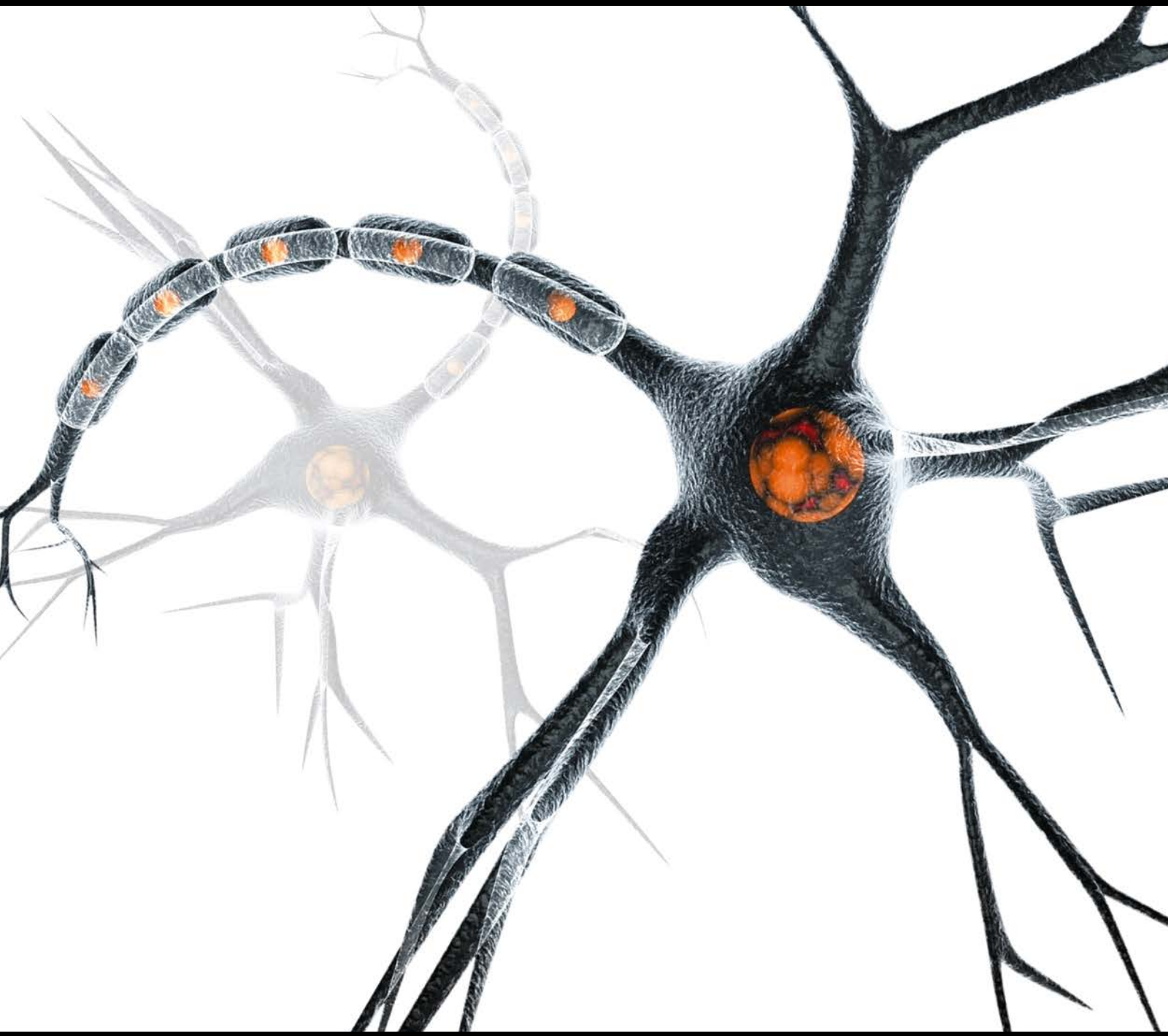
Neural Plasticity

Gene-Environment Interactions in Neurodevelopmental Disorders

Guest Editors: Susanna Pietropaolo, Wim E. Crusio, and Joram Feldon



Gene-Environment Interactions in Neurodevelopmental Disorders

Neural Plasticity

Gene-Environment Interactions in Neurodevelopmental Disorders

Guest Editors: Susanna Pietropaolo, Wim E. Crusio, and Joram Feldon

Editorial Board

Contents

Hindawi Publishing Corporation
Neural Plasticity
Volume 2017, Article ID 9272804, 2 pages
http://dx.doi.org/10.1155/2017/9272804

Editorial

Gene-Environment Interactions in Neurodevelopmental Disorders

Susanna Pietropaolo,[1,2] Wim E. Crusio,[1,2] and Joram Feldon[3]

[1] *University of Bordeaux, INCIA, Pessac Cedex, France*
[2] *CNRS, INCIA, UMR 5287, Pessac Cedex, France*
[3] *Department of Biology, The Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*

Correspondence should be addressed to Susanna Pietropaolo; susanna.pietropaolo@u-bordeaux.fr

Received 8 December 2016; Accepted 12 December 2016; Published 31 January 2017

Neurodevelopmental disorders (NDDs) include a huge variety of pathologies characterised by varying degrees of intellectual disability and behavioural dysfunction. Research during the last years has underlined the genetic nature of the aetiological factors involved in most NDDs, identifying in some cases (e.g., Fragile X and Rett syndromes) single gene mutations as the unique pathological cause, while determining in others multiple genetic risk factors (e.g., schizophrenia, autism spectrum disorders (ASDs)). The high interindividual variability in several key pathological aspects of NDDs, such as the severity of the behavioural symptoms and the age-related progression, has motivated researchers to direct their attention to environmental factors that may critically influence the expression of the genetic "determinants" of these pathologies. This research has led to several theoretical models describing the relationships between genetic and environmental insults in NDDs: these models have in turn emphasised the additive or synergistic interactions between genes and environment and increased the interest in better understanding the specific contributions of these interactions in the aetiopathology of NDDs.

Gene-environment interactions are obviously of relevance to most disorders of the nervous system but are especially important for developmental pathologies, because of the considerable plasticity of the developing brain and its critical responsiveness to environmental changes. Indeed, a large body of human and animal data has demonstrated that environmental stimulation/deprivation can, respectively, ameliorate or exacerbate the symptoms of many NDDs. Nonetheless, animal studies combining both genetic and environmental manipulations are still scarce, at least compared to the huge amount of research work that concentrates only on genetic effects. This special issue aims to attract attention to the importance of gene-environment interactions, which so far have often been ignored. Review and original research articles are combined to discuss the impact of the interactions between genetic and environmental interventions in both clinical and preclinical studies. A variety of NDDs are included, such as ASDs, schizophrenia, Fragile X, Down syndrome, and ADHD, and a multitude of genetic and environmental manipulations are discussed. Several environmental factors were studied, such as nutritional manipulations, immunological changes, environmental enrichment, stress, or social deprivation. These sometimes implied environmental adversity, but in other cases environmental stimulation, possibly supporting nonpharmacological therapies based on sensory-social or nutritional enrichment.

The review by C. Madore et al. investigates the relevance of nutritional factors in the aetiopathology of neurodevelopmental disorders. This article is focused in particular on the role of polyunsaturated fatty acids (PUFAs), because of the association between imbalances in PUFA levels and NDDs. Preclinical and clinical data are summarised, highlighting the anti-inflammatory properties of PUFAs and their impact on the microbiota, which is suggested as the main factor potentially linking inflammation, environmental adversity, and NDDs. P. Moran et al. instead provide a review of preclinical studies on gene-environment interactions in

schizophrenia using genetic animal models. This review discusses the synergistic effects of genetic and environmental risk factors on the expression of those endophenotypes that are relevant to schizophrenia, thus supporting the validity of multifactorial preclinical models.

The research article by E. Aronoff et al. provides original clinical data on the therapeutic impact of environmental enrichment in autistic children. Indeed, a therapy based on daily sensory enrichment is shown to ameliorate children's behavioural problems in multiple domains, including cognitive, emotional, social, and sensorial abilities. Interestingly, these effects were largely independent of the subjects' gender, nationality, or initial severity levels of the pathology. The article by L. Garbugino et al. parallels this human study, providing original data on the effects of early enrichment in an animal model of social dysfunction, the KO mouse for the μ-opioid receptor gene (*Oprm1*$^{-/-}$). Early environmental stimulation of the highly immature mouse pups using an enrichment protocol providing additional maternal care was applied and both short- and long-term behavioural effects of this manipulation were detected. The research article by E. Burrows et al. focuses its attention instead on environmental adversity, evaluating the behavioural effects of social isolation housing in a mouse model for autism, the Neuroligin-3 (NL3) mouse. The data presented here also highlight the importance of the choice of the procedures for behavioural testing in research on genetic models of neurodevelopmental disorders, since the effects of the environmental and genetic manipulations differed depending on the specific social test used.

The review by I. De Toma et al. expands the evaluation of the effects of gene-environment interactions in NDDs by assessing the role of epigenetic mechanisms in the aetiopathology of these diseases. The link between global and local epigenetic alterations, such as anomalies in DNA methylation, histone modifications or chromatin remodelling, and developmental disorders, is discussed, with a focus on two examples of developmental pathologies characterised by cognitive impairments, Down syndrome (DS) and Fragile X syndrome (FXS). This article also directly describes therapeutic approaches using epigenetic drugs that can act as cognitive enhancers in DS and FXS.

In conclusion, this special issue emphasises the importance of continuing and extending neurobehavioural studies on NDDs combining genetic with environmental manipulations and suggests that a multifactorial approach is most likely to identify novel therapeutic approaches and to advance our understanding of the aetiopathology of these complex disorders.

Susanna Pietropaolo
Wim E. Crusio
Joram Feldon

Hindawi Publishing Corporation
Neural Plasticity
Volume 2017, Article ID 8361290, 9 pages
http://dx.doi.org/10.1155/2017/8361290

Research Article

Social Isolation Alters Social and Mating Behavior in the R451C Neuroligin Mouse Model of Autism

E. L. Burrows,[1] A. F. Eastwood,[1] C. May,[1] S. C. Kolbe,[2] T. Hill,[1] N. M. McLachlan,[3] L. Churilov,[4] and A. J. Hannan[1,2]

[1]*Florey Institute of Neuroscience and Mental Health, Melbourne Brain Centre, University of Melbourne, Parkville, VIC 3010, Australia*
[2]*Department of Anatomy and Neuroscience, University of Melbourne, Parkville, VIC 3010, Australia*
[3]*Psychological Sciences, University of Melbourne, Parkville, VIC 3010, Australia*
[4]*Florey Institute of Neuroscience and Mental Health, 245 Burgundy St, Heidelberg, VIC 3084, Australia*

Correspondence should be addressed to E. L. Burrows; emma.burrows@florey.edu.au

Received 16 May 2016; Revised 28 October 2016; Accepted 22 November 2016; Published 31 January 2017

Academic Editor: Wim E. Crusio

Autism spectrum disorder (ASD) is a neurodevelopmental disorder typified by impaired social communication and restrictive and repetitive behaviors. Mice serve as an ideal candidate organism for studying the neural mechanisms that subserve these symptoms. The Neuroligin-3 (NL3) mouse, expressing a R451C mutation discovered in two Swedish brothers with ASD, exhibits impaired social interactions and heightened aggressive behavior towards male mice. Social interactions with female mice have not been characterized and in the present study were assessed in male $NL3^{R451C}$ and WT mice. Mice were housed in social and isolation conditions to test for isolation-induced increases in social interaction. Tests were repeated to investigate potential differences in interaction in naïve and experienced mice. We identified heightened interest in mating and atypical aggressive behavior in $NL3^{R451C}$ mice. $NL3^{R451C}$ mice exhibited normal social interaction with WT females, indicating that abnormal aggressive behavior towards females is not due to altered motivation to engage. Social isolation rearing heightened interest in social behavior in all mice. Isolation housing selectively modulated the response to female pheromones in $NL3^{R451C}$ mice. This study is the first to show altered mating behavior in the $NL3^{R451C}$ mouse and has provided new insights into the aggressive phenotype in this model.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social communication and repetitive and restrictive behavior [1]. Reflecting the diverse clinical presentation, ASDs are no longer thought to have a single causal factor. ASDs have a high heritability and 10–25% of individuals with the condition possess an associated genetic disorder [2]. This genetic link is further strengthened by studies that demonstrate a familial concordance ranging from 60 to 90% for ASDs between monozygotic twins [3, 4]. Of the associated genetic variants and mutations, many affect proteins involved in synapse function and development [5]. In particular, mutations in single genes that encode cell-adhesion molecules such as the neuroligin/neurexin complexes have been identified [6–10]. Neuroligins are proteins localized to the postsynaptic membrane [11, 12] and function as ligands to presynaptic neurexins, forming dynamic transsynaptic neurexin/neuroligin complexes, which putatively subserve synaptic formation [12–15] and function [16–18]. Disruption to the regulation of these pivotal synaptic proteins may provide insight into dysregulated synaptic mechanisms in ASD [11, 19–21]. Notwithstanding these strong genetic bases, there is evidence for a role of environmental modulation in the etiology of ASDs. Discrete modules of coexpressed ASD-associated genes specifically enriched in high-throughput RNA-sequencing but not GWAS implicate a nongenetic causative factor and potential interplay between genetic predisposition and the environment [22].

The identification of many genetic mutations associated with ASDs prompts the use of mouse models to further our understanding of how these may lead to the underlying

physiological mechanisms. Aberrant reciprocal social interactions can be probed in mouse models using assays that measure tendency to spend time with an unfamiliar mouse [20, 23]. The NL3 R451C mutation, discovered in two Swedish brothers with ASDs [6], results in only 10% of the functional protein being incorporated into the synaptic architecture [10] and affects the binding of NL3 to its presynaptic neurexin ligand [24]. $NL3^{R451C}$ mice show a diverse range of behavioral abnormalities, including altered social interaction, restrictive and repetitive behaviors, and synaptic dysfunction as shown by increased cortical inhibition together with enhanced hippocampal excitation in brain slices [25–32]. Inconsistencies between investigations of social interaction in $NL3^{R451C}$ mice have been reported [27–29]. These discrepancies have been said to be largely due to different genetic backgrounds, experimental conditions, and tests conducted by different laboratories [27, 33]. Heightened aggression towards younger sexually mature male mice has also been reported using a resident-intruder assay where the animals were permitted free interaction [31]. No aggression has been noted during free interaction juvenile social interaction testing, suggesting that the heightened aggression expressed in the adult $NL3^{R451C}$ mouse could be territorial in nature [27, 31]. Aggression in mice is a robust, innate, social behavior and serves to assist the acquisition of social ranking and resources from the environment, including female mates. Alterations in this behavior add to the understanding of the $NL3^{R451C}$ mouse social phenotype. Overlapping neural circuits control aggression and mating behavior in mice; however, it is not known if $NL3^{R451C}$ mice show any differences in social or mating behavior towards female mice [34, 35]. In order to determine whether the NL3 R451C mutation impacts social and mating behavior in male-female diads, we assessed interactions with female mice in male mutant and WT mice. The aim of the present study was also to address how context influences the behavior of adult male mice during social interaction. Mice were housed in social isolation from weaning to probe if this increased social interactions differentially in mice. Isolation housing has been previously shown to potentiate social interaction in mice [36]. Furthermore, tests were repeated to investigate whether experience altered social behavior between groups.

2. Methods

2.1. Animals and Housing. B6;129-*Nlgn3*^{tm1Sud}/J mice were obtained from Jackson Laboratories (Bar Harbor, Maine, USA) and backcrossed beyond generation F10 on a C57BL6 background. $NL3^{R451C}$ and WT animals were derived by mating heterozygous females with $NL3^{R451C}$ males, which produced 50 : 50 WT and $NL3^{R451C}$ male offspring (Y/+ and Y/R451C) that were genotyped as previously described [28]. All mice were socially housed until weaning at postnatal day 28 in conventional open-top cages ($31 \times 16 \times 10$ cm) with basic nesting materials (pine bedding and tissue paper) maintained at a constant temperature ($22 \pm 1^{\circ}$C). After weaning, all mice were transitioned from a standard 12-hour light-dark cycle (light: 07:00–19:00) to another 12-hour reverse-cycle room (light: 19:00–07:00) over three days (4-hour cycle shift per day). Food and water were available ad libitum. C57Bl/6J female mice were housed in groups of 5-6 individuals. At weaning, mice were pseudorandomly allocated to mixed housing (3-4 individuals) or social isolation, ensuring equal WT and $NL3^{R451C}$ mice in each mixed housing condition and that litters were spread over all conditions. All socially housed mice were individually housed following the first Male-Female Social Interaction Test (MFSIT; aged 13–19 weeks), to avoid excessive aggression, previously reported in $NL3^{R451C}$ adult male mice [31]. Experiments occurred between 08:00 and 18:00, during the dark cycle under red light (4 lux) at 55.0% humidity; mice were habituated to experimental rooms ($22 \pm 1^{\circ}$C) for at least 30 minutes prior to testing, from which all strong odors were eliminated. All experiments were approved by the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee.

2.2. Estrus Cycle Determination. Adult wild-type C57Bl/6J female mice were pap-smeared on the morning of each experimental day in order to determine the respective stage in the murine estrus cycle. Only those determined to be in estrus on the same experimental day were used either for urine collection or as a stimulus mouse. Animals were held by the base of their tails, with their hind limbs raised to evert their genital region and vaginal epithelial and blood cells were collected using a cotton-tipped applicator and smeared on the surface of a sterile glass microscopy slide and allowed to dry. Estrus phase was determined by staining cells using Thermo Scientific™ Shandon™ Kwik-Diff™ staining kit (Thermo Fisher Scientific Inc., USA).

2.3. Female Urine Sniffing Test (FUST). The Female Urine Sniffing Test has been described previously [37]. In brief, for at least one hour prior to the test, 8–10-week-old mice were habituated to a sterile cotton-tipped applicator suspended from the ceiling of a clean cage in the reverse light cycle dim red light illumination (4 lux). Urine from C57Bl/6J estrus female mice was thawed from a -80°C freezer to room temperature and combined into a single vial. The same urine combination was used within each experimental day. Urine (10 μL) was pipetted onto another sterile cotton-tipped applicator and suspended into the cage for 3 minutes. Latency and duration of sniffing were recorded using a digital camera (Panasonic, Secaucus, NJ, USA), positioned 30 cm from the cage, and were scored manually post hoc by a single independent observer blinded to genotype and housing condition.

2.4. Male-Female Social Interaction Test (MFSIT). Social behavior between socially and isolation-housed male mice, aged 14–20 weeks of age, towards a novel sexually mature female was assessed using a previously described protocol [38]. Each male experienced the MFSIT twice, one week apart, to probe for the effect of sexual experience. Females

(in estrus, determined same day of testing) were pseudo-randomly paired with the subject male, ensuring that each dyadic interaction was novel and that each female was paired with only one male per day. For at least one hour prior to the test, all mice were habituated to the experimental room. Male mice were additionally habituated to clean, transparent Perspex open-top cages (31 × 16 × 15 cm) with fresh, odorless pine litter. In Phase 1, the stimulus female was placed into the cage with the male for a 5 min period of free interaction. Following this, the female was removed and placed into a separate clean standard open-top cage. After 3 mins, the same female was then recoupled with the male for a second 5 min bout of free interaction (Phase 2). The test was repeated one week later where males were paired with a different female. Behaviors were recorded using a digital visual camera (Panasonic, Secaucus, NJ, USA), positioned 30 cm above the cage. Sniffing, stalking, mounting, and attacking behaviors (previously defined in [31, 39]) were scored by a single observer blinded to genotype and housing condition post hoc using a key-sensitive timer program custom written in MATLAB®. For each behavior, latency, number of bouts, and total duration were analyzed. Sniffing behavior was recorded when mice made contact with the female with their nose and were stationary. Stalking was defined as slow deliberate chasing behavior. 5 trials were chosen at random and scored by an independent observer. High concordance between the behaviors scored was seen (Supplementary Figure 1 available online at https://doi.org/10.1155/2017/8361290).

2.5. Statistical Analyses. FUST data was normally distributed and variance comparable and two-way ANOVA followed by Bonferroni post hoc tests was applied. MFSIT data was not normally distributed and random effect regression models were applied to estimate the effect size of each behavioral measure. Animals were repeatedly tested over 2 phases and over 2 weeks; thus these observations are correlated within a given animal. In all analyses, phase, episode, housing, and gene were used as independent variables. Two-sided p values were reported together with appropriate effect size estimates and 95% confidence intervals (95% CI) to indicate the precision. Latency describes the time to a behavior and may be censored (e.g., when an animal does not attack during the 300 sec observation period). A shared frailty Cox regression model was used to estimate the treatment effect size, measured as the hazard ratio of the first sniff/mount/attack occurring at any time over the 300 sec observation period. A negative binomial regression model was used to estimate the differences in number of mounting episodes, measured as the ratio of expected number of mounts. Clustered median regressions were applied to the duration data of those animals engaged in the specific behavior. Results for MISFIT data are graphically presented as a box and whiskers plot showing the 25th to the 75th percentile and the minimum to maximum of the data range and median shown by a line. Data from FUST are shown as mean ± SEM. Significance was evaluated at $p < 0.05$. Statistical analyses were performed with STATA v13IC (StataCorp, College Station, TX, USA) and IBM SPSS Statistics v22 (IBM Corp, Armonk, NY) software.

3. Results

3.1. Social Isolation Potentiates Social Behavior in Both NL3 and WT Mice. $NL3^{R451C}$ and WT mice were assessed for social and mating behavior towards a female mouse, over two phases (first and second exposure, following a 3-minute separation) and across two weeks (naïve and experienced, one week apart). No group differences were seen in latency to sniff, with all mice making contact with the female in under 6 seconds regardless of exposure or week tested (Figure 1(a)). Mice housed in isolation showed increased interest in interacting with the female, spending more time sniffing their head (Figure 1(b); median difference in time between social and isolation housing = 19.01; $p = 0.005$; 95% CI: 5.9, 32.12) and body (Figure 1(c); median difference in time between social and isolation housing = 13.34; $p < 0.001$; 95% CI: 6.02, 20.65) compared to socially housed animals. Isolation housing had a selective effect on time spent sniffing the genital region of the female mouse in NL3 mice only (Figure 1(c); median regression, gene∗housing interaction: $p < 0.001$). NL3 mice were more likely to sniff the genital region of the female mouse when they were housed in isolation (Figure 1(c); median difference in time between social and isolation housing for NL3 only = 43.0; $p = 0.006$; 95% CI: 13.09, 72.92).

Mice were scored for latency to groom (Figure 2(a)) and, provided they groomed within the session, they were scored for time spent grooming their head and body (Figure 2(b)) and genitals (Figure 2(c)). No differences were seen between WT and NL3 mice and no effect of housing on any measure was evident indicating that any differences seen in dyadic interactions were not due to time spent self-grooming.

3.2. NL3 Mice Show Altered Mating Behavior and Isolation Housing Does Not Modify Behavior. $NL3^{R451C}$ and WT mice did not show any difference in latency to mount (Figure 3(a)). All mice were quicker to mount after the period of separation (Figure 3(a); hazard ratio of first mount in second phase compared to first = 3.09; $p < 0.001$; 95% CI: 1.99, 4.81) and slower when the test was repeated a week later (Figure 3(a); hazard ratio of first mount in second week compared to first = 0.51; $p = 0.004$; 95% CI: 0.33, 0.80). $NL3^{R451C}$ mice mounted a greater number of times compared to WT mice (Figure 3(b); ratio of expected number of mounts between WT and NL3 mice = 1.95; $p = 0.004$; 95% CI: 1.24, 3.06). A lack of interaction between housing and genotype meant that we were unable to ascertain if this effect was specific to one condition. A trend for $NL3^{R451C}$ mice to spend longer mounting the female mouse was also observed (Figure 3(c); median difference in time between WT and NL3 mice = 39.42; $p = 0.056$; 95% CI: −1.05, 79.89). Duration mounting increased in all mice when they were exposed to the same female after a brief period of separation (Supplementary Figure 2; median difference in time between first phase and second = 12.77; $p = 0.023$; 95% CI: 1.29, 1.44); however,

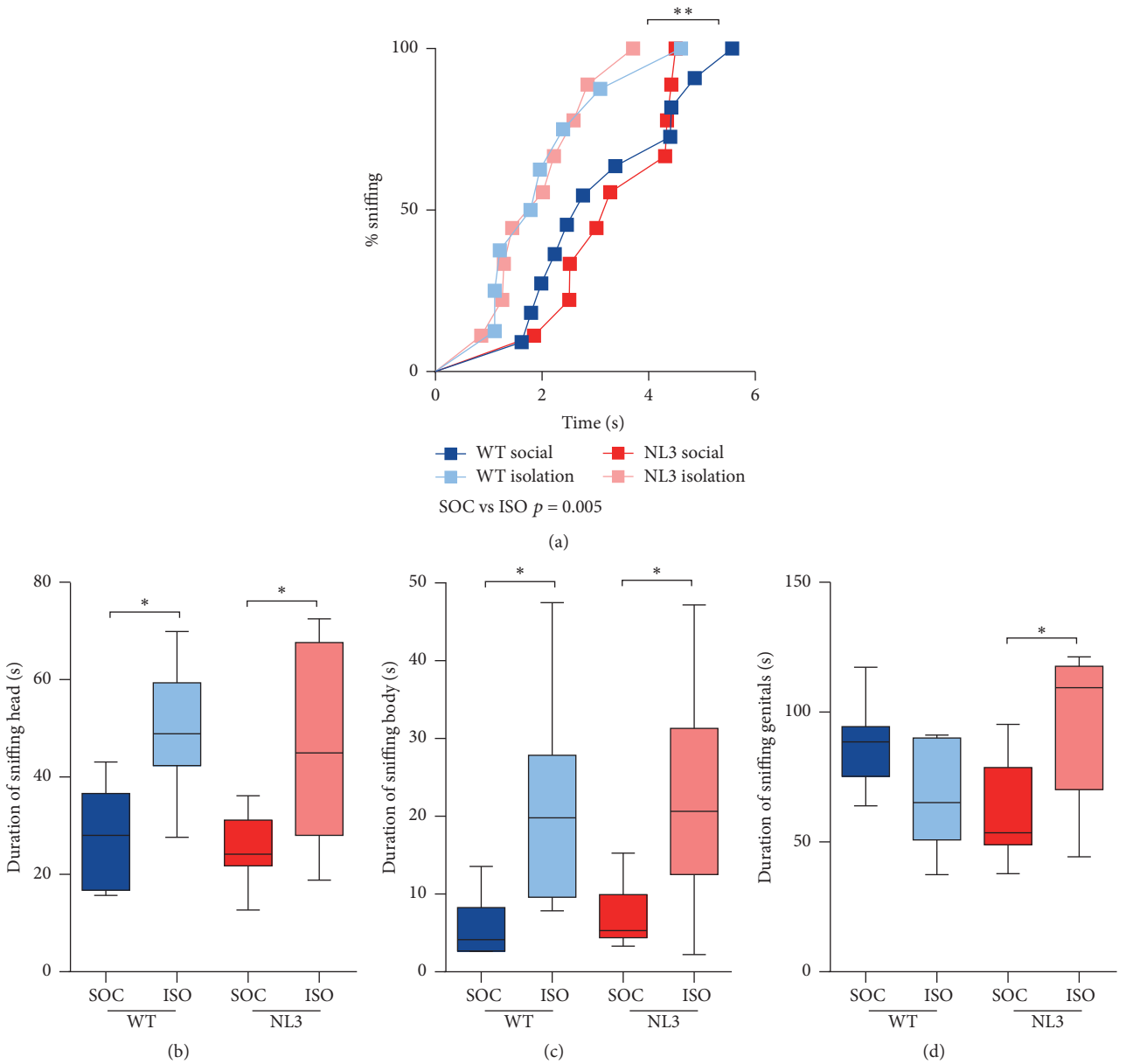


Figure 1: Isolation housing increased sociability in WT and NL3 mice. (a) Latency to sniff, (b) sniffing head of female, (c) sniffing body, and (d) sniffing genital region. Values are an average of 4 tests (2 phases, 2 weeks) and data in (b–d) are displayed as boxplots with median plus the 25th and 75th percentiles. Whiskers represent the minimum and maximum values. SOC = socially housed animals (WT: $n = 10$; NL3 = 10); ISO = isolation-housed (WT: $n = 9$; NL3 = 9) animals. $^{*}p < 0.05$ and $^{**}p < 0.01$.

mounting decreased when a novel female was placed in the test mouse's cage the following week (Supplementary Figure 2; median difference in time between first week and second = −19; $p = 0.004$; 95% CI: −31.89, −6.10).

3.3. NL3 Mice Are Aggressive towards Female Mice and Isolation Housing Reduces Incidence of Stalking. $NL3^{R451C}$ and WT mice were monitored for signs of aggression towards female mice during all phases of the test. While no genotype effect on stalking latency was seen (Figure 4(a)), $NL3^{R451C}$ mice stalked female mice for a longer duration (Figure 4(b); median difference in time between WT and NL3 mice = 9.42; $p = 0.038$; 95% CI: 0.53, 18.30). Regardless of genotype, mice housed in isolation were less likely to stalk the female, showing higher latencies to stalk (Figure 4(b); hazard ratio of first stalk in social compared to isolation housing = 0.92; $p = 0.021$; 95% CI: 0.21, 0.88) and reduced duration of stalking (Figure 4(c); median difference in time between social and isolation housing = −5.14; $p = 0.045$; 95% CI: −10.16, −0.11). $NL3^{R451C}$ mice were more likely to attack female mice, regardless of housing, exposure, or week of test (Figure 4(c); hazard ratio of first attack in WT compared to NL3 mice = 18.20; $p = 0.007$; 95% CI: 2.21, 149.67). With the exception

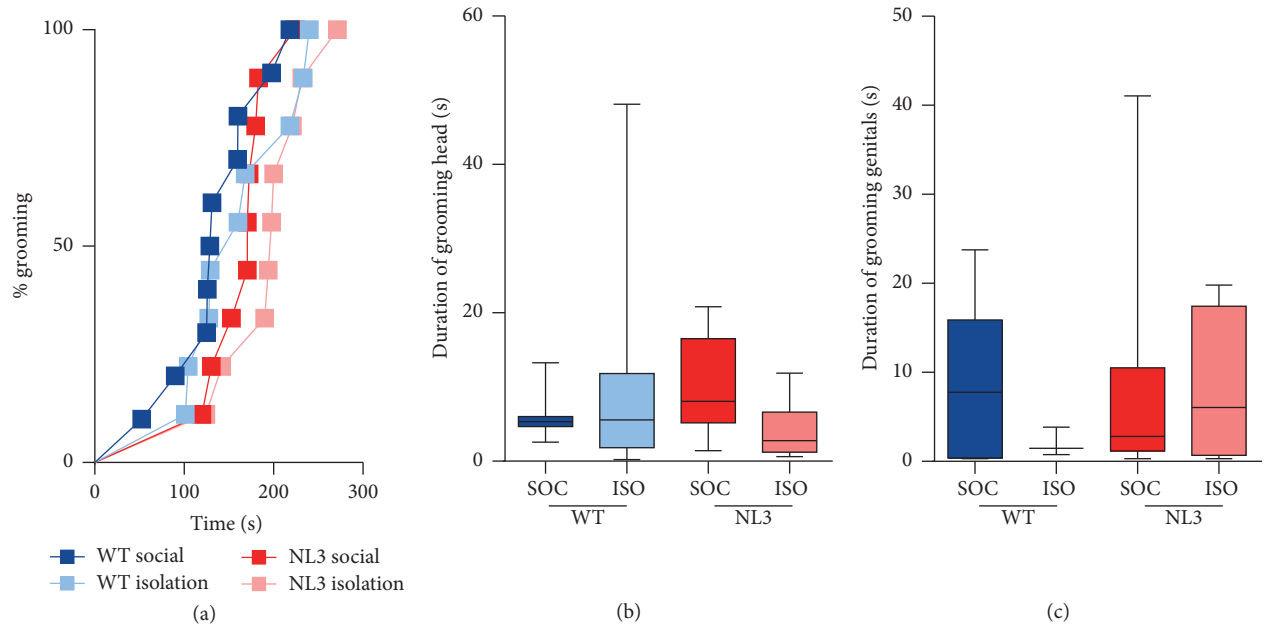


Figure 2: Self-grooming behavior in NL3 and WT mice. (a) Latency to groom, (b) time spent grooming head/body, and (c) genitals. Values are an average of 4 tests (2 phases, 2 weeks) and data in (b-c) are displayed as boxplots with median plus the 25th and 75th percentiles. Whiskers represent the minimum and maximum values. SOC = socially housed animals (WT: $n = 10$; NL3 = 10); ISO = isolation-housed (WT: $n = 9$; NL3 = 9) animals.

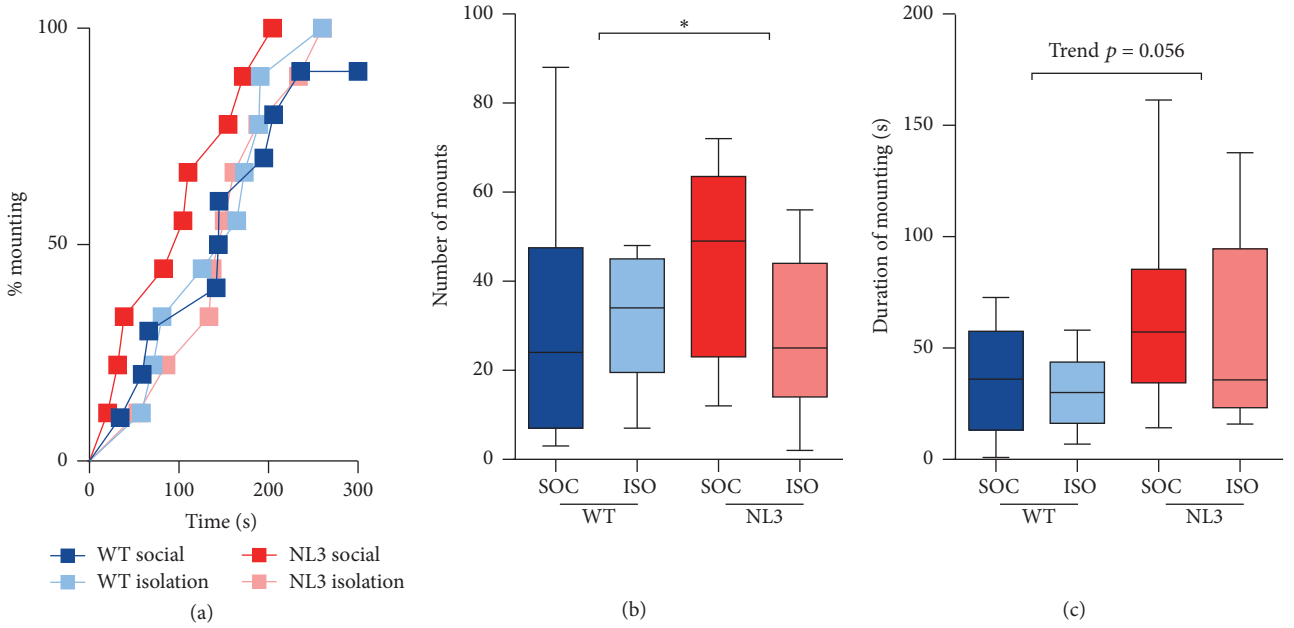


Figure 3: NL3 mice show heightened interest in mating compared to WT mice. (a) Time to first mount, (b) number of mounts, and (c) duration of mounting. Values are an average of 4 tests (2 phases, 2 weeks) and data in (b-c) are displayed as boxplots with median plus the 25th and 75th percentiles. Whiskers represent the minimum and maximum values. SOC = socially housed animals (WT: $n = 10$; NL3 = 10); ISO = isolation-housed (WT: $n = 9$; NL3 = 9) animals. $^{*}p < 0.05$.

of one WT mouse, only NL3^{R451C} mice attacked female mice. Attacks were brief and did not occur many times per mouse, limiting the analysis to latency.

Time spent investigating estrus female urine was measured in a naïve cohort of mice and was used as an index of arousal and interest in the female. Housing specifically modulated NL3 mouse sniffing behavior (Figure 5; two-way ANOVA, genotype∗housing interaction: $F_{1,33} = 11.264$; $p = 0.002$). Pairwise comparisons indicated that socially housed NL3 mice investigated the stimulus for less time compared to

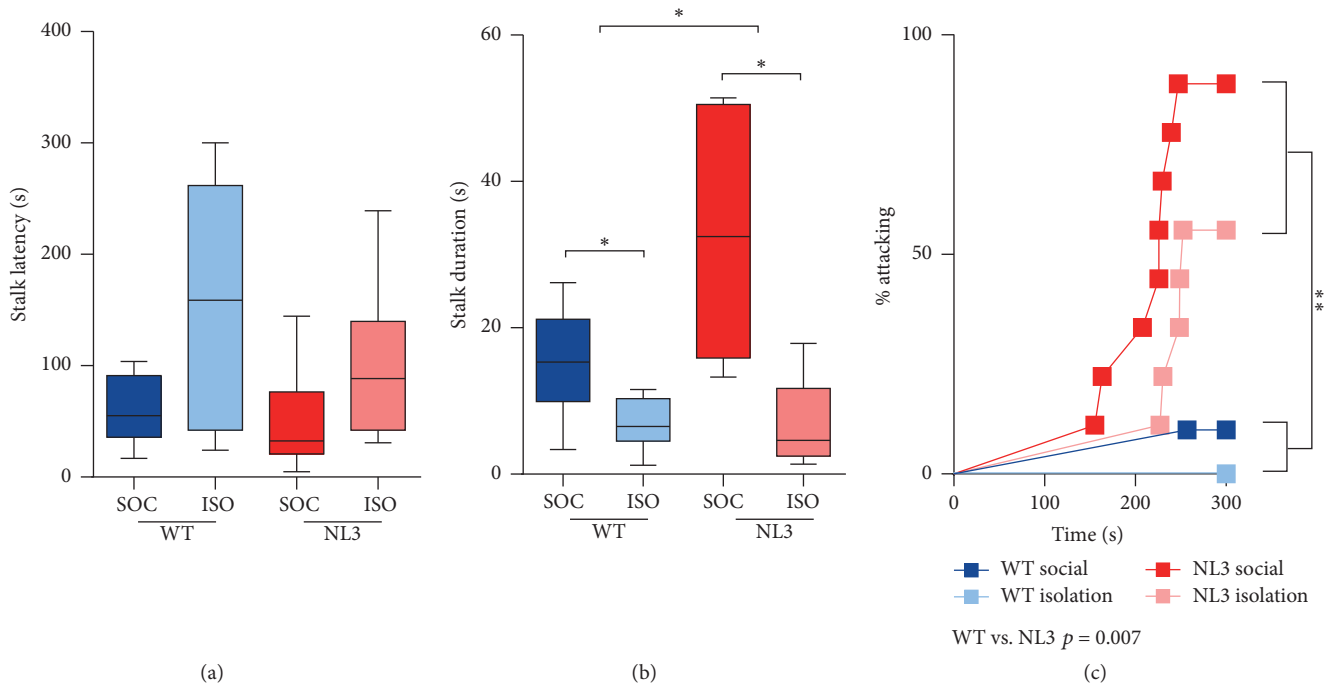


FIGURE 4: NL3 male mice show aggression towards female mice. (a) Latency to stalk female mouse, (b) duration of stalking female, and (c) percentage of male mice attacking female. Values are an average of 4 tests (2 phases, 2 weeks) and data in (a-b) are displayed as boxplots with median plus the 25th and 75th percentiles. Whiskers represent the minimum and maximum values. SOC = socially housed animals (WT: $n = 10$; NL3 = 10); ISO = isolation-housed (WT: $n = 9$; NL3 = 9) animals. $^{*}p < 0.05$ and $^{**}p < 0.01$.

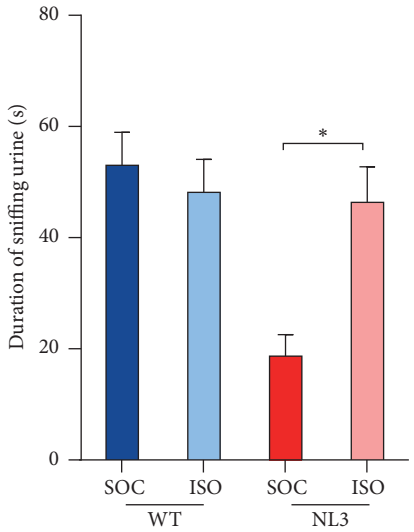


FIGURE 5: NL3 mice are less interested in female urine and social isolation increases interest to WT levels. Values are displayed as mean ± SEM. Asterisks represent a statistically significant difference between indicated groups. $^{*}p < 0.05$. SOC = socially housed animals (WT: $n = 10$; NL3 = 10); ISO = isolation-housed (WT: $n = 9$; NL3 = 9) animals.

their WT counterparts. Isolation housing increased investigation time in NL3 mice to levels comparable to WT mice.

4. Discussion

The present study identified heightened interest in mating and atypical aggressive behavior in NL3^{R451C} mice. NL3^{R451C} mice exhibit normal social interaction towards female mice, indicating that abnormal aggressive behavior is not due to altered motivation to engage in prosocial interactions. Isolation housing increased the time spent engaging in social interaction in all mice, in line with reports that social isolation increases motivation to engage in social communicative behaviors [40]. A selective effect of social isolation housing was seen on time spent investigating female pheromones in NL3^{R451C} mice. No difference in grooming was detected between NL3^{R451C} and WT mice, consistent with previous investigations into this behavior [33].

Heightened territorial aggression has previously been identified in NL3^{R451C} male mice utilizing the resident-intruder test whereby a male juvenile intruder mouse is introduced to the home cage of a test mouse [31]. The present study has shown that NL3^{R451C} mice also display aggressive behavior towards female mice. While aggression towards male mice is a robust, innate, social behavior to assist in the acquisition of social ranking and resources from the environment [41], aggression towards female mates is atypical. Studies employing similar paradigms to assess social interaction in male-female dyads have not shown aggression towards females in WT mice [42]. Rearing in social isolation leads to increased territorial aggression in adult mice [43, 44]. In the present study, socially housed

animals did not show an increase in aggression. This discrepancy could be due to a number of factors. The atypical aggression seen in $NL3^{R451C}$ mice may not be territorial in nature as the aggression is directed towards both males and females. Furthermore, testing was not conducted in the home cage of male test mice, reducing the likelihood and severity of aggression for those that showed the behavior. Using an assay designed to potentiate aggression would allow more thorough investigation of this atypical behavior in $NL3^{R451C}$ mice and also in isolation-housed mice.

Abnormal aggression towards female mice has been linked to altered levels of brain serotonin, with mice deficient in brain tryptophan hydroxylase 2 exhibiting hyperaggressive behavior towards their female interaction partners [45]. The hyperaggression in $NL3^{R451C}$ mice, previously identified towards male intruder mice, was mitigated following treatment with risperidone, an antagonist with high affinity for both serotonin and dopamine receptors [31]. Furthermore, overlapping neural circuits have been shown to control aggression and mating behavior in mice [46]. Specific activation of the ventral medial hypothalamus in male mice paired with a female, led to aggressive behavior in between bouts of mounting [35]. These findings provide a compelling reason to investigate both of these systems and brain regions in $NL3^{R451C}$ mice and to interrogate their role in mediating aggressive behavior. Furthermore, it will be of interest to explore the efficacy of risperidone treatment to reduce the aggression observed in $NL3^{R451C}$ mice towards female mice.

Since mice use pheromonal cues to identify individuals, we explored the possibility that pheromone detection may be altered in $NL3^{R451C}$ mice and could underlie their atypical aggression towards females. Unlike WT mice, $NL3^{R451C}$ mice showed reduced interest in exploring female urinary pheromones. Reduced interest could indicate that vomeronasal function may be compromised in $NL3^{R451C}$ mice; however, we have previously shown no impairment in olfactory discrimination of social and nonsocial odorants, including female urine [31]. Furthermore, isolation housing increased time spent sniffing urine in $NL3^{R451C}$ mice, indicating that the social housing was a likely factor in influencing interest in urinary pheromones. Social experience has been shown to modulate mating behavior, the production of vocalizations used during mating, and social interaction and response to pheromones [40, 47]. Mice normally live in large groups and exhibit social interactions that are dependent on the dynamics of multiple group members [48] and complex dominance hierarchies [49]. Further interrogation of dominance hierarchies in socially housed, mixed-genotype groups is therefore warranted.

In addition to aberrant aggression, $NL3^{R451C}$ mice showed heightened interest in mating with female mice. Increased mating drive could underlie this phenotype and future studies of this mouse model should assay for blood testosterone concentration differences from baseline following exposure to a female. Given that NL3 knockout mice have been reported to show reduced vocalizations during contact with a female mouse [50], investigation of social communication during mating in $NL3^{R451C}$ mice is also warranted.

This study identified heightened interest in mating and atypical aggressive behavior towards female mice in $NL3^{R451C}$ mice. This is the first investigation of social interactions in male-female dyads in $NL3^{R451C}$ mice and contributes to the full characterization of altered social behavior in this mouse model of ASD. Further investigation into the overlapping neural substrates underlying mating and aggressive behavior in the $NL3^{R451C}$ mouse may shed light into the role of Neuroligin-3 in mediating complex social behavior in mice. $NL3^{R451C}$ mice provide a very useful tool to model circuitry underlying abnormal social behavior in ASD.

Abbreviations

NL3: Neuroligin-3
R451C: Arginine to cysteine residue 451 substitution
WT: Wild-type.

Competing Interests

The authors declare that they have no conflicts of interest.

Authors' Contributions

E. L. Burrows conceived of the study, participated in its design and coordination, supervised all behavioral tests, collated data, and drafted the manuscript. A. F. Eastwood carried out the behavioral tests, scored all data, and drafted the manuscript. C. May and L. Churilov performed statistical analysis and revised the manuscript. S. C. Kolbe wrote MATLAB script for MISFIT analysis, assisted with data scoring and reliability scoring, and revised the manuscript. T. Hill assisted with validation of scoring. N. M. McLachlan participated in the study design and revised the manuscript. A. J. Hannan conceived of the study and participated in its design and coordination and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This work was funded by a Melbourne Neuroscience Institute Interdisciplinary Seed Grant and a Jack Brockhoff Foundation Medical Research Project Grant to A. J. Hannan and E. L. Burrows. E. L. Burrows is supported by a National Health and Medical Research Council-Australian Research Council (NHMRC-ARC) Dementia Research Development Fellowship. A. J. Hannan is an NHMRC Principal Research Fellow and has been supported by an ARC FT3 Future Fellowship (FT100100835). The authors thank past and present laboratory members for useful discussions. They also thank Daniel Drieberg, Ana Hudson, Brittany Cuic, Brett Purcell, and Craig Thomson for their technical assistance; Elisa Hill-Yardin for assistance in setting up the mouse colony; and

David Grayden for involvement in the Melbourne Neuroscience Institute application that provided funds for this study.

References

[1] APA, *Diagnostic and Statistical Manual of Mental Disorders*, American Psychiatric Publishing, Washington, DC, USA, 5th edition, 2013.

[2] B. S. Abrahams and D. H. Geschwind, "Advances in autism genetics: on the threshold of a new neurobiology," *Nature Reviews Genetics*, vol. 9, no. 5, pp. 341–355, 2008.

[3] A. Bailey, S. Palferman, L. Heavey, and A. Le Couteur, "Autism: the phenotype in relatives," *Journal of Autism and Developmental Disorders*, vol. 28, no. 5, pp. 369–392, 1998.

[4] S. Folstein and M. Rutter, "Infantile autism: a genetic study of 21 twin pairs," *Journal of Child Psychology and Psychiatry*, vol. 18, no. 4, pp. 297–321, 1977.

[5] I. Voineagu, X. Wang, P. Johnston et al., "Transcriptomic analysis of autistic brain reveals convergent molecular pathology," *Nature*, vol. 474, no. 7351, pp. 380–384, 2011.

[6] S. Jamain, H. Quach, C. Betancur et al., "Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism," *Nature Genetics*, vol. 34, no. 1, pp. 27–29, 2003.

[7] M. S. L. Ching, Y. Shen, W.-H. Tan et al., "Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders," *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, vol. 153, no. 4, pp. 937–947, 2010.

[8] F. Laumonnier, F. Bonnet-Brilhault, M. Gomot et al., "X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family," *The American Journal of Human Genetics*, vol. 74, no. 3, pp. 552–557, 2004.

[9] J. Yan, G. Oliveira, A. Coutinho et al., "Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric patients," *Molecular Psychiatry*, vol. 10, no. 4, pp. 329–332, 2005.

[10] A. A. Chubykin, X. Liu, D. Comoletti, I. Tsigelny, P. Taylor, and T. C. Südhof, "Dissection of synapse induction by neuroligins: effect of a neuroligin mutation associated with autism," *Journal of Biological Chemistry*, vol. 280, no. 23, pp. 22365–22374, 2005.

[11] C. Betancur, T. Sakurai, and J. D. Buxbaum, "The emerging role of synaptic cell-adhesion pathways in the pathogenesis of autism spectrum disorders," *Trends in Neurosciences*, vol. 32, no. 7, pp. 402–412, 2009.

[12] C. I. Nam and L. Chen, "Postsynaptic assembly induced by neurexin-neuroligin interaction and neurotransmitter," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 17, pp. 6137–6142, 2005.

[13] B. Chih, S. K. Afridi, L. Clark, and P. Scheiffele, "Disorder-associated mutations lead to functional inactivation of neuroligins," *Human Molecular Genetics*, vol. 13, no. 14, pp. 1471–1477, 2004.

[14] P. Scheiffele, J. Fan, J. Choih, R. Fetter, and T. Serafini, "Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons," *Cell*, vol. 101, no. 6, pp. 657–669, 2000.

[15] T. C. Südhof, "Neuroligins and neurexins link synaptic function to cognitive disease," *Nature*, vol. 455, no. 7215, pp. 903–911, 2008.

[16] A. A. Chubykin, D. Atasoy, M. R. Etherton et al., "Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2," *Neuron*, vol. 54, no. 6, pp. 919–931, 2007.

[17] M. Missier, W. Zhang, A. Rohlmann et al., "α-neurexins couple Ca^{2+} channels to synaptic vesicle exocytosis," *Nature*, vol. 423, no. 6943, pp. 939–948, 2003.

[18] F. Varoqueaux, G. Aramuni, R. L. Rawson et al., "Neuroligins determine synapse maturation and function," *Neuron*, vol. 51, no. 6, pp. 741–754, 2006.

[19] R. Anney, L. Klei, D. Pinto et al., "A genome-wide scan for common alleles affecting risk for autism," *Human Molecular Genetics*, vol. 19, no. 20, pp. 4072–4082, 2010.

[20] J. N. Crawley, "Mouse behavioral assays relevant to the symptoms of autism," *Brain Pathology*, vol. 17, no. 4, pp. 448–459, 2007.

[21] D. H. Geschwind and P. Levitt, "Autism spectrum disorders: developmental disconnection syndromes," *Current Opinion in Neurobiology*, vol. 17, no. 1, pp. 103–111, 2007.

[22] I. Voineagu, "Gene expression studies in autism: moving from the genome to the transcriptome and beyond," *Neurobiology of Disease*, vol. 45, no. 1, pp. 69–75, 2012.

[23] J. L. Silverman, M. Yang, C. Lord, and J. N. Crawley, "Behavioural phenotyping assays for mouse models of autism," *Nature Reviews Neuroscience*, vol. 11, no. 7, pp. 490–502, 2010.

[24] K. Ichtchenko, T. Nguyen, and T. C. Südhof, "Structures, alternative splicing, and neurexin binding of multiple neuroligins," *Journal of Biological Chemistry*, vol. 271, no. 5, pp. 2676–2682, 1996.

[25] M. R. Etherton, K. Tabuchi, M. Sharma, J. Ko, and T. C. Südhof, "An autism-associated point mutation in the neuroligin cytoplasmic tail selectively impairs AMPA receptor-mediated synaptic transmission in hippocampus," *EMBO Journal*, vol. 30, no. 14, pp. 2908–2919, 2011.

[26] C. Földy, R. C. Malenka, and T. C. Südhof, "Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling," *Neuron*, vol. 78, no. 3, pp. 498–509, 2013.

[27] T. C. Jaramillo, S. Liu, A. Pettersen, S. G. Birnbaum, and C. M. Powell, "Autism-related neuroligin-3 mutation alters social behavior and spatial learning," *Autism Research*, vol. 7, no. 2, pp. 264–272, 2014.

[28] K. Tabuchi, J. Blundell, M. R. Etherton et al., "A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice," *Science*, vol. 318, no. 5847, pp. 71–76, 2007.

[29] M. Etherton, C. Földy, M. Sharma et al., "Autism-linked neuroligin-3 R451C mutation differentially alters hippocampal and cortical synaptic function," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 33, pp. 13764–13769, 2011.

[30] P. E. Rothwell, M. V. Fuccillo, S. Maxeiner et al., "Autism-associated neuroligin-3 mutations commonly impair striatal circuits to boost repetitive behaviors," *Cell*, vol. 158, no. 1, pp. 198–212, 2014.

[31] E. L. Burrows, L. Laskaris, L. Koyama et al., "A neuroligin-3 mutation implicated in autism causes abnormal aggression and increases repetitive behavior in mice," *Molecular Autism*, vol. 6, article 62, 2015.

[32] M. Kumar, J. T. Duda, W.-T. Hwang et al., "High resolution magnetic resonance imaging for characterization of the neuroligin-3 knock-in mouse model associated with autism spectrum disorder," *PLoS ONE*, vol. 9, no. 10, Article ID e109872, 2014.

[33] K. K. Chadman, S. Gong, M. L. Scattoni et al., "Minimal aberrant behavioral phenotypes of neuroligin-3 R451C knockin mice," *Autism Research*, vol. 1, no. 3, pp. 147–158, 2008.

[34] H. Lee, D.-W. Kim, R. Remedios et al., "Scalable control of mounting and attack by Esr1+ neurons in the ventromedial hypothalamus," *Nature*, vol. 509, no. 7502, pp. 627–632, 2014.

[35] D. Lin, M. P. Boyle, P. Dollar et al., "Functional identification of an aggression locus in the mouse hypothalamus," *Nature*, vol. 470, no. 7333, pp. 221–226, 2011.

[36] M. E. Avale, J. Chabout, S. Pons et al., "Prefrontal nicotinic receptors control novel social interaction between mice," *FASEB Journal*, vol. 25, no. 7, pp. 2145–2155, 2011.

[37] O. Malkesman, M. L. Scattoni, D. Paredes et al., "The female urine sniffing test: a novel approach for assessing reward-seeking behavior in rodents," *Biological Psychiatry*, vol. 67, no. 9, pp. 864–871, 2010.

[38] M. Yang, D. Loureiro, D. Kalikhman, and J. N. Crawley, "Male mice emit distinct ultrasonic vocalizations when the female leaves the social interaction arena," *Frontiers in Behavioral Neuroscience*, 2013.

[39] T. E. McGill, "Sexual behavior of the mouse after long-term and short-term postejaculatory recovery periods," *Journal of Genetic Psychology*, vol. 103, no. 1, pp. 53–57, 1963.

[40] J. Chabout, P. Serreau, E. Ey et al., "Adult male mice emit context-specific ultrasonic vocalizations that are modulated by prior isolation or group rearing environment," *PLoS ONE*, vol. 7, no. 1, Article ID e29401, 2012.

[41] K. A. Miczek, S. C. Maxson, E. W. Fish, and S. Faccidomo, "Aggressive behavioral phenotypes in mice," *Behavioural Brain Research*, vol. 125, no. 1-2, pp. 167–181, 2001.

[42] M. L. Scattoni, L. Ricceri, and J. N. Crawley, "Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters," *Genes, Brain and Behavior*, vol. 10, no. 1, pp. 44–56, 2011.

[43] I. V. Karpova, V. V. Mikheev, V. V. Marysheva, E. R. Bychkov, and S. N. Proshin, "Oxytocin-induced changes in monoamine level in symmetric brain structures of isolated aggressive C57Bl/6 mice," *Bulletin of Experimental Biology and Medicine*, vol. 160, no. 5, pp. 605–609, 2016.

[44] M. Rodríguez-Arias, F. Navarrete, M. C. Blanco-Gandia et al., "Role of CB2 receptors in social and aggressive behavior in male mice," *Psychopharmacology*, vol. 232, no. 16, pp. 3019–3031, 2015.

[45] D. Beis, K. Holzwarth, M. Flinders, M. Bader, M. Wöhr, and N. Alenina, "Brain serotonin deficiency leads to social communication deficits in mice," *Biology Letters*, vol. 11, no. 3, 2015.

[46] K. Hashikawa, Y. Hashikawa, A. Falkner, and D. Lin, "The neural circuits of mating and fighting in male mice," *Current Opinion in Neurobiology*, vol. 38, pp. 27–37, 2016.

[47] F. I. Roullet, M. Wöhr, and J. N. Crawley, "Female urine-induced male mice ultrasonic vocalizations, but not scent-marking, is modulated by social experience," *Behavioural Brain Research*, vol. 216, no. 1, pp. 19–28, 2011.

[48] Y. Shemesh, Y. Sztainberg, O. Forkosh, T. Shlapobersky, A. Chen, and E. Schneidman, "High-order social interactions in groups of mice," *eLife*, vol. 2013, no. 2, Article ID e00759, 2013.

[49] A. Weissbrod, A. Shapiro, G. Vasserman et al., "Automated long-term tracking and social behavioural phenotyping of animal colonies within a semi-natural environment," *Nature Communications*, vol. 4, article 2018, 2013.

[50] K. Radyushkin, K. Hammerschmidt, S. Boretius et al., "Neuroligin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit," *Genes, Brain and Behavior*, vol. 8, no. 4, pp. 416–425, 2009.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 3597209, 15 pages
http://dx.doi.org/10.1155/2016/3597209

Review Article

Neuroinflammation in Autism: Plausible Role of Maternal Inflammation, Dietary Omega 3, and Microbiota

Charlotte Madore,[1] Quentin Leyrolle,[2,3,4,5] Chloé Lacabanne,[2,3,6,7] Anouk Benmamar-Badel,[2,3,8] Corinne Joffre,[2,3,9] Agnes Nadjar,[2,3,9] and Sophie Layé[2,3,9]

[1]*Ann Romney Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*
[2]*Nutrition et Neurobiologie Intégrée, UMR 1286, INRA, 33000 Bordeaux, France*
[3]*Nutrition et Neurobiologie Intégrée, UMR 1286, Bordeaux University, 33000 Bordeaux, France*
[4]*Inserm, U1141, Hôpital Robert Debré, Paris, France*
[5]*Université Paris Diderot, Sorbonne Paris Cité, Paris, France*
[6]*Department of Psychiatry, McGill University, Montreal, QC, Canada*
[7]*Douglas Mental Health University Institute, Montreal, QC, Canada*
[8]*Département de Biologie, École Normale Supérieure de Lyon, Université de Lyon, UCB Lyon 1, Lyon, France*
[9]*OptiNutriBrain International Associated Laboratory (NutriNeuro France-INAF Canada), Bordeaux, France*

Correspondence should be addressed to Sophie Layé; sophie.laye@bordeaux.inra.fr

Received 30 May 2016; Revised 24 August 2016; Accepted 27 September 2016

Academic Editor: Bruno Poucet

Several genetic causes of autism spectrum disorder (ASD) have been identified. However, more recent work has highlighted that certain environmental exposures early in life may also account for some cases of autism. Environmental insults during pregnancy, such as infection or malnutrition, seem to dramatically impact brain development. Maternal viral or bacterial infections have been characterized as disruptors of brain shaping, even if their underlying mechanisms are not yet fully understood. Poor nutritional diversity, as well as nutrient deficiency, is strongly associated with neurodevelopmental disorders in children. For instance, imbalanced levels of essential fatty acids, and especially polyunsaturated fatty acids (PUFAs), are observed in patients with ASD and other neurodevelopmental disorders (e.g., attention deficit hyperactivity disorder (ADHD) and schizophrenia). Interestingly, PUFAs, and specifically n-3 PUFAs, are powerful immunomodulators that exert anti-inflammatory properties. These prenatal dietary and immunologic factors not only impact the fetal brain, but also affect the microbiota. Recent work suggests that the microbiota could be the missing link between environmental insults in prenatal life and future neurodevelopmental disorders. As both nutrition and inflammation can massively affect the microbiota, we discuss here how understanding the crosstalk between these three actors could provide a promising framework to better elucidate ASD etiology.

1. Introduction

Autism is a complex neurodevelopmental condition whose different forms are described in DSM-V as autism spectrum disorder (ASD). ASD affects almost 1 in 100 children [1] and is characterized, in varying degrees, by deficits in verbal and nonverbal communication, and is associated with repetitive behaviors [2]. Several forms of ASD have been described, such as Asperger syndrome [3] or Kanner-type autism [4], revealing that ASD is a highly heterogeneous disorder, likely with multiple underlying causes. Intense scientific work has been performed in recent years to understand the potential origin of ASD, revealing that this disorder arises from both genetic and environmental factors, especially those influencing fetal and early-life development [5].

Although ASD has been shown to be highly heritable (recent estimates 38–54%), several meta-analyses have highlighted that nongenetic prenatal causes of ASD exist, opening the door for further studies to investigate such mechanisms [6]. Approximately 10% of ASD cases are linked to disorders of genetic etiology, such as fragile X syndrome, tuberous sclerosis, and Rett disorder. Supporting the idea of heterogeneity of ASD, single genetic mutations account for only 1-2% of ASD cases [7], with the majority of cases remaining idiopathic. Mutations identified by genetic studies have revealed that some affected genes are involved in brain development from *in utero* through infancy. Frequent aberrations in brain cytoarchitectural organization and neuronal connectivity have been observed in the brains of ASD patients, leading to the concept that ASD is a synaptopathy [8]. Genes involved in synapse formation or brain connectivity (e.g., *fmr1*, *mecp2*, *shank3*, *tsc*, *neuroligin*, and *cntnap2*) have been repeatedly linked to ASD [9–11].

