 3, pp. 260–264, 2009.

[47] G. P. Owens, M. F. Steger, A. A. Whitesell, and C. J. Herrera, "Post-traumatic stress disorder, guilt, depression, and meaning in life among military veterans," *Journal of Traumatic Stress*, vol. 22, no. 6, pp. 654–657, 2009.

[48] Y.-Q. Deng, S. Li, and Y.-Y. Tang, "The relationship between wandering mind, depression and mindfulness," *Mindfulness*, vol. 5, no. 2, pp. 124–128, 2014.

[49] G. J. Diefenbach, M. E. McCarthy-Larzelere, D. A. Williamson, A. Mathews, G. M. Manguno-Mire, and B. G. Bentz, "Anxiety, depression, and the content of worries," *Depression and Anxiety*, vol. 14, no. 4, pp. 247–250, 2001.

[50] G. J. Diefenbach, M. A. Stanley, and J. G. Beck, "Worry content reported by older adults with and without generalized anxiety disorder," *Aging and Mental Health*, vol. 5, no. 3, pp. 269–274, 2001.

[51] Y. Chida and M. Hamer, "Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations," *Psychological Bulletin*, vol. 134, no. 6, pp. 829–885, 2008.

[52] S. S. Dickerson and M. E. Kemeny, "Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research," *Psychological Bulletin*, vol. 130, no. 3, pp. 355–391, 2004.

[53] H. Hori, T. Teraishi, M. Ota et al., "Psychological coping in depressed outpatients: association with cortisol response to the combined dexamethasone/CRH test," *Journal of Affective Disorders*, vol. 152–154, no. 1, pp. 441–447, 2014.

[54] M. Olff, J. F. Brosschot, G. Godaert et al., "Modulatory effects of defense and coping on stress-induced changes in endocrine and immune parameters," *International Journal of Behavioral Medicine*, vol. 2, no. 2, pp. 85–103, 1995.

[55] S. H. Golden, B. N. Sánchez, A. S. DeSantis et al., "Salivary cortisol protocol adherence and reliability by socio-demographic features: the Multi-Ethnic Study of Atherosclerosis," *Psychoneuroendocrinology*, vol. 43, pp. 30–40, 2014.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 4932128, 20 pages
http://dx.doi.org/10.1155/2016/4932128

Review Article

Stress Effects on Multiple Memory System Interactions

Deborah Ness and Pasquale Calabrese

Faculty of Psychology and Interdisciplinary Platform Psychiatry and Psychology, Division of Molecular and Cognitive Neuroscience, Neuropsychology and Behavioural Neurology Unit, University of Basel, Birmannsgasse 8, 4055 Basel, Switzerland

Correspondence should be addressed to Pasquale Calabrese; pasquale.calabrese@unibas.ch

Received 25 June 2015; Accepted 27 August 2015

Academic Editor: Laura Musazzi

Extensive behavioural, pharmacological, and neurological research reports stress effects on mammalian memory processes. While stress effects on memory quantity have been known for decades, the influence of stress on multiple memory systems and their distinct contributions to the learning process have only recently been described. In this paper, after summarizing the fundamental biological aspects of stress/emotional arousal and recapitulating functionally and anatomically distinct memory systems, we review recent animal and human studies exploring the effects of stress on multiple memory systems. Apart from discussing the interaction between distinct memory systems in stressful situations, we will also outline the fundamental role of the amygdala in mediating such stress effects. Additionally, based on the methods applied in the herein discussed studies, we will discuss how memory translates into behaviour.

1. Introduction

In the following, the biological correlates and mechanisms of stress and their influence on memory processing will be discussed in order to outline the basic mechanisms underlying the differential ways in which memory can be affected by a stressor.

1.1. The Biology of Stress and Emotional Arousal. Stress refers to an organism's physiological and psychological reaction triggered by an external or internal stressor, such as an environmental condition or a psychological stimulus. It is a state of mental or emotional strain or tension resulting from adverse or demanding circumstances and can last for just a few minutes to hours (acute stress) up to months or even years (chronic stress). Stress is a highly subjective experience in the sense that equal events are not perceived as equally stressful by different individuals. Thus, stress can be caused by numerous diverse events, including hassles of everyday life (e.g., time pressure) and life-threatening situations or circumstances (e.g., war or natural disasters). Whether a situation is experienced as stressful or not is determined by complex interactions between different brain regions, including the prefrontal cortex, the hippocampus, and the amygdala [1, 2]. The involvement of these structures in the process of appraisal is critical to the ability to link the currently experienced situation with one's past experiences in order to modulate adaptive behaviour.

The aforementioned limbic structures as well as the prefrontal cortex have connections to the hypothalamus which plays a crucial role concerning the activation of a physiological stress response induced by endocrinologic changes [2]. There are two main classes of stress hormones: (1) glucocorticoids (GCs; corticosterone in rodents, cortisol in humans) and (2) catecholamines (epinephrine and norepinephrine).

If a situation is perceived as stressful, neurons located in the paraventricular nucleus of the hypothalamus synthesize and release corticotropin-releasing hormone (CRH) which in turn triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary gland into the bloodstream. ACTH acts on the adrenal glands and thus induces the release of GCs from the adrenal cortices. GCs alter the function of multiple body tissues in order to mobilize or store energy to meet the demands of a stress challenge [3]. Through a negative feedback mechanism GCs inhibit CRH as well as ACTH secretion as they bind to GC receptors in the hypothalamus and the hippocampus, hence terminating

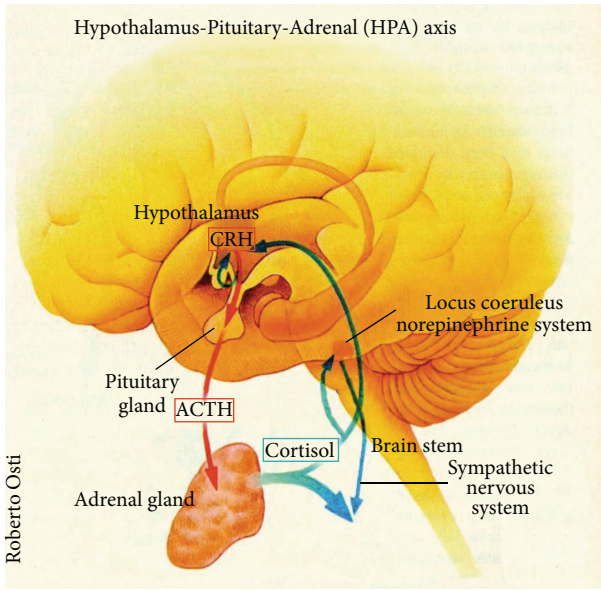


Figure 1: The Hypothalamus-Pituitary-Adrenal (HPA) axis. CRH = corticotropin-releasing hormone; ACTH = adrenocorticotropic hormone [6].

the stress response when the threatening situation is over [4, 5]. This stress system is known as the hypothalamic-pituitary-adrenal (HPA) axis (Figure 1).

There are two kinds of receptors which are targeted by GCs: (1) the low-affinity mineralocorticoid receptor (MR) and (2) the high-affinity glucocorticoid receptor (GR) [7]. While MRs are almost exclusively expressed in the hippocampal formation, GRs are widely distributed throughout the brain with especially high concentrations in the hippocampus, the amygdala, and the prefrontal cortex. As already mentioned, these brain regions play a critical role in the process of cognitive appraisal and are also actively involved in the negative feedback mechanism regulating HPA axis activation [8]. When there is no current stressor and the body is at rest, MRs are usually occupied, while GC levels are too low to bind to the low-affinity GRs. However, when GC levels rise, such as in the case of (chronic) stress, the GC concentration becomes high enough to activate the low-affinity GRs as well [3].

Apart from activating the HPA axis and its rather slow hormonal effects, the hypothalamus is also crucial for the activation of the autonomic nervous system in response to emotional arousal, which is produced by either aversive stressors or highly rewarding events [9]. This activation is initiated only seconds after a stressful event, whereas it takes up to 25 minutes until GC levels reach their peak. Autonomic nervous system activation triggers the release of epinephrine and norepinephrine from the adrenal medulla. Due to the characteristics of the blood brain barrier, catecholamines cannot enter the central nervous system, but they can indeed influence its activity through the complex connections between the autonomic and the central nervous system. Higher peripheral epinephrine levels induced by emotional arousal indirectly stimulate the release of central norepinephrine in the basolateral amygdala (BLA; Figure 2). Ascending fibres of the vagus nerve contain adrenergic receptors, which become activated through the binding of peripheral epinephrine in rats [10] and in humans [11]. These fibres transmit information regarding heightened activity in visceral sensory organs to the central nervous system, namely, to the nucleus tractus solitarius (NTS), which is located in the brainstem [12, 13]. In turn, NTS neurons activate the locus coeruleus (LC) through direct synapses and thus influence central norepinephrine activity [14]. Since most of the noradrenergic terminals in the BLA originate in the LC, the release of norepinephrine from the BLA seems to be mainly influenced by the activity of the LC [15–18]. Apart from its influence on the BLA, the LC also has extensive connections to the hippocampus and the prefrontal cortex, regions which are critically involved in memory processes (Figure 2). Norepinephrine is one of the primary neurotransmitters mediating the communication between these structures.

Apart from the above-described central effects of stress and emotional arousal, other sympathetic reactions, like elevated heart rate, blood pressure, and galvanic skin response, are also consistently reported [19–25]. Moreover, many other hormones, neurotransmitters, and neuropeptides are released after stressful experiences, which helps the organism to successfully adapt to the stressor and restore homeostasis [9].

Moreover, it has been proposed that increased plasticity provided by developing neurons in the hippocampus may increase an individual's capacity to adapt to a changing environment [26]. Recently, it has been theorized that the hippocampal formation, which is crucial to memory formation as well as spatial navigation, might also play an important role in stress regulation, possibly through the regulation of adult neurogenesis. For example, it has been found that artificially reduced neurogenesis through transgenic modifications or radiation exposure leads to an increased level of stress hormones following a stressful experience [27, 28]. This indicates that adult neurogenesis may be able to enhance the GC-mediated negative feedback mechanism of the HPA axis and thus could eventually act as a buffer to stress. In addition, GCs acting on GRs can result in the modulation of gene transcription through several complex molecular pathways, some of which are also involved in neurogenesis, namely, the forkhead box protein O3 (FOXO3A) pathway, which is activated, and the transforming growth factor β (TGFβ)-SMAD2-SMAD3 pathway and the Hedgehog pathway, which are inhibited [29–34].

Moreover, studies could show that stress also initiates the release of proinflammatory cytokines in the hippocampus and several other brain regions, where interleukin 1β levels are increased through a catecholamine-mediated mechanism [35]. Interestingly, stress also influences several neurotrophic factors, which are important for the growth and maintenance of neurons and thus their proper functioning. Among the neurotrophic factors sensible to stress are brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and neuregulin 1 (NRG1), which is part of the epidermal growth factor family of proteins [36].

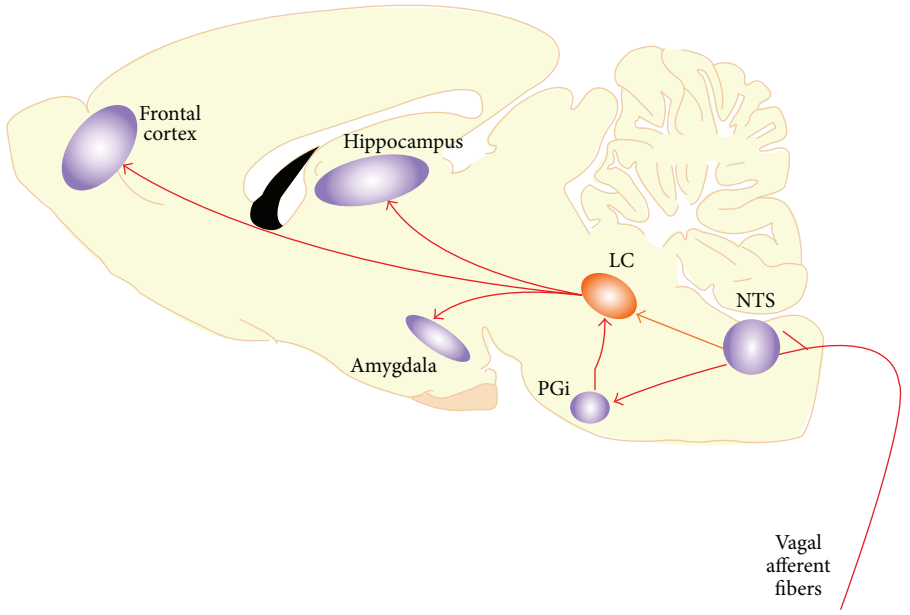


Figure 2: Schematic diagram depicting the activation of central structures through noradrenergic projections from the locus coeruleus (LC) in the rat brain. The nucleus tractus solitarius (NTS) receives peripheral input via the vagus nerve which is activated after stressful or emotionally arousing experiences [9].

However, we are still far from an integrative, complete understanding of the many effects stress has on a molecular level, especially when it comes to linking the knowledge to complex cognitive phenomena (e.g., memory) or even behavior. Since the neurobiological understanding of stress on a microlevel is still in its infancy and because it is beyond the scope of this review, we will focus on the above-described general biological stress response systems (HPA axis and autonomic nervous system), which are essential to understand the studies to be discussed in our review. However, it is of great importance to acknowledge that the correlates we describe in this paper (e.g., brain activity patterns) to explain the according behavioural findings *are themselves* the result of multiple complex interactions of different molecular pathways within each cell.

1.2. The Neuroanatomy of Multiple Memory Systems. Besides a chronological framework of memory processing (encoding, consolidation, and retrieval) a content-based subdivision of memory has been introduced and scientists became realerted to terms like conscious and nonconscious information processing. Whereas conscious memory processes are part of the declarative memory system, nonconscious information processes belong to the nondeclarative system. Since declarative memory allows for encoding the relationships between multiple items and events, it is considered to be representational, hence providing an internal model of the external world which is either true or false. Stored representations are highly flexible and thus able to guide performance in many different situations occurring in a changing environment [37]. In contrast, nondeclarative memory contents are not subject to conscious recollection, are not representational, and are thus neither true nor false. An important ability regarding nondeclarative memory processes lies in the extraction of common elements from a series of events. Nondeclarative memory formation can be described as modification of specialized performance systems, which become reactivated in situations similar to the original learning context [37].

Declarative memory is further subdivided into semantic and episodic memory (Figure 3). While episodic memory means context-based information processing with the possibility of "travelling back in time" and hence integrating a memory in its original spatiotemporal context, semantic memory (knowledge) is context-free [38]. Both of these declarative memory systems critically rely on brain structures in the medial temporal lobe (e.g., hippocampus) and the diencephalon. Additionally, other brain structures, such as the prefrontal lobe, also participate in episodic memory processes [39, 40].

Nondeclarative memory is further divided into four subsystems, which are responsible for functionally distinct processes: (1) procedural/habit memory, which refers to skill-based and largely automatic processes and is dependent on the striatum, (2) priming/perceptual learning—the phenomenon of an increased likelihood of reidentifying a previously subconsciously perceived stimulus/item—which is regulated by the neocortex, (3) conditioning, which involves the amygdala and the cerebellum, and (4) nonassociative learning, which operates over reflex pathways (Figure 3).

The described memory systems are simultaneously engaged in the parallel processing of information and can operate in a competitive or cooperative manner [41]. Seminal studies were able to show that memory operations in the mammalian brain do operate via anatomically distinct systems. In a study of Packard and coworkers [42] rats with fimbria-fornix lesions were impaired in a win-shift paradigm of the radial-maze test and caudate animals were unimpaired relative to controls. Conversely, rats with fimbria-fornix

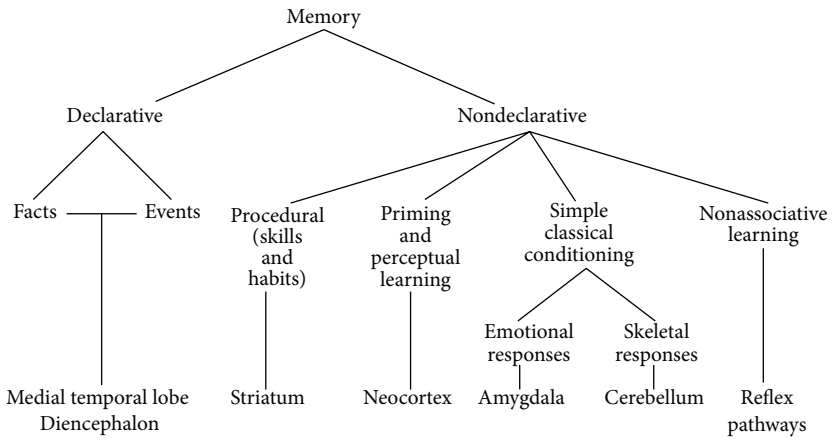


Figure 3: Taxonomy of multiple memory systems [37].

lesions were superior to controls in choice accuracy on the win-stay version radial-maze task, while caudate animals were impaired relative to control animals. This double dissociation indicated a different contribution of multiple memory systems which can be probed by different tests: (1) spatial navigation, (2) probabilistic classification learning (PCL), and (3) instrumental learning.

Spatial navigation tasks use single proximal as well as multiple distal cues, which help the subject to respond (Figure 4(a)). The hippocampal spatial memory system creates a cognitive map by associating multiple cues. In contrast, the procedural stimulus-response (S-R) memory system learns the association between a proximal cue/stimulus and a response. The S-R memory system is dependent on the dorsal striatum [2]. When subjects are trained in a spatial navigation task, subsequent behaviour in a test, where only the proximal cue is relocated, differs according to the used memory system. Going to the relocated proximal cue indicates S-R learning, whereas going to the originally reinforced target indicates multiple cue spatial learning.

In PCL tasks (e.g., the weather prediction task), subjects are trained in categorizing different stimuli (multiple cues on one to three out of four cards) to predict an outcome (sun versus rain) based on trial-by-trial feedback (Figure 4(b)). When learning has been controlled by the hippocampus-dependent declarative system during training, the explicit knowledge of the task is expected to be higher than after using the striatum-based procedural system.

Instrumental learning works with the paradigm of outcome devaluation (Figure 4(c)). Subjects are trained in two instrumental actions leading to distinct food rewards, one of which is subsequently devalued by unlimited access to this kind of food. If subjects use a goal-directed learning strategy dependent on the prefrontal cortex, they favour the food outcome which has not been devalued over the devalued food in a subsequent extinction test. A goal-directed learning strategy encodes the relationship between action and outcome and thus makes subjects sensitive to changes regarding the outcome value of their actions. In contrast, the lack of such behaviour indicates dorsolateral striatum-dependent S-R learning and is referred to as habit learning,

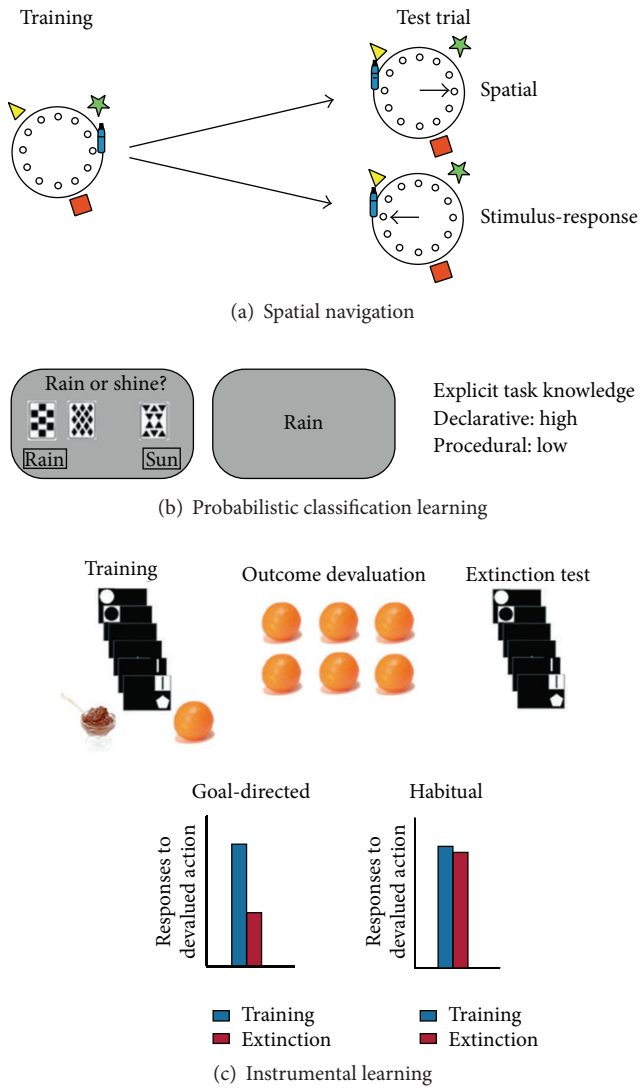


Figure 4: Behavioural tests to distinguish between multiple memory systems: (a) spatial navigation, (b) probabilistic classification learning, and (c) instrumental learning [2].

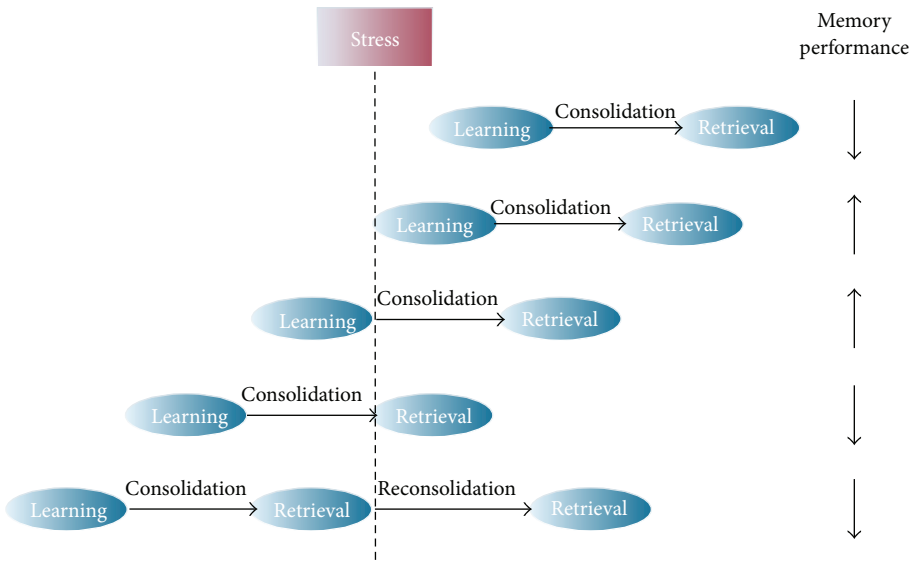


Figure 5: Stress affects memory performance in a different manner dependent on the timing of the stressor [2].

where the relationship between the stimulus and the response is encoded without a link to the actual outcome [2].

1.3. Memory Formation under Stress. The brain regions involved in a physiological stress response extensively overlap with the structures, which are critical for memory processes. A great amount of evidence from numerous studies confirms that stress and emotional arousal affect memory. In the following, the effects of stress on quantitative mostly time-dependent aspects and on qualitative aspects which involve multiple memory systems will be reported.

1.3.1. Stress Effects on Quantitative Memory Performance. Stress affects the amount of memory, that is, *how much* an individual remembers. These stress effects have been extensively studied with particular focus on hippocampus-dependent memory processing, showing time-dependent effects of the stressor on encoding, consolidation, and retrieval [8, 43]. The effects of stress on encoding are difficult to assess because they are always confounded with consolidation and retrieval processes. It is probably this difficulty which led to the highly conflicting results of different researchers studying stress effects on encoding. While some studies indicate enhancing effects of stress on memory encoding [44, 45], others reported impairing effects [46–49]. However, some of these contradictions might be due to differences in emotionality, which has been shown to critically influence memory processes. In a state of higher central norepinephrine triggered by emotional arousal, memory for negative information (e.g., negative pictures) appears to be enhanced in the presence of GCs. At the same time though, memory for neutral information seems to be impaired [50, 51].

The adrenal hormones released by emotional arousal thus seem to be important regulators of memory strength: the consolidation enhancing effects of GCs disappear without the cooccurrence of norepinephrine in the BLA. In line with this, lesions of the BLA or the administration of beta-adrenergic receptor antagonists in the BLA abolish the enhancing effects of high GC levels on memory consolidation [52, 53]. Furthermore, it has been shown that GR antagonists impair, whereas GCs enhance consolidation of emotionally relevant information [54–56]. This enhancement of the consolidation processes induced by stress hormones is also well supported in human studies [57]. In contrast, stress impairs retrieval of previously learned spatial material. Spatial memory in rodents is severely impaired when they are stressed or received GCs before retention testing [58, 59]. However, beta-adrenergic receptor antagonists or a lesion in the BLA abolishes the impairing effects of GCs on spatial memory retrieval [60, 61]. Thus, the effects of stress on memory retrieval also underlie the influence of noradrenergic activity in the BLA. This is also the case in humans: stress- or pharmacologically induced increases in GC levels do not impair memory retrieval in a nonarousing situation [62] or after blockage of the beta-adrenergic receptors in the BLA [63, 64]. It is important to recognize that there are also some studies which report contradicting results [65–67]. For example, Domes and colleagues [66] detected no global effect of cortisol on neither verbal nor nonverbal memory. However, it became evident that stress effects on memory depend on (1) the timing of the stress response as well as (2) the convergence of stress hormone activity [43, 68, 69]. In general, a physiological stress response is beneficial when occurring during the learning episode but impairs memory function when experienced during retrieval (Figure 5).

1.3.2. Stress Effects on Memory Quality. Apart from the stress effects on quantitative aspects of the memory, stress and emotional arousal also affect the processing mode. Rodents stressed before training in a spatial navigation task used the striatum-dependent S-R strategy more often than hippocampus-dependent spatial strategies in the test trial [47]. Moreover, anxiogenic drugs which induce strong

emotional arousal also facilitate the use of S-R strategies and reduce the use of spatial strategies [70, 71]. In humans, stress before training in a spatial navigation task also favoured the use of an S-R strategy over a spatial strategy [72]. Interestingly, according to the aforementioned studies, the adopted learning strategy does not necessarily influence the quantitative memory performance.

Moreover, when participants are stressed in an instrumental learning task, they are biased towards the use of a habitual strategy, which renders them insensitive to changes in the outcome value. In instrumental learning tasks as well as in spatial navigation tasks, stress prior to training appears to favour striatum-dependent S-R learning strategies over prefrontal cortex-dependent goal-directed or hippocampus-dependent spatial strategies, respectively [73].

There is evidence suggesting that chronic stress can have similar effects on the use of multiple memory systems. Like under acute stress, chronically stressed rodents favour the use of a habit strategy in spatial navigation [74] and instrumental responding [75]. Moreover, an enlargement of the dorsolateral striatum as well as medial prefrontal cortex-atrophy is associated with the switch from goal-directed to habit learning in chronically stressed rats [75]. An enlargement of the amygdala has also been reported in association with chronic stress [76], which is likely to also have an effect on the usage of multiple memory systems.

2. Comparison of Selected Studies

As mentioned before, stress effects on memory are evident for qualitative as well as for quantitative aspects of memory and their nature may be time-dependent. Stress effects on the engagement of multiple memory systems seem to favour striatum-dependent learning strategies and to render behaviour habitual. The studies discussed in this section will further address the question of whether the enhancement of striatum-based habit memory may be the result of an impaired hippocampus-based cognitive memory.

Moreover, in regard to the crucial role of the amygdala in the modulation of quantitative memory parameters under stress, the contribution of this brain region to the engagement of multiple memory systems is of interest. Studies investigating the role of the BLA in the switch from cognitive to habit memory will therefore also be included in this section.

The above-described tests for the study of multiple memory systems assess active behavioural choices. Therefore, the question of how memory translates into behaviour will also be discussed.