ASD brain transcriptome studies identify molecular abnormalities in synaptic and immune/microglia markers gene expression, with the former being downregulated and the latter upregulated [12]. Other genes related to inflammation (e.g., *il-1raplp1*, *il-1r2*, *c4b*, *met*, *mch2*, *par2*, *mtor1*, and *μpar*) have been reported to be differentially expressed in ASD as well [13, 14]. This is of particular interest as the perinatal environment generating chronic neuroinflammatory processes leads to the rapid development of ASD in susceptible children [15]. Indeed, maternal inflammation linked to infection, autoimmunity, obesity, or gestational diabetes during pregnancy is associated with a higher risk of neurodevelopmental disorders, in particular ASD [16], as reviewed by Estes et al. [15]. Many experimental studies have linked maternal immune activation (MIA) in the pathogenesis of ASD with neuroinflammatory events in the developing brain as an important component of brain malformation [17, 18]. Experimental studies also revealed that MIA induces long-lasting changes in immune system activity and microbiota, which are believed to be involved in behavioral alterations in offspring [19, 20]. Interestingly, the host microbiota has been shown to modulate local immune responses in the brain [21], and conversely neuroinflammation can influence the microbiota composition [19]. In addition to the microbiota, nutrition is an important component of inflammatory regulation and nutritional deficiency could also be an important risk factor for ASD [22]. Recent animal studies have revealed that maternal nutritional statuses in n-3 polyunsaturated fatty acids (PUFAs), essential fatty acids with anti-inflammatory properties that are present in the brain [22–24], regulate microglia activity in the developing brain [25] and influence ASD-like behavioral disorders [26]. Here, we discuss evidence of neuroimmune dysregulation in patients with ASD, along with the epidemiological, clinical, and experimental studies implicating MIA, gut microbiota, and lipid nutrition as environmental factors that can lead to sustained neuroinflammation and contribute to the etiology of ASD. Understanding these risk factors could contribute to the development of novel nutritional strategies for therapeutic interventions in ASD.

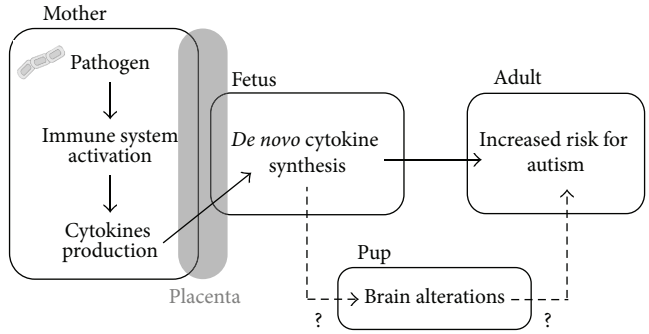


Figure 1: Association between prenatal infection and enhanced risk of neurodevelopmental disorders. During pregnancy, pathogens are thought to increase the risk of neurodevelopmental disorders in the offspring depending on the timing of infection and the magnitude of maternal immune response. Activation of the fetal immune system by *de novo* synthesis of cytokines sensitizes the brain to neurodevelopmental alterations. Interaction with other environmental and/or genetic factors also contributes to ASD etiology. Modeling prenatal immune activation represents a powerful tool to elucidate the relative contribution of these various factors for enhanced risk of ASD as well as other neurodevelopmental disorders.

2. Evidence of Neuroinflammatory Processes in Autism

Over the last 10 years, much evidence has accumulated pointing to inflammatory mechanisms as contributors to ASD, and intense research has been undertaken to determine exactly how immune dysregulation alters brain connectivity and function and plays a role in autism phenotypes [27] (Figure 1). The recent demonstration that microglia, the resident immune cells of the central nervous system (CNS), contribute not only to inflammatory events but also to neural development, has raised new hypotheses regarding their role in the etiology of autism. In addition to altered systemic immunity [28, 29], neuroinflammation has been observed in the brain of ASD patients. The presence of activated microglia has been reported in the dorsolateral prefrontal cortex of autistic patients [30]. Moreover, Positron Emission Tomography (PET) imaging studies have revealed an activation of microglia in other brain regions [31, 32]. Postmortem studies of individuals with ASD have also shown activation of microglia, as well as an increase in density [30, 33, 34]. Reinforcing the idea of immunological dysfunction in ASD [35–39], this activation of microglia is accompanied by increased expression of proinflammatory factors, such as cytokines and chemokines, in the brain and cerebrospinal fluid of ASD subjects [30, 34]. In particular, the proinflammatory cytokine IL-6 and the chemokines MCP-1 and RANTES have been reported in neonatal blood samples from ASD children [40]. Brain arginine vasopressin, which is released during inflammation and plays a role in social behavior in mammals, has also been associated with ASD [41] and is considered as a biomarker of the disease. Quinolinic acid and neopterin, which are activated by indoleamine 2,3-dioxygenase (IDO), an enzyme upregulated by inflammatory factors and involved in depression [42], are decreased in

the cerebrospinal fluid (CSF) of ASD patients [43]. This may reflect an inadequacy or lack of maturation of the immune system. Despite the lack of evidence in humans that neuroinflammation plays a direct role in the pathogenesis of autism, research in animal models strongly suggests this to be the case. Deficits in microglial activity during brain development have been shown to be deleterious toward the formation of mature synapses, leading to an increase of immature synapses that could account for cognitive and ASD-like behavioral deficits [44, 45]. Therefore, in addition to genetic risk factors for inflammation, environmental factors leading to neuroinflammatory events are receiving more scrutiny in the etiology of autism. In this review, we will particularly focus on maternal immune activation (MIA), PUFAs, and microbiota as environmental risk factors that may participate in the etiology of ASD in combination to genetic risk factors.

3. Risk Factors for Neuroinflammation and Autism

3.1. Maternal Infection during Pregnancy and Autism Epidemiological Studies. Epidemiological studies strongly support a link between maternal infection and the development of ASD [18]. Compelling evidence supporting this hypothesis comes from a study on babies born from mothers exposed to the 1964 rubella pandemic. An increased incidence of children suffering from autistic disorders of 8–13% (versus 0.05% in controls) was found in this ecological cohort [46, 47]. Since then, ASD has been associated with numerous types of infectious agents, including not only viral, but also bacterial and parasitic infections [18]. Data collected from a Danish register of one million children born between 1980 and 2005 showed an association between infection-driven hospitalizations of pregnant women and an increased prevalence of ASD diagnoses in children. Interestingly, the time-window of infection is critical for the association with ASD and is different depending on the pathogen. The first trimester has been identified as critical for viral infections whereas bacterial infections during the second trimester have been associated with [16]. These observations suggest that the maternal immune effectors synthesized during infection rather than infection *per se* would be responsible for cerebral changes in the offspring leading to ASD. Furthermore, in addition to the temporal window of infection, the magnitude of inflammation (i.e., fever duration and hospitalization) is crucial for the prognosis of children born from infected mothers. Of note, recent evidence, showing that infection with Zika virus (ZIKV) during pregnancy induces major brain damage and microencephaly, has led to speculation on the role of this virus in developmental diseases such as ASD [48–50]. ZIKV has been shown to directly infect neural cells and promote their death but could also activate the immune system and in turn affect neuronal network-building in the fetus brain [51, 52].

One plausible mechanism supporting the association between maternal infection and ASD is cytokine production in the fetal brain in response to maternal inflammatory reaction [53]. Such cytokine expression may affect normal brain development in the offspring. In 2013, Zerbo et al. [54] showed that maternal fever during pregnancy is associated with ASD outcomes in the offspring while the risk of developing autism is reduced when mothers take antipyretic medications [54]. Moreover, mothers of children with ASD present higher blood levels of interferon gamma (IFNγ), IL-4, and IL-5 amid pregnancy [55]. Recent case-control studies have shed light on the positive correlation between proinflammatory cytokines levels in the amniotic fluid and occurrence of ASD [28, 56] (recently reviewed in Bilbo and Schwarz, 2012 [57]). Remarkably, IFNγ is critical for social behavior and frontocortical brain regions, a hallmark of ASD, as demonstrated in mice deficient in adaptive immunity, further reinforcing the link between social behavior and this cytokine [58]. Altogether, these associations give rise to the hypothesis that maternal immune activation (MIA) irremediably impacts the developing brain, which contributes to the etiology of autism [18, 59–61].

3.1.1. Animal Models. The clinical evidence highlighting MIA as a risk factor for ASD has motivated the development of several animal models. In particular, infection of pregnant rodents with pathogens (virus and bacteria) relevant to human and activation of maternal immune system with viral or bacterial endotoxins in the absence of pathogen have been widely used (reviewed in Patterson, 2011 [18]). Interestingly, despite the fact that different molecular pathways are activated in these models, considerable overlaps have been found in behavioral impairment consistent with ASD symptoms.

3.1.2. Active Viral/Bacterial Infections. Attempts to model prenatal infection in animals led to exposing pregnant rodents to the human influenza virus. Prenatally exposed offspring presented typical signs of altered neuronal migration [62], as well as astrogliosis [63], mimicking alterations found in ASD patients [61, 64]. In another prenatal infection study, Fatemi and colleagues reported increased expression of *Vldlr* and *Foxp2*, also consistent with data from human ASD patients [65]. Behavioral assessments of murine offspring are designed to mirror as closely as possible those used to observe ASD patients [66, 67]. Deficits in sensorimotor gating are typically assessed by a prepulse inhibition (PPI) paradigm, in which a weak prestimulus inhibits the reaction for a subsequent stronger startling stimulus. Patients suffering from ASD display deficits of prepulse inhibition as a manifestation of their general inability to filter out unnecessary information. This has been linked to abnormalities of sensorimotor gating. Adult offspring that had been exposed to influenza early in their gestation exhibit PPI deficits and altered exploratory and social behavior [68]. Recently, the influenza model was used in rhesus monkeys, an animal model more relevant for human brain development. Flu infection early in the third trimester leads to reduced volume of cortical grey matter, decreased white matter in the parietal cortex, and neuronal alterations. Such aberrations of brain development are all characteristic of ASD [5].

Bacterial infections have also been shown to increase the risk of developing autism [18]. Live bacterial infection models

were developed in rodents by infecting dams with Group B *Streptococcus* (GBS), the most common human pathogen in fetal environments. When exposed to GBS during pregnancy, the offspring recapitulated numerous neurobiological and behavioral autistic-like symptoms. Moreover, a gender dichotomy appears in offspring, which is a cardinal feature of human ASD [69].

Taken together, findings obtained in animal models of viral and bacterial infections support the hypothesis of deleterious effects of a prenatal infection in ASD. Notably, viruses are never found in the brains of offspring, suggesting that the maternal immune response to infectious agents is more relevant than the agents themselves in the detrimental effects of prenatal immune challenges [68]. In fact, animal studies show that infectious agents do not usually reach fetal compartments; however, cytokines from the mother can still cross the placental barrier and stimulate *de novo* synthesis of cytokines in the fetal brain [70]. To test whether altered expression of maternal and/or fetal cytokines might play a role in linking maternal infection and development of autism, other models using immune-activating agents have been developed and are widely used in present-day studies.

3.1.3. Viral/Bacterial Mimics. Viral and bacterial mimics activate the maternal immune system to induce cytokine release without any intervention of active viruses or bacteria with poly(I:C) and lipopolysaccharide (LPS) being the most studied. Poly(I:C) models have been very useful in deciphering the critical time-windows of infection relevant to ASD [71]. Poly(I:C) administration at midgestational time points (E9, E12.5) recapitulates ASD-like behavior in offspring, including decreased social behavior, ultrasonic vocalization deficits, repetitive behaviors, increased anxiety, and deficits in PPI [17, 72, 73]. Impaired ability to filter stimuli has been mostly associated with schizophrenia-like phenotypes, especially in rodents, but human adults suffering from ASD have similar sensorimotor gating deficits [74]. In rhesus monkeys, poly(I:C) injection during the first trimester leads to impaired social interaction, social attention, and repetitive behavior [75, 76]. Most of the behavioral impairments in offspring from mothers treated with poly(I:C) are observed with LPS [77]. Interestingly, late gestation administration of LPS triggered PPI deficits and social behavior alterations in offspring in adulthood [78, 79], while behavioral deficits appeared in infancy when mothers receive LPS at an early stage of gestation [80, 81]. Very low doses of LPS administered to rhesus monkeys at the end of gestation also induce PPI impairment in offspring [82]. Of note, LPS administration in mice pups at 14 days of postnatal age can also trigger behavioral deficits, which differ from adolescence to adulthood, with anxiety-like behavior appearing at adolescence, while depressive-like behavior develops during adulthood only [83]. Indeed, the exposition to viral or bacterial mimics during the whole brain developmental period seems to be critical for later life behavioral deficits classically observed in ASD.

Neurobiological changes induced by viral and bacterial mimics also share common features such as altered dopaminergic neurotransmission [70, 84, 85], altered myelin properties within frontostriatal-limbic circuits [86], an increase in GFAP-positive cells, hippocampal disorganization [87–89], and synaptic density turnover and transmission abnormalities [71]. Such impairment could be linked to alterations in developmental processes such as neuronal migration, establishment of neuronal layers, synaptogenesis, and synaptic pruning [90, 91]. Indeed, large number of reelin-expressing and newly born neurons are decreased in the hippocampus of poly(I:C)-treated pups whereas the amount of apoptotic cells is increased [92]. The decreased number of reelin-positive cells, together with GAD67- and parvalbumin-positive cells, is found in the developing hippocampus and prefrontal cortex of offspring from LPS-injected mothers [93–96]. Interestingly, early pregnancy administration of LPS increases spine density in the hippocampus of offspring during development but decreases it in adulthood [97], suggesting a transient developmental effect on spines close to the inflammatory response window. This is consistent with the observed activation of microglia, the brain's innate immune cell recently highlighted as key in developmental brain wiring [98, 99], in the brain of pups from poly(I:C)-injected dams [73]. Therefore, it appears that immune challenges during pregnancy lead to the impairment of structural development and wiring. This could be linked to altered expression of neuronal migration genes [100] or to defects in synaptic pruning and synaptogenesis with a plausible involvement of microglia [45].

Numerous studies have highlighted that developmental impairment triggered by inflammatory mimics could involve cytokines [72]. Indeed, poly(I:C) is a synthetic double-stranded RNA that induces inflammatory responses by binding to Toll-Like Receptor- (TLR-) 3 [101]. Like viral particles, poly(I:C) is a potent inducer of not only classical interleukins (e.g., IL-1β and IL-6) or TNFα, but also type 1 IFN (α and β). LPS, a gram-negative bacteria cell wall component, activates TLR4. Most of the cytokines produced in response to poly(I:C) or LPS are quite similar, except for type 1 IFN release, which is only elicited by poly(I:C). In addition, LPS treatment leads to a longer and larger release of IL-6 [58], a cytokine consistently increased in ASD patients [60, 102, 103]. Prenatal administration of poly(I:C) and LPS activates inflammatory response not only in mothers, but also in the fetus [76, 104, 105]. Overall, data using manipulation of cytokines have reported that IL-6 is essential for MIA-induced abnormalities in offspring's brain and behavior [17, 18, 20, 70, 106] and supports evidence from human ASD patients [17, 60, 102]. Recent data pointed that IL-17, a cytokine found in the blood of ASD children [107, 108] and of animal model of MIA [109], is involved in some symptoms of MIA-induced ASD-like behavior [110] providing additional data on the role of cytokines in fetal brain development.

In summary, MIA triggered by active pathogens or noninfectious endotoxins (poly(I:C) and LPS) administered during pregnancy recapitulates ASD-like behaviors and neurobiological alterations in offspring. MIA-induced long-term deficits depend on the stage of pregnancy that is targeted, in accordance with observational studies in humans [79, 92]. Animal models of MIA offer the opportunity to better understand the mechanisms underlying MIA and autism-like disorders to develop specific anti-inflammatory strategies to protect mothers at risk of having children with ASD.

3.1.4. Interactions between ASD Risk Factor Genes and MIA. One important question arises from "inflammatory genes" × "inflammatory insults" as risk factors for autism. As previously described, MIA is an environmental risk factor for ASD that modulates the same inflammatory mediators identified as ASD susceptibility genes [111]. While many studies provide evidence for altered immune responses in patients with ASD [12, 111], recent transcriptome and protein interactome network analyses have revealed a direct link between genes implicated in ASD and immune signaling [112, 113]. Among the immunologic gene variants identified in ASD (e.g., *mecp2*, *il-1*, *mhc*, and *c4*), many are expressed by microglia or modulate their activity, especially during brain development. Of note, the deletion of mGluR5, whose expression is decreased in the brains of ASD patients, increased the number of microglia in mice [114]. Indeed, the contribution of genetic factors and environmental insults targeting the immune status to ASD risk could be of particular importance during the developing period. Studies using transgenic mice with ASD-associated mutations reported developmental defects in these animals. However, to our knowledge, the interaction between MIA and immunity risk variants in ASD in humans or animal models has not yet been reported.

Several studies have reported that early-life inflammation has differential effects in patients or in transgenic mice with targeted mutation of genes identified in ASD. Early prenatal inflammation in mice (E9) has been shown to trigger some behavioral and neurobiological abnormalities in mice expressing the human mutation of *disc1* [115]. Autism-like behaviors such as sensorimotor gating deficiencies and impaired social behavior were modified by MIA depending on the type of *disc1* mutation. One-half of patients with tuberous sclerosis have been shown to develop ASD. In a mouse model of tuberous sclerosis (*tsc2* haploinsufficiency), maternal immune challenge led to impaired social behavior in adult offspring. Moreover, the authors found that seasonal flu activity in late gestation and TSC mutations increased the risk of ASD in offspring. TSC is involved in the mTOR pathway as well as other ASD-associated genes, for instance, *pten*, *eif4e*, or *fmr1* [15]. In another recent study, alterations in sensorimotor gating and attention processes were observed in the offspring of *Nurr1* heterozygous mice undergoing prenatal immune challenge [116]. In another study, a positive association was found between copy number variants in some hot spots for ASD pathology and maternal infection or fever during pregnancy [117, 118]. Epigenetic changes after maternal immune activation have also been observed in the offspring's brains, including abnormalities in histone acetylation in genes known to be involved in neurodevelopment [119]. Another work has identified hypomethylation of ASD-related genes such as Mecp2 after MIA [120]. Altogether, these data strongly suggest that mutations in immune or nonimmune genes and environmental inflammatory insults are key in ASD. However, further studies are needed to understand how these factors converge on common molecular networks during brain development.

3.2. Gut Microbiota and Autism. Emerging evidences suggest that the microbiome plays an important role not only in immunity but also in neurodevelopmental disorders such as autism [19, 20]. Bacteria within the gut are complex ecosystems which produce metabolites, such as short-chain fatty acids (SCFAs), vitamins, and antimicrobial peptides [121]. The gut microbiota and its metabolites participate to the body physiology, including the brain [122], while microbiota alterations, often referred to as dysbiosis, participate to numerous pathologies, including neuropsychiatric disorder. Importantly, food composition influences gut microbiota composition and very recent data obtained in rodents causally linked maternal diet, gut microbial imbalance, and neurodevelopmental disorders [123]. Among the pathways through which gut microbiota influences brain functions, the immune system is particularly relevant to neuroinflammation and ASD [20].

3.2.1. Epidemiological Studies. Gut-brain interactions are now recognized to play a major role in neurodevelopment and in regulating behavior. In fact, ASD subjects often suffer from gastrointestinal distress [124], a comorbid factor for autism [125]. Gastrointestinal features include chronic abdominal pain and alterations in bowel habits, leading to questions about the nutritional status and the diet quality of children with ASD [125, 126]. Often, gastrointestinal symptoms remain mostly untreated and can give rise to behavioral alterations. Microbiome-related factors may also be responsible for increases in ASD prevalence [127]. Dysbiosis has been found in children with ASD compared to healthy controls [128, 129]. Gastrointestinal microflora is dysregulated in late onset autistic children [130], leading to alterations of microbial species density and variations of bacterial metabolites in feces and urine [131]. Studies have shown that the Clostridia species is consistently highly represented in feces from autistic children [129, 132]. There is also a greater abundance of *Bacteroides* and Firmicutes in severe ASD [130, 133]. Clostridia toxins are known to affect neurotransmitter functions that can possibly result in neurobehavioral changes. Dysregulated activity of the autonomic nervous system, associated with anxiety and stress-responsiveness, may also play a role in increased intestinal epithelial permeability in ASD subjects [134], leading to observed behavioral changes. Altered intestinal permeability could represent a possible explanation for behavioral abnormalities observed in ASD, as immune molecules or products of diverse microbial populations could more likely enter the blood circulation and affect the brain. Conversely, antibiotic therapy using vancomycin during a short period improved behavior [135]. Dysregulated gut-brain communications, in addition to genetic heritability, could account for some of the extreme diversity seen across wide spectrum for autism, depending on the severity of alterations of microbial communities.

3.2.2. Animal Models. Only a few studies have shown mechanistic connections between alterations of the gut microbiota and behavioral changes observed in ASD patients. Rodent models are useful for examining these interactions and to discover new targets from diet patterns to therapeutic treatment using probiotics instead of antibiotics.

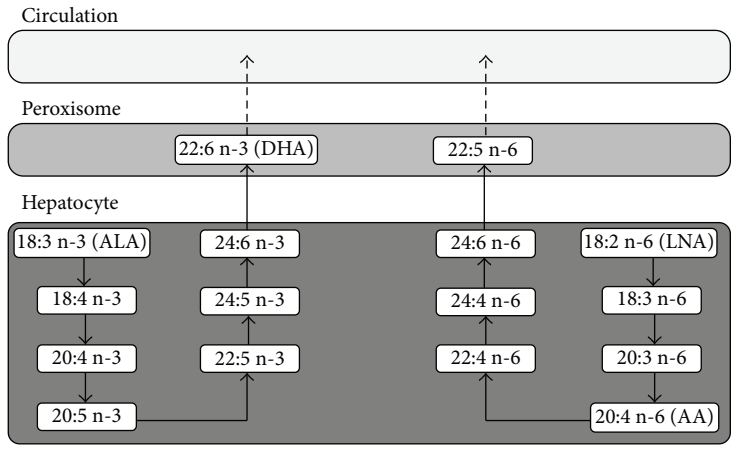


Figure 2: Synthesis of PUFAs in the liver. Precursors of n-3 and n-6 polyunsaturated fatty acid (PUFA) α-linolenic (ALA; 18:3 n-3) and linoleic acid (LNA; 18:2 n-6) can be desaturated and elongated. This leads to the synthesis of long-chain PUFAs, including docosahexaenoic acid (DHA; 22:6 n-3), but also arachidonic acid (AA; 20:4 n-6) which are carried into the blood as free forms or lipoproteins. Both, n-3 and n-6 long-chain PUFAs, compete for their synthesis (for desaturation and elongation), meaning that PUFAs intake significantly impacts their cerebral incorporation level.

Rodent models of ASD have been used to determine a link between alterations of the gut microbiome, associated changes in microbial factors, and their implication in behavioral changes observed in autistic-like behavior [136]. These changes were rapidly reversed by the use of probiotics in an MIA model [20]. Clostridia and *Bacteroides* species were the primary drivers of these microbiota differences. Offspring from an MIA model that received *Bacteroides fragilis* as a probiotic significantly recovered the abundance of some taxis. Moreover, *B. fragilis* dramatically attenuated altered behavior observed in offspring including communication, repetitive behaviors, and reduced anxiety. Animals subjected to valproic acid exposure *in utero*, a mouse model of ASD, show disturbed social interactions and increased expression of neuroinflammatory markers alongside intestinal inflammation [136]. Prenatal exposure to valproic acid has a transgenerational impact on the gut microbiota as observed by increased levels of short-chain fatty acids (SCFAs) like butyrate in the caecum of offspring [136]. Interestingly, SCFAs are considered neuroactive metabolites as they can cross the blood-brain barrier and modulate CNS function and behavior [137–139]. Interestingly, prenatal administration of propionic acid, a SCFA byproduct of enteric bacteria found in ASD subjects [140], triggers some of the ASD-like behavior [141]. Notably, propionic acid intracerebral administration activates microglia, suggesting a role of this SCFA in neuroinflammation [139]. Because the maternal transmission of immune factors induces specific changes in the gut microbiome, it could therefore affect the neurometabolites available to the offspring that could potentially lead to autistic-like behaviors or alterations of the gut epithelium. Further studies are needed to better understand whether changes in the gut microbiota of children could be a risk factor for dysbiosis, neuroinflammatory processes, and ASD.

3.2.3. Interactions between ASD Risk Factor Genes and Gut Microbiota. Abnormalities in immunity could be closely linked to the gut microbiota and dysbiosis in ASD. The gut microbiota stimulates both nonspecific and specific immunity in the first years of age [142] and has been recently suggested to regulate microglia activity [21]. After birth, the low-grade inflammation, although generally beneficial, triggered by the continuous immune stimulation provided by the gut microbiota [143] could be detrimental in children at risk for ASD because of genetic synaptic dysfunction. However, such a link has been poorly studied. Recently, transgenic mice with a defect in inflammasome/IL-1β production (i.e., caspase 1 KO mice) have been shown to have a different microbiota composition than wild-type mice, together with depressive-like behavior, suggesting that behavioral impairment linked to dysbiosis requires inflammasome activity [144, 145]. Whether a specific interaction between genes identified as risk factors for autism and dysbiosis/microbiota changes exists in patients with ASD is unknown. Further clinical and fundamental research on this issue is warranted.

3.3. Dietary N-3 Polyunsaturated Fatty Acids and Autism. Several studies have highlighted the fundamental role of lipids in neuronal processes and immune modulation, which are implicated in ASD. In particular, polyunsaturated fatty acids (PUFAs) are essential fatty acids required for brain development and maturation [22]. Because they need to be provided by alimentation (Figure 2), deficiencies or imbalances in these nutrients, both precursors and long chains strongly affect brain function, not only during development, but also throughout life and especially during periods of neuroinflammation. Recent evidence suggests that n-3 PUFA homeostasis may be altered in ASD, either as a result of nutritional imbalance or genetic defect [146].

3.3.1. Epidemiological Studies. Total n-3 PUFAs in the plasma of autistic children are decreased without any changes in the n-6 PUFAs family [147, 148]. A positive association between anti-myelin basic protein (MBP) antibodies and low levels

of the main n-3 PUFA found in the brain (docosahexaenoic acid, DHA) has been reported in autistic children [149]. Parental health questionnaires and red blood cell (RBC) fatty acid measurements have highlighted a decrease in DHA and total n-3 PUFAs in both autistic and Asperger patients. More recently, Al-Farsi and colleagues reported lower consumption of DHA foodstuff and a concomitant decrease in DHA levels in the plasma of children with ASD [150]. A case-control study in California measured fatty acids in the blood of 153 autistic children and 97 controls and showed that DHA is decreased in the phosphatidylethanolamine (PE) [151]. Another case-control study in Saudi Arabia showed altered phospholipid and fatty acid profiles in ASD patients [152]. Consistent with the idea of impaired PUFAs cerebral level and metabolism, Brigandi and colleagues uncovered a massive decrease in AA and DHA. They also found an increase in proinflammatory derivative Prostaglandin E2 in a subset of patients [153]. Interestingly, gene expression of FABP3, FABP5, and FABP7 has been shown to be modulated in psychiatric illnesses such as schizophrenia and ASD [154]. In the brains of ASD patients, FABP7, which binds DHA preferentially, was upregulated in both the frontal and parietal cortex [155]. As in schizophrenic patients, PUFA distribution and metabolism are markedly altered in ASD patients. Six weeks of DHA and eicosapentaenoic acid (EPA) supplementation in children with autism led to improvement of symptoms, especially stereotypy and hyperactivity [156]. A 12-week n-3 fatty acid dietary supplementation also led to the improvement of hyperactivity in autistic children [157]. Another study using a DHA, EPA, and AA dietary supplementation for 3 weeks in autistic children reported improved behavioral performance in two-thirds of children [158]. Recently, an open-label pilot study in Singapore found positive associations between blood fatty acid levels and changes in the core symptoms of ASD following a 12-week n-3 PUFA dietary supplementation [159]. However, several interventional studies with n-3 PUFAs failed to reproduce these beneficial effects [160–162]. Thus, larger cohorts and accurate ASD behavioral phenotypes are needed to clearly decipher the potential beneficial effects of n-3 PUFA dietary supplementation on behavioral deficits. In addition, the inflammatory status and/or the microbiota composition should be considered in interventional studies with n-3 PUFAs [15, 124].

3.3.2. Animal Models. Some human-like ASD alterations were observed in preclinical models of n-3 PUFA dietary deficit. Developmental n-3 PUFA depletion in rodents led to decreased levels of serotonin in the prefrontal cortex, as observed in autistic children [163, 164]. Numerous studies on n-3 PUFA deficiency models revealed profound alterations in GABAergic, dopaminergic, and cholinergic neurotransmission [165–168]. Importantly, long-term dietary n-3 PUFA deficiency triggers the development of ASD-like behavioral impairment in rodents, including reduced PPI [169], social interactions [170–174], and increased anxiety [171–173, 175]. Conversely, some studies investigated the possible beneficial role of n-3 PUFA dietary supplementation at weaning in different mice models of ASD. In the Fmr1-KO mice model of autism, n-3 PUFA supplementation rescues not only social defects but also memory impairments and some neurobiological imbalance [26]. In a model of prenatal inflammation by poly(I:C), DHA supplementation improves social interactions, decreases repetitive behaviors, and normalizes IL-6 levels after immune challenge [176]. A recent study on an early MIA model showed that n-3 PUFA-enriched diet alleviates ASD-like symptoms, altered GAD67 protein levels, metabolic changes, and PPI deficits [177]. As n-3 PUFAs potently regulate neuroinflammatory processes, microglia activity, and synaptic plasticity [24, 174, 178], their beneficial effects could be linked to their action on neuroinflammatory processes in the developing brain. Interestingly, n-3 PUFAs modify the gut microbiota composition, but their effect in ASD-like behavior has not yet been unraveled [179].

Taken together, both studies in humans and animals identify long-chain PUFAs, especially those from the n-3 series, as interesting candidates in curative strategies due to their ability to counteract some ASD-like symptoms and ameliorate inflammation. Several studies have also shown their ability to modulate the microbiota and vice versa. Indeed, one study reports that SCFA propionic acid administered into the brain of rats alters lipid metabolism, in particular the one of PUFAs [180]. According to a recent report, n-3 PUFA deficiency induces dysbiosis, with increased numbers of potential pathobionts, including bacteria from the Enterobacteriaceae family [181]. Conversely, n-3 PUFA supplementation prevents the bloom of Enterobacteriaceae, as well as the translocation of bacteria into the submucosal region, and instead promotes the enrichment of *Lactobacillus* and *Bifidobacterium* species [181, 182]. Using a genetic model of n-3 PUFA supplementation (Fat-1), Kaliannan et al. demonstrated that elevated n-3 PUFA levels enhance intestinal production and secretion of intestinal alkaline phosphatase (IAP), which induces changes in the gut bacteria composition, resulting in decreased LPS production and gut permeability and, ultimately, in reduced metabolic endotoxemia and inflammation [183]. N-3 PUFA deficiency during development (over gestation and lactation) also alters the normal trajectory of intestinal microbe establishment in the intestine of offspring, with lowered bacterial density, a decreased ratio of Firmicutes to Bacteroidetes, and a decrease in several other dominant microbes [184]. These data suggest that n-3 PUFA levels modulate microbiota composition and activity during development. However, more results are needed to unravel the underlying mechanisms.

N-3 PUFAs are likely to be taken up in large amounts by the brain during the end of gestation and the first month of life [185, 186]. The use of n-3 PUFA supplementation, especially during pregnancy and lactation, could help prevent ASD in children at risk. In this context, developmental animal studies giving n-3 PUFA supplementation from conception might be a fruitful line of investigation.

3.3.3. Interactions between ASD Risk Factor Genes and Dietary PUFAs. Genetic interactions and PUFAs content have been poorly studied in ASD. However, some links exist between lipid metabolism gene alleles, PUFA metabolism, and brain diseases. Indeed, the APOE4 allele, which is a well-known genetic risk factor of Alzheimer's disease, is involved in disrupted PUFA metabolism with a shift to long-chain n-3 PUFA

oxidation [187, 188]. Genetic variability in *fads* (desaturases involved in the metabolization of PUFAs) is involved in the bioavailability of long-chain PUFAs AA and DHA to the brain, as well as brain development and cognitive impairment [189–192]. Polymorphism of several genes involved in PUFA metabolism or inflammation is crucial in the efficacy of dietary n-3 PUFA supplementation on inflammation and triglyceride blood level [193, 194]. The relationship between PUFA metabolism genes, inflammation, and efficacy of n-3 PUFA dietary supplementation remains to be determined. This is of particular importance as concentration and expression of phospholipase A2, a phospholipase at the cross of PUFA metabolism and inflammation, are higher in ASD patients but are reduced by dietary supplementation with EPA [195–197]. N-3 PUFAs potently regulate not only neuroinflammatory pathways [24, 178, 198] but also synaptic plasticity [25, 173, 199–201]. These properties could be of high interest in correcting synaptic defects linked to genetic risk factors. Indeed, dietary n-3 PUFA supplementation rescues social behavioral impairment and neuroinflammation in a mouse model of fragile X syndrome [26].

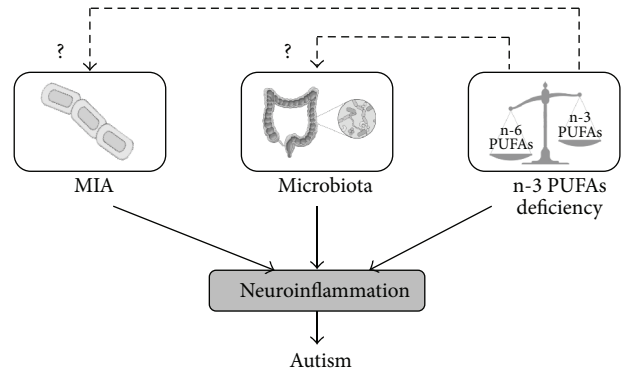


Figure 3: Environmental factors influencing neuroinflammation in autism. Early inflammation in the brain is a well-recognized risk factor for autism. Neuroinflammation is a process influenced by environmental factors such as MIA, microbiota, and n-3 PUFAs deficiency. However, crosstalks between these factors can make the situation increasingly complex. For instance, insufficient dietary n-3 PUFAs intake unavoidably impacts microbiota composition as well as MIA immunoreactivity possibly potentiating the proinflammatory response. This, in turn, can lead to increased risks for autism.

4. Conclusion

The pathogenesis of ASD is linked to maternal immune activation-triggered neuroinflammatory events in the developing brain of offspring, potentially in association with dysbiosis during pregnancy and/or infancy. The dysregulation of these components during early development leads to brain malformation and alterations that can be imprinted until adulthood. Thus, elucidating the brain-microbiota axis is critical for finding more effective strategies to prevent or treat ASD. In particular, nutritional interventions, especially those taking advantage of the anti-inflammatory properties of n-3 PUFAs, are promising candidates, as they would potentially modulate both neuroinflammatory components and microbiota dysbiosis in ASD (Figure 3). Further studies are therefore needed to decipher the mechanisms underlying the beneficial effect of n-3 PUFA diets in ASD.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Charlotte Madore and Quentin Leyrolle equally contributed to the work.

Acknowledgments

The authors apologize to those whose valuable work was not cited owing to space limitations. Quentin Leyrolle, Corinne Joffre, Chloé Lacabanne, Agnes Nadjar, and Sophie Layé are supported by Institut National de la Recherche Agronomique (INRA), Université de Bordeaux, Région Aquitaine, and Agence Nationale de la Recherche (ANR). Quentin Leyrolle is funded by the Institut National de la Santé et de la Recherche Médicale (INSERM), la Région Île de France, and the Cerebral Palsy Foundation. Chloé Lacabanne is supported by the Canadian Institutes of Health Research (CIHR) and ANR for the program Investissements d'Avenir d'Excellence (IDEX). The authors gratefully thank Dr. Lucien Garo and Dr. Kasia Szyszkowickz for English editing.

References

[1] M. D. Kogan, S. J. Blumberg, L. A. Schieve et al., "Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US, 2007," *Pediatrics*, vol. 124, no. 5, pp. 1395–1403, 2009.

[2] DSM-5 Overview: The Future Manual—APA DSM-5, http://www.dsm5.org/about/Pages/DSMVOverview.aspx.

[3] "neurodiversity.com-h. asperger: Die Autistischen Psychopathen' im Kindesalter," http://www.neurodiversity.com/library_asperger_1944.html.

[4] L. Kanner, "Problems of nosology and psychodynamics of early infantile autism," *American Journal of Orthopsychiatry*, vol. 19, no. 3, pp. 416–426, 1949.

[5] M.-C. Lai, M. V. Lombardo, J. Suckling et al., "Biological sex affects the neurobiology of autism," *Brain*, vol. 136, no. 9, pp. 2799–2815, 2013.

[6] J. Hallmayer, S. Cleveland, A. Torres et al., "Genetic heritability and shared environmental factors among twin pairs with autism," *Archives of General Psychiatry*, vol. 68, no. 11, pp. 1095–1102, 2011.

[7] B. S. Abrahams and D. H. Geschwind, "Advances in autism genetics: on the threshold of a new neurobiology," *Nature Reviews Genetics*, vol. 9, no. 5, pp. 341–355, 2008.

[8] H. Won, W. Mah, and E. Kim, "Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses," *Frontiers in Molecular Neuroscience*, vol. 6, p. 19, 2013.

[9] D. Oddi, W. E. Crusio, F. R. D'Amato, and S. Pietropaolo, "Monogenic mouse models of social dysfunction: Implications for autism," *Behavioural Brain Research*, vol. 251, pp. 75–84, 2013.

[10] A. Shcheglovitov, O. Shcheglovitova, M. Yazawa et al., "SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients," *Nature*, vol. 503, no. 7475, pp. 267–271, 2013.

[11] O. Peñagarikano, B. S. Abrahams, E. I. Herman et al., "Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits," *Cell*, vol. 147, no. 1, pp. 235–246, 2011.

[12] I. Voineagu, X. Wang, P. Johnston et al., "Transcriptomic analysis of autistic brain reveals convergent molecular pathology," *Nature*, vol. 474, no. 7351, pp. 380–386, 2011.

[13] R. P. Warren, V. K. Singh, P. Cole et al., "Increased frequency of the null allele at the complement C4b locus in autism," *Clinical and Experimental Immunology*, vol. 83, no. 3, pp. 438–440, 1991.

[14] D. Odell, A. Maciulis, A. Cutler et al., "Confirmation of the association of the C4B null allelle in autism," *Human Immunology*, vol. 66, no. 2, pp. 140–145, 2005.

[15] A. Estes, L. Zwaigenbaum, H. Gu et al., "Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2 years of life," *Journal of Neurodevelopmental Disorders*, vol. 7, no. 1, article 9117, 2015.

[16] H. Ó. Atladóttir, P. Thorsen, L. Østergaard et al., "Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders," *Journal of Autism and Developmental Disorders*, vol. 40, no. 12, pp. 1423–1430, 2010.

[17] S. E. P. Smith, J. Li, K. Garbett, K. Mirnics, and P. H. Patterson, "Maternal immune activation alters fetal brain development through interleukin-6," *The Journal of Neuroscience*, vol. 27, no. 40, pp. 10695–10702, 2007.

[18] P. H. Patterson, "Maternal infection and immune involvement in autism," *Trends in Molecular Medicine*, vol. 17, no. 7, pp. 389–394, 2011.

[19] J. F. Cryan and T. G. Dinan, "More than a gut feeling: the microbiota regulates neurodevelopment and behavior," *Neuropsychopharmacology*, vol. 40, no. 1, pp. 241–242, 2015.

[20] E. Y. Hsiao, S. W. McBride, S. Hsien et al., "Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders," *Cell*, vol. 155, no. 7, pp. 1451–1463, 2013.

[21] D. Erny, A. L. H. De Angelis, D. Jaitin et al., "Host microbiota constantly control maturation and function of microglia in the CNS," *Nature Neuroscience*, vol. 18, no. 7, pp. 965–977, 2015.

[22] R. P. Bazinet and S. Layé, "Polyunsaturated fatty acids and their metabolites in brain function and disease," *Nature Reviews Neuroscience*, vol. 15, no. 12, pp. 771–785, 2014.

[23] P. C. Calder, "Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?" *British Journal of Clinical Pharmacology*, vol. 75, no. 3, pp. 645–662, 2013.

[24] J.-C. Delpech, A. Thomazeau, C. Madore et al., "Dietary n-3 PUFAs deficiency increases vulnerability to inflammation-induced spatial memory impairment," *Neuropsychopharmacology*, vol. 40, no. 12, pp. 2774–2787, 2015.

[25] C. Madore, A. Nadjar, J.-C. Delpech et al., "Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system and neuronal plasticity-associated genes," *Brain, Behavior, and Immunity*, vol. 41, no. 1, pp. 22–31, 2014.

[26] S. Pietropaolo, M. 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.

[27] M. L. Estes and A. K. McAllister, "Maternal T_H17 cells take a toll on baby's brain," *Science*, vol. 351, no. 6276, pp. 919–920, 2016.

[28] P. Goines and J. Van de Water, "The immune system's role in the biology of autism," *Current Opinion in Neurology*, vol. 23, no. 2, pp. 111–117, 2010.

[29] J. Mead and P. Ashwood, "Evidence supporting an altered immune response in ASD," *Immunology Letters*, vol. 163, no. 1, pp. 49–55, 2015.

[30] J. T. Morgan, G. Chana, C. A. Pardo et al., "Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism," *Biological Psychiatry*, vol. 68, no. 4, pp. 368–376, 2010.

[31] K. Suzuki, G. Sugihara, Y. Ouchi et al., "Microglial activation in young adults with autism spectrum disorder," *Archives of General Psychiatry*, vol. 70, no. 1, pp. 49–58, 2013.

[32] T. Takano, "Role of microglia in autism: recent advances," *Developmental Neuroscience*, vol. 37, no. 3, pp. 195–202, 2015.

[33] N. A. Tetreault, A. Y. Hakeem, S. Jiang et al., "Microglia in the cerebral cortex in autism," *Journal of Autism and Developmental Disorders*, vol. 42, no. 12, pp. 2569–2584, 2012.

[34] D. L. Vargas, C. Nascimbene, C. Krishnan, A. W. Zimmerman, and C. A. Pardo, "Neuroglial activation and neuroinflammation in the brain of patients with autism," *Annals of Neurology*, vol. 57, no. 1, pp. 67–81, 2005.

[35] T. Cao, T. C. Thomas, J. M. Ziebell, J. R. Pauly, and J. Lifshitz, "Morphological and genetic activation of microglia after diffuse traumatic brain injury in the rat," *Neuroscience*, vol. 225, pp. 65–75, 2012.

[36] P. Ashwood, P. Krakowiak, I. Hertz-Picciotto, R. Hansen, I. Pessah, and J. Van de Water, "Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome," *Brain, Behavior, and Immunity*, vol. 25, no. 1, pp. 40–45, 2011.

[37] C. A. Molloy, A. L. Morrow, J. Meinzen-Derr et al., "Elevated cytokine levels in children with autism spectrum disorder," *Journal of Neuroimmunology*, vol. 172, no. 1-2, pp. 198–205, 2006.

[38] J. Li, D. Moazed, and S. P. Gygi, "Association of the histone methyltransferase Set2 with RNA polymerase II plays a role in transcription elongation," *Journal of Biological Chemistry*, vol. 277, no. 51, pp. 49383–49388, 2002.

[39] H. Wei, H. Zou, A. M. Sheikh et al., "IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation," *Journal of Neuroinflammation*, vol. 8, article 52, 2011.

[40] O. Zerbo, C. Yoshida, J. K. Grether et al., "Neonatal cytokines and chemokines and risk of Autism Spectrum Disorder: the Early Markers for Autism (EMA) study: a case-control study," *Journal of Neuroinflammation*, vol. 11, article 113, 2014.

[41] D. S. Carson, J. P. Garner, S. A. Hyde et al., "Arginine vasopressin is a blood-based biomarker of social functioning in children with autism," *PLoS ONE*, vol. 10, no. 7, Article ID e0132224, 2015.

[42] R. Dantzer, J. C. O'Connor, G. G. Freund, R. W. Johnson, and K. W. Kelley, "From inflammation to sickness and depression: when the immune system subjugates the brain," *Nature Reviews Neuroscience*, vol. 9, no. 1, pp. 46–56, 2008.

[43] A. W. Zimmerman, H. Jyonouchi, A. M. Comi et al., "Cerebrospinal fluid and serum markers of inflammation in autism," *Pediatric Neurology*, vol. 33, no. 3, pp. 195–201, 2005.

[44] R. C. Paolicelli, G. Bolasco, F. Pagani et al., "Synaptic pruning by microglia is necessary for normal brain development," *Science*, vol. 333, no. 6048, pp. 1456–1458, 2011.

[45] Y. Zhan, R. C. Paolicelli, F. Sforazzini et al., "Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior," *Nature Neuroscience*, vol. 17, no. 3, pp. 400–406, 2014.

[46] S. Chess, "Autism in children with congenital rubella," *Journal of Autism and Childhood Schizophrenia*, vol. 1, no. 1, pp. 33–47, 1971.

[47] S. Chess, "Follow-up report on autism in congenital rubella," *Journal of Autism and Childhood Schizophrenia*, vol. 7, no. 1, pp. 69–81, 1977.

[48] P. Brasil, J. P. Pereira Jr., C. Raja Gabaglia et al., "Zika virus infection in pregnant women in Rio de Janeiro—preliminary report," *The New England Journal of Medicine*, 2016.

[49] M. de Fatima Vasco Aragao, V. van der Linden, A. M. Brainer-Lima et al., "Clinical features and neuroimaging (CT and MRI) findings in presumed Zika virus related congenital infection and microcephaly: retrospective case series study," *British Medical Journal*, vol. 353, p. i1901, 2016.

[50] G. Gioula, M. L. Nunes, and D. I. Zafeiriou, "An emerging cause of concern in Europe: zika virus, the developing CNS and the pediatric neurologist," *European Journal of Paediatric Neurology*, vol. 20, no. 4, pp. 497–499, 2016.

[51] T. J. Nowakowski, A. A. Pollen, E. Di Lullo, C. Sandoval-Espinosa, M. Bershteyn, and A. R. Kriegstein, "Expression analysis highlights AXL as a candidate zika virus entry receptor in neural stem cells," *Cell Stem Cell*, vol. 18, no. 5, pp. 591–596, 2016.

[52] J. Dang, S. K. Tiwari, G. Lichinchi et al., "Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3," *Cell Stem Cell*, vol. 19, no. 2, pp. 258–265, 2016.

[53] M. V. Zaretsky, J. M. Alexander, W. Byrd, and R. E. Bawdon, "Transfer of inflammatory cytokines across the placenta," *Obstetrics & Gynecology*, vol. 103, no. 3, pp. 546–550, 2004.

[54] O. Zerbo, A.-M. Iosif, C. Walker, S. Ozonoff, R. L. Hansen, and I. Hertz-Picciotto, "Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (childhood autism risks from genetics and environment) study," *Journal of Autism and Developmental Disorders*, vol. 43, no. 1, pp. 25–33, 2013.

[55] P. E. Goines, L. A. Croen, D. Braunschweig et al., "Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A Case-Control Study," *Molecular Autism*, vol. 2, no. 1, article 13, 2011.

[56] M. W. Abdallah, N. Larsen, J. Grove et al., "Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders," *World Journal of Biological Psychiatry*, vol. 14, no. 7, pp. 528–538, 2013.

[57] S. D. Bilbo and J. M. Schwarz, "The immune system and developmental programming of brain and behavior," *Frontiers in Neuroendocrinology*, vol. 33, no. 3, pp. 267–286, 2012.

[58] A. J. Filiano, Y. Xu, N. J. Tustison et al., "Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour," *Nature*, vol. 535, no. 7612, pp. 425–429, 2016.

[59] P. H. Patterson, "Immune involvement in schizophrenia and autism: etiology, pathology and animal models," *Behavioural Brain Research*, vol. 204, no. 2, pp. 313–321, 2009.

[60] E. C. Parker-Athill and J. Tan, "Maternal immune activation and autism spectrum disorder: interleukin-6 signaling as a key mechanistic pathway," *NeuroSignals*, vol. 18, no. 2, pp. 113–128, 2010.

[61] I. Knuesel, L. Chicha, M. Britschgi et al., "Maternal immune activation and abnormal brain development across CNS disorders," *Nature Reviews Neurology*, vol. 10, no. 11, pp. 643–660, 2014.

[62] S. H. Fatemi, E. S. Emamian, D. Kist et al., "Defective corticogenesis and reduction in Reelin immunoreactivity in cortex and hippocampus of prenatally infected neonatal mice," *Molecular Psychiatry*, vol. 4, no. 2, pp. 145–154, 1999.

[63] S. H. Fatemi, E. S. Emamian, R. W. Sidwell et al., "Human influenza viral infection in utero alters glial fibrillary acidic protein immunoreactivity in the developing brains of neonatal mice," *Molecular Psychiatry*, vol. 7, no. 6, pp. 633–640, 2002.

[64] S. H. Fatemi, J. Earle, R. Kanodia et al., "Prenatal viral infection leads to pyramidal cell atrophy and macrocephaly in adulthood: implications for genesis of autism and schizophrenia," *Cellular and Molecular Neurobiology*, vol. 22, no. 1, pp. 25–33, 2002.

[65] S. H. Fatemi, T. J. Reutiman, T. D. Folsom et al., "Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorders," *Schizophrenia Research*, vol. 99, no. 1–3, pp. 56–70, 2008.

[66] J. L. Silverman, M. Yang, C. Lord, and J. N. Crawley, "Behavioural phenotyping assays for mouse models of autism," *Nature Reviews Neuroscience*, vol. 11, no. 7, pp. 490–502, 2010.

[67] E. Pasciuto, S. C. Borrie, A. K. Kanellopoulos et al., "Autism spectrum disorders: translating human deficits into mouse behavior," *Neurobiology of Learning and Memory*, vol. 124, pp. 71–87, 2015.

[68] L. Shi, S. H. Fatemi, R. W. Sidwell, and P. H. Patterson, "Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring," *The Journal of Neuroscience*, vol. 23, no. 1, pp. 297–302, 2003.

[69] S. M. Schaafsma and D. W. Pfaff, "Etiologies underlying sex differences in Autism Spectrum Disorders," *Frontiers in Neuroendocrinology*, vol. 35, no. 3, pp. 255–271, 2014.

[70] H. Ashdown, Y. Dumont, M. Ng, S. Poole, P. Boksa, and G. N. Luheshi, "The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia," *Molecular Psychiatry*, vol. 11, no. 1, pp. 47–55, 2006.

[71] P. Coiro, R. Padmashri, A. Suresh et al., "Impaired synaptic development in a maternal immune activation mouse model of neurodevelopmental disorders," *Brain, Behavior, and Immunity*, vol. 50, pp. 249–258, 2015.

[72] N. V. Malkova, C. Z. Yu, E. Y. Hsiao, M. J. Moore, and P. H. Patterson, "Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism," *Brain, Behavior, and Immunity*, vol. 26, no. 4, pp. 607–616, 2012.

[73] F. Zhu, Y. Zheng, Y. Liu, X. Zhang, and J. Zhao, "Minocycline alleviates behavioral deficits and inhibits microglial activation in the offspring of pregnant mice after administration of polyriboinosinic-polyribocytidilic acid," *Psychiatry Research*, vol. 219, no. 3, pp. 680–686, 2014.

[74] W. Perry, A. Minassian, B. Lopez, L. Maron, and A. Lincoln, "Sensorimotor gating deficits in adults with autism," *Biological Psychiatry*, vol. 61, no. 4, pp. 482–486, 2007.

[75] M. D. Bauman, A.-M. Iosif, S. E. P. Smith, C. Bregere, D. G. Amaral, and P. H. Patterson, "Activation of the maternal immune system during pregnancy alters behavioral development of rhesus monkey offspring," *Biological Psychiatry*, vol. 75, no. 4, pp. 332–341, 2014.

[76] C. J. Machado, A. M. Whitaker, S. E. P. Smith, P. H. Patterson, and M. D. Bauman, "Maternal immune activation in nonhuman primates alters social attention in juvenile offspring," *Biological Psychiatry*, vol. 77, no. 9, pp. 823–832, 2015.

[77] L. Harvey and P. Boksa, "Prenatal and postnatal animal models of immune activation: relevance to a range of neurodevelopmental disorders," *Developmental Neurobiology*, vol. 72, no. 10, pp. 1335–1348, 2012.

[78] G. Hava, L. Vered, M. Yael, H. Mordechai, and H. Mahoud, "Alterations in behavior in adult offspring mice following maternal inflammation during pregnancy," *Developmental Psychobiology*, vol. 48, no. 2, pp. 162–168, 2006.

[79] M.-E. Fortier, G. N. Luheshi, and P. Boksa, "Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy," *Behavioural Brain Research*, vol. 181, no. 2, pp. 270–277, 2007.

[80] T. B. Kirsten, M. Taricano, P. C. Maiorka, J. Palermo-Neto, and M. M. Bernardi, "Prenatal lipopolysaccharide reduces social behavior in male offspring," *NeuroImmunoModulation*, vol. 17, no. 4, pp. 240–251, 2010.

[81] T. B. Kirsten, G. P. Chaves-Kirsten, L. M. Chaible et al., "Hypoactivity of the central dopaminergic system and autistic-like behavior induced by a single early prenatal exposure to lipopolysaccharide," *Journal of Neuroscience Research*, vol. 90, no. 10, pp. 1903–1912, 2012.

[82] A. A. Willette, G. R. Lubach, R. C. Knickmeyer et al., "Brain enlargement and increased behavioral and cytokine reactivity in infant monkeys following acute prenatal endotoxemia," *Behavioural Brain Research*, vol. 219, no. 1, pp. 108–115, 2011.

[83] A.-L. Dinel, C. Joffre, P. Trifilieff et al., "Inflammation early in life is a vulnerability factor for emotional behavior at adolescence and for lipopolysaccharide-induced spatial memory and neurogenesis alteration at adulthood," *Journal of Neuroinflammation*, vol. 11, no. 1, article 155, 2014.

[84] L. Zuckerman, M. Rehavi, R. Nachman, and I. Weiner, "Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia," *Neuropsychopharmacology*, vol. 28, no. 10, pp. 1778–1789, 2003.

[85] C. Winter, A. Djodari-Irani, R. Sohr et al., "Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: implications for brain disorders of neurodevelopmental origin such as schizophrenia," *International Journal of Neuropsychopharmacology*, vol. 12, no. 4, pp. 513–524, 2009.

[86] Q. Li, C. Cheung, R. Wei et al., "Voxel-based analysis of postnatal white matter microstructure in mice exposed to immune challenge in early or late pregnancy," *NeuroImage*, vol. 52, no. 1, pp. 1–8, 2010.

[87] M. K. Paintlia, A. S. Paintlia, E. Barbosa, I. Singh, and A. K. Singh, "N-acetylcysteine prevents endotoxin-induced degeneration of oligodendrocyte progenitors and hypomyelination in developing rat brain," *Journal of Neuroscience Research*, vol. 78, no. 3, pp. 347–361, 2004.

[88] M. K. Paintlia, A. S. Paintlia, M. A. Contreras, I. Singh, and A. K. Singh, "Lipopolysaccharide-induced peroxisomal dysfunction exacerbates cerebral white matter injury: attenuation by N-acetyl cysteine," *Experimental Neurology*, vol. 210, no. 2, pp. 560–576, 2008.

[89] H. M. Golan, V. Lev, M. Hallak, Y. Sorokin, and M. Huleihel, "Specific neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy," *Neuropharmacology*, vol. 48, no. 6, pp. 903–917, 2005.

[90] B. E. Deverman and P. H. Patterson, "Cytokines and CNS development," *Neuron*, vol. 64, no. 1, pp. 61–78, 2009.

[91] S. E. Canetta and A. S. Brown, "Prenatal infection, maternal immune activation, and risk for schizophrenia," *Translational Neuroscience*, vol. 3, no. 4, pp. 320–327, 2012.

[92] U. Meyer, M. Nyffeler, A. Engler et al., "The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology," *Journal of Neuroscience*, vol. 26, no. 18, pp. 4752–4762, 2006.

[93] D. Nouel, M. Burt, Y. Zhang, L. Harvey, and P. Boksa, "Prenatal exposure to bacterial endotoxin reduces the number of GAD67- and reelin-immunoreactive neurons in the hippocampus of rat offspring," *European Neuropsychopharmacology*, vol. 22, no. 4, pp. 300–307, 2012.

[94] A. Basta-Kaim, K. Fijał, J. Ślusarczyk et al., "Prenatal administration of lipopolysaccharide induces sex-dependent changes in glutamic acid decarboxylase and parvalbumin in the adult rat brain," *Neuroscience*, vol. 287, pp. 78–92, 2015.

[95] L. Wischhof, E. Irrsack, F. Dietz, and M. Koch, "Maternal lipopolysaccharide treatment differentially affects 5-HT(2A) and mGlu2/3 receptor function in the adult male and female rat offspring," *Neuropharmacology*, vol. 97, pp. 275–288, 2015.

[96] K. Cui, H. Ashdown, G. N. Luheshi, and P. Boksa, "Effects of prenatal immune activation on hippocampal neurogenesis in the rat," *Schizophrenia Research*, vol. 113, no. 2-3, pp. 288–297, 2009.

[97] Y.-L. Lin and S. Wang, "Prenatal lipopolysaccharide exposure increases depression-like behaviors and reduces hippocampal neurogenesis in adult rats," *Behavioural Brain Research*, vol. 259, pp. 24–34, 2014.

[98] R. C. Paolicelli and C. T. Gross, "Microglia in development: linking brain wiring to brain environment," *Neuron Glia Biology*, vol. 7, no. 1, pp. 77–83, 2011.

[99] P. Squarzoni, G. Oller, G. Hoeffel et al., "Microglia modulate wiring of the embryonic forebrain," *Cell Reports*, vol. 8, no. 5, pp. 1271–1279, 2014.

[100] D. B. Oskvig, A. G. Elkahloun, K. R. Johnson, T. M. Phillips, and M. Herkenham, "Maternal immune activation by LPS selectively alters specific gene expression profiles of interneuron migration and oxidative stress in the fetus without triggering a fetal immune response," *Brain, Behavior, and Immunity*, vol. 26, no. 4, pp. 623–634, 2012.

[101] S. Akira and K. Takeda, "Toll-like receptor signalling," *Nature Reviews Immunology*, vol. 4, no. 7, pp. 499–511, 2004.

[102] H. Wei, I. Alberts, and X. Li, "Brain IL-6 and autism," *Neuroscience*, vol. 252, pp. 320–325, 2013.

[103] A. Masi, D. S. Quintana, N. Glozier, A. R. Lloyd, I. B. Hickie, and A. J. Guastella, "Cytokine aberrations in autism spectrum

disorder: a systematic review and meta-analysis," *Molecular Psychiatry*, vol. 20, no. 4, pp. 440–446, 2015.

[104] U. Meyer, "Prenatal Poly(I:C) exposure and other developmental immune activation models in rodent systems," *Biological Psychiatry*, vol. 75, no. 4, pp. 307–315, 2014.

[105] M. Kimura, L. A. Toth, H. Agostini, A. B. Cady, J. A. Majde, and J. M. Krueger, "Comparison of acute phase responses induced in rabbits by lipopolysaccharide and double-stranded RNA," *American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 267, no. 6, part 2, pp. R1596–R1605, 1994.

[106] U. Meyer, M. Nyffeler, S. Schwendener, I. Knuesel, B. K. Yee, and J. Feldon, "Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical dysfunction after in utero immune challenge," *Neuropsychopharmacology*, vol. 33, no. 2, pp. 441–456, 2008.

[107] G. A. Mostafa and L. Y. AL-Ayadhi, "Reduced serum concentrations of 25-hydroxy vitamin D in children with autism: relation to autoimmunity," *Journal of Neuroinflammation*, vol. 9, article 201, 2012.

[108] K. Suzuki, H. Matsuzaki, K. Iwata et al., "Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders," *PLoS ONE*, vol. 6, no. 5, article e20470, 2011.

[109] E. Y. Hsiao, S. W. McBride, J. Chow, S. K. Mazmanian, and P. H. Patterson, "Modeling an autism risk factor in mice leads to permanent immune dysregulation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 31, pp. 12776–12781, 2012.

[110] G. B. Choi, Y. S. Yim, H. Wong et al., "The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring," *Science*, vol. 351, no. 6276, pp. 933–939, 2016.

[111] M. L. Estes and A. K. McAllister, "Immune mediators in the brain and peripheral tissues in autism spectrum disorder," *Nature Reviews Neuroscience*, vol. 16, no. 8, pp. 469–486, 2015.

[112] Y. Sakai, C. A. Shaw, B. C. Dawson et al., "Protein interactome reveals converging molecular pathways among autism disorders," *Science Translational Medicine*, vol. 3, no. 86, Article ID 86ra49, 2011.

[113] M. N. Ziats and O. M. Rennert, "Expression profiling of Autism candidate genes during human brain development implicates central immune signaling pathways," *PLoS ONE*, vol. 6, no. 9, article e24691, 2011.

[114] G. Chana, L. Laskaris, C. Pantelis et al., "Decreased expression of mGluR5 within the dorsolateral prefrontal cortex in autism and increased microglial number in mGluR5 knockout mice: pathophysiological and neurobehavioral implications," *Brain, Behavior, and Immunity*, vol. 49, pp. 197–205, 2015.

[115] B. Abazyan, J. Nomura, G. Kannan et al., "Prenatal interaction of mutant DISC1 and immune activation produces adult psychopathology," *Biological Psychiatry*, vol. 68, no. 12, pp. 1172–1181, 2010.

[116] S. Vuillermot, E. Joodmardi, T. Perlmann, S. O. Ögren, J. Feldon, and U. Meyer, "Prenatal immune activation interacts with Genetic Nurr1 deficiency in the development of attentional impairments," *Journal of Neuroscience*, vol. 32, no. 2, pp. 436–451, 2012.

[117] V. Mazina, J. Gerdts, S. Trinh et al., "Epigenetics of autism-related impairment: copy number variation and maternal infection," *Journal of Developmental and Behavioral Pediatrics*, vol. 36, no. 2, pp. 61–67, 2015.

[118] W. Mandy and M.-C. Lai, "Annual research review: the role of the environment in the developmental psychopathology of autism spectrum condition," *Journal of Child Psychology and Psychiatry and Allied Disciplines*, vol. 57, no. 3, pp. 271–292, 2016.

[119] B. Tang, H. Jia, R. J. Kast, and E. A. Thomas, "Epigenetic changes at gene promoters in response to immune activation in utero," *Brain, Behavior, and Immunity*, vol. 30, pp. 168–175, 2013.

[120] P. Basil, Q. Li, E. L. Dempster et al., "Prenatal maternal immune activation causes epigenetic differences in adolescent mouse brain," *Translational Psychiatry*, vol. 4, article e434, 2014.

[121] E. Pessione, "Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows," *Frontiers in Cellular and Infection Microbiology*, vol. 2, article 86, 2012.

[122] M. G. Rooks and W. S. Garrett, "Gut microbiota, metabolites and host immunity," *Nature Reviews Immunology*, vol. 16, no. 6, pp. 341–352, 2016.

[123] S. A. Buffington, G. V. Di Prisco, T. A. Auchtung, N. J. Ajami, J. F. Petrosino, and M. Costa-Mattioli, "Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring," *Cell*, vol. 165, no. 7, pp. 1762–1775, 2016.

[124] M.-C. Lai, M. V. Lombardo, and S. Baron-Cohen, "Autism," *The Lancet*, vol. 383, no. 9920, pp. 896–910, 2014.

[125] D. L. Coury, P. Ashwood, A. Fasano et al., "Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda," *Pediatrics*, vol. 130, no. 2, pp. S160–S168, 2012.

[126] T. V. E. Kral, W. T. Eriksen, M. C. Souders, and J. A. Pinto-Martin, "Eating behaviors, diet quality, and gastrointestinal symptoms in children with autism spectrum disorders: a brief review," *Journal of Pediatric Nursing*, vol. 28, no. 6, pp. 548–556, 2013.

[127] Y. Wang and L. H. Kasper, "The role of microbiome in central nervous system disorders," *Brain, Behavior, and Immunity*, vol. 38, pp. 1–12, 2014.

[128] H. M. R. T. Parracho, M. O. Bingham, G. R. Gibson, and A. L. McCartney, "Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children," *Journal of Medical Microbiology*, vol. 54, part 10, pp. 987–991, 2005.

[129] Y. Song, C. Liu, and S. M. Finegold, "Real-time PCR quantitation of clostridia in feces of autistic children," *Applied and Environmental Microbiology*, vol. 70, no. 11, pp. 6459–6465, 2004.

[130] S. M. Finegold, D. Molitoris, Y. Song et al., "Gastrointestinal microflora studies in late-onset autism," *Clinical Infectious Diseases*, vol. 35, supplement 1, pp. S6–S16, 2002.

[131] C. S. Rosenfeld, "Microbiome disturbances and autism spectrum disorders," *Drug Metabolism and Disposition*, vol. 43, no. 10, pp. 1557–1571, 2015.

[132] G. Martirosian, A. Ekiel, M. Aptekorz et al., "Fecal lactoferrin and *Clostridium* spp. in stools of autistic children," *Anaerobe*, vol. 17, no. 1, pp. 43–45, 2011.

[133] B. L. Williams, M. Hornig, T. Buie et al., "Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances," *PLoS ONE*, vol. 6, no. 9, Article ID e24585, 2011.

[134] Å. V. Keita and J. D. Söderholm, "The intestinal barrier and its regulation by neuroimmune factors," *Neurogastroenterology and Motility*, vol. 22, no. 7, pp. 718–733, 2010.

[135] R. H. Sandler, S. M. Finegold, E. R. Bolte et al., "Short-term benefit from oral vancomycin treatment of regressive-onset

autism," *Journal of Child Neurology*, vol. 15, no. 7, pp. 429–435, 2000.

[136] C. G. M. De Theije, J. Wu, P. J. Koelink et al., "Autistic-like behavioural and neurochemical changes in a mouse model of food allergy," *Behavioural Brain Research*, vol. 261, pp. 265–274, 2014.

[137] D. F. MacFabe, "Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders," *Microbial Ecology in Health & Disease*, vol. 23, 2012.

[138] D. F. MacFabe, D. P. Cain, K. Rodriguez-Capote et al., "Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders," *Behavioural Brain Research*, vol. 176, no. 1, pp. 149–169, 2007.

[139] D. F. MacFabe, N. E. Cain, F. Boon, K.-P. Ossenkopp, and D. P. Cain, "Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: relevance to autism spectrum disorder," *Behavioural Brain Research*, vol. 217, no. 1, pp. 47–54, 2011.

[140] S. M. Finegold, S. E. Dowd, V. Gontcharova et al., "Pyrosequencing study of fecal microflora of autistic and control children," *Anaerobe*, vol. 16, no. 4, pp. 444–453, 2010.

[141] K. A. Foley, K.-P. Ossenkopp, M. Kavaliers, and D. F. MacFabe, "Pre- and neonatal exposure to lipopolysaccharide or the enteric metabolite, propionic acid, alters development and behavior in adolescent rats in a sexually dimorphic manner," *PLoS ONE*, vol. 9, no. 1, Article ID e87072, 2014.

[142] S. Nell, S. Suerbaum, and C. Josenhans, "The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models," *Nature Reviews Microbiology*, vol. 8, no. 8, pp. 564–577, 2010.

[143] S. Rakoff-Nahoum, J. Paglino, F. Eslami-Varzaneh, S. Edberg, and R. Medzhitov, "Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis," *Cell*, vol. 118, no. 2, pp. 229–241, 2004.

[144] B. M. Brinkman, F. Hildebrand, M. Kubica et al., "Caspase deficiency alters the murine gut microbiome," *Cell Death & Disease*, vol. 2, article e220, 2011.

[145] M. Wong, A. Inserra, M. D. Lewis et al., "Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition," *Molecular Psychiatry*, vol. 21, no. 6, pp. 797–805, 2016.

[146] C. M. Brown and D. W. Austin, "Autistic disorder and phospholipids: a review," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 84, no. 1-2, pp. 25–30, 2011.

[147] S. Vancassel, G. Durand, C. Barthélémy et al., "Plasma fatty acid levels in autistic children," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 65, no. 1, pp. 1–7, 2001.

[148] J. Jory, "Abnormal fatty acids in Canadian children with autism," *Nutrition*, vol. 32, no. 4, pp. 474–477, 2016.

[149] G. A. Mostafa, H. Y. El-Khashab, and L. Y. AL-Ayadhi, "A possible association between elevated serum levels of brain-specific auto-antibodies and reduced plasma levels of docosahexaenoic acid in autistic children," *Journal of Neuroimmunology*, vol. 280, pp. 16–20, 2015.

[150] Y. M. Al-Farsi, M. I. Waly, R. C. Deth et al., "Impact of nutrition on serum levels of docosahexaenoic acid among Omani children with autism," *Nutrition*, vol. 29, no. 9, pp. 1142–1146, 2013.

[151] M. M. Wiest, J. B. German, D. J. Harvey, S. M. Watkins, and I. Hertz-Picciotto, "Plasma fatty acid profiles in autism: a case-control study," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 80, no. 4, pp. 221–227, 2009.

[152] A. K. El-Ansary, A. G. Ben Bacha, and L. Y. Al- Ayahdi, "Plasma fatty acids as diagnostic markers in autistic patients from Saudi Arabia," *Lipids in Health and Disease*, vol. 10, article 62, 2011.

[153] S. A. Brigandi, H. Shao, S. Y. Qian, Y. Shen, B.-L. Wu, and J. X. Kang, "Autistic children exhibit decreased levels of essential fatty acids in red blood cells," *International Journal of Molecular Sciences*, vol. 16, no. 5, pp. 10061–10076, 2015.

[154] M. Maekawa, Y. Iwayama, R. Arai et al., "Polymorphism screening of brain-expressed FABP7, 5 and 3 genes and association studies in autism and schizophrenia in Japanese subjects," *Journal of Human Genetics*, vol. 55, no. 2, pp. 127–130, 2010.

[155] C. Shimamoto, T. Ohnishi, M. Maekawa et al., "Functional characterization of FABP3, 5 and 7 gene variants identified in schizophrenia and autism spectrum disorder and mouse behavioral studies," *Human molecular genetics*, vol. 23, no. 24, pp. 6495–6511, 2014.

[156] G. P. Amminger, G. E. Berger, M. R. Schäfer, C. Klier, M. H. Friedrich, and M. Feucht, "Omega-3 Fatty acids supplementation in children with autism: a double-blind randomized, Placebo-controlled Pilot Study," *Biological Psychiatry*, vol. 61, no. 4, pp. 551–553, 2007.

[157] S. Bent, K. Bertoglio, P. Ashwood, A. Bostrom, and R. L. Hendren, "A pilot randomized controlled trial of omega-3 fatty acids for autism spectrum disorder," *Journal of Autism and Developmental Disorders*, vol. 41, no. 5, pp. 545–554, 2011.

[158] N. A. Meguid, H. M. Atta, A. S. Gouda, and R. O. Khalil, "Role of polyunsaturated fatty acids in the management of Egyptian children with autism," *Clinical Biochemistry*, vol. 41, no. 13, pp. 1044–1048, 2008.

[159] Y. P. Ooi, S.-J. Weng, L. Y. Jang et al., "Omega-3 fatty acids in the management of autism spectrum disorders: findings from an open-label pilot study in Singapore," *European Journal of Clinical Nutrition*, vol. 69, no. 8, pp. 969–971, 2015.

[160] P. Politi, H. Cena, M. Comelli et al., "Behavioral effects of Omega-3 fatty acid supplementation in young adults with severe autism: An Open Label Study," *Archives of Medical Research*, vol. 39, no. 7, pp. 682–685, 2008.

[161] G. Dubnov-Raz, Z. Khoury, I. Wright, R. Raz, and I. Berger, "The effect of alpha-linolenic acid supplementation on ADHD symptoms in children: a randomized controlled double-blind study," *Frontiers in Human Neuroscience*, vol. 8, article 780, 2014.

[162] D. Mankad, A. Dupuis, S. Smile et al., "A randomized, placebo controlled trial of omega-3 fatty acids in the treatment of young children with autism," *Molecular Autism*, vol. 6, no. 1, article 18, 2015.

[163] S. M. Innis and S. de la Presa Owens, "Dietary fatty acid composition in pregnancy alters neurite membrane fatty acids and dopamine in newborn rat brain," *Journal of Nutrition*, vol. 131, no. 1, pp. 118–122, 2001.

[164] C. A. Pardo and C. G. Eberhart, "The neurobiology of autism," *Brain Pathology*, vol. 17, no. 4, pp. 434–447, 2007.

[165] 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.

[166] L. Zimmer, S. Delpal, D. Guilloteau, J. Aïoun, G. Durand, and S. Chalon, "Chronic n-3 polyunsaturated fatty acid deficiency

alters dopamine vesicle density in the rat frontal cortex," *Neuroscience Letters*, vol. 284, no. 1-2, pp. 25–28, 2000.

[167] S. Chalon, "Omega-3 fatty acids and monoamine neurotransmission," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 75, no. 4-5, pp. 259–269, 2006.

[168] S. Aïd, S. Vancassel, C. Poumès-Ballihaut, S. Chalon, P. Guesnet, and M. Lavialle, "Effect of a diet-induced n-3 PUFA depletion on cholinergic parameters in the rat hippocampus," *Journal of Lipid Research*, vol. 44, no. 8, pp. 1545–1551, 2003.

[169] I. Fedorova, A. R. Alvheim, N. Hussein, and N. Salem Jr., "Deficit in prepulse inhibition in mice caused by dietary n-3 fatty acid deficiency," *Behavioral Neuroscience*, vol. 123, no. 6, pp. 1218–1225, 2009.

[170] M. L. Jones, P. J. Mark, and B. J. Waddell, "Maternal omega-3 fatty acid intake increases placental labyrinthine antioxidant capacity but does not protect against fetal growth restriction induced by placental ischaemia-reperfusion injury," *Reproduction*, vol. 146, no. 6, pp. 539–547, 2013.

[171] 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, no. 9, article e437, 2014.

[172] 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.

[173] 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.

[174] 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.

[175] A. Moranis, J.-C. Delpech, V. De Smedt-Peyrusse et al., "Long term adequate n-3 polyunsaturated fatty acid diet protects from depressive-like behavior but not from working memory disruption and brain cytokine expression in aged mice," *Brain, Behavior, and Immunity*, vol. 26, no. 5, pp. 721–731, 2012.

[176] M. J. Weiser, B. Mucha, H. Denheyer et al., "Dietary docosahexaenoic acid alleviates autistic-like behaviors resulting from maternal immune activation in mice," *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 106, pp. 27–37, 2016.

[177] Q. Li, Y. O. Leung, I. Zhou et al., "Dietary supplementation with n-3 fatty acids from weaning limits brain biochemistry and behavioural changes elicited by prenatal exposure to maternal inflammation in the mouse model," *Translational Psychiatry*, vol. 5, no. 9, article e641, 2015.

[178] V. F. Labrousse, A. Nadjar, C. Joffre et al., "Short-term long chain Omega3 diet protects from neuroinflammatory processes and memory impairment in aged mice," *PLoS ONE*, vol. 7, no. 5, Article ID e36861, 2012.

[179] M. M. Pusceddu, S. El Aidy, F. Crispie et al., "N-3 Polyunsaturated Fatty Acids (PUFAs) reverse the impact of early-life stress on the gut microbiota," *PLoS ONE*, vol. 10, no. 10, Article ID e0139721, 2015.

[180] R. H. Thomas, M. M. Meeking, J. R. Mepham et al., "The enteric bacterial metabolite propionic acid alters brain and plasma phospholipid molecular species: further development of a rodent model of autism spectrum disorders," *Journal of Neuroinflammation*, vol. 9, article 153, 2012.

[181] S. Ghosh, E. Molcan, D. Decoffe, C. Dai, and D. L. Gibson, "Diets rich in n-6 PUFA induce intestinal microbial dysbiosis in aged mice," *British Journal of Nutrition*, vol. 110, no. 3, pp. 515–523, 2013.

[182] M. G. Gareau, P. M. Sherman, and W. A. Walker, "Probiotics and the gut microbiota in intestinal health and disease," *Nature Reviews. Gastroenterology & Hepatology*, vol. 7, no. 9, pp. 503–514, 2010.

[183] K. Kaliannan, B. Wang, X.-Y. Li, K.-J. Kim, and J. X. Kang, "A host-microbiome interaction mediates the opposing effects of omega-6 and omega-3 fatty acids on metabolic endotoxemia," *Scientific Reports*, vol. 5, Article ID 11276, 2015.

[184] D. L. Gibson, S. K. Gill, K. Brown et al., "Maternal exposure to fish oil primes offspring to harbor intestinal pathobionts associated with altered immune cell balance," *Gut Microbes*, vol. 6, no. 1, pp. 24–32, 2015.

[185] M. T. Clandinin, J. E. Chappell, S. Leong, T. Heim, P. R. Swyer, and G. W. Chance, "Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements," *Early Human Development*, vol. 4, no. 2, pp. 131–138, 1980.

[186] M. T. Clandinin, J. E. Chappell, S. Leong, T. Heim, P. R. Swyer, and G. W. Chance, "Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements," *Early Human Development*, vol. 4, no. 2, pp. 121–129, 1980.

[187] R. Chouinard-Watkins and M. Plourde, "Fatty acid metabolism in carriers of apolipoprotein E epsilon 4 allele: is it contributing to higher risk of cognitive decline and coronary heart disease?" *Nutrients*, vol. 6, no. 10, pp. 4452–4471, 2014.

[188] T. M. Dang, V. Conway, and M. Plourde, "Disrupted fatty acid distribution in HDL and LDL according to apolipoprotein E allele," *Nutrition*, vol. 31, no. 6, pp. 807–812, 2015.

[189] A. J. Yeates, T. M. Love, K. Engström et al., "Genetic variation in FADS genes is associated with maternal long-chain PUFA status but not with cognitive development of infants in a high fish-eating observational study," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 102-103, pp. 13–20, 2015.

[190] E. Morales, M. Bustamante, J. R. Gonzalez et al., "Genetic variants of the FADS gene cluster and ELOVL gene family, colostrums LC-PUFA levels, breastfeeding, and child cognition," *PLoS ONE*, vol. 6, no. 2, Article ID e17181, 2011.

[191] C. D. Steer, E. Lattka, B. Koletzko, J. Golding, and J. R. Hibbeln, "Maternal fatty acids in pregnancy, FADS polymorphisms, and child intelligence quotient at 8 y of age," *American Journal of Clinical Nutrition*, vol. 98, no. 6, pp. 1575–1582, 2013.

[192] B. Koletzko, E. Lattka, S. Zeilinger, T. Illig, and C. Steer, "Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children," *The American Journal of Clinical Nutrition*, vol. 93, no. 1, pp. 211–219, 2011.

[193] H. Cormier, I. Rudkowska, S. Lemieux, P. Couture, and M.-C. Vohl, "Expression and sequence variants of inflammatory genes; effects on plasma inflammation biomarkers following a 6-week supplementation with fish oil," *International Journal of Molecular Sciences*, vol. 17, no. 3, p. 375, 2016.

[194] B. L. Tremblay, H. Cormier, I. Rudkowska, S. Lemieux, P. Couture, and M.-C. Vohl, "Association between polymorphisms

in phospholipase A2 genes and the plasma triglyceride response to an n-3 PUFA supplementation: a clinical trial," *Lipids in Health and Disease*, vol. 14, no. 1, article 12, 2015.

[195] J. G. Bell, J. R. Sargent, D. R. Tocher, and J. R. Dick, "Red blood cell fatty acid compositions in a patient with autistic spectrum disorder: a characteristic abnormality in neurodevelopmental disorders?" *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 63, no. 1-2, pp. 21–25, 2000.

[196] M. H. F. D. S. Tostes, H. C. Polonini, R. Mendes, M. A. F. Brandão, W. F. Gattaz, and N. R. B. Raposo, "Fatty acid and phospholipase A2 plasma levels in children with autism," *Trends in Psychiatry and Psychotherapy*, vol. 35, no. 1, pp. 76–80, 2013.

[197] L. F. Ning, Y. Q. Yu, E. T. Guoji et al., "Meta-analysis of differentially expressed genes in autism based on gene expression data," *Genetics and Molecular Research*, vol. 14, no. 1, pp. 2146–2155, 2015.

[198] J.-C. Delpech, C. Madore, C. Joffre et al., "Transgenic increase in n-3/n-6 fatty acid ratio protects against cognitive deficits induced by an immune challenge through decrease of neuroinflammation," *Neuropsychopharmacology*, vol. 40, no. 3, pp. 525–536, 2015.

[199] A. Thomazeau, C. Bosch-Bouju, O. Manzoni, and S. Layé, "Nutritional n-3 PUFA deficiency abolishes endocannabinoid gating of hippocampal long-term potentiation," *Cerebral Cortex*, 2016.

[200] D. Yamada, J. Takeo, P. Koppensteiner, K. Wada, and M. Sekiguchi, "Modulation of fear memory by dietary polyunsaturated fatty acids via cannabinoid receptors," *Neuropsychopharmacology*, vol. 39, no. 8, pp. 1852–1860, 2014.

[201] F. Calon, G. P. Lim, F. Yang et al., "Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model," *Neuron*, vol. 43, no. 5, pp. 633–645, 2004.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 2173748, 23 pages
http://dx.doi.org/10.1155/2016/2173748

Review Article

Gene × Environment Interactions in Schizophrenia: Evidence from Genetic Mouse Models

Paula Moran,[1] Jennifer Stokes,[2] Julia Marr,[2] Gavin Bock,[2] Lieve Desbonnet,[3] John Waddington,[4,5] and Colm O'Tuathaigh[2]

[1]*School of Psychology, University of Nottingham, Nottingham NG7 2RD, UK*
[2]*School of Medicine, University College Cork, Brookfield Health Sciences Complex, College Road, Cork, Ireland*
[3]*School of Life Sciences, University of Glasgow, Room 220, Bower Building, Glasgow G12 8QQ, UK*
[4]*Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2, Ireland*
[5]*Jiangsu Key Laboratory of Translational Research & Therapy for Neuropsychiatric Disorders and Department of Pharmacology, College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, China*

Correspondence should be addressed to Colm O'Tuathaigh; c.otuathaigh@ucc.ie

Received 27 March 2016; Revised 20 July 2016; Accepted 21 August 2016

Academic Editor: Joram Feldon

The study of gene × environment, as well as epistatic interactions in schizophrenia, has provided important insight into the complex etiopathologic basis of schizophrenia. It has also increased our understanding of the role of susceptibility genes in the disorder and is an important consideration as we seek to translate genetic advances into novel antipsychotic treatment targets. This review summarises data arising from research involving the modelling of gene × environment interactions in schizophrenia using preclinical genetic models. Evidence for synergistic effects on the expression of schizophrenia-relevant endophenotypes will be discussed. It is proposed that valid and multifactorial preclinical models are important tools for identifying critical areas, as well as underlying mechanisms, of convergence of genetic and environmental risk factors, and their interaction in schizophrenia.

1. Introduction

Schizophrenia is a psychotic illness characterised by multifaceted psychopathology and dysfunction [1–5], with a European prevalence estimate for psychotic disorders (including schizophrenia) of 1.2% [6, 7]. This debilitating disorder is characterised by heterogeneous display of positive symptoms (hallucinations, delusions, and thought disorder), negative symptoms (avolition, restricted affect, poverty of speech, and social withdrawal), and cognitive dysfunction (e.g., working memory deficits, executive function, and attentional dysfunction), which typically emerge during late adolescence and young adulthood. Antipsychotic drugs which are currently available and commonly prescribed are efficacious against positive symptoms including hallucinations and delusions but are associated with significant side effects which negatively impact on compliance [4, 8], have little beneficial effect against the negative or cognitive symptoms, and moreover are not effective in all patients [9].

Schizophrenia is also a highly heritable disorder of neurodevelopment, where the development and expression of positive or psychotic symptoms are best viewed as signifying the outcome of a pathobiological cascade which originates in early brain development [4, 10]. Research over the past decade has significantly advanced our understanding of the genetic basis of schizophrenia, identifying risk loci, and suggesting biologically plausible mechanisms by which genetic risk is conferred [11], but much is still unknown [9]. A multitude of factors including, but not restricted to, gene × environment (G × E) and gene × gene (G × G) interactions, epigenetic modifications, and considerable heterogeneity at a genetic and phenotypic level, complicate our understanding of the role of these genes in the disorder and the translation of genetic advances into novel biological treatment targets [12, 13]. G × E interactions in schizophrenia might reflect genetic control of responses to protective or adverse environmental factors, as well as context-dependent phenotypic expression.

However, recent articles have highlighted the challenges associated with selecting appropriate statistical methods for identifying G × E interactions in schizophrenia and other neuropsychiatric disorders [14, 15]

Adoption and twin studies have confirmed that schizophrenia has a significant heritability component [16] with risk to develop schizophrenia or a related psychotic disorder positively correlated with degree of genetic similarity [17]. However, twin studies conducted in schizophrenic patients indicate that genes contribute no more than 50% to aetiology suggesting that developmental and environmental factors also have a major role to play [9]. Epidemiological studies have suggested that a diversity of factors including prenatal infection/immune activation, paternal age, malnutrition, hypoxia-related obstetric complications, and childhood/adolescence social stress and cannabis abuse are associated with increased risk for development of this disorder [2]. A "multihit" model has been proposed, and two crucial time windows associated with early brain development and maturation during adolescence have been identified as particularly sensitive periods for exposure to adverse environmental events, which could eventually trigger schizophrenia-relevant biological sequelae [18].

Recent genomewide association study (GWAS) analyses have identified multiple common schizophrenia risk alleles, each contributing a small effect, although they have provided mixed support for some of the more prominent common risk alleles identified in case-control and family-based genetic association studies [5, 19, 20]. Additionally, the discovery of microRNA changes and copy number variations (CNVs) in schizophrenia highlight the contribution and impact of rare and highly penetrant alleles in conferring genetic risk for schizophrenia [21, 22]. If a multiple-hit hypothesis is in fact an underlying model for the majority of cases for schizophrenia, it is likely to involve a combination of single nucleotide polymorphisms (SNPs), rare penetrant mutations, and environmental factors [23]. A large number of G × E interaction studies in patients with schizophrenia have focused on one candidate gene interacting with a specific environmental exposure. Since these studies have a specific prior hypothesis, they can be investigated with a modest sample size. Recent reviews of G × E interactions across clinical and preclinical studies in schizophrenia have however highlighted the relative paucity of relevant clinical data, noting that several of the animal models discussed in the present review have consequently selected G × E manipulations based on either combinations of genetic and environmental factors which have been (a) independently associated with schizophrenia, but not in combination, and/or (b) target common biological pathways implicated in schizophrenia, for example, disturbance of dopaminergic (DA) transmission [24–26].

G × E interactions in schizophrenia may also take the form of environmental factors impacting on DNA methylation, producing changes in gene expression through epimutations [27, 28]. Epigenetic factors represent an important mechanism whereby the adverse effects of environmental risk factors may impact gene expression. This topic has previously been discussed in more detail in relation to schizophrenia (e.g., [27]) and genetic models of schizophrenia [28]. One notable example is advanced paternal age (APA), which has been shown to be a risk factor for schizophrenia [29] as well as a host of other adverse neurodevelopmental outcomes (attention deficit hyperactivity disorder (ADHD), 30; autistic spectrum disorder (ASD), [30]). The predominant hypothesis in the field postulates age-related accumulation of *de novo* mutations in paternal sperm DNA [31], with a growing body of evidence suggesting that epigenetic changes in these cells could also be implicated [32, 33].

The present review will seek to summarise recent research which has been conducted on modelling of G × E interactions in schizophrenia using preclinical genetic models, primarily constitutive knockout or transgenic lines. There will be an emphasis placed on summarising evidence for psychosis-relevant features in the models, together with any evidence for mechanistic-based interrogation of the underlying pathophysiology.

2. Genetic Basis of Schizophrenia

Meta-analyses of twin and adoption studies have shown that heritability accounts for approximately 70% of disease risk in schizophrenia [34], where the magnitude of risk varies widely, from relatively modest odds for common genetic variants to substantial risks due to rare variants. Rare chromosomal deletions and duplications can increase risk for the disorder, with the magnitude of the increase in risk substantially greater than that observed for common variants [35–37].

GWAS data has implicated several candidate genes with a strong link to the pathophysiology of the disorder, while questioning the impact of hitherto prominent susceptibility targets (e.g., *disrupted-in-schizophrenia-1* (*DISC1*), *neuregulin-1* (*NRG1*)) [38]. The most recent analysis has identified 108 agreed loci that contribute to risk for schizophrenia; specifically, the Psychiatric Genomics Consortium (PGC) collaborative molecular genetic study of almost 37,000 patients with schizophrenia and 113,000 healthy controls identified 83 novel risk markers and replicated 25 existing markers [39]. The study pointed particularly to genes involved in neurodevelopment, the immune and stress response, glutamatergic neurotransmission, and DA D2 receptor activity.

CNV analyses which detect structural variants in the form of submicroscopic deletions and duplications of DNA have identified rare *de novo* and inherited variants that confer high risk for schizophrenia (Odds Ratio = 3–20) [40]. An exome-sequencing study involving 2536 schizophrenia cases and 2543 controls demonstrated a polygenic burden primarily arising from rare (less than 1 in 10,000), disruptive mutations distributed across many genes [41]. These authors were able to detect several small and highly enriched sets, notably of genes related to N-methyl-D-aspartate (NMDA) receptor-associated postsynaptic density-95 (PSD-95) protein complexes, activity-regulated cytoskeleton- (ARC-) associated interacting proteins and fragile × mental retardation protein (FMRP) targets [42].

Importantly, some of the genetic factors linked with increased risk for schizophrenia also display association to broader phenotypes including bipolar disorder, as well as major depression, ADHD, and autism [43], suggesting that

clinical overlap between these disorders may in part reflect a shared genetic basis. In a recent combined GWAS of 19779 bipolar disorder and schizophrenia cases versus 19423 controls, in addition to a direct comparison GWAS of 7129 schizophrenia cases versus 9252 bipolar disorder cases, the authors identify five previously identified regions reaching genome-wide significance as well as a novel locus [44]. These authors reported a significant correlation between a bipolar disorder polygenic risk score and the clinical dimension of mania in patients with schizophrenia. Overlapping disease pathways may, in part, explain shared symptoms across diagnoses, as well as multiple diagnoses within patients [45].

3. Mutant Models of G × E Interactions in Schizophrenia

Interactions between genetic risk and environmental stressors at various stages of life appear important in the development of schizophrenia [46–48]. Preclinical genetic models provide tools for assessing the relative contribution of genes, exposure to environmental pathogens, and their interaction, on the development of schizophrenia-relevant phenotypes [25, 48, 49]. Preclinical modelling of G × E interactions related to schizophrenia has typically involved examining the phenotypic consequences of epidemiologically relevant but also translationally valid, experimental manipulations in various candidate risk gene mutant models [50, 51]. Combining an environmental challenge with a genetic mutation can produce both protective and adverse effects. It has been noted that the potential to generate such results should be incorporated within the study design and that exclusively focusing on a limited set of prespecified outcome measures may exclude the possibility of reporting such unexpected and complex bidirectional results [28]. Particularly in the context of evidence for a shared genetic basis underlying several major neuropsychiatric disorders, the discovery of novel behavioural phenotypes in preclinical models of G × E interactions has the potential to inform us about the role of the environment in evoking diverse clinical outcomes in patients with the same mutation.

Timing of the environmental insult is an important factor that needs to be considered during the development and evaluation of the G × E model. Mutant modelling of G × E interactions in schizophrenia studies has typically involved environmental manipulations at particular periods of brain development (e.g., early pregnancy or adolescence) which are regarded as important to the pathogenesis of schizophrenia. These critical periods of brain development correspond to early life (pre-, peri-, and early postnatal period) or later (adolescent) stages in humans [52, 53].

While many of the studies discussed below, which aim to simulate G × E interactions implicated in psychosis in rodent models, consist mostly of descriptive analyses, a growing number of studies are starting to provide important mechanistic insight into the molecular/cellular basis underlying such interactions. Elucidating the biological mechanisms underlying synergistic G × E effects on emergence of neuropsychiatric phenotypes necessitates interrogation of the molecular basis of the observed phenotypes.

4. Modelling Schizophrenia in Rodents

While it is impossible to model schizophrenia *per se* in mice or other rodents, three important criteria need to be satisfied in order for any experimental model to claim validity for the disorder. Firstly, the model should reflect, at least in part, the etiopathological basis of the disorder. Secondly, while research has emphasized the neurodevelopmental aspect of schizophrenia, its clinical onset is postpubertal. This fact emphasises the importance of examining the data of young animals as part of any G × E interaction modelling effort, so that the trajectory from insult during early development or young adulthood to the emergence of adult phenotypes can be established. Thirdly, the experimental model should reflect endophenotypes relevant to schizophrenia in adulthood. Endophenotypes are quantifiable, intermediate disease features that bridge the gap between the overt manifestations of schizophrenia and underlying risk genes [54]. Earlier reviews have highlighted the value of utilising endophenotypic endpoints in preclinical genetic studies, where intermediate biological or behavioural phenotypes are less susceptible to confounding influences and are therefore easier to investigate [26]. Schizophrenia-relevant endophenotypes include behavioural deficits (e.g., working memory impairment, deficits in sensory or sensorimotor gating, and social withdrawal) and several histological/structural changes such as enlarged lateral ventricles and deficits in a specific subtype of interneurons in the cortex.

Recently, efforts have been made to identify equivalent behavioural domains and functional assays between humans and animals, including the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) and Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS). More recently, the Research Domain Criteria (RDoC) initiative from the National Institute of Mental Health (NIMH) aims to reclassify psychiatric disorders according to basic dimensions of functioning, where each behavioural domain is studied across multiple levels of analysis, from genes to neural circuits to behaviour in both animal models and humans, assuming that these behavioural domains share more or less similar underlying mechanism across species [55]. RDoC includes the following domains: negative valence systems (fear, anxiety, and loss), positive valence system (reward learning, reward evaluation), cognitive systems (attention, perception, working memory, and cognitive control), systems for social processes (attachment formation, social communication, perception of self, and perception of others), and arousal/modulatory systems (arousal, circadian rhythms, sleep, and wakefulness).

5. Modelling Environmental Risk Factors Relevant to Schizophrenia in Rodents

There is a general consensus among schizophrenia researchers that diverse biological, environmental, and psychosocial insults, across the lifespan, accumulate in their adverse impact on an already developmentally compromised brain to result in the development of psychotic illness [26, 48].

Consistent with the well-considered "stress-vulnerability" aetiological model, these extend from early biological and psychosocial insults during the prenatal or perinatal period (including winter birth, maternal infections or immune challenge, and other obstetric complications [2, 48]), through exposure to adversity during infancy and childhood (e.g. societal factors, childhood abuse; [56]), to pathogenic factors present during adolescence and young adulthood (exposure to psychosocial stressors, prolonged exposure to drugs of abuse including cannabis [57]). As noted above in relation to genetic factors, numerous environmental factors associated with schizophrenia and other psychotic disorders are also associated with a range of other neurodevelopmental and neuropsychiatric outcomes, including autistic spectrum disorder, attention-deficit hyperactivity disorder, and epilepsy [20], leading some authors to propose that schizophrenia is best conceptualised as one of a spectrum of clinical outcomes that result from exposure to selected genetic or environmental factors, or both [20]. Translational efforts to model such factors on rodents have generally sought to develop ethologically appropriate (e.g., maternal deprivation, postweaning social isolation, or social defeat during adolescence to study the effects of psychosocial stress on neurobehavioural measures across development in mice/rats) or practicable and exposure-relevant biological manipulations (e.g., inflammatory responses after infection and cytokine-mediated effects on brain development using polyinosinic-polycytidylic acid (Poly I:C) and lipopolysaccharide (LPS) in rats/mice) to investigate the biological underpinnings of G × E interactions.

6. Infection and Schizophrenia

It is well established that prenatal influenza exposure is associated with increased risk of developing schizophrenia in the offspring [58, 59]. This risk liability has been shown to extend to other viral and bacterial agents, as well as exposure to parasitic agents such as *Toxoplasma gondii* [2, 60]. The emergence of schizophrenic symptomatology in adult offspring has been shown to be dependent upon maternal infection at different gestational points throughout pregnancy [61, 62], which is an important consideration when developing valid animal models of maternal infection in schizophrenia. While a multitude of infectious agents have been associated with increased risk for schizophrenia, it is proposed that the common pathophysiological mechanism underlying their "schizophregenicity" involves activation of the maternal immune system [2, 62].

Preclinical experimental models have been developed which involve prenatal exposure to infection, immune activation, or another relevant biological insult. These models have included gestational exposure to human influenza virus, the bacterial endotoxin LPS, and Poly I:C, a synthetic analogue of double-stranded RNA which is recognized as an infectious pathogen by the human immune system [63]. In the rodent prenatal Poly I:C model, administration of Poly I:C to pregnant dams causes elevations in maternal serum cytokines that are accompanied by emergence in adulthood of behavioural and neural phenotypes related to those evident in schizophrenia [64]. Timing of immune challenge is a significant determinant of brain and behaviour outcomes in subsequent offspring. It has been shown that the effects of maternal immune challenge during gestation between early (gestational day [GD] [9]) and late (GD17) pregnancy periods in mice are dissociable in terms of foetal brain cytokine responses to maternal inflammation and subsequent functional effects [65, 66]. These challenge periods correspond to the end of the first trimester (GD9) and middle/late phase of the second trimester (GD17) in humans [67, 68].

Poly I:C treatment during early pregnancy is associated with schizophrenia-related endophenotypes in adult offspring including deficits in prepulse inhibition (PPI [67, 68]) as well as latent inhibition (LI [69]), two measures of preattentional and selective attention processes, respectively, which are disturbed in schizophrenia. Across various measures of social interaction, both early and late gestational treatment Poly I:C in dams has been shown to disrupt sociability and social cognition [66, 70, 71]. Similarly, offspring of Poly I:C-treated dams display a hyperexploratory phenotype in a novel environment [64], as well as increased behavioural sensitivity to DA agonists and NMDA receptor antagonists [72, 73]; both of these features are considered proxy measures for the positive symptoms of schizophrenia. Structural brain endophenotypes associated with schizophrenia have also been demonstrated in the brains of adult offspring of Poly I:C treated mice; these include lateral ventricular enlargement and decreased hippocampal volume [74, 75].

As the majority of individuals exposed to neurodevelopmental insults such as infections do not develop schizophrenia in adulthood, it is important to assess the additive and interactive effects of infection and genetic vulnerability on the development of schizophrenia-relevant endophenotypes.

6.1. NRG1 × Immune Challenge. *Neuregulin-1* (*NRG1*) is putative risk gene which has been widely studied in relation to its association with schizophrenia [76–78]. In meta-analysis, the association between the *NRG1* schizophrenia-associated risk haplotype (HapICE, first reported by Stefansson et al. [76]) and schizophrenia has proved replicable [77]. NRG1 belongs to a family of growth factors which are encoded by four genes (*NRG1-4*); it has greater than 30 isoforms, grouped into six "types" (I–VI) that are differentiated on the basis of N-terminal sequence, expression of the α or β epidermal growth factor- (EGF-) like domain, and presence of a transmembrane (TM) region [79, 80]. NRG1 proteins are ligands for ErbB receptor tyrosine kinases; this, in turn, activates intracellular signalling pathways that are known to play a prominent role in diverse developmental processes implicated in schizophrenia [79, 80]. NRG1 is expressed in diverse brain areas, including the PFC, hippocampus, cerebellum, and substantia nigra in both humans and rodents [80]. NRG1 isoforms differ in domain structure and expression levels in various tissues/cells during brain development and, later, in adulthood; isoform-specific roles and properties, particularly in relation to the NRG1-schizophrenia association, remain poorly understood [80]. This level of genetic complexity highlights the difficulty associated with generating accurate preclinical

genetic models of NRG1 dysfunction in schizophrenia. Clinical genetic analyses have supported the association between *NRG1* variation, inflammatory function, and neurogenesis. Interaction between the genes encoding the proinflammatory cytokine interleukin 1β (IL-1β) and *NRG1* genotype increases the risk of schizophrenia and shortens the age of onset for the disorder [81]. Additionally, a missense mutation in *NRG1* has been reported to increase activation of proinflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and interleukin 8 (IL-8) in patients with schizophrenia [82].

Various *NRG1* knockout and transgenic mouse lines have been developed to study the relationship between altered NRG1 signalling and impact on behavioural and brain endophenotypes relevant to schizophrenia [26, 83]. Mice with heterozygous knockout of the transmembrane- (TM-) domain truncation of exon 11 *NRG1*, which is associated with the disruption of several *NRG1* splice variants, display increased novelty-induced hyperactivity, which is reversed by antipsychotic treatment [76, 84, 85]. Disruption to PPI has also been reported in the TM-domain *NRG1* mutant line [86–88], and they also display deficits in social interaction [85].

Comparisons with alternative TM-domain or more isoform-specific *NRG1* deletions indicates that differences in the targeting strategy, as it relates to the *NRG1* gene, can produce very different effects across various neurobehavioural measures. For example, in contrast with the exon 11 TM-domain lines, mutant mice with targeted disruption of type I/type II *NRG1* do not show a hyperactive phenotype [89, 90]. Similarly, no significant behavioural impairments, aside from mild cognitive deficits, were observed in a TM-domain mutant line with a truncation from exon 9 [91].

O'Leary et al. [92] examined the unique and combined effects of prenatal immune challenge (*via* administration of Poly I:C at GD9) and postnatal cross-fostering (a control procedure which can also act as a stressor, where offspring are separated from dams and raised by surrogate mothers) in mice with partial TM-domain (exon 9) deletion of *NRG1*. In this study, distinct phenotypic effects across schizophrenia-related behavioural measures (social interaction, PPI, and open-field exploration) were observed for both individual environmental variables as well as interactions between these factors and genotype [92]. *NRG1* mutants demonstrated impaired social novelty preference, PPI, and a sex-specific (females only) decrease in spatial working memory performance, irrespective of exposure to the stressor. Poly I:C treatment also disrupted PPI and working memory performance across both genotypes. Combining *NRG1* disruption and prenatal immune challenge caused deficits in social behaviour and spatial working memory, whereas combining *NRG1* disruption with the early life stressor (cross-fostering) impaired social novelty preference, a measure of social cognition. No synergistic effect of *NRG1* disruption and prenatal immune challenge was observed in relation to PPI, which may be attributable to a masking effect of *NRG1*-related PPI disruption on potential *NRG1* × prenatal immune challenge interactions on sensorimotor gating. However, the combination of prenatal immune challenge and cross-fostering (i.e., E × E) also produced several behavioural deficits in the open field, social behaviour, and PPI. The results of this study suggest that the emergence of schizophrenia-relevant endophenotypes can arise from multiple, often very complicated, interactions involving individual genes interacting with several biological and psychosocial factors.

6.2. DISC1 × Immune Challenge. *DISC1* is a prominent schizophrenia risk gene, which was originally identified at the breakpoint of a balanced chromosomal translocation cosegregating with mental disorders in a large Scottish kindred [93]. Subsequent clinical genetic studies have identified evidence for involvement of common and rare risk variants at this locus in the etiology of a range of neuropsychiatric disorders, including schizophrenia, schizoaffective disorder, bipolar disorder, and recurrent depressive disorder [94, 95]. DISC1 is an essential synaptic protein, which interacts with a wider molecular network to mediate processes associated with cellular and synaptic function [96]. Mutant models of *DISC1* gene function display anatomical, behavioural, and pharmacological phenotypes relevant to several neuropsychiatric disorders, including schizophrenia and depression [97–103]. As with the *NRG1* mutant data, these *DISC1* mutant phenotypic analyses again illustrate how different mutations in the same gene can result in divergent phenotypic outcomes. For example, a transgenic line with inducible and reversible expression of a DISC1 C-terminal fragment under the calcium/calmodulin-dependent protein kinase II alpha (αCAMKII) promoter demonstrated impaired social functioning and disruption of spatial working memory [99]. In a transgenic line with expression of a dominant-negative truncated form of DISC1 under the αCaMKII promoter, mutants exhibited novelty-induced hyperactivity but no other major phenotypes [98]. Double transgenic mice expressing human *DISC1* under the cytomegalovirus (CMV) promoter with tetracycline under the αCaMKII promoter showed a hyperactive phenotype, as well as deficits in social interaction and spatial memory [101]. Another group described two mouse line carrying point mutations in *DISC1* (L100P and Q31L), where abnormalities associated with schizophrenia were observed in the L100P line; these included deficits in PPI and LI, as well as working memory, many of which were shown to be reversible by antipsychotic administration [97].

Employing the Poly I:C immune challenge procedure, Lipina et al. [104] demonstrated that the mutant offspring of L100P dams who had been given a single injection of Poly I:C on GD9 demonstrated more prominent PPI and LI deficits, as well as impaired working memory and sociability, relative to L100P controls or both challenged and unchallenged wildtype controls, where moderate deficits in these tasks were already observed following the genetic or environmental manipulation alone. Coadministration of an IL-6 antagonist blocked the disruptive effects of prenatal Poly I:C on PPI and LI performance in L100P mice, providing a direct link between Poly I:C treatment and behavioural disruption in these mice.

The phenotypic effects of combining prenatal immune challenge with *DISC1* disruption were also described in a study conducted in mice with inducible expression of mutant *hDISC1* in forebrain neurons [50, 101]. Poly I:C treatment increased anxiety in mutants and controls in the open field,

and both challenged and nonchallenged *DISC1* mutants displayed lateral ventricular enlargement relative to controls. Male *DISC1* mutant offspring of dams treated with Poly I:C at GD9 demonstrated decreased social approach behaviours, as well as an anxiogenic phenotype (less time in the open arms of the elevated plus maze) and depression-like behaviours (i.e., decreased latency to immobility in the forced swim test). These behavioural deficits were accompanied by altered serotonergic neurotransmission in the hippocampus, decreased hypothalamic-pituitary-adrenal (HPA) axis reactivity and attenuation of genotypic enlargement of the lateral ventricles, as well as differential modulation of secretion of inflammatory cytokines [50].

Another study examined the interaction of *DISC1* mutation with *neonatal* treatment with Poly I:C between postnatal days 2 and 6 [105]. While neither the *DISC1* mutation nor neonatal immune challenge were independently associated with any phenotypic effects, transgenic mice expressing a dominant-negative form of *DISC1* displayed a pronounced schizophrenia-related phenotype across several cognitive endophenotypes (spontaneous Y-maze alternation [which measures working memory processes], recognition memory, and contextual fear memory) following neonatal immune challenge. Social interaction and MK-801-induced hyperactivity were also selectively altered in Poly I:C-treated *DISC1* mutants. These behavioural deficits were accompanied by a decrease in parvalbumin-positive interneurons in the medial prefrontal cortex (a cellular endophenotype for schizophrenia) of *DISC1* × neonatal immune challenge mutants. It was later shown, employing the same experimental design, that the antipsychotic drug clozapine successfully reversed the recognition memory deficits in *DISC1* mutants exposed to neonatal Poly I:C [106].

A recent study examined the interaction between *DISC1* genotype, employing the transgenic model of inducible expression of dominant-negative mutant human *DISC1*, and prenatal exposure to the toxin lead (Pb2+), to assess the development of neuropsychiatric phenotypes in resultant lead-exposed offspring [107]. Lead exposure was associated with the expression of increased anxiety, disruption of PPI, increased responsivity to the NMDA receptor antagonist MK-801, and ventricular enlargement (also observed in nonstressed *DISC1* mutants versus controls). The authors reported several, often sex-specific, synergistic effects, demonstrating more pronounced PPI deficits, heightened MK-801 responsivity, and alterations in exploratory activity and ventricular volume in *DISC1* mice exposed to lead.

6.3. Nurr1 × Immune Challenge. Nurr1 is a member of the orphan steroid hormone receptor family which is involved in key processes including differentiation, migration, and survival of midbrain DA neurons [108], as well as regulation of the expression of genes which are crucial for DA neurotransmission [109]. The combination of partial knockout of *Nurr1* and prenatal immune activation *via* late gestational Poly I:C administration resulted in additive effects on locomotor hyperactivity in a novel environment and PPI disruption, where deficits across both measures were already observed following genetic disruption of *Nurr1* or exposure to Poly I:C alone. In contrast, multiplicative disruptive effects of both genetic and environmental manipulations were observed for measures of attentional function including LI persistence and a measure of sustained attention [110]. Synergistic interactions between *Nurr1* haploinsufficiency and prenatal immune activation on DA D2 receptor density in the nucleus accumbens core and shell were also reported, as well as a significant decrease and increase in tyrosine hydroxylase and catechol-O-methyltransferase (COMT) density, respectively, in the medial prefrontal cortex [110].

7. Cannabis Use and Schizophrenia

Recent epidemiological surveys have calculated mean estimates of lifetime prevalence of cannabis use of 25% and 35.8% among youth aged 15-16 in the UK and USA, respectively [111, 112]. Therefore, a significant number of young people are exposed to cannabis during an important neurodevelopmental stage characterised by maturation of neural circuitry across several brain areas implicated in schizophrenia and other neuropsychiatric disorders. Lifetime cannabis use increases risk for developing a psychotic disorder [113, 114], where the risk quotient is highest among individuals who use cannabis during adolescence [115–118]. However, despite high prevalence estimates for lifetime cannabis use, a relatively small proportion of cannabis users go on to develop subclinical symptoms or a clinical psychotic disorder [119]. This may be explained by the interaction between cannabis-related psychosis risk and genetic disposition, as well as the copresence of other adverse environmental conditions [120]. It may also reflect differential concentrations of delta-9-tetrahydrocannabinol (THC) and cannabidiol in cannabis products. THC is the principal psychotomimetic ingredient of cannabis; cannabidiol, in contrast, is a cannabinoid which can exert anxiolytic and potentially antipsychotic effects [117, 119].

A recent analysis, conducted in a population-based sample, revealed a negative association between cannabis use in early adolescence and cortical thickness (a morphological endophenotype for schizophrenia) in male adolescents with a high genetic risk for schizophrenia, as indicated by their risk profiles across 108 genetic loci identified by the Psychiatric Genomics Consortium in a large genome-wide comparison of patients with schizophrenia and control individuals [121]. G × E studies examining the link between cannabis and psychosis in humans face the challenge of conclusively excluding the possibility that individuals with a particular genotype or profile of exposure to environmental adversity may be more likely to use cannabis, as opposed to cannabis exposure independently affecting the pathway to psychosis [122]. Delta-9-tetrahydrocannabinol (THC) is the principal psychotomimetic ingredient of cannabis; cannabidiol, in contrast, is another component of cannabis which is thought to exert anxiolytic effects [123]. Prolonged exposure to THC during the period corresponding to adolescence in rats and mice is associated with the emergence of deficits across several schizophrenia-related endophenotypes, including attentional and memory function (PPI, recognition memory), novelty-induced hyperactivity [123], and deterioration in

reinforcement learning performance [124]. It is also accompanied by neuronal hyperactivity in the mesocorticolimbic DA pathway as well as modification of prefrontal cortical molecular pathways [125]. Cannabinoid modulation of activity of DA projections from the brain stem to the striatum, in particular, has been linked with the development of cannabis-induced psychosis [126].

7.1. NRG1 × Cannabis Exposure during Adolescence. A putative association between *NRG1* genotype and cannabis-related psychosis has not yet been examined in clinical samples. A genome-wide linkage scan, and follow-up association analysis, for cannabis dependence in African-American and European-American families, revealed that *NRG1* variation was associated with increased risk for cannabis dependence in African-Americans, and this effect was pronounced in females [127].

Male TM-domain *NRG1* mutant mice have shown increased susceptibility to several of the neurobehavioural effects of acute THC relative to wildtype controls. These genotypic effects have included greater sensitivity to the PPI-enhancing and anxiogenic effects of THC, as well as its locomotor activity suppressing effects [128–130]. These authors also observed that THC-induced increase in immediate early gene (*c-fos*) expression was greater in the shell of the nucleus accumbens, central nucleus of the amygdala, paraventricular nucleus, and dorsolateral bed nucleus of the stria terminalis of *TM-NRG1* mutants relative to controls [129]. Adding complexity to the interpretation of G × E effects in this model and suggesting the presence of second-level E × E interactions, this genotype-dependent increase in *c-fos* expression was only observed in mice who had been subjected to behavioural assessments. In a complementary manner, *TM-NRG1* mutants also demonstrated increased tolerance to the locomotor suppressant and anxiogenic effects of the synthetic cannabinoid CP 55,940, administered during adulthood [131]. *TM-NRG1* mutants were also resistant to the cannabinoid-induced decrease in investigative social behaviours compared to controls [132]. The latter study also showed that several of adolescent THC effects on cannabinoid receptor 1 (CB1R) and 5-HT2A receptor binding (decreased in *TM-NRG1* mutants, increased in wildtypes) in the substantia nigra and insular cortex were genotype-dependent. Adolescent THC also selectively increased NMDA receptor binding in the auditory cortex, cingulate cortex, and hippocampus of *TM-NRG1* mutants [132], as well as inducing differential expression of proteins implicated in NMDA receptor trafficking and glutamatergic function in the hippocampus of adolescent THC-treated *TM-NRG1* mutants versus controls [133].

Cannabidiol is another psychoactive component of cannabis which has been reported to possess anxiolytic [134] and putative antipsychotic properties [135]. Long and colleagues examined the neurobehavioural effects of chronic cannabidiol during adulthood in TM-NRG1 mutants relative to controls [136]. Chronic cannabidiol selectively enhanced social interaction and increased $GABA_A$ receptor binding in the granular retrosplenial cortex in *TM-NRG1* mutants but had no effect on PPI or novelty-induced exploratory activity [136]. Collectively, studies conducted on THC, synthetic cannabinoid, and cannabidiol effects in TM-domain *NRG1* mutants would indicate altered sensitivity to the neurobehavioural effects of this class of drugs, in a manner which is dependent upon timing and duration of treatment.

7.2. DISC1 × Cannabis Exposure during Adolescence. A recent study investigated the interaction, at a preclinical level, between mutation in *DISC1* and the effects of chronic adolescent administration of THC [137]. In this model, a putative dominant-negative form of *DISC1* (*DN-DISC1*) which is expressed under the control of the alpha-CAMKII promoter in forebrain pyramidal neurons, chronic treatment with THC during adolescence (postnatal days 28–48) worsened deficits in cue-dependent fear memory in *DN-DISC1* mice, while neuronal activation induced by fear memory retrieval was also selectively impaired in *DN-DISC1* mice. *DN-DISC1* mice also demonstrated deficits in contextual fear memory irrespective of treatment condition. The combinatorial effect of adolescent THC exposure and *DN-DISC1* expression on the endocannabinoid system was also indicated by a synergistic reduction in synaptic CB1R expression in the prefrontal cortex, hippocampus, and amygdala.

7.3. COMT × Cannabis Exposure during Adolescence. COMT is an enzyme involved in the catabolism of catecholamines and is the principal enzyme controlling the metabolism of DA in the prefrontal cortex [138]. A common functional polymorphism in the *COMT* gene, the Val158Met variant, has been associated with differential reactivity to stressful stimuli. Individuals with the *COMT* Val/Val (high enzyme activity) genotype exhibit decreased affective reactivity to stress relative to carriers of Met/Met, the low enzyme activity allele [139]. Studies have shown that the disruptive effects of childhood abuse on adult emergence of cognitive deficits [140] and frequency of self-reported psychotic experiences [141] are present only in *COMT* Met/Met carriers. In one of the first clinical G × E reports reported for schizophrenia, risk to develop psychosis was shown to be highest among those who used cannabis during adolescence and were *COMT* Val/Val carriers [142]. Preclinical genetic studies employing a constitutive *COMT* gene knockout model, which looked at the interaction between chronic intermittent THC and Win 55,212 (a synthetic CB1R agonist) exposure during adolescence and *COMT* deletion, demonstrated that *COMT* genotype modulated responsivity to adolescent cannabinoid effects in relation to hyperactivity in a novel environment, working memory, and PPI [123, 143]. Specifically, THC treatment reversed enhancement of working memory in *COMT* knockout mice and produced changes in exploratory activity and PPI that were not observed following COMT knockout or THC treatment alone These deficits were accompanied in a genotype-dependent manner by changes across morphological measures of DA-ergic and GABA-ergic function [144].

8. Social Stress and Schizophrenia

Exposure to psychosocial stressors, particularly at developmentally important time points, has been shown to both play a role in the development of a psychotic disorder and

precipitate the onset of psychotic illness when the stressful experience occurs closer to the onset of the disorder [144–147]. One particular social stressor which has been both linked with increased risk for schizophrenia and modelled in preclinical assays is social defeat, which refers to the defeated feeling of subordination which is experienced following an adverse social encounter [148, 149]. Animals studies have consistently shown that exposure to social defeat is associated with changes across several schizophrenia-related endophenotypes, as well as HPA axis function, and corticolimbic DA neurotransmission (see [150] for detailed review of evidence). Generally, rats or mice subjected to social defeat demonstrate impaired social behaviour, as well as increased behavioural signs of anxiety and depression [151, 152].

8.1. NRG1 × Social Stress. The combined effect of *NRG1* heterozygous knockout and chronic social defeat stress (via intermittent access to an aggressive CD1 strain conspecific) during adolescence produced genotype-dependent working memory deficits and elevated basal cytokine levels during adulthood in *TM-NRG1* mutant mice relative to controls [86]. *TM-NRG1* mutants displayed a genotypic increase in novelty-induced activity, disruption of PPI and social novelty preference, and decreased anxiety relative to wildtypes. However, the combination of repeated social defeat stress and partial *NRG1* knockout produced deficits in the Y-maze spontaneous alternation task (a measure of working memory), which were not observed in stressed wildtype controls. In contrast, in the sucrose preference test (a measure which is utilised to model anhedonia in rodents), stressed control mice displayed reduced sucrose preference (i.e., an "anhedonic" profile), whereas no such effect was observed in stressed *NRG1* mutants. Another recent study which compared the effects of acute and chronic exposure to a nonsocial stressor, restraint stress, during adolescence in *TM-NRG1* mutants versus controls reported increased sensitivity to the anxiogenic effects of acute stress exposure in mutants [153]. Chronic intermittent stress during adolescence also produced deficits in PPI in *NRG1* mutants relative to both stressed wildtypes and nonstressed mice belonging to both genotypes. *NRG1* mutants also demonstrated decreased corticosterone levels, as well as increased apical dendritic spine density and decreased apical dendritic lengths and complexity in layer II/III pyramidal neurons of the medial prefrontal cortex, following chronic restraint stress.

8.2. DISC1 × Social Stress. The phenotypic effects of social defeat stress during adulthood in mice were examined in DISC1 L100P and Q31L (a DISC1 line which demonstrates more affective disorder-related phenotypes and fewer psychosis-relevant phenotypes than the L100P line) mutants [154]. They reported decreased vertical activity levels during exploration in a novel environment, as well as social interaction in mice with heterozygous mutation in *DISC1* (L100P) following exposure to social defeat. While L100P mice displayed a deficit in PPI, and both L100P and Q31L mice displayed disruptions in LI, social defeat did not worsen deficits in these tasks for any group. Social defeat stress during adulthood was also associated with increased immobility in the forced swim test, as well as an anhedonic profile in the sucrose consumption test, but these effects were not genotype-dependent.

Another study employed the C′-truncated *DN-DISC1* model, where expression is under the control of the widely expressed prion protein promoter. Mutants and controls were subjected to three weeks of social isolation during middle and late adolescence (postnatal days 35–56). This manipulation resulted in the emergence of schizophrenia-related behavioural deficits, including PPI disruption, increased immobility in a forced swim test (a measure of behavioural despair which has been used to model apathy), and increased methamphetamine-induced locomotion, in mutants relative to isolated wildtypes and nonisolated mice of both genotypes [155]. *DN-DISC1* × isolation mice also displayed decreased tyrosine hydroxylase expression, total tissue DA levels, and basal extracellular DA in the frontal cortex relative to all other genotype and environmental conditions. The same genotype-dependent effect of increased DA release was observed in the nucleus accumbens of isolated *DN-DISC1* mutants relative to all other groups. The observed behavioural and cellular endophenotypes were rescued by administration of the glucocorticoid receptor antagonist RU-486, suggesting that the heightened stress-induced corticosterone response in *DN-DISC1* × isolation mice might represent the mechanism underlying the schizophrenia-relevant behavioural and cellular phenotypes. A recent follow-up study which assessed DNA methylation of HPA-axis/glucocorticoid-related genes in the mesocortical DA-ergic neurons of *DN-DISC1* × isolation mice revealed altered DNA methylation of *tyrosine hydroxylase, brain-derived neurotrophic factor* (*BDNF*) and *FK506 binding protein 5* genes [53]; these epigenetic changes were once again reversed by glucocorticoid receptor antagonist treatment.

9. Other Genes Implicated in Pathogenesis of Schizophrenia: Evidence for G × E Interactions

9.1. Dystrobrevin Binding Protein 1 (DTNBP1). Several studies have identified *DTNBP1* (or *dysbindin-1*) as a potential risk gene for schizophrenia [156–158]. Genetic association studies have shown that variations in this gene are associated with abnormal prefrontal cortical function in patients with schizophrenia, as well as episodic and working memory performance in healthy subjects [159–161]. The relevance of regionally specific loss of *DTNBP1* expression to the pathophysiology of this neurodevelopmental disorder is highlighted by postmortem studies revealing a decrease in *DTNBP1* expression in neurons of the dorsolateral prefrontal cortex and hippocampus [162, 163]. At a cellular level, DTNBP1 is mainly expressed in synaptic sites and plays an important role in synaptic homeostasis by regulating neurotransmitter vesicle exocytosis and vesicle biogenesis in neurons. DTNBP1 is also found in the nucleus, where it is reported to regulate transcription factor NF-kappa B activity to promote the expression of matrix metalloproteinase

protein-9 (MMP-9), a matrix metalloproteinase that influences synaptic plasticity and learning and memory, and TNF-α [164]. In mice containing a loss-of-function mutation in *DTNBP1* (sandy, *sdy*), they demonstrate hyperactivity, deficits in spatial learning and memory ability that are indicative of disrupted hippocampal function, and disruption of DA-ergic, glutamatergic, and GABA-ergic transmission in the prefrontal cortex [165–171]. While genetic background does appear to be an important factor in determining whether specific schizophrenia-related phenotypes are reported for the *sdy* mouse, memory impairment is a consistent phenotypic trait of DTNBP1-deficient mice irrespective of the mouse strain adopted [166, 172, 173].

Clinical studies provide some evidence indicating potentially significant associations between *DTNBP1* gene variation and the impact of adverse environmental risk factors on risk to develop schizophrenia [174, 175]. A study which examined potential interactions between *DTNBP1* variation and serious obstetric complications in a cohort of schizophrenia patients reported that the interaction of both factors influenced risk for schizophrenia [174]. It is also suggested that a common underlying molecular defect involving *DTNBP1* contribution to the development of anxiety and stress-related disorders may involve changes in glutamatergic neurotransmission or DA-ergic function [175]. Indeed, characterisation of the behavioural phenotype of the *sdy* mouse revealed enhanced anxiety in these mutants, as indicated by a reduced habituation to novelty, reduced locomotor activity and time spent in the center of an open field test, and fewer open arm entries in the elevated plus maze test [166, 176]. It is possible, therefore, that *DTNBP1* mutation directly or indirectly affects neuronal circuitry subserving anxiety behaviours and stress responsivity, meriting further examination of potential interactions between stress-related environmental risk factors in schizophrenia and *DTNBP1* gene abnormalities.

The timing of environmental insults during development and specific genetic vulnerability are important considerations in determining susceptibility to neurodevelopmental disorders and could differentially affect the degree to which *DTNBP1* mutations impact on structural and functional properties of neuronal cells, circuit connectivity, and overt behavioural phenotypes such as cognition, anxiety, and affective behaviour, leading to heterogeneous clinical phenotypes in schizophrenia [2, 25]. Evidence indicates that endogenous levels of the dysbindin protein in the mouse brain are higher during embryonic and early postnatal ages [177] suggesting adverse experiences during these vulnerable periods are more likely to affect the developmental course of dysbindin protein expression than those experienced during later stages of development. These findings highlight the critical nature of the temporal expression of *DTNBP1* in the brain and suggest that environmental factors experienced in early postnatal life and in adolescence may significantly impact on the trajectory of brain development and susceptibility to schizophrenia in those with *DTNBP1*-related genetic vulnerability.