2.1. Competition of Multiple Memory Systems. In order to study which memory system or which learning strategy *is favoured* under stress, a classical spatial navigation task can be used. However, such a dual-solution task cannot be used to investigate the influence stress has on habit and cognitive memory separately. To be able to explore the potentially distinct effects stress may have on hippocampal cognitive and striatal habit memory, two single-solution tasks are required. Both of the studies conducted by Wingard and Packard [77] and Packard and Gabriele [78] made use of this advantage of applying two single-solution tasks. In both experiments, adult male rats were trained in a water-maze plus task which required either the use of cognitive "place" or habitual "response" learning. Rats trained in the place learning version always had to go to the same arm of the maze (e.g., the west arm) independently of the start position to reach the escape platform, which reinforced the use of a spatial strategy. In contrast, in the habit learning version of the maze, only a turn in the same direction leads to the escape platform, which reinforced the use of a habit strategy.

In the study by Wingard and Packard [77], a physiological stress response was induced after training by injections of a beta-adrenergic antagonist in the BLA, which leads to an increase of central norepinephrine levels. As discussed earlier, a rise in central norepinephrine levels is a hallmark of the physiological effects triggered by stressful or emotionally arousing experiences. The effects of this pharmacologically induced stress response on the acquisition of either space or habit learning strategies is revealing: while performance in the place learning version was significantly impaired, performance in the habit learning version was dramatically enhanced under stress compared to the control condition (Figure 6). Interestingly, whereas these effects were present after an immediate infusion of the drug after learning, a 2 h delayed infusion remained ineffective [77]. These results suggest that higher norepinephrine levels due to stress or emotional arousal seem to time-dependently affect consolidation processes of multiple memory systems.

In a subsequent study [78], these results could be replicated using peripheral injections of RS 79948 instead of intra-BLA infusions of the drug. How peripheral stress hormone levels can affect central noradrenergic activity has been described previously. These pathways elegantly explain why peripheral administration of the drug results in similar central activity patterns as intra-BLA infusions and is thus also able to distinctly affect consolidation processes of multiple memory systems. The findings suggest that the switch to striatum-dependent habit learning strategies under stress in classical dual-solution tasks appears to be a result of both enhanced habit memory and impaired cognitive memory.

Additional evidence regarding this idea comes from a study using functional magnetic resonance imaging (fMRI) by Schwabe and Wolf [79], where 60 healthy participants solved a PCL task in a scanner. Participants who were stressed with the socially evaluated cold pressure test (SECPT) before the PCL task were biased towards the use of a striatum-dependent multicue learning strategy at the expense of a hippocampus-dependent single-cue learning strategy. As expected, using multicue learning strategies was correlated with more activity in the neostriatum (putamen and caudate nucleus), while the use of single-cue learning strategies has been associated with increased activity in the hippocampal formation [79]. Neuroimaging data revealed further that the striatum was significantly activated during PCL in both groups, but that there was no significant activation of medial temporal lobe structures in the stress group (Figure 7). This is in line with the idea that the hippocampal declarative memory system is impaired under stress.

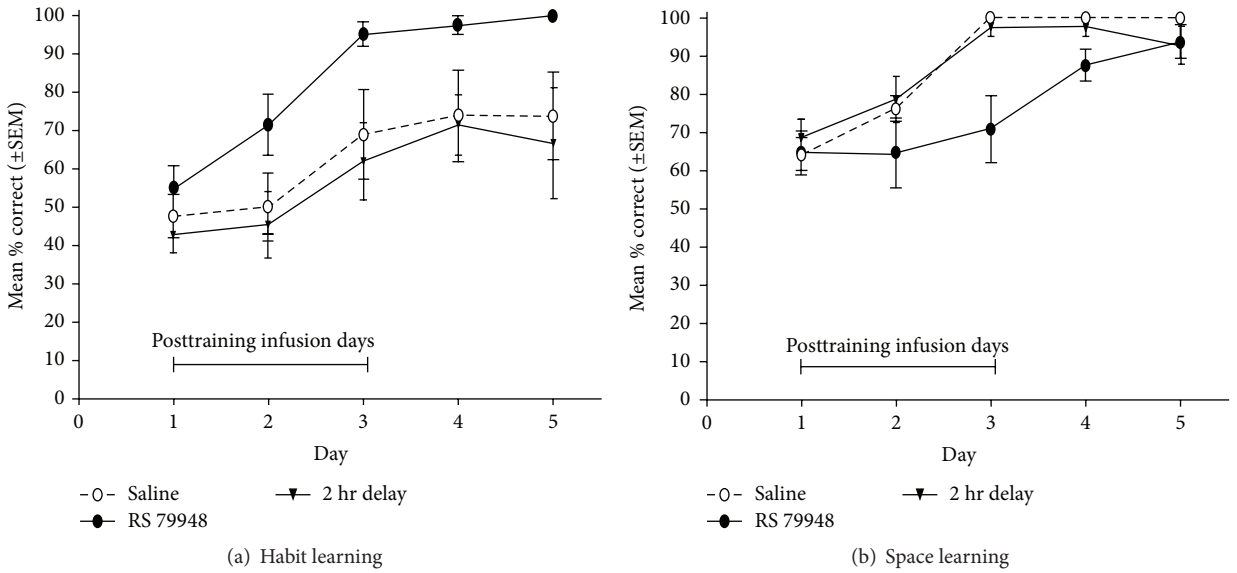


Figure 6: Intra-BLA infusions of the beta-adrenergic antagonist RS 79948 (a) enhance the acquisition of habit learning and (b) impair the acquisition of space learning strategies in comparison to a control condition (saline) and a 2 h delayed administration of RS 79948 [77].

Interestingly, PCL performance in the control group was correlated with activity in the left hippocampus but not with striatal activity. In contrast, PCL performance in the stress group was positively correlated with activity in the right caudate nucleus and the left putamen, but negatively correlated with activity in the left hippocampus [79]. However, the learning curves of stress and control groups were comparable: over time an increase of the percentage of correct responses and a decrease of reaction time were observed across groups (Figure 8). This indicates that stress is not hindering the acquisition of the PCL task but rather changes the applied learning strategy from hippocampus-dependent declarative to striatum-dependant procedural learning.

These data demonstrate how a shift from cognitive to habit memory systems under stress can rescue task performance. Because the hippocampal memory system is impaired under stress its inhibitory or competitive effects on the striatal memory system are reduced or abolished. Importantly, the negative correlation between left hippocampal activity and task performance after stress suggests that attempts to engage the declarative memory system during a stressful experience even disrupts task performance [79]. Thus, cognitive and habit memory systems seem to interact in a primarily competitive manner.

2.2. The Role of the Amygdala. As mentioned beforehand, in the study conducted by Wingard and Packard [77], infusions of the beta-adrenergic receptor antagonist RS 79948 right into the BLA impaired hippocampus-dependent memory and enhanced striatum-dependent memory in rodents. Thus, the amygdala seems to play a crucial role when it comes to the modulation of multiple memory systems. The rise of central norepinephrine levels may affect the efferent projections of the BLA in a manner that impairs synaptic plasticity in

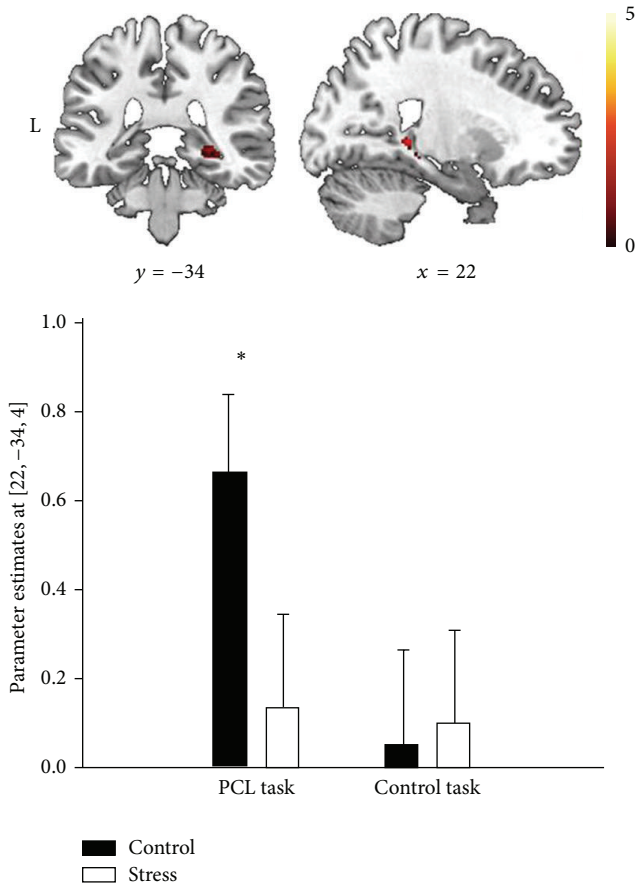


Figure 7: Neuroimaging data assessed during PCL shows less right hippocampal activity during PCL in the stress group compared to the control group [79].

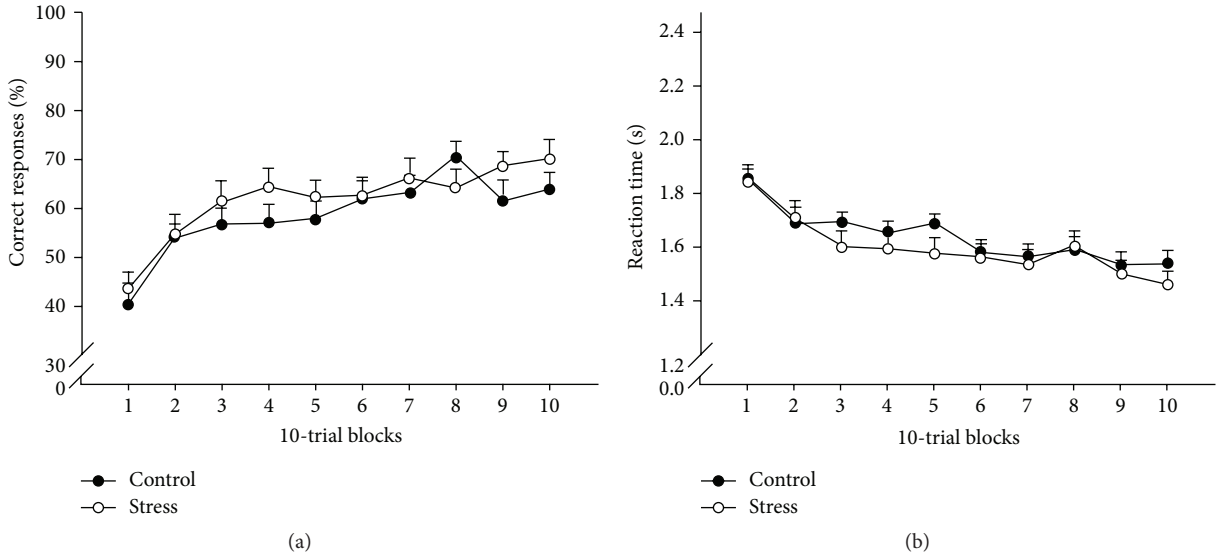


Figure 8: Comparable learning curves in the PCL task of the stress group and the control group: gradual improvement of classification performance across training. (a) Percentage of correct responses and (b) reaction time in seconds [79].

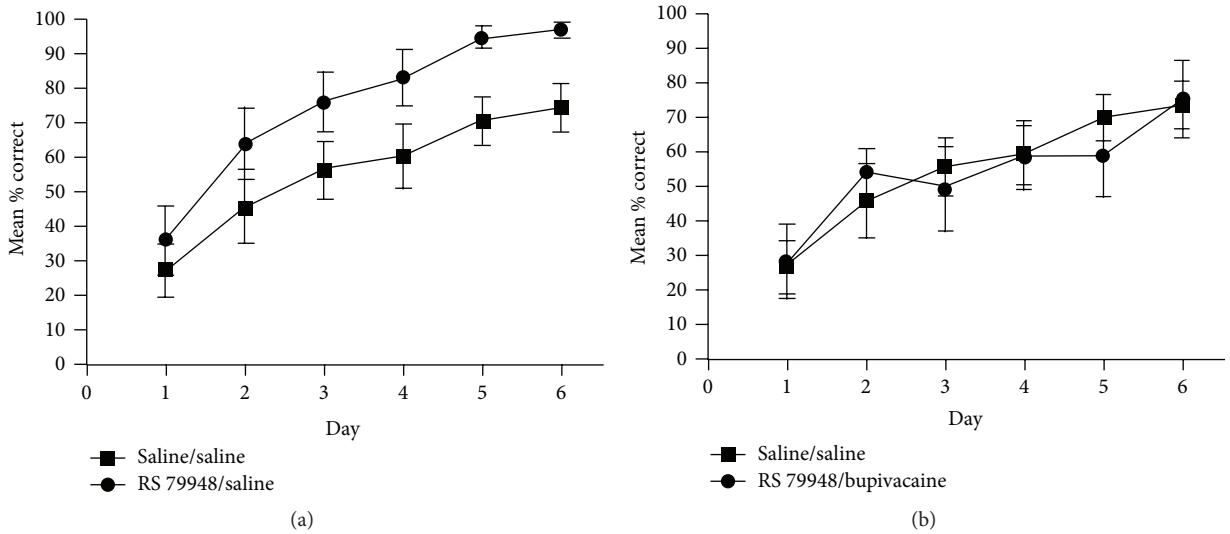


Figure 9: (a) Posttraining peripheral injections of RS 7948 enhance habit memory, but (b) BLA inactivation with bupivacaine blocks this enhancement [78].

the hippocampus, which consequently results in impaired cognitive memory.

The study by Packard and Gabriele [78] further investigated whether the functional integrity of the BLA is necessary in order for norepinephrine levels to affect the engagement of cognitive and habit memory. Therefore, the functional integrity of the BLA was disrupted using direct injections of the sodium channel blocker bupivacaine. As previously mentioned, posttraining peripheral injections of RS 79948 enhanced response learning and impaired place learning [78]. Importantly, these enhancing and impairing effects were abolished when the BLA had been inactivated (Figures 9 and 10).

Importantly, Packard and Gabriele [78] also showed in their experiment that an inactivation of the BLA without peripheral injections of RS 79948 affected neither place nor response learning. This means that the functional integrity of the BLA is not necessary for the acquisition of either learning strategy. However, the impairing and enhancing effects of peripheral stress hormone levels on hippocampus-dependent cognitive and striatum-dependent habit memory, respectively, require an intact BLA.

Although Schwabe and Wolf [79] did not find significant differences regarding the activation of the amygdala during the PCL and the visuomotor control task for the stress and the control group, the authors suggest that the enhancing and impairing effects of SECPT-induced stress on habit and cognitive memory could nevertheless be mediated by the amygdala. Considering this possibility, a reasonable explanation of the findings might be that the mere fact that the task

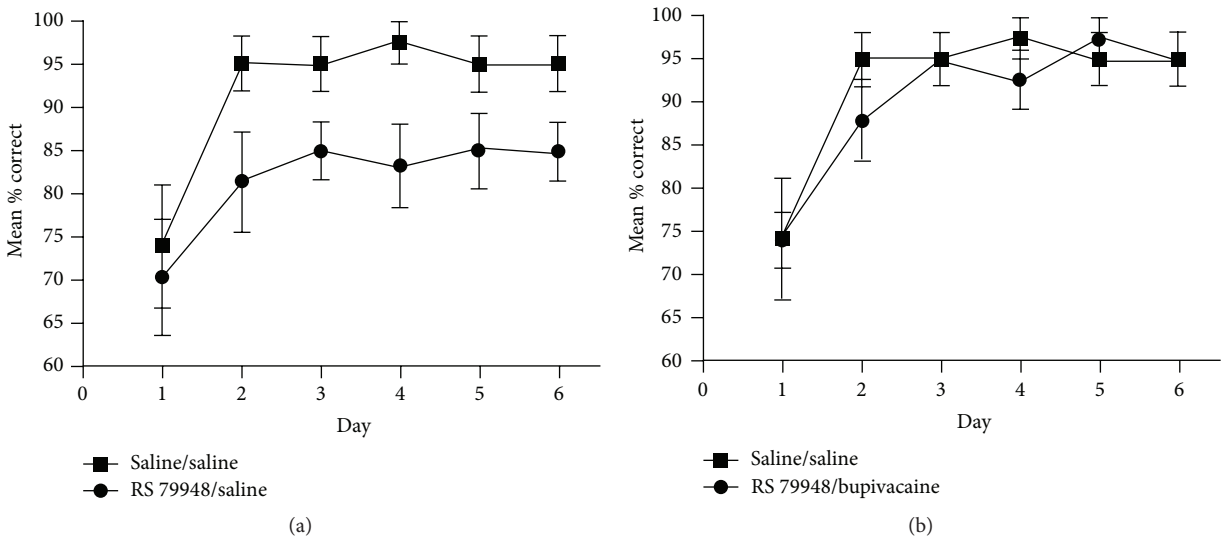


Figure 10: (a) Posttraining peripheral injections of RS 7948 impair cognitive memory, but (b) BLA inactivation with bupivacaine blocks this impairment [78].

had to be performed in a scanner could have been arousing to some extent, leading to enough activity in the amygdala in order for the SECPT-induced stress hormones to unfold their effects.

The role of the amygdala in emotionally arousing situations has been described earlier in this paper. Regarding the results of the herein discussed studies, it can thus be concluded that the emotional state can modulate the degree of interference between cognitive and habit memory systems and even release habit memory from competing/inhibitory influences of cognitive memory.

2.3. How Memory Translates into Behaviour. Free recall or recognition tasks, which are often used to study quantitative parameters of memory, directly inspect an individual's mere *ability to remember* previously learned, mostly declarative material. On the other hand, the tests used in studies examining qualitative aspects of memory assess an individual's *behavioural choices* in order to clarify which memory system is engaged in a task. This is especially clear in the case of instrumental learning tasks, where participants are trained in two instrumental actions, as described above. Because the engagement of different memory systems is able to modulate an individual's choice of a particular action, we will subsequently discuss how memory may translate into behaviour.

In a study conducted by Schwabe and Wolf [80], participants were trained in two instrumental actions leading to two different food rewards (chocolate/oranges). One of the instrumental actions led to a high probability of receiving a particular food reward, while the other instrumental action never led to the rewarding food outcome but instead induced a low probability of receiving a common outcome (peppermint tea). After the training session, participants were invited to eat one of the rewarding foods to satiety in order to devalue this particular food outcome. Participants underwent a SECPT or control procedure before the subsequent extinction test, where only the common outcome was delivered. Hunger and pleasantness ratings revealed that devaluation was successful across groups because both the stress group and the control group ranked the pleasantness of the devalued food significantly lower before the extinction test [80]. However, while control participants chose the high probability action of the devalued food outcome significantly less often during the extinction test than during training, this behaviour was not observed in stressed participants (Figure 11). These results indicate the use of the dorsal-striatum-dependent habit memory after an acute stressor. They are in alignment with the studies by Wingard and Packard [77] and Packard and Gabriele [78] which both reported enhanced response learning after stress, as well as with the fMRI study by Schwabe and Wolf [79], which found a correlation between such procedural learning processes and striatal activity in a PCL task.

In the experiment by Schwabe and Wolf [80], the stress-induced use of the habit system thus made participant's behaviour insensitive to changes in outcome value. It is of utter importance to notice that the relative engagement of multiple memory systems during encoding and consolidation processes has not been stress-manipulated in this study [80]. The originally used learning strategy can therefore be at least partly neglected when retrieval processes are sufficiently affected by stress.

Since the quality of retrieved information plays an important role in the cognitive appraisal of a situation, stress prior to retrieval can influence subsequent behavioural choices. In the instrumental learning task used by Schwabe and Wolf [80], the retrieved information under stress was dependent on the dorsolateral striatum-based habit memory, which contains information about the S-R relationship but is ignorant of the outcome value of an action. Therefore, the behaviour of stressed participants in the extinction test did not reflect participant's decreased pleasantness rankings of the devalued outcome [80]. Participant's behavioural choices were thus

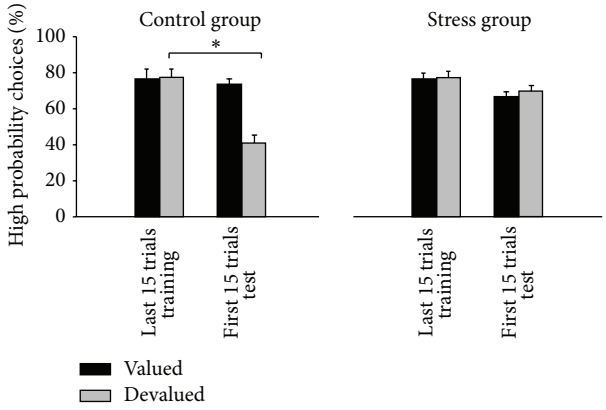


Figure 11: Percent of high probability actions of controls and stressed participants in the last 15-trial block of training and the first 15-trial block of extinction testing [80].

affected by stress effects on the quality of the retrieved information.

In the control condition on the other hand, participant's behavioural choices were congruent with the value they associated to a particular outcome [80]. This goal-directed behaviour indicates that the retrieved information in the extinction test contains sufficient knowledge about the task to establish action-outcome relationships and thus allows the individual to accordingly adapt behavioural choices. However, the assessment of explicit task knowledge after the extinction test revealed no significant differences between the stress and the control groups [80]. This may at first seem to contradict the idea that procedural memory only contains information about the relationship between a stimulus and a response. Interestingly, explicit task knowledge was examined after the extinction test, when salivary cortisol levels of the stress and the control groups were comparable [80]. Thus, it can be assumed that, during the test assessing task knowledge, participants of the control group as well as of the stress group were able to retrieve information concerning the action-outcome relationship. During the extinction test, however, this information was accessible only to the control group, but not to the stress group due to stressed participant's higher cortisol levels.

This is in line with the findings concerning task knowledge acquisition reported in the fMRI study by Schwabe and Wolf [79]. In this study, the authors could show that stress previous to a PCL task reduced participant's explicit knowledge of the task. Cortisol levels at the time of examination of task knowledge were not significantly different in the stress and the control groups. Taken together, these results suggest that the stress-induced enhancement of the striatum-dependent procedural memory system during encoding or consolidation process renders subsequent behavioural choices habitual because only S-R relationships have been learned and can thus be retrieved. Interestingly, stress solely affecting retrieval processes also makes behaviour habitual [80]. This suggests that the quality of retrieved information which is used to make behavioural choices, critically depends on (1) the primarily engaged memory system during the learning process and (2) the memory system engaged in retrieval. It seems that during encoding and consolidation at resting-state both memory systems separately and simultaneously store information, but the subsequent access to either of these systems and its information is affected by an individual's emotional state during retrieval.

3. Discussion

An overwhelming number of studies have reported stress effects on memory formation. This evidence can be explained regarding the fact that the brain regions involved in a physiological stress response extensively overlap with the structures which are critical for memory processes. A stressful or emotionally arousing experience can (1) activate the HPA axis which results in rising GC levels and (2) lead to an increase in central norepinephrine levels. There is ample evidence that these stress hormones interact in the BLA, which then distinctly affects memory processes in other regions of the brain, such as the hippocampus [53, 81]. For example, stress can enhance memory consolidation, particularly for emotionally relevant information, as well as impair memory retrieval in both rodents and humans [54–59, 62–64].

Moreover, stress modulates the engagement of multiple memory systems and favours striatum-dependent habit memory over hippocampus-dependent cognitive memory in rodents and humans, without necessarily affecting task performance in a spatial navigation task [47, 70, 72]. Additional evidence suggests that stress also favours the striatum-dependent habit system in instrumental learning tasks [73]. Thus, stress appears to favour S-R learning strategies dependent on the striatum over prefrontal cortex-dependent goal-directed or hippocampus-dependent spatial strategy, respectively.

The studies discussed in this paper further show that while habit memory appears to be enhanced under stress, cognitive memory seems to be impaired [77, 78]. These distinct effects of stress on multiple memory systems were found using a single-solution spatial navigation task and they imply that striatum-dependent habit memory enhancement may even come at the expense of hippocampus-dependent cognitive memory. In line with this idea are the neuroimaging data from the study by Schwabe and Wolf [79], which revealed that the striatum gets significantly activated during a PCL task in both a stress and a control group, but that there was no significant activation of medial temporal lobe structures in the stress group. Importantly, left hippocampal activity was even negatively correlated with PCL performance in the stress group [79]. Since no differences in task performance were found between the stress and the control groups, it can be concluded that (1) a shift from cognitive to habit memory systems under stress can rescue task performance and (2) the attempt to engage the declarative memory system during the experience of stress even disrupts task performance. The interaction between the hippocampus-based memory system and the striatum-based memory system thus seems to be of a primarily competitive manner.

Investigating the role of the BLA concerning the shift from cognitive to habit memory, the results of

the above-discussed studies indicate that the interaction of GCs and norepinephrine affects the engagement of multiple memory systems and the quantitative parameters of memory in a similar manner. Peripheral injections as well as intra-BLA infusions of the beta-adrenergic receptor antagonist RS 79948 impaired hippocampus-dependent memory and enhanced striatum-dependent memory [77, 78]. Similarly, many studies on quantitative memory parameters found that the process of memory retrieval under stress is impaired in spatial memory tasks [58, 59], as well as in declarative memory tasks [62–64], which may be explained by the fact that both spatial and declarative memory rely on the hippocampus. However, these studies on quantitative memory parameters report that the impairing effects of stress on spatial or declarative memory retrieval are abolished after blockage of beta-adrenergic receptors in the BLA, which leads to an increase in norepinephrine levels [60–64]. The same principle appears to be true for the engagement of multiple memory systems: higher norepinephrine levels due to injections of RS 79948 allow impairing effects on hippocampus-dependent spatial memory, whereas a simultaneous inactivation of the BLA abolishes this impairment [78]. These results suggest that the functional integrity of the BLA is of utter importance for stress to affect quantitative and qualitative parameters of hippocampus-based memory processes.

The behavioural tests which were developed for the study of multiple memory systems assess behavioural choices, which indicate the engagement of either procedural or declarative memory system in learning processes. Schwabe and Wolf [80] reported that the stress-induced use of the habit system can render participant's behaviour insensitive to changes in outcome value in an instrumental learning task. As discussed above, the quality of available information is critical for the process of cognitive appraisal of a situation. Taken together, the studies discussed in this review suggest that the quality of consolidated material is dependent on the engaged memory system during the learning process as well as during retrieval. If an S-R learning strategy is used and thus the striatum-dependant memory system is controlling the acquisition of a task, the subsequent behavioural choices have a habitual character [77, 78]. On the other hand, if a spatial strategy is used and thus the hippocampus-dependent declarative system is controlling consolidation processes, the subsequently retrieved information may come in the form of an (either true or false) internal representation of the external world. This cognitive model integrates and compares learned associations between multiple items and events and thus subsequent behavioural choices are based on a cognitive evaluation of the different available actions in respect of a desired outcome. Moreover, the discussed findings indicate that the memory system engaged in retrieval processes can modulate behaviour [80]. The retrieved information using a hippocampus-dependent strategy contains knowledge about action-outcome relationships which allows participants to choose goal-directed actions. A goal-directed behavioural strategy is dependent on different brain regions, including the prefrontal cortex, which also plays an important role in the cognitive appraisal of a situation. Moreover, coping with a stressor can increase cognitive load and thus limit available cognitive resources. If cognitive resources are reduced during the process of retrieval, the careful evaluation of hippocampus-based declarative memory is impaired [80]. Because under stress the prefrontal cortex seems to be already "busy" with a different task (coping), this brain area cannot be sufficiently engaged in the processes of retrieval and cognitive appraisal in order to enable the use of a goal-directed strategy. The subsequent switch to striatum-dependent habit system may result in actions, which do not lead to desired outcomes because the retrieved information only consists of S-R relationships and is thus insensitive to changes in the value of an outcome.

Interestingly, the S-R learning strategy refers to the procedural process of extracting common elements from a series of events, which results in the use of a heuristic rule-of-thumb in order to guide behaviour in a state of decreased cognitive resources. Behavioural choices on the basis of heuristic processing do not necessarily lead to adverse decisions but rather are adaptive mechanisms in order to operate effectively when cognitive load is high.