9.2. SNAP-25. Synaptosomal-associated protein of 25 kDa (*SNAP-25*) is a gene associated with both synaptic transmission [178] and increased risk for schizophrenia [179, 180]. Mice containing a point mutation in the *SNAP-25* gene display several schizophrenia-associated endophenotypes including hyperactivity and increased behavioural sensitivity to psychostimulants, which are both mediated through DA D2 receptor activation [181, 182]. *SNAP-25* mutants were demonstrated to be particularly sensitive to the disruptive effects of variable prenatal stress on social novelty preference [183]. In the same study, both the point mutation and variable prenatal stress independently produced disruption of PPI. In a recent study, prenatal exposure to nicotine throughout gestation and early perinatal development in mice with partial loss of function of *SNAP-25* resulted in increased hyperactivity, social interaction deficits, and deficits in long-term depression, which are paralleled by changes in the affinity of the DA D2 receptor [184].

9.3. BDNF. Brain-derived neurotrophic factor (BDNF) is implicated in diverse neurodevelopmental processes, including neuronal differentiation and survival, and plasticity, and may be important to the pathophysiology of schizophrenia [162, 185]. Theleritis et al. [186] demonstrated that *BDNF* genotype is related to childhood trauma but not to cognitive deficits in first episode schizophrenia. Exposure of pregnant mice to restraint stress was associated with increased *BDNF* expression in the frontal cortex and hippocampus of adult offspring [187]. A recent study evaluated the interaction between prolonged adolescent exposure to escalating doses of methamphetamine and heterozygous disruption of *BDNF* in mice and demonstrated that decreased *BDNF* expression may alter sensitivity to psychostimulant exposure at important developmental periods [188]. Methamphetamine-treated wild-type mice, but not *BDNF* heterozygous mice, showed locomotor sensitization to acute 3 mg/kg D-amphetamine, and this study also demonstrated increased sensitivity to amphetamine-induced disruption of PPI in *BDNF* heterozygotes [189].

9.4. RELN. Reelin is a protein that is involved in brain development and synaptic plasticity; Reelin-mediated signalling pathway dysfunction has been linked with the pathophysiology of schizophrenia [190, 191]. *Reeler* is an autosomal recessive mutant mouse containing a mutation in the *RELN* gene, and several studies have examined the phenotypic consequences of interaction between early life adversity and the heterozygous *reeler* mouse phenotype. Interestingly, *reeler* mutants who were prenatally exposed to the neurotoxin chlorpyrifos [192] or early maternal separation [193] demonstrated a reversal of genotypic deficits across a number of schizophrenia-relevant endophenotypes; these included abnormalities in ultrasonic vocalisations and exploratory behaviour, as well as social interaction [193]. Neither chlorpyrifos exposure nor maternal separation alone exerted any effects on offspring behaviour. A recent study examined the phenotypic consequences of prenatal hypoxia on schizophrenia-related phenotypes in heterozygous *reeler* mice [194]. Exposure to prenatal hypoxia at embryonic day 17

(E17) was associated with a genotype-independent increase in anxiety (measured in the open-field test). No effect of genotype on PPI was observed, but a small treatment-related increase in PPI across both genotypes was reported [194]. *RELN* genotype × prenatal hypoxia interaction was found in relation to frontal cortex volume, which was increased in wildtypes, but the genotypic increase in *RELN* mutants was decreased following prenatal hypoxia exposure. A selective reduction in glucocorticoid receptor protein levels in the hippocampus of stressed *RELN* mutants was also observed.

10. Discussion

The current review provides a summary of findings arising from the growing body of research on the generation of animal models of schizophrenia based on the interaction of genetic mutations and well-characterised environmental factors ([28, 49, 195]; see Tables 1 and 2 for summary of G × E findings related to selected schizophrenia-associated genes). These findings support the proposed "multihit" diathesis-stress model, whereby vulnerability to schizophrenia involves both the independent contribution and synergistic convergence of temporally sensitive biological and environmental factors across development. Identification of biological and environmental influences across critical developmental periods and the mechanistic basis for their interaction may eventually result in enhanced identification of schizophrenia risk and the development of suitable preventative strategies.

A number of caveats and methodological considerations arise from our review of preclinical G × E models relevant to schizophrenia. Firstly, the heuristic value of a G × E model depends upon the level of construct validity possessed by the experimental model of the environmental stressor. Translation of epidemiologically appropriate environmental factors into current animal models of G × E interactions constitutes a particular challenge for models of G × E interplay in schizophrenia [196]. Secondly, it has to be noted that the majority of the studies outlined above have been conducted using rodent models involving a single gene mutation, while schizophrenia is a polygenic disorder [5]. Thirdly, much of the evidence outlined in the preceding sections is essentially descriptive, or the studies cited have focused on a limited number of molecular markers; more detailed molecular interrogation of phenotypic effects, at different time points, is required. In particular, neural circuits in animal models of G × E interactions will need to be examined with respect to behavioural changes, with a particular attention to the pathological trajectory from early development to the emergence and expression of the specified disease-relevant endophenotypes in adulthood [5]. These mechanistic studies will provide a solid basis for the development and evaluation of targeted preventative or rescue strategies. Lastly, several of the G × E models discussed have demonstrated that the effects of coexposure to a genetic mutation and an environmental stressor can result in modification of the phenotypic effects of one factor or the other but may also produce phenotypic effects, both protective and adverse, which may not be observed following exposure to any one factor alone [28].

It has been suggested that genetic risk to develop a psychotic disorder may be expressed as altered responsivity to everyday stressful situations [197], such that idiopathic responsivity to stressors may be an important determinant of induction of psychosis. At a phenotypic level, both the human genetic and preclinical G × E data related to schizophrenia have highlighted the importance of incorporating behavioural and physiological measures of stress responsivity in any phenotyping strategy. Both streams of evidence have clearly shown that it represents a modulating trait which might increase risk for schizophrenia [198, 199] and modulate the expression or severity of schizophrenia-relevant endophenotypes in preclinical G × E models (e.g., [24]).

As evident in the above description of G × E interaction in relevant mutant models, sex-specific effects are commonly observed, even allowing for the limited number of studies which have examined such effects in both sexes. Gender differences in schizophrenia have been noted across such domains as symptomatology and course of illness. Males show lower premorbid functioning, earlier age of onset, more severe cognitive deficits, and poorer prognosis at an earlier age of onset, and a poorer course of illness [200, 201]. There is sufficient evidence to conclude that independent and interactive effects of genetic and environmental manipulations on behavioural indices can differ between the sexes. Therefore, there is a requirement for G × E models to be validated for both sexes.

Despite the difficulty in interpreting the evidence for first- and second-order interactions arising from multifactorial G × E studies conducted in nonhumans, some authors have proposed common biological mechanisms or processes which might underlie such interactions [5]. One such mechanism is a disturbance in glutamatergic function, which may be related to dysfunction of parvalbumin-positive interneurons in the cerebral cortex and hippocampus, which are sensitive to alterations in NMDA-type glutamate receptors [202]. One of the common findings in both animal models and postmortem tissue from patients with schizophrenia is a reduction of mRNA or protein levels of the calcium-binding protein parvalbumin in cortical fast-spiking (FS) interneurons. Both preclinical genetic and environmentally based models using schizophrenia risk genes or stressors, respectively, have consistently observed a decreased number or impaired function of parvalbumin-positive interneurons in the hippocampus or cortex [91, 203]. A different model has suggested that genetic risk factors interact with social environmental risk factors (including early life adversity and psychosocial stress) to impact on the DA system, increasing its response to environmental stressors and to the abuse of drugs such as cannabis and psychostimulants [204, 205]. There are various strands of evidence to support this theory, including the well-characterised impact of acute and long-term exposure to stress and drugs of abuse on mesolimbic DA-ergic pathway dysfunction, and the fact that many of the genetic risk factors implicated in schizophrenia are associated with underlying alterations in the DA system [206]. Mesolimbic DA-ergic dysregulation is posited to be a fluid and dynamic process that may be reactive to acute and chronic stressors, including early brain insult, prolonged exposure to

Table 1: Summary of evidence for gene, environment, and gene × environment effects in mutant models for selected genes associated with schizophrenia.

Gene target	Environmental exposure	Reference(s)	Impact on schizophrenia-relevant behavioural endophenotypes			Use of preventative or rescue strategy
			Genetic manipulation	Environmental manipulation	Gene × environment	
NRG1	Prenatal Poly I:C	[92]	Decreased social novelty preference and PPI; sex-specific (females only) decrease in working memory	Disruption of working memory and PPI	Decreased sociability in Poly I:C × WT mice only; sex-specific decrease in alternation (i.e., working memory) following Poly I:C treatment was attenuated in female *NRG1* mutants	—
NRG1	Acute Δ-9 THC during adulthood	[128, 129]	Increased novelty-induced activity; decreased anxiety in the elevated plus maze and light-dark test; increased *c-fos* expression in the lateral septum and nucleus accumbens	Decreased novelty-induced activity; increased anxiety in the elevated plus maze; enhanced PPI; decreased social interaction; increased *c-fos* expression in the dorsolateral part of the bed nucleus of the stria terminalis and central nucleus of the amygdala	Increased sensitivity to locomotor suppressant effects of THC in *NRG1* mutants; greater PPI enhancement in *NRG1* mutants; greater increase in *c-fos* expression in the dorsolateral part of the bed nucleus of the stria terminalis and central nucleus of the amygdala, and paraventricular nucleus of the hypothalamus in *NRG1* mutants	—
NRG1	Subchronic CP 55, 940 [CB1R agonist] during adulthood	[131]	Increased novelty-induced activity	Decreased novelty-induced activity; increased anxiety in elevated plus maze and open field	Increased tolerance to CP55,940-induced anxiolytic and locomotor suppressant effects in *NRG1* mutants; increased *c-fos* expression in lateral septum in treated *NRG1* mutants	—
NRG1	Subchronic Δ-9-THC during adolescence	[132]	Increased novelty-induced activity	Decreased novelty-induced activity	Decreased anxiogenic effects of THC in *NRG1* mutants; decreased social investigative behaviours in WT only; disruption of PPI in THC-treated *NRG1* mutants	—
NRG1	Subchronic cannabidiol during adulthood	[136]	Increased novelty-induced activity; disrupted PPI; decreased 5-HT_{2a} receptor binding in substantia nigra	Enhanced PPI after acute cannabidiol; increased social interaction following chronic cannabidiol	Decreased sensitivity to anxiolytic effects of cannabidiol in mutants; selective enhancement of social interaction and PPI in *NRG1* mutants; selective enhancement of $GABA_A$ receptor binding in the granular retrosplenial cortex of *NRG1* mutants and reduction of 5-HT_{2a} receptor binding in the substantia nigra of WT	—

Table 1: Continued.

Gene target	Environmental exposure	Reference(s)	Impact on schizophrenia-relevant behavioural endophenotypes			Use of preventative or rescue strategy
			Genetic manipulation	Environmental manipulation	Gene × environment	
NRG1	Subchronic Δ-9 THC during adolescence	[133]	Altered expression of proteins involved in vesicular release of neurotransmitters, 5-HT neurotransmission, and growth factor expression	Reduced hippocampal expression of heat shock proteins and oxidative stress	Altered expression of proteins implicated in NMDA-mediated glutamatergic neurotransmission	—
NRG1	Social defeat during adolescence	[86]	Increased novelty-induced activity; decreased social novelty preference; PPI disruption; decreased anxiety	—	Selective decrease in anxiety and working memory in stressed *NRG1* mutants; protective effect of *NRG1* genotype on disruption of sucrose preference following social defeat	—
NRG1	Chronic restraint stress during adolescence	[153]	—	Increased NMDA receptor binding in ventral part of the lateral septum and dentate gyrus	PPI disruption in *NRG1* only following chronic stress exposure; altered patterns of NMDA receptor binding in infralimbic subregion of medial prefrontal cortex and dentate gyrus; decreased corticosterone levels, as well as increased apical dendritic spine density and decreased apical dendritic lengths and complexity in layer II/III pyramidal neurons of the medial prefrontal cortex	—
DISC1	Prenatal Poly I:C	[50]	Enlargement of the lateral ventricles	Increased anxiety in open field; decreased volume of amygdala and left/right periaqueductal grey; decrease in linear density of spines in pyramidal neurons of the CA1 region	Increase in anxiety in elevated plus maze and increased immobility in forced swim test in *DISC1* mutants; decreased social interaction in challenged *DISC1* offspring; decreased linear spine density on dendrites of granule cells of the dentate gyrus in *DISC1* mutants only; opposite effects on lateral ventricle volume (increased in WT, decreased in mutants)	—

Table 1: Continued.

Gene target	Environmental exposure	Reference(s)	Impact on schizophrenia-relevant behavioural endophenotypes			Use of preventative or rescue strategy
			Genetic manipulation	Environmental manipulation	Gene × environment	
DISC1	Prenatal Poly I:C	[104]	Decreased PPI in *DISC1* Q31L mutant; decreased LI and social affiliative behaviour in *DISC1* L100P line	Decreased PPI and LI; disruption of spatial discrimination and object exploration	More prominent PPI and LI deficits in L100P mutants; impaired working memory and sociability in challenged *DISC1* offspring; increase of Poly I:C-induced increase in IL-6 in brains of *DISC1* mutants	Coadministration of IL-6 antagonist with Poly I:C reversed Poly I:C-related deficits in mutants and controls
DISC1	Neonatal Poly I:C	[105, 106]	—	—	Selective deficits in short-term memory and object recognition memory in *DISC1* mutants; increased behavioural sensitivity to MK-801 in *DISC1* mice exposed to Poly I:C; selective decrease in parvalbumin-positive interneurons in the medial prefrontal cortex	Cognitive deficits in Poly I:C-treated DISC1 mutants improved by clozapine while haloperidol had no effect; clozapine suppressed the augmentation of MK-801-induced hyperactivity
DISC1	Prenatal lead exposure	[107]	Enlargement of lateral ventricles; decreased anxiety in open field	Increased anxiety in open field; increased anxiety in elevated plus maze; increased MX-801 responsivity; decreased PPI; enlargement of lateral ventricles	Heightened responsivity to the NMDAR antagonist MK-801 and increased PPI disruption in female *DISC1* mice; synergistic decrease in exploratory activity and synergistic increase in lateral ventricular volume in *DISC1* mutants	Systemic administration of D-serine, a coagonist at the NMDA receptor, reversed PPI deficits in female lead-exposed mutants
DISC1	Subchronic Δ-9 THC during adolescence	[137]	Decrease in contextual fear memory; decreased synaptic CB1R expression in the prefrontal cortex, hippocampus, and amygdala	Decrease in synaptic CB1R expression in the prefrontal cortex, hippocampus, and amygdala	Disruption in cue-dependent fear memory	—
DISC1	Social defeat during adulthood	[154]	Decreased PPI in *DISC1* L100P; impaired LI in L100P and *DISC1* Q31L; decreased sociability and social novelty in Q31L mutants	Increased immobility in forced swim test; decreased sucrose intake in the sucrose consumption test	Decrease in exploratory activity and sociability and social novelty in L100P; increase in anxiety in the elevated plus maze in L100P but not Q31L mutants exposed to social defeat	—

Table 1: Continued.

Gene target	Environmental exposure	Reference(s)	Impact on schizophrenia-relevant behavioural endophenotypes			Use of preventative or rescue strategy
			Genetic manipulation	Environmental manipulation	Gene × environment	
DISC1	Prolonged social isolation during adolescence	[51, 155]	—	—	PPI disruption, forced swim immobility, and methamphetamine-induced locomotion, in isolated *DISC1* mutants; decreased tyrosine hydroxylase expression, total tissue DA levels, and DA in the frontal cortex; increased DA release in the nucleus accumbens; altered DNA methylation of *tyrosine hydroxylase*, *BDNF*, and *FK506 binding protein 5* genes	RU-486 normalized basal and methamphetamine-induced extracellular DA, tyrosine hydroxylase, and DA D2 receptor levels in G × E model; RU-486 also reversed PPI, forced swim test deficits, and changes in amphetamine-induced activity in this model
COMT	Subchronic Δ-9 THC during adolescence	[123]	Improved spatial working memory in *COMT* KO males	Decreased object recognition, social novelty preference, and anxiety	Increased hyperactivity and greater disruption of working memory in THC-treated *COMT* KO mice	—
COMT	Subchronic Win 55,212 [CB1R agonist] during adolescence	[143]	—	Decreased social novelty preference; decreased anxiety in the light-dark test	Selective disruption of PPI in cannabinoid-treated *COMT* mutants; decreased sensitivity to disruptive effects on sociability in mutants relative to WT	—
COMT	Subchronic Δ-9 THC during adolescence	[144]	Increased CB1R intensity in the prefrontal cortex; decreased CB1R intensity in the hippocampus; parvalbumin cell size decreased in *COMT* heterozygotes	Decreased cell density in the VTA	Decreased parvalbumin cell intensity in the prefrontal cortex; decreased DA cell size in VTA; increased CB1R intensity in hippocampus of THC-treated *COMT* mutants	—

BDNF, brain-derived neurotrophic factor; CB1R, cannabinoid receptor 1; *COMT*, catechol-*O*-methyltransferase; DA, dopamine; Δ-9 THC, delta-9-tetrahydrocannabinol; *DISC1*, disrupted in schizophrenia 1; $GABA_A$, gamma-aminobutyric acid type A receptor; IL-6, interleukin 6; KO, knockout; LI, latent inhibition; NMDA receptor, N-methyl-D-aspartate receptor; NRG1, neuregulin-1; PPI, prepulse inhibition; $5\text{-}HT_{2A}$, serotonin 2_A receptor; VTA, ventral tegmental area.

Table 2: Summary of evidence for gene, environment, and gene × environment effects in mutant models for selected genes associated with schizophrenia.

Gene target	Environmental exposure	Reference(s)	Impact on schizophrenia-relevant behavioural endophenotypes			Use of preventative or rescue strategy
			Genetic manipulation	Environmental manipulation	Gene × environment	
Nurr1	Prenatal Poly I:C	[110]	Increased novelty-induced activity; decreased PPI, reduction in tyrosine hydroxylase-positive cells in the substantia nigra	Increased novelty-induced activity; decreased PPI; spatial working memory deficits; increase in tyrosine hydroxylase-positive cells in the VTA	Additive effects on novelty-induced hyperactivity; synergistic reduction in attentional shifting and sustained attention; decrease in DA D2 receptor immunoreactivity in the nucleus accumbens	—
Snap-25	Variable prenatal stress	[183]	Decreased PPI in the *blind-drunk* point mutant	PPI disruption	Decreased social novelty preference	Clozapine and haloperidol (to a lesser extent) reversal of PPI deficits was most pronounced in G × E group
Snap-25	Prenatal nicotine exposure	[184]	Increased novelty-induced activity and decreased social interaction	—	More pronounced novelty-induced hyperactivity and greater disruption of social interaction; deficits in DA D2 receptor-dependent induction of long-term synaptic depression	—
BDNF	Chronic methamphetamine exposure	[188]	—	Locomotor sensitisation and increased entropy	Decreased locomotor sensitisation and entropy in *BDNF* heterozygotes	—
BDNF	Chronic methamphetamine exposure	[189]	Decreased PPI and increased acoustic startle reactivity in *BDNF* heterozygotes	Locomotor sensitisation; increased sensitivity to MK-801 and amphetamine-induced PPI disruption	Increased sensitivity to amphetamine-induced PPI disruption in preexposed *BDNF* heterozygotes	—
RELN	Maternal separation	[193]	Decreased frequency of ultrasonic vocalisations; decreased activity in a novel environment	—	Decreased sensitivity to disruptive effects of maternal separation in heterozygous *RELN* mutants	—
RELN	Prenatal exposure to the pesticide chlorpyrifos Maternal separation	[192]	Decreased frequency of ultrasonic vocalisations	—	Prenatal chlorpyrifos: selective increase in ultrasonic vocalisation in *RELN* mutants; disrupted behavioural response to acute scopolamine Maternal separation: decreased social motivation in WT but not *RELN* mutants	—

Table 2: Continued.

Gene target	Environmental exposure	Reference(s)	Impact on schizophrenia-relevant behavioural endophenotypes			Use of preventative or rescue strategy
			Genetic manipulation	Environmental manipulation	Gene × environment	
RELN	Prenatal hypoxia	[194]	Increase in frontal cortex volume in *RELN* mutants	Reduction in glucocorticoid receptor protein levels in frontal cortex	Increase in frontal cortex volume in WT but opposite effect observed in *RELN* mutants; selective reduction in glucocorticoid receptor protein levels in hippocampus of *RELN* mutants; selective changes in brain expression of hypoxia-related proteins in mutants	—

BDNF, brain-derived neurotrophic factor; DA, dopamine; Δ-9 THC, delta-9-tetrahydrocannabinol; NURR1, nuclear receptor related 1 protein; PPI, prepulse inhibition; *RELN*, reelin; SNAP-25, synaptosome associated protein 25 kDa; VTA, ventral tegmental area; WT, wildtype.

drugs of abuse, and psychosocial stress, across the lifespan of the individual. Another theory places a greater emphasis on the convergence of genetic and environmental factors upon regulation of synaptic plasticity and function, as well as the stabilisation of cortical microcircuitry [42, 207]. It has been observed that intact synaptic function depends on a large number of molecular pathways which will be affected by several environmental factors throughout brain development. Additionally, stress-associated signalling cascades are well known to modulate the development and maintenance of synaptic connectivity [5].

What existing animal studies of G × E interactions relevant to schizophrenia highlight is that developing valid multifactorial models which are amenable to investigations not yet possible in clinical studies will become increasingly important in determining the mechanisms underlying convergence of genetic and environmental risk factors and their interaction.

Competing Interests

The authors declare that they have no competing interests.

References

[1] A. S. Brown, "Prenatal infection as a risk factor for schizophrenia," *Schizophrenia Bulletin*, vol. 32, no. 2, pp. 200–202, 2006.

[2] A. S. Brown, "The environment and susceptibility to schizophrenia," *Progress in Neurobiology*, vol. 93, no. 1, pp. 23–58, 2011.

[3] P. D. Harvey, A. P. Wingo, K. E. Burdick, and R. J. Baldessarini, "Cognition and disability in bipolar disorder: lessons from schizophrenia research," *Bipolar Disorders*, vol. 12, no. 4, pp. 364–375, 2010.

[4] J. L. Waddington, R. J. Hennessy, C. M. P. O'Tuathaigh et al., "Schizophrenia and the lifetime trajectory of psychotic illness: developmental neuroscience and pathobiology, redux," in *The Origins of Schizophrenia*, A. S. Brown and P. H. Patterson, Eds., pp. 3–21, Columbia University Press, New York, NY, USA, 2012.

[5] M. J. Owen, A. Sawa, and P. B. Mortensen, "Schizophrenia," *The Lancet*, vol. 388, no. 10039, pp. 86–97, 2016.

[6] A. Gustavsson, M. Svensson, F. Jacobi et al., "Cost of disorders of the brain in Europe 2010," *European Neuropsychopharmacology*, vol. 21, no. 10, pp. 718–779, 2011.

[7] H. U. Wittchen, F. Jacobi, J. Rehm et al., "The size and burden of mental disorders and other disorders of the brain in Europe 2010," *European Neuropsychopharmacology*, vol. 21, no. 9, pp. 655–679, 2011.

[8] S. Leucht and S. Heres, "Epidemiology, clinical consequences, and psychosocial treatment of nonadherence in schizophrenia," *Journal of Clinical Psychiatry*, vol. 67, no. 5, pp. 3–8, 2006.

[9] P. J. Harrison, "Recent genetic findings in schizophrenia and their therapeutic relevance," *Journal of Psychopharmacology*, vol. 29, no. 2, pp. 85–96, 2015.

[10] J. L. Rapoport, J. N. Giedd, and N. Gogtay, "Neurodevelopmental model of schizophrenia: update 2012," *Molecular Psychiatry*, vol. 17, no. 12, pp. 1228–1238, 2012.

[11] J. Gratten, N. R. Wray, M. C. Keller, and P. M. Visscher, "Large-scale genomics unveils the genetic architecture of psychiatric disorders," *Nature Neuroscience*, vol. 17, no. 6, pp. 782–790, 2014.

[12] M. Burmeister, "Basic concepts in the study of diseases with complex genetics," *Biological Psychiatry*, vol. 45, no. 5, pp. 522–532, 1999.

[13] M. Burmeister, M. G. McInnis, and S. Zöllner, "Psychiatric genetics: progress amid controversy," *Nature Reviews Genetics*, vol. 9, no. 7, pp. 527–540, 2008.

[14] S. Zammit, M. J. Owen, and G. Lewis, "Misconceptions about gene-environment interactions in psychiatry," *Evidence-Based Mental Health*, vol. 13, no. 3, pp. 65–68, 2010.

[15] M. R. Munafò, S. Zammit, and J. Flint, "Practitioner Review: a critical perspective on gene-environment interaction models—what impact should they have on clinical perceptions and practice?" *Journal of Child Psychology and Psychiatry and Allied Disciplines*, vol. 55, no. 10, pp. 1092–1101, 2014.

[16] R. A. Shih, P. L. Belmonte, and P. P. Zandi, "A review of the evidence from family, twin and adoption studies for a genetic

contribution to adult psychiatric disorders," *International Review of Psychiatry*, vol. 16, no. 4, pp. 260–283, 2004.

[17] C. A. Prescott and I. I. Gottesman, "Genetically mediated vulnerability to schizophrenia," *Psychiatric Clinics of North America*, vol. 16, no. 2, pp. 245–267, 1993.

[18] M. S. Keshavan, "Development, disease and degeneration in schizophrenia: a unitary pathophysiological model," *Journal of Psychiatric Research*, vol. 33, no. 6, pp. 513–521, 1999.

[19] S. Tosato, P. Dazzan, and D. Collier, "Association between the neuregulin 1 gene and schizophrenia: a systematic review," *Schizophrenia Bulletin*, vol. 31, no. 3, pp. 613–617, 2005.

[20] M. J. Owen, "Implications of genetic findings for understanding schizophrenia," *Schizophrenia Bulletin*, vol. 38, no. 5, pp. 904–907, 2012.

[21] C. A. Ross, R. L. Margolis, S. A. J. Reading, M. Pletnikov, and J. T. Coyle, "Neurobiology of schizophrenia," *Neuron*, vol. 52, no. 1, pp. 139–153, 2006.

[22] M. C. O'Donovan, N. J. Craddock, and M. J. Owen, "Genetics of psychosis; insights from views across the genome," *Human Genetics*, vol. 126, no. 1, pp. 3–12, 2009.

[23] D. S. Rudd, M. Axelsen, E. A. Epping, N. C. Andreasen, and T. H. Wassink, "A genome-wide CNV analysis of schizophrenia reveals a potential role for a multiple-hit model," *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, vol. 165, no. 8, pp. 619–626, 2014.

[24] T. Karl, "Neuregulin 1: a prime candidate for research into gene-environment interactions in schizophrenia? Insights from genetic rodent models," *Frontiers in Behavioral Neuroscience*, vol. 7, article 106, 2013.

[25] G. Kannan, A. Sawa, and M. V. Pletnikov, "Mouse models of gene-environment interactions in schizophrenia," *Neurobiology of Disease*, vol. 57, pp. 5–11, 2013.

[26] C. M. O'Tuathaigh and J. L. Waddington, "Closing the translational gap between mutant mouse models and the clinical reality of psychotic illness," *Neuroscience and Biobehavioral Reviews*, vol. 58, pp. 19–35, 2015.

[27] V. Labrie, S. Pai, and A. Petronis, "Epigenetics of major psychosis: progress, problems and perspectives," *Trends in Genetics*, vol. 28, no. 9, pp. 427–435, 2012.

[28] Y. Ayhan, R. McFarland, and M. V. Pletnikov, "Animal models of gene-environment interaction in schizophrenia: a dimensional perspective," *Progress in Neurobiology*, vol. 136, pp. 1–27, 2016.

[29] D. Malaspina, "Paternal factors and schizophrenia risk: de novo mutations and imprinting," *Schizophrenia Bulletin*, vol. 27, no. 3, pp. 379–393, 2001.

[30] A. Reichenberg, R. Gross, S. Sandin, and E. S. Susser, "Advancing paternal and maternal age are both important for autism risk," *American Journal of Public Health*, vol. 100, no. 5, pp. 772–773, 2010.

[31] A. Kong, M. L. Frigge, G. Masson et al., "Rate of de novo mutations and the importance of father's age to disease risk," *Nature*, vol. 488, no. 7412, pp. 471–475, 2012.

[32] M. C. Perrin, A. S. Brown, and D. Malaspina, "Aberrant epigenetic regulation could explain the relationship of paternal age to schizophrenia," *Schizophrenia Bulletin*, vol. 33, no. 6, pp. 1270–1273, 2007.

[33] R. G. Smith, A. Reichenberg, R. L. Kember et al., "Advanced paternal age is associated with altered DNA methylation at brain-expressed imprinted loci in inbred mice: implications for neuropsychiatric disease," *Molecular Psychiatry*, vol. 18, no. 6, pp. 635–636, 2013.

[34] P. F. Sullivan, K. S. Kendler, and M. C. Neale, "Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies," *Archives of General Psychiatry*, vol. 60, no. 12, pp. 1187–1192, 2003.

[35] International Schizophrenia Consortium, "Rare chromosomal deletions and duplications increase risk of schizophrenia," *Nature*, vol. 455, pp. 237–241, 2008.

[36] H. Stefansson, D. Rujescu, S. Cichon et al., "Large recurrent microdeletions associated with schizophrenia," *Nature*, vol. 455, pp. 232–236, 2008.

[37] G. Kirov, D. Rujescu, A. Ingason, D. A. Collier, M. C. O'Donovan, and M. J. Owen, "Neurexin 1 (NRXN1) deletions in schizophrenia," *Schizophrenia Bulletin*, vol. 35, no. 5, pp. 851–854, 2009.

[38] P. V. Gejman, A. R. Sanders, and K. S. Kendler, "Genetics of schizophrenia: new findings and challenges," *Annual Review of Genomics and Human Genetics*, vol. 12, pp. 121–144, 2011.

[39] Schizophrenia Working Group of the Psychiatric Genomics Consortium, "Biological insights from 108 schizophrenia-associated genetic loci," *Nature*, vol. 511, pp. 421–427, 2014.

[40] C. L. Winchester, J. A. Pratt, and B. J. Morris, "Risk genes for schizophrenia: translational opportunities for drug discovery," *Pharmacology and Therapeutics*, vol. 143, no. 1, pp. 34–50, 2014.

[41] S. M. Purcell, J. L. Moran, M. Fromer et al., "A polygenic burden of rare disruptive mutations in schizophrenia," *Nature*, vol. 506, no. 7487, pp. 185–190, 2014.

[42] J. Hall, S. Trent, K. L. Thomas, M. C. O'Donovan, and M. J. Owen, "Genetic risk for schizophrenia: convergence on synaptic pathways involved in plasticity," *Biological Psychiatry*, vol. 77, no. 1, pp. 52–58, 2015.

[43] Cross-Disorder Group of the Psychiatric Genomics Consortium, "Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs," *Nature Genetics*, vol. 45, pp. 984–994, 2013.

[44] D. M. Ruderfer, A. H. Fanous, S. Ripke et al., "Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia," *Molecular Psychiatry*, vol. 19, no. 9, pp. 1017–1024, 2014.

[45] S. A. McCarroll, G. Feng, and S. E. Hyman, "Genome-scale neurogenetics: methodology and meaning," *Nature Neuroscience*, vol. 17, no. 6, pp. 756–763, 2014.

[46] F. Cirulli, N. Francia, A. Berry, L. Aloe, E. Alleva, and S. J. Suomi, "Early life stress as a risk factor for mental health: role of neurotrophins from rodents to non-human primates," *Neuroscience and Biobehavioral Reviews*, vol. 33, no. 4, pp. 573–585, 2009.

[47] E. J. Nestler and S. E. Hyman, "Animal models of neuropsychiatric disorders," *Nature Neuroscience*, vol. 13, no. 10, pp. 1161–1169, 2010.

[48] J. van Os, G. Kenis, and B. P. F. Rutten, "The environment and schizophrenia," *Nature*, vol. 468, no. 7321, pp. 203–212, 2010.

[49] L. Gray and A. J. Hannan, "Dissecting cause and effect in the pathogenesis of psychiatric disorders: genes, environment and behaviour," *Current Molecular Medicine*, vol. 7, no. 5, pp. 470–478, 2007.

[50] B. Abazyan, J. Nomura, G. Kannan et al., "Prenatal interaction of mutant DISC1 and immune activation produces adult psychopathology," *Biological Psychiatry*, vol. 68, no. 12, pp. 1172–1181, 2010.

[51] M. Niwa, R. S. Lee, T. Tanaka, K. Okada, S. Kano, and A. Sawa, "A critical period of vulnerability to adolescent stress: epigenetic

mediators in mesocortical dopaminergic neurons," *Human Molecular Genetics*, vol. 25, no. 7, pp. 1370–1381, 2016.

[52] T. D. Cannon, T. G. M. Van Erp, C. E. Bearden et al., "Early and late neurodevelopmental influences in the prodrome to schizophrenia: contributions of genes, environment, and their interactions," *Schizophrenia Bulletin*, vol. 29, no. 4, pp. 653–669, 2003.

[53] C. M. O'Tuathaigh, L. Desbonnet, P. M. Moran, and J. L. Waddington, "Susceptibility genes for schizophrenia: mutant models, endophenotypes and psychobiology," *Current Topics in Behavioural Neuroscience*, vol. 12, pp. 209–250, 2012.

[54] D. Braff, N. J. Schork, and I. I. Gottesman, "Endophenotyping schizophrenia," *American Journal of Psychiatry*, vol. 164, no. 5, pp. 705–707, 2007.

[55] B. N. Cuthbert and T. R. Insel, "Toward the future of psychiatric diagnosis: the seven pillars of RDoC," *BMC Medicine*, vol. 11, article 126, 2013.

[56] B. D. Kelly, E. O'Callaghan, J. L. Waddington et al., "Schizophrenia and the city: a review of literature and prospective study of psychosis and urbanicity in Ireland," *Schizophrenia Research*, vol. 116, no. 1, pp. 75–89, 2010.

[57] P. Casadio, C. Fernandes, R. M. Murray, and M. Di Forti, "Cannabis use in young people: the risk for schizophrenia," *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 8, pp. 1779–1787, 2011.

[58] J. J. McGrath, M. R. Pemberton, J. L. Welham, and R. M. Murray, "Schizophrenia and the influenza epidemics of 1954, 1957 and 1959: a southern hemisphere study," *Schizophrenia Research*, vol. 14, no. 1, pp. 1–8, 1994.

[59] M. Cannon, D. Cotter, V. P. Coffey et al., "Prenatal exposure to the 1957 influenza epidemic and adult schizophrenia: a follow-up study," *British Journal of Psychiatry*, vol. 168, pp. 368–371, 1996.

[60] P. B. Mortensen, C. B. Pedersen, T. Westergaard et al., "Effects of family history and place and season of birth on the risk of schizophrenia," *The New England Journal of Medicine*, vol. 340, no. 8, pp. 603–608, 1999.

[61] S. L. Buka, T. D. Cannon, E. F. Torrey, and R. H. Yolken, "Maternal exposure to herpes simplex virus and risk of psychosis among adult offspring," *Biological Psychiatry*, vol. 63, no. 8, pp. 809–815, 2008.

[62] A. S. Brown and E. J. Derkits, "Prenatal infection and schizophrenia: a review of epidemiologic and translational studies," *The American Journal of Psychiatry*, vol. 167, no. 3, pp. 261–280, 2010.

[63] U. Meyer, J. Feldon, M. Schedlowski, and B. K. Yee, "Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia," *Neuroscience and Biobehavioral Reviews*, vol. 29, no. 6, pp. 913–947, 2005.

[64] U. Meyer, J. Feldon, M. Schedlowski, and B. K. Yee, "Immunological stress at the maternal-foetal interface: a link between neurodevelopment and adult psychopathology," *Brain, Behavior, and Immunity*, vol. 20, no. 4, pp. 378–388, 2006.

[65] U. Meyer, P. J. Murray, A. Urwyler, B. K. Yee, M. Schedlowski, and J. Feldon, "Adult behavioral and pharmacological dysfunctions following disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling," *Molecular Psychiatry*, vol. 13, no. 2, pp. 208–221, 2008.

[66] B. K. Bitanihirwe, D. Peleg-Raibstein, F. Mouttet, J. Feldon, and U. Meyer, "Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia," *Neuropsychopharmacology*, vol. 35, no. 12, pp. 2462–2478, 2010.

[67] L. Shi, S. H. Fatemi, R. W. Sidwell, and P. H. Patterson, "Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring," *The Journal of Neuroscience*, vol. 23, no. 1, pp. 297–302, 2003.

[68] U. Meyer, M. Nyffeler, A. Engler et al., "The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology," *The Journal of Neuroscience*, vol. 26, no. 18, pp. 4752–4762, 2006.

[69] L. Zuckerman, M. Rehavi, R. Nachman, and I. Weiner, "Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia," *Neuropsychopharmacology*, vol. 28, no. 10, pp. 1778–1789, 2003.

[70] S. E. P. Smith, J. Li, K. Garbett, K. Mirnics, and P. H. Patterson, "Maternal immune activation alters fetal brain development through interleukin-6," *The Journal of Neuroscience*, vol. 27, no. 40, pp. 10695–10702, 2007.

[71] N. V. Malkova, C. Z. Yu, E. Y. Hsiao, M. J. Moore, and P. H. Patterson, "Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism," *Brain, Behavior, and Immunity*, vol. 26, no. 4, pp. 607–616, 2012.

[72] L. Zuckerman and I. Weiner, "Post-pubertal emergence of disrupted latent inhibition following prenatal immune activation," *Psychopharmacology*, vol. 169, no. 3-4, pp. 308–313, 2003.

[73] K. Zavitsanou, C. K. Lim, T. Purves-Tyson et al., "Effect of maternal immune activation on the kynurenine pathway in preadolescent rat offspring and on MK801-induced hyperlocomotion in adulthood: amelioration by COX-2 inhibition," *Brain, Behavior, and Immunity*, vol. 41, no. 1, pp. 173–181, 2014.

[74] Y. Piontkewitz, M. Arad, and I. Weiner, "Abnormal trajectories of neurodevelopment and behavior following in utero insult in the rat," *Biological Psychiatry*, vol. 70, no. 9, pp. 842–851, 2011.

[75] S. Reisinger, D. Khan, E. Kong, A. Berger, A. Pollak, and D. D. Pollak, "The Poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery," *Pharmacology and Therapeutics*, vol. 149, pp. 213–226, 2015.

[76] H. Stefansson, E. Sigurdsson, V. Steinthorsdottir et al., "Neuregulin 1 and susceptibility to schizophrenia," *American Journal of Human Genetics*, vol. 71, no. 4, pp. 877–892, 2002.

[77] Y. G. Gong, C. N. Wu, Q. H. Xing, X. Z. Zhao, J. Zhu, and L. He, "A two-method meta-analysis of Neuregulin 1(NRG1) association and heterogeneity in schizophrenia," *Schizophrenia Research*, vol. 111, no. 1–3, pp. 109–114, 2009.

[78] H. C. Loh, P. Y. Tang, S. F. Tee et al., "Neuregulin-1 (NRG-1) and its susceptibility to schizophrenia: a case-control study and meta-analysis," *Psychiatry Research*, vol. 208, no. 2, pp. 186–188, 2013.

[79] P. J. Harrison and A. J. Law, "Neuregulin 1 and schizophrenia: genetics, gene expression, and neurobiology," *Biological Psychiatry*, vol. 60, no. 2, pp. 132–140, 2006.

[80] L. Mei and W.-C. Xiong, "Neuregulin 1 in neural development, synaptic plasticity and schizophrenia," *Nature Reviews Neuroscience*, vol. 9, no. 6, pp. 437–452, 2008.

[81] K. Hänninen, H. Katila, M. Saarela et al., "Interleukin-1 beta gene polymorphism and its interactions with neuregulin-1 gene polymorphism are associated with schizophrenia," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 258, no. 1, pp. 10–15, 2007.

[82] K. Marballi, M. P. Quinones, F. Jimenez et al., "In vivo and in vitro genetic evidence of involvement of neuregulin 1 in immune system dysregulation," *Journal of Molecular Medicine*, vol. 88, no. 11, pp. 1133–1141, 2010.

[83] T. Kato, A. Kasai, M. Mizuno et al., "Phenotypic characterization of transgenic mice overexpressing neuregulin-1," *PLoS ONE*, vol. 5, no. 12, Article ID e14185, 2010.

[84] T. Karl, L. Duffy, A. Scimone, R. P. Harvey, and P. R. Schofield, "Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia," *Genes, Brain and Behavior*, vol. 6, no. 7, pp. 677–687, 2007.

[85] C. M. P. O'Tuathaigh, D. Babovic, G. J. O'Sullivan et al., "Phenotypic characterization of spatial cognition and social behavior in mice with 'knockout' of the schizophrenia risk gene neuregulin 1," *Neuroscience*, vol. 147, no. 1, pp. 18–27, 2007.

[86] L. Desbonnet, C. O'Tuathaigh, G. Clarke et al., "Phenotypic effects of repeated psychosocial stress during adolescence in mice mutant for the schizophrenia risk gene neuregulin-1: a putative model of gene × environment interaction," *Brain, Behavior, and Immunity*, vol. 26, no. 4, pp. 660–671, 2012.

[87] M. van den Buuse, L. Wischhof, R. X. Lee, S. Martin, and T. Karl, "Neuregulin 1 hypomorphic mutant mice: enhanced baseline locomotor activity but normal psychotropic drug-induced hyperlocomotion and prepulse inhibition regulation," *International Journal of Neuropsychopharmacology*, vol. 12, no. 10, pp. 1383–1393, 2009.

[88] T. Karl, T. H. J. Burne, M. Van den Buuse, and R. Chesworth, "Do transmembrane domain neuregulin 1 mutant mice exhibit a reliable sensorimotor gating deficit?" *Behavioural Brain Research*, vol. 223, no. 2, pp. 336–341, 2011.

[89] M. Rimer, D. W. Barrett, M. A. Maldonado, V. M. Vock, and F. Gonzalez-Lima, "Neuregulin-1 immunoglobulin-like domain mutant mice: clozapine sensitivity and impaired latent inhibition," *NeuroReport*, vol. 16, no. 3, pp. 271–275, 2005.

[90] Y.-J. J. Chen, M. A. Johnson, M. D. Lieberman et al., "Type III neuregulin-1 is required for normal sensorimotor gating, memory-related behaviors, and corticostriatal circuit components," *The Journal of Neuroscience*, vol. 28, no. 27, pp. 6872–6883, 2008.

[91] J.-C. Pei, C.-M. Liu, and W.-S. Lai, "Distinct phenotypes of new transmembrane-domain neuregulin 1 mutant mice and the rescue effects of valproate on the observed schizophrenia-related cognitive deficits," *Frontiers in Behavioral Neuroscience*, vol. 8, article 126, 2014.

[92] C. O'Leary, L. Desbonnet, N. Clarke et al., "Phenotypic effects of maternal immune activation and early postnatal milieu in mice mutant for the schizophrenia risk gene neuregulin-1," *Neuroscience*, vol. 277, pp. 294–305, 2014.

[93] D. H. R. Blackwood, A. Fordyce, M. T. Walker, D. M. St. Clair, D. J. Porteous, and W. J. Muir, "Schizophrenia and affective disorders—cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family," *American Journal of Human Genetics*, vol. 69, no. 2, pp. 428–433, 2001.

[94] N. A. Sachs, A. Sawa, S. E. Holmes, C. A. Ross, L. E. DeLisi, and R. L. Margolis, "A frameshift mutation in disrupted in schizophrenia 1 in an American family with schizophrenia and schizoaffective disorder," *Molecular Psychiatry*, vol. 10, no. 8, pp. 758–764, 2005.

[95] N. J. Bradshaw and D. J. Porteous, "DISC1-binding proteins in neural development, signalling and schizophrenia," *Neuropharmacology*, vol. 62, no. 3, pp. 1230–1241, 2012.

[96] N. J. Brandon and A. Sawa, "Linking neurodevelopmental and synaptic theories of mental illness through DISC1," *Nature Reviews Neuroscience*, vol. 12, no. 12, pp. 707–722, 2011.

[97] S. J. Clapcote, T. V. Lipina, J. K. Millar et al., "Behavioral phenotypes of Disc1 missense mutations in mice," *Neuron*, vol. 54, no. 3, pp. 387–402, 2007.

[98] T. Hikida, H. Jaaro-Peled, S. Seshadri et al., "Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 36, pp. 14501–14506, 2007.

[99] W. Li, Y. Zhou, J. D. Jentsch et al., "Specific developmental disruption of disrupted-in-schizophrenia-1 function results in schizophrenia-related phenotypes in mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 46, pp. 18280–18285, 2007.

[100] M. Kvajo, H. McKellar, P. A. Arguello et al., "A mutation in mouse Disc1 that models a schizophrenia risk allele leads to specific alterations in neuronal architecture and cognition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 19, pp. 7076–7081, 2008.

[101] M. V. Pletnikov, Y. Ayhan, O. Nikolskaia et al., "Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia," *Molecular Psychiatry*, vol. 13, no. 2, pp. 173–186, 2008.

[102] S. Shen, B. Lang, C. Nakamoto et al., "Schizophrenia-related neural and behavioral phenotypes in transgenic mice expressing truncated DISC1," *The Journal of Neuroscience*, vol. 28, no. 43, pp. 10893–10904, 2008.

[103] Y. Mao, X. Ge, C. L. Frank et al., "Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3β/β-catenin signaling," *Cell*, vol. 136, no. 6, pp. 1017–1031, 2009.

[104] T. V. Lipina, C. Zai, D. Hlousek, J. C. Roder, and A. H. C. Wong, "Maternal immune activation during gestation interacts with Disc1 point mutation to exacerbate schizophrenia-related behaviors in mice," *The Journal of Neuroscience*, vol. 33, no. 18, pp. 7654–7666, 2013.

[105] D. Ibi, T. Nagai, Y. Kitahara et al., "Neonatal polyI:C treatment in mice results in schizophrenia-like behavioral and neurochemical abnormalities in adulthood," *Neuroscience Research*, vol. 64, no. 3, pp. 297–305, 2009.

[106] T. Nagai, Y. Kitahara, D. Ibi, T. Nabeshima, A. Sawa, and K. Yamada, "Effects of antipsychotics on the behavioral deficits in human dominant-negative DISC1 transgenic mice with neonatal polyI:C treatment," *Behavioural Brain Research*, vol. 225, no. 1, pp. 305–310, 2011.

[107] B. Abazyan, J. Dziedzic, K. Hua et al., "Chronic exposure of mutant DISC1 mice to lead produces sex-dependent abnormalities consistent with schizophrenia and related mental disorders: a gene-environment interaction study," *Schizophrenia Bulletin*, vol. 40, no. 3, pp. 575–584, 2014.

[108] B. Kadkhodaei, T. Ito, E. Joodmardi et al., "Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons," *The Journal of Neuroscience*, vol. 29, no. 50, pp. 15923–15932, 2009.

[109] P. Rojas, E. Joodmardi, Y. Hong, T. Perlmann, and S. O. Ögren, "Adult mice with reduced Nurr1 expression: an animal model

for schizophrenia," *Molecular Psychiatry*, vol. 12, no. 8, pp. 756–766, 2007.

[110] S. Vuillermot, E. Joodmardi, T. Perlmann, S. O. Ögren, J. Feldon, and U. Meyer, "Prenatal immune activation interacts with genetic Nurr1 deficiency in the development of attentional impairments," *The Journal of Neuroscience*, vol. 32, no. 2, pp. 436–451, 2012.

[111] B. Hibell, U. Guttormsson, S. Ahlström et al., *The 2011 ESPAD Report. Substance Use Among Students in 36 European Countries*, The Swedish Council for Information on Alcohol and Other Drugs (CAN), Stockholm, Sweden, 2012.

[112] L. D. Johnston, P. M. O'Malley, J. G. Bachman et al., *Monitoring the Future National Survey Results on Drug Use, 1975–2013: Volume I, Secondary School Students*, Institute for Social Research, The University of Michigan, Ann Arbor, Mich, USA, 2014.

[113] D. Linszen and T. van Amelsvoort, "Cannabis and psychosis: an update on course and biological plausible mechanisms," *Current Opinion in Psychiatry*, vol. 20, no. 2, pp. 116–120, 2007.

[114] T. H. Moore, S. Zammit, A. Lingford-Hughes et al., "Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review," *The Lancet*, vol. 370, no. 9584, pp. 319–328, 2007.

[115] D. M. Fergusson, L. J. Horwood, and N. R. Swain-Campbell, "Cannabis dependence and psychotic symptoms in young people," *Psychological Medicine*, vol. 33, no. 1, pp. 15–21, 2003.

[116] L. Arseneault, M. Cannon, R. Poulton, R. Murray, A. Caspi, and T. E. Moffitt, "Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study," *British Medical Journal*, vol. 325, no. 7374, pp. 1212–1213, 2002.

[117] L. Arseneault, M. Cannon, J. Witton, and R. M. Murray, "Causal association between cannabis and psychosis: examination of the evidence," *British Journal of Psychiatry*, vol. 184, pp. 110–117, 2004.

[118] J. McGrath, J. Welham, J. Scott et al., "Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults," *Archives of General Psychiatry*, vol. 67, no. 5, pp. 440–447, 2010.

[119] J. Decoster, J. van Os, I. Myin-Germeys, M. De Hert, and R. van Winkel, "Genetic variation underlying psychosis-inducing effects of cannabis: critical review and future directions," *Current Pharmaceutical Design*, vol. 18, no. 32, pp. 5015–5023, 2012.

[120] R. Van Winkel, G. Esquivel, G. Kenis et al., "Genome-wide findings in schizophrenia and the role of gene-environment interplay," *CNS Neuroscience and Therapeutics*, vol. 16, no. 5, pp. e185–e192, 2010.

[121] L. French, C. Gray, G. Leonard et al., "Early cannabis use, polygenic risk score for schizophrenia and brain maturation in adolescence," *JAMA Psychiatry*, vol. 72, no. 10, pp. 1002–1011, 2015.

[122] D. Goldman, "America's cannabis experiment," *JAMA Psychiatry*, vol. 72, no. 10, pp. 969–970, 2015.

[123] C. M. P. O'Tuathaigh, M. Hryniewiecka, A. Behan et al., "Chronic adolescent exposure to δ-9-tetrahydrocannabinol in COMT mutant mice: impact on psychosis-related and other phenotypes," *Neuropsychopharmacology*, vol. 35, no. 11, pp. 2262–2273, 2010.

[124] M. Schneider and M. Koch, "Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats," *Neuropsychopharmacology*, vol. 28, no. 10, pp. 1760–1769, 2003.

[125] J. Renard, L. G. Rosen, M. Loureiro et al., "Adolescent cannabinoid exposure induces a persistent sub-cortical hyper-dopaminergic state and associated molecular adaptations in the prefrontal cortex," *Cerebral Cortex*, 2016.

[126] P. D. Morrison and R. M. Murray, "From real-world events to psychosis: the emerging neuropharmacology of delusions," *Schizophrenia Bulletin*, vol. 35, no. 4, pp. 668–674, 2009.

[127] S. Han, B.-Z. Yang, H. R. Kranzler et al., "Linkage analysis followed by association show NRG1 associated with cannabis dependence in African Americans," *Biological Psychiatry*, vol. 72, no. 8, pp. 637–644, 2012.

[128] A. A. Boucher, G. E. Hunt, T. Karl, J. Micheau, I. S. McGregor, and J. C. Arnold, "Heterozygous neuregulin 1 mice display greater baseline and Δ9-tetrahydrocannabinol-induced c-Fos expression," *Neuroscience*, vol. 149, no. 4, pp. 861–870, 2007.

[129] A. A. Boucher, J. C. Arnold, L. Duffy, P. R. Schofield, J. Micheau, and T. Karl, "Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects of Δ^9-tetrahydrocannabinol," *Psychopharmacology*, vol. 192, no. 3, pp. 325–336, 2007.

[130] L. E. Long, R. Chesworth, J. C. Arnold, and T. Karl, "A follow-up study: acute behavioural effects of Δ^9-THC in female heterozygous neuregulin 1 transmembrane domain mutant mice," *Psychopharmacology*, vol. 211, no. 3, pp. 277–289, 2010.

[131] A. A. Boucher, G. E. Hunt, J. Micheau et al., "The schizophrenia susceptibility gene neuregulin 1 modulates tolerance to the effects of cannabinoids," *International Journal of Neuropsychopharmacology*, vol. 14, no. 5, pp. 631–643, 2011.

[132] L. E. Long, R. Chesworth, X.-F. Huang, I. S. McGregor, J. C. Arnold, and T. Karl, "Transmembrane domain Nrg1 mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence," *International Journal of Neuropsychopharmacology*, vol. 16, no. 1, pp. 163–175, 2013.

[133] J. R. Spencer, K. M. E. Darbyshire, A. A. Boucher et al., "Novel molecular changes induced by Nrg1 hypomorphism and Nrg1-cannabinoid interaction in adolescence: a hippocampal proteomic study in mice," *Frontiers in Cellular Neuroscience*, vol. 7, article 15, 2013.

[134] J. A. S. Crippa, G. N. Derenusson, T. B. Ferrari et al., "Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report," *Journal of Psychopharmacology*, vol. 25, no. 1, pp. 121–130, 2011.

[135] F. M. Leweke, J. K. Mueller, B. Lange et al., "Therapeutic potential of cannabinoids in psychosis," *Biological Psychiatry*, vol. 79, no. 7, pp. 604–612, 2015.

[136] L. E. Long, R. Chesworth, X.-F. Huang et al., "Distinct neurobehavioural effects of cannabidiol in transmembrane domain neuregulin 1 mutant mice," *PLoS ONE*, vol. 7, no. 4, Article ID e34129, 2012.

[137] M. D. Ballinger, A. Saito, B. Abazyan et al., "Adolescent cannabis exposure interacts with mutant DISC1 to produce impaired adult emotional memory," *Neurobiology of Disease*, vol. 82, pp. 176–184, 2015.

[138] E. M. Tunbridge, D. R. Weinberger, and P. J. Harrison, "A novel protein isoform of catechol O-methyltransferase (COMT): brain expression analysis in schizophrenia and bipolar disorder and effect of Val^{158}Met genotype," *Molecular Psychiatry*, vol. 11, no. 2, pp. 116–117, 2006.

[139] D. Collip, R. Van Winkel, O. Peerbooms et al., "COMT Val158Met-stress interaction in psychosis: role of background psychosis risk," *CNS Neuroscience and Therapeutics*, vol. 17, no. 6, pp. 612–619, 2011.

[140] X. Goldberg, M. Fatjó-Vilas, S. Alemany, I. Nenadic, C. Gastó, and L. Fañanás, "Gene-environment interaction on cognition: a twin study of childhood maltreatment and COMT variability," *Journal of Psychiatric Research*, vol. 47, no. 7, pp. 989–994, 2013.

[141] S. Alemany, B. Arias, M. Fatjó-Vilas et al., "Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes," *Acta Psychiatrica Scandinavica*, vol. 129, no. 1, pp. 54–62, 2014.

[142] A. Caspi, T. E. Moffitt, M. Cannon et al., "Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction," *Biological Psychiatry*, vol. 57, no. 10, pp. 1117–1127, 2005.

[143] C. M. P. O'Tuathaigh, G. Clarke, J. Walsh et al., "Genetic vs. pharmacological inactivation of COMT influences cannabinoid- induced expression of schizophrenia-related phenotypes," *International Journal of Neuropsychopharmacology*, vol. 15, no. 9, pp. 1331–1342, 2012.

[144] Á. T. Behan, M. Hryniewiecka, C. M. P. O'Tuathaigh et al., "Chronic adolescent exposure to delta-9-tetrahydrocannabinol in COMT mutant mice: impact on indices of dopaminergic, endocannabinoid and GABAergic pathways," *Neuropsychopharmacology*, vol. 37, no. 7, pp. 1773–1783, 2012.

[145] L. J. Phillips, P. D. McGorry, B. Garner et al., "Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: implications for the development of psychotic disorders," *Australian and New Zealand Journal of Psychiatry*, vol. 40, no. 9, pp. 725–741, 2006.

[146] K. D. Tessner, V. Mittal, and E. F. Walker, "Longitudinal study of stressful life events and daily stressors among adolescents at high risk for psychotic disorders," *Schizophrenia Bulletin*, vol. 37, no. 2, pp. 432–441, 2011.

[147] F. Varese, E. Barkus, and R. P. Bentall, "Dissociation mediates the relationship between childhood trauma and hallucination-proneness," *Psychological Medicine*, vol. 42, no. 5, pp. 1025–1036, 2012.

[148] J.-P. Selten and E. Cantor-Graae, "Hypothesis: social defeat is a risk factor for schizophrenia?" *The British Journal of Psychiatry*, vol. 191, no. 51, pp. s9–s12, 2007.

[149] J.-P. Selten, E. van der Ven, B. P. F. Rutten, and E. Cantor-Graae, "The social defeat hypothesis of schizophrenia: an update," *Schizophrenia Bulletin*, vol. 39, no. 6, pp. 1180–1186, 2013.

[150] C. Hammels, E. Pishva, J. De Vry et al., "Defeat stress in rodents: from behavior to molecules," *Neuroscience and Biobehavioral Reviews*, vol. 59, pp. 111–140, 2015.

[151] O. Berton, C. A. McClung, R. J. DiLeone et al., "Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress," *Science*, vol. 311, no. 5762, pp. 864–868, 2006.

[152] V. Krishnan, M. 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.

[153] T. W. Chohan, A. A. Boucher, J. R. Spencer et al., "Partial genetic deletion of neuregulin 1 modulates the effects of stress on sensorimotor gating, dendritic morphology, and HPA axis activity in adolescent mice," *Schizophrenia Bulletin*, vol. 40, no. 6, pp. 1272–1284, 2014.

[154] F. N. Haque, T. V. Lipina, J. C. Roder, and A. H. C. Wong, "Social defeat interacts with Disc1 mutations in the mouse to affect behavior," *Behavioural Brain Research*, vol. 233, no. 2, pp. 337–344, 2012.

[155] 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.

[156] R. E. Straub, Y. Jiang, C. J. MacLean et al., "Genetic variation in the 6p22.3 Gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia," *American Journal of Human Genetics*, vol. 71, no. 2, pp. 337–348, 2002.

[157] B. Riley, P.-H. Kuo, B. S. Maher et al., "The dystrobrevin binding protein 1 (DTNBP1) gene is associated with schizophrenia in the Irish Case Control Study of Schizophrenia (ICCSS) sample," *Schizophrenia Research*, vol. 115, no. 2-3, pp. 245–253, 2009.

[158] M. Ayalew, H. Le-Niculescu, D. F. Levey et al., "Convergent functional genomics of schizophrenia: from comprehensive understanding to genetic risk prediction," *Molecular Psychiatry*, vol. 17, no. 9, pp. 887–905, 2012.

[159] K. E. Burdick, T. E. Goldberg, B. Funke et al., "DTNBP1 genotype influences cognitive decline in schizophrenia," *Schizophrenia Research*, vol. 89, no. 1–3, pp. 169–172, 2007.

[160] A. J. Fallgatter, A.-C. Ehlis, M. J. Herrmann et al., "DTNBP1 (dysbindin) gene variants modulate prefrontal brain function in schizophrenic patients—support for the glutamate hypothesis of schizophrenias," *Genes, Brain and Behavior*, vol. 9, no. 5, pp. 489–497, 2010.

[161] J.-P. Zhang, K. E. Burdick, T. Lencz, and A. K. Malhotra, "Meta-analysis of genetic variation in DTNBP1 and general cognitive ability," *Biological Psychiatry*, vol. 68, no. 12, pp. 1126–1133, 2010.

[162] C. S. Weickert, D. A. Rothmond, T. M. Hyde, J. E. Kleinman, and R. E. Straub, "Reduced DTNBP1 (dysbindin-1) mRNA in the hippocampal formation of schizophrenia patients," *Schizophrenia Research*, vol. 98, no. 1–3, pp. 105–110, 2008.

[163] J. Tang, R. P. LeGros, N. Louneva et al., "Dysbindin-1 in dorsolateral prefrontal cortex of schizophrenia cases is reduced in an isoform-specific manner unrelated to dysbindin-1 mRNA expression," *Human Molecular Genetics*, vol. 18, no. 20, pp. 3851–3863, 2009.

[164] C. Fu, D. Chen, R. Chen, Q. Hu, G. Wang, and K.-L. Lim, "The schizophrenia-related protein dysbindin-1A is degraded and facilitates NF-Kappa B activity in the nucleus," *PLoS ONE*, vol. 10, no. 7, Article ID e0132639, 2015.

[165] R. T. Swank, H. O. Sweet, M. T. Davisson, M. Reddington, and E. K. Novak, "Sandy: a new mouse model for platelet storage pool deficiency," *Genetical Research*, vol. 58, no. 1, pp. 51–62, 1991.

[166] K. Takao, K. Toyama, K. Nakanishi et al., "Impaired long-term memory retention and working memory in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia," *Molecular brain*, vol. 1, article 11, 2008.

[167] J. D. Jentsch, H. Trantham-Davidson, C. Jairl, M. Tinsley, T. D. Cannon, and A. Lavin, "Dysbindin modulates prefrontal cortical glutamatergic circuits and working memory function in mice," *Neuropsychopharmacology*, vol. 34, no. 12, pp. 2601–2608, 2009.

[168] K. H. Karlsgodt, K. Robleto, H. Trantham-Davidson et al., "Reduced dysbindin expression mediates N-methyl-D-aspartate receptor hypofunction and impaired working memory performance," *Biological Psychiatry*, vol. 69, no. 1, pp. 28–34, 2011.

[169] F. Papaleo, F. Yang, S. Garcia et al., "Dysbindin-1 modulates prefrontal cortical activity and schizophrenia-like behaviors via dopamine/D2 pathways," *Molecular Psychiatry*, vol. 17, no. 1, pp. 85–98, 2012.

[170] W. B. Glen Jr., B. Horowitz, G. C. Carlson et al., "Dysbindin-1 loss compromises NMDAR-dependent synaptic plasticity and

contextual fear conditioning," *Hippocampus*, vol. 24, no. 2, pp. 204–213, 2014.