These theoretical considerations of the relationship between memory and behaviour may remind us of the dual-system theory proposed by Kahneman [82]. System 1, which is also referred to as intuition, results in a gut feeling regarding the current situation and can potentially bias people towards making irrational choices [82]. Moreover, system 1 operates in a parallel and automatic manner and is fast, effortless, and inflexible. All these characteristics are also attributable to the striatum-dependent habit memory system, which has been extensively discussed in this paper. On the other hand, the proposed system 2, which is also referred to as reasoning, operates in a controlled, slow, and serial manner and is effortful and flexible [82]. The hippocampus-dependant cognitive system as discussed in this paper thus seems to correspond to system 2. These similarities might be of valuable interest in the research on decision making. It is tempting to speculate that stress and emotional arousal can mediate the engagement of system 1 and system 2 in subsequent decision-making processes by modulating effects on multiple memory systems. It might thus be the case that the engagement of hippocampus-based cognitive and striatum-based habit memory during learning can determine subsequent behavioural choices because of qualitative aspects of retrieved information.

4. Conclusion

In mammal evolution, multiple memory systems have developed to serve different functions. On the one hand, incremental habit memory formation has evolved as a consequence of specialized performance systems which are reactivated in similar situations, thus allowing to react fast, albeit they are rather inflexible. On the other hand, a memory system has developed to serve everyday memory performance for unique episodes. Both systems are subserved by different neuroanatomical networks. As we have outlined in this review, the interaction between these memory systems is modulated by stress hormones: a physiological stress response leads to enhanced striatal habit memory and

Table 1

	Wingard and Packard [77]	Packard and Gabriele [78]	Schwabe and Wolf [80]	Schwabe and Wolf [79]
Task	Spatial navigation	Spatial navigation	Instrumental learning	Probabilistic Classification Learning (PCL)
Involved multiple memory systems	Hippocampus-dependent "cognitive" memory (place learning) Dorsal striatum-dependent "habit" memory (response learning)	Hippocampus-dependent "cognitive" memory (place learning) Dorsal striatum-dependent "habit" memory (response learning)	Goal-directed system (action-outcome learning): prefrontal cortex, dorsomedial thalamus, and dorsomedial striatum Habit system (S-R learning): dorsolateral striatum	Hippocampus-dependent declarative memory Striatum-dependent procedural memory
Hypothesis	Intra-BLA administration of an anxiogenic biases rats towards the use of habit memory Intra-BLA infusion of RS-79948 is anxiogenic	Peripheral administration of an anxiogenic drug enhances and impairs response and place learning, respectively The functional integrity of the BLA may be critical for these effects	Acute stress favours habits over goal-directed actions when it is administered before the extinction test (after learning)	Stress may modulate the engagement of hippocampus-based declarative and striatum-based procedural memory systems in classification learning This may be observable in functional activity patterns assessed with fMRI
Sample	Rodents n = 59 male Charles River Long-Evans rats	Rodents n = 87 male Charles River Long-Evans rats	Humans n = 68 students (34 men, 34 women; 18–32 years) Exclusion of 17 subjects	Humans n = 60 students (30 men, 30 women; 18–30 years) Exclusion of 1 subject

Table 1: Continued.

	Wingard and Packard [77]	Packard and Gabriele [78]	Schwabe and Wolf [80]	Schwabe and Wolf [79]
Methods	Training (5 consecutive days, six trials/day) in single-solution water plus maze task (hippocampus-dependent place learning versus dorsal striatum-dependent response learning) Intra-BLA infusions of RS-79948 (place task $n = 8$, response task $n = 7$) or saline (place task $n = 7$, response task $n = 7$) immediately following training on days 1–3 Additional groups (place task $n = 7$, response task $n = 7$) received intra-BLA infusions of RS-79948 2 h after training Anxiogenic potential of RS-79948: standard anxiety test with an automated elevated plus maze apparatus after intra-BLA infusions of RS 79948 ($n = 8$) or saline ($n = 8$)	Training (6 consecutive days, six trials/day) in single-solution water plus maze task (hippocampus-dependent place learning versus dorsal striatum-dependent response learning) Infusions of either peripheral and intra-BLA saline (place task $n = 7$, response task $n = 9$), peripheral RS-79948 and intra-BLA saline (place task $n = 9$, response task $n = 8$), peripheral saline and intra-BLA bupivacaine (place task $n = 7$, response task $n = 7$), or peripheral RS 79948 and intra-BLA bupivacaine (place task $n = 8$, response task $n = 7$) immediately following training on days 1–3	Training in two instrumental actions (high versus low probability) leading to a food outcome: three randomized trial types with different outcomes: (i) Chocolate (75 trials) (ii) Orange (75 trials) (iii) Neutral (75 trials) Selective devaluation of one outcome (chocolate or orange) through eating to satiety Socially evaluated cold pressor test (SECPT) versus control condition Subjective stress ratings immediately after SECPT or control condition Extinction test with 75 trials of the three trial types (25 min after SECPT/control condition and approximately 40 min after training) Assessment of explicit task knowledge after extinction test (free recall and multiple-choice questionnaire) Hunger and pleasantness ratings before learning, before and after devaluation, and before the extinction test	SECPT/control condition before learning Subjective stress ratings immediately after SECPT or control condition Weather-prediction task (PCL task) and visuomotor control task (25 min after SECPT/control condition) in the scanner: 200 randomized trials (100 trials per task; 1 out of 14 different cue patterns is presented per trial) Assessment of explicit task knowledge (10 items questionnaire) outside the scanner Learning strategy analysis with a mathematical model (comparison of a participant's actual responses and the expected responses using a declarative or procedural strategy)
Induction of stress/ arousal	BLA: injection of beta-adrenergic antagonist (RS 79948)	Peripheral injection of beta-adrenergic antagonist (RS 79948)	Socially evaluated cold pressure test (SECPT)	Socially evaluated cold pressure test (SECPT)
Physiological stress parameters	None measured	None measured	Blood pressure before, during, and after the SECPT or control condition Cortisol: saliva samples after arrival at the laboratory, just before, just after, and 20 and 50 min after the SECPT or control condition	Blood pressure before, during, and after the SECPT or control condition Cortisol: saliva samples before and immediately after the SECPT or control condition as well as before and after the learning task (25 and 90 min after the SECPT/control condition, resp.)

Table 1: Continued.

	Wingard and Packard [77]	Packard and Gabriele [78]	Schwabe and Wolf [80]	Schwabe and Wolf [79]
Affected memory phase	Consolidation	Consolidation	Retrieval	Encoding/consolidation
Behavioural results	Posttraining immediate intra-BLA infusions of RS-79948 (relative to delayed infusion or saline) impaired acquisition of the place task and enhanced acquisition of the response task Anxiogenic potential of RS 79948: intra-BLA infusions of RS 79948 (relative to saline) lead to more and less time spent in the closed and open arms of the maze, respectively	Posttraining peripheral RS 79948 injections enhance response learning BLA inactivation blocks the enhancement of response learning Posttraining peripheral RS 79948 injections impair place learning BLA inactivation blocks the impairment of place learning	SECPT increased subjective stress ratings Habitual behaviour after SECPT: participants indicated that they do not want the devalued outcome any more but still chose the referring action Goal-directed behaviour after control condition: control participants did not prefer the devalued food anymore; thus they did not prefer the associated action anymore No stress effects on explicit task knowledge	SECPT increased subjective stress ratings Comparable learning curves in the PCL task between SECPT/control condition: gradual improvement of classification performance across training Stress effects on learning strategy during PCL: decreased use of single-cue-based strategies (hippocampus-dependent) and increased use of multicue-based strategies (striatum-dependent) Stress effects on explicit task knowledge: participants in the SECPT condition remembered fewer details of the PCL task
Physiological results (stress parameters)	None measured	None measured	Increase in blood pressure during SECPT Increased salivary cortisol before extinction (20 min after SECPT) The SECPT-induced increase in cortisol levels (baseline-peak) is associated with habit performance	Increase in blood pressure during SECPT Systolic blood pressure during the SECPT/control condition correlates with the use of multicue-based strategies Increased salivary cortisol immediately before the PCL task in the scanner (25 min after SECPT) Salivary cortisol levels (across both groups and for all time points) correlate with PCL performance The SECPT-induced increase in cortisol levels (baseline-peak) is not associated with PCL performance Salivary cortisol levels 25 and 90 min after the SECPT correlate with the use of multicue-based strategies

Table 1: Continued.

	Wingard and Packard [77]	Packard and Gabriele [78]	Schwabe and Wolf [80]	Schwabe and Wolf [79]
Neuroimaging results	None	None	None	Activated brain areas during the PCL task: caudate nucleus, putamen, hippocampus, parahippocampal cortex, orbitofrontal cortex, cingulate cortex, and inferior frontal cortex Activity of the hippocampus correlated with the use of single-cue strategies, and activity of the putamen and the caudate nucleus correlated with multicue strategies during the PCL task Activation of the striatum during PCL in both groups, but no significant activation of medial temporal lobe structures in the stress group Caudate nucleus activity correlates with salivary cortisol levels (across both groups and for all time points), but not with the increase in cortisol levels (baseline-peak) after SECPT PCL performance in the stress group is positively correlated with activity in the right caudate nucleus and the left putamen, but negatively correlated with activity in the left hippocampus PCL performance in the control group is correlated with activity in the left hippocampus, but not with striatal activity

Table 1: Continued.

	Wingard and Packard [77]	Packard and Gabriele [78]	Schwabe and Wolf [80]	Schwabe and Wolf [79]
Conclusions	Intra-BLA infusions of RS 79948 can bias rats towards using habit memory by impairing cognitive memory Intra-BLA infusions of RS 79948 exert an anxiogenic effect at the same dose that impairs and enhances cognitive and habit memory, respectively Emotional state can modulate the degree of interference between cognitive and habit memory systems (release habit memory from competing/inhibitory influences of cognitive memory)	The functional integrity of the BLA is not necessary for the acquisition of place and response learning The functional integrity of the BLA is critical in order for peripheral injections of RS 79948 to impair hippocampus-dependent cognitive memory and enhance dorsal striatum-dependent habit memory, respectively	Acute stress before extinction testing can abolish sensitivity of performance to outcome value Acute stress can make behaviour habitual without affecting processes involved in learning (encoding, consolidation)	Stress does not affect the acquisition of the PCL task, but it changes the nature of classification learning from flexible, declarative learning to inflexible, procedural learning Stress impairs the hippocampus-dependent system and allows the striatum to control behaviour, which rescues task performance Attempts to engage the declarative system in PCL after stress disrupt performance

impaired hippocampal cognitive memory. Furthermore, we could show that the functional integrity of the amygdala is of utter importance in mediating this interaction, particularly concerning the impairing effects of stress on hippocampus-based memories.

These distinct memory systems are differentially involved in the learning process in different situations (e.g., stressful versus nonstressful) and help us to focus either on particular elements of a given situation in order to make quick decisions and "survive" (at the expense of overlooking some peripheral nonsalient details), or on the situation as a whole, in order to guide "rational" decision-making. Since the quality of consolidated memories depends on the engaged memory systems during learning as well as retrieval, it is argued that optimal memory functioning and decision-making require a balanced interaction between different neuroanatomical networks and their modulating stress hormones.

The aim of this contribution was to review and discuss the neural correlates of mammalian multiple memory systems and how they are affected by stress. We recapitulated the biological systems involved in a stress response, which are fundamental for the understanding of the herein discussed studies, on a macrolevel. However, before drawing conclusions, one should bear in mind that the behavioural and imaging results reported in our paper can only account for understanding part of the complex nature of memory. While the presented data helps pointing out this complexity, it certainly cannot fully explain it. Further research should focus on stress effects on a cellular level in order to gain a deeper understanding of the exact mechanisms responsible for the engagement of multiple memory systems. By integrating our knowledge concerning molecular and transcriptional alterations under stress into the herein proposed cognitive theories, we will hopefully be able to shed light onto the complex interactions between and within an organism's central and autonomic nervous systems and the endocrinological system during stressful experiences.

5. Overview of Presented Studies

See Table 1.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank Micha Eichmann for his professional assistance with some technical issues. Moreover they thank the reviewers for their critical comments which improved the paper. This work was supported by the Swiss Multiple Sclerosis Society (SMSG).

References

[1] M. G. Packard and J. Goodman, "Emotional arousal and multiple memory systems in the mammalian brain," *Frontiers in Behavioral Neuroscience*, vol. 6, article 14, 2012.

[2] L. Schwabe and O. T. Wolf, "Stress and multiple memory systems: from 'thinking' to 'doing'.," *Trends in cognitive sciences*, vol. 17, no. 2, pp. 60–68, 2013.

[3] E. R. De Kloet and R. Derijk, "Signaling pathways in brain involved in predisposition and pathogenesis of stress-related disease: genetic and kinetic factors affecting the MR/GR balance," *Annals of the New York Academy of Sciences*, vol. 1032, pp. 14–34, 2004.

[4] S. J. Lupien, I. Oueliet-Morin, A. Hupbach et al., "Beyond the stress concept: allostatic load—a developmental biological and cognitive perspective," in *Developmental Psychopathology, Volume 2: Developmental Neuroscience*, D. Cicchetti and D. J. Cohen, Eds., pp. 578–628, John Wiley & Sons, Hoboken, NJ, USA, 2006.

[5] A. M. Bao, G. Meynen, and D. F. Swaab, "The stress system in depression and neurodegeneration: focus on the human hypothalamus," *Brain Research Reviews*, vol. 57, no. 2, pp. 531–553, 2008.

[6] J. E. Sherin and C. B. Nemeroff, "Post-traumatic stress disorder: the neurobiological impact of psychological trauma," *Dialogues in Clinical Neuroscience*, vol. 13, no. 3, pp. 263–278, 2011.

[7] J. M. H. M. Reul and E. R. de Kloet, "Anatomical resolution of two types of corticosterone receptor sites in rat brain with in vitro autoradiography and computerized image analysis," *Journal of Steroid Biochemistry*, vol. 24, no. 1, pp. 269–272, 1986.

[8] B. Roozendaal, "Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval," *Neurobiology of Learning and Memory*, vol. 78, no. 3, pp. 578–595, 2002.

[9] C. K. McIntyre, J. L. McGaugh, and C. L. Williams, "Interacting brain systems modulate memory consolidation," *Neuroscience and Biobehavioral Reviews*, vol. 36, no. 7, pp. 1750–1762, 2012.

[10] J. Schreurs, T. Seelig, and H. Schulman, "β_2-adrenergic receptors on peripheral nerves," *Journal of Neurochemistry*, vol. 46, no. 1, pp. 294–296, 1986.

[11] A. J. Lawrence, D. Watkins, and B. Jarrott, "Visualization of beta-adrenoceptor binding sites on human inferior vagal ganglia and their axonal transport along the rat vagus nerve," *Journal of Hypertension*, vol. 13, no. 6, pp. 631–635, 1995.

[12] M. Kalia and J. M. Sullivan, "Brainstem projections of sensory and motor components of the vagus nerve in the rat," *Journal of Comparative Neurology*, vol. 211, no. 3, pp. 248–264, 1982.

[13] K. K. Sumal, W. W. Blessing, T. H. Joh, D. J. Reis, and V. M. Pickel, "Synaptic interaction of vagal afferents and catecholaminergic neurons in the rat nucleus tractus solitarius," *Brain Research*, vol. 277, no. 1, pp. 31–40, 1983.

[14] E. J. Van Bockstaele, J. Peoples, and P. Telegan, "Efferent projections of the nucleus of the solitary tract to peri-locus coeruleus dendrites in rat brain: evidence for a monosynaptic pathway," *Journal of Comparative Neurology*, vol. 412, no. 3, pp. 410–428, 1999.

[15] E. Asan, *The Catecholaminergic Innervation of the Rat Amygdala*, vol. 142 of *Advances in Anatomy, Embryology and Cell Biology*, Springer, Berlin, Germany, 1998.

[16] J. H. Fallon, D. A. Koziell, and R. Y. Moore, "Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal

cortex and entorhinal cortex," *Journal of Comparative Neurology*, vol. 180, no. 3, pp. 509–523, 1978.

[17] S. E. Loughlin, S. L. Foote, and F. E. Bloom, "Efferent projections of nucleus locus coeruleus: topographic organization of cells of origin demonstrated by three-dimensional reconstruction," *Neuroscience*, vol. 18, no. 2, pp. 291–306, 1986.

[18] J. A. Ricardo and E. T. Koh, "Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat," *Brain Research*, vol. 153, no. 1, pp. 1–26, 1978.

[19] H. C. Abercrombie, A. S. Chambers, L. Greischar, and R. M. Monticelli, "Orienting, emotion, and memory: phasic and tonic variation in heart rate predicts memory for emotional pictures in men," *Neurobiology of Learning and Memory*, vol. 90, no. 4, pp. 644–650, 2008.

[20] A. K. Anderson, Y. Yamaguchi, W. Grabski, and D. Lacka, "Emotional memories are not all created equal: evidence for selective memory enhancement," *Learning & Memory*, vol. 13, no. 6, pp. 711–718, 2006.

[21] L. Cahill and M. T. Alkire, "Epinephrine enhancement of human memory consolidation: interaction with arousal at encoding," *Neurobiology of Learning and Memory*, vol. 79, no. 2, pp. 194–198, 2003.

[22] L. Cahill, B. Prins, M. Weber, and J. L. McGaugh, "β-Adrenergic activation and memory for emotional events," *Nature*, vol. 371, no. 6499, pp. 702–704, 1994.

[23] H. D. Critchley, C. J. Mathias, and R. J. Dolan, "Fear conditioning in humans: the influence of awareness and autonomic arousal on functional neuroanatomy," *Neuron*, vol. 33, no. 4, pp. 653–663, 2002.

[24] T. Moor, L. Mundorff, A. Bohringer et al., "Evidence that baroreflex feedback influences long-term incidental visual memory in men," *Neurobiology of Learning and Memory*, vol. 84, no. 3, pp. 168–174, 2005.

[25] K. A. Nielson, D. Yee, and K. I. Erickson, "Memory enhancement by a semantically unrelated emotional arousal source induced after learning," *Neurobiology of Learning and Memory*, vol. 84, no. 1, pp. 49–56, 2005.

[26] G. Kempermann, "New neurons for 'survival of the fittest'," *Nature Reviews Neuroscience*, vol. 13, no. 10, pp. 727–736, 2012.

[27] J. S. Snyder, A. Soumier, M. Brewer, J. Pickel, and H. A. Cameron, "Adult hippocampal neurogenesis buffers stress responses and depressive behaviour," *Nature*, vol. 476, no. 7361, pp. 458–461, 2011.

[28] A. Surget, A. Tanti, E. D. Leonardo et al., "Antidepressants recruit new neurons to improve stress response regulation," *Molecular Psychiatry*, vol. 16, no. 12, pp. 1177–1188, 2011.

[29] C. Anacker, A. Cattaneo, A. Luoni et al., "Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis," *Neuropsychopharmacology*, vol. 38, no. 5, pp. 872–883, 2013.

[30] V. M. Renault, V. A. Rafalski, A. A. Morgan et al., "$FoxO_3$ regulates neural stem cell homeostasis," *Cell Stem Cell*, vol. 5, no. 5, pp. 527–539, 2009.

[31] Y. Wu, H. Peng, M. Cui, N. P. Whitney, Y. Huang, and J. C. Zheng, "CXCL12 increases human neural progenitor cell proliferation through Akt-1/FOXO3a signaling pathway," *Journal of Neurochemistry*, vol. 109, no. 4, pp. 1157–1167, 2009.

[32] M. Graciarena, A. M. Depino, and F. J. Pitossi, "Prenatal inflammation impairs adult neurogenesis and memory related behavior through persistent hippocampal TGFbeta1 downregulation," *Brain, Behavior, and Immunity*, vol. 24, no. 8, pp. 1301–1309, 2010.

[33] S. Ahn and A. L. Joyner, "In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog," *Nature*, vol. 437, no. 7060, pp. 894–897, 2005.

[34] Y. He, H. Zhang, A. Yung et al., "ALK5-dependent TGF-β 2 signaling is a major determinant of late-stage adult neurogenesis," *Nature Neuroscience*, vol. 17, no. 7, pp. 943–952, 2014.

[35] J. D. Johnson, J. Campisi, C. M. Sharkey et al., "Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines," *Neuroscience*, vol. 135, no. 4, pp. 1295–1307, 2005.

[36] I. Mahar, F. R. Bambico, N. Mechawar, and J. N. Nobrega, "Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects," *Neuroscience and Biobehavioral Reviews*, vol. 38, pp. 173–192, 2014.

[37] L. R. Squire, "Memory systems of the brain: a brief history and current perspective," *Neurobiology of Learning and Memory*, vol. 82, no. 3, pp. 171–177, 2004.

[38] E. Tulving, *Elements of Episodic Memory*, Oxford University Press, Cambridge, UK, 1983.

[39] A. P. Shimamura and L. R. Squire, "A neuropsychological study of fact memory and source amnesia," *Journal of Experimental Psychology: Learning, Memory, and Cognition*, vol. 13, no. 3, pp. 464–473, 1987.

[40] E. Tulving, "Remembering and knowing the past," *American Scientist*, vol. 77, no. 4, pp. 361–367, 1989.

[41] J. J. Kim and M. G. Baxter, "Multiple brain-memory systems: the whole does not equal the sum of its parts," *Trends in Neurosciences*, vol. 24, no. 6, pp. 324–330, 2001.

[42] M. G. Packard, R. Hirsh, and N. M. White, "Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems," *The Journal of Neuroscience*, vol. 9, no. 5, pp. 1465–1472, 1989.

[43] M. Joëls, Z. Pu, O. Wiegert, M. S. Oitzl, and H. J. Krugers, "Learning under stress: how does it work?" *Trends in Cognitive Sciences*, vol. 10, no. 4, pp. 152–158, 2006.

[44] U. M. Nater, C. Moor, U. Okere et al., "Performance on a declarative memory task is better in high than low cortisol responders to psychosocial stress," *Psychoneuroendocrinology*, vol. 32, no. 6, pp. 758–763, 2007.

[45] L. Schwabe, A. Bohringer, M. Chatterjee, and H. Schachinger, "Effects of pre-learning stress on memory for neutral, positive and negative words: different roles of cortisol and autonomic arousal," *Neurobiology of Learning and Memory*, vol. 90, no. 1, pp. 44–53, 2008.

[46] B. M. Elzinga, A. Bakker, and J. D. Bremner, "Stress-induced cortisol elevations are associated with impaired delayed, but not immediate recall," *Psychiatry Research*, vol. 134, no. 3, pp. 211–223, 2005.

[47] J. J. Kim, H. J. Lee, J.-S. Han, and M. G. Packard, "Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning," *The Journal of Neuroscience*, vol. 21, no. 14, pp. 5222–5228, 2001.

[48] C. Kirschbaum, O. T. Wolf, M. May, W. Wippich, and D. H. Hellhammer, "Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults," *Life Sciences*, vol. 58, no. 17, pp. 1475–1483, 1996.

[49] S. J. Lupien, S. Gaudreau, B. M. Tchiteya et al., "Stress-induced declarative memory impairment in healthy elderly

subjects: relationship to cortisol reactivity," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 7, pp. 2070–2075, 1997.

[50] J. D. Payne, E. D. Jackson, L. Ryan, S. Hoscheidt, W. J. Jacobs, and L. Nadel, "The impact of stress on neutral and emotional aspects of episodic memory," *Memory*, vol. 14, no. 1, pp. 1–16, 2006.

[51] M. Tops, G. van der Pompe, D. Baas et al., "Acute cortisol effects on immediate free recall and recognition of nouns depend on stimulus valence," *Psychophysiology*, vol. 40, no. 2, pp. 167–173, 2003.

[52] B. Roozendaal and J. L. McGaugh, "Basolateral amygdala lesions block the memory-enhancing effect of glucocorticoid administration in the dorsal hippocampus of rats," *European Journal of Neuroscience*, vol. 9, no. 1, pp. 76–83, 1997.

[53] B. Roozendaal, S. Okuda, E. A. Van Der Zee, and J. L. McGaugh, "Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 17, pp. 6741–6746, 2006.

[54] M. S. Oitzl and E. R. de Kloet, "Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning," *Behavioral Neuroscience*, vol. 106, no. 1, pp. 62–71, 1992.

[55] C. Sandi and S. P. R. Rose, "Corticosteroid receptor antagonists are amnestic for passive avoidance learning in day-old chicks," *European Journal of Neuroscience*, vol. 6, no. 8, pp. 1292–1297, 1994.

[56] B. Roozendaal and J. L. McGaugh, "Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task," *Neurobiology of Learning and Memory*, vol. 65, no. 1, pp. 1–8, 1996.

[57] H. C. Abercrombie, N. S. Speck, and R. M. Monticelli, "Endogenous cortisol elevations are related to memory facilitation only in individuals who are emotionally aroused," *Psychoneuroendocrinology*, vol. 31, no. 2, pp. 187–196, 2006.

[58] D. J.-F. de Quervain, B. Roozendaal, and J. L. McGaugh, "Stress and glucocorticoids impair retrieval of long-term spatial memory," *Nature*, vol. 394, no. 6695, pp. 787–790, 1998.

[59] D. M. Diamond, A. M. Campbell, C. R. Park et al., "Influence of predator stress on the consolidation versus retrieval of long-term spatial memory and hippocampal spinogenesis," *Hippocampus*, vol. 16, no. 7, pp. 571–576, 2006.

[60] B. Roozendaal, Q. K. Griffith, J. Buranday, D. J.-F. De Quervain, and J. L. McGaugh, "The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: dependence on the basolateral amygdala," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 3, pp. 1328–1333, 2003.

[61] B. Roozendaal, E. L. Hahn, S. V. Nathan, D. J.-F. De Quervain, and J. L. McGaugh, "Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala," *Journal of Neuroscience*, vol. 24, no. 37, pp. 8161–8169, 2004.

[62] S. Kuhlmann and O. T. Wolf, "A non-arousing test situation abolishes the impairing effects of cortisol on delayed memory retrieval in healthy women," *Neuroscience Letters*, vol. 399, no. 3, pp. 268–272, 2006.

[63] D. J.-F. de Quervain, A. Aerni, and B. Roozendaal, "Preventive effect of β-adrenoceptor blockade on glucocorticoid-induced memory retrieval deficits," *The American Journal of Psychiatry*, vol. 164, no. 6, pp. 967–969, 2007.

[64] L. Schwabe, M. S. Oitzl, S. Richter, and H. Schächinger, "Modulation of spatial and stimulus-response learning strategies by exogenous cortisol in healthy young women," *Psychoneuroendocrinology*, vol. 34, no. 3, pp. 358–366, 2009.

[65] T. W. Buchanan and D. Tranel, "Stress and emotional memory retrieval: effects of sex and cortisol response," *Neurobiology of Learning and Memory*, vol. 89, no. 2, pp. 134–141, 2008.

[66] G. Domes, J. Rothfischer, U. Reichwald, and M. Hautzinger, "Inverted-U function between salivary cortisol and retrieval of verbal memory after hydrocortisone treatment," *Behavioral Neuroscience*, vol. 119, no. 2, pp. 512–517, 2005.

[67] L. Schwabe, S. Römer, S. Richter, S. Dockendorf, B. Bilak, and H. Schächinger, "Stress effects on declarative memory retrieval are blocked by a beta-adrenoceptor antagonist in humans," *Psychoneuroendocrinology*, vol. 34, no. 3, pp. 446–454, 2009.

[68] E. R. de Kloet, M. S. Oitzl, and M. Joëls, "Stress and cognition: are corticosteroids good or bad guys?" *Trends in Neurosciences*, vol. 22, no. 10, pp. 422–426, 1999.

[69] D. M. Diamond, A. M. Campbell, C. R. Park, J. Halonen, and P. R. Zoladz, "The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law," *Neural Plasticity*, vol. 2007, Article ID 60803, 33 pages, 2007.

[70] M. G. Packard and J. C. Wingard, "Amygdala and 'emotional' modulation of the relative use of multiple memory systems," *Neurobiology of Learning and Memory*, vol. 82, no. 3, pp. 243–252, 2004.

[71] A. E. Elliott and M. G. Packard, "Intra-amygdala anxiogenic drug infusion prior to retrieval biases rats towards the use of habit memory," *Neurobiology of Learning and Memory*, vol. 90, no. 4, pp. 616–623, 2008.

[72] L. Schwabe, M. S. Oitzl, C. Philippsen et al., "Stress modulates the use of spatial versus stimulus-response learning strategies in humans," *Learning and Memory*, vol. 14, no. 1, pp. 109–116, 2007.

[73] L. Schwabe and O. T. Wolf, "Stress prompts habit behavior in humans," *The Journal of Neuroscience*, vol. 29, no. 22, pp. 7191–7198, 2009.

[74] L. Schwabe, S. Dalm, H. Schächinger, and M. S. Oitzl, "Chronic stress modulates the use of spatial and stimulus-response learning strategies in mice and man," *Neurobiology of Learning and Memory*, vol. 90, no. 3, pp. 495–503, 2008.

[75] E. Dias-Ferreira, J. C. Sousa, I. Melo et al., "Chronic stress causes frontostriatal reorganization and affects decision-making," *Science*, vol. 325, no. 5940, pp. 621–625, 2009.

[76] A. Vyas, R. Mitra, B. S. S. Rao, and S. Chattarji, "Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons," *The Journal of Neuroscience*, vol. 22, no. 15, pp. 6810–6818, 2002.

[77] J. C. Wingard and M. G. Packard, "The amygdala and emotional modulation of competition between cognitive and habit memory," *Behavioural Brain Research*, vol. 193, no. 1, pp. 126–131, 2008.

[78] M. G. Packard and A. Gabriele, "Peripheral anxiogenic drug injections differentially affect cognitive and habit memory: role of basolateral amygdala," *Neuroscience*, vol. 164, no. 2, pp. 457–462, 2009.

[79] L. Schwabe and O. T. Wolf, "Stress modulates the engagement of multiple memory systems in classification learning," *Journal of Neuroscience*, vol. 32, no. 32, pp. 11042–11049, 2012.

[80] L. Schwabe and O. T. Wolf, "Socially evaluated cold pressor stress after instrumental learning favors habits over goal-directed action," *Psychoneuroendocrinology*, vol. 35, no. 7, pp. 977–986, 2010.

[81] B. Roozendaal, B. S. McEwen, and S. Chattarji, "Stress, memory and the amygdala," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 423–433, 2009.

[82] D. Kahneman, "Maps of bounded rationality: a perspective on intuitive judgement and choice," in *Les Prix Nobel: The Nobel Prizes 2002*, T. Frangsmyr, Ed., pp. 449–489, The Nobel Foundation, Stockholm, Sweden, 2003.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 7215684, 12 pages
http://dx.doi.org/10.1155/2016/7215684

Research Article

Acute Psychological Stress Modulates the Expression of Enzymes Involved in the Kynurenine Pathway throughout Corticolimbic Circuits in Adult Male Rats

Haley A. Vecchiarelli,[1,2,3] Chaitanya P. Gandhi,[1,2,3] and Matthew N. Hill[1,2,4,5]

[1] *Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1*
[2] *Mathison Centre for Mental Health Research and Education, Cumming School of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1*
[3] *Department of Neuroscience, Cumming School of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1*
[4] *Department of Cell Biology and Anatomy, Cumming School of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1*
[5] *Department of Psychiatry, Cumming School of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1*

Correspondence should be addressed to Matthew N. Hill; mnhill@ucalgary.ca

Received 6 July 2015; Revised 26 August 2015; Accepted 2 September 2015

Academic Editor: Jordan Marrocco

Tryptophan is an essential dietary amino acid that is necessary for protein synthesis, but also serves as the precursor for serotonin. However, in addition to these biological functions, tryptophan also serves as a precursor for the kynurenine pathway, which has neurotoxic (quinolinic acid) and neuroprotective (kynurenic acid) metabolites. Glucocorticoid hormones and inflammatory mediators, both of which are increased by stress, have been shown to bias tryptophan along the kynurenine pathway and away from serotonin synthesis; however, to date, there is no published data regarding the effects of stress on enzymes regulating the kynurenine pathway in a regional manner throughout the brain. Herein, we examined the effects of an acute psychological stress (120 min restraint) on gene expression patterns of enzymes along the kynurenine pathway over a protracted time-course (1–24 h post-stress termination) within the amygdala, hippocampus, hypothalamus, and medial prefrontal cortex. Time-dependent changes in differential enzymes along the kynurenine metabolism pathway, particularly those involved in the production of quinolinic acid, were found within the amygdala, hypothalamus, and medial prefrontal cortex, with no changes seen in the hippocampus. These regional differences acutely may provide mechanistic insight into processes that become dysregulated chronically in stress-associated disorders.

1. Introduction

Tryptophan is an essential dietary amino acid. It is well known that it serves as the precursor for serotonin, but what is less well known is that the majority (90–95%) of tryptophan besides that utilized for protein synthesis, is shuttled to the kynurenine pathway, the end point of which is the generation of nicotinamide adenine dinucleotide (NAD+). Metabolites along the kynurenine pathway both are synthesized in the brain and can travel into the brain [1]. Furthermore, upregulation of the kynurenine pathway has been demonstrated in depressive disorders, and the intensity of depressive symptoms has been found to correlate with the upregulation of kynurenine pathway [2–10]. The enhancement of this differential metabolic route of tryptophan has been hypothesized to contribute to potential alterations in serotonergic function in depression, such that an increase shuttling of tryptophan to the kynurenine pathway could result in a deficit in serotonin synthesis [11].

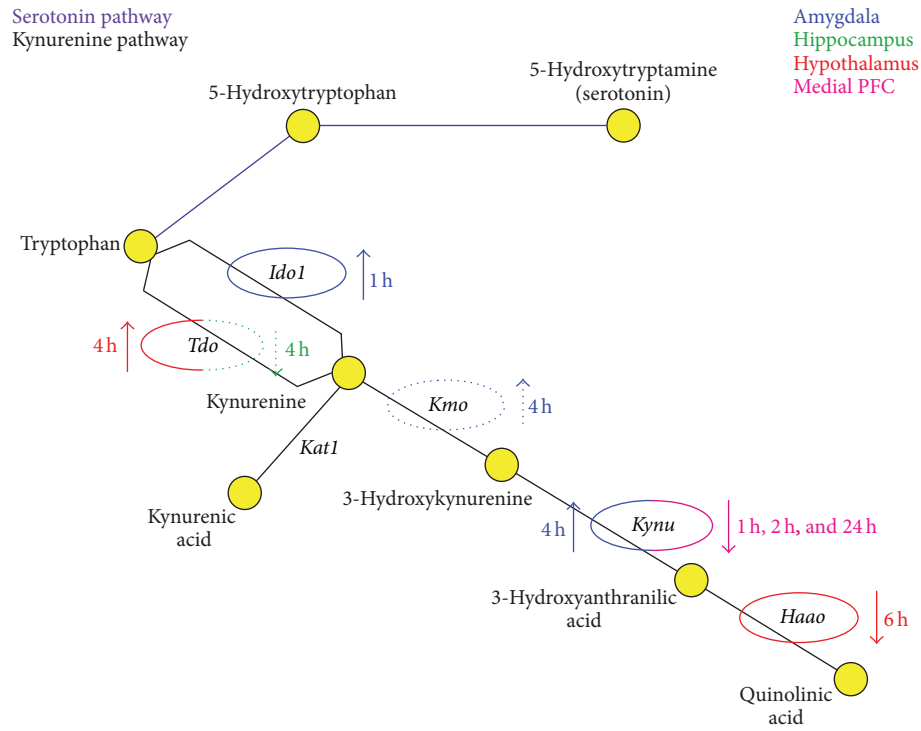


Figure 1: Schematic representation of tryptophan metabolism and its influence by stress. This figure shows the multiple ways that tryptophan can be metabolized. The classic, well-known pathway is its conversion to serotonin (purple). The primary pathway of tryptophan metabolism, however, is the kynurenine pathway (black), by which tryptophan is converted to kynurenine via tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase 1 (IDO1). From there, it can be converted into the neuroprotective kynurenic acid (KNYA) via kynurenine aminotransferase (KAT1) or to quinolinic acid (QUIN) via a multistep process involving the enzymes kynurenine 3-monooxygenase (KMO), kynureninase (KYNU), and 3-hydroxyanthranilate 3,4-dioxygenase (3-HOA). An analysis of mRNA expression levels of tryptophan 2,3-dioxygenase (*Tdo*), indoleamine 2,3-dioxygenase 1 (*Ido1*), kynurenine aminotransferase (*Kat1*), kynurenine 3-monooxygenase (*Kmo*), kynureninase (*Kynu*), and 3-hydroxyanthranilate 3,4-dioxygenase (*Haao*) revealed increases in *Ido1*, *Kmo*, and *Kynu* mRNA expression in the amygdala (blue), decreases in *Tdo* mRNA expression in the hippocampus (green), an upregulation of *Tdo*, and downregulation of *Haao* mRNA expression in the hypothalamus (red) and a downregulation of *Kynu* mRNA expression in the medial prefrontal cortex (pink) (solid lines indicate significant changes, while dashed lines indicate trending changes). This figure summates the regional and temporal effects of acute stress on the mRNA expression of kynurenine pathway enzymes.

The rate-limiting step in this pathway is the conversion of tryptophan to kynurenine by either tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase 1 (IDO1). Kynurenine can then be metabolized along a variety of pathways. One pathway results in the conversion of kynurenine to kynurenic acid (KYNA) by kynurenine aminotransferase1 (KAT1). KYNA is an N-methyl-D-aspartate receptor (NMDAR) and α7 nicotinic acetylcholine receptor (α7nAChR) antagonist and is thought to play a neuroprotective role through inhibiting glutamate signaling [1]. The other pathway, which results in the generation of quinolinic acid (QUIN), has multiple steps, by which kynurenine is converted to intermediates through kynurenine 3-monooxygenase (KMO) and kynureninase (KYNU) and, finally, by 3-hydroxyanthranilic acid 3,4-dioxygenase (3-HOA) to QUIN (Figure 1) [1]. As a highly selective NMDAR agonist, QUIN can induce excitotoxicity [1]. When taken altogether, the balance of the kynurenine metabolites, particularly KYNA and QUIN, could play an important role in central nervous system functioning, and that disruption of this balance could be important for a number of neurological disorders, including neurodegenerative and affective disorders [1].

It has been well established that inflammation can affect the activity of enzymes regulating the kynurenine pathway [12]. Specifically, IDO1 is upregulated by proinflammatory cytokines, such as TNF-α and IFNγ, and it has also been shown to be downregulated by anti-inflammatory cytokines, such as IL-4 [12]. Furthermore, there is evidence that stress, and specifically the major output of the HPA axis, glucocorticoids, can upregulate TDO [12]. Taken together, these data suggest that stress and/or inflammation have the ability to upregulate levels of kynurenine. Consistent with this, kynurenine levels are elevated in diseases that have elevated glucocorticoid output and inflammatory mediators, such as major depression and coronary heart disease [1, 2]. This increased pool of kynurenine may serve as a precursor for the generation of downstream metabolites, and in the context of disease states, it may amplify the production of the neurotoxic QUIN, which has been hypothesized to contribute to structural alterations and loss of neuropil in disease states such as major depression [8, 12, 13].

It is now well established that acute stress upregulates inflammatory mediators, both in humans, peripherally, and in rodents, both peripherally and in the brain [14, 15]. Recent work from our group has demonstrated that stress-induced changes in inflammatory mediators within the brain are not uniform and exhibit significant spatiotemporal specificity, with the amygdala appearing to be highly sensitive to induction of proinflammatory molecules following exposure to acute psychological stress [16]. Given that stress increases inflammatory cytokines and glucocorticoid levels, both of which have been shown to increase kynurenine levels, it seems highly plausible that stress could exert robust and dynamic effects on the pathway regulating kynurenine synthesis. Consistent with this hypothesis, it has been shown that various acute stressors can lead to an upregulation of the kynurenine pathway [17–19]; however, only one of these studies actually examined expression levels of the enzymes involved in kynurenine metabolism and did so in a whole brain manner without any regional specificity [17]. Therefore, the goal of this study was to investigate whether acute stress exposure affected gene expression levels of the enzymes in the kynurenine pathway, particularly investigating the key corticolimbic brain regions involved in regulating the stress response, the amygdala, hippocampus, hypothalamus, and medial prefrontal cortex, in a time-dependent manner.

2. Materials and Methods

2.1. Animals. Adult (approximately 70 postnatal days old, 300 g), male, Sprague Dawley rats ($N = 48$), obtained from Charles River (St. Constant, QC, Canada), were used in the following experiment. Animals were allowed to acclimate for at least one week prior to testing and kept on a 12:12 hour (h) light/dark cycle with *ad libitum* access to food and water. The experiment was conducted during the light phase of the cycle. The University of Calgary Animal Care Committee and the Canadian Council for Animal Care approved all animal use.

2.2. Experimental Design. Animals were subjected to 120 minutes (min) of restraint stress within the first 2 h of the light cycle, using clear, Plexiglas, Broome Style restrainers (Plas-Labs, Lansing, MI, United States). Rats were then decapitated 1 h, 2 h, 4 h, 6 h, or 24 h following stress termination ($n = 6$ for all time points). A nonstressed basal group ($n = 6$), designated as B, was implemented to allow for comparison of relative mRNA expression. However, given that circadian variations in inflammatory mediators exist in rats [20–22], two additional basal groups, referred to as 4B and 6B, were implemented for the 4 h ($n = 6$) and 6 h ($n = 6$) time points. Hence, there were three control groups: B, 4B, and 6B, which were used as controls for animals in the 1 h, 2 h, and 24 h groups, the 4 h group, and the 6 h group, respectively.

Following animal sacrifice, trunk blood was collected in Vacutainer blood collection tubes containing K2 ethylenediaminetetraacetic acid (EDTA) (BD, Mississauga, ON, Canada) and placed on ice for a maximum of 30 min before centrifugation at 10,000 g and 4°C for 20 min. Plasma was aliquoted and stored at −80°C for the measurement of corticosterone. Brains were extracted and the amygdala, hippocampus, hypothalamus, and medial prefrontal cortex were dissected out as previously described [23]. Following dissection, brain regions were immediately flash frozen on dry ice and stored in 2 mL RNase-free microtubes (Diamed, Mississauga, ON, Canada) at −80°C for further processing. Tissue collection areas and tools were sterilized with 70% ethanol and RNaseZAP (Sigma-Aldrich, Oakville, ON, Canada) between dissections to prevent RNase activity.

2.3. Corticosterone ELISA. Plasma corticosterone levels were measured in duplicate using a commercially available enzyme linked immunosorbent assay (ELISA) kit (Cayman Chemical Company, Ann Arbor, MI, United States), according to the manufacturer's protocol. The limit of detection for this assay at 80% binding/maximal binding is 30 pg/mL, while the standard curve ranges from 8.3 pg/mL to 5,000 pg/mL. Intra- and interassay variability are less than 10% at the middle points of the standard curve. All plasma samples were diluted 1 : 1000 so that values fell within the standard curve.

2.4. Tissue Processing for mRNA Isolation and cDNA Synthesis. Throughout the entirety of the mRNA isolation procedure, 70% ethanol and RNaseZAP (Sigma-Aldrich, Oakville, ON, Canada) were used to ensure that contamination by RNases did not occur. mRNA was isolated from each sample using the RNeasy Plus Universal Mini kit (Qiagen, Toronto, ON, Canada) and the QIAcube (Qiagen), according to the manufacturer's protocols. Briefly, samples were placed in 900 μL of QIAzol lysis reagent (Qiagen), containing phenol and guanidine thiocyanate, and homogenized for 2 min at 50 Hz using 50 mm steel beads (Qiagen) with a TissueLyser LT homogenizer (Qiagen). After lysis, 100 μL of gDNA eliminator solution and 180 μL of chloroform were added to the samples, followed by centrifugation for 15 min at 4°C and 12,000 g. The aqueous phase containing mRNA was transferred to new microcentrifuge tubes and placed in the QIAcube to carry out the RNeasy Plus Universal program. In this automated protocol, 600 μL of 70% ethanol is added to the samples, which are transferred to spin columns and centrifuged for 15 sec at 20°C and 8,000 g. The centrifugation process is repeated and followed by buffer rinses, to aid in the removal of non-mRNA biomolecules. Finally, the spin column is placed in a 1.5 mL collection tube and nuclease-free H_2O (Qiagen) was added to the samples so that they were eluted to a final volume of 100 μL. A Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) was used to measure mRNA concentration and purity (using the A_{260}/A_{280} ratio). Samples were then aliquoted and frozen at −80°C. All samples had an A_{260}/A_{280} ratio value between 1.9 and 2.1, indicating high mRNA purity.

In order to synthesize cDNA, qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, United States) was used, according to manufacturer's protocols. To summarize, 1 μg of mRNA was added to 4 μL of a reaction buffer ($MgCl_2$, dNTPs, recombinant RNase inhibitor protein, qScript reverse transcriptase, random primers, oligo(dT) primer, and stabilizers) and nuclease-free H_2O to result in a solution with a final volume of 20 μL. Samples were then incubated for

Table 1: Primer sequences used for control and kynurenine pathway enzymes.

Name	Forward 5′-3′	Reverse 5′-3′
	Control primers	
Rplp2 [16]	CGC TAC GTT GCC TCT TAT CT	GCC CAC GCT GTC TAG TAT TT
B2m [16]	CAG TTC CAC CCA CCT CAA ATA G	GTG TGA GCC AGG ATG TAG AAA G
	Kynurenine pathway enzyme primers	
Tdo	GAA CTT CCT CTC CTC CTT AGA	GGC CAT TCA CAC ACT CAT TA
Ido1	AGG AAC AGA TGG CAG AGT	CAG TCG TCG TTC ACC TTT AC
Kat1	CAG ACA TCT CAG ACT TCA AGA G	CCA CCA AGC CCA TGT TT
Kmo	TCC ACT TTC ATC CCT CTC TAT	GAG TCC TCT GTT TAT CAC CTT T
Kynu	CAG ACT GCT TAC TGC CAT AC	CCC AGT GTG TGA GAT TTA CTT
Haao	TGA TTG AGA GAA GGC GAA TG	CCT TAC AGT GGA ACC ATT TCT

5 min at 25°C, 30 min at 42°C, and 5 min at 85°C and held at 4°C. As with mRNA isolation, cDNA samples were read on a Nanodrop spectrophotometer to measure cDNA content and stored at −20°C. Prior to quantitative PCR (qPCR) analysis, cDNA aliquots were diluted in nuclease-free H_2O to a final concentration of 25 ng/μL.

2.5. Quantitative PCR. Primers were first designed using the IDTDNA software (Coralville, IA, United States), PrimerQuest. Although all efforts were taken to check the literature for duplication of previous primers, any replication of previously published primers is purely accidental and likely due to using the same software program. mRNA sequences for genes of interest were obtained using NCBI Nucleotide searches. The program was then instructed to construct primers that would result in products of no longer than 200 base pairs (bp) and had annealing temperatures (T_m) of 52–55°C. Primer pairs that met these criteria were then analyzed with the NCBI BLAST tool to check for specificity. Finally, primers were reconstituted and diluted in nuclease-free H_2O to obtain a final stock concentration of 10 μM. For a list of primer sequences, see Table 1.

Quantitative PCR (qPCR) was performed using the Quanta PerfeCTa SYBR Green FastMix (Quanta Biosciences) on a Rotor-Gene Q (Qiagen), according to manufacturer's instructions. Briefly, 50 ng of cDNA, primers (final concentration of 1 μM for forward and reverse each), and nuclease-free H_2O were added to a reaction buffer ($MgCl_2$, dNTPs, AccuFast Taq DNA Polymerase, SYBR Green I dye, and stabilizers). First, each primer was analyzed in a serially diluted standard curve to assess proper reaction conditions (number of cycles and annealing temperature) and to check for primer reaction efficiency (must be between 80 and 120%). All primers were assayed using the following specifications: 1 min at 90°C, 40 cycles of 95°C for 5 sec and 60°C for 30 sec. This was followed by a melt step (72°C to 95°C) to assess for single primer products. Each gene of interest's primer pair was compared with all reference primer pairs to ascertain which ones have the most similar reaction efficiencies. For each gene of interest, one reference primer was chosen that matched its efficiency.

Since all samples per brain region could not fit in one qPCR assay, they were divided as follows: assays contained at least one sample from the B, 1 h, 2 h, and 24 h groups, from the 4B and 4 h group, or from the 6B and 6 h group. For each run, a standard curve for each primer was run to continually validate primer efficiencies. Likewise, for each sample, control primers and primers of interest were assayed in triplicate, while no template controls (containing nuclease-free H_2O) were assayed singularly.

The C_t value, or the point at which fluorescent detection crosses the threshold, was assessed for each well. The three C_t values for each sample were averaged for both the reference primer and primer of interest. The difference between the reference primer and primer of interest, ΔC_t, was calculated for each sample. Subsequently, the difference between the average ΔC_t value for the basal groups (B, 4B, and 6B) and each sample (from the 1 h, 2 h, 24 h, 4 h, and 6 h groups), $\Delta\Delta C_t$, was calculated. Finally, the $2^{-\Delta\Delta C_t}$ values for each sample were calculated and the data was normalized so that the averages $2^{-\Delta\Delta C_t}$ of the basal groups were 1.

2.6. Statistical Analysis. All data were analyzed using GraphPad Prism software (GraphPad, La Jolla, CA, United States). Before any analyses were performed, outliers were removed using the robust regression and outlier removal (ROUT) method in the software. It involves using nonlinear regression to fit a curve that is not influenced by outliers [25]. This is followed by the identification of outliers using the residuals of the robust fit.

One-way analyses of variance (ANOVA), and if applicable *post hoc* analyses with Bonferroni's correction for multiple comparisons, was used to compare the means of the 1 h, 2 h, and 24 h groups to the B group, while two-tailed Student's t-tests were used to compare the relative mRNA expression levels of the 4 h and 6 h groups to the 4B and 6B group, respectively. F- or t-values, p-values, and eta square (R^2) values are reported for all data. All data are reported as means ± standard error of the mean (SEM) and $p < 0.05$ was considered the threshold for statistical significance.

3. Results

3.1. Effects of Acute Psychological Stress on Plasma Corticosterone Concentrations. Two hours of restraint stress reliably elevates plasma corticosterone in our hands [16], but to determine if stress had residual effects on plasma corticosterone at

Table 2: Circulating corticosterone.

Time post-stress termination	Plasma corticosterone (ng/mL)
B	79.53 ± 21.29
1 h	80.94 ± 21.09
2 h	168.40 ± 74.35
4B	63.97 ± 4.27
4 h	112.00 ± 24.25
6B	**248.90 ± 61.55***
6 h	267.90 ± 99.53
24	33.74 ± 7.59

Effects of acute psychological restraint stress on plasma corticosterone (ng/mL) concentrations at varying times post-stress termination. All data are represented as means ± SEM (n = 5-6/group). * $p < 0.05$ compared to B group.

the time points we examined gene expression, we measured plasma corticosterone levels at all time points. Plasma corticosterone levels were not altered 1 h, 2 h, or 24 h post-stress termination ($F(3, 19) = 1.75$, $p > 0.05$, and $R^2 = 0.22$), compared to their time-matched basal (B) group (Table 2). Similarly, corticosterone levels remained the same at both the 4 h ($t(9) = 2.15$, $p > 0.05$, and $R^2 = 0.34$) and 6 h time points post-stress termination ($t(9) = 0.17$, $p > 0.05$, and $R^2 = 0.003$), relative to their time-matched controls (Table 2). Finally, two-tailed t-tests showed a significant increase in the 6B group compared to the B group ($t(10) = 2.60$, $p = 0.03$, and $R^2 = 0.40$) but not between the 4B and B ($t(10) = 0.72$, $p > 0.05$, and $R^2 = 0.05$), which is indicative of circadian changes in basal levels of corticosterone (Table 2). This suggests that stress-induced corticosterone levels had recovered to basal levels at all time points examined in this study.

3.2. Acute Psychological Stress Upregulates Ido1, Kmo, and Kynu mRNA Expression in the Amygdala. 120 min of restraint stress altered amygdalar mRNA expression of *Ido1*, *Kmo*, and *Kynu* but not of *Tdo*, *Kat1*, or *Haao* (3-HOA) (Figure 2). There was a significant effect of stress on amygdalar *Ido1* mRNA expression ($F(3, 17) = 5.71$, $p = 0.007$), $R^2 = 0.50$, and subsequent *post hoc* analysis demonstrated that a significant difference between the B group and the 1 h group existed ($p < 0.05$) but not between the B and the 2 h or 24 h groups (Figure 2). There were no significant increases or decreases at the 4 h ($t(10) = 0.19$, $p = 0.86$, and $R^2 = 0.003$) or 6 h ($t(10) = 0.61$, $p = 0.56$, and $R^2 = 0.04$) time point with regard to *Ido1* mRNA expression (Figure 2). Interestingly, acute restraint stress also produced a trending increase in *Kmo* ($t(7) = 2.01$, $p = 0.09$, and $R^2 = 0.36$) and a significant increase in *Kynu* ($t(7) = 3.03$, $p = 0.02$, and $R^2 = 0.57$) mRNA expression at the 4 h time point (Figure 2). There were no differences in *Kmo* mRNA expression at the 1 h, 2 h, 24 h ($F(3, 17) = 1.80$, $p = 0.19$, and $R^2 = 0.24$), or 6 h time point ($t(10) = 0.01$, $p = 0.99$, and $R^2 = 0.000009$), compared to their time-matched basal controls. Likewise, *Kynu* mRNA expression at the 1 h, 2 h, 24 h ($F(3, 16) = 2.63$, $p = 0.09$, and $R^2 = 0.33$), and 6 h time points ($t(7) = 0.17$, $p = 0.87$, and $R^2 = 0.004$) remained the same as time-matched controls (Figure 2).

At all time points examined (1 h, 2 h, and 24 h; 4 h; 6 h), there were no significant changes compared to time-matched basal controls (B, 4B, and 6B, resp.) with *Tdo* ($F(3, 19) = 0.94$, $p = 0.44$, and $R^2 = 0.13$; $t(10) = 0.41$, $p = 0.69$, and $R^2 = 0.02$; $t(10) = 0.78$, $p = 0.45$, and $R^2 = 0.06$), *Kat1* ($F(3, 18) = 0.43$, $p = 0.74$, and $R^2 = 0.07$; $t(10) = 0.44$, $p = 0.67$, and $R^2 = 0.02$; $t(10) = 0.87$, $p = 0.41$, and $R^2 = 0.07$), and *Haao* ($F(3, 17) = 0.89$, $p = 0.47$, and $R^2 = 0.14$; $t(10) = 0.85$, $p = 0.41$, and $R^2 = 0.07$; $t(10) = 1.03$, $p = 0.33$, and $R^2 = 0.10$) gene expression (Figure 2).

3.3. Acute Psychological Stress Does Not Alter Expression of Kynurenine Pathway Enzymes in the Hippocampus. There were no significant alterations of mRNA expression in any of the kynurenine pathway enzymes; however, hippocampal *Tdo* expression was trending towards a decrease at the 4 h time point ($t(9) = 1.95$, $p = 0.08$, and $R^2 = 0.30$), when compared to its time-matched control (Figure 3). There were no significant differences between the 1 h, 2 h, and 24 h time points and the B group ($F(3, 12) = 0.85$, $p = 0.49$, and $R^2 = 0.18$) or the 6 h time point and the 6B group ($t(9) = 0.50$, $p = 0.623$, and $R^2 = 0.03$) for *Tdo* (Figure 3).

As mentioned above, at all time points examined (1 h, 2 h, and 24 h; 4 h; 6 h), there were no significant changes compared to time-matched basal controls (B, 4B, and 6B, resp.) in gene expression of *Ido1* ($F(3, 14) = 1.40$, $p = 0.29$, and $R^2 = 0.23$; $t(9) = 1.24$, $p = 0.25$; $t(9) = 0.70$, $p = 0.50$, and $R^2 = 0.05$), *Kat1* ($F(3, 11) = 0.72$, $p = 0.56$, and $R^2 = 0.16$; $t(9) = 0.30$, $p = 0.77$, and $R^2 = 0.01$; $t(10) = 1.61$, $p = 0.14$, and $R^2 = 0.21$), *Kmo* ($F(3, 15) = 1.13$, $p = 0.37$, and $R^2 = 0.18$; $t(7) = 0.65$, $p = 0.54$, and $R^2 = 0.06$; $t(8) = 0.43$, $p = 0.68$, and $R^2 = 0.02$), *Kynu* ($F(3, 9) = 1.07$, $p = 0.41$, and $R^2 = 0.26$; $t(7) = 0.45$, $p = 0.67$, and $R^2 = 0.03$; $t(8) = 0.23$, $p = 0.83$, and $R^2 = 0.006$), and *Haao* ($F(3, 11) = 1.20$, $p = 0.35$, and $R^2 = 0.25$; $t(9) = 0.13$, $p = 0.90$, and $R^2 = 0.002$; $t(8) = 0.27$, $p = 0.79$, and $R^2 = 0.009$) (Figure 3).