[171] S. K. Bhardwaj, R. T. Ryan, T. P. Wong, and L. K. Srivastava, "Loss of dysbindin-1, a risk gene for schizophrenia, leads to impaired group 1 metabotropic glutamate receptor function in mice," *Frontiers in Behavioral Neuroscience*, vol. 9, article 72, 2015.

[172] F. Papaleo and D. R. Weinberger, "Dysbindin and Schizophrenia: it's dopamine and glutamate all over again," *Biological Psychiatry*, vol. 69, no. 1, pp. 2–4, 2011.

[173] M. M. Cox, A. M. Tucker, J. Tang et al., "Neurobehavioral abnormalities in the dysbindin-1 mutant, sandy, on a C57BL/6J genetic background," *Genes, Brain and Behavior*, vol. 8, no. 4, pp. 390–397, 2009.

[174] K. K. Nicodemus, S. Marenco, A. J. Batten et al., "Serious obstetric complications interact with hypoxia-regulated/vascular-expression genes to influence schizophrenia risk," *Molecular Psychiatry*, vol. 13, no. 9, pp. 873–877, 2008.

[175] J. Voisey, C. D. Swagell, I. P. Hughes et al., "A polymorphism in the dysbindin gene (DTNBP1) associated with multiple psychiatric disorders including schizophrenia," *Behavioral and Brain Functions*, vol. 6, article 41, 2010.

[176] S. Hattori, T. Murotani, S. Matsuzaki et al., "Behavioral abnormalities and dopamine reductions in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia," *Biochemical and Biophysical Research Communications*, vol. 373, no. 2, pp. 298–302, 2008.

[177] C. A. Ghiani, M. Starcevic, I. A. Rodriguez-Fernandez et al., "The dysbindin-containing complex (BLOC-1) in brain: developmental regulation, interaction with SNARE proteins and role in neurite outgrowth," *Molecular Psychiatry*, vol. 15, no. 2, pp. 204–215, 2010.

[178] T. C. Südhof and J. E. Rothman, "Membrane fusion: grappling with SNARE and SM proteins," *Science*, vol. 323, no. 5913, pp. 474–477, 2009.

[179] C. M. Lewis, D. F. Levinson, L. H. Wise et al., "Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia," *American Journal of Human Genetics*, vol. 73, no. 1, pp. 34–48, 2003.

[180] J. Lochman, V. J. Balcar, F. Šťastný, and O. Šerý, "Preliminary evidence for association between schizophrenia and polymorphisms in the regulatory regions of the ADRA2A, DRD3 and SNAP-25 genes," *Psychiatry Research*, vol. 205, no. 1-2, pp. 7–12, 2013.

[181] X. Fan and E. J. Hess, "D2-like dopamine receptors mediate the response to amphetamine in a mouse model of ADHD," *Neurobiology of Disease*, vol. 26, no. 1, pp. 201–211, 2007.

[182] X. Fan, M. Xu, and E. J. Hess, "D2 dopamine receptor subtype-mediated hyperactivity and amphetamine responses in a model of ADHD," *Neurobiology of Disease*, vol. 37, no. 1, pp. 228–236, 2010.

[183] P. L. Oliver and K. E. Davies, "Interaction between environmental and genetic factors modulates schizophrenic endophenotypes in the Snap-25 mouse mutant blind-drunk," *Human Molecular Genetics*, vol. 18, no. 23, pp. 4576–4589, 2009.

[184] M. Baca, A. M. Allan, L. D. Partridge, and M. C. Wilson, "Gene-environment interactions affect long-term depression (LTD) through changes in dopamine receptor affinity in Snap25 deficient mice," *Brain Research*, vol. 1532, pp. 85–98, 2013.

[185] T. Niitsu, T. Ishima, T. Yoshida et al., "A positive correlation between serum levels of mature brain-derived neurotrophic factor and negative symptoms in schizophrenia," *Psychiatry Research*, vol. 215, no. 2, pp. 268–273, 2014.

[186] C. Theleritis, H. L. Fisher, I. Shäfer et al., "Brain derived neurotropic factor (BDNF) is associated with childhood abuse but not cognitive domains in first episode psychosis," *Schizophrenia Research*, vol. 159, no. 1, pp. 56–61, 2014.

[187] E. Dong, P. Tueting, F. Matrisciano, D. R. Grayson, and A. Guidotti, "Behavioral and molecular neuroepigenetic alterations in prenatally stressed mice: relevance for the study of chromatin remodeling properties of antipsychotic drugs," *Translational Psychiatry*, vol. 6, article e711, 2016.

[188] E. E. Manning, A. L. Halberstadt, and M. van den Buuse, "BDNF-deficient mice show reduced psychosis-related behaviors following chronic methamphetamine," *International Journal of Neuropsychopharmacology*, vol. 19, no. 4, 2016.

[189] E. E. Manning and M. van den Buuse, "BDNF deficiency and young-adult methamphetamine induce sex-specific effects on prepulse inhibition regulation," *Frontiers in Cellular Neuroscience*, vol. 7, article 92, 2013.

[190] S. H. Fatemi, J. A. Earle, and T. McMenomy, "Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression," *Molecular Psychiatry*, vol. 5, no. 6, pp. 654–663, 2000.

[191] I. Knuesel, "Reelin-mediated signaling in neuropsychiatric and neurodegenerative diseases," *Progress in Neurobiology*, vol. 91, no. 4, pp. 257–274, 2010.

[192] G. Laviola, E. Ognibene, E. Romano, W. Adriani, and F. Keller, "Gene-environment interaction during early development in the heterozygous reeler mouse: clues for modelling of major neurobehavioral syndromes," *Neuroscience and Biobehavioral Reviews*, vol. 33, no. 4, pp. 560–572, 2009.

[193] E. Ognibene, W. Adriani, S. Macrì, and G. Laviola, "Neurobehavioural disorders in the infant reeler mouse model: interaction of genetic vulnerability and consequences of maternal separation," *Behavioural Brain Research*, vol. 177, no. 1, pp. 142–149, 2007.

[194] K. R. Howell and A. Pillai, "Effects of prenatal hypoxia on schizophrenia-related phenotypes in heterozygous reeler mice: a gene × environment interaction study," *European Neuropsychopharmacology*, vol. 24, no. 8, pp. 1324–1336, 2014.

[195] Y. Ayhan, A. Sawa, C. A. Ross, and M. V. Pletnikov, "Animal models of gene-environment interactions in schizophrenia," *Behavioural Brain Research*, vol. 204, no. 2, pp. 274–281, 2009.

[196] E. L. Burrows, C. E. McOmish, and A. J. Hannan, "Gene-environment interactions and construct validity in preclinical models of psychiatric disorders," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 35, no. 6, pp. 1376–1382, 2011.

[197] I. Myin-Germeys, P. Delespaul, and J. van Os, "Behavioural sensitization to daily life stress in psychosis," *Psychological Medicine*, vol. 35, no. 5, pp. 733–741, 2005.

[198] R. D. Goodwin, X. F. Amador, D. Malaspina, S. A. Yale, R. R. Goetz, and J. M. Gorman, "Anxiety and substance use comorbidity among inpatients with schizophrenia," *Schizophrenia Research*, vol. 61, no. 1, pp. 89–95, 2003.

[199] I. Myin-Germeys, M. Oorschot, D. Collip, J. Lataster, p. delespaul, and J. van Os, "Experience sampling research in psychopathology: opening the black box of daily life," *Psychological Medicine*, vol. 39, no. 9, pp. 1533–1547, 2009.

[200] J. M. Goldstein, L. J. Seidman, J. M. Goodman et al., "Are there sex differences in neuropsychological functions among patients

with schizophrenia?" *American Journal of Psychiatry*, vol. 155, no. 10, pp. 1358–1364, 1998.

[201] M. Han, X.-F. Huang, D. C. Chen et al., "Gender differences in cognitive function of patients with chronic schizophrenia," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 39, no. 2, pp. 358–363, 2012.

[202] D. A. Lewis, A. A. Curley, J. R. Glausier, and D. W. Volk, "Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia," *Trends in Neurosciences*, vol. 35, no. 1, pp. 57–67, 2012.

[203] S. Canetta, S. Bolkan, N. Padilla-Coreano et al., "Maternal immune activation leads to selective functional deficits in offspring parvalbumin interneurons," *Molecular Psychiatry*, vol. 21, no. 7, pp. 956–968, 2016.

[204] J. van Os and S. Kapur, "Schizophrenia," *The Lancet*, vol. 374, no. 9690, pp. 635–645, 2009.

[205] R. M. Murray, L. Sideli, C. La Cascia, and D. La Barbera, "Bridging the gap between research into biological and psychosocial models of psychosis," *Shanghai Archives of Psychiatry*, vol. 27, no. 3, pp. 139–143, 2015.

[206] O. D. Howes, C. McDonald, M. Cannon, L. Arseneault, J. Boydell, and R. M. Murray, "Pathways to schizophrenia: the impact of environmental factors," *International Journal of Neuropsychopharmacology*, vol. 7, no. 1, pp. S7–S13, 2004.

[207] P. J. Harrison and D. R. Weinberger, "Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence," *Molecular Psychiatry*, vol. 10, no. 1, pp. 40–68, 2005.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 2734915, 23 pages
http://dx.doi.org/10.1155/2016/2734915

Research Article

Environmental Enrichment Therapy for Autism: Outcomes with Increased Access

Eyal Aronoff,[1] Robert Hillyer,[1] and Michael Leon[2]

[1]*Mendability, LLC, 915 South 500 East, American Fork, UT 84003, USA*
[2]*Center for Autism Research and Translation, Center for the Neurobiology of Learning and Memory, Department of Neurobiology and Behavior, 2205 McGaugh Hall, The University of California Irvine, Irvine, CA 92697-4550, USA*

Correspondence should be addressed to Michael Leon; mleon@uci.edu

Received 30 March 2016; Revised 20 June 2016; Accepted 23 August 2016

Academic Editor: Susanna Pietropaolo

We have previously shown in two randomized clinical trials that environmental enrichment is capable of ameliorating symptoms of autism spectrum disorder (ASD), and in the present study, we determined whether this therapy could be effective under real-world circumstances. 1,002 children were given daily Sensory Enrichment Therapy, by their parents, using personalized therapy instructions given over the Internet. Parents were asked to assess the symptoms of their child every 2 weeks for up to 7 months. An intention-to-treat analysis showed significant overall gains for a wide range of symptoms in these children, including learning, memory, anxiety, attention span, motor skills, eating, sleeping, sensory processing, self-awareness, communication, social skills, and mood/autism behaviors. The children of compliant caregivers were more likely to experience a significant improvement in their symptoms. The treatment was effective across a wide age range and there was equal progress reported for males and females, for USA and international subjects, for those who paid and those who did not pay for the therapy, and for individuals at all levels of initial symptom severity. Environmental enrichment, delivered via an online system, therefore appears to be an effective, low-cost means of treating the symptoms of ASD.

1. Introduction

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition, presenting in early childhood in 1 of 45 children in the USA [1], and it appears to arise from a complex interaction between genetic and environmental factors [2–4]. Social interaction and communication skills are impaired in this disorder, and individuals with ASD also have unusual repetitive behaviors and/or narrow interests. The condition can persist for life, with major implications for the individual, the family, and the healthcare system [5]. There are currently limited medical treatments for individuals with ASD [6], and while there are several behavioral therapies available for treatment, these programs are inaccessible to many [7, 8], are often costly [5, 9], are typically less effective as patients age [10], are not reliably effective [11], and may address a narrow range of symptoms [12–15]. A treatment that successfully addresses the limitations of current therapies therefore would be of great value.

How much can the environment affect the expression of ASD symptoms? After reviewing the animal literature regarding the substantial benefits of environmental enrichment for animal models of autism, Reynolds et al. [16] noted that the key aspects of environmental enrichment appear to include novel and diverse sensorimotor experiences. They went on to propose that environmental enrichment might be a useful means of treating children with autism, presumably by suppressing the expression of ASD symptoms through neural compensatory mechanisms that would be evoked by the environmental stimulation. In other words, the gene x environment interaction that produced the expression of autism symptoms may be shifted by changing the environmental input to the children with ASD and thereby reducing the expression of those symptoms.

To test that hypothesis, we conducted a randomized clinical trial in which environmental enrichment was given to 6–13-year-old children with classic autism for 6 months by their parents [17]. The therapy included about three-dozen

novel sensory exercises that were given to the children in the morning and evening. The children were assessed at baseline and after 6 months by the same psychologists who were unaware of the group assignment of the children. We found clinically significant improvements in autism symptom severity in 42% of the children in the enriched group, as revealed by the Childhood Autism Rating Scale, while only 7% of the standard-care controls experienced such an improvement. We also reported improved cognition following environmental enrichment, with enriched children scoring 11.3 points higher than controls on the objective Leiter International Performance Scale (Leiter-R) after 6 months of therapy.

In a second randomized clinical trial, environmental enrichment was used to treat 3–6-year-old children with classic autism, and their assessments were also completed by experienced psychologists who were blind to group assignment. Woo et al. [18] again found significant improvements in the cognitive scores of enriched children over 6 months using the Leiter-R, with enriched children gaining 8.42 IQ points, while the standard-care group gained 1.53 points, a statistically significant difference. A significant improvement for the enriched children was also found in receptive language, using the Reynell Developmental Language Scales, another objective test of symptom improvement. Enriched children gained 7.42 points on the receptive language scale, whereas the standard-care group had an average increase of 3.63 points on that assessment. Improvements for the enriched children also outpaced that of controls in the reduction of abnormal sensory responsiveness, as assessed by their parents with the Short Sensory Profile [19]. Enriched children gained 11.36 points on that survey, whereas the standard-care children improved by 2.85 points. Finally, we found that 21% of the children who were initially classified as having autism with the objective Autism Diagnostic Observation Schedule fell below the autism classification cutoff using that same test after six months of environmental enrichment. None of the children in the standard-care control group improved to that extent. In both of these studies, standard care included various combinations of speech therapy, occupational therapy, Applied Behavioral Analysis, social skills therapy, adapted physical education, and physical therapy.

We then wanted to determine whether this therapy could be provided to a large number of individuals with autism via a telehealth system under real-world conditions. Indeed, several forms of behavioral therapy have been made available to relatively small numbers of parents via the Internet with encouraging outcomes. Parental instruction for autism behavioral therapies such as Pivotal Response Training, Applied Behavior Analysis, and the Early Start Denver Protocol have been made available over the Internet and these at-home therapies have shown significant improvements in parental confidence and parental treatment fidelity, as well as improvements in spontaneous imitation skills, communication skills, problem behaviors, and anxiety in children with ASD [20–28].

Given the efficacy of environmental enrichment for treatment of ASD symptom in our randomized clinical trials and given its potential for increasing access to treatment for children with ASD, the enrichment therapy has been adapted for real-world circumstances and made available on the Internet by Mendability, LLC, as a paid online service, which has been accredited for the provision of behavioral healthcare by the Joint Commission on Accreditation of Healthcare Organizations. We set out to determine the efficacy of environmental enrichment as provided via this telehealth service. Unlike the therapy used in the two clinical trials, the therapeutic exercises from this system were individualized, based upon the specific challenges, age, abilities, and progress of the children. In addition, while both of the clinical trials included only children 3–12 years old with classic autism, this study extended both the range of ages of the subjects who received the therapy and the range of ASD severity of those treated by including self-selected individuals across the entire autism spectrum.

2. Materials and Methods

2.1. Intervention. All research activities adhered to the Health Insurance Portability and Accountability Act and were in compliance with its privacy, security, and electronic transaction guidelines. The data set used in the study was stripped of personal information prior to analysis.

The study was a nonconcurrent single-subject, multiple-baseline design initiated by a retrospective review of the behavioral assessments of parents regarding their children at the start of their treatment and over the course of their treatment. The individuals who received environmental enrichment were given instructions for daily exposure to multiple sensorimotor exercises via customized worksheets that were generated by the online software after completion of an extensive questionnaire. As the parents were delivering the stimulation, there was also an increase in social interactions for the enriched children. Licensed, experienced occupational therapists reviewed the worksheets to ensure that the computer-generated exercises were within the capability of each subject. These therapists made any adjustments to the therapy if needed, but such interventions were rare. The occupational therapists were also available for consultations with the parents via email, phone, and video over the course of the therapeutic intervention

Environmental enrichment, in the form of Sensory Enrichment Therapy, pairs different types of sensory and motor exercises on a daily basis. Varied textures, such as plastic turf doormats, aluminum foil, sponges, artificial flowers, adhesive tape, and bubble wrap, were used to stimulate the sense of touch. For object manipulation, there were beads to sort and arrange, discs to insert or pull, and rice or toothpicks to insert into foam or Play-Doh, which was also used to squeeze and shape. Thermal stimulation came from different temperatures of water, spoons, or mugs. Visual stimulation came in the form of fine art, photos, and other images. Auditory stimulation came in the form of classical music or sound makers. Proprioceptive and vestibular stimulation came in the form of various exercises requiring walking or ascending and descending stairs while carrying an object overhead. Balance skills were elicited on a raised or angled beam, and different movements were performed in place with a blindfold. Pleasant scents provided olfactory stimulation.

A partial list of the exercises available to the children in this study can be found in previous reports [17, 18]. The online system selected exercises from a database of more than 400 different sensory exercises, which allowed a new individualized therapy worksheet to be developed for each 2-week period.

The therapeutic exercises were administered once or twice a day, with each session lasting 10–15 minutes. In addition, there were 4–6 daily pairings of olfactory stimuli and gentle tactile stimuli for 30–60 seconds. Every 2 weeks, after the detailed parental assessments of their child's symptoms were completed, the expert system software assigned new exercises that were delivered in a new worksheet for the next 2-week period. The enrichment therapy was added to any other therapies in which the child was engaged.

2.2. Assessments. Parents were initially asked to complete an online assessment with 301 potential questions that probed the behavioral symptoms associated with autism (a list of which can be found in the Appendix). Care was given to avoid questions that could be extracted from higher-level questions. For example, if the parent reported that the child had good reading comprehension, the system did not ask if she/he knew the letters of the alphabet. Furthermore, parents were presented only age-appropriate questions. For example, if the parent reported that the child was 3 years old, a question about reading comprehension would not be presented. The parents were asked to assess each aspect of their behavior with these descriptors: 0 = could not be worse, 1 = severe problem, 2 = big problem, 3 = bit of a problem, 4 = maybe a problem, and 5 = not a problem. A progress bar was displayed for each question to help the parent set the level of improvement on the scale.

In the initial assessment, the system presented an average of 280.46 (SD = 22.43, CI = 279.07–281.85) questions. Whenever the parent rated a question as "not a problem," (mean = 153 or 55% of the questions, SD = 48.81, CI = 150–156) or when the parent was not able to generate an answer to a question for various reasons (mean = 8 questions or 3% of the questions, SD = 16, CI = 7–9), those questions were then omitted from the subsequent questionnaires.

2.3. Participation. 1,002 subjects were recruited to initiate the treatment, using Google ads, TEDx ads, Facebook ads, and email messages. They had a mean age of 7.37 years (SD = 3.83, CI = 7.14–7.61) and ranged in age between 1 and 18 years. There were 796 males and 206 females, 752 of whom were from the USA, 239 were international residents, and 11 were of unknown geographic location. There were 835 children whose parents paid for the therapy and 167 children who received the therapy at no charge. There were 559 parents who indicated that their child had received a diagnosis of autism, 41 children who had a diagnosis of Asperger's syndrome, and 30 who had probable autism. In addition, 31 children were regarded as having pervasive developmental disorder, 18 were regarded as having ADHD, 10 were described as having global developmental delay, 42 were described as having other disorders, and there were 271 children whose parents did not provide a formal diagnosis. However, rather than focusing on their presumptive diagnoses, we focused on improvement in the symptoms that were revealed by the answers to the assessment questions.

2.4. Calculating Composite Score. The parents completed these questionnaires a mean of 1.75 times each month. The mean number of questions to which the parents responded over the course of their participation was 119 questions at each assessment (SD = 43.54, CI = 116–122). The final scores were recorded for each individual whenever they stopped their participation and the change in symptom severity was then calculated for each subject for all answered questions in an intention-to-treat analysis. The range of scores for the individual behavioral components was 0 to 5. The mean of all answered questions was then calculated for a composite score that characterized the mean change in symptom severity for each child, as assessed by their parent. Questions that were not age-appropriate or were initially answered as "not a problem" or "cannot measure" were not subsequently presented and were therefore not included in the calculations. If no assessment was taken in any month, we interpolated the results by time-weighted averaging between the last assessment and the next closest assessment. In addition, we calculated the change in assessments for specific categories of symptoms: anxiety, attention span, communication, eating, learning, memory, mood/behavior, motor skills, self-awareness, sensory processing, sleep, and social skills. We further clustered those categories into basic skills, complex skills, and personality traits for analysis. We used paired t-tests for two sample means to compare symptom severity before and after Sensory Enrichment Therapy, and we calculated R^2 for evaluating correlations.

3. Results and Discussion

174 participants answered the questionnaire for 1 month, 144 for two months, 81 for 3 months, 65 for 4 months, 79 for 5 months, 59 for 6 months, and 400 for more than 6 months. In all, we collected more than 650,000 answers to the questions that are shown in the Appendix, along with the proportion of subjects who indicated a problem in that area, the mean initial score, and the mean change in that score at the final assessment.

Figure 1 shows the correlation of the time spent in environmental enrichment therapy in months, relative to the mean difference in the composite score of the participants from the initiation of the therapy to their final assessment: $R^2 = 0.14$ ($p < 0.05$). The mean initial symptom severity score for all subjects was 2.48 (SD = 0.60, CI = 2.44–2.51) and the mean final score for all subjects improved to 2.93 (SD = 0.78, CI = 2.88–2.98; df = 1,001, $t = -26.58$, $p < 0.00001$). The effect size should be considered to be large (Cohen's $d = -1.68$).

The change in symptom severity as a function of parental engagement with the therapy, as determined by the number of sensory exercise worksheets that the parents downloaded, is shown in Figures 2 and 3. The more assumed parental engagement, the better outcome for the children ($R^2 = 0.26$, $p < 0.05$). Indeed, only 5.93% (2,036 out of 35,055) of questions answered by the 295 parents who downloaded 1–3

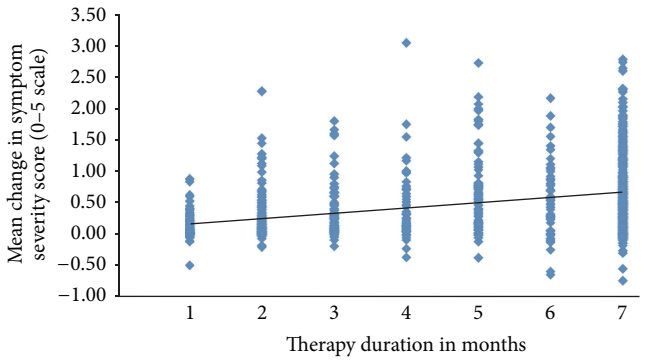


Figure 1: Mean change in symptom severity score as a function of therapy duration in months ($R^2 = 0.14$). Symptom severity score was the change on a 0–5 scale for all answered questions for each subject.

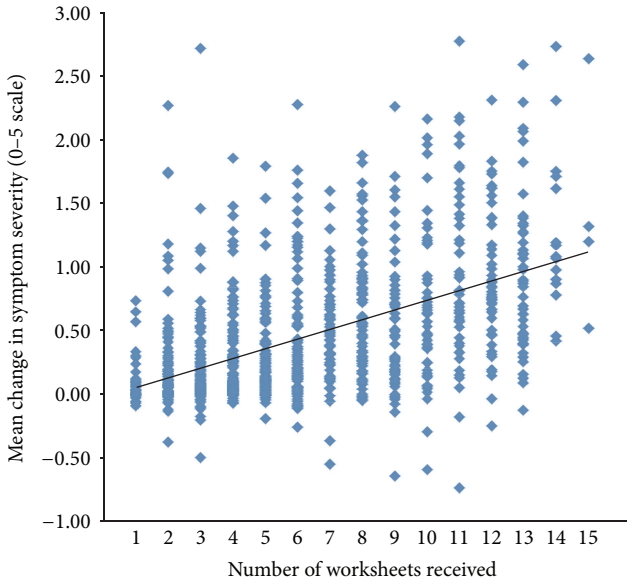


Figure 2: Mean change in symptom severity, as determined by all answered questions on a 0–5 scale from the initial to the final assessment as a function of the number of worksheets received, which served as a reflection of parental engagement with the therapy ($R^2 = 0.26$).

worksheets had experienced an improvement of at least 1 point on the symptom severity scale, while 46.09% (11,467 out of 24,852) of questions answered by the 217 parents who downloaded at least 10 worksheets had such an improvement. Even though the children of parents who downloaded 1–3 exercise sheets had a significant improvement in their progress on their composite scores of symptom severity (mean = 0.18, SD = 0.34, CI = 0.14–0.22, $t = -9.00$, df = 294, $p < 0.00001$), those subjects whose parents downloaded 10 or more exercise sheets had a mean improvement of 0.90 (SD = 0.63, CI = 0.81–0.98, $t = -20.95$, df = 216, $p < 0.00001$). When the symptom improvement for both of these groups was compared directly with an unpaired t-test with unequal variances, the children of the compliant parents

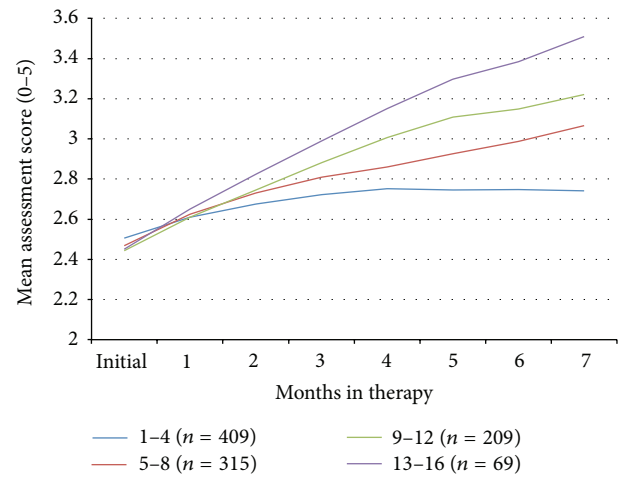


Figure 3: Mean composite assessment score (scale 0–5) over time for subjects whose parents downloaded different numbers of exercise worksheets.

had a significantly better outcome than the children of the noncompliant parents ($t = -15.19$, df = 309, $p < 0.00001$).

To understand the impact of the passage of time on symptom severity, we looked at the progress of 93 subjects whose parents had completed the assessments for 6 or more months but only downloaded 1–3 worksheets. These data differ from the analysis above in that we only looked at those children who had the therapy for at least 6 months, whereas the above comparison included the outcomes of all the children in these groups, regardless of the time that they remained in the study. This noncompliant group had a mean age of 7.77 (SD = 3.93, CI = 6.96–8.58) and a mean initial severity of 2.53 (SD = 0.65, CI = 2.40–2.67) and the percent of questions that were initially marked as "not a problem" was 54% (SD = 17%, CI = 51%–58%), with mean composite symptom improvement of 0.24 (SD = 0.35, CI = 0.17–0.31, $t = -6.72$, df = 92, $p < 0.00001$). Due to the low compliance levels, this group likely reflects the improvement of symptoms over time without significant Sensory Enrichment Therapy, compared to the 182 subjects whose parents completed assessments for 6 or more months and downloaded 10 or more worksheets. That group had similar mean age: 7.46 (SD = 3.87, CI = 6.89–8.02), similar mean initial severity: 2.45 (SD = 0.57, CI = 2.36–2.53), and similar percent of initial questions that were marked as "not a problem": 55% (SD = 16%, CI = 52%–57%), but they had a mean composite symptom improvement of 0.91 (SD = 0.63, CI = 0.82–1.00, $t = -19.40$, df = 181, $p < 0.00001$). A direct comparison of these groups showed that the compliant parents had children who experienced much larger symptom improvements than the noncompliant parents even when time is kept constant ($t = -11.28$, df = 272, $p < 0.00001$).

The change in symptom severity as a function of initial age is shown in Figures 4 and 5 and there was no statistically significant difference between these factors. Initial symptom severity also did not affect the eventual outcomes (Figure 6). Subjects with a composite symptom severity score < 3 (mean

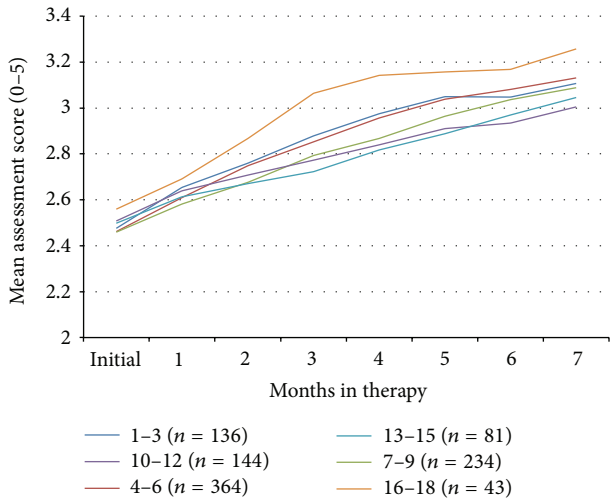


FIGURE 4: Mean composite assessment score (scale 0–5) over time for subjects of different ages.

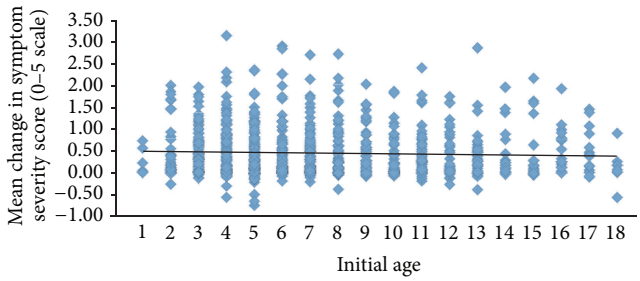


FIGURE 5: Mean change in symptom severity score as a function of initial age ($R^2 = 0.002$). Symptom severity score was the change on a 0–5 scale for all answered questions for each subject.

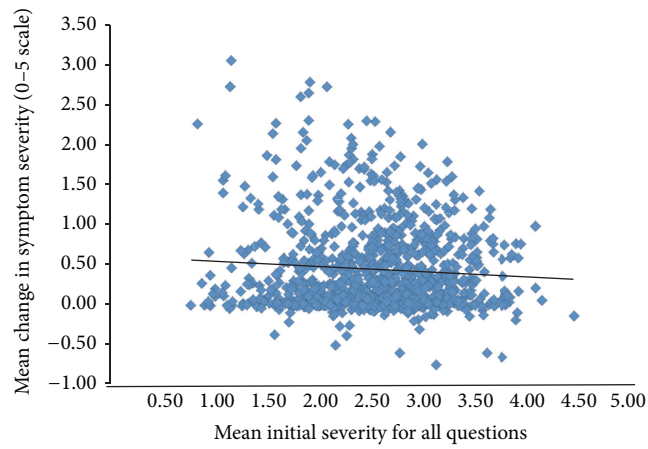


FIGURE 6: Mean change in symptom severity, determined by all answered questions on a 0–5 scale as a function of initial symptom severity ($R^2 = 0.005$).

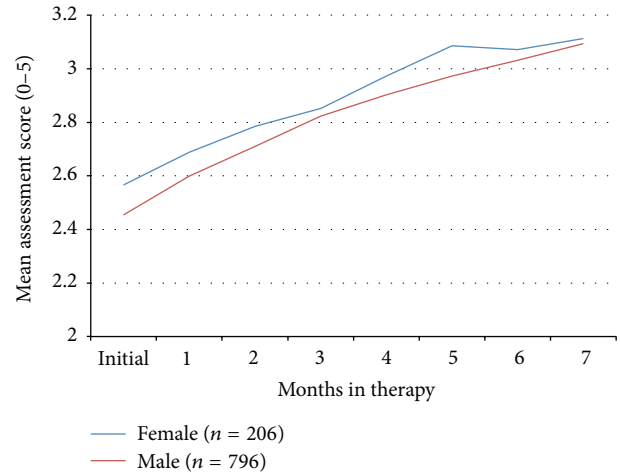


FIGURE 7: Mean composite assessment score (scale 0–5) over time for male and female subjects.

change = 0.47, SD = 0.56, CI = 0.43–0.51, $t = -23.56$, df = 802, $p < 0.00001$) and subjects with a composite symptom severity score > 3 (mean change = 0.40, SD = 0.44, CI = 0.33–0.46, $t = -12.75$, df = 198, $p < 0.00001$) had similar significant improvements in their symptoms. Both American subjects (mean = 0.45, SD = 0.55, CI = 0.41–0.49, $t = -22.53$, df = 751, $p < 0.00001$) and international subjects (mean = 0.45, SD = 0.51, CI = 0.38–0.51, $t = -13.54$, df = 238, $p < 0.00001$), along with both males (mean = 0.46, SD = 0.55, CI = 0.42–0.50, $t = -23.74$, df = 795, $p < 0.00001$) and females (mean = 0.43, SD = 0.52, CI = 0.36–0.50, $t = -11.86$, df = 205, $p < 0.00001$), experienced similar improvements in their symptoms (Figure 7).

Those symptoms that we regarded as basic skills improved by a mean of 0.49 (SD = 0.55, CI = 0.46–0.53, $t = -28.19$, df = 1,001, $p < 0.00001$), complex skills by 0.42 (SD = 0.55, CI = 0.38–0.45, $t = -23.99$, df = 1,000, $p < 0.00001$), and personality traits by 0.35 (SD = 0.59, CI = 0.32–0.39, $t = -18.87$, df = 990, $p < 0.00001$).

We found statistically significant improvements for all symptom categories: anxiety (mean score improvement = 0.43, SD = 0.67, CI = 0.38–0.47, $t = -18.79$, df = 864), attention span (mean = 0.42, SD = 0.68, CI = 0.38–0.46, $t = -19.12$, df = 955), communication (mean = 0.51, SD = 0.65, CI = 0.47–0.55, $t = -23.55$, df = 920), eating (mean = 0.48, SD = 0.68, CI = 0.43–0.52, $t = -19.66$, df = 782), learning (mean = 0.47, SD = 0.63, CI = 0.43–0.51, $t = -22.77$, df = 935), memory (mean = 0.41, SD = 0.69, CI = 0.35–0.47, $t = -14.38$, df = 576), mood/behavior (mean = 0.40, SD = 0.61, CI = 0.36–0.43, $t = -20.28$, df = 970), motor skills (mean = 0.45, SD = 0.58, CI = 0.41–0.49, $t = -22.82$, df = 875), self-awareness (mean = 0.50, SD = 0.72, CI = 0.45–0.55, $t = -20.75$, df = 882), sensory processing (mean = 0.49, SD = 0.62, CI = 0.45–0.53, $t = -24.24$, df = 954), sleep (mean = 0.47, SD = 0.67, CI = 0.42–0.53, $t = -18.19$, df = 654), and social skills (mean = 0.42, SD = 0.61, CI = 0.38–0.45, $t = -21.40$, df = 972). All p's < 0.00001. The n differs in these comparisons because not all subjects had problems in all areas of concern.

There was no significant difference in the initial characteristics between the children whose parents paid for the treatment ($n = 835$, mean age = 7.21 years, SD = 3.76, CI = 6.96–7.47), initial composite symptom severity (mean = 2.48,

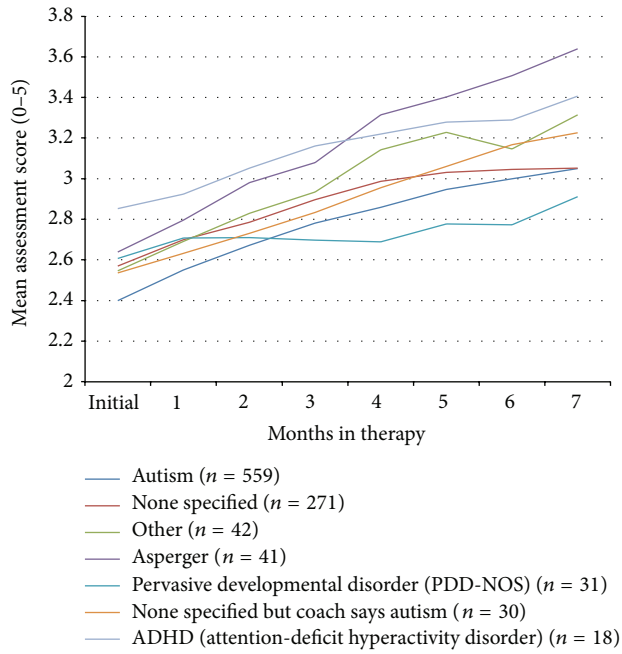


Figure 8: Mean composite assessment score (scale 0–5) over time for subjects with different reported diagnoses.

SD = 0.59, CI = 2.44–2.52) and percent of questions marked as "not a problem" (mean = 55%, SD = 17%, CI = 53%–56%), and those who did not pay for the treatment (n = 167, mean age = 8.17 years, SD = 4.08, CI = 7.54–8.79), mean initial severity (mean = 2.47, SD = 0.65, CI = 2.37–2.57) and percent of questions marked as "not a problem" (mean = 54%, SD = 17%, CI = 52%–57%). Similarly, there was no significant difference in improvement between the paying group and the nonpaying group. The mean change in composite scores was 0.45 (n = 835, SD = 0.55, CI = 0.41–0.49, t = −23.79, df = 834, $p < 0.00001$) for those who paid and 0.47 (n = 167, SD = 0.51, CI = 0.39–0.55, t = −11.90, df = 166, $p < 0.00001$) for those who did not pay for the treatment.

The progress of those subjects with different reported diagnoses is shown in Figure 8. Those reported to have ADHD had a mean improvement in their symptoms of 0.33 (SD = 0.41, CI = 0.13–0.54, t = −3.46, df = 17, $p < 0.003$). Those described as having Asperger's syndrome had a significant improvement in their symptoms (mean improvement = 0.54, SD = 0.61, CI = 0.34–0.73, t = −5.63, df = 40, $p < 0.00001$), those with ASD diagnoses improved by 0.47 (SD = 0.58, CI = 0.43–0.52, t = −19.25, df = 558, $p < 0.00001$), and those who were regarded as having ASD by the occupational therapists (coach) improved by 0.70 (SD = 0.46, CI = 0.53–0.87, t = −8.40, df = 29, $p < 0.0001$). Those with global developmental delay also benefitted from the treatment, with a mean composite severity score improvement of 0.56 (SD = 0.77, CI = 0.01–1.11, t = −2.31, df = 9, $p < 0.05$). Those individuals who were described as having PDD-NOS improved by 0.33 (SD = 0.36, CI = 0.20–0.46, t = −5.18, df = 30, $p < 0.0001$). Subjects without a reported diagnostic category improved by 0.39 (SD = 0.46, CI = 0.33–0.44, t = −13.69, df = 270, $p < 0.00001$) and those who were regarded as being in a variety of diagnostic categories improved by 0.47 (SD = 0.44, CI = 0.33–0.60, t = −6.95, df = 41, $p < 0.00001$).

3.1. Treatment Outcomes. Environmental enrichment in the form of Sensory Enrichment Therapy, provided through an online portal, appears to be effective in supporting a broad range of symptomatic improvements in individuals with ASD over a wide range of ages and symptom severity, as well as across geographic locations, and for both genders. There was also a significant association between treatment compliance and therapeutic effectiveness.

The online treatment effects even appear to be stronger than what was observed in the randomized clinical trials, where the treatment effects based on Cohen's d for the standardized mean difference for the within-subject change on the Leiter, Short Sensory Profile, and Reynell Receptive Language assessments were 0.54, 0.51, and 0.45, respectively [18], outcomes that should be regarded as producing medium/large effects. The magnitude of the effect for improvement in composite scores using the online system for all participants was 1.68, a large effect. The improvement in effect size over the randomized clinical trials, all things being equal, raises the possibility that the personalized sensorimotor exercises used in the online system may have been superior to the standardized exercises that were used in the randomized clinical trials. The advice and guidance of occupational therapists also may have contributed to the efficacy of the online system. Parents may also have been hopeful for a positive outcome for their child and that hope may have been reflected in their assessments. It will be important, therefore, to conduct a further study to test children treated with the online system with objective, validated assessments.

The outcomes in this study compare well with other parent-mediated therapies, particularly for those therapies geared for children and adolescents [29–34]. Moreover, environmental enrichment appears to benefit both core ASD symptoms and symptoms that are typically comorbid with autism [35, 36]. Indeed, 92% of children with ASD have at least two cooccurring mental health problems [37].

It is also of interest that children who were at different levels of symptom severity at the initiation of the therapy were able to benefit equally from this therapeutic approach. Similarly, both males and females benefitted from this approach to the same extent. Perhaps the most compelling finding is that older individuals benefitted to the same extent as younger subjects. Given that standard-care interventions are typically effective principally for young children [10, 38, 39], it is encouraging to have a therapy that is effective over a wide range of ages.

3.2. Sensory Impairment and ASD. We have again shown that enhanced sensorimotor experiences appear to ameliorate ASD symptoms. Conversely, it also appears that a degradation of sensory experiences may increase the risk of autism. For example, congenitally blind children have a 42% elevated probability of having an ASD diagnosis [40]. Even children with less serious ophthalmic problems have a 19% elevated risk of ASD [41]. Indeed, 69% of children with ASD were reported to have abnormal visual acuity [42]. Individuals

with autism also have deficits in visual motion processing, as assessed by fMRI responses, which accompany deficits in both primary visual cortex and extrastriate cortex [43]. Similarly, visually evoked electrophysiological potentials reveal neural responses early in the visual pathway that are compromised in individuals with ASD [44].

It is also the case that degradation of auditory stimulation is associated with an increased risk of ASD. Up to 7% of deaf children are diagnosed with ASD [45, 46] and 10% of individuals with ASD were reported to have hearing problems [47].

Möbius syndrome typically involves both hearing loss and visual difficulties, and 45% of these children are diagnosed with ASD [48, 49]. The congenital oculo-auriculovertebral spectrum disorder also involves loss of vision and audition and 42% of those individuals are given an ASD diagnosis [50]. Furthermore, 68% of children with CHARGE syndrome have ASD diagnoses, which involves an even greater multisensory loss (hearing, olfaction, and vision) [51, 52]. Sensory loss therefore is associated with an increased risk of the expression of ASD symptoms and greater sensory loss is associated with a higher ASD risk.

Individuals with ASD also have problems integrating multisensory information into a single percept [53–56]. Using diffusion tensor imaging fiber tractography, Chang et al. [57] evaluated the structural connectivity of white matter tracts in individuals with ASD and they found that they had decreased connectivity relative to controls in parietooccipital tracts involved in sensory perception and multisensory integration.

Since the loss of sensory stimulation due to neural anomalies or damage to sensory systems is associated with the increased expression of ASD symptoms, it seems possible that environmental restriction of sensory stimuli would have a similar effect. Indeed, a significant proportion of children who were raised in orphanages with very little sensory or social stimulation develop what has been called postinstitutional autistic syndrome [58]. Such children display symptoms similar to children with ASD: they have stereotypic behaviors, an inability to identify human emotions, disordered social communication, abnormal language, poor cognition, abnormal executive function, altered theory of mind, poor sensory integration, poor motor behavior, and abnormal attachment responses [58–63].

These children also share some of the same neurological abnormalities as children with autism. For example, both groups have depressed activity in their orbitofrontal cortex/amygdala circuit, areas associated with social cognition and emotion [64–67]. Children raised in orphanages and children with ASD also have diminished white-matter connectivity in the uncinate fasciculus [68–71], which is a major pathway for communication between the amygdala and orbitofrontal cortex. Both socially/sensory deprived children and children with ASD do not have the right-hemisphere specialization for their neurophysiological response to human faces [72–76]. Finally, neither children with ASD nor institutionalized children have the normal increase in ventral striatum activity as they anticipate a reward [77–81].

The importance of sensory/social stimulation was underlined in the deprived children when they were placed in foster homes or were given environmental enrichment in their orphanage. In these new circumstances, many of their symptoms were greatly ameliorated and their cognitive abilities improved significantly [82–84]. Moreover, the quality of the foster care correlates with better improvements in their outcomes, as does their early transfer into foster care [59, 85, 86]. Sensory deprivation therefore appears to be associated with an increased risk of expressing ASD symptoms and sensory enrichment seems to be able to ameliorate those symptoms.

3.3. Normal Sensory Stimulation and the Maintenance of Brain Health. If individuals with ASD need enhanced sensory stimulation to experience typical neurobehavioral responses, is it the case that neurotypical individuals also need a high level of sensory stimulation to sustain normal brain function? In fact, the loss of sensory input is associated with a decline in higher-order functioning, including both facilitating a cognitive decline in older adults and increasing the risk of intellectual disability in children [87, 88]. For example, visual impairment and hearing impairment are associated with cognitive dysfunction in humans [89–92]. Similarly, mastication problems are also associated with cognitive loss [93, 94].

Longitudinal studies of older adults without initial cognitive impairment found that the failure to identify odors predicted the onset of mild cognitive impairment within five years [95–98]. In another study of nondemented older adults, poor odor identification, along with aging and having the ApoE-4 allele, predicted an increased cognitive decline over five years that could not be predicted by performance on a vocabulary test [99]. Even within a three-year period, poor olfactory discrimination predicted a significant cognitive decline [100]. Similarly, self-reports of poor olfactory function predicted the onset of dementia over a ten-year period [101]. Among those individuals who already had mild cognitive impairment, poor olfactory abilities predicted the onset of dementia [102, 103].

Anosmic individuals experience a loss of gray matter in their medial prefrontal cortex, the subcallosal gyrus, the nucleus accumbens, the dorsolateral prefrontal cortex, cerebellum, occipital gyrus, piriform cortex, anterior insular cortex, orbital frontal cortex, supramarginal gyrus, precuneus, hippocampus, and parahippocampal region, brain areas that include those involved in cognitive function [104]. The longer the olfactory loss, the more severe the loss of gray matter in these areas. Peng et al. [105] similarly showed extensive loss of both gray and white matter in the brains of anosmics, a neural loss which was exacerbated with increased duration of the sensory loss. When hyposmic individuals who have impaired olfactory function were examined, their gray matter was diminished in the insular cortex, anterior cingulate cortex, orbitofrontal cortex, cerebellum, fusiform gyrus, precuneus, middle temporal gyrus, and piriform cortex. In addition, their white matter was diminished underneath the insular cortex, in the cerebellum, and in the middle frontal gyrus [106]. Even distorted olfactory experience in the case of paranosmia is associated with a diminishment of gray matter in a variety of brain areas [107].

While sensory loss and cognitive decline may be independent of each other, there is at least some reason to believe

that their relationship can be causal: that sensory loss can speed cognitive decline. For example, diminished olfactory and auditory abilities accurately predict subsequent cognitive decline in prospective studies [100, 108]. In addition, the use of hearing aids or the provision of cochlear implants can induce cognitive gains in people with hearing loss [109, 110]. Prevention of vision loss in a mouse model of glaucoma prevented its cognitive decline [111] and active mastication improved cognitive function in humans following its diminishment with the inability to chew normally [112].

3.4. Animal Models of Autism Respond to Environmental Enrichment. Animal models of syndromic forms of autism have shown that enriched environments can ameliorate the autism-like symptoms that are seen under low levels of sensory stimulation. An enriched environment for experimental animals allows for increased social interactions in a large cage, along with the opportunity to engage with a variety of inanimate objects and to have the ability to exercise [113]. There is a mouse model of autism that mimics Rett syndrome, with the same gene deletion as humans who have the syndrome. When these mice are housed in an enriched sensorimotor environment, their autism-like symptoms, motor coordination, memory, and anxiety improve [114–116]. Moreover, an enriched environment normalizes the excitatory and inhibitory synaptic densities in their cerebellum and cortex [116], restores cortical long-term potentiation, increases cortical brain-derived neurotrophic factor, and improves the expression of synaptic markers [114, 116].

Fragile X syndrome results from a mutation of the FMR1 gene and often produces children with symptoms that are characteristic of autism. Sensorimotor enrichment similarly rescues FMR1 knockout mice from cognitive deficiencies, reduces anxiety, and increases their exploratory behavior [117].

Most humans with Potocki-Lupski syndrome are diagnosed with autism [118, 119] and the mouse model of this syndrome also has autism-like symptoms, including abnormal ultrasonic vocalizations, perseverative/stereotypic behaviors, anxiety, deficits in learning and memory, and motor deficits when they are housed in a low-stimulation environment [120]. Mice with this genetic anomaly living in an enriched sensorimotor environment have improved motor skills, improved learning and memory, reduced aggressive behavior, and reduced anxiety, although it did not improve their social abnormalities or their abnormal vocalizations [120]. As a whole, these data point to the conclusion that these genetic anomalies are only capable of producing their autism-like syndrome under limited environmental stimulation.

There are three other animal models of autism that also respond well to environmental enrichment. BTBR mice have been differentially bred to express what appear to be core symptoms of autism. Specifically, they have impaired social interactions, deficits in communication, poor social transmission of food preferences, and repetitive behaviors [121–123]. This model of autism also responds well to environmental enrichment, normalizing their repetitive grooming behaviors and their repetitive exploration of objects, as well as their cognitive ability [124, 125]. When BTBR mice were given social enrichment by housing them with a very social mouse strain, the BTBR mice showed improved sociability, but it did not normalize their repetitive behaviors [126].

Deer mice who are kept isolated in a small cage engage in repetitive, stereotyped behavior that resembles ASD behavioral patterns. Such behavior is normalized in an enriched environment [127].

Fetal exposure to valproic acid increases the expression of ASD symptoms in humans, and it has similar effects in rats [128]. Animals exposed to valproic acid in fetal life have a suppressed pain response, increased anxiety, hypersensitivity to sensory stimuli, repetitive and stereotyped behaviors, decreased exploration, and limited social interactions [129]. In addition, these rats have lower acoustic prepulse inhibition, which is involved in adaptation to sensory input [129]. Again, sensory enrichment in such rats ameliorated their autism-like symptoms, including decreased repetitive activity and anxiety, while increasing normal exploratory activity and social behaviors [129].

In addition to ASD, environmental enrichment is effective in ameliorating the symptoms of a large number of neurological disorders [130–134] and it seems quite possible that other neurological disorders can be treated with this approach. Indeed, we have initiated an effort to determine the efficacy of environmental enrichment for the treatment of other developmental neurobehavioral disorders.

3.5. Sensory Abnormalities in ASD. How might increased sensorimotor experiences ameliorate the symptoms of autism? Up to 95% of children with autism have sensory processing abnormalities that include increased sensory seeking behavior, avoidance or diminished responses to some sensory stimuli, and enhanced perceptual abilities [135–142]. Indeed, abnormal sensory reactivity is included in the current DSM-5 diagnostic criteria for ASD [143].

Some of the sensory abnormalities that have been described in ASD occur early in neural sensory processing and therefore raise the possibility that the core symptoms of ASD may be responses to abnormal sensory input [144–146]. For example, the strength of perceptual binding of audiovisual speech observed in individuals with ASD is strongly related to their low-level multisensory temporal processing abilities, suggesting that sensory problems may underlie core elements of their disorder [147, 148]. Alternatively, the anxiety evoked by abnormal sensory responses may be ameliorated by engaging in repetitive behaviors and/or rituals [149]. Indeed, anxiety in preschoolers with ASD increases the probability of their engaging in rituals [150]. Differences in temperament, personality, language, and social development of children with ASD also appear to be related to their sensory problems [151, 152]. Environmental enrichment decreases abnormal sensory responses [18] and this ability may underlie part of its effectiveness in reducing other symptoms of autism.

3.6. Study Limitations. While these data suggest the feasibility of a real-world online treatment for ASD using environmental enrichment, there are several limitations of this study. At the same time, it is important to point out

that these data are quite consistent with the outcomes of the two randomized clinical trials that evaluated environmental enrichment for the treatment of ASD [17, 18].

Because parents self-selected the number of worksheets that they received, there are limits regarding the interpretation of these findings, as there may have been other variables associated with that behavior that may have actually caused the lower level of improvement in the children of those parents who appeared to be unengaged with the therapy.

Another factor is that most parents were paying for this therapy and financial considerations may well have been a factor in determining the length of time that parents were willing to participate in the program, or it may have affected their evaluation of the outcomes for their child. However, there was no difference in outcomes reported by parents who were paying and parents who were scholarship recipients and were not paying for the treatment. It also should be noted that an even lower-cost alternative payment plan has recently been instituted for this online therapy and this change has reduced patient dropout from the program. In addition, while finances may have been a variable in determining the length of treatment, the fact that some children experienced a rapid, large improvement in their symptoms raises the possibility that their parents may have stopped treatment because their child had made good progress on the therapy, rather than stopping due to financial reasons or dissatisfaction with the therapy.

An additional limitation of this study is that there were no professional diagnoses for the subjects. The subjects in this study had a variety of reported diagnoses or no reported diagnosis. However, diagnostic categorization of psychiatric disorders does not correlate well with the biological bases of the disorders [153], and the National Institute for Mental Health has concluded that it makes more sense to evaluate psychiatric issues based on individual symptoms, as we have done, rather than relying on diagnostic categories to describe subjects in clinical trials [153].

Parents were also the only source of information regarding the outcomes for their children. However, most assessments of treatments for ASD rely on parental feedback for the determination of symptom improvement, including the Strengths and Difficulties Questionnaire, the Vineland Adaptive Behavior Scale, the Childhood Autism Rating Scale, the Aberrant Behavior Checklist, the Short Sensory Profile, the Modified Checklist of Autism in Toddlers, the Autism Diagnostic Interview, the Social Communication Questionnaire, the Autism Behavior Checklist, the Gilliam Autism Rating Scale, the Parent Interview for Autism, the Asperger Syndrome Diagnostic Scale, the Autism Spectrum Screening Questionnaire, the PDD Behavior Inventory, the Children's Communication Checklist, and the Childhood Autism Spectrum Test. Direct observation, using, for example, both the Autism Diagnostic Observation Schedule and the Global Clinical Impression Scale, relies on limited time spent with the child under atypical conditions. These assessment tools are often inadequate, on their own, to reveal reliable changes in outcomes over time. The former test requires an evaluator to determine whether or not the subject's highly variable behavior is typical or atypical in sessions six months apart and the latter asks the assessor to compare the behavior of the child at the initiation of the therapy to the behavior shown after 6 months of therapy. The reality is that it is difficult to obtain critical information about the progress of ASD children without being able to observe their behavior on a daily basis. While the parents were the only source of assessment in this study, their conclusions were consistent with both the objective and subjective measures used in our previous two randomized clinical trials that showed improvements for children with ASD after Sensory Enrichment Therapy.

This study also did not have a control group to compare to those given Sensory Enrichment Therapy, and it is therefore possible that the benefits of this therapy may have been seen simply with the passage of time. On the other hand, the noncompliant parents who continued their assessments for at least 6 months had a much smaller improvement in their child's symptoms than compliant parents. These data suggest that there was a critical difference in the outcomes that depended on the intensity of the treatment. It is also the case that the outcomes of children treated with Sensory Enrichment Therapy appear to be much better than the developmental trajectories of 6,975 children with autism, aged 2–14, who were assessed repeatedly over a long period of time [154]. They found that children with ASD who did not have access to this therapy had heterogeneous developmental pathways. Unlike our treated children, the children that they followed with a low initial ASD severity score tended to have the greatest improvements over time, and few of the children that they followed experienced a major improvement in their symptoms, particularly over the initial 7 months.

There was also limited demographic information of subjects and their parents in our study, aside from age, gender, and geographic origin. Neither was there information collected regarding their concurrent use of pharmaceuticals, concurrent behavioral/medical treatments, or the training level of concurrent treatment providers. The diagnoses and patient age were heterogeneous, as one would expect in the real world, but that enhances the generalizability of our conclusions. In addition, while there was objective evidence of whether the parents downloaded worksheets, there was no objective assessment of the fidelity with which they administered the treatment to their children. We also do not know whether the same parent completed all of the assessments.

There is always a trade-off between the internal validity provided by well-run randomized clinical trials in evaluating the efficacy of a treatment and the external validity evaluating the effectiveness of the treatment when it is given to a broad variety of individuals under real-world circumstances. In this study, we have evaluated the effectiveness of Sensory Enrichment Therapy with a large number of diverse individuals and have shown that the efficacy previously demonstrated for this therapy in clinical trials can also be seen to be effective in the real world.

3.6.1. Gene-Environment Interactions in Autism. The genetic underpinnings of autism spectrum disorder have been established with studies of twins, families, and populations [3, 155–158], but these data also make it clear that there is also a significant environmental risk for the expression of autism

Table 1: Specific questions for parental assessment.

Questions regarding symptom severity	% affected	Initial score	Change
Social skills			
Basic skill			
Sitting still and waiting	89%	2.02	0.49
Eye contact	84%	2.46	0.59
Waiting for a turn	82%	2.45	0.50
Interrupting a lot	80%	2.03	0.35
Acknowledging people around him/her	75%	2.50	0.62
Ability to entertain himself/herself	51%	2.45	0.51
Complex skill			
Making friends	93%	1.38	0.36
Seems unable to pick up common social cues	93%	1.55	0.30
Playing with other persons of the same age	92%	1.45	0.43
Awkward in social situations	88%	1.88	0.30
Can remember people's names	61%	2.37	0.47
Inappropriate signs of affection to loved ones	51%	2.55	0.41
Showing inappropriate signs of affection to strangers	49%	2.54	0.34
Can remember people's faces	45%	3.14	0.43
Lying or stealing	25%	3.03	0.49
Making threats	23%	2.57	0.43
Personality trait			
Stubborn, cannot let it go	74%	2.35	0.33
Inflexible opinions	73%	2.16	0.39
Seeking attention	72%	2.35	0.33
Sharing toys	71%	2.50	0.38
Seems to not think before speaking	70%	2.43	0.31
Obsessed with being in control	62%	2.38	0.27
Shy	59%	2.67	0.41
Being a sore loser	54%	2.40	0.45
Suspicious or mistrusting of others	36%	2.88	0.30
Attention span			
Basic skill			
Completes instructions	84%	2.13	0.51
Attention span	84%	2.02	0.48
Can keep focus	83%	1.88	0.44
Able to concentrate	83%	2.09	0.51
Squirms or fidgets	82%	2.00	0.41
Pacing	61%	2.19	0.40
Complex skill			
Needs reminders	85%	1.81	0.33
Cannot sit through something boring	83%	1.82	0.36
Gets bored easily	83%	2.27	0.40
Planning ahead	83%	1.46	0.40
Finishes what they started	82%	1.77	0.40
Can finish lengthy projects	82%	1.29	0.32
Organizing self for an activity	82%	1.51	0.45
Independently prepares for things	81%	1.45	0.44
Cannot sit still	81%	2.09	0.41
Accomplishes complicated tasks	80%	1.63	0.36
Absent-minded	79%	1.83	0.43
Constantly on the move	78%	2.00	0.38
Loses or misplaces things	73%	2.09	0.37
Has trouble deciding things	71%	2.18	0.48

Table 1: Continued.

Questions regarding symptom severity	% affected	Initial score	Change
Communication			
Basic skill			
Sharing thoughts with words	83%	1.44	0.56
Speaking in sentences	79%	1.34	0.48
Pronunciation	75%	1.82	0.49
Vocabulary	75%	1.53	0.51
Repeating things over and over	70%	1.93	0.35
Communicating needs with or without words	66%	2.56	0.63
Uses the wrong words for things	57%	2.54	0.35
Responding to his/her name	46%	2.96	0.55
Stuttering	18%	2.41	0.48
Complex skill			
Understands what others are saying	74%	2.64	0.59
Seems to just repeat what he/she heard	69%	2.08	0.43
Uncontrolled swearing	9%	2.26	0.33
Learning			
Basic skill			
Dressing self	66%	2.47	0.53
Learning new concepts	62%	2.40	0.62
Relating things together	61%	2.38	0.60
Identifying patterns	53%	2.33	0.47
Knows numbers	31%	2.22	0.49
Knows the alphabet letters	27%	1.96	0.51
Knows colors	24%	2.34	0.50
Complex skill			
Retells stories	78%	1.39	0.33
Understands what is going on in a story	77%	2.01	0.42
Follows a plot	73%	1.71	0.29
Reads with expression	72%	1.55	0.28
Tying shoelaces	71%	1.27	0.23
Guesses words instead of sounding them out	65%	1.76	0.30
Can learn abstract concepts	64%	1.61	0.41
Understands concepts of time	60%	1.67	0.43
Enjoys being read to	60%	2.14	0.40
Understands denominations of money have different value	60%	1.49	0.36
Basic math skills	55%	1.85	0.42
Spelling	54%	1.84	0.41
Understands what is not seen still exists	45%	2.19	0.47
Mood & behavior			
Basic skill			
Giving in to cravings	74%	2.32	0.30
Cannot interrupt favorite activities	71%	2.68	0.45
Frequency of tantrums	70%	2.87	0.44
Expressing emotion	66%	2.55	0.49
Duration of tantrums	59%	2.94	0.50
Unexplained bursts of laughter	59%	2.59	0.32
Thrashing	43%	2.67	0.52
Low energy levels	38%	2.82	0.50
Hates spills on their clothes	37%	2.81	0.47
Aggressive toward self	37%	2.85	0.43
Cannot be away from primary caregiver	32%	2.93	0.53

Table 1: Continued.