3.4. Acute Psychological Stress Upregulates Tdo and Downregulates Haao Expression in the Hypothalamus. 120 min of restraint stress significantly altered hypothalamic mRNA expression of *Tdo* and *Haao* but not of *Ido1*, *Kat1*, *Kynm*, or *Kynu* (Figure 4). *Tdo* mRNA levels were significantly elevated in the hypothalamus at the 4 h time point ($t(6) = 2.48$, $p = 0.05$, and $R^2 = 0.51$) but not at the 1 h, 2 h, and 24 h ($F(3, 17) = 0.79$, $p = 0.52$, and $R^2 = 0.12$) or 6 h time point ($t(8) = 1.81$, $p = 0.11$, and $R^2 = 0.29$) (Figure 4). Furthermore, even though *Haao* mRNA expression was not significantly altered at the 1 h, 2 h, or 24 h time point ($F(3, 17) = 0.07$, $p = 0.97$, and $R^2 = 0.01$) or the 4 h time point ($t(9) = 0.10$, $p = 0.93$, and $R^2 = 0.001$), it was significantly downregulated 6 h post-stress termination ($t(8) = 2.41$, $p = 0.04$, and $R^2 = 0.42$) (Figure 4).

Alterations in *Kat1* mRNA expression trended toward significance between the 1 h, 2 h, and 24 h and its time-matched basal control ($F(3, 17) = 2.51$, $p = 0.09$, and $R^2 = 0.31$),

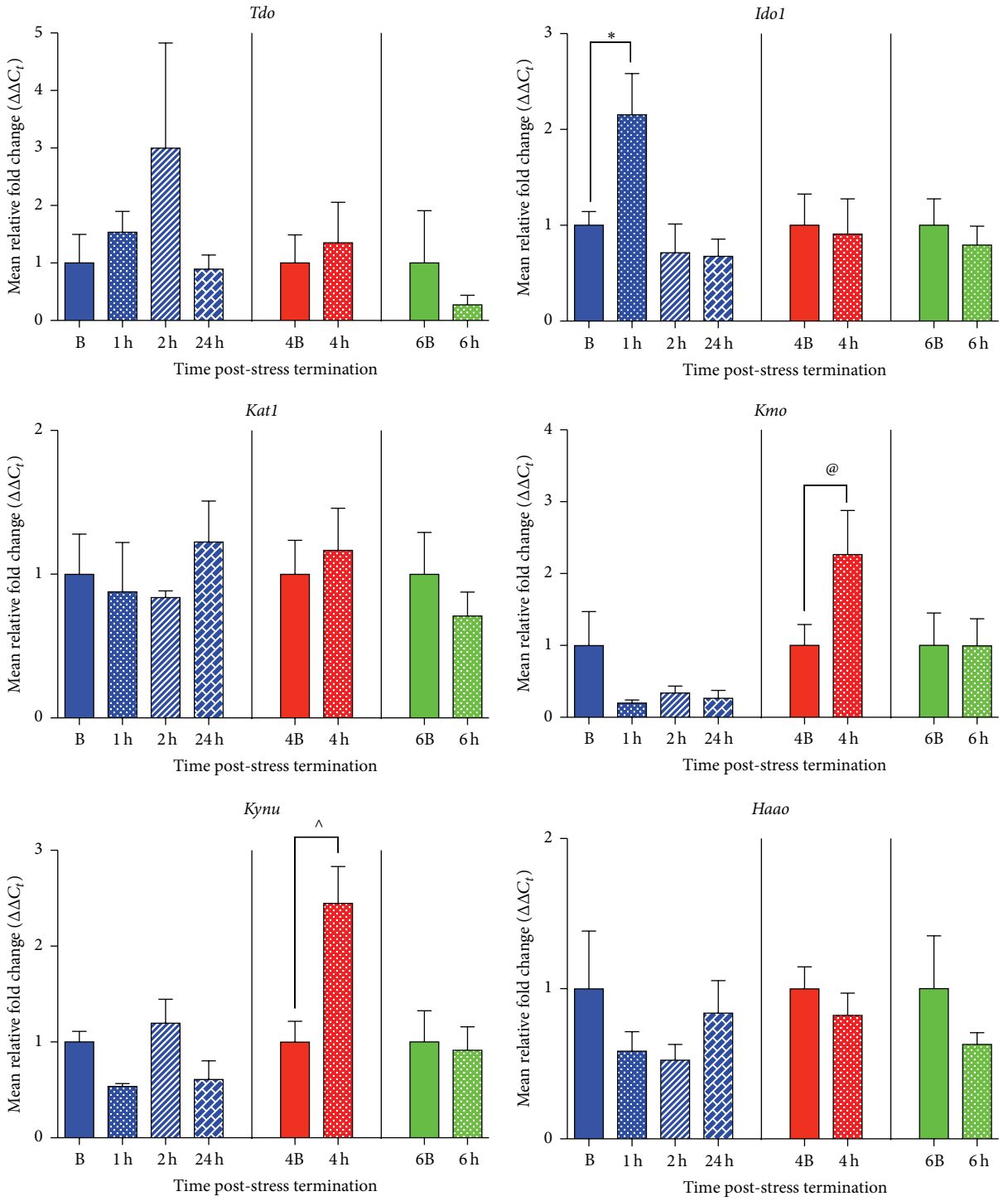


Figure 2: Effects of acute restraint stress on enzymes regulating the kynurenine pathway in the amygdala. mRNA expression levels of tryptophan 2,3-dioxygenase (*Tdo*), indoleamine 2,3-dioxygenase 1 (*Ido1*), kynurenine aminotransferase1 (*Kat1*), kynurenine 3-monooxygenase (*Kmo*), kynureninase (*Kynu*), and 3-hydroxyanthranilate 3,4-dioxygenase (*Haao*) were examined 1–24 h post-stress termination. All data are represented as means ± SEM (n = 3–6/group). $^{*}p < 0.05$ (in comparison to the B group), $^{\wedge}p < 0.05$ (in comparison to the 4B group), and $^{@}p < 0.10$ (in comparison to the 4B group).

but *post hoc* analysis showed that this was not between the basal groups and any of the stress groups but between the stress groups; furthermore, there were no changes at the 4 h or 6 h post-stress termination time point with *Kat1* mRNA expression compared to their time-matched basal controls ($t(8) = 0.66$, $p = 0.53$, and $R^2 = 0.05$; $t(8) = 0.25$, $p = 0.81$, and $R^2 = 0.008$). There were also no significant differences with regard to *Ido1* ($F(3, 17) = 1.61$, $p = 0.22$, and $R^2 = 0.22$;

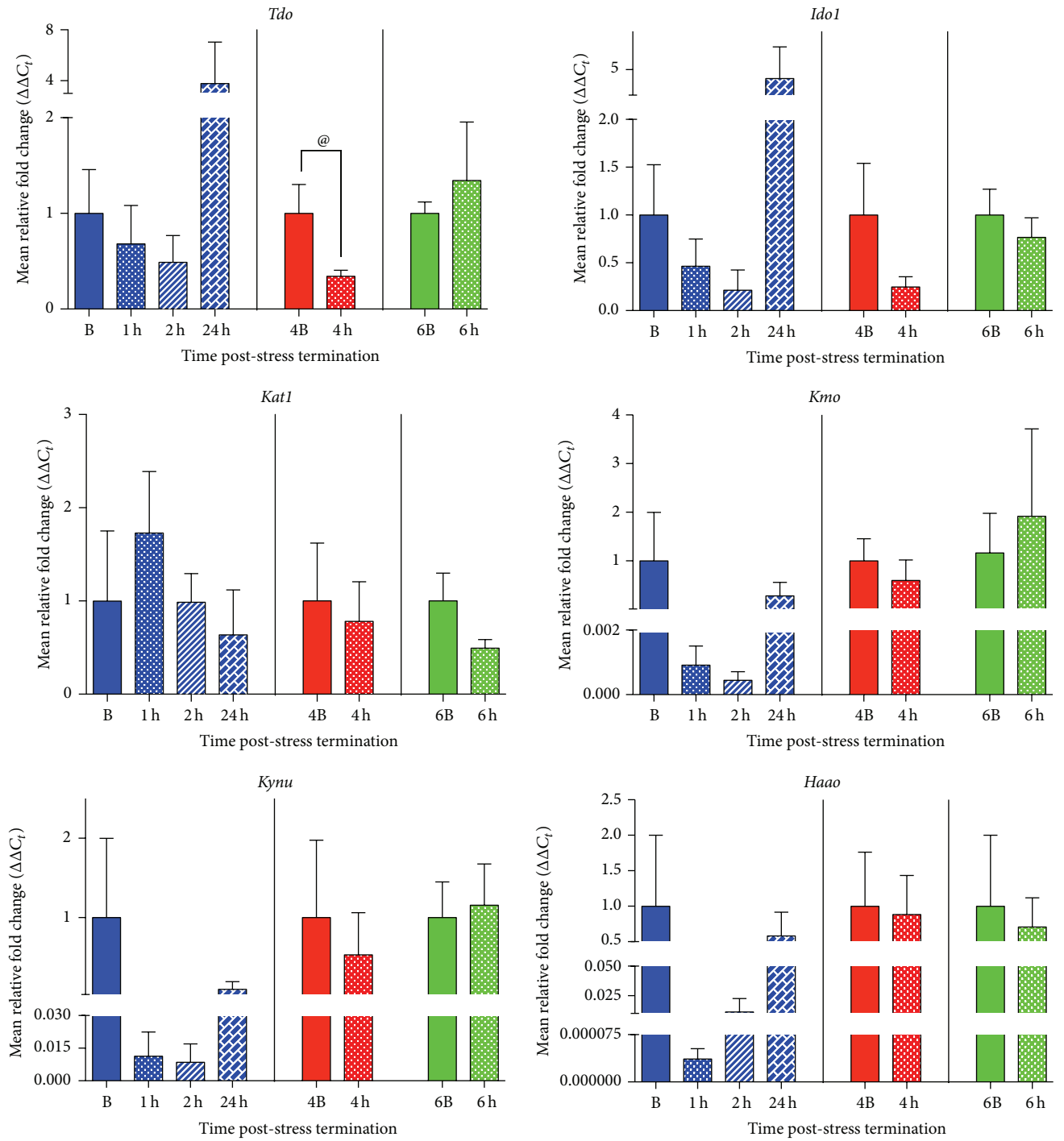


Figure 3: Effects of acute restraint stress on enzymes regulating the kynurenine pathway in the hippocampus. mRNA expression levels of tryptophan 2,3-dioxygenase (*Tdo*), indoleamine 2,3-dioxygenase 1 (*Ido1*), kynurenine aminotransferase (*Kat1*), kynurenine 3-monooxygenase (*Kmo*), kynureninase (*Kynu*), and 3-hydroxyanthranilate 3,4-dioxygenase (*Haao*) were examined 1–24 h post-stress termination. All data are represented as means ± SEM (n = 3–6/group). $^{@}p < 0.10$ (in comparison to the 4B group).

$t(7) = 0.31$, $p = 0.77$, and $R^2 = 0.01$; $t(8) = 0.65$, $p = 0.54$, and $R^2 = 0.05$), *Kmo* ($F(3, 20) = 1.10$, $p = 0.37$, and $R^2 = 0.14$; $t(10) = 0.09$, $p = 0.93$, and $R^2 = 0.0009$; $t(10) = 0.11$, $p = 0.91$, and $R^2 = 0.001$), or *Kynu* ($F(3, 19) = 0.92$, $p = 0.45$, and $R^2 = 0.13$); 4 h ($t(10) = 0.86$, $p = 0.41$, and $R^2 = 0.07$); 6 h ($t(9) = 1.03$, $p = 0.33$, and $R^2 = 0.10$) hypothalamic mRNA expression at any time point examined compared to time-matched basal controls (Figure 4).

3.5. Acute Psychological Stress Downregulates Kynu Expression in the Medial Prefrontal Cortex. 120 min of acute restraint stress significantly altered mRNA expression of *Kynu* but not

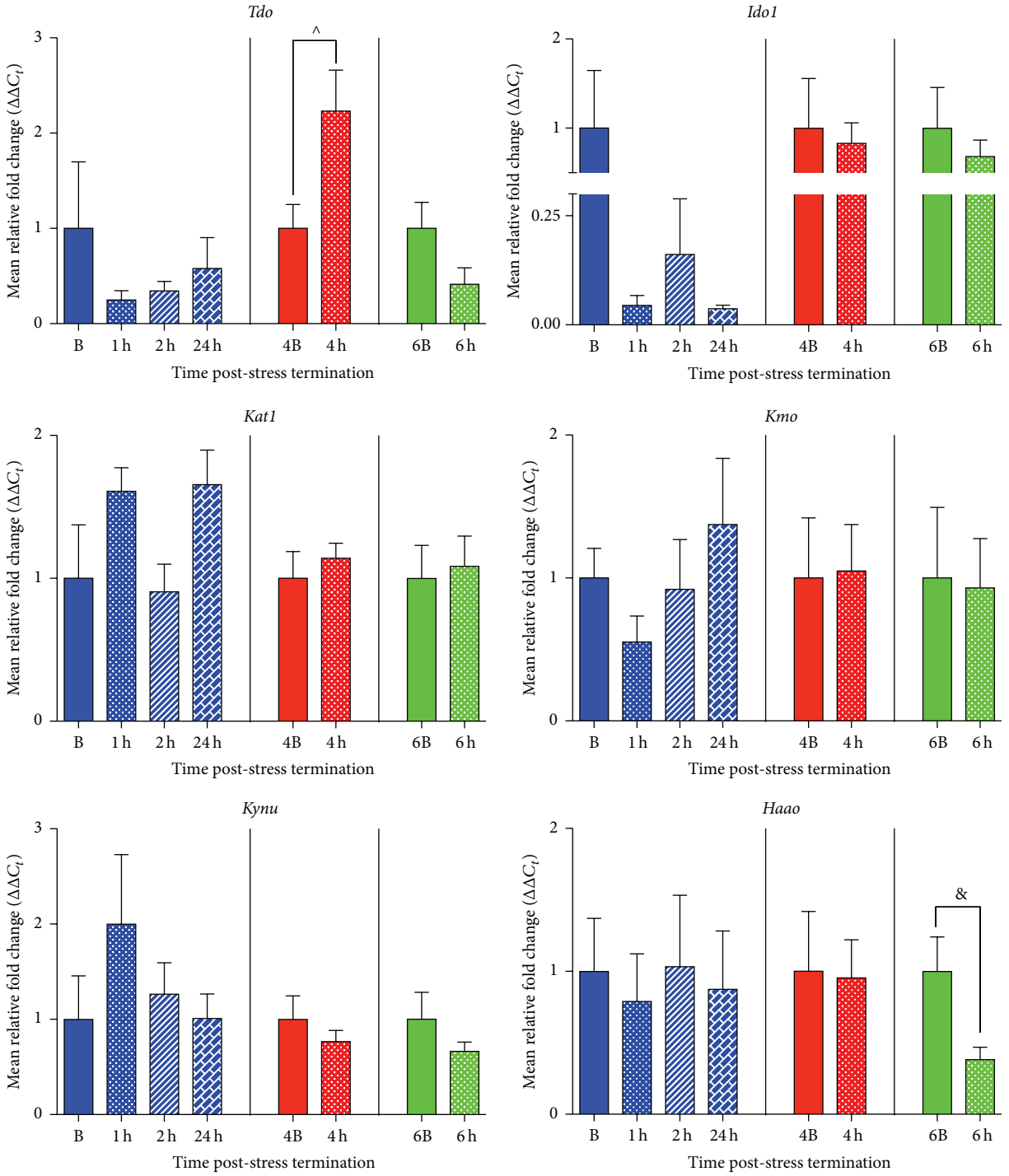


Figure 4: Effects of acute restraint stress on enzymes regulating the kynurenine pathway in the hypothalamus. mRNA expression levels of tryptophan 2,3-dioxygenase (*Tdo*), indoleamine 2,3-dioxygenase 1 (*Ido1*), kynurenine aminotransferase (*Kat1*), kynurenine 3-monooxygenase (*Kmo*), kynureninase (*Kynu*), and 3-hydroxyanthranilate 3,4-dioxygenase (*Haao*) were examined 1–24 h post-stress termination. All data are represented as means ± SEM (n = 3–6/group). $^{\wedge}p < 0.05$ (in comparison to the 4B group); $^{\&}p < 0.05$ (compared to 6B group).

of *Tdo*, *Ido1*, *Kat1*, *Kmo*, or *Haao* in the medial prefrontal cortex (Figure 5). Restraint stress altered *Kynu* mRNA expression between the 1 h, 2 h, and 24 h groups and their time-matched control B ($F(3, 16) = 5.70$, $p = 0.008$, and $R^2 = 0.52$), and *post hoc* analysis revealed that *Kynu* mRNA was downregulated at 1 h ($p < 0.01$), 2 h ($p < 0.01$), and 24 h ($p < 0.05$), in comparison to the B group. This decrease was not present at the 4 h ($t(7) = 0.62$, $p = 0.55$, and $R^2 = 0.05$) or 6 h post-stress ($t(10) = 0.60$, $p = 0.56$, and $R^2 = 0.03$) time point, when compared to their basal controls (Figure 5).

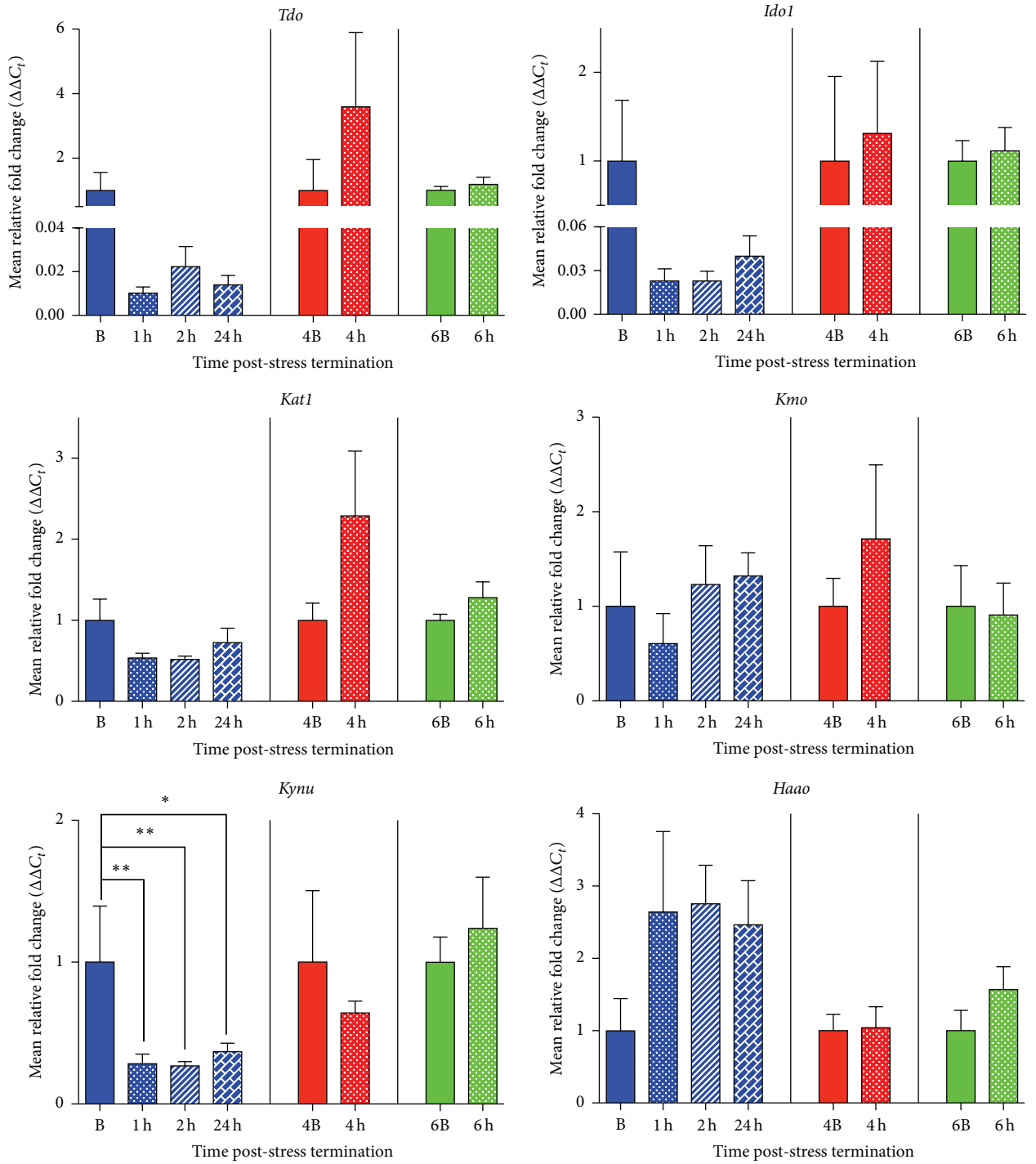


Figure 5: Effects of acute restraint stress on enzymes regulating the kynurenine pathway in the medial prefrontal cortex. mRNA expression levels of tryptophan 2,3-dioxygenase (*Tdo*), indoleamine 2,3-dioxygenase 1 (*Ido1*), kynurenine aminotransferase (*Kat1*), kynurenine 3-monooxygenase (*Kmo*), kynureninase (*Kynu*), and 3-hydroxyanthranilate 3,4-dioxygenase (*Haao*) were examined 1–24 h post-stress termination. All data are represented as means ± SEM (n = 3–6/group). $^{*}p < 0.05$ (in comparison to the B group); $^{**}p < 0.01$ (compared to 3B group).

Despite trending differences in *Tdo* ($F(3, 19) = 2.88$, $p = 0.06$, and $R^2 = 0.31$) expression between the B, 1 h, 2 h, and 24 h groups, *post hoc* analysis revealed that *Tdo* expression was not significantly different between the 1 h, 2 h, and 24 h post-stress termination groups when compared to their time-matched basal control. Furthermore, there were no differences between the 4 h ($t(10) = 1.03$, $p = 0.33$, and $R^2 = 0.10$) and 6 h ($t(10) = 0.74$, $p = 0.48$, and

R^2 = 0.05) termination groups when compared to their time-matched basal controls. For the other enzymes in the kynurenine pathway, at all time points examined (1 h, 2 h, and 24 h; 4 h; 6 h), there were no significant changes compared to time-matched basal controls (B, 4B, and 6B, resp.) in gene expression of *Ido1* ($F(3, 18) = 2.33$, $p = 0.11$, and $R^2 = 0.28$; $t(10) = 0.25$, $p = 0.81$, and $R^2 = 0.006$; $t(10) = 0.33$, $p = 0.75$, and $R^2 = 0.01$), *Kat1* ($F(3, 19) = 1.79$, $p = 0.18$, and $R^2 = 0.22$; $t(9) = 1.42$, $p = 0.19$, and $R^2 = 0.18$; $t(10) = 1.32$, $p = 0.22$, and $R^2 = 0.15$), *Kmo* ($F(3, 15) = 0.88$, $p = 0.48$, and $R^2 = 0.15$; $t(7) = 1.06$, $p = 0.33$, and $R^2 = 0.14$; $t(10) = 0.17$, $p = 0.87$, and $R^2 = 0.003$), and *Haao* ($F(3, 16) = 0.81$, $p = 0.51$, and $R^2 = 0.13$; $t(7) = 0.12$, $p = 0.91$, and $R^2 = 0.002$; $t(8) = 1.34$, $p = 0.22$, and $R^2 = 0.18$) (Figure 5).

4. Discussion

Our results are the first to show that acute stress alters gene expression of enzymes that regulate the kynurenine pathway in a regionally dependent manner (Figure 1). Specifically, we showed that in the amygdala there is an increase in *Ido1*, *Kmo*, and *Kynu* mRNA expression following acute stress exposure (Figure 2). Interestingly, these increases occur along the pathway in a temporal manner, with *Ido1* mRNA expression coming up 1 h post-stress termination followed by *Kmo* and *Kynu* mRNA expression increases at 4 h post-stress termination (Figure 1). The only other enzyme that was increased by acute stress exposure was *Tdo* mRNA expression in the hypothalamus (Figure 4). In addition to these increases, it was also found that within the hypothalamus there was a delayed decrease in *Haao* mRNA expression (Figure 4). Decreases in the gene expression of enzymes in the kynurenine pathway were also found in the medial prefrontal cortex, specifically in *Kynu* mRNA expression, which was found to decrease at 1 h, 2 h, and 24 h post-stress termination time points (Figure 5). Finally, there were essentially no alterations from stress exposure on kynurenine pathway enzymes in the hippocampus, aside from a trending decrease in *Tdo* mRNA expression (Figure 3). Taken altogether this work showed that acute stress induces temporal and regional alterations in mRNA levels of enzymes along the kynurenine pathway; specifically, enzymes that promote the excitotoxic NMDAR agonist quinolinic acid are upregulated in the amygdala following acute stress exposure, and quinolinic pathway enzymes are decreased in the medial prefrontal cortex (Figure 1). These regional differences have been highlighted before, both in terms of acute stress-induced inflammation [16] and also in kynurenine pathway metabolites in response to chronic stress [26, 27]. Furthermore, given the ability of quinolinic acid to act as a NMDAR agonist and thus potentially increase excitability, these regional differences in the kynurenine pathway may somewhat underlie the regional changes in neuronal activity observed as a result of chronic stress or psychiatric illness, such as depression, specifically, hyperexcitable amygdala, and hypoexcitable cortex [1, 4–10].

It should be noted that there is some variability in the mRNA expression data. In spite of this, there were significant changes in enzymes, such that along the QUIN neurotoxic arm of the kynurenine pathway several enzymes are increased within the amygdala, while a downregulation of mRNA expression of these enzymes was seen in the medial prefrontal cortex, following exposure to acute stress (Figure 1). It is unclear what could be cause of this variability; however, it has been previously reported that genes that are regulated by stress or inflammation exhibit a high degree of expression variability [26, 28]. It is possible that this variability of gene expression is caused by differences in gene expression regulators (e.g., transcription factors, upstream activators) across individuals [27, 28].

As inflammation is known to induce enzymes along the kynurenine pathway, specifically, upregulation of IDO1 and enzymes that increase QUIN, a neurotoxic kynurenine metabolite [1, 13, 29], this regional specificity in response to acute restraint stress is aligned with recent work from our group demonstrating that acute stress produces regional effects on inflammatory mediators in the brain. Specifically, we found that acute restraint stress leads to an increase of inflammatory mediators in circulation and the amygdala but a decrease in the medial prefrontal cortex [16]. These regionally specific changes in inflammatory cytokine levels following acute stress parallel, to some degree, the region-specific changes we found herein. Given the ability of inflammatory cytokines to modulate tryptophan metabolism along the kynurenine pathway, it seems quite plausible that stress modulates the expression of these enzymes in a regional manner through the regulation of inflammatory mediators. Consistent with this, previous reports have demonstrated that glucocorticoids and inflammation can modulate the kynurenine pathway [12, 29, 30] and these data suggest that this may occur, in part, through alterations in the expression of the enzymes involved in the kynurenine metabolism. Whole brain alterations of kynurenine metabolites and enzymes following stress exposure require TNF-α and IFNγ [17]. As we have recently shown that acute psychological stress reliably increases TNF-α levels in the amygdala [16], it is quite likely that stress-induced TNF-α signalling mediates the documented changes here in enzymes involved in kynurenine metabolism. Having established this pattern, future work will seek to determine the discrete role of both TNF-α and glucocorticoids in this process.

Given that the hippocampus has traditionally been studied as a region highly sensitive to the effects of stress, it is interesting to note that this was the only brain region examined in which we did not find any significant changes in the expression of enzymes involved in kynurenine metabolism. This is consistent with the apparent lack of effect we previously reported of acute stress on inflammatory mediators in the hippocampus [16] and parallels studies demonstrating a lack of effect of chronic stress on downstream by-products of kynurenine metabolism within the hippocampus [31, 32]. As our analysis did not subdivide the hippocampus into distinct regions (e.g., CA1, CA3, and dentate gyrus), these data do not preclude the possibility of discrete subregion changes in these enzymes within the hippocampus, a hypothesis which is consistent with other data indicating that there are differences in cytokine responses within hippocampal subregions [33].

With regard to cellular sources of kynurenine metabolites, there are numerous reports that have shown that KYNA producing enzymes are primarily localized to astrocytes in the brain, while QUIN producing enzymes are primarily localized to microglia [1, 24]. As it has been reported that stress can increase microglial activation [34, 35], it is possible that these observed effects of stress on QUIN producing enzymes are due to increased microglial activation and the subsequent upregulation of gene transcription within these cells. Future work should investigate the cell-type specific origins of kynurenine pathway metabolites and enzymes in response to stress.

These regional differences in enzymes regulating the kynurenine pathway, in response to acute stress, could underlie changes that others have observed with chronic stress paradigms. Specifically, previous reports have documented an upregulation of QUIN pathway metabolites in subcortical regions (i.e., the amygdala) and downregulation of these metabolites in the cortex (cingulate cortex) [31, 32], which parallels the increase and decrease we documented of enzymes in these cascades, respectively. The functional relevance of these changes has yet to be established, but given that QUIN can activate NMDA channels and promote excitotoxicity, it is interesting to note that biochemical indications of hyperexcitability and structural damage have been found within the amygdala following exposure to chronic stress and in depression [36].

To summarize, these data present the first investigation of the effects of acute stress on regional changes in mRNA expression of enzymes regulating the kynurenine pathway. Specifically, we found changes in enzymes along the QUIN neurotoxic arm of the kynurenine pathway are increased within the amygdala, while a downregulation of mRNA expression of these enzymes was seen in the medial prefrontal cortex, following exposure to acute stress (Figure 1). More so, it seems quite plausible that these changes are a consequence of increased inflammatory mediators in discrete brain regions and could provide a mechanistic pathway linking stress-induced inflammation to alterations in cellular integrity within the brain. The data herein, demonstrating acute stress-induced changes in the gene expression of enzymes leading to kynurenine metabolites that alter excitatory signalling, may may provide insight into processes that can become dysregulated during chronic stress conditions, which could, in turn, contribute to some of the neuropathological effects documented following stress or inflammatory-related illnesses, particularly major depression.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Haley A. Vecchiarelli and Chaitanya P. Gandhi contributed equally to this work.

Acknowledgments

The authors would like to thank Drs. J. Megan Gray and Maria Morena for their thoughtful comments and suggestions and Kowther Hassan for her technical assistance. The authors also acknowledge the University of Calgary's Health Sciences Animal Resource Centre for animal care. Chaitanya P. Gandhi has received funding from Alberta Innovates Health Solutions (AI-HS) and Natural Sciences and Engineering Research Council of Canada (NSERC); Haley A. Vecchiarelli has received a studentship from the Hotchkiss Brain Institute and stipend funding from the University of Calgary. This research was supported by operating funds to Matthew N. Hill from the Canadian Institute of Health Research (CIHR) and NSERC and performed with equipment purchased through the Canada Foundation for Innovation (CFI).

References

[1] R. Schwarcz, J. P. Bruno, P. J. Muchowski, and H.-Q. Wu, "Kynurenines in the mammalian brain: when physiology meets pathology," *Nature Reviews Neuroscience*, vol. 13, no. 7, pp. 465–477, 2012.

[2] L. Capuron, A. Ravaud, P. J. Neveu, A. H. Miller, M. Maes, and R. Dantzer, "Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy," *Molecular Psychiatry*, vol. 7, no. 5, pp. 468–473, 2002.

[3] C. Kohl, T. Walch, R. Huber et al., "Measurement of tryptophan, kynurenine and neopterin in women with and without postpartum blues," *Journal of Affective Disorders*, vol. 86, no. 2-3, pp. 135–142, 2005.

[4] B. E. Leonard and A. Myint, "Changes in the immune system in depression and dementia: causal or coincidental effects?" *Dialogues in Clinical Neuroscience*, vol. 8, no. 2, pp. 163–174, 2006.

[5] M. Maes, R. Verkerk, S. Bonaccorso, W. Ombelet, E. Bosmans, and S. Scharpé, "Depressive and anxiety symptoms in the early puerperium are related to increased degradation of tryptophan into kynurenine, a phenomenon which is related to immune activation," *Life Sciences*, vol. 71, no. 16, pp. 1837–1848, 2002.

[6] A. H. Miller, S. Ancoli-Israel, J. E. Bower, L. Capuron, and M. R. Irwin, "Neuroendocrine-immune mechanisms of behavioral comorbidities in patients with cancer," *Journal of Clinical Oncology*, vol. 26, no. 6, pp. 971–982, 2008.

[7] H. Miura, N. Ozaki, M. Sawada, K. Isobe, T. Ohta, and T. Nagatsu, "A link between stress and depression: shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression," *Stress*, vol. 11, no. 3, pp. 198–209, 2008.

[8] A.-M. Myint, Y. K. Kim, R. Verkerk, S. Scharpé, H. Steinbusch, and B. Leonard, "Kynurenine pathway in major depression: evidence of impaired neuroprotection," *Journal of Affective Disorders*, vol. 98, no. 1-2, pp. 143–151, 2007.

[9] W. Swardfager, N. Herrmann, Y. Dowlati et al., "Indoleamine 2,3-dioxygenase activation and depressive symptoms in patients with coronary artery disease," *Psychoneuroendocrinology*, vol. 34, no. 10, pp. 1560–1566, 2009.

[10] P. A. Zunszain, C. Anacker, A. Cattaneo, L. A. Carvalho, and C. M. Pariante, "Glucocorticoids, cytokines and brain abnormalities in depression," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 35, no. 3, pp. 722–729, 2011.

[11] B. Leonard and M. Maes, "Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression," *Neuroscience and Biobehavioral Reviews*, vol. 36, no. 2, pp. 764–785, 2012.

[12] A. M. Myint, "Kynurenines: from the perspective of major psychiatric disorders," *The FEBS Journal*, vol. 279, no. 8, pp. 1375–1385, 2012.

[13] J. Steiner, B. Bogerts, Z. Sarnyai et al., "Bridging the gap between the immune and glutamate hypotheses of schizophrenia and major depression: potential role of glial NMDA receptor modulators and impaired blood-brain barrier integrity," *The World Journal of Biological Psychiatry*, vol. 13, no. 7, pp. 482–492, 2012.

[14] T. Deak, K. A. Bordner, N. K. McElderry et al., "Stress-induced increases in hypothalamic IL-1: a systematic analysis of multiple stressor paradigms," *Brain Research Bulletin*, vol. 64, no. 6, pp. 541–556, 2005.

[15] N. Rohleder, "Stimulation of systemic low-grade inflammation by psychosocial stress," *Psychosomatic Medicine*, vol. 76, no. 3, pp. 181–189, 2014.

[16] H. A. Vecchiarelli, C. P. Gandhi, J. M. Gray, M. Morena, K. I. Hassan, and M. N. Hill, "Divergent responses of inflammatory mediators within the amygdala and medial prefrontal cortex to acute psychological stress," *Brain, Behavior, and Immunity*, 2015.

[17] C. Kiank, J.-P. Zeden, S. Drude et al., "Psychological stress-induced, IDO1-dependent tryptophan catabolism: implications on immunosuppression in mice and humans," *PLoS ONE*, vol. 5, no. 7, Article ID e11825, 2010.

[18] H. Miura, Y. Ando, Y. Noda, K. Isobe, and N. Ozaki, "Long-lasting effects of inescapable-predator stress on brain tryptophan metabolism and the behavior of juvenile mice," *Stress*, vol. 14, no. 3, pp. 262–272, 2011.

[19] D. Pawlak, Y. Takada, T. Urano, and A. Takada, "Serotonergic and kynurenic pathways in rats exposed to foot shock," *Brain Research Bulletin*, vol. 52, no. 3, pp. 197–205, 2000.

[20] C. Cearley, L. Churchill, and J. M. Krueger, "Time of day differences in IL1β and TNFα mRNA levels in specific regions of the rat brain," *Neuroscience Letters*, vol. 352, no. 1, pp. 61–63, 2003.

[21] A. Arjona and D. K. Sarkar, "Circadian oscillations of clock genes, cytolytic factors, and cytokines in rat NK cells," *Journal of Immunology*, vol. 174, no. 12, pp. 7618–7624, 2005.

[22] A. Nakao, "Temporal regulation of cytokines by the circadian clock," *Journal of Immunology Research*, vol. 2014, Article ID 614529, 4 pages, 2014.

[23] M. N. Hill, I. N. Karatsoreos, C. J. Hillard, and B. S. McEwen, "Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones *in vivo*," *Psychoneuroendocrinology*, vol. 35, no. 9, pp. 1333–1338, 2010.

[24] R. Schwarcz and R. Pellicciari, "Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities," *Journal of Pharmacology and Experimental Therapeutics*, vol. 303, no. 1, pp. 1–10, 2002.

[25] H. J. Motulsky and R. E. Brown, "Detecting outliers when fitting data with nonlinear regression—a new method based on robust nonlinear regression and the false discovery rate," *BMC Bioinformatics*, vol. 7, article 123, 2006.

[26] C. C. Pritchard, L. Hsu, J. Delrow, and P. S. Nelson, "Project normal: defining normal variance in mouse gene expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 23, pp. 13266–13271, 2001.

[27] U. Alon, "Network motifs: theory and experimental approaches," *Nature Reviews Genetics*, vol. 8, no. 6, pp. 450–461, 2007.

[28] J. W. K. Ho, M. Stefani, C. G. dos Remedios, and M. A. Charleston, "Differential variability analysis of gene expression and its application to human diseases," *Bioinformatics*, vol. 24, no. 13, pp. i390–i398, 2008.

[29] G. I. Byrne, L. K. Lehmann, J. G. Kirschbaum, E. C. Borden, C. M. Lee, and R. R. Brown, "Induction of tryptophan degradation *in vitro* and *in vivo*: a γ-interferon stimulated activity," *Journal of Interferon Research*, vol. 6, no. 4, pp. 389–396, 1986.

[30] A. R. Green, T. L. Sourkes, and S. N. Young, "Liver and brain tryptophan metabolism following hydrocortisone administration to rats and gerbils," *British Journal of Pharmacology*, vol. 53, no. 2, pp. 287–292, 1975.

[31] A. Laugeray, J.-M. Launay, J. Callebert, A. Surget, C. Belzung, and P. R. Barone, "Peripheral and cerebral metabolic abnormalities of the tryptophan-kynurenine pathway in a murine model of major depression," *Behavioural Brain Research*, vol. 210, no. 1, pp. 84–91, 2010.

[32] A. Laugeray, J.-M. Launay, J. Callebert, A. Surget, C. Belzung, and P. R. Barone, "Evidence for a key role of the peripheral kynurenine pathway in the modulation of anxiety- and depression-like behaviours in mice: focus on individual differences," *Pharmacology Biochemistry and Behavior*, vol. 98, no. 1, pp. 161–168, 2011.

[33] S. Rosi, V. Ramirez-Amaya, A. Vazdarjanova, P. F. Worley, C. A. Barnes, and G. L. Wenk, "Neuroinflammation alters the hippocampal pattern of behaviorally induced Arc expression," *Journal of Neuroscience*, vol. 25, no. 3, pp. 723–731, 2005.

[34] S. Sugama, M. Fujita, M. Hashimoto, and B. Conti, "Stress induced morphological microglial activation in the rodent brain: involvement of interleukin-18," *Neuroscience*, vol. 146, no. 3, pp. 1388–1399, 2007.

[35] E. S. Wohleb, N. D. Powell, J. P. Godbout, and J. F. Sheridan, "Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior," *The Journal of Neuroscience*, vol. 33, no. 34, pp. 13820–13833, 2013.

[36] E. Sibille, Y. Wang, J. Joeyen-Waldorf et al., "A molecular signature of depression in the amygdala," *The American Journal of Psychiatry*, vol. 166, no. 9, pp. 1011–1024, 2009.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 2762518, 13 pages
http://dx.doi.org/10.1155/2016/2762518

Research Article

Behavioral Deficits in Juveniles Mediated by Maternal Stress Hormones in Mice

Jamie Maguire[1] and Istvan Mody[2]

[1]*Department of Neuroscience, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA*
[2]*Departments of Neurology and Physiology, The David Geffen School of Medicine, University of California, 635 Charles Young Dr. South, Los Angeles, CA 90095, USA*

Correspondence should be addressed to Jamie Maguire; jamie.maguire@tufts.edu

Received 18 June 2015; Revised 14 August 2015; Accepted 25 August 2015

Academic Editor: Laura Musazzi

Maternal depression has been shown to negatively impact offspring development. Investigation into the impact of maternal depression and offspring behavior has relied on correlative studies in humans. Further investigation into the underlying mechanisms has been hindered by the lack of useful animal models. We previously characterized a mouse model which exhibits depression-like behaviors restricted to the postpartum period and abnormal/fragmented maternal care ($Gabrd^{-/-}$ mice). Here we utilized this unique mouse model to investigate the mechanism(s) through which maternal depression-like behaviors adversely impact offspring development. Cross-fostering experiments reveal increased anxiety-like and depression-like behaviors in mice reared by $Gabrd^{-/-}$ mothers. Wild type and $Gabrd^{-/-}$ mice subjected to unpredictable stress during late pregnancy exhibit decreased pup survival and depression-like behavior in the postpartum period. Exogenous corticosterone treatment in wild type mice during late pregnancy is sufficient to decrease pup survival and induce anxiety-like and depression-like behaviors in the offspring. Further, the abnormal behaviors in juvenile mice reared by $Gabrd^{-/-}$ mice are alleviated by treatment of the mothers with the corticotropin-releasing hormone (CRH) antagonist, Antalarmin. These studies suggest that hyperresponsiveness of the HPA axis is associated with postpartum depression and may mediate the adverse effects of maternal depression on offspring behavior.

1. Introduction

Maternal depression has adverse effects on infant behavioral, emotional, and cognitive development [1–7]. Studies investigating the impact of postpartum depression on child development have largely relied on correlative studies in humans. However, investigation into the mechanisms mediating the transmission of negative affect from depressed mother to child has been impeded by the lack of useful animal models. We previously characterized a mouse model with deficits in maternal care which exhibits depression-like behaviors during the postpartum period ($Gabrd^{-/-}$ mice) [8]. Here we utilize this mouse model to investigate the mechanisms underlying the negative impact of maternal depression-like behaviors on offspring development.

Deficits in offspring development associated with maternal depression are correlated with elevated levels of stress hormones in both the mother and the fetus [9]. Treatment of pregnant women with exogenous glucocorticoids results in deficits in child development similar to those related to postpartum depression [10], suggesting that the stress response may mediate the adverse effects of maternal depression on offspring development (for review see [11]). The body's physiological response to stress is mediated by the hypothalamic-pituitary-adrenal (HPA) axis and involves the release of CRH from the hypothalamus, which triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary, ultimately resulting in glucocorticoid (corticosterone in mice and cortisol in humans) release from the adrenal gland. Although basal levels of corticosterone increase throughout pregnancy and lactation, stress-induced elevations in stress hormones are suppressed (for review see [12]), which is thought to protect the fetus from the negative effects of exposure to high levels of glucocorticoids [13, 14].

Accumulating evidence suggests that dysregulation of the HPA axis plays a role in postpartum depression. The HPA axis may be hyperresponsive in postpartum depression, as indicated by elevated levels of cortisol [15], although these findings are controversial [16, 17]. More convincing evidence exists for increased levels of CRH [18, 19] and ACTH [18] associated with postpartum depression. Accordingly, it has been suggested that CRH levels are increased in women with postpartum depression and may even be used as a diagnostic criteria for postpartum depression [19]. Elevated levels of stress hormones in the depressed mother result in elevated levels of stress hormones in the infant [20] and higher levels of stress hormones are associated with decreased maternal care and offspring anxiety [21]. Therefore, it is reasonable to hypothesize that dysregulation of the HPA axis may play a role not only in postpartum depression but also in the negative impact of maternal depression on offspring development. Consistent with this hypothesis administration of exogenous corticosterone to the dams during the postpartum period induced behavioral abnormalities in the offspring [22].

The activity of the HPA axis is governed by CRH neurons in the paraventricular nucleus (PVN) of the hypothalamus (for review see [23]). The activity of these neurons, and thus activity of the HPA axis, is tightly regulated by robust GABAergic inhibition (for review see [24–26]), including tonic GABAergic inhibition mediated by δ subunit-containing $GABA_ARs$ [27]. Interestingly, we demonstrated that a mouse model deficient in the $GABA_AR$ δ subunit ($Gabrd^{-/-}$ mice) exhibit depression-like behaviors exclusively during the postpartum period and deficits in maternal behaviors [8]. A recent study confirmed these findings demonstrating that $Gabrd^{-/-}$ dams provide fragmented maternal care and their offspring exhibit phenotypes similar to those subjected to early life stress [28].

In this study, we utilized this mouse model ($Gabrd^{-/-}$ mice), which exhibit abnormal/fragmented maternal care and depression-like behavior during the postpartum period [8, 28], to investigate the impact of maternal depression-like behavior on offspring development and the involvement of stress-related steroid hormones. It is well known that maternal depression negatively impacts child development in humans [1–7]. Here we reproduce analogous deficits in offspring development associated with maternal depression-like behavior in $Gabrd^{-/-}$ dams which can be mimicked with unpredictable stress or exogenous corticosterone administration in wild type mice. Further, blocking CRH signaling with Antalarmin during pregnancy in $Gabrd^{-/-}$ mice prevents the adverse behavioral effects on the juvenile offspring. This study demonstrates the utility of $Gabrd^{-/-}$ mice in investigating the pathophysiological mechanisms of postpartum depression and implicates hyperexcitability of the HPA axis in postpartum depression-like behavior and the negative impact on offspring development.

2. Materials and Methods

2.1. Animal Handling and Treatment. Adult (3 months old) C57/Bl6 and $Gabrd^{-/-}$ mice were housed at the University of California, Los Angeles (UCLA), Division of Laboratory Animal Medicine. The animals (4/cage) were housed in clear plastic cages in a temperature- and humidity-controlled environment with a 12 h light/dark cycle (light on at 6 a.m.) and were maintained on an *ad libitum* diet of lab chow and water. Animals were handled according to protocols approved by the UCLA, Chancellor's Animal Research Committee (ARC). For the pregnancy experiments, C57/Bl6 and $Gabrd^{-/-}$ adult female mice were exposed to a male mouse for a single dark cycle. The female was checked for a vaginal plug and placed into a separated home cage. The pregnant female was individually housed with the single litter until the pups were weaned. For the cross-fostering experiments, the mothers were removed from the native litter and swapped with a surrogate (either wild type or $Gabrd^{-/-}$) immediately following delivery. The mothers were exchanged to minimize the disturbance and handling of the pups. The juvenile offspring remained in the home cage with the mother (or surrogate) and littermates until all behavioral testing was complete.

2.2. Unpredictable Stress Paradigm. This study utilized a stress paradigm devised by another research team, which they termed "chronic ultramild stress" [29, 30] and has been shown to elevate corticosterone levels during pregnancy [29, 30]. In the current study, this stress paradigm is referred to as unpredictable stress (US). Wild type and $Gabrd^{-/-}$ mice were subjected to unpredictable stress from D14 to D21 of pregnancy as previously described [29, 30]. Wild type and $Gabrd^{-/-}$ mice at D14 of pregnancy were randomly assigned to two groups: Group 1 (stressed) were subjected to an unpredictable stressor (cage tilt, confinement in a small cage, overnight light exposure for a single night, soiled cage for a single 24-hour period, and difficult access to food) during the dark period for 7 consecutive days until D21 of pregnancy unless parturition occurred before at which time subjection to the stressors was immediately ceased. The stressors were alternated to prevent habituation to a single stressor. The periods of stress were separated by stress-free intervals of at least 12 hours. Group 2 (controls) were maintained in their home cage without subjection to the unpredictable stressors.

2.3. Behavioral Tests. Behavioral tests were performed on wild type and $Gabrd^{-/-}$ dams at 48 hrs postpartum, a time point which has previously been demonstrated to be associated with abnormal postpartum and maternal behaviors in $Gabrd^{-/-}$ mice [8]. Behavioral tests in juvenile mice began at P21 with the open field test followed by the more stressful forced swim test 24 hrs later. P21 was chosen as the time point to test the impact of maternal behavior on the behavior of the offspring since this time point is prior to weaning and at this time the offspring still share a home cage with the mother.

2.3.1. Depression Assay: Porsolt Forced Swim Test. Depression-like behavior was assessed in wild type and $Gabrd^{-/-}$ dams subjected to unpredictable stress (US) at 48 hrs postpartum and in cross-fostered, CORT, and Antalarmin-treated

offspring at approximately P21 using the forced swim test as previously described [8, 31]. Briefly, each mouse was placed individually in a glass cylinder (21 cm × 12 cm), containing 9 cm of room temperature water (22–25°C), in which there is no escape. The latency to immobility and the total duration of immobility throughout the 6 min forced swim test were measured. The mouse was considered to be immobile when it ceased swimming and remained floating motionless, except for infrequent movements of a single hindlimb to maintain being afloat. All tests were videotaped and subsequently analyzed and scored.

2.3.2. Anxiety Assay: Open Field Test. Anxiety-like behavior was assessed in cross-fostered, CORT, and Antalarmin-treated juvenile offspring at P21 using the open field test. Animals were tested in the same testing area, under bright light, with no visual cues. The apparatus was cleaned with ethanol and water in between animals to prevent olfactory cues. The mice were placed into the center of the open field, which consists of a plexiglass container (40 × 30 × 40 cm) with a grid of squares (10 × 7) on the bottom. The amount of time spent in the center (6 × 3) squares was measured over a single 10 min testing period. The total number of lines crossed (beam breaks) during the 10 min test was also counted.

2.4. Acute Stress Paradigm. The acute stress paradigm was utilized to measure stress-induced elevations in corticosterone in virgin and postpartum wild type and *Gabrd*$^{-/-}$ mice. An adapted protocol of the CO_2 exposure paradigm [32] in adult female wild type and *Gabrd*$^{-/-}$ mice was used as an acute stressor. Exposure to 35% CO_2 for 2 min was used to produce marked increases in circulating corticosterone levels [33]. Virgin and postpartum (48 hrs) wild type and *Gabrd*$^{-/-}$ mice were randomly assigned to two groups: Group 1 (stressed) which were subjected to a single episode of CO_2 stress (35% for 2 min) and Group 2 (controls) which were handled in a similar way as Group 1 except they received air instead of air enriched with CO_2. All animals were handled similarly in which their home cage was inserted into a larger ventilation box where CO_2 (or air in the case of controls) was administered. The animals were allowed to recover for 30 min prior to blood collection.

2.5. Steroid Hormone Concentration Determination. Blood was collected for corticosterone measurements from wild type and *Gabrd*$^{-/-}$ mice 30 min following acute CO_2 stress and compared to controls. Mice were anesthetized with isoflurane before whole blood was collected from experimental groups by retro-orbital bleeding between 12 and 14 hrs. Plasma was immediately isolated by high speed centrifugation and stored at −20° until use. Corticosterone levels were measured by enzyme immunoassay according to manufacturer's specifications (Enzo Life Sciences) as described previously [27, 34–36]. Briefly, triplicate 5 μL plasma samples were assayed and compared to a standard curve using a spectrophotometer (at 450 nm). Intra-assay variability of the corticosterone assay was 7.8 ng/mL between paired samples and the interassay variability was 4.4 ng/mL for the same samples between assays.

2.6. Corticosterone Implantation. Wild type mice at day 14 (D14) of pregnancy were briefly anaesthetized with halothane until unresponsive to a foot pinch and were either sham implanted or implanted with a 21-day release 10 mg corticosterone pellet (Innovative Research of America, Sarasota, FL). The hair from the incision site on the back of the neck was clipped and swabbed with ethanol and iodine prior to making the incision. A small 1 cm incision was made on the back of the neck and a small slow-release pellet (or nothing for sham) was placed underneath the skin using forceps without touching the external area. The incision was then closed with sutures. Pup survival was determined at postnatal day 7 (P7) and offspring behavior was assessed in the juveniles at P21. Two mice per litter were randomly selected for behavioral analysis.

2.7. Antalarmin Treatment. Antalarmin was administered to *Gabrd*$^{-/-}$ mice from D14 to D21 of pregnancy in the drinking water to minimize the handling of the animals. At day 14 of pregnancy, the normal drinking water was replaced with the Antalarmin solution (10 mg Antalarmin/10 μL ethanol/100 mL drinking water). The animals were maintained on either vehicle (10 μL ethanol/100 mL drinking water) or Antalarmin until D21 of pregnancy or immediately after parturition (if before D21) at which time the animals were returned to normal drinking water. This treatment strategy was previously shown to block elevations in corticosterone levels [35, 36]. Pup survival was assessed at P7 and offspring behavior was assessed at P21.

2.8. Statistics. A one-way ANOVA with Tukey's post hoc multiple comparisons test was used to determine statistical significance for comparing more than two experimental groups. Student's t-test was used to determine statistical significance between two experimental groups. All statistical tests were carried out using Graphpad 6.0 (Prism).