Questions regarding symptom severity	% affected	Initial score	Change
Complex skill			
Severity of tantrums	66%	2.78	0.46
Feels like mind is in a fog	62%	2.47	0.44
Seems obsessed with one topic	58%	2.43	0.36
Unreasonable fears	42%	2.96	0.39
Unable to discard broken or worthless things	36%	2.70	0.50
Cannot part with favorite blanket or object	27%	2.88	0.49
Compulsive spending	25%	2.49	0.49
Preoccupied with germs	9%	2.91	0.58
Suicidal thoughts/side effects	5%	3.41	0.75
Personality trait			
Impulsive	79%	2.05	0.29
Shouting instead of verbalizing	66%	2.35	0.34
Feeling appropriate emotions	66%	2.77	0.38
Getting overexcited easily	65%	2.57	0.30
Screaming and screeching	62%	2.35	0.38
Becoming discouraged easily	62%	2.57	0.35
Ability to relax	62%	2.62	0.42
Whining and complaining	59%	2.67	0.30
Feeling serene	59%	2.72	0.38
Crying	57%	2.96	0.35
Aggressive toward others	55%	2.88	0.38
Gets angry quickly and a lot	53%	2.79	0.38
Cannot snap out of a bad mood	43%	3.01	0.43
Regularly changes between overenthusiastic and miserable	42%	2.86	0.37
Tendency to feel depressed	33%	3.08	0.37
Obsessed with perfection	29%	2.98	0.41
Panic attacks	25%	3.03	0.36
Feeling guilty for no real reason	22%	3.16	0.33
Preoccupied with tidiness	20%	3.04	0.37
Obsession with death	10%	3.26	0.29
Anxiety			
Basic skill			
Repetitive mannerisms	73%	2.20	0.32
Watching the same show over and over	70%	2.01	0.42
Repetitive motion all the time	61%	2.33	0.36
Flaps hands when excited	59%	2.20	0.47
Repetitive motion can be interrupted	51%	2.96	0.45
Taps, clicks, pops, sniffs, or other tics	43%	2.37	0.43
Grinding teeth	42%	2.58	0.67
Twitches or other motor tics	35%	2.65	0.36
Rocking back and forth	28%	2.68	0.55
Sensory processing			
Basic skill			
Sensitive to loudness	78%	2.35	0.47
Sensitive to busy loud crowds	76%	2.25	0.50
Cannot handle transitions	75%	2.58	0.50
Does not prepare for cold or warm	68%	2.38	0.44
Sensitive to certain tastes	63%	2.64	0.38
Accepts to wear a blindfold	60%	2.18	0.48

Table 1: Continued.

Questions regarding symptom severity	% affected	Initial score	Change
Sensitive to electric motor sound	59%	2.41	0.51
Sensitive to certain voices	57%	2.47	0.42
Sensitive to certain textures	57%	2.81	0.57
Hates brushing his/her teeth	53%	2.57	0.60
Seems insensitive to cold	48%	2.77	0.51
Bothered by water in his/her ears	48%	2.68	0.53
Sensitive to clothing	46%	2.86	0.52
Sensitive to light	45%	2.98	0.44
Does not seem to feel pain	44%	2.89	0.56
Sensitive to certain smells	43%	2.98	0.46
Sensitive when touched	42%	3.01	0.50
Quality of the sense of smell	40%	3.22	0.63
Sensitive to feeling motion	37%	2.98	0.48
Can discern different flavors	36%	3.13	0.53
Can breathe in deeply	33%	2.97	0.54
Hates wearing shoes	32%	2.80	0.66
Sensitive to moving objects	32%	3.12	0.47
Sensitive to dark	31%	3.06	0.43
Ability to detect sounds	28%	3.16	0.52
Looks flushed and overheated	26%	3.25	0.44
Can feel and locate light touch	26%	3.23	0.54
Sensitive to silence	19%	3.17	0.44
Experiences unexplained tingling sensation	16%	3.50	0.06
Can see well (with vision aids if needed)	14%	3.41	0.34
Sweating suddenly for no reason	13%	3.08	0.54
Complex skill			
Seems unaware of threatening situations	75%	2.21	0.45
Sensitive to certain pitches	72%	2.31	0.38
Hates having haircuts	64%	2.13	0.56
Scared of heights	42%	3.01	0.43
Hears things that are not there	19%	3.08	0.48
Sees things that are not there	19%	3.10	0.51
Eating			
Basic skill			
Tries new foods	62%	1.92	0.57
Tolerates different food textures	62%	2.15	0.43
Leaves dinner table	60%	2.17	0.34
Use of utensils	59%	2.56	0.42
Eats a variety of foods	58%	2.05	0.46
Accepts food	45%	2.47	0.58
Consistency of BM	40%	2.70	0.46
Regular bowel movements	36%	2.72	0.52
Gagging	34%	2.66	0.59
Flatulence	33%	2.98	0.33
Eats too little	30%	2.55	0.44
Can feed himself/herself	28%	2.93	0.51
Eats too much	22%	2.93	0.34
Ability to chew	20%	2.88	0.56
Ability to swallow	13%	3.15	0.58

Table 1: Continued.

Questions regarding symptom severity	% affected	Initial score	Change
Self-awareness			
Basic skill			
Imaginary play	61%	1.96	0.53
Unaware of surroundings	57%	2.76	0.55
Unaware of self	53%	2.82	0.50
Talking to himself/herself	52%	2.32	0.36
Bladder control at night	44%	1.94	0.51
Daytime bladder control	34%	2.02	0.64
Daytime bowel movement control	34%	1.76	0.63
Does not want to shower or bathe	31%	2.83	0.55
Bowel movement control at night	23%	1.71	0.58
Complex skill			
Grooming and caring for appearing neat	68%	2.32	0.26
Things do not seem real to him/her	35%	3.07	0.30
Experiences "déja-vu"	26%	2.76	0.41
Real life seems like it is a dream	26%	3.05	0.30
Talking to people who are not there	19%	2.68	0.45
Personality trait			
Daydreaming	53%	2.52	0.28
Memory			
Basic skill			
Can remember instructions	54%	2.22	0.45
Can remember what happened yesterday	42%	2.25	0.47
Visual memory	32%	2.99	0.37
Complex skill			
Can give directions to where they put something	50%	1.42	0.33
Can remember directions to go find something	46%	2.17	0.43
Can remember dates	44%	1.43	0.34
Can remember facts	43%	1.91	0.40
Can remember events	40%	2.15	0.41
Can remember important events years ago	38%	1.86	0.29
Can remember sequence of numbers	36%	2.29	0.38
Can remember sequence of letters	34%	2.17	0.37
Can remember songs	28%	2.83	0.53
Motor skills			
Basic skill			
Balance on the left leg	53%	2.57	0.45
Balance on the right leg	52%	2.58	0.43
Balance in general	44%	2.95	0.54
Tongue control	44%	2.69	0.45
Accident prone	43%	2.86	0.43
Walks into things	43%	2.87	0.47
Tripping	41%	3.01	0.48
Falling	36%	3.05	0.50
Can keep his/her eyes on a moving target	35%	3.16	0.41
Muscles are limp	31%	2.76	0.53
Can scan with his/her eyes, left to right, top to bottom	31%	3.10	0.46
Jumping	30%	2.69	0.48
Strength of right arm	28%	3.01	0.43
Strength of left arm	28%	3.03	0.43

Table 1: Continued.

Questions regarding symptom severity	% affected	Initial score	Change
Walks on tippy toes	27%	2.45	0.51
Control of fingers of left hand	26%	2.89	0.49
Control of fingers of right hand	26%	2.89	0.51
Strength of right leg	26%	3.08	0.42
Strength of left leg	26%	3.10	0.44
Running	22%	3.01	0.38
Strength of neck	21%	3.21	0.43
Involuntary movement occurs in the body in general	20%	2.93	0.58
Right leg muscles are always tight	20%	2.92	0.43
Left leg muscles are always tight	19%	2.94	0.44
Control of right hand	19%	3.11	0.46
Control of left hand	19%	3.08	0.47
Drooling	17%	2.92	0.62
Control of right leg	16%	3.10	0.51
Control of left leg	16%	3.10	0.51
Can go downstairs	16%	2.91	0.59
Control of right arm	15%	3.09	0.49
Control of left arm	15%	3.15	0.45
Right arm muscles are always tight	14%	3.24	0.41
Left arm muscles are always tight	14%	3.28	0.41
Involuntary movements occur in fingers of right hand	14%	2.93	0.50
Involuntary movements occur in fingers of left hand	13%	2.95	0.49
Involuntary movements occur in right hand	13%	2.95	0.52
Involuntary movements occur in left hand	13%	3.03	0.57
Can go upstairs	12%	2.95	0.70
Control of neck	12%	3.12	0.47
Involuntary movements occur in right arm	12%	3.03	0.55
Involuntary movements occur in left arm	12%	3.10	0.53
Involuntary movements occur in right leg	10%	3.22	0.55
Involuntary movements occur in left leg	10%	3.22	0.54
Sitting up	10%	3.03	0.56
Shakes all the time	9%	3.14	0.58
Crawling	8%	2.96	0.49
Walking	6%	2.89	0.63
Standing on own	4%	2.36	0.61
Standing being supported	3%	2.97	0.79
Complex skill			
Writing penmanship	77%	1.73	0.44
Stays in the lines when coloring	74%	1.74	0.41
Drawing ability	73%	1.85	0.46
Scissor control	71%	2.23	0.43
Catching with one hand	66%	1.89	0.27
Catching with two hands	58%	2.47	0.45
Ability to do push-ups	57%	2.13	0.25
Clumsiness	56%	2.74	0.45
Throwing skill	55%	2.70	0.44
Ability to do sit-ups	51%	2.26	0.24
Riding a bicycle	49%	1.69	0.36
Control of facial expression	48%	2.78	0.39
Kicking a ball	48%	2.78	0.40
Posture	45%	2.91	0.37
Ability to do squats	44%	2.51	0.30

Table 1: Continued.

Questions regarding symptom severity	% affected	Initial score	Change
Strength of left hand	37%	2.89	0.40
Strength of right hand	36%	2.86	0.43
Riding a tricycle	36%	2.12	0.44
Climbing skills	32%	2.97	0.47
Control of right ankle	17%	3.03	0.41
Control of left ankle	17%	3.02	0.42
Sleep			
Basic skill			
Falls asleep right away	53%	2.55	0.41
Has difficulty going back to sleep	48%	2.66	0.48
Will not stay asleep	43%	2.75	0.46
Wakes up grumpy	34%	3.04	0.55
Sleeps in own bed	30%	2.22	0.46
Sleeps in	28%	2.85	0.43
Wakes up screaming at night	18%	3.21	0.61
Falls asleep unexpectedly	7%	3.20	0.57
Complex skill			
Has bad dreams	29%	3.41	0.44
Other			
Is in physical pain	17%	3.27	0.19
Frequency of absence episodes	5%	3.22	0.79
Duration of absence episode	5%	3.57	0.47
Difficulty to interrupt an absence episode	4%	3.03	0.77
Frequency of convulsions	3%	2.93	0.77
Intensity of convulsions	3%	3.24	0.80
Unexplained body stiffening episodes	3%	3.24	0.22
Duration of convulsions	2%	3.57	0.62
Recovery time after an absence episode	2%	3.32	0.74
Recovery time after a convulsion	2%	3.53	0.57
Unexplained eye-rolling episodes	2%	3.18	0.68
Unexpected loss of muscle tone	1%	2.86	0.76
Feeling outside of body	1%	3.82	−0.30
Unexplained buzzing feeling	1%	3.71	−0.43
Unexpected loss of consciousness	0%	3.25	0.47
Unexpected blackouts	0%	3.50	1.00

symptoms. The heterogeneity of both the symptoms and the genetics are high, but the phenotypic heterogeneity does not correlate well with the genetic heterogeneity [159]. There is extraordinary complexity in the underlying genetics, with hundreds of common and rare genetic variants increasing the risk for ASD, with the preponderance of risk due to common variations [158]. In addition, the total burden of these genetic variants is correlated with the expression of ASD symptoms [158, 160]. Moreover, the same single-nucleotide polymorphisms can be shared with ASD, attention-deficit hyperactivity disorder, bipolar disorder, major depressive disorder, or schizophrenia [161].

Although the early estimates from twin studies of the relative contribution of genes and environment greatly favored the role of genes in elevating ASD risk [155–157], more recent studies using genome-wide estimates have about equal risk assigned to genes and environment [3, 158]. This change in the relative importance of genes and environment may be due to a number of variables, including changes in the ways by which ASD is diagnosed, with the diagnostic category expanding to include Asperger's syndrome. Another possibility is that those individuals with syndromic ASD are less likely to be included in recent studies, as differential diagnoses of these disorders has improved. Differences in statistical modeling of the data may also have contributed to this shift.

There are a number of risk factors that suggest an interaction between genes and environment in ASD. For example, there is a strong relationship between paternal age and ASD risk [162], perhaps due to an increase in genetic anomalies with age [163]. Importantly, Hultman et al. [162] showed that it was not due to having a father who had ASD-like symptoms and was unable to find a mate earlier

in life. To the contrary, they showed that, in families with one child diagnosed with ASD, that child was likely to be born to the father when he was older than when the children without autism were born. In addition, the time since the birth of one child predicted the occurrence of ASD in the other child. Advanced paternal age also predicted a higher concordance rate for ASD between both monozygotic and dizygotic twins, suggesting that the additional genetic anomalies that come with advancing paternal age may add to other genetic anomalies to result in an ASD diagnosis [164]. Finally, Frans et al. [165] showed that increasing age of the grandfather also predicted increased risk for ASD in the grandchildren, suggesting that environmental experiences well in advance of the child's conception appear to increase the risk of ASD.

Older mothers also have children with an increased ASD risk [166], even where various other factors involved with pregnancy and birth are considered. While younger mothers have eggs that respond to DNA damage by arresting at metaphase of the first meiosis, thereby preventing abnormal embryos, older mothers have a reduced ability to engage this developmental control point and therefore are more likely to have increased chromosomal anomalies in embryos [167]. Such anomalies may result in an increased risk of ASD.

In another example of gene x environment interaction, valproic acid has been given to pregnant women for the treatment of epilepsy, migraine, or bipolar disorder. This drug inhibits histone deacetylase, which impacts gene transcription [168], and it induces DNA demethylation [169], which dysregulates the Wnt/b-catenin signaling pathway that is involved in brain development [170]. There is also an increased risk of ASD in their children [170]. Recall that when fetal rats are exposed to valproic acid, they develop autism-like symptoms that are greatly ameliorated by living in an enriched sensorimotor environment [114–116]. These data show that the probability of expressing autism symptoms can be increased or decreased, depending on environmental experiences. Decreased sensory stimulation, along with valproic acid exposure, increases the expression of ASD symptoms and increased sensory stimulation decreases the expression of those symptoms in the animal model.

There are other environmental factors during fetal life that can increase the risk of ASD. A clear example of a gene x environment interaction can be found in the study of the risk of ASD with exposure to air pollution. Specifically, children who were exposed to high levels of air pollution either during pregnancy or as infants are at increased risk for ASD [171–174]. Air pollution appears to interact with the MET receptor tyrosine kinase gene, which is involved in mediating brain development. A variant of this gene that disrupts MET transcription is associated with an increased risk of ASD [175] and children exposed to high levels of air pollution only had an elevated risk for ASD if they also had this genetic variant [176].

4. Conclusions

Environmental enrichment in the form of Sensory Enrichment Therapy provided online shows promise as an effective approach for treatment of a wide range of symptoms in individuals with autism. This therapy appears to be an effective, low-cost means of treating ASD symptoms and associated symptoms across different ages, geographic location, gender, and symptom severity under real-world conditions.

Appendix

See Table 1.

Competing Interests

Eyal Aronoff is a founder and the majority shareholder of Mendability, LLC, which funded this project, and Robert Hillyer is an employee of Mendability, LLC. Michael Leon is an independent researcher with no financial ties to Mendability.

Acknowledgments

The authors would like to acknowledge Claudie Gordon-Pomares for her pioneering work on Sensory Enrichment Therapy and her ongoing contribution to the development of the therapy and Cynthia Woo for her help with manuscript preparation.

References

[1] B. Zablotsky, L. I. Black, M. J. Maenner, L. A. Schieve, and S. J. Blumberg, "Estimated prevalence of autism and other developmental disabilities following questionnaire; Changes in the 2014 National Health Interview Survey," *National Health Statistics Report*, vol. 87, pp. 1–20, 2015.

[2] E. Colvert, B. Tick, F. McEwen et al., "Heritability of autism spectrum disorder in a UK population-based twin sample," *JAMA Psychiatry*, vol. 72, no. 5, pp. 415–423, 2015.

[3] S. Sandin, P. Lichtenstein, R. Kuja-Halkola, H. Larsson, C. M. Hultman, and A. Reichenberg, "The familial risk of autism," *The Journal of the American Medical Association*, vol. 311, no. 17, pp. 1770–1777, 2014.

[4] B. Tick, P. Bolton, F. Happé, M. Rutter, and F. Rijsdijk, "Heritability of autism spectrum disorders: a meta-analysis of twin studies," *Journal of Child Psychology and Psychiatry*, vol. 57, no. 5, pp. 585–595, 2015.

[5] T. A. Lavelle, M. C. Weinstein, J. P. Newhouse, K. Munir, K. A. Kuhlthau, and L. A. Prosser, "Economic burden of childhood autism spectrum disorders," *Pediatrics*, vol. 133, no. 3, pp. e520–e529, 2014.

[6] N. Klein and K. J. Kemper, "Integrative approaches to caring for children with autism," *Current Problems in Pediatric and Adolescent Health Care*, vol. 46, no. 6, pp. 195–201, 2016.

[7] T. W. Benevides, H. J. Carretta, and S. J. Lane, "Unmet need for therapy among children with autism spectrum disorder: results from the 2005-2006 and 2009-2010 national survey of children with special health care needs," *Maternal and Child Health Journal*, vol. 20, no. 4, pp. 878–888, 2016.

[8] R. Pennington, C. Horn, and A. Berrong, "An evaluation of the differences between big city and small town special education services for students with low incidence disabilities in Kentucky," *Rural Special Education Quarterly*, vol. 28, no. 4, pp. 3–9, 2009.

[9] M. L. Ganz, "The lifetime distribution of the incremental societal costs of autism," *Archives of Pediatrics and Adolescent Medicine*, vol. 161, no. 4, pp. 343–349, 2007.

[10] R. MacDonald, D. Parry-Cruwys, S. Dupere, and W. Ahearn, "Assessing progress and outcome of early intensive behavioral intervention for toddlers with autism," *Research in Developmental Disabilities*, vol. 35, no. 12, pp. 3632–3644, 2014.

[11] H.-C. Steinhausen, C. Mohr Jensen, and M. B. Lauritsen, "A systematic review and meta-analysis of the long-term overall outcome of autism spectrum disorders in adolescence and adulthood," *Acta Psychiatrica Scandinavica*, vol. 133, no. 6, pp. 1–8, 2016.

[12] S. Eikeseth, T. Smith, E. Jahr, and S. Eldevik, "Intensive behavioral treatment at school for 4- to 7-year-old children with autism. A 1-year comparison controlled study," *Behavior Modification*, vol. 26, no. 1, pp. 49–68, 2002.

[13] H. Goldstein, "Communication intervention for children with autism: a review of treatment efficacy," *Journal of Autism and Developmental Disorders*, vol. 32, no. 5, pp. 373–396, 2002.

[14] J. Case-Smith and M. Arbesman, "Evidence-based review of interventions for autism used in or of relevance to occupational therapy," *American Journal of Occupational Therapy*, vol. 62, no. 4, pp. 416–429, 2008.

[15] A. Bitterman, T. C. Daley, S. Misra, E. Carlson, and J. Markowitz, "A national sample of preschoolers with autism spectrum disorders: special education services and parent satisfaction," *Journal of Autism and Developmental Disorders*, vol. 38, no. 8, pp. 1509–1517, 2008.

[16] S. Reynolds, S. J. Lane, and L. Richards, "Using animal models of enriched environments to inform research on sensory integration intervention for the rehabilitation of neurodevelopmental disorders," *Journal of Neurodevelopmental Disorders*, vol. 2, no. 3, pp. 120–132, 2010.

[17] C. C. Woo and M. Leon, "Environmental enrichment as an effective treatment for autism: a randomized controlled trial," *Behavioral Neuroscience*, vol. 127, no. 4, pp. 487–497, 2013.

[18] C. C. Woo, J. H. Donnelly, R. Steinberg-Epstein, and M. Leon, "Environmental enrichment as a therapy for autism: a clinical trial replication and extension," *Behavioral Neuroscience*, vol. 129, no. 4, pp. 412–422, 2015.

[19] W. Dunn, *The Sensory Profile Manual*, The Psychological Corporation, San Antonio, Tex, USA, 1999.

[20] C. D. Hamad, R. W. Serna, L. Morrison, and R. Fleming, "Extending the reach of early intervention training for practitioners: a preliminary investigation of an online curriculum for teaching behavioral intervention knowledge in autism to families and service providers," *Infants & Young Children*, vol. 23, no. 3, pp. 195–208, 2010.

[21] S. L. Hepburn, A. Blakeley-Smith, B. Wolff, and J. A. Reaven, "Telehealth delivery of cognitive-behavioral intervention to youth with autism spectrum disorder and anxiety: a pilot study," *Autism*, vol. 20, no. 2, pp. 207–218, 2016.

[22] B. Ingersoll and N. I. Berger, "Parent engagement with a telehealth-based parent-mediated intervention program for children with autism spectrum disorders: predictors of program use and parent outcomes," *Journal of Medical Internet Research*, vol. 17, no. 10, article e227, 2015.

[23] J. Jang, D. R. Dixon, J. Tarbox, D. Granpeesheh, J. Kornack, and Y. de Nocker, "Randomized trial of an eLearning program for training family members of children with autism in the principles and procedures of applied behavior analysis," *Research in Autism Spectrum Disorders*, vol. 6, no. 2, pp. 852–856, 2012.

[24] S. Lindgren, D. Wacker, A. Suess et al., "Telehealth and autism: treating challenging behavior at lower cost," *Pediatrics*, vol. 137, supplement 2, pp. S167–S175, 2016.

[25] N. Nefdt, R. Koegel, G. Singer, and M. Gerber, "The use of a self-directed learning program to provide introductory training in pivotal response treatment to parents of children with autism," *Journal of Positive Behavior Interventions*, vol. 12, no. 1, pp. 23–32, 2010.

[26] L. A. Vismara, G. S. Young, and S. J. Rogers, "Telehealth for expanding the reach of early autism training to parents," *Autism Research and Treatment*, vol. 2012, Article ID 121878, 12 pages, 2012.

[27] L. A. Vismara, C. McCormick, G. S. Young, A. Nadhan, and K. Monlux, "Preliminary findings of a telehealth approach to parent training in autism," *Journal of Autism and Developmental Disorders*, vol. 43, no. 12, pp. 2953–2969, 2013.

[28] A. L. Wainer and B. R. Ingersoll, "Increasing access to an ASD imitation intervention via a telehealth parent training program," *Journal of Autism and Developmental Disorders*, vol. 45, no. 12, pp. 3877–3890, 2015.

[29] A. S. Carter, D. S. Messinger, W. L. Stone, S. Celimli, A. S. Nahmias, and P. Yoder, "A randomized controlled trial of Hanen's 'More Than Words' in toddlers with early autism symptoms," *Journal of Child Psychology and Psychiatry*, vol. 52, no. 7, pp. 741–752, 2011.

[30] S. J. Rogers, A. Estes, C. Lord et al., "Effects of a brief early start Denver model (ESDM)-based parent intervention on toddlers at risk for autism spectrum disorders: a randomized controlled trial," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 51, no. 10, pp. 1052–1065, 2012.

[31] H. H. Schertz, S. L. Odom, K. M. Baggett, and J. H. Sideris, "Effects of Joint Attention Mediated Learning for toddlers with autism spectrum disorders: an initial randomized controlled study," *Early Childhood Research Quarterly*, vol. 28, no. 2, pp. 249–258, 2013.

[32] M. Siller and M. Sigman, "The behaviors of parents of children with autism predict the subsequent development of their children's communication," *Journal of Autism and Developmental Disorders*, vol. 32, no. 2, pp. 77–89, 2002.

[33] H. McConachie and T. Diggle, "Parent implemented early intervention for young children with autism spectrum disorder: a systematic review," *Journal of Evaluation in Clinical Practice*, vol. 13, no. 1, pp. 120–129, 2007.

[34] I. P. Oono, E. J. Honey, and H. McConachie, "Parent-mediated early intervention for young children with autism spectrum disorders (ASD)," *The Cochrane Database of Systematic Reviews*, no. 4, Article ID CD009774, 2013.

[35] O. T. Leyfer, S. E. Folstein, S. Bacalman et al., "Comorbid psychiatric disorders in children with autism: interview development and rates of disorders," *Journal of Autism and Developmental Disorders*, vol. 36, no. 7, pp. 849–861, 2006.

[36] K. P. Maski, S. S. Jeste, and S. J. Spence, "Common neurological co-morbidities in autism spectrum disorders," *Current Opinion in Pediatrics*, vol. 23, no. 6, pp. 609–615, 2011.

[37] M. Posserud, M. Hysing, W. Helland, C. Gillberg, and A. J. Lundervold, "Autism traits: the importance of 'co-morbid' problems for impairment and contact with services. Data from the Bergen Child Study," *Research in Developmental Disabilities*, 2016.

[38] N. Peters-Scheffer, R. Didden, H. Korzilius, and P. A. Sturmey, "A meta-analytic study on the effectiveness of comprehensive

ABA-based early intervention programs for children with autism spectrum disorders," *Research in Autism Spectrum Disorders*, vol. 5, no. 1, pp. 60–69, 2011.

[39] A. S. Weitlauf, M. L. McPheeters, B. Peters et al., *Therapies for Children With Autism Spectrum Disorder: Behavioral Interventions Update*, Comparative Effectiveness Review no. 137, AHRQ Publication no. 14-EHC036-EF, Agency for Healthcare Research and Quality, Rockville, Md, USA, 2014.

[40] R. Brown, R. P. Hobson, A. Lee, and J. Stevenson, "Are there 'autistic-like' features in congenitally blind children?" *Journal of Child Psychology and Psychiatry and Allied Disciplines*, vol. 38, no. 6, pp. 693–703, 1997.

[41] N. M. Mukaddes, A. Kilincaslan, G. Kucukyazici, T. Sevketoglu, and S. Tuncer, "Autism in visually impaired individuals," *Psychiatry and Clinical Neurosciences*, vol. 61, no. 1, pp. 39–44, 2007.

[42] S. Steffenburg, "Neuropsychiatric assessment of children with autism: a population-based study," *Developmental Medicine and Child Neurology*, vol. 33, no. 6, pp. 495–511, 1991.

[43] C. E. Robertson, C. Thomas, D. J. Kravitz et al., "Global motion perception deficits in autism are reflected as early as primary visual cortex," *Brain*, vol. 137, no. 9, pp. 2588–2599, 2014.

[44] F. Pei, S. Baldassi, and A. M. Norcia, "Electrophysiological measures of low-level vision reveal spatial processing deficits and hemispheric asymmetry in autism spectrum disorder," *Journal of Vision*, vol. 14, article 3, 2014.

[45] A. I. Donaldson, K. S. Heavner, and T. A. Zwolan, "Measuring progress in children with autism spectrum disorder who have cochlear implants," *Archives of Otolaryngology—Head and Neck Surgery*, vol. 130, no. 5, pp. 666–671, 2004.

[46] A. Daneshi and S. Hassanzadeh, "Cochlear implantation in prelingually deaf persons with additional disability," *The Journal of Laryngology & Otology*, vol. 121, no. 7, pp. 635–638, 2007.

[47] U. Rosenhall, V. Nordin, M. Sandström, G. Ahlsén, and C. Gillberg, "Autism and hearing loss," *Journal of Autism and Developmental Disorders*, vol. 29, no. 5, pp. 349–357, 1999.

[48] M. Johansson, E. Wentz, E. Fernell, K. Strömland, M. T. Miller, and C. Gillberg, "Autistic spectrum disorders in Möbius sequence: a comprehensive study of 25 individuals," *Developmental Medicine and Child Neurology*, vol. 43, no. 5, pp. 338–345, 2001.

[49] M. Johansson, M. Råstam, E. Billstedt et al., "Autism spectrum disorders and underlying brain pathology in CHARGE association," *Developmental Medicine and Child Neurology*, vol. 48, no. 1, pp. 40–50, 2006.

[50] M. Johansson, C. Gillberg, and M. Råstam, "Autism spectrum conditions in individuals with Möbius sequence, CHARGE syndrome and oculo-auriculo-vertebral spectrum: diagnostic aspects," *Research in Developmental Disabilities*, vol. 31, no. 1, pp. 9–24, 2010.

[51] T. S. Hartshorne, T. L. Grialou, and K. R. Parker, "Autistic-like behavior in CHARGE syndrome," *American Journal of Medical Genetics Part A*, vol. 133, no. 3, pp. 257–261, 2005.

[52] M. Johansson, E. Billstedt, S. Danielsson et al., "Autism spectrum disorder and underlying brain mechanism in the oculoauriculovertebral spectrum," *Developmental Medicine and Child Neurology*, vol. 49, no. 4, pp. 280–288, 2007.

[53] J. H. Foss-Feig, L. D. Kwakye, C. J. Cascio et al., "An extended multisensory temporal binding window in autism spectrum disorders," *Experimental Brain Research*, vol. 203, no. 2, pp. 381–389, 2010.

[54] L. D. Kwakye, J. H. Foss-Feig, C. J. Cascio, W. L. Stone, and M. T. Wallace, "Altered auditory and multisensory temporal processing in autism spectrum disorders," *Frontiers in Integrative Neuroscience*, vol. 4, article 129, 2011.

[55] A. B. Brandwein, J. J. Foxe, J. S. Butler et al., "Neurophysiological indices of atypical auditory processing and multisensory integration are associated with symptom severity in autism," *Journal of Autism and Developmental Disorders*, vol. 45, no. 1, pp. 230–244, 2015.

[56] A. B. Brandwein, J. J. Foxe, J. S. Butler et al., "The development of multisensory integration in high-functioning autism: high-density electrical mapping and psychophysical measures reveal impairments in the processing of audiovisual inputs," *Cerebral Cortex*, vol. 23, no. 6, pp. 1329–1341, 2013.

[57] Y.-S. Chang, J. P. Owen, S. S. Desai et al., "Autism and sensory processing disorders: shared white matter disruption in sensory pathways but divergent connectivity in social-emotional pathways," *PLoS ONE*, vol. 9, no. 7, Article ID e103038, 2014.

[58] R. Hoksbergen, J. ter Laak, K. Rijk, C. van Dijkum, and F. Stoutjesdijk, "Post-institutional autistic syndrome in Romanian adoptees," *Journal of Autism and Developmental Disorders*, vol. 35, no. 5, pp. 615–623, 2005.

[59] B. H. Ellis, P. A. Fisher, and S. Zaharie, "Predictors of disruptive behavior, developmental delays, anxiety, and affective symptomatology among institutionally reared Romanian children," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 43, no. 10, pp. 1283–1292, 2004.

[60] A. R. Levin, C. H. Zeanah Jr., N. A. Fox, and C. A. Nelson, "Motor outcomes in children exposed to early psychosocial deprivation," *The Journal of Pediatrics*, vol. 164, no. 1, pp. 123–129.e1, 2014.

[61] L. Miller, W. Chan, K. Comfort, and L. Tirella, "Health of children adopted from Guatemala: comparison of orphanage and foster care," *Pediatrics*, vol. 115, no. 6, pp. e710–e717, 2005.

[62] A. B. Wismer Fries, T. E. Ziegler, J. R. Kurian, S. Jacoris, and S. D. Pollak, "Early experience in humans is associated with changes in neuropeptides critical for regulating social behavior," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 47, pp. 17237–17240, 2005.

[63] E. Colvert, M. Rutter, J. Kreppner et al., "Do theory of mind and executive function deficits underlie the adverse outcomes associated with profound early deprivation?: findings from the English and Romanian adoptees study," *Journal of Abnormal Child Psychology*, vol. 36, no. 7, pp. 1057–1068, 2008.

[64] J. Bachevalier and K. A. Loveland, "The orbitofrontal-amygdala circuit and self-regulation of social-emotional behavior in autism," *Neuroscience and Biobehavioral Reviews*, vol. 30, no. 1, pp. 97–117, 2006.

[65] S. Baron-Cohen, H. A. Ring, E. T. Bullmore, S. Wheelwright, C. Ashwin, and S. C. R. Williams, "The amygdala theory of autism," *Neuroscience and Biobehavioral Reviews*, vol. 24, no. 3, pp. 355–364, 2000.

[66] S. Baron-Cohen, H. A. Ring, S. Wheelwright et al., "Social intelligence in the normal and autistic brain: an fMRI study," *European Journal of Neuroscience*, vol. 11, no. 6, pp. 1891–1898, 1999.

[67] H. T. Chugani, M. E. Behen, O. Muzik, C. Juhász, F. Nagy, and D. C. Chugani, "Local brain functional activity following early deprivation: a study of postinstitutionalized Romanian orphans," *NeuroImage*, vol. 14, no. 6, pp. 1290–1301, 2001.

[68] T. J. Eluvathingal, H. T. Chugani, M. E. Behen et al., "Abnormal brain connectivity in children after early severe socioemotional

deprivation: a diffusion tensor imaging study," *Pediatrics*, vol. 117, no. 6, pp. 2093–2100, 2006.

[69] Y. Aoki, O. Abe, Y. Nippashi, and H. Yamasue, "Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies," *Molecular Autism*, vol. 4, no. 1, article 25, 2013.

[70] M. Pardini, M. Elia, F. G. Garaci et al., "Long-term cognitive and behavioral therapies, combined with augmentative communication, are related to uncinate fasciculus integrity in autism," *Journal of Autism and Developmental Disorders*, vol. 42, no. 4, pp. 585–592, 2012.

[71] M. Pardini, F. G. Garaci, L. Bonzano et al., "White matter reduced streamline coherence in young men with autism and mental retardation," *European Journal of Neurology*, vol. 16, no. 11, pp. 1185–1190, 2009.

[72] L. J. Carver and G. Dawson, "Development and neural bases of face recognition in autism," *Molecular Psychiatry*, vol. 7, no. 2, pp. S18–S20, 2002.

[73] J. P. McCleery, N. Akshoomoff, K. R. Dobkins, and L. J. Carver, "Atypical face versus object processing and hemispheric asymmetries in 10-month-old infants at risk for autism," *Biological Psychiatry*, vol. 66, no. 10, pp. 950–957, 2009.

[74] J. C. McPartland, G. Dawson, S. J. Webb, H. Panagiotides, and L. J. Carver, "Event-related brain potentials reveal anomalies in temporal processing of faces in autism spectrum disorder," *Journal of Child Psychology and Psychiatry*, vol. 45, no. 7, pp. 1235–1245, 2004.

[75] S. W. Parker, C. A. Nelson, C. H. Zeanah et al., "An event-related potential study of the impact of institutional rearing on face recognition," *Development and Psychopathology*, vol. 17, no. 3, pp. 621–639, 2005.

[76] A. Senju, Y. Tojo, K. Yaguchi, and T. Hasegawa, "Deviant gaze processing in children with autism: an ERP study," *Neuropsychologia*, vol. 43, no. 9, pp. 1297–1306, 2005.

[77] R. Boecker, N. E. Holz, A. F. Buchmann et al., "Impact of early life adversity on reward processing in young adults: EEG-fMRI results from a prospective study over 25 years," *PLoS ONE*, vol. 9, no. 8, Article ID e104185, 2014.

[78] S. Delmonte, J. H. Balsters, J. McGrath et al., "Social and monetary reward processing in autism spectrum disorders," *Molecular Autism*, vol. 3, no. 1, article 7, 2012.

[79] G. Kohls, M. Schulte-Rüther, B. Nehrkorn et al., "Reward system dysfunction in autism spectrum disorders," *Social Cognitive and Affective Neuroscience*, vol. 8, no. 5, pp. 565–572, 2013.

[80] M. A. Mehta, E. Gore-Langton, N. Golembo, E. Colvert, S. C. R. Williams, and E. Sonuga-Barke, "Hyporesponsive reward anticipation in the basal ganglia following severe institutional deprivation early in life," *Journal of Cognitive Neuroscience*, vol. 22, no. 10, pp. 2316–2325, 2010.

[81] A. A. Scott-Van Zeeland, M. Dapretto, D. G. Ghahremani, R. A. Poldrack, and S. Y. Bookheimer, "Reward processing in autism," *Autism Research*, vol. 3, no. 2, pp. 53–67, 2010.

[82] M. J. Bakermans-Kranenburg, M. H. van Ijzendoorn, and F. Juffer, "Earlier is better: a meta-analysis of 70 years of intervention improving cognitive development in institutionalized children," *Monographs of the Society for Research in Child Development*, vol. 73, no. 3, pp. 279–293, 2008.

[83] K. Bos, C. H. Zeanah, N. A. Fox, S. S. Drury, K. A. McLaughlin, and C. A. Nelson, "Psychiatric outcomes in young children with a history of institutionalization," *Harvard Review of Psychiatry*, vol. 19, no. 1, pp. 15–24, 2011.

[84] C. A. Nelson III, C. H. Zeanah, N. A. Fox, P. J. Marshall, A. T. Smyke, and D. Guthrie, "Cognitive recovery in socially deprived young children: the Bucharest early intervention project," *Science*, vol. 318, no. 5858, pp. 1937–1940, 2007.

[85] C. L. M. Petrenko, "A review of intervention programs to prevent and treat behavioral problems in young children with developmental disabilities," *Journal of Developmental and Physical Disabilities*, vol. 25, no. 6, pp. 651–679, 2013.

[86] R. E. Vanderwert, P. J. Marshall, C. A. Nelson III, C. H. Zeanah, and N. A. Fox, "Timing of intervention affects brain electrical activity in children exposed to severe psychosocial neglect," *PLoS ONE*, vol. 5, no. 7, Article ID e11415, 2010.

[87] R. Kiani and H. Miller, "Sensory impairment and intellectual disability," *Advances in Psychiatric Treatment*, vol. 16, no. 3, pp. 228–235, 2010.

[88] J. M. Pinto, D. W. Kern, K. E. Wroblewski, R. C. Chen, L. P. Schumm, and M. K. McClintock, "Sensory function: insights from wave 2 of the national social life, health, and aging project," *Journals of Gerontology, Series B: Psychological Sciences and Social Sciences*, vol. 69, supplement 2, pp. S144–S153, 2014.

[89] J. Gallacher, V. Ilubaera, Y. Ben-Shlomo et al., "Auditory threshold, phonologic demand, and incident dementia," *Neurology*, vol. 79, no. 15, pp. 1583–1590, 2012.

[90] F. R. Lin, K. Yaffe, J. Xia et al., "Hearing loss and cognitive decline in older adults," *JAMA Internal Medicine*, vol. 173, no. 4, pp. 293–299, 2013.

[91] D. R. Moore, M. Edmondson-Jones, P. Dawes et al., "Relation between speech-in-noise threshold, hearing loss and cognition from 40–69 years of age," *PLoS ONE*, vol. 9, no. 9, Article ID e107720, 2014.

[92] J. van Splunder, J. S. Stilma, R. M. D. Bernsen, and H. M. Evenhuis, "Prevalence of visual impairment in adults with intellectual disabilities in the Netherlands: cross-sectional study," *Eye*, vol. 20, no. 9, pp. 1004–1010, 2006.

[93] R. A. F. Weijenberg and F. Lobbezoo, "Chew the pain away: oral habits to cope with pain and stress and to stimulate cognition," *BioMed Research International*, vol. 2015, Article ID 149431, 7 pages, 2015.

[94] H. Chen, M. Iinuma, M. Onozuka, and K.-Y. Kubo, "Chewing maintains hippocampus-dependent cognitive function," *International Journal of Medical Sciences*, vol. 12, no. 6, pp. 502–509, 2015.

[95] A. B. Graves, J. D. Bowen, L. Rajaram et al., "Impaired olfaction as a marker for cognitive decline: interaction with apolipoprotein E $\varepsilon 4$," *Neurology*, vol. 53, no. 7, pp. 1480–1487, 1999.

[96] G. E. Swan and D. Carmelli, "Impaired olfaction predicts cognitive decline in nondemented older adults," *Neuroepidemiology*, vol. 21, no. 2, pp. 58–67, 2002.

[97] R. S. Wilson, J. A. Schneider, S. E. Arnold, Y. Tang, P. A. Boyle, and D. A. Bennett, "Olfactory identification and incidence of mild cognitive impairment in older age," *Archives of General Psychiatry*, vol. 64, no. 7, pp. 802–808, 2007.

[98] C. R. Schubert, L. L. Carmichael, C. Murphy, B. E. K. Klein, R. Klein, and K. J. Cruickshanks, "Olfaction and the 5-year incidence of cognitive impairment in an epidemiological study of older adults," *Journal of the American Geriatrics Society*, vol. 56, no. 8, pp. 1517–1521, 2008.

[99] J. K. Olofsson, M. Rönnlund, S. Nordin, L. Nyberg, L.-G. Nilsson, and M. Larsson, "Odor identification deficit as a predictor of five-year global cognitive change: interactive effects with age and ApoE-$\varepsilon 4$," *Behavior Genetics*, vol. 39, no. 5, pp. 496–503, 2009.

[100] H. R. Sohrabi, K. A. Bates, M. G. Weinborn et al., "Olfactory discrimination predicts cognitive decline among community-dwelling older adults," *Translational Psychiatry*, vol. 2, no. 5, article e118, 2012.

[101] I. Stanciu, M. Larsson, S. Nordin, R. Adolfsson, L.-G. Nilsson, and J. K. Olofsson, "Olfactory impairment and subjective olfactory complaints independently predict conversion to dementia: a longitudinal, population-based study," *Journal of the International Neuropsychological Society*, vol. 20, no. 2, pp. 209–217, 2014.

[102] D. P. Devanand, K. S. Michaels-Marston, X. Liu et al., "Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up," *The American Journal of Psychiatry*, vol. 157, no. 9, pp. 1399–1405, 2000.

[103] D. P. Devanand, M. H. Tabert, K. Cuasay et al., "Olfactory identification deficits and MCI in a multi-ethnic elderly community sample," *Neurobiology of Aging*, vol. 31, no. 9, pp. 1593–1600, 2010.

[104] T. Bitter, H. Gudziol, H. P. Burmeister, H.-J. Mentzel, O. Guntinas-Lichius, and C. Gaser, "Anosmia leads to a loss of gray matter in cortical brain areas," *Chemical Senses*, vol. 35, no. 5, pp. 407–415, 2010.

[105] P. Peng, H. Gu, W. Xiao et al., "A voxel-based morphometry study of anosmic patients," *British Journal of Radiology*, vol. 86, no. 1032, Article ID 20130207, 2013.

[106] T. Bitter, J. Brüderle, H. Gudziol, H. P. Burmeister, C. Gaser, and O. Guntinas-Lichius, "Gray and white matter reduction in hyposmic subjects—a voxel-based morphometry study," *Brain Research*, vol. 1347, pp. 42–47, 2010.

[107] T. Bitter, H. Gudziol, H. P. Burmeister et al., "Gray matter alterations in parosmia," *Neuroscience*, vol. 177, pp. 177–182, 2011.

[108] R. K. Gurgel, P. D. Ward, S. Schwartz, M. C. Norton, N. L. Foster, and J. T. Tschanz, "Relationship of hearing loss and dementia: a prospective, population-based study," *Otology and Neurotology*, vol. 35, no. 5, pp. 775–781, 2014.

[109] P. Dawes, R. Emsley, K. J. Cruickshanks et al., "Hearing loss and cognition: the role of hearing aids, social isolation and depression," *PLoS ONE*, vol. 10, no. 3, Article ID e0119616, 2015.

[110] I. Mosnier, J.-P. Bebear, M. Marx et al., "Improvement of cognitive function after cochlear implantation in elderly patients," *JAMA Otolaryngology—Head and Neck Surgery*, vol. 141, no. 5, pp. 442–450, 2015.

[111] A. A. Wong and R. E. Brown, "Prevention of vision loss protects against age-related impairment in learning and memory performance in DBA/2J mice," *Frontiers in Aging Neuroscience*, vol. 5, article 52, 2013.

[112] Y. Ono, T. Yamamoto, K.-Y. Kubo, and M. Onozuka, "Occlusion and brain function: mastication as a prevention of cognitive dysfunction," *Journal of Oral Rehabilitation*, vol. 37, no. 8, pp. 624–640, 2010.

[113] M. R. Rosenzweig and E. L. Bennett, "Psychobiology of plasticity: effects of training and experience on brain and behavior," *Behavioural Brain Research*, vol. 78, no. 1, pp. 57–65, 1996.

[114] B. Kerr, P. A. Silva, K. Walz, and J. I. Young, "Unconventional transcriptional response to environmental enrichment in a mouse model of Rett syndrome," *PLoS ONE*, vol. 5, no. 7, Article ID e11534, 2010.

[115] M. Kondo, L. J. Gray, G. J. Pelka, J. Christodoulou, P. P. L. Tam, and A. J. Hannan, "Environmental enrichment ameliorates a motor coordination deficit in a mouse model of Rett syndrome—Mecp2 gene dosage effects and BDNF expression," *European Journal of Neuroscience*, vol. 27, no. 12, pp. 3342–3350, 2008.

[116] G. Lonetti, A. Angelucci, L. Morando, E. M. Boggio, M. Giustetto, and T. Pizzorusso, "Early environmental enrichment moderates the behavioral and synaptic phenotype of MeCP2 null mice," *Biological Psychiatry*, vol. 67, no. 7, pp. 657–665, 2010.

[117] L. Restivo, F. Ferrari, E. Passino et al., "Enriched environment promotes behavioral and morphological recovery in a mouse model for the fragile X syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 32, pp. 11557–11562, 2005.

[118] N. Nag, J. M. Moriuchi, C. G. K. Peitzman, B. C. Ward, N. H. Kolodny, and J. E. Berger-Sweeney, "Environmental enrichment alters locomotor behaviour and ventricular volume in Mecp2lox mice," *Behavioural Brain Research*, vol. 196, no. 1, pp. 44–48, 2009.

[119] L. Potocki, W. Bi, D. Treadwell-Deering et al., "Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype," *American Journal of Human Genetics*, vol. 80, no. 4, pp. 633–649, 2007.

[120] M. Lacaria, C. Spencer, W. Gu, R. Paylor, and J. R. Lupski, "Enriched rearing improves behavioral responses of an animal model for CNV-based autistic-like traits," *Human Molecular Genetics*, vol. 21, no. 14, pp. 3083–3096, 2012.

[121] H. G. McFarlane, G. K. Kusek, M. Yang, J. L. Phoenix, V. J. Bolivar, and J. N. Crawley, "Autism-like behavioral phenotypes in BTBR T + tf/J mice," *Genes, Brain and Behavior*, vol. 7, no. 2, pp. 152–163, 2008.

[122] S. S. Moy, J. J. Nadler, M. D. Poe et al., "Development of a mouse test for repetitive, restricted behaviors: relevance to autism," *Behavioural Brain Research*, vol. 188, no. 1, pp. 178–194, 2008.

[123] S. S. Moy, J. J. Nadler, N. B. Young et al., "Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains," *Behavioural Brain Research*, vol. 176, no. 1, pp. 4–20, 2007.

[124] B. L. Pearson, R. L. H. Pobbe, E. B. Defensor et al., "Motor and cognitive stereotypies in the BTBR T+tf/J mouse model of autism," *Genes, Brain and Behavior*, vol. 10, no. 2, pp. 228–235, 2011.

[125] S. Reynolds, M. Urruela, and D. P. Devine, "Effects of environmental enrichment on repetitive behaviors in the BTBR T+tf/J mouse model of autism," *Autism Research*, vol. 6, no. 5, pp. 337–343, 2013.

[126] P. MacPherson, R. McGaffigan, D. Wahlsten, and P. V. Nguyen, "Impaired fear memory, altered object memory and modified hippocampal synaptic plasticity in split-brain mice," *Brain Research*, vol. 1210, pp. 179–188, 2008.

[127] M. Yang, K. Perry, M. D. Weber, A. M. Katz, and J. N. Crawley, "Social peers rescue autism-relevant sociability deficits in adolescent mice," *Autism Research*, vol. 4, no. 1, pp. 17–27, 2011.

[128] C. A. Turner and M. H. Lewis, "Environmental enrichment: effects on stereotyped behavior and neurotrophin levels," *Physiology and Behavior*, vol. 80, no. 2-3, pp. 259–266, 2003.

[129] T. Schneider and R. Przewłocki, "Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism," *Neuropsychopharmacology*, vol. 30, no. 1, pp. 80–89, 2005.

[130] T. Schneider, J. Turczak, and R. Przewłocki, "Environmental enrichment reverses behavioral alterations in rats prenatally exposed to valproic acid: issues for a therapeutic approach in autism," *Neuropsychopharmacology*, vol. 31, no. 1, pp. 36–46, 2006.

[131] L. Baroncelli, C. Braschi, M. Spolidoro, T. Begenisic, A. Sale, and L. Maffei, "Nurturing brain plasticity: impact of environmental enrichment," *Cell Death and Differentiation*, vol. 17, no. 7, pp. 1092–1103, 2010.

[132] A. J. Hannan, "Review: environmental enrichment and brain repair: harnessing the therapeutic effects of cognitive stimulation and physical activity to enhance experience-dependent plasticity," *Neuropathology and Applied Neurobiology*, vol. 40, no. 1, pp. 13–25, 2014.

[133] J. Nithianantharajah and A. J. Hannan, "Enriched environments, experience-dependent plasticity and disorders of the nervous system," *Nature Reviews Neuroscience*, vol. 7, no. 9, pp. 697–709, 2006.

[134] T. Y. C. Pang and A. J. Hannan, "Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity," *Neuropharmacology*, vol. 64, pp. 515–528, 2013.

[135] A. Ben-Sasson, L. Hen, R. Fluss, S. A. Cermak, B. Engel-Yeger, and E. Gal, "A meta-analysis of sensory modulation symptoms in individuals with autism spectrum disorders," *Journal of Autism and Developmental Disorders*, vol. 39, no. 1, pp. 1–11, 2009.

[136] C. L. Hilton, J. D. Harper, R. H. Kueker et al., "Sensory responsiveness as a predictor of social severity in children with high functioning autism spectrum disorders," *Journal of Autism and Developmental Disorders*, vol. 40, no. 8, pp. 937–945, 2010.

[137] J. K. Kern, M. H. Trivedi, B. D. Grannemann et al., "Sensory correlations in autism," *Autism*, vol. 11, no. 2, pp. 123–134, 2007.

[138] S. R. Leekam, C. Nieto, S. J. Libby, L. Wing, and J. Gould, "Describing the sensory abnormalities of children and adults with autism," *Journal of Autism and Developmental Disorders*, vol. 37, no. 5, pp. 894–910, 2007.

[139] L. Mottron, M. Dawson, I. Soulières, B. Hubert, and J. Burack, "Enhanced perceptual functioning in autism: an update, and eight principles of autistic perception," *Journal of Autism and Developmental Disorders*, vol. 36, no. 1, pp. 27–43, 2006.

[140] S. J. Rogers and S. Ozonoff, "Annotation: what do we know about sensory dysfunction in autism? A critical review of the empirical evidence," *Journal of Child Psychology and Psychiatry*, vol. 46, no. 12, pp. 1255–1268, 2005.

[141] S. D. Tomchek and W. Dunn, "Sensory processing in children with and without autism: a comparative study using the short sensory profile," *American Journal of Occupational Therapy*, vol. 61, no. 2, pp. 190–200, 2007.

[142] R. L. Watling, J. Deitz, and O. White, "Comparison of sensory profile scores of young children with and without autism spectrum disorders," *American Journal of Occupational Therapy*, vol. 55, no. 4, pp. 416–423, 2001.

[143] American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders*, American Psychiatric Association, Washington, DC, USA, 5th edition, 2013.

[144] M. Behrmann, G. Avidan, G. L. Leonard et al., "Configural processing in autism and its relationship to face processing," *Neuropsychologia*, vol. 44, no. 1, pp. 110–129, 2006.

[145] M.-J. Caron, L. Mottron, C. Berthiaume, and M. Dawson, "Cognitive mechanisms, specificity and neural underpinnings of visuospatial peaks in autism," *Brain*, vol. 129, no. 7, pp. 1789–1802, 2006.

[146] R. L. Gabriels, J. A. Agnew, L. J. Miller et al., "Is there a relationship between restricted, repetitive, stereotyped behaviors and interests and abnormal sensory response in children with autism spectrum disorders?" *Research in Autism Spectrum Disorders*, vol. 2, no. 4, pp. 660–670, 2008.

[147] T. Kujala, T. Lepistö, and R. Näätänen, "The neural basis of aberrant speech and audition in autism spectrum disorders," *Neuroscience and Biobehavioral Reviews*, vol. 37, no. 4, pp. 697–704, 2013.

[148] R. A. Stevenson, J. K. Siemann, B. C. Schneider et al., "Multisensory temporal integration in autism spectrum disorders," *The Journal of Neuroscience*, vol. 34, no. 3, pp. 691–697, 2014.

[149] E. J. Marco, L. B. N. Hinkley, S. S. Hill, and S. S. Nagarajan, "Sensory processing in autism: a review of neurophysiologic findings," *Pediatric Research*, vol. 69, no. 5, pp. 48R–54R, 2011.

[150] S. Wigham, J. Rodgers, M. South, H. McConachie, and M. Freeston, "The interplay between sensory processing abnormalities, intolerance of uncertainty, anxiety and restricted and repetitive behaviours in autism spectrum disorder," *Journal of Autism and Developmental Disorders*, vol. 45, no. 4, pp. 943–952, 2015.

[151] D. W. Evans, F. L. Gray, and J. F. Leckman, "The rituals, fears and phobias of young children: insights from development, psychopathology and neurobiology," *Child Psychiatry and Human Development*, vol. 29, no. 4, pp. 261–276, 1999.

[152] M. E. Brock, A. Freuler, G. T. Baranek, L. R. Watson, M. D. Poe, and A. Sabatino, "Temperament and sensory features of children with autism," *Journal of Autism and Developmental Disorders*, vol. 42, no. 11, pp. 2271–2284, 2012.

[153] M. J. Owen, "New approaches to psychiatric diagnostic classification," *Neuron*, vol. 84, no. 3, pp. 564–571, 2014.

[154] C. Fountain, A. S. Winter, and P. S. Bearman, "Six developmental trajectories characterize children with autism," *Pediatrics*, vol. 129, no. 5, pp. e1112–e1120, 2012.

[155] A. Bailey, A. Le Couteur, I. Gottesman et al., "Autism as a strongly genetic disorder: evidence from a British twin study," *Psychological Medicine*, vol. 25, no. 1, pp. 63–77, 1995.

[156] S. Folstein and M. Rutter, "Infantile autism: a genetic study of 21 twin pairs," *Journal of Child Psychology and Psychiatry and Allied Disciplines*, vol. 18, no. 4, pp. 297–321, 1977.

[157] E. R. Ritvo, B. J. Freeman, A. Mason-Brothers, A. Mo, and A. M. Ritvo, "Concordance for the syndrome of autism in 40 pairs of afflicted twins," *American Journal of Psychiatry*, vol. 142, no. 1, pp. 74–77, 1985.

[158] T. Gaugler, L. Klei, S. J. Sanders et al., "Most genetic risk for autism resides with common variation," *Nature Genetics*, vol. 46, no. 8, pp. 881–885, 2014.

[159] P. Chaste, L. Klei, S. J. Sanders et al., "A genome-wide association study of autism using the Simons Simplex Collection: does reducing phenotypic heterogeneity in autism increase genetic homogeneity?" *Biological Psychiatry*, vol. 77, no. 9, pp. 775–784, 2015.

[160] L. Klei, S. J. Sanders, M. T. Murtha et al., "Common genetic variants, acting additively, are a major source of risk for autism," *Molecular Autism*, vol. 3, article 9, 2012.

[161] Cross-Disorder Group of the Psychiatric Genomics Consortium, "Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis," *The Lancet*, vol. 381, no. 9875, pp. 1371–1379, 2013.

[162] C. M. Hultman, S. Sandin, S. Z. Levine, P. Lichtenstein, and A. Reichenberg, "Advancing paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies," *Molecular Psychiatry*, vol. 16, no. 12, pp. 1203–1212, 2011.

[163] R. Sharma, A. Agarwal, V. K. Rohra, M. Assidi, M. Abu-Elmagd, and R. F. Turki, "Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring," *Reproductive Biology and Endocrinology*, vol. 13, no. 1, article 35, 2015.

[164] S. Lundström, C. M. A. Haworth, E. Carlström et al., "Trajectories leading to autism spectrum disorders are affected by paternal age: findings from two nationally representative twin studies," *Journal of Child Psychology and Psychiatry and Allied Disciplines*, vol. 51, no. 7, pp. 850–856, 2010.

[165] E. M. Frans, S. Sandin, A. Reichenberg et al., "Autism risk across generations: a population-based study of advancing grandpaternal and paternal age," *JAMA Psychiatry*, vol. 70, no. 5, pp. 516–521, 2013.

[166] S. Sandin, C. M. Hultman, A. Kolevzon, R. Gross, J. H. MacCabe, and A. Reichenberg, "Advancing maternal age is associated with increasing risk for autism: a review and meta-analysis," *Journal of the American Academy of Child & Adolescent Psychiatry*, vol. 51, no. 5, pp. 477–486.e1, 2012.

[167] P. Marangos, M. Stevense, K. Niaka et al., "DNA damage-induced metaphase I arrest is mediated by the spindle assembly checkpoint and maternal age," *Nature Communications*, vol. 6, article 8706, 2015.

[168] R. X. Moldrich, G. Leanage, D. She et al., "Inhibition of histone deacetylase in utero causes sociability deficits in postnatal mice," *Behavioural Brain Research*, vol. 257, pp. 253–264, 2013.

[169] H. O. Kalkman, "A review of the evidence for the canonical Wnt pathway in autism spectrum disorders," *Molecular Autism*, vol. 3, no. 1, article 10, 2012.

[170] N. C. Inestrosa and L. Varela-Nallar, "Wnt signalling in neuronal differentiation and development," *Cell and Tissue Research*, vol. 359, no. 1, pp. 215–223, 2015.

[171] H. E. Volk, I. Hertz-Picciotto, L. Delwiche, F. Lurmann, and R. McConnell, "Residential proximity to freeways and autism in the CHARGE study," *Environmental Health Perspectives*, vol. 119, no. 6, pp. 873–877, 2011.

[172] H. E. Volk, F. Lurmann, B. Penfold, I. Hertz-Picciotto, and R. McConnell, "Traffic-related air pollution, particulate matter, and autism," *JAMA Psychiatry*, vol. 70, no. 1, pp. 71–77, 2013.

[173] C.-R. Jung, Y.-T. Lin, and B.-F. Hwang, "Air pollution and newly diagnostic autism spectrum disorders: a population-based cohort study in Taiwan," *PLoS ONE*, vol. 8, no. 9, Article ID e75510, 2013.

[174] A. E. Kalkbrenner, G. C. Windham, M. L. Serre et al., "Particulate matter exposure, prenatal and postnatal windows of susceptibility, and autism spectrum disorders," *Epidemiology*, vol. 26, no. 1, pp. 30–42, 2015.

[175] D. B. Campbell, J. S. Sutcliffe, P. J. Ebert et al., "A genetic variant that disrupts MET transcription is associated with autism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 45, pp. 16834–16839, 2006.

[176] H. E. Volk, T. Kerin, F. Lurmann, I. Hertz-Picciotto, R. McConnell, and D. B. Campbell, "Interaction of the MET receptor tyrosine kinase gene and air pollution exposure in autism spectrum disorder," *Epidemiology*, vol. 25, no. 1, pp. 44–47, 2014.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 4235898, 20 pages
http://dx.doi.org/10.1155/2016/4235898

Review Article

Where Environment Meets Cognition: A Focus on Two Developmental Intellectual Disability Disorders

I. De Toma,[1,2] L. Manubens Gil,[1,2] S. Ossowski,[2,3] and M. Dierssen[1,2]

[1]*Cellular and Systems Neurobiology, Systems Biology Program, Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, 08003 Barcelona, Spain*
[2]*Pompeu Fabra University, 08003 Barcelona, Spain*
[3]*Genomic and Epigenomic Variation in Disease Group, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, 08003 Barcelona, Spain*

Correspondence should be addressed to I. De Toma; ilario.detoma@crg.eu and M. Dierssen; mara.dierssen@crg.eu

Received 1 February 2016; Accepted 3 April 2016

Academic Editor: Susanna Pietropaolo

One of the most challenging questions in neuroscience is to dissect how learning and memory, the foundational pillars of cognition, are grounded in stable, yet plastic, gene expression states. All known epigenetic mechanisms such as DNA methylation and hydroxymethylation, histone modifications, chromatin remodelling, and noncoding RNAs regulate brain gene expression, both during neurodevelopment and in the adult brain in processes related to cognition. On the other hand, alterations in the various components of the epigenetic machinery have been linked to well-known causes of intellectual disability disorders (IDDs). Two examples are Down Syndrome (DS) and Fragile X Syndrome (FXS), where global and local epigenetic alterations lead to impairments in synaptic plasticity, memory, and learning. Since epigenetic modifications are reversible, it is theoretically possible to use epigenetic drugs as cognitive enhancers for the treatment of IDDs. Epigenetic treatments act in a context specific manner, targeting different regions based on cell and state specific chromatin accessibility, facilitating the establishment of the lost balance. Here, we discuss epigenetic studies of IDDs, focusing on DS and FXS, and the use of epidrugs in combinatorial therapies for IDDs.

1. Epigenetics and Cognition

Intellectual disability disorders (IDDs) are complex multifactorial illnesses involving chronic alterations in neural circuit structure and function as well as likely abnormalities in glial cells. Converging evidence indicates that epigenetic control of gene expression is pivotal to learning and memory, as underscored also by the range of intellectual disabilities and behavioural deficits increasingly traced to a staggering number of epigenetic modulators. This review focuses on the importance of epigenomics in neuroscience, especially in neurodevelopment and cognition. Since epigenetic mechanisms are reversible, they are targets of interest in conceiving new therapies for the treatment of IDDs. We will specifically address two genetic intellectual disabilities, Down Syndrome (DS), caused by trisomy 21 [1], and Fragile X Syndrome (FXS), caused by the absence of FMRP protein upon a "CGG" triplet expansion at the 5′-UTR of the FMR1 gene [2]. Both IDDs show epigenetic dysregulation and, despite the differences in their neuropathological signs, share disturbances in the molecular events that regulate the way nerve cells develop dendritic spines.

1.1. Epigenetic Mechanisms Regulate Neurodevelopment and Cognition. Since the first definition of epigenetics [3] the meaning of this term has broadened to include several mechanisms of gene expression regulation not interfering with the DNA sequence but regulating the chromatin state. These include DNA chemical modifications, histone post-translational modifications, chromatin remodelling, and the expression of noncoding RNAs (ncRNAs). Even though these mechanisms are quite different, they have in common interfering with chromatin compaction. Nuclear proteins and DNA compose chromatin that can be more condensed impairing transcription, or more loose, facilitating gene expression. The notion that experience modulates cognitive

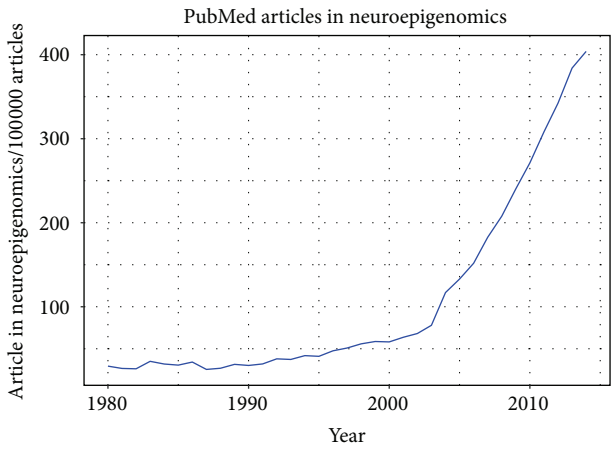


Figure 1: Trends in publications in the field of neuroepigenetics. The plot shows the number of publications on *PubMed* by year, normalized by the total of number of articles. The x-axis represents the years, and the y-axis plots the number of articles in neuroepigenetics per 100.000 articles.

function and development has become an accepted tenet of modern neuroscience. However, the precise molecular mechanisms by which the environment modulates neurological development are still to be elucidated. One such mechanism is cognitive-activity-dependent gene expression [4]. Epigenetics mediates the interaction between the environment and the genome and, therefore, epigenetic control of gene expression is pivotal to learning and memory and can explain brain plasticity, the capacity of neurons to remodel their structures based on external inputs. This is important for two well-studied aspects in neuroscience: neurodevelopment and cognition (e.g., memory and learning), two components that are somehow interconnected as highlighted by the common mechanisms that underlie developmental and adult experience/learning associated synapse addition. In neurodevelopmental disorders such DS or FXS, problems in neural development come along with the adult cognitive impairment [1] but while dendritic spine numbers are lower and dendritic tree is affected in DS [5], FXS appears to be the only form of intellectual disability that exhibit increased numbers of dendritic spines without alterations in the dendritic arbour [6]. Recent studies established that neuronal activity triggers local de novo synthesis of proteins in the dendrites of the affected postsynaptic neurons, and the concept of a dynamic proteome at the synapse is beginning to emerge [7]. In fact, the number of papers dealing with both epigenetics and neuroscience has started to grow steadily especially after the establishment of next-generation sequencing techniques in 2004, reaching over 400 publications every 100,000 on PubMed (Figure 1). This has led to the definition of a new emerging field termed "neuroepigenetics" [8] or "neuroepigenomics" [9]. Since epigenetic mechanisms are important regulators in both neurodevelopment and cognition, we believe that these neuroepigenomics studies will be crucial in understanding the pathogenesis of neurodevelopmental IDDs, where both defects in brain development and cognition coexist. This review collects recent evidence confirming this hypothesis, pointing out how tackling epigenetic deregulation could be an ideal therapeutic approach for restoring the phenotype in neurodevelopmental IDDs.

1.1.1. Chemical Modifications of DNA. The family of enzymes called DNA methyltransferases (DNMTs) catalyse the most studied modification of DNA. DNMTs transfer a methyl group from S-adenyl methionine (SAM) to a cytosine residue to form 5-methyl-cytosine (5mC). Cytosine methylation occurs especially at CG dinucleotides (CpG sites) which are underrepresented in the genome since 5mC tends to deaminate into thymine [10]. Those sites are usually methylated with the exception of CpG islands, ≈0.2–1 kb conserved regions with higher density (>50%) of CpG sites, which are usually found on gene promoters [11]. Generally speaking, this modification represses transcription both by sterically interfering with transcription factor binding and especially by recruiting repressive complexes upon binding to proteins with methyl binding domains [12].

In mammals, three main DNMTs exist: DNMT1 is called the "maintenance DNMT" since it usually binds to hemimethylated sites avoiding passive demethylation during DNA synthesis and DNMT3a and DNMT3b are the so-called de novo DNMTs [13]. Interestingly, DNMTs are highly expressed in the brain not only during neurodevelopment but also in postmitotic neurons [14], suggesting a role for DNA methylation beyond development, which is connected to brain functions in the adult. As a matter of fact, although DNA methylation has been thought to be a static epigenetic mark that could be lost only by passive demethylation during cell division, nowadays it is known that DNA methylation is dynamic and can be also actively regulated. TET enzymes initially oxidize 5mC, and, in a second phase, it can be deaminated by AID/Apobec enzymes or further TET-oxidized. Finally, the oxidation products are repaired by the base excision repair (BER) [15, 16].

Several studies highlight regulation by DNA methylation at the promoters of key genes involved in cognition. Interestingly, following contextual fear conditioning, one of the most used models for studying memory in rodent models, DNMTs are upregulated in the hippocampus during memory formation and this results in an increase in DNA methylation at the promoter of the memory suppressor gene PP1 and a decrease in the methylation at the promoter of the synaptic plasticity gene RELN during memory consolidation. Accordingly, inhibition of DNMTs resulted in PP1 demethylation and problems in memory consolidation [17]. The same is true for the BDNF gene, where DNA methylation regulation upon the learning task results in the specific increase in BDNF exons I and IV mRNA transcript during consolidation of fear memory [18]. Those changes in DNA methylation were dynamic, acute (40 minutes), and transient, being reverted in 24 hours. This finally contradicts the dogma depicting DNA methylation as a static mark and supports the hippocampus' role in memory formation and consolidation.

Moreover, the brain shows particularly high levels of two other methylation types: non-CpG methylation (mCH, where H stands for adenine A, thymine T, or cytosine C) and

hydroxymethylation (5hmC), suggesting a specific neural role for these modifications [19, 20]. While mCH is absent in the foetal cortex, it accumulates in neurons during early postnatal development becoming the main form of DNA methylation and repressing critical genes during development. In this context DNMT3a seems to play a critical role. Of note, neurons show higher mCH levels than glial cells, but neuron-specific genes are repressed and methylated at the level of CH in glial cells [21]. As regards hydroxymethylation, recent studies suggest that 5hmC is not a simple intermediate product in the oxidative cytosine demethylation pathway as it was initially thought, but it is involved in keeping gene promoters ready for gene activation, preventing their DNA methylation. In agreement with this, TET1 overexpression resulted in impaired contextual fear conditioning during memory formation [22], while TET1 knockout results in defects in memory extinction and synaptic plasticity [23].

Heyward and Sweatt [51] proposed a very appealing model according to which in basal state conditions memory promoting genes are methylated and kept silenced while memory suppressor genes are basally expressed. Upon learning, both TET proteins and DNMTs are induced, the former derepressing memory promoting genes and the latter silencing memory suppressor genes. After sufficient time, the basal state is restored probably through TET-mediated derepression of memory suppressor genes and DNMT remethylation of memory promoting genes. However, what mechanisms give rise to the basal state differences in gene promoter methylation is still not known.

But how can this transient mark lead to memory storage, where memories can last a lifetime? There should be a self-perpetuating mechanism. Many studies on DNA methylation investigated the hippocampal role in memory formation and consolidation but not the further consolidation of this information in remote memory. According to a well established model, bursts of activity called "sharp-waves" would promote cortical plasticity, transferring memories from the hippocampus to the neocortex [52]. Heyward and Sweatt speculate that these waves would result in the epigenetic storing of the learning event in cortical cells, probably through double strand DNA methylation, which would be highly resistant to erasure thanks to the self-perpetuating action of DNMT1, which recognizes the hemimethylated helix and methylates the unmethylated strand [51]. Supporting the role of DNA methylation in maintaining memories, the CaN (calcineurin) gene showed delayed (1 day) and persistent (>30 days) DNA methylation in cortical neurons upon contextual fear memory even after protein levels returned to baseline, during the process of transition of contextual fear memory from "transient" (hippocampus) to "remote" (prefrontal cortex) [24].

1.1.2. Histone Modifications. Histones are the main protein component of the chromatin and come in 4 flavours: H2A, H2B, H3, and H4. These basic proteins strongly associate with the DNA forming an octamer called nucleosome, along which 147 bp of the DNA helix wrap around. Additional compaction is performed by the H1 linker histone, which binds the nucleosome at its entry and exit site. Importantly, long protruding tails depart from each histone core and their posttranslational modifications (PTMs) regulate the level of chromatin compaction [53]. There are several PMTs acting on histone tails such as acetylation, methylation, phosphorylation, SUMOylation, and ADP-ribosylation. However, we only need a minimal set of epigenomic features to define chromatin states and most studies focus on specific and recurrent histone modifications [54].

Histone acetylation has a positive effect on transcription by relaxing the chromatin compaction. The acetyl group neutralizes the positive charges on Lysine (K) and Arginine (R) residues, decreasing the electrostatic interactions between the nucleosome and the DNA. The writers of this epigenetic modification are called histone acetyl transferases (HATs), while the erasers are called histone deacetylases (HDACs) [27].

Histone acetylation has emerged as a key mechanism of memory regulation. One of the first studies showed how novel tastes induce long-lasting Lysine acetylation through ERK/MAP pathway activation in the insular cortex [55]; the same was true for contextual fear conditioning during memory formation [56]. Several subsequent studies showed that global HDACs inhibitors (HDADi) improve cognitive impairments and boost learning and memory [27]. Acetylation occurs in several K residues such as H3K9/14/27 and H4K12 but also in H2B and many other sites. According to the current view, these modifications play an important role in establishing a permissive transcription, preparing cells to activate gene expression upon specific stimuli [57]. Even though it was initially thought that HDAC inhibitors enhanced gene expression globally and nonspecifically, it is now clear that specific molecules, such as the CREB transcription factor, regulate their action. CREB recruits the coactivator CBP that through its HAT domain increases acetylation at the level of the genes involved in memory consolidation [28].

Several HDAC isoforms can regulate histone acetylation levels in the adult brain. For instance, while HDAC5 is important in the nucleus accumbens, the reward centre of the brain and its disruption result in a hypersensitive response to chronic drug abuse [30]; HDAC2 was found to negatively deregulate memory formation and synaptic plasticity [58], and HDAC3 inhibition enhanced long-term object memory formation [29]. While, generally speaking, the effect of HDAC inhibition is positive for cognitive activities, this is not the case for the sirtuin family of HDACs, where SIRT1 obliteration impairs hippocampal memory formation, a defect that can be explained by decreased dendritic branching and spines, which are specialized structure for cognition [59]. Subsequent studies showed also how HDCA1 is required for fear extinction learning through a mechanism involving H3K9 deacetylation [31] and HDAC4 is required for synaptic plasticity and memory formation [60].

Histone acetylation often correlates with histone phosphorylation. For example, H3 phosphorylation at serine (S) 10 (H3S10P) together with acetylation of H3K9 is induced during spatial memory formation and facilitates the early gene activation (c-Fos, Erg1, and Arc) of the ERK/MAPK pathway [61].

The second most studied histone modification is methylation. While histone acetylation always results in transcriptional activation, histone methylation effects depend on the protein complexes docking on the different modifications. For example, H3K4 methylation and monomethylation of H3K9 (H3K9me1) result in transcriptional activation, whereas H3K9me2 and H3K9me3 result in transcriptional silencing. Histone methylation can occur at either Lysine (K) or Arginine (R) and is performed by a group of proteins containing SET domains called histone methyl transferases (HMTs). Despite being conceived initially as a static histone modification, whose half-life coincides with the histone turn over itself, histone methylation has shown to be dynamically regulated through the action of histone demethylases (HDMs) such as LSD1 for H3K4me and H3K4me2 and JMJD1a for H3K9me and H3K9me2 [62].

H3K4me3 is usually present in the proximity of the transcription start site of active genes and it has been shown to be induced one hour after contextual fear conditioning, activating promoter regions of memory genes such as ZIF268 and BDNF, to return to baseline levels at 24 hours, underlining a role in memory formation. A similar dynamic was observed for the transcriptional repressive H3K9me2 mark. Interestingly, mice deficient in *Mll*, a H3K4 methyltransferase, show defects in contextual fear memory formation [34]. In parallel, GLP/G9a, an H3K9me2 methyltransferase, is extremely important for cognition. H3K9me2 is a "switching chromatin signal" [63], acting during both development and cognition and modulating gene expression by recruiting reader, writer, and eraser enzymes. This complex is required during memory consolidation both in the hippocampus and in the entorhinal cortex [35]. Moreover, H3K9me2 is induced from 1 hour up to 25 hours upon fear conditioning, and fear memory is enhanced when inhibiting both its demethylation (LSD1-mediated) and its methylation (GLP/G9a-mediated) [64]. Finally, GLP/G9a is also important in adaptive behaviour since its deficiency leads to defects in learning, motivation, and environmental adaptation [37].

Although less studied, several other histone methylation marks play an important role during cognitive processes. For example, H3K36me3, marking the $3'$ end of transcribed genes, is immediately induced during object recognition memory in both the hippocampus and prefrontal cortex and is reactivated after activation of recent (24 h) and remote (7 days) memory, with hypermethylation of the ZIF268 promoter [36].

Future research should focus on integrative analysis showing how those marks crosstalk and what is the precise dynamic of their activation.

1.1.3. Chromatin Remodelling. Nucleosome remodelling complexes (NRCs) alter nucleosome positioning in an ATP-dependent way, promoting nucleosome sliding, eviction, or histone variants exchange. In the brain the most studied NRC is the neuron-specific Brg1/hBrm Associated Factor (nBAF) complex, a multiprotein complex belonging to the SWI/SNF family that regulates gene expression in both development and adult cognition. Of particular importance in neurodevelopment is the upregulation of the BAF45b and BAF45c subunits and the switch between the BAF53a and BAF53b, which begins at E12.5 and is exclusive to postmitotic neurons, being essential for BRG1's ATPase activity [65]. This complex has shown to be important in cognition since BAF53b deficient mice showed large impairments in long-term memory formation [46].

1.1.4. Noncoding RNAs (ncRNAs). Noncoding RNAs (ncRNAs) are transcripts that are not translated into a protein. They include two broad categories: small RNAs and long noncoding RNAs (lncRNAs). The first comprehends microRNAs (miRNAs) that generally inhibit gene expression by complementarity to their targets and PIWI interacting RNAs (piRNAs), involved in transposon repression through RNA mediated DNA methylation. The function of long noncoding RNAs is less known; while initially thought to be "transcriptional noise," recent studies suggest that lncRNAs can regulate gene expression by acting as "guide" or scaffold RNAs, targeting epigenetic changes to specific genomic locations. Many noncoding RNAs have been identified in the brain, and approximately 40% of them are not found in other tissues [66]. While for most of the lncRNA the mechanism remains elusive, extensive evidences suggest they have important roles in neural development, synaptogenesis, and synaptic plasticity [67]. Notably, TUNA, RMST, and DALI regulate neural differentiation by directing transcription factors, chromatin-remodelling machineries, and DNMTs to important genomic loci [41, 68, 69].

The complex picture of epigenetic regulation in the brain can be puzzling, with some epigenetic changes enhancing cognition and other impairing neural activities. However the take-home message is that every kind of epigenetic change has been found associated with neural activity, indicating that a correct balance of the epigenetic machinery is needed for a proper neural function. Moreover, epigenetic changes should not be seen as distinct and isolated events. Repressive modifications tend to occur together and the same is true for permissive modifications. As an example, several methyl binding proteins recruit HDACs allowing cytosine methylation and histone deacetylation to act in concert to repress gene transcription [70]. That means that epigenetic mechanisms orchestrate the specific gene expression activated upon brain activity.

2. Epigenetic Dysregulation in Intellectual Disabilities

Many intellectual disability disorders arise from mutations affecting the function of the epigenetic regulators discussed in Section 1, underlining the importance of a correct balance between readers and erasers of epigenetic modification for a proper brain function (Table 1).

Besides the epigenetic syndromes arising by direct perturbation in the functions of key epigenetic molecules, several, if not all, other syndromes and IDDs have probably an epigenetic component or origin. Epigenetics means dynamics and reversibility, and thus a lack of epigenetic coordination may lead to defects in neurodevelopment with consequent defects in cognition. In this context it is obvious that an

Table 1: Epigenetic mechanisms in cognition and IDDs.

Epigenetic changes	Writers	Erasers	Readers	Effect on gene expression	Effects on cognition	IDDs involved
5mC	DNMTs	TETs AID/Apobec	MBPs	Repression	Memory formation [17, 18] Memory consolidation [24]	Rett Syndrome [25]
mCH	DNMT3a	TETs AID/Apobec	MBPs (e.g., MeCP2)	Repression		Rett Syndrome [26]
5hmC	TETs			Activation	Memory formation [22] Memory extinction [23] LTD [23]	
Histone acetylation	HATs	HDACs (HDAC1–5, SIRT1)	CPB BRDs	Activation	Memory formation [27] Memory consolidation [28, 29] Depression [30] Fear extinction [31]	Rubinstein-Taybi syndrome [32, 33]
Histone methylation	HMTs (MLL, GLP/G9a)	HDMs (LSD1, JMJD1a)	Chromodomains (e.g., HP1)	Activation/repression	Memory formation [34] Memory consolidation [35] Adaptive behaviour [36]	Kleefstra syndrome [37, 38] Sotos syndrome [39] Wolf-Hirschhorn syndrome [40] Kabuki syndrome [41, 42] Weaver syndrome 2 [43] Claes-Jensen-type syndromic X-linked ID [44] Siderius X-linked ID [45]
Chromatin remodelling			nBAF, ATRX	Activation/repression	Long-term memory formation [46]	Coffin-Siris Syndrome [47] Nicolaides-Baraitser syndrome [48] ATRX syndrome [49] ASD [50]

early therapeutic intervention is preferential, but given the reversibility of epigenetic processes, it is in theory possible to restore a proper neuronal function ameliorating the cognitive impairment in this developmental IDDs. To what extent this is feasible, together with the efficacy and duration of the effect for this approach, remains to be elucidated. The best therapeutic strategies will likely consist in combinatorial therapies using both neuromodulators and "epidrugs" as cognitive enhancers.

In the end of the section we will specifically focus on two developmental genetic disorders, DS and FXS, showing how both development and cognition are interconnected and how epigenetic regulation is essential in both processes as a gateway for processing inputs received from the environment.

2.1. Cognitive Function, Synaptic Plasticity, and Epigenetics. Epigenetic effectors involved in intellectual disability developmental disorders are likely interacting with fundamental players in neuronal maturation. For instance, nBAF complexes regulate genes essential for dendritic outgrowth and spine formation [71], and the activity of GLP/G9A, MeCP2, and ncRNAs affects the regulation of BDNF expression whose role in neuritogenesis, synaptogenesis, spine maturation, and axonal arborisation has been thoroughly assessed and is reviewed elsewhere [72, 73]. BDNF is of specific importance for initiating guided branching in the cell membrane [74] and for completing spine maturation [75].

Converging evidence indicates that epigenetic control of gene expression is also pivotal to learning and memory through its crosstalk with neuronal activity and synaptic plasticity mechanisms, as underscored also by the range of intellectual disabilities and behavioural deficits increasingly traced to a staggering number of epigenetic modulators. Specifically, several epigenetic modifications act as key signalling relay in the integration of synaptic inputs, as vividly shown for histone acetylation in CREB-dependent changes triggered during NMDA-receptor mediated long-term potentiation (LTP) [76], but also more recently for the rapid surges of DNA methylation and demethylation and 5-hydroxymethylation in response to neuronal activity [21]. Some epigenetic marks, including DNA methylation and histone methylation on Lysine 9 and Lysine 27 of histone H3, can be stably propagated over extended periods of time, in proliferating and postmitotic cells, implying alternative neuronal activity-dependent plasticity mechanisms putatively involved in learning and memory. Through recruitment mechanisms still poorly understood, epigenetic modifiers can exert genome-wide but also highly gene-specific effects. These features have made epigenetics the focal point of the grounding in molecular terms of how Hebbian (i.e., synapse-specific) and non-Hebbian (i.e., neuron-wide) mechanisms of LTP integrate information processing [77]. Indeed, one of the most thought-provoking hypotheses recently put forward is that genome-wide epigenetic changes may bias neurons towards cell-wide thresholds or set points, consequently modifying their susceptibility to Hebbian plasticity mechanisms and finally orchestrating a neuron's global response to the variety of molecular events involved in synaptic plasticity, suggesting a role in plasticity regulation and homeostasis [8]. The challenge is to functionally validate the relevance of specific epigenetic axes in learning and memory.

The amount of information obtained from cellular and molecular neuroscience of cognitive processes is overwhelming. However, the connection between this information and mechanistic conclusions at the cognitive level relies on important assumptions and generalizations. For example, while several molecular events in neurons signal plasticity mechanisms, the link between these mechanisms and the formation and loss of memories is only correlational. Even so, to verify whether the studied mechanisms are involved in cognition we still use behavioural tests in normal and diseased animal models. Thus, critical aspects for the assessment of rodent models of IDDs and the study of the underlying molecular mechanisms are face and predictive validity of the tests [78, 79]. In relation to DS and FXS, some tests on the best characterized mouse models (Ts65Dn [80] and Fmr1 KO [81]) stand out as widely accepted and relevant. In relation to long-term memory acquisition, consolidation, and retrieval, impaired in both syndromes, fear conditioning tests [82] and the Morris water maze [83] in combination with pharmacological interventions have shown face and predictive validity for both syndromes [84, 85]. However, construct validity and differences between the underlying biological causes of the syndromes are not well understood. To this end, more disease specific tests that provide experimental tools for preclinical therapeutic studies are required. In this regard, the development of behavioural paradigms, such as the touch-screen based tasks, can reproduce in mice the paradigms of The Cambridge Neuropsychological Test Automated Battery *(CANTAB)*, originally developed at the University of Cambridge in the 1980s [86]. Deepening on construct validity by the accurate assessment of cognitive domains provided by CANTAB-based touch-screen tasks in mice will allow not only improving the treatments of the syndromes, but also better understanding the biological substrates of cognition.

2.2. IDDs by Direct Mutation of Epigenetic Genes. In the last decade, the discovery of mutations in the various components of the epigenetic machinery (writers, erasers, readers, and remodellers) has been linked to a number of well-known causes of IDDs [43, 87]. Intellectual disability is generally defined as deficits of intellectual function and adaptive behaviour that occur during the developmental period (see, e.g., http://aaidd.org/) and epigenetic disturbances are expected to have widespread downstream consequences (Figure 2). Rett Syndrome (RTT) is one of the most studied of such disorders, an X-linked dominant neurodevelopmental disorders, arising from mutations in a DNA methylation reader: the methyl-DNA-binding protein MeCP2. RTT patients show, next to morphological defects, a progressive cognitive impairment, autistic behaviour, and language and social impairments probably due to dendritic and spine atrophy [25]. MeCP2 normally results in transcriptional repression due to binding to methylated CpG (mCG) or CpA (mCA) dinucleotides, followed by HDAC recruitment [88]. However, MeCP2 can also result in transcriptional activation when binding to the promoters of some genes in association

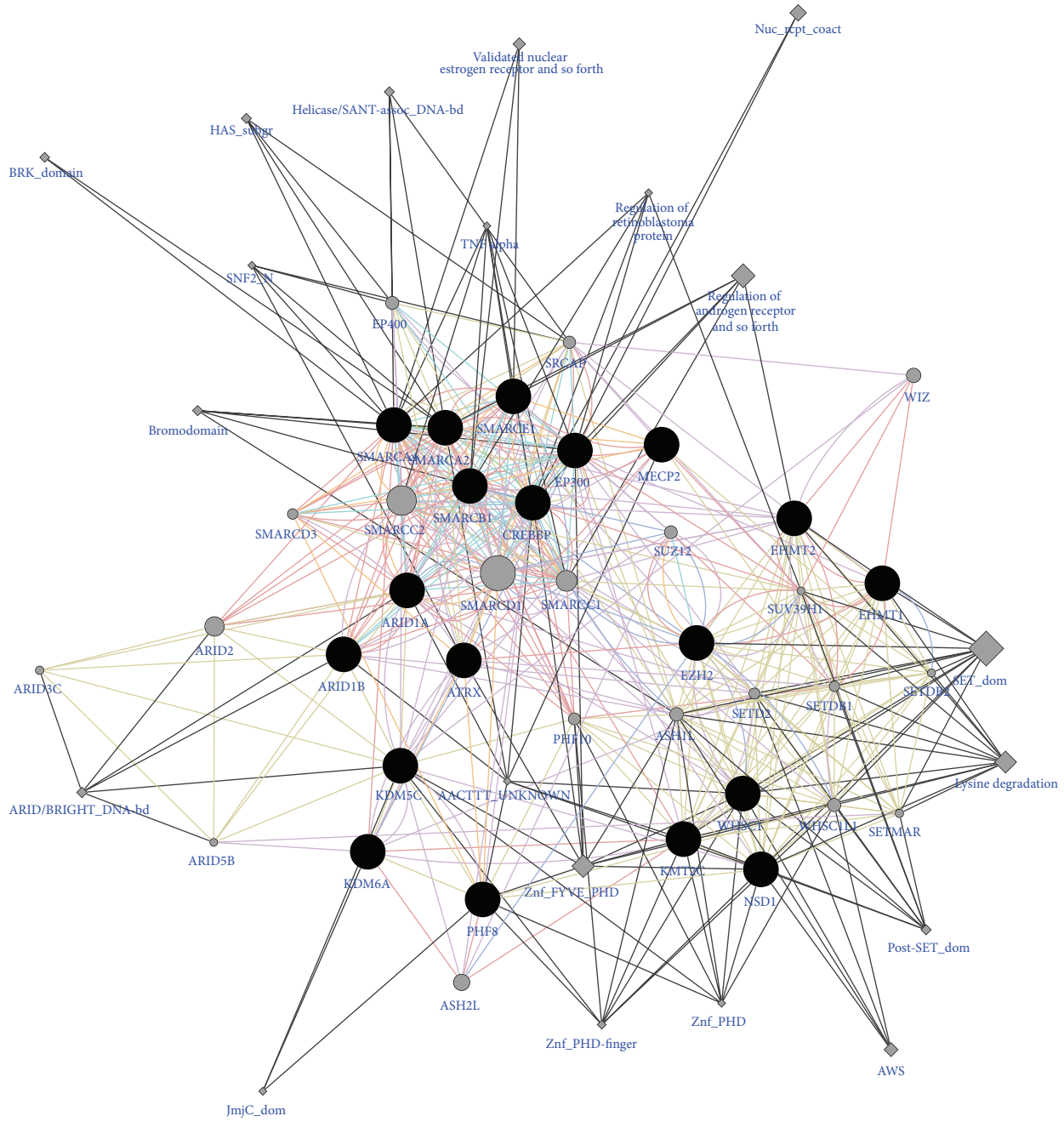


Figure 2: Protein-protein interactions of key epigenetic genes involved in genetic IDDs. Network of interactions of genes involved in the genetic IDDs arising from mutations in the epigenetic machinery (black nodes). Nodes sizes are related to the degree of the nodes (connectivity). Edge colours indicate coexpression (violet), colocalization (blue), genetic interactions (green), pathway (cyan), physical interaction (pink), predicted (orange), and shared protein domain (beige).

with the transcriptional activator CREB1 [89]. For instance, MeCP2 regulates the activity-dependent gene BDNF, keeping it switched off in absence of neuronal activity. Upon brain activity, MeCP2 gets phosphorylated and is released from BDNF promoter, enabling its expression [90]. Interestingly, it has been shown that longer genes have specific functions in the nervous system and tend to have a higher density of mCA. As a consequence, these genes are the most upregulated by MeCP2 knockout.

Another IDD directly arising from mutations in epigenetic players is Rubinstein-Taybi syndrome (RTS). Most of the RTS patients have mutations in the gene encoding for the cyclic AMP-responsive element binding protein (CREB) binding protein (CBP) [32], while in a minority of cases the mutations are in the gene encoding for p300 [33]. CBP and p300 are transcriptional coactivators with HAT activity, involved in development and cognition [91]. Interestingly, mouse model of RTS (CBP +/− mice) shows defects in

synaptic plasticity due to impaired late phase long-term potentiation, with consequent defects in long-term memory. At the epigenetic level these mice show decreased histone acetylation that can be reversed by HDAC inhibition ameliorating the phenotype [92].

Several HMTs have been associated with congenital IDDs. A deletion containing the GLP/EHMT1 gene (euchromatin histone methyltransferase 1) causes Kleefstra syndrome, a developmental severe IDD, with defects in learning, motivation, and environmental adaptation. GLP/G9a is essential for regulating H3K9 dimethylation levels and regulates brain function through maintenance of the transcriptional homeostasis in adult neurons [37]. In minor cases Kleefstra syndrome is due to a de novo point mutation in the MLL3 gene, encoding for a H4K4 HMT [38]. Impaired H3K36 methylation is observed in two learning disabilities: Sotos Syndrome, due to NSD1 deletion [39], and Wolf-Hirschhorn syndrome, due to NSD2 deletion [40]. Mutation for the MLL2 gene, with reduced H3K4 methylation, is responsible for the Kabuki syndrome 1, with impaired hippocampus-dependent memory and developmental disorders [41], while de novo mutation at the EZH2 gene results in Weaver Syndrome 2, with impaired H3K27 methylation and consequent defects in neural differentiation [43]. Similarly, mutations in histone demethylases result in IDDs. Impaired H3K4me2/3 demethylation due to mutation in the KDM5C gene causes an autistic disorder called Claes-Jensen-type syndromic X-linked ID, with impaired brain development and plasticity [44]. Finally, mutations in the gene encoding the H3K9 demethylase PHF8 account for Siderius X-linked ID syndrome [44], while mutation in the gene KDM6A, H3K9 demethylases, gives Kabuki syndrome 2, with very similar clinical picture to Kabuki syndrome 1 [42].

Several mutations have been described in subunits of the nuclear remodelling complex nBAF, which are linked to IDDs and autism spectrum disorders (ASDs) [50]. The most affected genes belong to the SMARC and ARID families, the first having helicase and ATPase activity, the latter conferring DNA recognition binding sites. Examples of these IDDs are Coffin-Siris Syndrome (CSS) [47] and the Nicolaides-Baraitser syndrome (NBS) [48]. Another example of IDDs arising from mutation in chromatin remodelling components is the X-linked form of syndromic mental retardation associated with alpha thalassemia (ATRX syndrome), caused by point mutations in the ATRX gene, SWI/SNF chromatin remodelling containing an ATPase/helicase domain. These mutations have been shown to cause diverse changes in the pattern of DNA methylation, which may provide a link between chromatin remodelling, DNA methylation, and gene expression in developmental processes [49].

Since affecting epigenetic mechanisms, these mutations would lead theoretically to the deregulation of a very broad and nonspecific set of genes; however they surprisingly give rise to well defined syndromes, suggesting that they conversely lead to specific dysregulation of key genes. However, all these IDDs share common clinical features, indicating that they share common molecular pathways, deregulated upon epigenetic imbalance, which could be targeted therapeutically.

Note that even though these IDDs arise from mutations/deletions in specific components of the epigenetic machinery, the common molecular phenotype is a global epigenetic imbalance, affecting several epigenetic mechanisms. Histone modification and/or DNA modifications always occur in concert, with nuclear remodelling complexes bringing various epigenetic players at the regulatory regions of the genome. Moreover, several other disorders, even if not arising from direct impairment of the epigenetic machinery, show a strong epigenetic component, such as foetal alcohol spectrum disorders, neurodegenerative diseases (Alzheimer Disease, dementia, and Parkinson disease), poly-Q disorders (Huntington disease, spinal and bulbar muscular atrophy, and spinocerebellar ataxia type 3), autism spectrum disorders (ASDs), addiction, schizophrenia, stress, and Friedrich ataxia [91, 93, 94]. This suggests that epigenetics plays an important role in all neurological disorders characterized by defects in neurodevelopment and/or cognition. The establishment of a proper epigenetic balance could be the key in the treatment of these disorders.

2.3. Down Syndrome: A Global Epigenetic Perturbation. Down Syndrome (DS) is the most common genetic intellectual disability arising from the total of partial trisomy of chromosome 21, leading to a developmental disorder characterized by various defects, including impairments in language, memory, learning, and a higher frequency of developing Alzheimer Disease (AD) [1]. While DS would theoretically lead to 1.5-fold upregulation of all HSA21 genes, transcriptomic studies revealed that genes were differentially expressed on all chromosomes forming the so-called gene expression dysregulation domains (GEDDs), pattern of chromosome regions showing up- or downregulation of transcription in the trisomic cell along the whole genome. Interestingly, in DS actively transcribed regions are less expressed, while lowly transcribed regions are more expressed, leading to "flattening" of gene expression profiles. Further analysis of these data (GSE55504 [95]) has shown that even though the trisomic chromosome shows the highest fraction of deregulated genes, DS genes are distributed among all chromosomes (Figure 3(a), Ilario De Toma).

Epigenetic deregulation due to triplicated genes could explain the genome-wide change of gene expression, as chromosome 21 contains genes regulating all epigenetic aspects discussed in Section 1, and their overexpression due to the trisomy would easily affect the epigenetic balance, as will be reviewed in the following paragraphs.

2.3.1. DNA Chemical Modifications in DS. DNMT3L is encoded on chromosome 21 and stimulates the activity of DNMT3a and DNMT3b [96]. Several studies have shown a deregulation in DNA methylation patterns in DS individuals, with a genome-wide hypermethylation [97–99], probably due to DNMT3L overexpression [100]. Some of the differentially methylated genes actually correlated with the cognitive impairment level in DS patients, such as TSC2, which has also been associated with the tau pathology in Alzheimer Disease [99]. The same widespread DNA hypermethylation was observed also in DS placenta, underlining

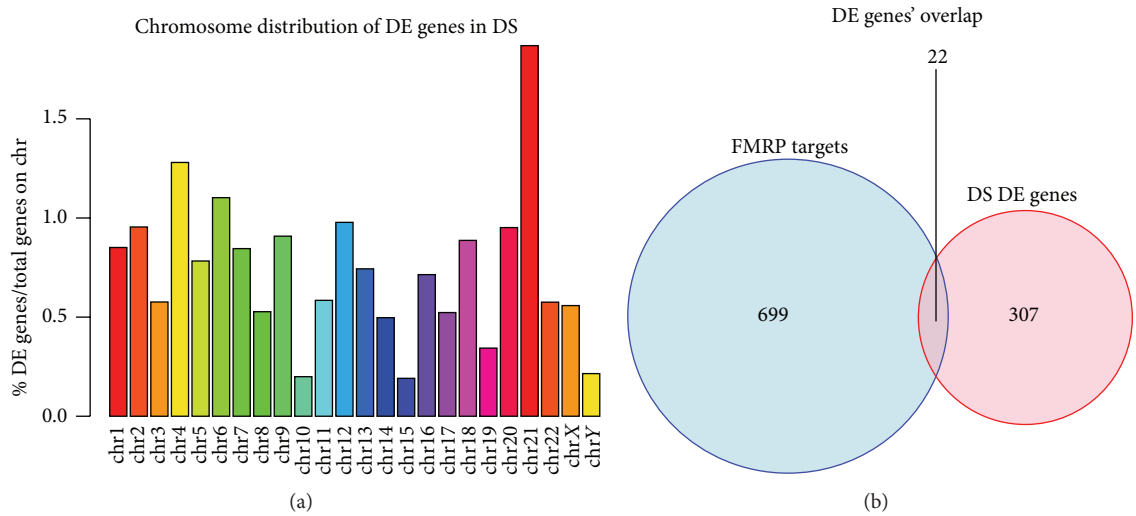


FIGURE 3: Differentially expressed (DE) genes in Down Syndrome (DS). (a) Bar plot showing the percentage of differentially expressed (DE) genes in DS over the total genes of each chromosome. (b) Venn Diagram showing the overlap among FMRP targets and a list of genes consistently expressed differentially in DS.

the importance of epigenetic balance already at the foetal stage [101]. Conversely to the genomic hypermethylation, mitochondrial hypomethylation was seen in DS, probably due to reduced levels of the methyl donor SAM, leading to mitochondrial dysfunction [102]. Interestingly, mitochondrial dysfunction might affect histone modifications since the mitochondria are the source of high energy intermediates which are necessary for histone acetylation, deacetylation, methylation, and phosphorylation [103]. Finally, TET proteins are downregulated in DS by DNA methylation of the promoter of the genes from which they are encoded [97], resulting in a decrease in 5hmC levels and genomic hypermethylation [101].

2.3.2. Histone Modifications and Chromatin Remodelling in DS. Many HSA21 genes influence specific histone modifications. An example is the phosphotyrosine kinase DYRK1A, which is able to regulate several proteins involved in epigenetic mechanisms. It promotes both histone deacetylation by phosphorylating SIRT1 [104] and histone acetylation by phosphorylating the CREB transcription factor, resulting in its binding with the HAT CBP [105]. DYRK1A also interferes with chromatin remodelling by binding nBAF and reducing the levels of the NRSF/REST neuron-restrictive silencing factor which is essential for neural differentiation [106]. Other HSA21 genes regulate the same key proteins: ETS2 [107] and the constitutive chromatin protein HMGN1 [108] influence the activity of CBP enhancing the H3K14 activity, while the activity of nBAF is modulated also by BRWD1 [109], a bromodomain containing protein recruiting nBAF to acetylated histones, and RUNX1 [110], which forms complexes that are associated with the active mark H3K4me3 and H4 acetylation. Moreover, HMGN1 not only interferes with histone acetylation, but inhibits phosphorylation of H3S10 and H3S28 [108] and inhibits the methyl binding protein MeCP2 by modifying the chromatin structure at the level of its promoter [111]. Finally, HSA21 encodes for two histone pseudogenes (H2AFZP and H2BFS) whose roles have not been elucidated yet, and the chromatin assembly factor 1B (CHAF1B) that forms a complex with the methyl-CpG binding protein MBD1 and the heterochromatin protein HP1 to favour chromatin repression through 5mC and H3K9me3 [112].

2.3.3. ncRNAs in DS. HSA21 encodes for five miRNAs: mir-99a, mir125b2, mir155, mir802, and let-7c. Interestingly, mir155 and mir802 downregulate the methyl binding protein MeCP2 [113]. Furthermore, mir155 is also involved in synaptic dysfunction since it results in the downregulation of SNX27, a key component in the endosomal pathway that assures the glutamate receptor recycling [114]. Of note, mir125b levels increase also in AD brains [115]. Thus, besides the well-known role of APP overexpression, epigenetics could directly link AD and DS since a disturbance of epigenetic balance has also been observed in AD [116], partly explaining the higher frequency of early-onset AD in DS patients [1]. As regards long noncoding RNAs they constitute almost 35% of HSA21 annotated genes (GRCh38 assembly), making HSA21 the second chromosome with the highest percentage of long noncoding RNA after HSA18 (Ilario De Toma, personal communication). Future studies are needed to elucidate the role in epigenetics and cognition of these long noncoding RNAs [117].

Summing up, even though DS is caused by a precise genetic defect (trisomy 21), epigenetic mechanisms are globally dysregulated through various mechanisms. One of the main problems for obtaining a complete picture of epigenetic contributions in DS is that different studies performed analyses on different cell types and tissues, in different developmental stages (embryonic fibroblasts, neurons, blood cells, etc.). Since epigenetics is involved in differentiation and cell fate, this has led to different results that are often difficult to

compare, as the epigenetic differences related to development and cell differentiation could mask the differences due to the trisomy.

2.4. FXS: Not Only Local Epigenetic Perturbation. Fragile X Syndrome (FXS) is the most common monogenic cause of intellectual disability, where a "CGG" triplet expansion at the 5′-UTR of the FMR1 gene is responsible for the loss of the Fragile X mental retardation protein (FMRP), a synaptically expressed RNA-binding protein regulating translation [2]. FMRP acts on its RNA targets in various ways: it influences RNAs stability, preventing or sustaining mRNA decay [118]; it transports RNAs from the cell body to synapses [119]; and it inhibits mRNA translation both by stalling ribosomes on their target mRNAs [2] and by inhibiting translation initiation [120].

When the CGG repeat expands between 56 and 200 (permutation), the FMR1 gene is upregulated with increased histone acetylation in the promoter [121], while in full mutation patients (>200 repeats) the FMR1 locus is transcriptionally repressed through cytosine methylation directed towards the repeats and the nearby sites constituting the CpG island. This results in demethylation and deacetylation of H3K4, methylation of H3K9 and H4K20, and trimethylation of H3K27 [122], with final transcriptional repression of the whole region. The failure in the heterochromatinization of the FMR1 locus in subjects with over 200 repeats translates in the complete lack of penetrance of the syndrome. These healthy carriers are called unmethylated full mutation (UFM) carriers and have a normal epigenetic profile (with the exception of partial H3K9 methylation), with a 30–40% increase in FMRP levels (as in permutation carriers) [123].

Interestingly, DNA demethylation with demethylating agents such as 5-azadeoxycytidine (5-azadC) reactivates FMR1 transcription in full mutation patients, with restoration of euchromatic marks (H3K4 methylation and acetylation) and partial reduction of the repressive H3K9 methylation [124, 125]. Even though 5-azadC was enough to reactivate the FMR1 locus, costimulation with HDAC inhibitors revealed synergic effect, yet ineffective alone [124]. However, inhibiting specifically the class III HDAD SIRT1 is effective in reactivating the FMR1 locus with an increase in H3K9 and H4K16 acetylation, while leaving unaltered DNA methylation. Since DNA demethylation leads to acetylation of H4K16 but not H3K9, it could be that H3K9 deacetylation is an early event, which is followed by DNA methylation and H4K16 deacetylation [126]. Similarly to some other long noncoding RNAs, the FMR1 transcript plays a direct role in gene silencing by directing the recruitment of repressive complexes like PRC2 to the locus, with consequent histone H3K27 methylation. This could be important in the beginning of the process of FMR1 repression [122]. The mechanism is still not known but could involve R loops made by the FMR1 transcript, particular conformations due to the repeat expansion [127].

One of the most debated questions is if this epigenetic deregulation has a genome-wide effect or affects only the FMR1 locus. A recent work succeeded in detecting differential DNA methylation only at the FMR1 locus, which is wholly affected. However, the study does not discriminate DNA methylation and hydroxyl-methylation (which are often diametrically regulated) and used the HumanMethylation450 BeadChip kit, taking into account just a specific subset of the genome [128].

Indeed, a global deregulation is possible since a lot of FMRP target mRNAs are involved in chromatin remodelling such as *HDAC4/5*, *NCOR1–3*, and *CBP* [2] and several ncRNAs [129, 130], whose transcript and protein levels are presumably altered by FRMP absence. Moreover, in the complex FMR1 locus, several ncRNAs are encoded, but most of them have not been characterized yet. One of these ncRNAs is FMR4, which is switched off similarly to FMR1 in full-length expansions. This lncRNA regulates target genes at distal locations such as the methyl-CpG-binding domain protein 4 (MBD4), hampering neural differentiation in FXS [131]. Interestingly, FMRP target genes are enriched in long genes and significantly overlap with MeCP2-repressed genes. As we said these genes are enriched in mCA and are important for brain function [90]. Once again this is emblematic of the molecular pathway commonalities across IDDs involving epigenetic mechanisms (in this case FXS and RTT).

FMRP has been shown to be involved in dendritic mRNA localization, synaptic protein synthesis, and synaptic plasticity. The mechanism relies on mGluR signalling in glutamatergic postsynaptic sites. When mGluR channels are active in a synapse, a phosphorylation cascade is triggered that affects the LTP pathway and triggers rapid local protein synthesis of preexisting dendritic mRNAs, including FMRP, around the active synapse [132]. As a result of FMRP regulation, proper tuning of the translation dynamics involved in mGluR-dependent LTD is established in active synapses. Even though the mechanism of dendritic spine maturation is not fully elucidated, recent observations suggest that the proper pruning and maturation of synaptic spines (impaired in FXS) rely on the interplay between local dendritic BDNF mRNA translation and secretion, with FMRP playing a key role in the regulation of these local events [133]. How the inactivation of FMRP and its effects on local translation interact with actin polymerization, or proteins such as cofilin, myosin, Arp2/3, and profilin [134] is an open question.

2.5. DS and FXS, Differences and Similarities. DS and FXS show striking similarities and differences. Both intellectual disabilities are common genetic developmental disorders characterized by specific defects in structural and synaptic plasticity due to alterations in specific molecular pathways. However, those alterations are often opposite, with the common final outcome of cognitive impairment [135]. DS patients show reduced dendritic branching and complexity in pyramidal neurons along with fewer and abnormal spines with enlarged heads that could explain the cognitive deficits [5]. This goes along, at the molecular level, with alterations in synaptic plasticity molecular pathways: long-term potentiation (LTP), the ability of the neuron to strengthen its synapses, is suppressed in DS mouse models [136], while long-term depression (LTD), the ability to weaken unused synapses, is enhanced [137]. Conversely, in FXS patients the cognitive impairment goes along with an increased density of thin and elongated spines in the same neurons [138]. Looking

at the regulation of molecular pathways in FXS, while the role of LTP is controversial [139, 140], LTD is strongly induced, due to the overactivation of glutamate receptors [132].

Although presenting opposite phenotypes, DS and FXS share defects in dendritic spine morphology due to alterations in local protein synthesis. Both HSA21 RCAN1 and FMRP regulate calcineurin (CaN) activation, which is important for cofilin dephosphorylation. RCAN1 normally keeps calcineurin inactive; this increases phosphorylated cofilin that facilitates actin polymerization at the spine level. The enlarged spine heads observed in DS patient are probably due to RCAN1 overexpression. As a matter of fact, RCAN1-overexpressing mice show a phenotype similar to DS, with reduced volume and neuron number in the hippocampus, defective neurogenesis, enlarged spine heads, enhanced local protein synthesis of dendra, and impaired LTP [141, 142]. On the contrary in FXS patients, the silencing of the FMR1 locus results in the increase of the FMRP target PP2AC [143], phosphatase that dephosphorylates cofilin. This leads to the formation of long and thin filopodia-like spine heads, hallmark of FXS, due to defective actin polymerization. Calcineurin activates also local protein synthesis by dephosphorylating FMRP and allowing in this way the translation of the FMRP targets required for local protein synthesis and synaptic plasticity such as αCaMKII [142]. The ability of RCAN1 to bind and inhibit CaN is modulated also by the HSA21 gene DYRK1A, a serine threonine kinase important for synaptogenesis and spine actin dynamics [144]. This further links DS and FXS deregulation in the pathway of local protein synthesis.

Recent analyses from our group (Ilario De Toma, personal communication) show the link between DS deregulated molecular pathways and affected proteins in FXS by comparing 324 genes found to be consistently deregulated in DS in a published meta-analysis [145], with a list of FMRP targets [2]. The overlap was extremely significant ($p < 0.0005$, hypergeometric test) and included 9 HSA21 genes. Among those genes, *APP* is involved in Alzheimer Disease, which as we already stated has an early onset in DS patients [146, 147]; *SYNJ1/synaptojanin* regulates neurotransmission together with two other HSA21 genes, intersectin/DAP160 and RCAN1 [148], and is involved in learning and memory; *Tiam1* and *Ttc3* are involved in neurogenesis [149]; and NRIP1 is needed for cognition and recruits HDACs [150] (Figure 3(b)). Finally, even though not present in our list of genes consistently deregulated in DS, the HSA21 gene DSCAM is a FMRP target and is involved in neural development [151].

One interesting question that needs to be unravelled is whether the epigenetic deregulation is upstream of the deregulation of those molecular pathways. This would allow a common therapy for both disorders to rescue the epigenetic imbalance at the base of their aetiology.

3. Restoring a Balanced Epigenetic State for the Treatment of ID

Historically the treatment of DS and FXS has focused on restoring the neurotransmitter balance that is compromised in the two disorders or on replacing deficits in different systems. As mentioned before, in FXS there is global hyperexcitation due to overactivation of the glutamatergic pathway, while in DS there is an overinhibition due to the predominance of the GABA inhibitory pathway. Therefore in the attempt to restore the neurotransmitter balance, agonist and antagonist for both glutamate and GABA receptors have undergone clinical trials. However results have been unsuccessful by now, due to lack of efficacy and or safety [152, 153]. For instance, inhibiting the GABA pathway in DS may increase the susceptibility of DS patients to epileptic seizures, together with side effects in various developmental processes [154].

In the US, commercial formulations aimed at ameliorating the DS phenotype are composed mainly of antioxidant and folates. The rationale behind this is that DS patients overexpress two HSA21 encoded enzymes, SOD-1, leading to an increase in reactive oxygen species production, and cystathionine β-synthase, resulting in folate deficiency. However clinical trials showed that this approach is ineffective [155].

None of these traditional approaches have been revealed as safe and effective in the treatment of IDs. However, a possible future therapy based on the direct or indirect modulation of epigenetic mechanisms is promising. Restoring a balanced epigenetic state will be key to renormalize the altered expression in master regulator genes involved in the cognitive problems (Figure 4).

3.1. Environmental Enrichment: An "Epigenetic" Treatment. As we have previously pointed out in this review, the environment is a main driver of epigenetic modifications. During development, the microenvironment allows the genome to be interpreted differently by different cell types and in different developmental stages and contexts. The effect of the environment on gene expression is particularly evident in the case of monozygotic twins that are genetically identical but phenotypically and epigenetically different, especially when grown apart [156]. Environmental Enrichment (EE) is an effective protocol used in rodent models to boost learning and memory. The paradigm consists in keeping laboratory mice in a so-called enriched environment with respect to laboratory standards: larger cages, larger groups, various stimulatory objects such as toys of all sort, and running wheels. The aim is to provide the animals every kind of sensory, cognitive, and motor stimuli such as the possibility to establish more complex social interactions, to explore and play with new objects, and the opportunity for voluntary physical activity. Interestingly, EE improves learning and memory, enhancing long-term potentiation [157], and delays or rescues deficits in a variety of mouse models of neurological disorders [158]. Of note, EE is effective in both FXS and DS models. In *Fmr1* KO mice, EE rescued behavioural and neuronal abnormalities, activating the glutamatergic signalling and increasing dendritic branching, spine number, and maturation. Interestingly, EE acts independently of FMRP expression in Fmr1 KO mice, as it did not affect FMRP levels [159], but translates in reduced FMRP protein in mouse model of fragile X premutation [160]. Similarly, EE protocols increased dendritic branching and spines in DS models [161], probably

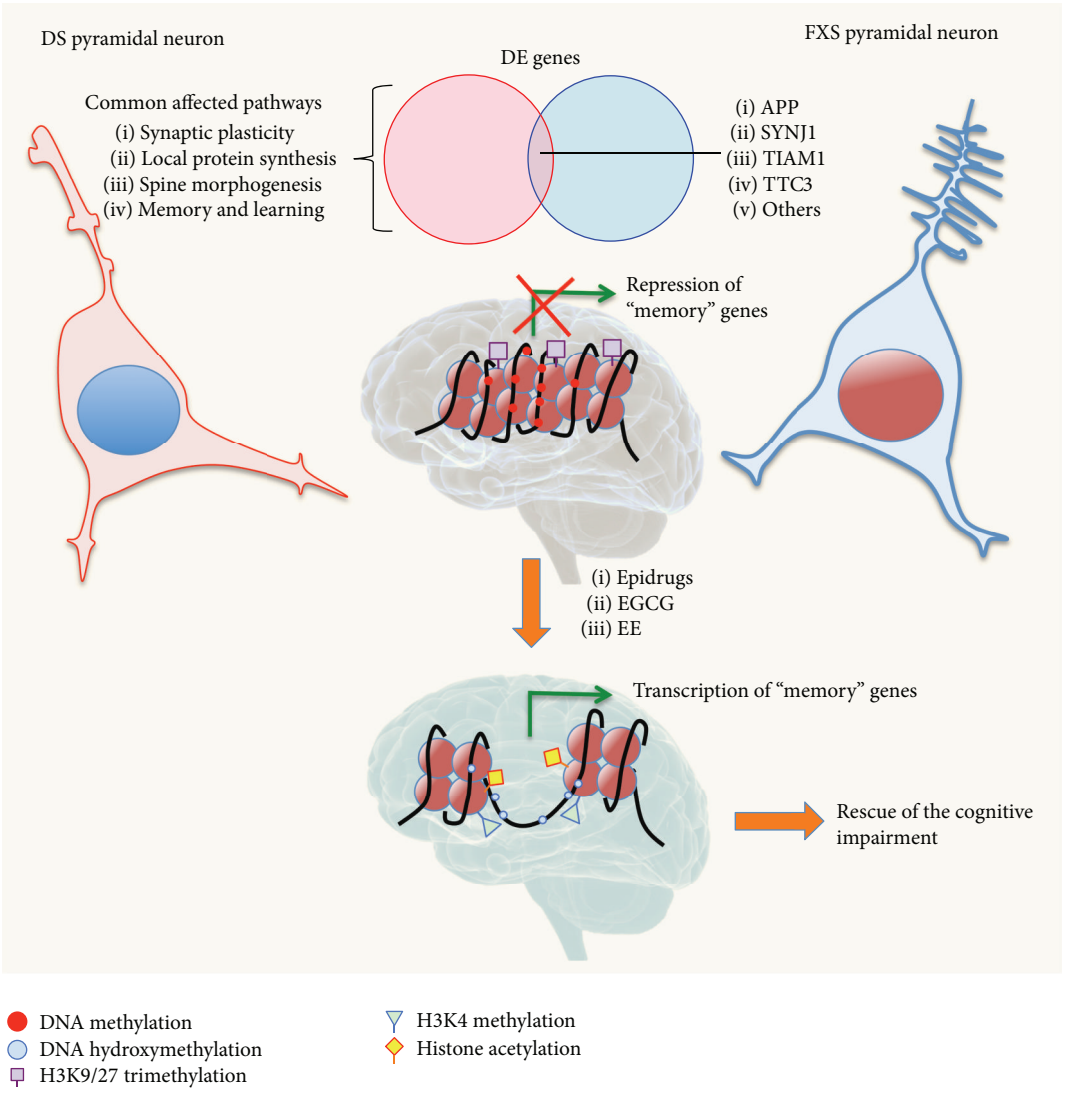


Figure 4: Reestablishing a balanced epigenetic state to rescue the cognitive impairment in Down Syndrome (DS) and Fragile X Syndrome (FXS). Cartoon representation of a DS (left) and FXS (right) pyramidal neuron. In both syndromes alterations in synaptic plasticity, local protein synthesis, spine morphogenesis, and memory and learning contribute to the cognitive impairment. However the structural phenotype is distinct: DS neurons have large and *stubby* spines, while FXS neurons have long immature filopodia-like spines. Key genes involved in these pathways (e.g., APP, SYNJ1, TIAM1, and TTC3) are commonly deregulated due to epigenetic modifications at the chromatin level. The cartoon shows the repression of "memory" genes by DNA methylation (red circles) and H3K9 and H3K27 trimethylation (violet squares) in DS and FXS, with consequent chromatin compaction. Conversely, epigallocatechin gallate (EGCG) and Environmental Enrichment (EE) or directly *epidrugs* can reactivate the chromatin state at the level of "memory" gene by DNA hydroxymethylation (blue circles), H3K4 methylation (green triangles), and histone acetylation (yellow squares), rescuing the cognitive deficits.

by normalizing DYRK1A levels [162]. Epigenetic mechanism could be involved in the effects of EE in IDDs, since EE-induced benefits are long-lasting (at least 3-4 weeks) and are supported by a specific EE-dependent transcriptional profile, which is likely activated through epigenetic mechanisms [163]. Four-week housing in EE conditions, while rescuing impaired memories in both contextual fear conditioning and water maze assays, was associated with enhanced histone acetylation on several residues. This effect was mimicked by daily injection of HDAC inhibitors in the murine peritoneum [164]. Another interesting experiment used mice deficient in CBP, a transcriptional coactivator with histone acetyltransferase activity. EE improved some defects in behaviour and cognition caused by CBP deficiency and promoted synaptic growth. However, its ability to enhance spatial navigation and pattern separation and to induce neurogenesis was severely compromised in absence of CBP, with attenuation of the transcriptional profile normally associated with EE due to decreased acetylation of the promoter regions of genes involved in cognition [165], suggesting that CBP contributes to EE ability to activate gene expression through histone acetylation.

Understanding the full epigenetic, genetic, and molecular mechanisms of Environmental Enrichment will guide the

development of a new class of therapeutics called "enviromimetics" for the treatment of IDDs. Enviromimetics are compounds aimed to mimic the beneficial effect of EE on cognition. An important unanswered question is how EE results in mouse models relate to human living experience, since most humans already do experience high levels of complexity and novelty in their natural environments. However, individuals vary widely for the kind and amount of mental exercise and physical activity performed. It will be extremely important in order to improve existing therapeutic approaches to closely reproduce in animal models the environmental factors relevant to human conditions [158]. Moreover research on EE paves the way for nonpharmacological treatment with promising outcomes in disorders such as DS and FXS if used in synergy with other cognitive enhancers.

3.2. Epigenetic Drugs in Intellectual Disabilities. It is now increasingly thought that approaches aimed at reestablishing a proper gene expression profile, especially of key genes impaired in cognitive disabilities, are the future of therapy [152, 153]. An efficient way to induce long-lasting transcriptional changes is to modulate epigenetic players, a field that is booming in cancer research [166]. Epigenetic changes are reversible and therefore are suitable to alleviate certain features in IDDs that originate from epigenetic alterations. Insights from cancer research could directly be conveyed to new "cognitive epigenetics." As a matter of fact, FDA has approved four epidrugs against cancer, two DNMT inhibitors (5-azacytidine and decitabine) and two HDAC inhibitors [167]. Moreover valproic acid, which has already been used against epilepsy and bipolar disorders, has shown HDAC inhibitory and anticarcinogenic activity, being the first epidrug approved for neurological disorders [168]. One of the concerns related to the use of epidrugs is their potential genome-wide and nonchromatin effect, since, for example, HDACs can also act on nonhistone proteins. Although these unwanted effects are less severe than one might expect, a technology currently in development called "epigenetic editing" will allow specifically targeting epigenetic drugs to the gene(s) of interest, thanks to the usage of DNA binding domains such as zinc finger proteins [169].

3.3. Epigallocatechin-3-gallate (EGCG): Panacea for IDDs? The flavonoid epigallocatechin-3-gallate (EGCG) is the most abundant polyphenol extracted from green tea. Strikingly, this molecule is effective in both mouse models of AD and DS. In AD mice, EGCG decreased beta-amyloid levels and plaques via ADAM10-mediated promotion of the alpha-secretase proteolytic pathway and modulates tau-profiles with final cognitive improvements [170]. Similarly, in DS models, EGCG recovered cognitive and neural plasticity phenotypes, a result that was replicated in a pilot clinical trial in humans [171]. Strikingly, a pilot clinical trial is undergoing for EGCG treatment of FXS individuals (https://clinicaltrials.gov/ct2/show/NCT01855971). EGCG has a plethora of different effects and has thus been investigated in studies from various research areas, including cancer research [172]. However, the heterogeneous effects make it difficult to fully identify and understand the underlying molecular therapeutic mechanisms. Among its properties, it has antioxidant and anti-inflammatory effects and is able to regulate several enzymes by modulating their kinase activity. Moreover, EGCG interferes at various levels with epigenetic mechanisms, affecting the chromatin state. EGCG inhibits both DNA methyltransferases [173] and class I histone deacetylases (HDAC 1, 2, 3, and 8) [174, 175]; it reduces the level of H327me3 and H2AK119 ubiquitination by reducing polycomb protein levels [176] and affects miRNAs expression [177]. The property of EGCG of modulating epigenetic changes makes it an ideal candidate for the treatment of IDDs including DS and FXS. Its widespread epigenetic effect might reestablish the lost epigenetic balance, acting in a context specific way and resulting in being effective in several IDDs, even if the source and kind of epigenetic dysregulation are different. Many properties of EGCG would contribute to its efficacy. For instance, besides its epigenetic effect, EGCG inhibition of DYRK1A kinase activity results in normalization of this gene which is crucial for DS pathology [178]. In addition, EGCG could also act by rescuing DS mitochondrial dysfunction as it stimulates mitochondrial biogenesis and rescues oxidative phosphorylation [179]. Remarkably, DYRK1A is also involved in epigenetic regulation (see Section 2), suggesting that EGCG could both directly and indirectly regulate the epigenetic state. This interconnection is even stronger if we consider that, similarly to EGCG effect, enriched environments rescue defects in DS, normalize DYRK1A levels, and modulate epigenetic modifications. EGCG can thus be considered an "enviromimetics." Of note, a recent study showed that the combination of EE and EGCG acts synergistically in ameliorating learning alterations and age-related cognitive decline in DS [180], underlining the potential of combinatorial therapeutic approaches.

4. Conclusion and Future Perspectives

Developmental disorders are often characterized by intellectual disability due to defects in structural and synaptic plasticity, with impaired activity-dependent cognitive-related molecular processes such as local protein synthesis, long-term potentiation, and long-term depression. In this context, it is difficult to discern what can still be rescued in cognitive developmental disorders and what is irreversibly lost. Epigenetics is not only indirectly needed for cognition by regulating neurodevelopment, but, as we amply discussed in this review, directly regulates experience-based cognitive processes. Epigenetics intercalates in development, cognition, and aging/neurodegeneration, playing a key regulatory role in all these processes. For instance, DNA methylation allows cells to be "programmed" and differentiate during development, is dynamically regulated during cognitive processes, and increases gradually with aging. In contrast to genetic alterations, epigenetic modifications are reversible and this gives a great therapeutic potential to epigenetic drugs to at least partially revert the phenotype associated with IDDs. We have reviewed how epigenetic treatments restore cognitive deficits in various models of cognitive impairment, restoring a correct balance among writers and erasers of epigenetic

modification. Of course an early treatment will maximize the efficacy of epidrugs, since the more differentiated a tissue, the less reversible the phenotypes.

Two main concerns are associated with epigenetic treatment: genome-wide nonspecific effects and toxicity. As regards the genome-wide effects, it would be worth conceiving ways to deliver epigenetic drugs such as DNMTi and HDACi to the cell types that actually show the epigenetic imbalance. Moreover, the epigenetic editing approach might be a promising solution, allowing directing the epigenetic drug to the loci of the key genes involved in the IDD. Noticeably, the genome-wide action of epigenetic drugs may also be an advantage, since it makes it possible to restore the epigenetic balance in disorders such as DS and FXS, where a similar cognitive impairment is originated by different morphologic phenotypes affecting common altered synaptic plasticity pathways. In this case the same molecule will work in a context specific manner on different loci, since the "substrate" of their action (the syndromic chromatin state) will be different in both cases, restoring the impaired epigenetic balance.