3. Results

3.1. Impact of Maternal Depression-Like Behaviors in Gabrd$^{-/-}$ *Mice on Offspring Behavior.* Here we utilized *Gabrd*$^{-/-}$ mice [8] to investigate the impact of maternal depression-like behaviors on offspring behavior. To determine if the maternal behavior, *per se*, directly mediates the deficits in offspring behavior, we performed cross-fostering experiments. Immediately following delivery, the natural mothers of wild type or *Gabrd*$^{-/-}$ litters were replaced with a surrogate wild type or *Gabrd*$^{-/-}$ mother and the behavior of the cross-fostered animals was then assessed in the juvenile offspring at age P21 (Figure 1(a)). Our data demonstrate that juvenile wild type or *Gabrd*$^{-/-}$ mice reared by mice exhibiting depression-like behavior during the postpartum period (*Gabrd*$^{-/-}$ mice) exhibit increased anxiety-like behavior in the open field test compared to juvenile wild type or *Gabrd*$^{-/-}$ mice reared by

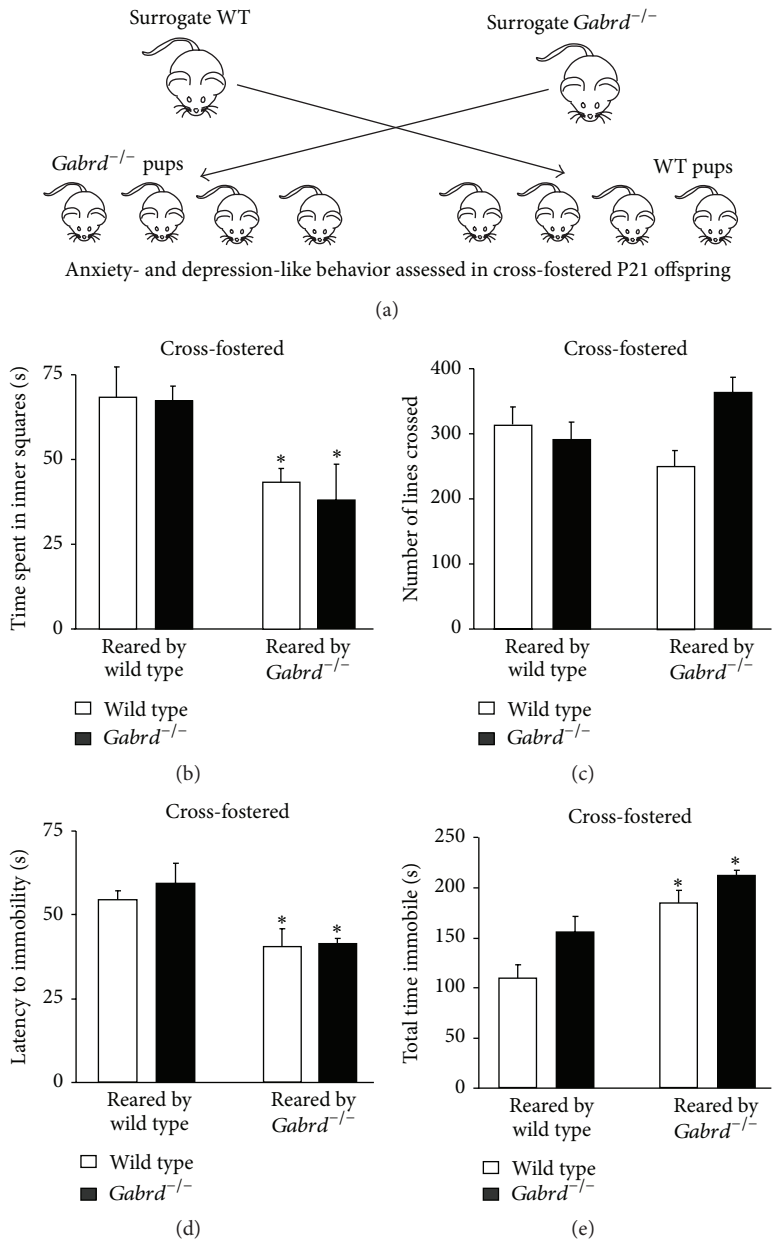


Figure 1: Maternal depression-like behaviors negatively impact offspring behavior. (a) A diagram outlining the experimental design for the cross-fostering experiments. Wild type and *Gabrd*$^{-/-}$ mice were reared by either a surrogate wild type or surrogate *Gabrd*$^{-/-}$ mother. Juvenile wild type and *Gabrd*$^{-/-}$ offspring reared by surrogate mothers exhibiting depression-like behavior (*Gabrd*$^{-/-}$ mice) exhibit anxiety-like behavior in the open field test, evident by a significant decrease in the time spent in the center squares (b) with no change in the number of lines crossed (c) during the 10 min test. n = 12–17 mice per experimental group; $*$ denotes $p < 0.05$ using a one-way ANOVA with Tukey's multiple comparisons test. Juvenile wild type and *Gabrd*$^{-/-}$ offspring reared by surrogate mothers exhibiting depression-like behavior (*Gabrd*$^{-/-}$ mice) also exhibit depression-like behavior, assessed using the forced swim test. Offspring reared by surrogate *Gabrd*$^{-/-}$ mothers exhibit a significant decreased latency to immobility (d) and an increase in the total time spent immobile (e) compared to offspring reared by surrogate wild type mothers. n = 10–12 mice per experimental group; $*$ denotes $p < 0.05$ using one-way ANOVA with Tukey's multiple comparisons test.

surrogate wild type mothers (Table 1; Figure 1(b)). Juvenile mice reared by *Gabrd*$^{-/-}$ mice spent less time in the center squares of the open field test compared to juvenile mice reared by wild type mothers (Table 1; Figure 1(b)) (n = 12–17 mice per experimental group; $*$ denotes $p < 0.05$ using a one-way ANOVA with Tukey's multiple comparisons test; $F(3, 54) = 7.778$). However, there is no significant difference in the locomotor behavior, indicated by the number of lines crossed, between offspring reared by wild type and *Gabrd*$^{-/-}$ mothers (Table 1; Figure 1(c)) (n = 12–17 mice per

Table 1: Summary of experimental results.

	Surrogate	Pup survival	Open field		Forced swim test	
		%	Time in center (s)	Beam breaks	Latency to immobility (s)	Total time immobile (s)
Dams						
Wild type		92.0 ± 1.0			70.7 ± 6.2	148.3 ± 26.7
Wild type CUS		56.2 ± 0.9*			49.5 ± 8.2*	245.6 ± 3.3*
Gabrd$^{-/-}$		65.5 ± 0.9*			47.7 ± 2.2*	205.5 ± 9.8*
Gabrd$^{-/-}$ CUS		41.7 ± 1.1*			55.8 ± 5.2*	222.8 ± 8.1*
Wild type sham		88.9 ± 2.7				
Wild type CORT		20.5 ± 4.4*				
Gabrd$^{-/-}$ vehicle		51.9 ± 6.2				
Gabrd$^{-/-}$ Antalarmin		75.0 ± 5.1*				
Offspring (P21)						
Cross-fostered wild type	WT surrogate		82.9 ± 12.6	324.1 ± 29.1	53.6 ± 2.7	107.1 ± 13.7
Cross-fostered *Gabrd*$^{-/-}$ mice	WT surrogate		78.6 ± 10.6	331.5 ± 32.4	58.5 ± 6.0	152.9 ± 15.5
Cross-fostered wild type	*Gabrd*$^{-/-}$ surrogate		43.3 ± 4.3**	250.5 ± 22.8	39.9 ± 5.3**	181.4 ± 12.3**
Cross-fostered *Gabrd*$^{-/-}$ mice	*Gabrd*$^{-/-}$ surrogate		40.7 ± 4.2**	360.4 ± 24.4	40.9 ± 1.4**	208.4 ± 5.0**
Wild type sham			65.0 ± 11.2	338.7 ± 20.4	53.0 ± 7.5	124.5 ± 38.2
Wild type CORT			37.0 ± 4.0**	546.9 ± 18.1**	41.5 ± 4.5	204.4 ± 11.1**
Gabrd$^{-/-}$ vehicle			45.1 ± 7.1	560.3 ± 43.9	49.1 ± 4.1	201.0 ± 3.3
Gabrd$^{-/-}$ Antalarmin			96.7 ± 12.8**	567.0 ± 65.4	71.0 ± 9.3**	100.0 ± 23.1**

*Significance $p < 0.05$ compared to control, sham, or vehicle.
**Significance $p < 0.05$ compared to wild type, sham, or vehicle.

experimental group; ∗ denotes $p < 0.05$ using a one-way ANOVA with Tukey's multiple comparisons test; $F(3, 54) = 2.819$). These results suggest that maternal depression-like behaviors negatively impact offspring development, resulting in increased anxiety-like behavior. In addition, both wild type and *Gabrd*$^{-/-}$ juvenile mice, reared by *Gabrd*$^{-/-}$ mothers, exhibit an increase in depression-like behavior compared to mice reared by surrogate wild type mothers. Juvenile mice reared by *Gabrd*$^{-/-}$ mice exhibit a decreased latency to immobility and an increase in the total time spent immobile in the forced swim test compared to juvenile mice reared by wild type mothers (Table 1; Figures 1(d) and 1(e)) (n = 10–12 mice per experimental group; ∗ denotes $p < 0.05$ using a one-way ANOVA with Tukey's multiple comparisons test; latency: $F(3, 39) = 4.386$; total time: $F(3, 39) = 12.61$). These data demonstrate that maternal depression-like behaviors in *Gabrd*$^{-/-}$ mice during the postpartum period negatively impact offspring development and validate the use of this model for investigating the mechanisms mediating the negative impact of maternal depression on offspring development.

3.2. Role of Stress in Abnormal Maternal and Postpartum Behaviors. We proposed that the abnormal postpartum behaviors in *Gabrd*$^{-/-}$ mice may be associated with altered stress reactivity during the postpartum period. Postpartum *Gabrd*$^{-/-}$ mice exhibit an increase in corticosterone levels following acute CO_2 stress (384.6 ± 27.6 ng/mL) compared to postpartum wild type mice (81.0 ± 10.4 ng/mL), virgin wild type mice (241.0 ± 45.3 ng/mL), or virgin *Gabrd*$^{-/-}$ mice (225.6 ± 32.7 ng/mL) (Figure 2). However, there is no significant difference in circulating corticosterone levels between unstressed postpartum *Gabrd*$^{-/-}$ mice (20.5 ± 4.5 ng/mL), postpartum wild type mice (16.8 ± 4.1 ng/mL), virgin *Gabrd*$^{-/-}$ mice (59.9 ± 10.6 ng/mL), or virgin wild type mice (30.7 ± 5.3 ng/mL) (Figure 2) (n = 7–17 mice per experimental group; ∗ denotes $p < 0.05$ using a one-way ANOVA with Tukey's multiple comparisons test; $F(8, 93) = 20.94$).

If altered stress reactivity plays a role in maternal depression-like behaviors in *Gabrd*$^{-/-}$ mice, then we hypothesized that chronic stress would be sufficient to induce the same behavioral disturbances in wild type mice during the postpartum period. Wild type mice subjected to unpredictable stress (US) from D14 to D21 of pregnancy (Figure 3(a)) exhibit a decrease in the survival rate of their pups compared to unstressed controls (Table 1; Figure 3(b)) (n: wild type = 12 mothers, 100 pups; wild type US = 9 mothers, 73 pups; ∗ denotes $p < 0.05$ using Student's t-test). Decreased pup survival is exacerbated in *Gabrd*$^{-/-}$

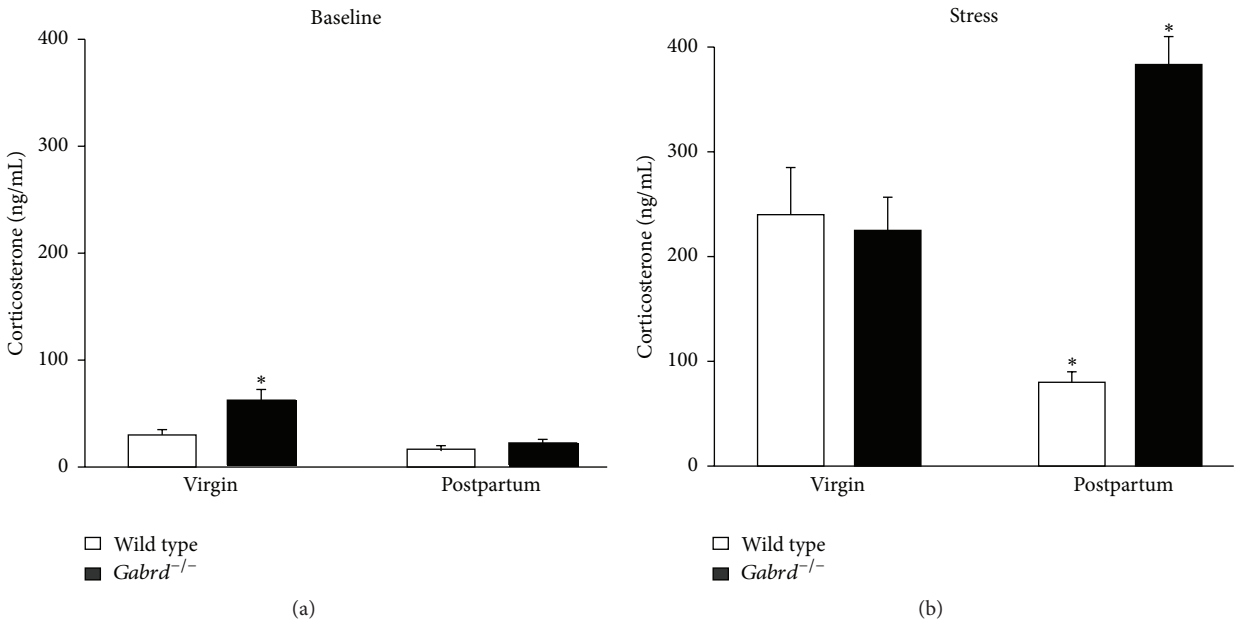
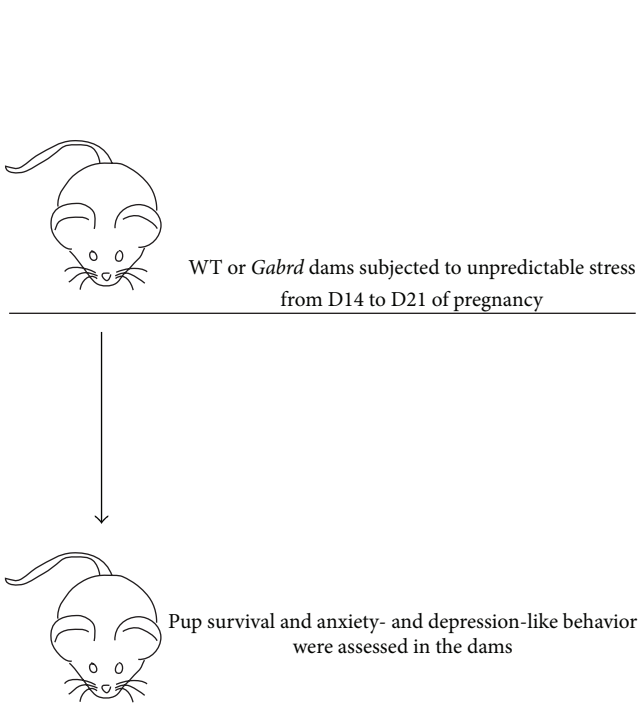


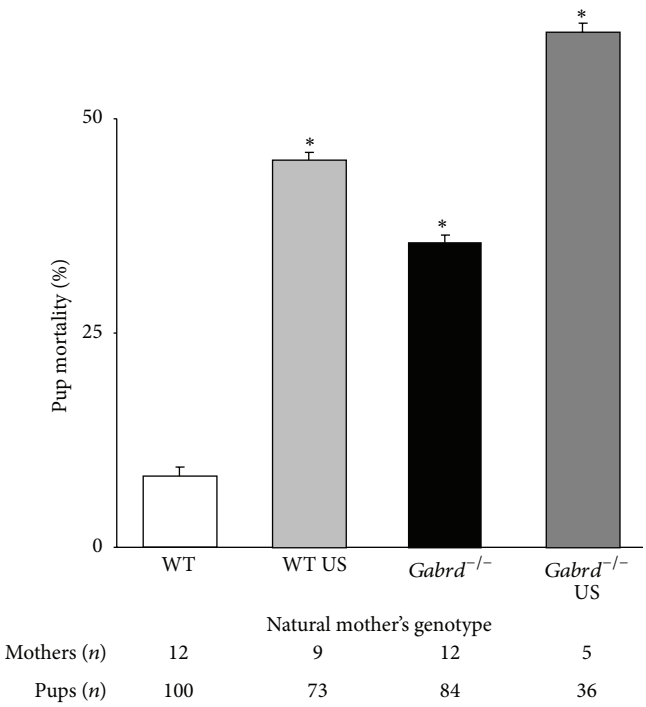
Figure 2: HPA axis hyperexcitability in postpartum $Gabrd^{-/-}$ mice. (a) Baseline corticosterone levels measured in the plasma of virgin and postpartum wild type and $Gabrd^{-/-}$ mice. (b) Average circulating corticosterone levels following CO_2 stress in virgin and postpartum wild type and $Gabrd^{-/-}$ mice. (n = 7–17 mice per experimental group; $*$ denotes $p < 0.05$ using one-way ANOVA with Tukey's multiple comparisons test).

mice subjected to US compared to unstressed $Gabrd^{-/-}$ mice (Table 1; Figure 3(b)) (n: $Gabrd^{-/-}$ = 12 mothers, 84 pups; $Gabrd^{-/-}$ US: 5 mothers, 36 pups; $*$ denotes $p < 0.05$ using Student's t-test). Dams subjected to US also fail to build a nest and keep the pups at an increased distance from the mother (data not shown), similar to $Gabrd^{-/-}$ mice [8]. These abnormal maternal behaviors in wild type mice subjected to the US paradigm are associated with depression-like behaviors in the dams (Figures 3(c)-3(d)). Wild type mice subjected to unpredictable stress exhibit a decreased latency to immobility and an increased total time spent immobile in the forced swim test at 48 hours postpartum compared to unstressed postpartum wild type mice (Table 1; Figures 3(c)-3(d)) (n = 5–9 for each experimental group; $*$ denotes $p < 0.05$ using a one-way ANOVA with Tukey's multiple comparisons test; latency: $F(3, 22) = 2.729$; total time: $F(3, 22) = 7.874$). These data are consistent with the hypothesis that altered stress reactivity plays a role in mediating abnormal postpartum behaviors.

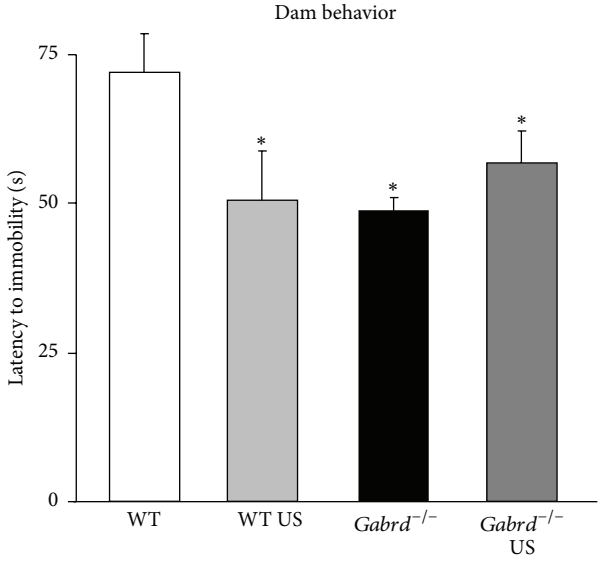
3.3. Role of Maternal Corticosterone on Offspring Behavior. To investigate whether altered stress reactivity in $Gabrd^{-/-}$ mice plays a role in mediating the negative impact of maternal depression-like behaviors on offspring development, we either sham-implanted wild type mice or implanted them with a slow-release 10 mg corticosterone pellet on day 14 of pregnancy and assessed offspring behavior at P21 (Figure 4(a)). We determined that corticosterone treatment does not interfere with pup delivery or litter size (7.3 ± 0.9 pups) compared to sham implanted mice (6.0 ± 0.8 pups) (n: sham = 9 mothers, 54 pups; CORT: 10 mothers, 73 pups; $*$ denotes $p < 0.05$ using Student's t-test). Note: litter sizes were determined at the time of delivery since there is a decreased survival rate of the pups born to corticosterone implanted mice (Figure 4(b)); however, all pups were alive at the time of delivery. Corticosterone levels are significantly elevated in the corticosterone implanted dams at 48 hours postpartum (192.8 ± 50.3 ng/mL) compared to sham implanted mice (29.8 ± 3.2 ng/mL), postpartum wild type controls (16.8 ± 4.1 ng/mL), or stressed postpartum wild type mice (81.0 ± 10.4 ng/mL) (n = 10–15 mice per experimental group; $*$ denotes $p < 0.05$ using Student's t-test). Corticosterone treatment in the mothers at D14 was sufficient to induce abnormal postpartum behaviors in wild type mice, such as inability to build a proper nest (data not shown) and an increase in pup mortality rate due to cannibalism or neglect (Table 1; Figure 4(b)), similar to that seen in $Gabrd^{-/-}$ mice [8] (n: sham = 9 mothers, 54 pups; CORT: 10 mothers, 73 pups; $*$ denotes $p < 0.05$ using Student's t-test). Corticosterone treatment in the mother was also sufficient to induce behavioral deficits in their juvenile offspring. Juvenile mice (P21) reared by mothers treated with corticosterone spent less time in the center squares of the open field test compared to juvenile mice reared by sham implanted wild type mothers (Table 1; Figure 4(c)) (n = 8 mice per experimental group, 2 mice per litter in 4 different litters; $*$ denotes $p < 0.05$ using Student's t-test), indicative of anxiety-like behavior. In addition, corticosterone treatment alters locomotor behavior in the offspring of corticosterone-treated mothers, evident from the increased number of lines crossed in the open field test compared to sham implanted mothers (Table 1; Figure 4(c)) (n = 8 mice per experimental



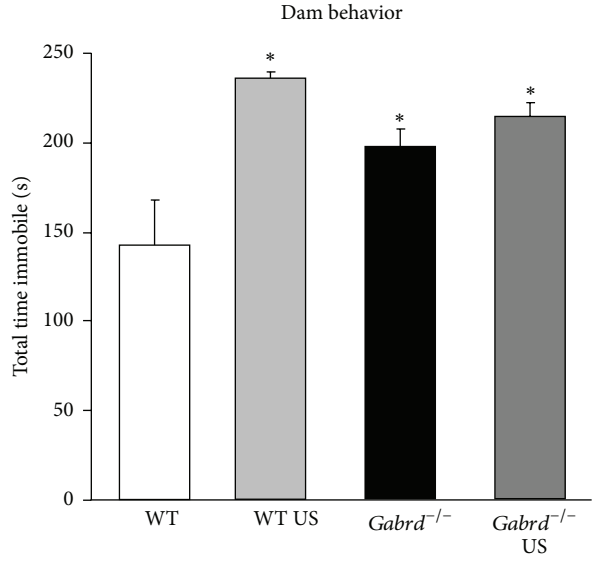
(a)



(b)



(c)



(d)

Figure 3: Unpredictable stress during pregnancy results in decreased pup survival and depression-like behavior in the dams. (a) A diagram outlining the experimental design. Wild type and *Gabrd*$^{-/-}$ dams were subjected to unpredictable stress from D14 to D21 of pregnancy and pup survival and depression-like behavior were assessed in the dams at 48 hrs postpartum. (b) Wild type and *Gabrd*$^{-/-}$ mice subjected to unpredictable stress from D14 to D21 of pregnancy exhibit a decrease in pup survival compared to control wild type and *Gabrd*$^{-/-}$ mice. *n*: wild type = 12 mothers, 100 pups; wild type US = 9 mothers, 73 pups; *Gabrd*$^{-/-}$ = 12 mothers, 84 pups; *Gabrd*$^{-/-}$ US = 5 mothers, 36 pups; $*$ denotes $p < 0.05$ using Student's *t*-test. Wild type and *Gabrd*$^{-/-}$ dams subjected to unpredictable stress from D14 to D21 of pregnancy exhibit a decreased latency to immobility (c) and an increased total time spent immobile (d) in the forced swim test at 48 hrs postpartum. $n = 5$–9 for each experimental group; $*$ denotes $p < 0.05$ using one-way ANOVA with Tukey's multiple comparisons test.

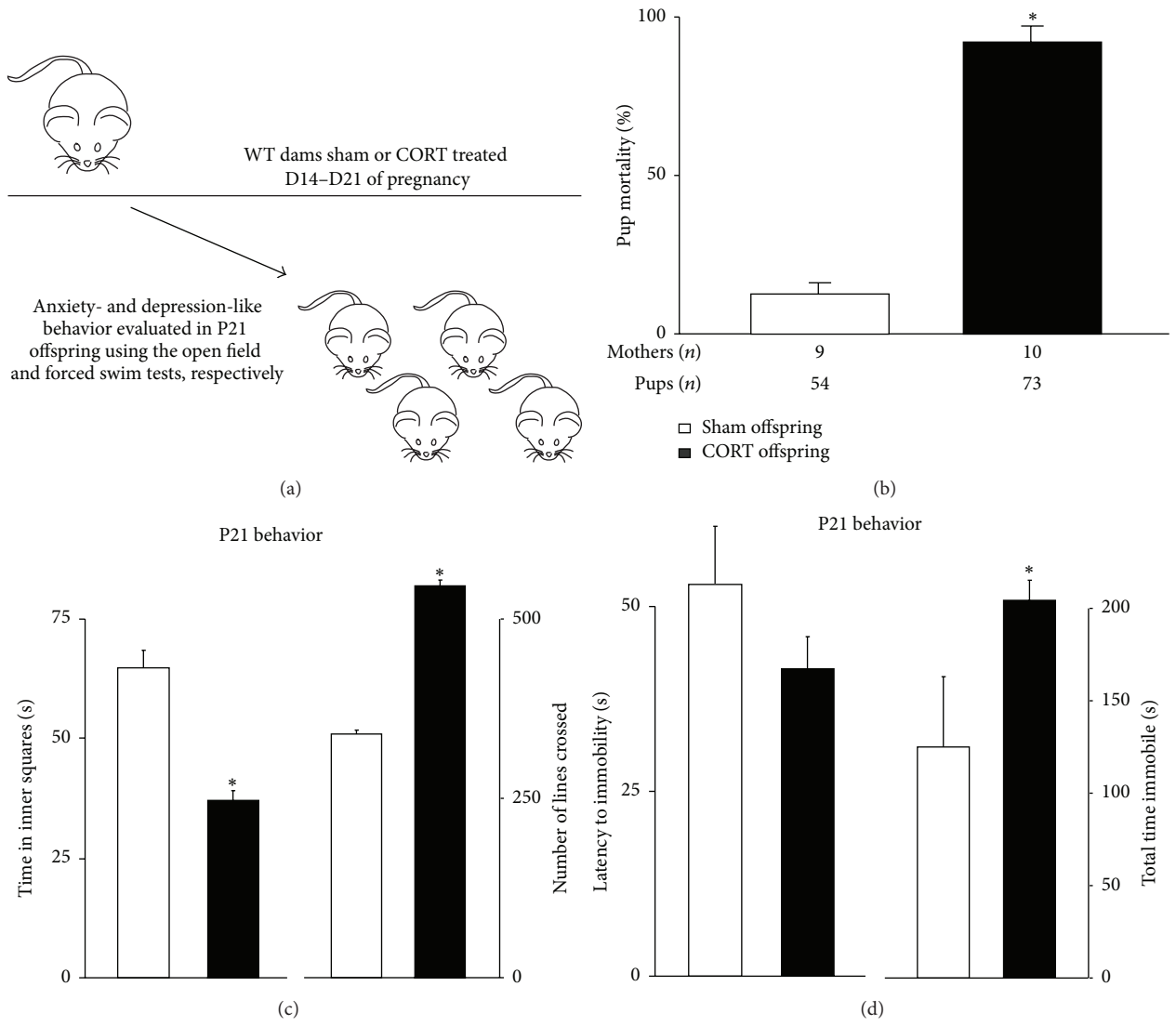


Figure 4: Corticosterone treatment in wild type mice during pregnancy decreases pup survival and induces deficits in offspring behavior. (a) A diagram outlining the experimental design. Wild type dams were implanted with a 10 mg, slow-release corticosterone pellet at D14 of pregnancy and pup survival and offspring behavior in the juveniles were assessed at P21. (b) Corticosterone-treated wild type mice exhibit a decrease in pup survival compared to sham implanted wild type mice. *n*: sham = 9 mothers, 54 pups; CORT: 10 mothers, 73 pups; ∗ denotes $p < 0.05$ using Student's *t*-test. (c-d) Juvenile offspring reared by wild type mothers implanted with a 10 mg, slow-release corticosterone pellet at D14 of pregnancy, exhibit anxiety-like and depression-like behaviors at P21. (c) Juvenile mice reared by corticosterone-treated mothers spent a decreased amount of time in the center of the open field test and an increase in locomotor activity. (d) Juvenile mice reared by corticosterone-treated mothers exhibited a decreased latency to immobility and an increase in the total time spent immobile in the forced swim test ($n = 8$ mice per experimental group; ∗ denotes $p < 0.05$ using Student's *t*-test).

group, 2 mice per litter in 4 different litters; ∗ denotes $p < 0.05$ using Student's *t*-test). Corticosterone treatment in wild type mothers also induced depression-like behavior in the offspring. Offspring reared by corticosterone implanted wild type mothers spend an increased total time immobile during the forced swim test compared to juvenile mice reared by sham implanted wild type mothers (Table 1; Figure 4(d)) ($n = 8$ mice per experimental group, 2 mice per litter in 4 different litters; ∗ denotes $p < 0.05$ using Student's *t*-test). These data support the hypothesis that stress hormones, specifically corticosterone, mediate the negative impact of maternal depression-like behaviors on offspring behavior in the mouse.

If altered stress reactivity in $Gabrd^{-/-}$ mothers plays a role in the impact of maternal depression-like behaviors on offspring development, we hypothesized that inhibiting the stress response in the mother with the corticotropin-releasing hormone (CRH) antagonist, Antalarmin, would decrease the anxiety- and depression-like behaviors in the juvenile offspring (Figure 5(a)). We did not observe any changes in litter size associated with Antalarmin treatment (7.6 ± 0.9 pups) compared to vehicle treatment (6.5 ± 0.8

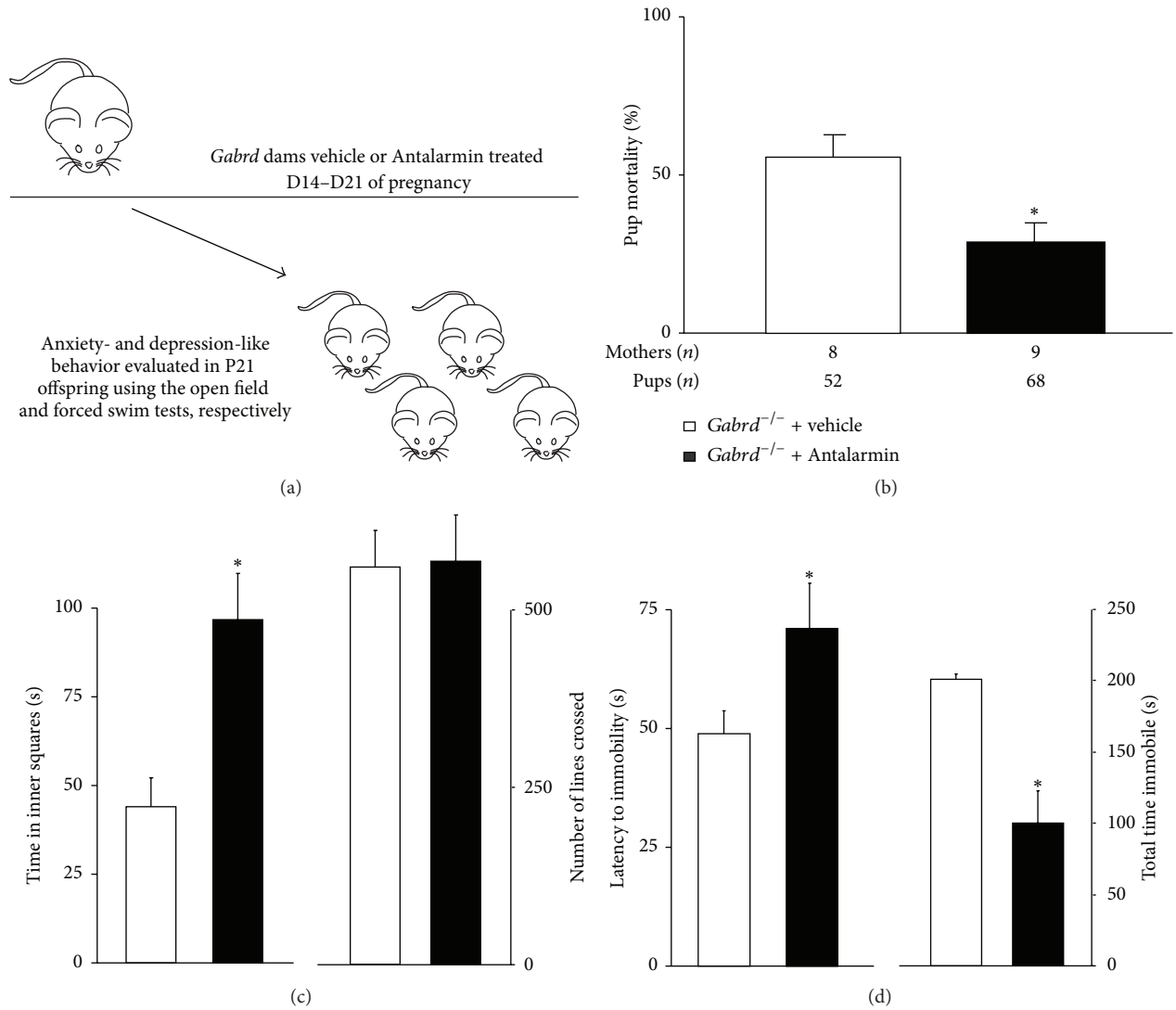


FIGURE 5: Blocking CRH signaling in $Gabrd^{-/-}$ mice during pregnancy increased pup survival and diminished the negative impact on offspring behavior. (a) A diagram outlining the experimental design. $Gabrd^{-/-}$ dams were treated with Antalarmin from D14 to D21 of pregnancy and pup survival and offspring behavior in the juveniles were assessed at P21. (b) Antalarmin-treated $Gabrd^{-/-}$ mothers exhibit an increase in pup survival compared to vehicle-treated controls. *n*: $Gabrd^{-/-}$ vehicle = 8 mothers, 52 pups; $Gabrd^{-/-}$ + Antalarmin: 9 mothers, 68 pups; significance was determined as $p < 0.05$ using Student's *t*-test. (c-d) Juvenile mice reared by $Gabrd^{-/-}$ mothers treated with Antalarmin exhibit a decrease in anxiety-like and depression-like behavior at P21. (c) Offspring of Antalarmin-treated $Gabrd^{-/-}$ mothers spend an increased amount of time in the center of the open field with no change in activity compared to vehicle-treated controls. (d) Offspring of Antalarmin-treated $Gabrd^{-/-}$ mothers exhibited an increased latency to immobility and a decreased total time spent immobile in the forced swim test (n = 8–10 mice per experimental group; $*$ denotes $p < 0.05$ using Student's *t*-test).

pups). This dose of Antalarmin was sufficient to decrease the stress-induced circulating corticosterone levels in postpartum $Gabrd^{-/-}$ mice (101.5 ± 12.0 ng/mL) to levels similar to postpartum wild type mice (81.0 ± 10.4 ng/mL) which is significantly lower than the stress-induced levels in postpartum $Gabrd^{-/-}$ mice (384.6 ± 27.6 ng/mL). Antalarmin treatment ameliorated the abnormal postpartum behaviors in postpartum $Gabrd^{-/-}$ mice. $Gabrd^{-/-}$ dams treated with Antalarmin exhibit an increase in pup survival compared to controls (Table 1; Figure 5(b)) (n: $Gabrd^{-/-}$ vehicle = 8 mothers, 52 pups; $Gabrd^{-/-}$ + Antalarmin: 9 mothers, 68 pups; significance was determined as $p < 0.05$ using Student's *t*-test). Further, Antalarmin treatment in the mother was also sufficient to ameliorate the mood disorders in juvenile mice reared by $Gabrd^{-/-}$ mothers. Juvenile mice (P21) reared by $Gabrd^{-/-}$ mothers treated with Antalarmin spent more time in the center squares of the open field test, which is indicative of decreased anxiety levels, compared to juvenile mice reared by vehicle-treated $Gabrd^{-/-}$ mothers (Table 1; Figure 5(c)) (n = 8–10 offspring per experimental group,

2 mice per litter in 4-5 different litters; significance was determined as $p < 0.05$ using Student's t-test). There was no significant difference in the number of lines crossed in the open field test between the offspring of Antalarmin-treated and vehicle-treated *Gabrd*$^{-/-}$ mothers (Table 1; Figure 5(c)). Similarly, offspring reared by Antalarmin-treated *Gabrd*$^{-/-}$ mothers exhibit decreased depression-like behavior, evident by an increased latency to immobility and decreased total time spent immobile compared to offspring reared by vehicle-treated *Gabrd*$^{-/-}$ mothers (Table 1; Figure 5(d)) ($n = 8$–10 mice per experimental group, 2 mice per litter in 4-5 different litters; significance was determined as $p < 0.05$ using Student's t-test). These data demonstrate that inhibiting the stress response, such as with the CRH antagonist, Antalarmin, is therapeutic in ameliorating the abnormal postpartum behaviors in *Gabrd*$^{-/-}$ mothers as well as preventing the negative impact of maternal depression-like behaviors on offspring development.

4. Discussion

This study highlights the utility of a unique mouse model to investigate the underlying mechanisms mediating the pathophysiology of postpartum depression and the mechanism(s) through which postpartum depression negatively impacts offspring development. It is generally accepted that both genetic and environmental factors play a role in the pathophysiology of postpartum depression. However, either the current animal models exhibit a genetic predisposition for depression-like behavior or the behavior is environmentally induced [37]. Here we describe a genetic mouse model exhibiting depression-like behavior that is restricted to the postpartum period [8], which is aggravated by environmental stress similar to the human condition [38]. This is the first genetic mouse model which exhibits a predisposition to postpartum depression-like behavior in which there is also an environmental component. Therefore, we feel that this is a useful model for studying the mechanisms mediating postpartum depression-like behavior and the accompanying deficits in offspring development.

Clearly, hormone changes throughout pregnancy and the postpartum period trigger the onset of postpartum depression. However, gonadal hormone levels do not appear to be significantly altered in women with postpartum depression [39–41], suggesting that women must be predisposed to the disorder. During pregnancy, levels of estrogen and progesterone steadily increase due to placental production of these hormones, which decrease abruptly with the removal of the placenta. However, no change in estrogen or progesterone levels has consistently been shown to be associated with postpartum depression [41]. There are numerous other hormonal changes that occur during pregnancy, including changes in oxytocin, prolactin, and cortisol levels (for review see [42]). However, no alterations out of the physiological range were found for prolactin [41, 43], oxytocin, or vasopressin [44] associated with postpartum depression. Hypercortisolism has been suggested to play a role in the pathophysiology of postpartum depression [45], since major depression is also associated with hypercortisolism [46]. Normally, the stress-induced activation of the HPA axis is suppressed during pregnancy ([47], for review see [12]), consistent with our observations in postpartum wild type mice (Figure 2). Altered levels of cortisol [11, 18, 48], ACTH [18], and CRH [19] have been associated with postpartum depression. Researchers have gone so far as to say that elevated CRH levels may be used as a diagnostic criterion for postpartum depression [19]. However, other studies have failed to reproduce these results ([41, 43], for review see [42]). Here we demonstrate hyperresponsivity of the HPA axis associated with depression-like behaviors during the postpartum period in a mouse model, similar to what has been observed in women with postpartum depression [49].

Consistent with a role for HPA axis hyperresponsiveness in postpartum depression-like behaviors, this study supports a role for elevated corticosterone in the pathophysiology of postpartum mood disorders, since physiological stress is sufficient to induce depression-like behavior during the postpartum period in mice (Figure 3). Previous studies have demonstrated that corticosterone alters maternal care and induces postpartum depression-like behaviors in the dams [50]. Here we demonstrate that exogenous corticosterone treatment in wild type mice during pregnancy results in a robust decrease in pup survival (Figure 4). This has previously been observed [51], albeit not to the same extent as in the current study. The discrepancy may be due to a prolonged exposure in our study to levels of corticosterone normally found in stress. However, we cannot rule out potential abnormalities in the pups due to corticosterone exposure which may impact pup mortality. Interestingly, our study demonstrates that physiological stress in the dams is sufficient to increase pup mortality in both wild type and *Gabrd*$^{-/-}$ mice (Figure 3(b)). However, unpredictable stress does not alter depression-like behaviors in postpartum *Gabrd*$^{-/-}$ although it increases depression-like behaviors in wild type mice (Figures 3(c) and 3(d)). We interpret these data to indicate that physiological stress is incapable of altering depression-like behaviors in postpartum *Gabrd*$^{-/-}$ mice in which corticosterone levels are already elevated, demonstrating a potential ceiling effect. Similarly, our results demonstrate that the CRH antagonist, Antalarmin, increases pup survival in *Gabrd*$^{-/-}$ mice (Figure 5). These data demonstrate a direct role of stress hormones in postpartum mood disorders and may contribute to the negative impact of maternal depression on offspring development.

Children exposed to mothers with postpartum depression exhibit deficits in cognitive development, motor, and emotional development (for review see [52]). Many mechanisms have been proposed to mediate the negative association between maternal depression and offspring development, including environmental and genetic components. It is also possible that there is a direct, biochemical component of maternal depression which impacts offspring development. Deficits in child development associated with maternal depression are correlated with elevated cortisol levels in the mother [53, 54], suggesting that the stress hormones may play a role in the negative impact of maternal depression on child

development. Consistent with this theory, we demonstrate behavioral deficits in mice reared by *Gabrd*$^{-/-}$ mothers, which exhibit hyperresponsiveness of the HPA axis, and offspring reared by wild type mice subjected to US or treated with exogenous corticosterone. Further, corticosterone treatment in dams has previously been shown to result in adverse behavioral effects in the offspring [22], which could be a direct effect of corticosterone levels in the offspring which may alter subsequent HPA axis activity [55]. These data support a direct role of stress hormones in both the pathophysiology of postpartum depression and the negative impact of maternal depression on offspring development.

In the current study, the mouse models which exhibit abnormal postpartum behaviors and a negative impact offspring development, including *Gabrd*$^{-/-}$ mice and wild type dams subjected to unpredictable stress or treated with exogenous corticosterone, exhibit a high degree of pup mortality due to cannibalism and/or neglect. This is in contrast to the human condition of postpartum depression which is not typically associated with infant mortality or infanticide. Neonaticide and infanticide are more commonly associated with postpartum psychosis [56]. It is possible that postpartum *Gabrd*$^{-/-}$ mice more accurately model postpartum psychosis. However, this is difficult to assess in mice and requires further study.

Due to the high level of pup mortality in the mouse models exhibiting abnormal postpartum behaviors and a negative impact offspring development, including *Gabrd*$^{-/-}$ mice and wild type dams subjected to unpredictable stress or treated with exogenous corticosterone, we cannot rule out both the impact of changing litter size on maternal behaviors and the impact on offspring development. Relevant to the current study, smaller litter size is associated with more directed maternal care [57, 58]. Further, mice reared in smaller litters also exhibit decreased anxiety-like behaviors during adulthood [58]. These findings are in contrast to the current study where we observe a decreased litter size in *Gabrd*$^{-/-}$ litters associated with abnormal maternal care [8] and increased anxiety- and depression-like behaviors in the offspring reared by *Gabrd*$^{-/-}$ mice (Figure 1). Thus, it does not appear that litter size impacts the findings in the current study.

This study directly demonstrates the negative impact of postpartum depression-like behavior and deficits in maternal care on offspring behavior. Further, our data suggest a role for HPA axis dysfunction in mediating the negative impact of maternal mood on offspring behavior.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by Grants from the NIH/NIMH MH076994 (Istvan Mody), NIH/NINDS 073574 (Jamie Maguire), and the Charles H. Hood Foundation (Jamie Maguire). The investigators would like to thank Reyes Main Lazaro for his expertise in the timed pregnancies and the blood sample collections. His assistance on this project was greatly appreciated. The authors would also like to acknowledge the technical contribution of Isabella Ferando and Charlotte Simonsen on this study.

References

[1] R. Feldman, A. Granat, C. Pariente, H. Kanety, J. Kuint, and E. Gilboa-Schechtman, "Maternal depression and anxiety across the postpartum year and infant social engagement, fear regulation, and stress reactivity," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 48, no. 9, pp. 919–927, 2009.

[2] S. L. Grace, A. Evindar, and D. E. Stewart, "The effect of postpartum depression on child cognitive development and behavior: a review and critical analysis of the literature," *Archives of Women's Mental Health*, vol. 6, no. 4, pp. 263–274, 2003.

[3] K. Lyons-Ruth, D. Zoll, D. Connell, and H. U. Grunebaum, "The depressed mother and her one-year-old infant: environment, interaction, attachment, and infant development," *New Directions for Child Development*, no. 34, pp. 61–82, 1986.

[4] L. Murray, "The impact of postnatal depression on infant development," *Journal of Child Psychology and Psychiatry and Allied Disciplines*, vol. 33, no. 3, pp. 543–561, 1992.

[5] L. Murray, A. Fiori-Cowley, R. Hooper, and P. Cooper, "The impact of postnatal depression and associated adversity on early mother-infant interactions and later infant outcome," *Child Development*, vol. 67, no. 5, pp. 2512–2526, 1996.

[6] L. Murray, D. Sinclair, P. Cooper, P. Ducournau, P. Turner, and A. Stein, "The socioemotional development of 5-year-old children of postnatally depressed mothers," *Journal of Child Psychology and Psychiatry*, vol. 40, no. 8, pp. 1259–1271, 1999.

[7] M. Righetti-Veltema, A. Bousquet, and J. Manzano, "Impact of postpartum depressive symptoms on mother and her 18-month-old infant," *European Child and Adolescent Psychiatry*, vol. 12, no. 2, pp. 75–83, 2003.

[8] J. Maguire and I. Mody, "$GABA_AR$ plasticity during pregnancy: relevance to postpartum depression," *Neuron*, vol. 59, no. 2, pp. 207–213, 2008.

[9] R. Gitau, A. Cameron, N. M. Fisk, and V. Glover, "Fetal exposure to maternal cortisol," *The Lancet*, vol. 352, no. 9129, pp. 707–708, 1998.

[10] D. Owen, M. H. Andrews, and S. G. Matthews, "Maternal adversity, glucocorticoids and programming of neuroendocrine function and behaviour," *Neuroscience & Biobehavioral Reviews*, vol. 29, pp. 209–226, 2005.

[11] M. Bloch, R. C. Daly, and D. R. Rubinow, "Endocrine factors in the etiology of postpartum depression," *Comprehensive Psychiatry*, vol. 44, no. 3, pp. 234–246, 2003.

[12] D. A. Slattery and I. D. Neumann, "No stress please! Mechanisms of stress hyporesponsiveness of the maternal brain," *The Journal of Physiology*, vol. 586, no. 2, pp. 377–385, 2008.

[13] P. J. Brunton and J. A. Russell, "Attenuated hypothalamo-pituitary-adrenal axis responses to immune challenge during pregnancy: the neurosteroid-opioid connection," *Journal of Physiology*, vol. 586, no. 2, pp. 369–375, 2008.

[14] P. J. Brunton, J. A. Russell, and A. J. Douglas, "Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis

during pregnancy and lactation," *Journal of Neuroendocrinology*, vol. 20, no. 6, pp. 764–776, 2008.

[15] S. L. Handley, T. L. Dunn, G. Waldron, and J. M. Baker, "Tryptophan, cortisol and puerperal mood," *British Journal of Psychiatry*, vol. 136, no. 5, pp. 498–508, 1980.

[16] A. Feksi, B. Harris, R. F. Walker, D. Riad-Fahmy, and R. G. Newcombe, "'Maternity blues' and hormone levels in saliva," *Journal of Affective Disorders*, vol. 6, no. 3-4, pp. 351–355, 1984.

[17] V. Kuevi, R. Causon, A. F. Dixson et al., "Plasma amine and hormone changes in 'post-partum blues,'" *Clinical Endocrinology*, vol. 19, no. 1, pp. 39–46, 1983.

[18] M.-A. Magiakou, G. Mastorakos, D. Rabin, B. Dubbert, P. W. Gold, and G. P. Chrousos, "Hypothalamic corticotropin-releasing hormone suppression during the postpartum period: implications for the increase in psychiatric manifestations at this time," *Journal of Clinical Endocrinology and Metabolism*, vol. 81, no. 5, pp. 1912–1917, 1996.

[19] I. S. Yim, L. M. Glynn, C. D. Schetter, C. J. Hobel, A. Chicz-DeMet, and C. A. Sandman, "Risk of postpartum depressive symptoms with elevated corticotropin—releasing hormone in human pregnancy," *Archives of General Psychiatry*, vol. 66, no. 2, pp. 162–169, 2009.

[20] P. A. Brennan, R. Pargas, E. F. Walker, P. Green, D. J. Newport, and Z. Stowe, "Maternal depression and infant cortisol: influences of timing, comorbidity and treatment," *Journal of Child Psychology and Psychiatry*, vol. 49, no. 10, pp. 1099–1107, 2008.

[21] K. Priebe, R. D. Romeo, D. D. Francis et al., "Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: a cross-fostering study," *Developmental Psychobiology*, vol. 47, pp. 398–407, 2005.

[22] S. Brummelte, S. E. Lieblich, and L. A. M. Galea, "Gestational and postpartum corticosterone exposure to the dam affects behavioral and endocrine outcome of the offspring in a sexually-dimorphic manner," *Neuropharmacology*, vol. 62, no. 1, pp. 406–418, 2012.

[23] W. E. Cullinan, D. R. Ziegler, and J. P. Herman, "Functional role of local GABAergic influences on the HPA axis," *Brain Structure and Function*, vol. 213, no. 1-2, pp. 63–72, 2008.

[24] J. P. Herman and W. E. Cullinan, "Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis," *Trends in Neurosciences*, vol. 20, no. 2, pp. 78–84, 1997.

[25] J. P. Herman, N. K. Mueller, and H. Figueiredo, "Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration," *Annals of the New York Academy of Sciences*, vol. 1018, pp. 35–45, 2004.

[26] J. P. Herman, J. G. Tasker, D. R. Ziegler, and W. E. Cullinan, "Local circuit regulation of paraventricular nucleus stress integration: glutamate-GABA connections," *Pharmacology Biochemistry and Behavior*, vol. 71, no. 3, pp. 457–468, 2002.

[27] J. Sarkar, S. Wakefield, G. MacKenzie, S. J. Moss, and J. Maguire, "Neurosteroidogenesis is required for the physiological response to stress: role of neurosteroid-sensitive GABA A receptors," *The Journal of Neuroscience*, vol. 31, no. 50, pp. 18198–18210, 2011.

[28] B. G. Gunn, L. Cunningham, M. A. Cooper et al., "Dysfunctional astrocytic and synaptic regulation of hypothalamic glutamatergic transmission in a mouse model of early-life adversity: relevance to neurosteroids and programming of the stress response," *Journal of Neuroscience*, vol. 33, no. 50, pp. 19534–19554, 2013.

[29] D. Misdrahi, M. C. Pardon, F. Pérez-Diaz, N. Hanoun, and C. Cohen-Salmon, "Prepartum chronic ultramild stress increases corticosterone and estradiol levels in gestating mice: implications for postpartum depressive disorders," *Psychiatry Research*, vol. 137, no. 1-2, pp. 123–130, 2005.

[30] M.-C. Pardon, P. Gérardin, C. Joubert, F. Pérez-Diaz, and C. Cohen-Salmon, "Influence of prepartum chronic ultramild stress on maternal pup care behavior in mice," *Biological Psychiatry*, vol. 47, no. 10, pp. 858–863, 2000.

[31] R. D. Porsolt, A. Bertin, and M. Jalfre, "Behavioral despair in mice: a primary screening test for antidepressants," *Archives Internationales de Pharmacodynamie et de Therapie*, vol. 229, no. 2, pp. 327–336, 1977.

[32] M. L. Barbaccia, G. Roscetti, M. Trabucchi et al., "Time-dependent changes in rat brain neuroactive steroid concentrations and GABA(A) receptor function after acute stress," *Neuroendocrinology*, vol. 63, no. 2, pp. 166–172, 1996.

[33] J. Maguire and I. Mody, "Neurosteroid synthesis-mediated regulation of GABAA receptors: relevance to the ovarian cycle and stress," *The Journal of Neuroscience*, vol. 27, no. 9, pp. 2155–2162, 2007.

[34] V. Lee, J. Sarkar, and J. Maguire, "Loss of Gabrd in CRH neurons blunts the corticosterone response to stress and diminishes stress-related behaviors," *Psychoneuroendocrinology*, vol. 41, pp. 75–88, 2014.

[35] S. Miller and J. Maguire, "Deficits in KCC2 and activation of the HPA axis lead to depressionlike behavior following social defeat," *Hormonal Studies*, vol. 2, no. 1, 2014.

[36] K. K. O'Toole, A. Hooper, S. Wakefield, and J. Maguire, "Seizure-induced disinhibition of the HPA axis increases seizure susceptibility," *Epilepsy Research*, vol. 108, no. 1, pp. 29–43, 2014.

[37] E. J. Nestler, E. Gould, H. Manji et al., "Preclinical models: status of basic research in depression," *Biological Psychiatry*, vol. 52, pp. 503–528, 2002.

[38] J. W. Rich-Edwards, K. Kleinman, A. Abrams et al., "Sociodemographic predictors of antenatal and postpartum depressive symptoms among women in a medical group practice," *Journal of Epidemiology and Community Health*, vol. 60, no. 3, pp. 221–227, 2006.

[39] C. B. Ballinger, M. C. K. Browning, and A. H. W. Smith, "Hormone profiles and psychological symptoms in peri-menopausal women," *Maturitas*, vol. 9, no. 3, pp. 235–251, 1987.

[40] A. Heidrich, M. Schleyer, H. Spingler et al., "Postpartum blues: relationship between not-protein bound steroid hormones in plasma and postpartum mood changes," *Journal of Affective Disorders*, vol. 30, no. 2, pp. 93–98, 1994.

[41] M. W. O'Hara, J. A. Schlechte, D. A. Lewis, and M. W. Varner, "Controlled prospective study of postpartum mood disorders: psychological, environmental, and hormonal variables," *Journal of Abnormal Psychology*, vol. 100, no. 1, pp. 63–73, 1991.

[42] V. Hendrick, L. L. Altshuler, and R. Suri, "Hormonal changes in the postpartum and implications for postpartum depression," *Psychosomatics*, vol. 39, no. 2, pp. 93–101, 1998.

[43] B. Harris, S. Johns, H. Fung et al., "The hormonal environment of post-natal depression," *British Journal of Psychiatry*, vol. 154, pp. 660–667, 1989.

[44] G. Stein, J. Morton, A. Marsh, J. Hartshorn, J. Ebeling, and U. Desaga, "Vasopressin and mood during the puerperium," *Biological Psychiatry*, vol. 19, no. 12, pp. 1711–1717, 1984.

[45] G. P. Chrousos, D. J. Torpy, and P. W. Gold, "Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications," *Annals of Internal Medicine*, vol. 129, no. 3, pp. 229–240, 1998.

[46] B. J. Carroll, F. Cassidy, D. Naftolowitz et al., "Pathophysiology of hypercortisolism in depression," *Acta Psychiatrica Scandinavica*, vol. 115, no. 433, pp. 90–103, 2007.

[47] A. J. Douglas, S. L. Meddle, N. Toschi, O. J. Bosch, and I. D. Neumann, "Reduced activity of the noradrenergic system in the paraventricular nucleus at the end of pregnancy: implications for stress hyporesponsiveness," *Journal of Neuroendocrinology*, vol. 17, no. 1, pp. 40–48, 2005.

[48] Z. N. Stowe and C. B. Nemeroff, "Women at risk for postpartum-onset major depression," *American Journal of Obstetrics and Gynecology*, vol. 173, no. 2, pp. 639–645, 1995.

[49] L. K. Takahashi, J. G. Turner, and N. H. Kalin, "Prolonged stress-induced elevation in plasma corticosterone during pregnancy in the rat: implications for prenatal stress studies," *Psychoneuroendocrinology*, vol. 23, no. 6, pp. 571–581, 1998.

[50] S. Brummelte and L. A. M. Galea, "Chronic corticosterone during pregnancy and postpartum affects maternal care, cell proliferation and depressive-like behavior in the dam," *Hormones and Behavior*, vol. 58, no. 5, pp. 769–779, 2010.

[51] H. Zahwa, J. L. Yorty, and R. H. Bonneau, "Elevated maternal corticosterone during lactation hinders the neonatal adaptive immune response to herpes simplex virus (HSV) infection," *Brain, Behavior, and Immunity*, vol. 22, no. 3, pp. 339–353, 2008.

[52] L. Murray and P. J. Cooper, "Effects of postnatal depression on infant development," *Archives of Disease in Childhood*, vol. 77, no. 2, pp. 99–101, 1997.

[53] C. de Weerth, Y. van Hees, and J. K. Buitelaar, "Prenatal maternal cortisol levels and infant behavior during the first 5 months," *Early Human Development*, vol. 74, no. 2, pp. 139–151, 2003.

[54] S. L. Halligan, J. Herbert, I. M. Goodyer, and L. Murray, "Exposure to postnatal depression predicts elevated cortisol in adolescent offspring," *Biological Psychiatry*, vol. 55, no. 4, pp. 376–381, 2004.

[55] S. Brummelte, K. L. Schmidt, M. D. Taves, K. K. Soma, and L. A. M. Galea, "Elevated corticosterone levels in stomach milk, serum, and brain of male and female offspring after maternal corticosterone treatment in the rat," *Developmental Neurobiology*, vol. 70, no. 10, pp. 714–725, 2010.

[56] T. Pearlstein, M. Howard, A. Salisbury, and C. Zlotnick, "Postpartum depression," *American Journal of Obstetrics and Gynecology*, vol. 200, no. 4, pp. 357–364, 2009.

[57] R. Priestnall, "Effects of litter size on the behaviour of lactating female mice (*Mus musculus*)," *Animal Behaviour*, vol. 20, no. 2, pp. 386–394, 1972.

[58] P. F. D. Seitz, "The effects of infantile experiences upon adult behavior in animal subjects: I. Effects of litter size during infancy upon adult behavior in the rat," *The American Journal of Psychiatry*, vol. 110, no. 12, pp. 916–927, 1954.