However, the epigenetic reversibility property is a double-edged sword. Since epigenetic changes are reversible, some epigenetic drug formulations are simply not long lasting. That would, for example, account for what has been found in the pilot clinical trial involving EGCG and the rescue of the cognitive impairment in DS patients, where stopping EGCG treatment leads to the reappearance of the impaired phenotype [171]. To overcome this problem the future of therapeutic treatment for cognitive disorders should focus in potentiating and extending the effect of epidrugs, while at the same time reducing the toxicity associated with chronic treatment. In this sense, combinatorial therapy could play an important role, having synergic effect as it has been shown in mouse models for EE and EGCG [180] or in the synergic effect of DNA demethylation and histone hyperacetylation in the reactivation of the FMR1 gene in human cell cultures [124]. Moreover this approach could be combined to conventional treatments, such as neuromodulators aimed at restoring the neurotransmitter balance in DS and FXS. Tackling the epigenetic deregulation from many sides, together with the targeting of specific molecular pathways, will allow both to reduce the dose and thus the toxicity of the drugs in the formulation and to extend their efficacy.

To this end a deeper understanding of all the epigenetic, transcriptional, and molecular cascades activating upon cognition in both physiological and pathological contexts is needed. Future integrative studies will combine epigenetic data, transcriptional data, and molecular data to get new insights into the pathogenesis of IDDs, focusing both on common altered pathways and to specific mechanisms. The development of new technologies and the increase in high-throughput data will allow in a near future elucidating the cognitive processes that are dysregulated in IDDs. Most has yet to come. For instance, as regards DNA methylation, one of the first epigenetic modifications that has been studied, it would be important to discriminate among 5mC and 5hmC, since both are highly present in the adult brain and have opposite outcomes on transcriptional regulation. A new technique called TET Assisted Bisulfite sequencing (TAB-seq) in conjunction with bisulfite sequencing, allows discerning between 5mC and 5hmC at single base resolution [181]; however very few datasets of this kind are available by now due to cost-related issues.

The main problem with previous studies is that they lack cell-specificity. For example, many studies on DS focus on different types of cells (e.g., blood cell, fibroblast) and different animal models, taking cells at different developmental stages, with results that are difficult to compare. Moreover, the brain is probably the most complicated organ, where several different cell types such as excitatory pyramidal cells, inhibitory interneurons, and glial cells compose even a reasonably delimited part, such as the hippocampus. Since epigenetic changes are responsible for cell fate, the epigenetic variations associated with cell type determination will sum up to the epigenetic changes associated with the syndromic state or to the cognitive processes, and this will dramatically reduce the power of the studies. Differences in cell type composition in compared samples of complex tissues will result in difficulties to distinguish treatment or disease specific changes from "epigenetic noise" caused by cell type specific marks varying based on cell type fractions. Therefore, cell type specific studies using techniques such as cell sorting to focus on single cell populations will have an increased power in detecting epigenetic differences and underpinning new key mechanism of regulation.

We speculate that epigenetic drugs, such as EGCG in combination with other cognitive enhancers and specific drugs interfering with the cell and disorder specific molecular targets, will allow the recovery of the epigenetic balance lost in IDDs such as DS and FXS, making the healing of the cognitive impairment possible.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

M. Dierssen and I. De Toma devised the structure and decided the figures. L. Manubens contributed to Sections 2.1 and 2.5. I. De Toma wrote the rest of the main text and prepared pictures. S. Ossowski and M. Dierssen revised the manuscript.

Acknowledgments

The research leading to the results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007–2013) under REA Grant Agreement no. 608959. The work of the lab of M. Dierssen presented in the text is funded by FRAXA Foundation, Fondation Jerome Lejeune, Ministerio de Economia y Competitividad (SAF2013-49129-C2-1-R and "Centro de Excelencia Severo Ochoa 2013–2017," SEV-2012-0208). The laboratory of M. Dierssen is supported by DIUE

de la Generalitat de Catalunya (Grups consolidats SGR 2014/1125).

References

[1] M. Dierssen, "Down syndrome: the brain in trisomic mode," *Nature Reviews Neuroscience*, vol. 13, no. 12, pp. 844–858, 2012.

[2] J. C. Darnell, S. J. Van Driesche, C. Zhang et al., "FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism," *Cell*, vol. 146, no. 2, pp. 247–261, 2011.

[3] C. H. Waddington, "Genetic assimilation of the bithorax phenotype," *Evolution*, vol. 10, no. 1, pp. 1–13, 1956.

[4] E. Benito and A. Barco, "The neuronal activity-driven transcriptome," *Molecular Neurobiology*, vol. 51, no. 3, pp. 1071–1088, 2015.

[5] M. Suetsugu and P. Mehraein, "Spine distribution along the apical dendrites of the pyramidal neurons in Down's syndrome. A quantitative golgi study," *Acta Neuropathologica*, vol. 50, no. 3, pp. 207–210, 1980.

[6] S. A. Irwin, R. Galvez, and W. T. Greenough, "Dendritic spine structural anomalies in fragile-X mental retardation syndrome," *Cerebral Cortex*, vol. 10, no. 10, pp. 1038–1044, 2000.

[7] I. J. Cajigas, T. Will, and E. M. Schuman, "Protein homeostasis and synaptic plasticity," *The EMBO Journal*, vol. 29, no. 16, pp. 2746–2752, 2010.

[8] J. D. Sweatt, "The emerging field of neuroepigenetics," *Neuron*, vol. 80, no. 3, pp. 624–632, 2013.

[9] I. Maze, L. Shen, B. Zhang et al., "Analytical tools and current challenges in the modern era of neuroepigenomics," *Nature Neuroscience*, vol. 17, no. 11, pp. 1476–1490, 2014.

[10] A. P. Bird, "DNA methylation and the frequency of CpG in animal DNA," *Nucleic Acids Research*, vol. 8, no. 7, pp. 1499–1504, 1980.

[11] A. Bird, M. Taggart, M. Frommer, O. J. Miller, and D. Macleod, "A fraction of the mouse genome that is derived from islands of nonmethylated, CpG-rich DNA," *Cell*, vol. 40, no. 1, pp. 91–99, 1985.

[12] X. Nan, R. R. Meehan, and A. Bird, "Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2," *Nucleic Acids Research*, vol. 21, no. 21, pp. 4886–4892, 1993.

[13] M. Okano, D. W. Bell, D. A. Haber, and E. Li, "DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development," *Cell*, vol. 99, no. 3, pp. 247–257, 1999.

[14] J. Feng, H. Chang, E. Li, and G. Fan, "Dynamic expression of de novo DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system," *Journal of Neuroscience Research*, vol. 79, no. 6, pp. 734–746, 2005.

[15] J. U. Guo, Y. Su, C. Zhong, G.-L. Ming, and H. Song, "Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain," *Cell*, vol. 145, no. 3, pp. 423–434, 2011.

[16] S. Ito, L. Shen, Q. Dai et al., "Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine," *Science*, vol. 333, no. 6047, pp. 1300–1303, 2011.

[17] C. A. Miller and J. D. Sweatt, "Covalent modification of DNA regulates memory formation," *Neuron*, vol. 53, no. 6, pp. 857–869, 2007.

[18] F. D. Lubin, T. L. Roth, and J. D. Sweatt, "Epigenetic regulation of bdnf gene transcription in the consolidation of fear memory," *The Journal of Neuroscience*, vol. 28, no. 42, pp. 10576–10586, 2008.

[19] K. E. Varley, J. Gertz, K. M. Bowling et al., "Dynamic DNA methylation across diverse human cell lines and tissues," *Genome Research*, vol. 23, no. 3, pp. 555–567, 2013.

[20] C.-X. Song, K. E. Szulwach, Y. Fu et al., "Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine," *Nature Biotechnology*, vol. 29, no. 1, pp. 68–75, 2011.

[21] R. Lister, E. A. Mukamel, J. R. Nery et al., "Global epigenomic reconfiguration during mammalian brain development," *Science*, vol. 341, no. 6146, Article ID 1237905, 2013.

[22] G. Kaas, C. Zhong, D. Eason et al., "TET1 controls CNS 5-methylcytosine hydroxylation, active DNA demethylation, gene transcription, and memory formation," *Neuron*, vol. 79, no. 6, pp. 1086–1093, 2013.

[23] A. Rudenko, M. Dawlaty, J. Seo et al., "Tet1 is critical for neuronal activity-regulated gene expression and memory extinction," *Neuron*, vol. 79, no. 6, pp. 1109–1122, 2013.

[24] C. A. Miller, C. F. Gavin, J. A. White et al., "Cortical DNA methylation maintains remote memory," *Nature Neuroscience*, vol. 13, no. 6, pp. 664–666, 2010.

[25] M. Chahrour and H. Y. Zoghbi, "The story of rett syndrome: from clinic to neurobiology," *Neuron*, vol. 56, no. 3, pp. 422–437, 2007.

[26] H. W. Gabel, B. Kinde, H. Stroud et al., "Disruption of DNA-methylation-dependent long gene repression in Rett syndrome," *Nature*, vol. 522, no. 7554, pp. 89–93, 2015.

[27] J. P. Lopez-Atalaya and A. Barco, "Can changes in histone acetylation contribute to memory formation?" *Trends in Genetics*, vol. 30, no. 12, pp. 529–539, 2014.

[28] E. Korzus, M. G. Rosenfeld, and M. Mayford, "CBP histone acetyltransferase activity is a critical component of memory consolidation," *Neuron*, vol. 42, no. 6, pp. 961–972, 2004.

[29] S. C. McQuown, R. M. Barrett, D. P. Matheos et al., "HDAC3 is a critical negative regulator of long-term memory formation," *Journal of Neuroscience*, vol. 31, no. 2, pp. 764–774, 2011.

[30] 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.

[31] S. Bahari-Javan, A. Maddalena, C. Kerimoglu et al., "HDAC1 regulates fear extinction in mice," *The Journal of Neuroscience*, vol. 32, no. 15, pp. 5062–5073, 2012.

[32] F. Petrif, R. H. Giles, H. G. Dauwerse et al., "Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP," *Nature*, vol. 376, no. 6538, pp. 348–351, 1995.

[33] J. H. Roelfsema, S. J. White, Y. Ariyürek et al., "Genetic heterogeneity in rubinstein-taybi syndrome: mutations in both the CBP and EP300 genes cause disease," *American Journal of Human Genetics*, vol. 76, no. 4, pp. 572–580, 2005.

[34] S. Gupta, S. Y. Kim, S. Artis et al., "Histone methylation regulates memory formation," *The Journal of Neuroscience*, vol. 30, no. 10, pp. 3589–3599, 2010.

[35] S. Gupta-Agarwal, A. V. Franklin, T. DeRamus et al., "G9a/GLP histone lysine dimethyltransferase complex activity in the hippocampus and the entorhinal cortex is required for gene activation and silencing during memory consolidation," *Journal of Neuroscience*, vol. 32, no. 16, pp. 5440–5453, 2012.

[36] J. Gräff, B. T. Woldemichael, D. Berchtold, G. Dewarrat, and I. M. Mansuy, "Dynamic histone marks in the hippocampus and cortex facilitate memory consolidation," *Nature communications*, vol. 3, article 991, 2012.

[37] A. Schaefer, S. C. Sampath, A. Intrator et al., "Control of cognition and adaptive behavior by the GLP/G9a epigenetic suppressor complex," *Neuron*, vol. 64, no. 5, pp. 678–691, 2009.

[38] T. Kleefstra, J. M. Kramer, K. Neveling et al., "Disruption of an EHMT1-associated chromatin-modification module causes intellectual disability," *American Journal of Human Genetics*, vol. 91, no. 1, pp. 73–82, 2012.

[39] M. P. Pasillas, M. Shah, and M. P. Kamps, "NSD1 PHD domains bind methylated H3K4 and H3K9 using interactions disrupted by point mutations in human sotos syndrome," *Human Mutation*, vol. 32, no. 3, pp. 292–298, 2011.

[40] N. M. C. Maas, G. Van Buggenhout, F. Hannes et al., "Genotype-phenotype correlation in 21 patients with Wolf-Hirschhorn syndrome using high resolution array comparative genome hybridisation (CGH)," *Journal of Medical Genetics*, vol. 45, no. 2, pp. 71–80, 2008.

[41] C. Kerimoglu, R. C. Agis-Balboa, A. Kranz et al., "Histone-methyltransferase MLL2 (KMT2B) is required for memory formation in mice," *The Journal of Neuroscience*, vol. 33, no. 8, pp. 3452–3464, 2013.

[42] N. Miyake, S. Mizuno, N. Okamoto et al., "KDM6A point mutations cause Kabuki syndrome," *Human Mutation*, vol. 34, no. 1, pp. 108–110, 2013.

[43] W. T. Gibson, R. L. Hood, S. H. Zhan et al., "Mutations in EZH2 cause weaver syndrome," *American Journal of Human Genetics*, vol. 90, no. 1, pp. 110–118, 2012.

[44] S. Iwase, F. Lan, P. Bayliss et al., "The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases," *Cell*, vol. 128, no. 6, pp. 1077–1088, 2007.

[45] Y. Qiao, X. Liu, C. Harvard et al., "Autism-associated familial microdeletion of Xp11.22," *Clinical Genetics*, vol. 74, no. 2, pp. 134–144, 2008.

[46] A. Vogel-Ciernia, D. P. Matheos, R. M. Barrett et al., "The neuron-specific chromatin regulatory subunit BAF53b is necessary for synaptic plasticity and memory," *Nature Neuroscience*, vol. 16, no. 5, pp. 552–561, 2013.

[47] B. T. Staahl and G. R. Crabtree, "Creating a neural specific chromatin landscape by npBAF and nBAF complexes," *Current Opinion in Neurobiology*, vol. 23, no. 6, pp. 903–913, 2013.

[48] P. Nicolaides and M. Baraitser, "An unusual syndrome with mental retardation and sparse hair," *Clinical Dysmorphology*, vol. 2, no. 3, pp. 232–236, 1993.

[49] D. J. Picketts, D. R. Higgs, S. Bachoo, D. J. Blake, O. W. J. Quarrell, and R. J. Gibbons, "ATRX encodes a novel member of the SNF2 family of proteins: mutations point to a common mechanism underlying the ATR-X syndrome," *Human Molecular Genetics*, vol. 5, no. 12, pp. 1899–1907, 1996.

[50] B. M. Neale, Y. Kou, L. Liu et al., "Patterns and rates of exonic *de novo* mutations in autism spectrum disorders," *Nature*, vol. 485, no. 7397, pp. 242–245, 2012.

[51] F. D. Heyward and J. D. Sweatt, "DNA methylation in memory formation: emerging insights," *Neuroscientist*, vol. 21, no. 5, pp. 475–489, 2015.

[52] B. J. Wiltgen, R. A. M. Brown, L. E. Talton, and A. J. Silva, "New circuits for old memories: the role of the neocortex in consolidation," *Neuron*, vol. 44, no. 1, pp. 101–108, 2004.

[53] A. J. Bannister and T. Kouzarides, "Regulation of chromatin by histone modifications," *Cell Research*, vol. 21, no. 3, pp. 381–395, 2011.

[54] J. Shin, G.-L. Ming, and H. Song, "Seeking a roadmap toward neuroepigenetics," *Neuron*, vol. 86, no. 1, pp. 12–15, 2015.

[55] M. W. Swank and J. D. Sweatt, "Increased histone acetyltransferase and lysine acetyltransferase activity and biphasic activation of the ERK/RSK cascade in insular cortex during novel taste learning," *The Journal of Neuroscience*, vol. 21, no. 10, pp. 3383–3391, 2001.

[56] J. M. Levenson, K. J. O'Riordan, K. D. Brown, M. A. Trinh, D. L. Molfese, and J. D. Sweatt, "Regulation of histone acetylation during memory formation in the hippocampus," *The Journal of Biological Chemistry*, vol. 279, no. 39, pp. 40545–40559, 2004.

[57] J. P. Lopez-Atalaya, S. Ito, L. M. Valor, E. Benito, and A. Barco, "Genomic targets, and histone acetylation and gene expression profiling of neural HDAC inhibition," *Nucleic Acids Research*, vol. 41, no. 17, pp. 8072–8084, 2013.

[58] J.-S. Guan, S. J. Haggarty, E. Giacometti et al., "HDAC2 negatively regulates memory formation and synaptic plasticity," *Nature*, vol. 459, no. 7243, pp. 55–60, 2009.

[59] S. Michán, Y. Li, M. M.-H. Chou et al., "SIRT1 is essential for normal cognitive function and synaptic plasticity," *The Journal of Neuroscience*, vol. 30, no. 29, pp. 9695–9707, 2010.

[60] M.-S. Kim, M. W. Akhtar, M. Adachi et al., "An essential role for histone deacetylase 4 in synaptic plasticity and memory formation," *Journal of Neuroscience*, vol. 32, no. 32, pp. 10879–10886, 2012.

[61] S. D. Carter, K. R. Mifsud, and J. M. H. 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.

[62] F. D. Lubin, S. Gupta, R. R. Parrish, N. M. Grissom, and R. L. Davis, "Epigenetic mechanisms: critical contributors to long-term memory formation," *Neuroscientist*, vol. 17, no. 6, pp. 616–632, 2011.

[63] M. Benevento, M. van de Molengraft, R. van Westen, H. van Bokhoven, and N. N. Kasri, "The role of chromatin repressive marks in cognition and disease: a focus on the repressive complex GLP/G9a," *Neurobiology of Learning and Memory*, vol. 124, pp. 88–96, 2015.

[64] S. Gupta-Agarwal, T. J. Jarome, J. Fernandez, and F. D. Lubin, "NMDA receptor- and ERK-dependent histone methylation changes in the lateral amygdala bidirectionally regulate fear memory formation," *Learning and Memory*, vol. 21, no. 7, pp. 351–362, 2014.

[65] I. Olave, W. Wang, Y. Xue, A. Kuo, and G. R. Crabtree, "Identification of a polymorphic, neuron-specific chromatin remodeling complex," *Genes and Development*, vol. 16, no. 19, pp. 2509–2517, 2002.

[66] X. Cao, G. Yeo, A. R. Muotri, T. Kuwabara, and F. H. Gage, "Noncoding RNAs in the mammalian central nervous system," *Annual Review of Neuroscience*, vol. 29, pp. 77–103, 2006.

[67] J. A. Briggs, E. J. Wolvetang, J. S. Mattick, J. L. Rinn, and G. Barry, "Mechanisms of long non-coding RNAs in mammalian nervous system development, plasticity, disease, and evolution," *Neuron*, vol. 88, no. 5, pp. 861–877, 2015.

[68] N. Lin, K.-Y. Chang, Z. Li et al., "An evolutionarily conserved long noncoding RNA TUNA controls pluripotency and neural lineage commitment," *Molecular Cell*, vol. 53, no. 6, pp. 1005–1019, 2014.

[69] V. Chalei, S. N. Sansom, L. Kong et al., "The long non-coding RNA Dali is an epigenetic regulator of neural differentiation," *eLife*, vol. 3, Article ID e04530, 2014.

[70] T. Vaissiere, C. Sawan, and Z. Herceg, "Epigenetic interplay between histone modifications and DNA methylation in gene silencing," *Mutation Research*, vol. 659, no. 1-2, pp. 40–48, 2008.

[71] J. I. Wu, J. Lessard, I. A. Olave et al., "Regulation of dendritic development by neuron-specific chromatin remodeling complexes," *Neuron*, vol. 56, no. 1, pp. 94–108, 2007.

[72] D. K. Binder and H. E. Scharfman, "Mini review," *Growth Factors*, vol. 22, no. 3, pp. 123–131, 2004.

[73] K. Deinhardt and M. V. Chao, "Shaping neurons: Long and short range effects of mature and proBDNF signalling upon neuronal structure," *Neuropharmacology*, vol. 76, pp. 603–609, 2014.

[74] H. W. Horch and L. C. Katz, "BDNF release from single cells elicits local dendritic growth in nearby neurons," *Nature Neuroscience*, vol. 5, no. 11, pp. 1177–1184, 2002.

[75] M. Kaneko, Y. Xie, J. J. An, M. P. Stryker, and B. Xu, "Dendritic BDNF synthesis is required for late-phase spine maturation and recovery of cortical responses following sensory deprivation," *The Journal of Neuroscience*, vol. 32, no. 14, pp. 4790–4802, 2012.

[76] J. J. Day and J. D. Sweatt, "Epigenetic mechanisms in cognition," *Neuron*, vol. 70, no. 5, pp. 813–829, 2011.

[77] H. K. Kato, A. M. Watabe, and T. Manabe, "Non-Hebbian synaptic plasticity induced by repetitive postsynaptic action potentials," *The Journal of Neuroscience*, vol. 29, no. 36, pp. 11153–11160, 2009.

[78] F. J. van der Staay, S. S. Arndt, and R. E. Nordquist, "Evaluation of animal models of neurobehavioral disorders," *Behavioral and Brain Functions*, vol. 5, article 11, 2009.

[79] J. N. Crawley, "Designing mouse behavioral tasks relevant to autistic-like behaviors," *Mental Retardation and Developmental Disabilities Research Reviews*, vol. 10, no. 4, pp. 248–258, 2004.

[80] R. H. Reeves, N. G. Irving, T. H. Moran et al., "A mouse model for Down syndrome exhibits learning and behaviour deficits," *Nature Genetics*, vol. 11, no. 2, pp. 177–184, 1995.

[81] K. M. Huber, S. M. Gallagher, S. T. Warren, and M. F. Bear, "Altered synaptic plasticity in a mouse model of fragile X mental retardation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 11, pp. 7746–7750, 2002.

[82] M. S. Fanselow, "Conditional and unconditional components of post-shock freezing," *The Pavlovian Journal of Biological Science*, vol. 15, no. 4, pp. 177–182, 1980.

[83] 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.

[84] T. M. Kazdoba, P. T. Leach, J. L. Silverman, and J. N. Crawley, "Modeling fragile X syndrome in the Fmr1 knockout mouse," *Intractable & Rare Diseases Research*, vol. 3, no. 4, pp. 118–133, 2014.

[85] N. Rueda, J. Flórez, and C. Martínez-Cué, "Mouse models of Down syndrome as a tool to unravel the causes of mental disabilities," *Neural Plasticity*, vol. 2012, Article ID 584071, 26 pages, 2012.

[86] A. E. Horner, C. J. Heath, M. Hvoslef-Eide et al., "The touchscreen operant platform for testing learning and memory in rats and mice," *Nature Protocols*, vol. 8, no. 10, pp. 1961–1984, 2013.

[87] T. Kleefstra, H. G. Brunner, J. Amiel et al., "Loss-of-function mutations in Euchromatin histone methyl transferase 1 (EHMT1) cause the 9q34 subtelomeric deletion syndrome," *The American Journal of Human Genetics*, vol. 79, no. 2, pp. 370–377, 2006.

[88] T. Nikitina, X. Shi, R. P. Ghosh, R. A. Horowitz-Scherer, J. C. Hansen, and C. L. Woodcock, "Multiple modes of interaction between the methylated DNA binding protein MeCP2 and chromatin," *Molecular and Cellular Biology*, vol. 27, no. 3, pp. 864–877, 2007.

[89] M. Chahrour, Y. J. Sung, C. Shaw et al., "MeCP2, a key contributor to neurological disease, activates and represses transcription," *Science*, vol. 320, no. 5880, pp. 1224–1229, 2008.

[90] W. G. Chen, Q. Chang, Y. Lin et al., "Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2," *Science*, vol. 302, no. 5646, pp. 885–889, 2003.

[91] L. M. Valor, J. Viosca, J. P. Lopez-Atalaya, and A. Barco, "Lysine acetyltransferases CBP and p300 as therapeutic targets in cognitive and neurodegenerative disorders," *Current Pharmaceutical Design*, vol. 19, no. 28, pp. 5051–5064, 2013.

[92] J. M. Alarcón, G. Malleret, K. Touzani et al., "Chromatin acetylation, memory, and LTP are impaired in $CBP^{+/-}$ mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration," *Neuron*, vol. 42, no. 6, pp. 947–959, 2004.

[93] S. Parkel, J. P. Lopez-Atalaya, and A. Barco, "Histone H3 lysine methylation in cognition and intellectual disability disorders," *Learning and Memory*, vol. 20, no. 10, pp. 570–579, 2013.

[94] Y. J. Loke, A. J. Hannan, and J. M. Craig, "The role of epigenetic change in autism spectrum disorders," *Frontiers in Neurology*, vol. 6, article 107, Article ID 00107, 2015.

[95] A. Letourneau, F. A. Santoni, X. Bonilla et al., "Domains of genome-wide gene expression dysregulation in Down's syndrome," *Nature*, vol. 508, no. 7496, pp. 345–350, 2014.

[96] K. Gardiner and M. Davisson, "The sequence of human chromosome 21 and implications for research into Down syndrome," *Genome Biology*, vol. 1, no. 2, 2000.

[97] M. G. Bacalini, D. Gentilini, A. Boattini et al., "Identification of a DNA methylation signature in blood cells from persons with down syndrome," *Aging*, vol. 7, no. 2, pp. 82–96, 2015.

[98] S. Horvath, P. Garagnani, M. G. Bacalini et al., "Accelerated epigenetic aging in Down syndrome," *Aging Cell*, vol. 14, no. 3, pp. 491–495, 2015.

[99] M. J. Jones, P. Farré, L. M. McEwen et al., "Distinct DNA methylation patterns of cognitive impairment and trisomy 21 in down syndrome," *BMC Medical Genomics*, vol. 6, no. 1, article 58, 2013.

[100] J. Lu, M. Mccarter, G. Lian et al., "Global hypermethylation in fetal cortex of Down syndrome due to DNMT3L overexpression," *Human Molecular Genetics*, vol. 25, no. 9, pp. 1714–1727, 2016.

[101] S. Jin, Y. K. Lee, Y. C. Lim et al., "Global DNA hypermethylation in Down syndrome placenta," *PLoS Genetics*, vol. 9, no. 6, Article ID e1003515, 2013.

[102] V. Infantino, A. Castegna, F. Iacobazzi et al., "Impairment of methyl cycle affects mitochondrial methyl availability and glutathione level in Down's syndrome," *Molecular Genetics and Metabolism*, vol. 102, no. 3, pp. 378–382, 2011.

[103] D. C. Wallace and W. Fan, "Energetics, epigenetics, mitochondrial genetics," *Mitochondrion*, vol. 10, no. 1, pp. 12–31, 2010.

[104] X. Guo, J. G. Williams, T. T. Schug, and X. Li, "DYRK1A and DYRK3 promote cell survival through phosphorylation and activation of SIRT1," *The Journal of Biological Chemistry*, vol. 285, no. 17, pp. 13223–13232, 2010.

[105] E. J. Yang, Y. S. Ahn, and K. C. Chung, "Protein kinase Dyrk1 activates cAMP response element-binding protein during neuronal differentiation in hippocampal progenitor cells," *Journal of Biological Chemistry*, vol. 276, no. 43, pp. 39819–39824, 2001.

[106] A.-M. Lepagnol-Bestel, A. Zvara, G. Maussion et al., "DYRK1A interacts with the REST/NRSF-SWI/SNF chromatin remodelling complex to deregulate gene clusters involved in the neuronal phenotypic traits of Down syndrome," *Human Molecular Genetics*, vol. 18, no. 8, pp. 1405–1414, 2009.

[107] H.-J. Sun, X. Xu, X.-L. Wang et al., "Transcription factors Ets2 and Sp1 act synergistically with histone acetyltransferase p300 in activating human interleukin-12 p40 promoter," *Acta Biochimica et Biophysica Sinica*, vol. 38, no. 3, pp. 194–200, 2006.

[108] T. Ueda, Y. V. Postnikov, and M. Bustin, "Distinct domains in high mobility group N variants modulate specific chromatin modifications," *Journal of Biological Chemistry*, vol. 281, no. 15, pp. 10182–10187, 2006.

[109] H. Huang, I. Rambaldi, E. Daniels, and M. Featherstone, "Expression of the *Wdr9* gene and protein products during mouse development," *Developmental Dynamics*, vol. 227, no. 4, pp. 608–614, 2003.

[110] R. Bakshi, M. Q. Hassan, J. Pratap et al., "The human SWI/SNF complex associates with RUNX1 to control transcription of hematopoietic target genes," *Journal of Cellular Physiology*, vol. 225, no. 2, pp. 569–576, 2010.

[111] L. Abuhatzira, A. Shamir, D. E. Schones, A. A. Schäffer, and M. Bustin, "The chromatin-binding protein HMGN1 regulates the expression of Methyl CpG-binding Protein 2 (MECP2) and affects the behavior of mice," *Journal of Biological Chemistry*, vol. 286, no. 49, pp. 42051–42062, 2011.

[112] B. E. Reese, K. E. Bachman, S. B. Baylin, and M. R. Rountree, "The methyl-CpG binding protein MBD1 interacts with the p150 subunit of chromatin assembly factor 1," *Molecular and Cellular Biology*, vol. 23, no. 9, pp. 3226–3236, 2003.

[113] R. C. Samaco and J. L. Neul, "Complexities of rett syndrome and MeCP2," *The Journal of Neuroscience*, vol. 31, no. 22, pp. 7951–7959, 2011.

[114] X. Wang, Y. Zhao, X. Zhang et al., "Loss of sorting nexin 27 contributes to excitatory synaptic dysfunction by modulating glutamate receptor recycling in Down's syndrome," *Nature Medicine*, vol. 19, no. 4, pp. 473–480, 2013.

[115] W. J. Lukiw, "Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus," *NeuroReport*, vol. 18, no. 3, pp. 297–300, 2007.

[116] C. S. Veerappan, S. Sleiman, and G. Coppola, "Epigenetics of Alzheimer's disease and frontotemporal dementia," *Neurotherapeutics*, vol. 10, no. 4, pp. 709–721, 2013.

[117] D. Bhartiya, K. Pal, S. Ghosh et al., "LncRNome: a comprehensive knowledgebase of human long noncoding RNAs," *Database*, vol. 2013, Article ID bat034, 2013.

[118] S. De Rubeis and C. Bagni, "Fragile X mental retardation protein control of neuronal mRNA metabolism: insights into mRNA stability," *Molecular and Cellular Neuroscience*, vol. 43, no. 1, pp. 43–50, 2010.

[119] J. B. Dictenberg, S. A. Swanger, L. N. Antar, R. H. Singer, and G. J. Bassell, "A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome," *Developmental Cell*, vol. 14, no. 6, pp. 926–939, 2008.

[120] I. Napoli, V. Mercaldo, P. P. Boyl et al., "The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP," *Cell*, vol. 134, no. 6, pp. 1042–1054, 2008.

[121] P. K. Todd, S. Y. Oh, A. Krans et al., "Histone deacetylases suppress CGG repeat-induced neurodegeneration via transcriptional silencing in models of fragile X tremor ataxia syndrome," *PLoS Genetics*, vol. 6, no. 12, Article ID e1001240, 2010.

[122] D. Kumari and K. Usdin, "The distribution of repressive histone modifications on silenced FMR1 alleles provides clues to the mechanism of gene silencing in fragile X syndrome," *Human Molecular Genetics*, vol. 19, no. 23, pp. 4634–4642, 2010.

[123] R. Pietrobono, E. Tabolacci, F. Zalfa et al., "Molecular dissection of the events leading to inactivation of the *FMR1* gene," *Human Molecular Genetics*, vol. 14, no. 2, pp. 267–277, 2005.

[124] P. Chiurazzi, M. G. Pomponi, R. Pietrobono, C. E. Bakker, G. Neri, and B. A. Oostra, "Synergistic effect of histone hyperacetylation and DNA demethylation in the reactivation of the FMR1 gene," *Human Molecular Genetics*, vol. 8, no. 12, pp. 2317–2323, 1999.

[125] E. Tabolacci, R. Pietrobono, U. Moscato, B. A. Oostra, P. Chiurazzi, and G. Neri, "Differential epigenetic modifications in the FMR1 gene of the fragile X syndrome after reactivating pharmacological treatments," *European Journal of Human Genetics*, vol. 13, no. 5, pp. 641–648, 2005.

[126] R. Biacsi, D. Kumari, and K. Usdin, "SIRT1 inhibition alleviates gene silencing in Fragile X mental retardation syndrome," *PLoS Genetics*, vol. 4, no. 3, Article ID e1000017, 2008.

[127] E. W. Loomis, L. A. Sanz, F. Chédin, and P. J. Hagerman, "Transcription-associated R-loop formation across the human FMR1 CGG-repeat region," *PLoS Genetics*, vol. 10, no. 4, Article ID e1004294, 2014.

[128] R. S. Alisch, T. Wang, P. Chopra, J. Visootsak, K. N. Conneely, and S. T. Warren, "Genome-wide analysis validates aberrant methylation in fragile X syndrome is specific to the FMR1 locus," *BMC Medical Genetics*, vol. 14, article 18, 2013.

[129] C. Lacoux, D. Di Marino, P. P. Boyl et al., "BC1-FMRP interaction is modulated by 2′-O-methylation: RNA-binding activity of the tudor domain and translational regulation at synapses," *Nucleic Acids Research*, vol. 40, no. 9, pp. 4086–4096, 2012.

[130] P. Jin, D. C. Zarnescu, S. Ceman et al., "Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway," *Nature Neuroscience*, vol. 7, no. 2, pp. 113–117, 2004.

[131] V. J. Peschansky, C. Pastori, Z. Zeier et al., "Changes in expression of the long non-coding RNA FMR4 associate with altered gene expression during differentiation of human neural precursor cells," *Frontiers in Genetics*, vol. 6, article 263, 2015.

[132] M. F. Bear, K. M. Huber, and S. T. Warren, "The mGluR theory of fragile X mental retardation," *Trends in Neurosciences*, vol. 27, no. 7, pp. 370–377, 2004.

[133] L. L. Orefice, C.-C. Shih, H. Xu, E. G. Waterhouse, and B. Xu, "Control of spine maturation and pruning through proBDNF synthesized and released in dendrites," *Molecular and Cellular Neuroscience*, vol. 71, pp. 66–79, 2016.

[134] P. Hotulainen and C. C. Hoogenraad, "Actin in dendritic spines: connecting dynamics to function," *The Journal of Cell Biology*, vol. 189, no. 4, pp. 619–629, 2010.

[135] K. T. Chang, H. Ro, W. Wang, and K.-T. Min, "Meeting at the crossroads: common mechanisms in Fragile X and Down syndrome," *Trends in Neurosciences*, vol. 36, no. 12, pp. 685–694, 2013.

[136] A. M. Kleschevnikov, P. V. Belichenko, A. J. Villar, C. J. Epstein, R. C. Malenka, and W. C. Mobley, "Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of down syndrome," *Journal of Neuroscience*, vol. 24, no. 37, pp. 8153–8160, 2004.

[137] J. J. Scott-McKean and A. C. S. Costa, "Exaggerated NMDA mediated LTD in a mouse model of Down syndrome and pharmacological rescuing by memantine," *Learning and Memory*, vol. 18, no. 12, pp. 774–778, 2011.

[138] B. C. McKinney, A. W. Grossman, N. M. Elisseou, and W. T. Greenough, "Dendritic spine abnormalities in the occipital

cortex of C57BL/6 Fmr1 knockout mice," *American Journal of Medical Genetics—Neuropsychiatric Genetics*, vol. 136, no. 1, pp. 98–102, 2005.

[139] J. Larson, R. E. Jessen, D. Kim, A.-K. S. Fine, and J. Du Hoffmann, "Age-dependent and selective impairment of long-term potentiation in the anterior piriform cortex of mice lacking the fragile X mental retardation protein," *Journal of Neuroscience*, vol. 25, no. 41, pp. 9460–9469, 2005.

[140] H. Hu, Y. Qin, G. Bochorishvili, Y. Zhu, L. Van Aelst, and J. J. Zhu, "Ras signaling mechanisms underlying impaired GluR1-dependent plasticity associated with fragile X syndrome," *The Journal of Neuroscience*, vol. 28, no. 31, pp. 7847–7862, 2008.

[141] K. R. Martin, A. Corlett, D. Dubach et al., "Over-expression of RCAN1 causes down syndrome-like hippocampal deficits that alter learning and memory," *Human Molecular Genetics*, vol. 21, no. 13, pp. 3025–3041, 2012.

[142] W. Wang, J. Z. Zhu, K. T. Chang, and K.-T. Min, "DSCR1 interacts with FMRP and is required for spine morphogenesis and local protein synthesis," *EMBO Journal*, vol. 31, no. 18, pp. 3655–3666, 2012.

[143] M. Castets, C. Schaeffer, E. Bechara et al., "FMRP interferes with the Rac1 pathway and controls actin cytoskeleton dynamics in murine fibroblasts," *Human Molecular Genetics*, vol. 14, no. 6, pp. 835–844, 2005.

[144] M. Martinez de Lagran, R. Benavides-Piccione, I. Ballesteros-Yañez et al., "Dyrk1A influences neuronal morphogenesis through regulation of cytoskeletal dynamics in mammalian cortical neurons," *Cerebral Cortex*, vol. 22, no. 12, pp. 2867–2877, 2012.

[145] M. Vilardell, A. Rasche, A. Thormann et al., "Meta-analysis of heterogeneous Down Syndrome data reveals consistent genome-wide dosage effects related to neurological processes," *BMC Genomics*, vol. 12, article 229, 2011.

[146] I. T. Lott and E. Head, "Down syndrome and alzheimer's disease: a link between development and aging," *Mental Retardation and Developmental Disabilities Research Reviews*, vol. 7, no. 3, pp. 172–178, 2001.

[147] K. J. Lee, C. E. H. Moussa, Y. Lee et al., "Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines," *Neuroscience*, vol. 169, no. 1, pp. 344–356, 2010.

[148] K. T. Chang and K.-T. Min, "Upregulation of three *Drosophila* homologs of human chromosome 21 genes alters synaptic function: implications for Down syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 40, pp. 17117–17122, 2009.

[149] M. Perluigi, F. Di Domenico, and D. A. Buttterfield, "Unraveling the complexity of neurodegeneration in brains of subjects with Down syndrome: insights from proteomics," *Proteomics—Clinical Applications*, vol. 8, no. 1-2, pp. 73–85, 2014.

[150] F. Duclot, M. Lapierre, S. Fritsch et al., "Cognitive impairments in adult mice with constitutive inactivation of RIP140 gene expression," *Genes, Brain and Behavior*, vol. 11, no. 1, pp. 69–78, 2012.

[151] K. Yamakawa, Y.-K. Huo, M. A. Haendel et al., "DSCAM: a novel member of the immunoglobulin superfamily maps in a Down syndrome region and is involved in the development of the nervous system," *Human Molecular Genetics*, vol. 7, no. 2, pp. 227–237, 1998.

[152] T. L. Schaefer, M. H. Davenport, and C. A. Erickson, "Emerging pharmacologic treatment options for fragile X syndrome," *Application of Clinical Genetics*, vol. 8, pp. 75–93, 2015.

[153] R. de la Torre and M. Dierssen, "Therapeutic approaches in the improvement of cognitive performance in Down syndrome: past, present, and future," *Progress in Brain Research*, vol. 197, pp. 1–14, 2012.

[154] D. F. Owens and A. R. Kriegstein, "Developmental neurotransmitters?" *Neuron*, vol. 36, no. 6, pp. 989–991, 2002.

[155] J. M. Ellis, K. T. Hooi, R. E. Gilbert et al., "Supplementation with antioxidants and folinic acid for children with Down's syndrome: randomised controlled trial," *The British Medical Journal*, vol. 336, no. 7644, pp. 594–597, 2008.

[156] J. E. Castillo-Fernandez, T. D. Spector, and J. T. Bell, "Epigenetics of discordant monozygotic twins: implications for disease," *Genome Medicine*, vol. 6, no. 7, article 60, 2014.

[157] M. Grilli, S. Zappettini, A. Zanardi et al., "Exposure to an enriched environment selectively increases the functional response of the pre-synaptic NMDA receptors which modulate noradrenaline release in mouse hippocampus," *Journal of Neurochemistry*, vol. 110, no. 5, pp. 1598–1606, 2009.

[158] J. Nithianantharajah and A. J. Hannan, "Enriched environments, experience-dependent plasticity and disorders of the nervous system," *Nature Reviews Neuroscience*, vol. 7, no. 9, pp. 697–709, 2006.

[159] L. Restivo, F. Ferrari, E. Passino et al., "Enriched environment promotes behavioral and morphological recovery in a mouse model for the fragile X syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 32, pp. 11557–11562, 2005.

[160] R. E. von Leden, L. C. Curley, G. D. Greenberg, M. R. Hunsaker, R. Willemsen, and R. F. Berman, "Reduced activity-dependent protein levels in a mouse model of the fragile X premutation," *Neurobiology of Learning and Memory*, vol. 109, pp. 160–168, 2014.

[161] M. Dierssen, R. Benavides-Piccione, C. Martínez-Cué et al., "Alterations of neocortical pyramidal cell phenotype in the Ts65Dn mouse model of Down syndrome: effects of environmental enrichment," *Cerebral Cortex*, vol. 13, no. 7, pp. 758–764, 2003.

[162] M. Pons-Espinal, M. M. de Lagran, and M. Dierssen, "Environmental enrichment rescues DYRK1A activity and hippocampal adult neurogenesis in TgDyrk1A," *Neurobiology of Disease*, vol. 60, pp. 18–31, 2013.

[163] S. S. Shin, J. W. Bales, H. Q. Yan et al., "The effect of environmental enrichment on substantia nigra gene expression after traumatic brain injury in rats," *Journal of Neurotrauma*, vol. 30, no. 4, pp. 259–270, 2013.

[164] A. Fischer, F. Sananbenesi, X. Wang, M. Dobbin, and L.-H. Tsai, "Recovery of learning and memory is associated with chromatin remodelling," *Nature*, vol. 447, no. 7141, pp. 178–182, 2007.

[165] J. P. Lopez-Atalaya, A. Ciccarelli, J. Viosca et al., "CBP is required for environmental enrichment-induced neurogenesis and cognitive enhancement," *EMBO Journal*, vol. 30, no. 20, pp. 4287–4298, 2011.

[166] M. A. Dawson and T. Kouzarides, "Cancer epigenetics: from mechanism to therapy," *Cell*, vol. 150, no. 1, pp. 12–27, 2012.

[167] A. Nebbioso, V. Carafa, R. Benedetti, and L. Altucci, "Trials with 'epigenetic' drugs: an update," *Molecular Oncology*, vol. 6, no. 6, pp. 657–682, 2012.

[168] A. Papi, A. M. Ferreri, P. Rocchi, F. Guerra, and M. Orlandi, "Epigenetic modifiers as anticancer drugs: effectiveness of valproic acid in neural crest-derived tumor cells," *Anticancer Research*, vol. 30, no. 2, pp. 535–540, 2010.

[169] M. L. de Groote, P. J. Verschure, and M. G. Rots, "Epigenetic editing: targeted rewriting of epigenetic marks to modulate expression of selected target genes," *Nucleic Acids Research*, vol. 40, no. 21, pp. 10596–10613, 2012.

[170] K. Rezai-Zadeh, G. W. Arendash, H. Hou et al., "Green tea epigallocatechin-3-gallate (EGCG) reduces β-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice," *Brain Research*, vol. 1214, pp. 177–187, 2008.

[171] R. De la Torre, S. De Sola, M. Pons et al., "Epigallocatechin-3-gallate, a DYRK1A inhibitor, rescues cognitive deficits in Down syndrome mouse models and in humans," *Molecular Nutrition and Food Research*, vol. 58, no. 2, pp. 278–288, 2014.

[172] E.-S. Scarpa and P. Ninfali, "Phytochemicals as innovative therapeutic tools against cancer stem cells," *International Journal of Molecular Sciences*, vol. 16, no. 7, pp. 15727–15742, 2015.

[173] M. Z. Fang, Y. Wang, N. Ai et al., "Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines," *Cancer Research*, vol. 63, no. 22, pp. 7563–7570, 2003.

[174] S. N. Saldanha, R. Kala, and T. O. Tollefsbol, "Molecular mechanisms for inhibition of colon cancer cells by combined epigenetic-modulating epigallocatechin gallate and sodium butyrate," *Experimental Cell Research*, vol. 324, no. 1, pp. 40–53, 2014.

[175] V. S. Thakur, K. Gupta, and S. Gupta, "Green tea polyphenols increase p53 transcriptional activity and acetylation by suppressing class I histone deacetylases," *International Journal of Oncology*, vol. 41, no. 1, pp. 353–361, 2012.

[176] S. R. Choudhury, S. Balasubramanian, Y. C. Chew, B. Han, V. E. Marquez, and R. L. Eckert, "(-)-Epigallocatechin-3-gallate and DZNep reduce polycomb protein level via a proteasome-dependent mechanism in skin cancer cells," *Carcinogenesis*, vol. 32, no. 10, pp. 1525–1532, 2011.

[177] D. Milenkovic, C. Deval, E. Gouranton et al., "Modulation of miRNA expression by dietary polyphenols in apoE deficient mice: a new mechanism of the action of polyphenols," *PLoS ONE*, vol. 7, no. 1, Article ID e29837, 2012.

[178] J. Bain, H. McLauchlan, M. Elliott, and P. Cohen, "The specificities of protein kinase inhibitors: an update," *Biochemical Journal*, vol. 371, no. 1, pp. 199–204, 2003.

[179] D. Valenti, D. De Rasmo, A. Signorile et al., "Epigallocatechin-3-gallate prevents oxidative phosphorylation deficit and promotes mitochondrial biogenesis in human cells from subjects with Down's syndrome," *Biochimica et Biophysica Acta (BBA)—Molecular Basis of Disease*, vol. 1832, no. 4, pp. 542–552, 2013.

[180] S. Catuara-Solarz, J. Espinosa-Carrasco, I. Erb et al., "Principal component analysis of the effects of environmental enrichment and (-)-epigallocatechin-3-gallate on age-associated learning deficits in a mouse model of down syndrome," *Frontiers in Behavioral Neuroscience*, vol. 9, article 330, 2015.

[181] M. Yu, G. C. Hon, K. E. Szulwach et al., "Base-resolution analysis of 5-hydroxymethylcytosine in the mammalian genome," *Cell*, vol. 149, no. 6, pp. 1368–1380, 2012.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2016, Article ID 5346161, 11 pages
http://dx.doi.org/10.1155/2016/5346161

Research Article

Early Social Enrichment Improves Social Motivation and Skills in a Monogenic Mouse Model of Autism, the *Oprm1*$^{-/-}$ Mouse

Luciana Garbugino,[1] Eleonora Centofante,[1,2] and Francesca R. D'Amato[1]

[1]*CNR, Institute of Cell Biology and Neurobiology, IRCCS Santa Lucia Foundation, Via del Fosso di Fiorano 64, 00143 Roma, Italy*
[2]*F. M. Kirby Neurobiology Center, Department of Neurology, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA*

Correspondence should be addressed to Francesca R. D'Amato; francesca.damato@cnr.it

Received 14 January 2016; Revised 11 March 2016; Accepted 5 April 2016

Academic Editor: Joram Feldon

Environmental enrichment has been proven to have positive effects on both behavioral and physiological phenotypes in rodent models of mental and neurodevelopmental disorders. In this study, we used mice lacking the μ-opioid receptor gene (*Oprm1*$^{-/-}$), which has been shown to have deficits in social competence and communication, to assess the hypothesis that early enrichment can ameliorate sociability during development and adulthood. Due to the immaturity of sensory-motor capabilities of young pups, we chose as environmental stimulation a second lactating female, who provided extra maternal care and stimulation from birth. The results show that double mothering normalized the abnormal response to maternal separation in *Oprm1*$^{-/-}$ pups and increased social motivation in juveniles and adult knockout mice. Additionally, we observed that *Oprm1*$^{-/-}$ mice act as less attractive social partners than wild types, which suggests that social motivation can be modulated by the stimulus employed. This experiment supports previous findings suggesting that early social environmental stimulation has profound and long-term beneficial effects, encouraging the use of nonpharmacological interventions for the treatment of social defects in neurodevelopmental diseases.

1. Introduction

Modeling neuropsychiatric disorders in animals is an extremely challenging task because of the peculiarity of human symptoms, the lack of biomarkers, and the early state of knowledge of the relevant neurobiology and genetics. Defective social behaviors are among the symptoms shared by different neuropsychiatric disorders such as depression, schizophrenia, and autism. Social malfunctioning is the common trait of different mouse models of psychopathologies, deriving from genetic and environmental factors, and mainly consists in altered motor skills and sensory inputs and motivational defects.

Autism spectrum disorder (ASD) is a developmental disorder defined by impairments in social communication and social interactions and a restricted repertoire of activities and interests [1]. ASD is not associated with a single specific mutation, but several and different genetic abnormalities were found to be associated with the syndrome, frequently acting in combination and, possibly, interacting with environmental factors. Parallel to the investigation of the aetiology of ASD, emphasis has recently been placed on therapeutic approaches that can improve and/or delay the development of symptoms.

Pharmacological as well as environmental treatments have been applied and some of them proved to have positive outcomes, reducing the severity of some ASD symptoms. As an example of pharmacological treatments, the atypical antipsychotic risperidone reduces some symptoms in affected children [2, 3]. In mouse models as well, acute and chronic pharmacological treatments suggest improvement for some symptoms in young adult individuals, but long-lasting effects are rarely reported [4]. As far as behavioral therapies are concerned, it is well known that early treatments in ASD children show greater success in delaying and slowing down some symptoms.

It is difficult to model behavioral therapies in preclinical research, and environmental enrichment is the most common nonpharmacological treatment used in mouse models

of several disorders (anxiety, depression, etc.). Environmental enrichment is usually applied from weaning onward and has almost always beneficial effects. Social and physical enrichment promote not only cognitive skills but also social interactions in several animal models [5–7]. Again, more pronounced and stable effects are associated with precocious exposure to the experimental environmental conditions [8]. Environmental enrichment usually involves housing conditions consisting of larger enclosures combined with more complex and variable physical and social environment. Exposing newborns to such an enriched environment, with them being deaf, blind, and almost immobile, could be useless because of the immaturity of their sensory-motor capabilities. Moreover, an early environment characterized by great variability could also represent a stressor for pups that need a secure (and stable) attachment basis to properly develop from an emotional point of view. The mother usually represents this stable basis: as a matter of fact, cross-fostering during the first days of life has a deleterious effect on pups' development [9–12].

The positive effects of environmental enrichment could reach the pups through the mother. Dams' behavior and physiology is affected by different housing conditions and this, in turn, can affect pups' development. Wild mice usually rear their pups in communal nests, where females, usually related, nurse and care for pups indiscriminately. To investigate the effects of such complex early environment, Branchi and coworkers performed a series of studies exploring the neurobiological and behavioral effects of being reared in communal nest, rather than in standard laboratory conditions, and reported more elaborated social competences in these mice [13–15]. Using a different experimental approach, we provided additional stimulation to both mothers and pups by housing the dam and her litter with a second (lactating or nonlactating) female allomother from birth onward. This socially enriched environment resulted in increased care for the pups and revealed positive outcome in terms of cognitive performance in outbred mice [16] and reduction of ASD symptoms in the fragile X syndrome mouse model [17].

The aim of this paper is to evaluate the possibility that the additional social stimulation, provided by the presence of a second lactating female from birth onward, can increase affiliative motivation in an animal model of social deficits. To this purpose, we used mice lacking the μ-opioid receptor gene (*Oprm1*$^{-/-}$) that have been proposed as a monogenic model of autism [18]. These animals show deficits in social behavior and communication from infancy onward, together with anatomical, neurochemical, and genetic landmarks of the disease [19–21]. The disruption of the μ-opioid signaling during development was able to induce deficits in infant-mother attachment, as well as in social interactions that persisted till adulthood. We hypothesized that the presence of a second female, which provides additional social stimulation, can improve social interactions in these μ-opioid knockout mice. For this purpose, we measured ultrasounds at postnatal day 8 (PND8) during isolation and sociability at weaning (PND28–30). Adult animals were then tested for emotionality, social recognition, and social preference. We expected that the early social enriched environment might affect social motivation and social-related behaviors in deficient μ-KO mice.

2. Materials and Methods

2.1. Subjects and Rearing Conditions. μ-opioid receptor knockout mice (*Oprm1*$^{-/-}$ and μ-KO) and their wild type controls (*Oprm1*$^{+/+}$ and WT), born from a colony raised in our animal facility, were used in this study. The generation of mice lacking μ-opioid receptors has been described and well characterized elsewhere [19, 22]. The colony was housed under constant temperature (20–22°C) and humidity conditions under a 12 h light/dark cycle with lights on at 7 am. Food and water were provided ad libitum. After weaning (PND28–30), animals were housed with same sex/genotype in standard mice cages containing 3–5 animals. WT and μ-KO subjects of these experiments were derived from homozygous breeding pairs.

Mating protocol consisted in housing two multiparous females (4-month-old) with one male of the same line for 15 days. After males were removed, according to reproductive status inferred from body weight increase (>35% from premating weight) and abdominal bulges presence (usually appearance on days 16-17 of gestation), females were assigned to one of the following experimental conditions: pregnant female housed alone (P) or two pregnant females housed together (P+P).

Around the expected day of partum, cages were inspected twice a day. After delivery, the number of pups in the P condition and the identity of the mother in the P+P cages were recorded. In the P+P condition, after at least 1 day (but no more than 5) elapsed between the two births, the younger litter was left in the cage while the older one was removed. P+P cages with females delivering on the same day or at a time interval superior to 5 days were discarded. In all conditions, cages with fewer than 4 pups were also discarded. The day of delivery was considered as PND0 and experimental conditions (P and P+P) were referred to hereafter as L and L+L since former pregnant (P) females were now lactating (L) females. Pups from the same litter were weaned, separated by sex, and housed in standard cages of 3–5 at PND28. Experimental animals derived from 7 μ-KO-L, 7 μ-KO-L+L, 7 WT-L, and 7 WT-L+L litters, with litter sizes between 4 and 8.

21–28-day-old male and female NMRI mice (Harlan) were used as stimulus partners in the Social Approach-Avoidance Test.

All experiments were conducted under license from the Italian Department of Health and in accordance with the Italian regulations on the use of animals for research (legislation DL 116/92 and 26/2014) and European guidelines on animal care.

2.2. Behavioral Tests during Development and at Adulthood. All experiments were carried out in enclosed rooms located in a sector outside the animal facility, to which animals were transferred approximately 1 h before the experiments started. The experimental rooms were kept under temperature and luminosity conditions equal to those of the animal facility

(unless differently specified). With the exception of pups' ultrasonic vocalizations (USVs), all experimental sessions were video recorded and behavioral data were subsequently collected using The Observer (Noldus, Netherlands) or SMART (Panlab, Harvard Apparatus) software. As for the USVs, Avisoft technology (Avisoft Bioacoustics, Berlin, Germany) has been used to record and analyze the number of ultrasounds emitted by pups. Experiments were performed during the light phase between approximately 11 am and 4 pm. At the end of the test, cages were brought back to the breeding facility. Body weight of tested mice was measured at the end of the behavioral tests and the effects of genotype, rearing condition, and sex were evaluated during development (PND8), at weaning (PND28–30), and at adulthood (PND75–80).

2.2.1. Ultrasonic Calls Emission. Pups' behavior was evaluated at PND8 by measuring USVs emitted during 5 minutes of isolation [21, 23]. After 1 h of acclimatization to the experimental room, the mother was removed and transferred into a clean cage, while pups were left in their home-cage, on a warm plate set at the temperature of 35°C to prevent cooling. No more than 4 pups/litter were employed. Pups were then individually placed into a beaker, containing own-cage (one male and one female) or clean bedding (one male and one female), and the vocalizations were recorded. Ultrasonic vocalizations were recorded using an UltraSoundGate Condenser Microphone (CM16, Avisoft Bioacoustics, Berlin, Germany) lowered 1 cm above the top of the isolation beaker containing the pup. The microphone was sensitive to frequencies of 15–180 kHz with a flat frequency response (±6 dB) between 25 and 140 kHz. It was connected via an UltraSoundGate USB Audio device to a personal computer, where acoustic data were recorded as wav files at 250,000 Hz in 16-bit format. Sound files were transferred to SasLab Pro (version 4.40; Avisoft Bioacoustics) for sonographic analysis and a fast Fourier transformation was conducted (512 FFT-length, 100% frame, Hamming window, and 75% time window overlap). Further details on this procedure, the device used, and the analysis of data can be found in our previous papers [16, 20]. The total number of ultrasounds emitted by each pup was analyzed by a 4-way ANOVA, the factors being genotype (μ-KO versus WT), early rearing condition (L versus L+L), bedding (clean versus home-cage), and sex (male versus females). For the sake of simplicity, a three-way ANOVA will follow in case of no effect of sex as independent variable, according to our previous results [20].

2.2.2. Social Approach-Avoidance Test. Animals were tested immediately before weaning (PND28–30) in a gray Plexiglas rectangular box (60 × 40 × 24 cm) consisting of three same-size interconnected chambers. Two identical clear Plexiglas cylinders (8 cm in diameter) with multiple small holes were placed, one in each end chamber of the apparatus. During the habituation session (10 min), the mouse was placed in the central chamber and allowed to freely explore the whole apparatus. During the test session, a stimulus NMRI mouse, age/sex matched, was introduced into one cylinder (pseudo-randomly chosen), whereas a white object was introduced into the other cylinder. Both 10 min sessions were recorded by a video camera and the time the subject mouse spent in each chamber and in proximity of each cylinder (2 cm: time close) was measured by a video-tracking system (SMART 1.1). After each test, the entire apparatus was carefully cleaned with 10% ethanol. Time spent in each chamber during habituation was scored to exclude any basal preference for one of the two lateral chambers. Time spent in proximity of each cylinder was analyzed by a 4-way ANOVA for repeated measures, the factors being genotype (μ-KO versus WT), rearing condition (L versus L+L), sex of the experimental subject (male versus female), and, as within factor, the stimulus in the cylinder (object versus mouse). For the sake of simplicity, a three-way ANOVA will follow in case of no effect of sex as independent variable, as expected by our previous results [20].

2.2.3. Emotionality. Male and female mice were tested in the elevated plus maze at PND75–90 for emotionality. The elevated plus maze consisted of 2 open (5 cm wide and 30 cm long) and 2 closed arms (5 cm wide and 30 cm long, enclosed by a wall of 14 cm in height) arranged in a plus configuration, joined by a central square of 5 cm × 5 cm.

The apparatus was made of opaque Plexiglas and kept on a base 40 cm above the floor. Mice were exposed to a test of standard 5 min duration. At the beginning of the test, each mouse was placed individually in the center of the maze, with the head facing an open arm (the same for all mice). All tests were conducted between 13:00 h and 15:00 h and recorded by a video camera. The animals were initially accustomed to the experimental room for at least 1 hour before the experiment.

The time spent in the different arms of the apparatus was evaluated by automatic software analysis (Panlab SMART 1.1, Harvard Apparatus) and the total time spent on all four arms (Time Open + Time Closed: TO + TC), the number of entries (Entries Open: EO), and percentage of time spent in open arms (%TO = 100 × Time Open/(Time Open + Time Closed)) were used as behavioral indices of emotionality in three-way ANOVAs, the factors being genotype (μ-KO versus WT), rearing condition (L versus L+L), and sex of the subject (male versus female).

2.2.4. Social Recognition (PND90–110). The social recognition test has been used in previous studies [24, 25] as a social memory test to assess the ability of rodents to recognize animals they have been previously exposed to: mice show a characteristic decline in the time spent investigating a partner, with a full recovery following the introduction of a new conspecific. Subject mice were housed individually in clean cages for two days before test and served as residents. During test, which was performed in a soundproof cabin, a same sex/genotype, standard reared partner was introduced into the resident's cage. Partners were younger mice (PND45–70) housed in standard cages in groups of four/five mice. The partner remained in the resident's cage for one minute and the behavior of mice was video recorded. The partner was removed and returned into a clean home-cage for

Table 1: Mean (±SEM) body weight (gr.) of mice (males and females) belonging to the four experimental groups.

Experimental groups	*Oprm1*$^{-/-}$		*Oprm1*$^{+/+}$		ANOVA		
	L	L+L	L	L+L	$F_{1/68}$ G	$F_{1/68}$ RC	$F_{1/68}$ S
Day 8							
Males	4.68 ± 0.15 [9]	5.99 ± 0.23 [8]	4.60 ± 0.24 [13]	6.49 ± 0.10 [9]	0.78	152.02***	0.14
Females	4.68 ± 0.20 [7]	6.31 ± 0.17 [10]	4.65 ± 0.17 [11]	6.40 ± 0.16 [9]			
					ANOVA		
					$F_{1/56}$ G	$F_{1/56}$ E	$F_{1/56}$ S
Days 28–30							
Males	16.32 ± 0.48 [8]	14.08 ± 0.51 [10]	14.86 ± 0.79 [8]	15.03 ± 0.89 [8]	1.69	1.41	0.21
Females	15.64 ± 0.44 [6]	15.06 ± 0.56 [8]	14.17 ± 0.29 [8]	14.54 ± 0.66 [8]			
					ANOVA		
					$F_{1/66}$ G	$F_{1/66}$ E	$F_{1/66}$ S
Days 75–80							
Males	27.17 ± 0.59 [11]	28.06 ± 0.41 [5]	25.75 ± 0.87 [14]	26.68 ± 0.54 [6]	0.68	0.53	90.42**
Females	21.36 ± 0.31 [9]	21.75 ± 0.30 [6]	22.39 ± 0.82 [12]	21.74 ± 0.53 [11]			

G: genotype; RC: rearing condition; S: sex. ** $p < 0.001$, *** $p < 0.0001$.
Sample sizes are reported in brackets.

a 10-minute break. This procedure was repeated for a total of four identical sessions. During the fifth session, an unfamiliar, same sex/genotype and standard reared mouse partner from a new social cage was introduced and the behavior was video recorded for one minute. Video recordings were analyzed afterwards using The Observer software and the time spent by the resident male in social investigation (SI: sniffing and following the partner), as well as agonistic behaviors, was measured. A four-way ANOVA for repeated measures was applied to evaluate the effects of genotype (μ-KO versus WT), rearing condition (L versus L+L), sex (male versus female), and session (sessions A, B, C, D, and E) on the total amount of partner investigation during each 1 min session. For the sake of simplicity, separate three-way ANOVAs will follow in case of significant sex differences.

2.2.5. Partner Preference (PND90–110). This test was performed in a batch of adult animals naïve to the Social Approach-Avoidance Test. The same apparatus and the same procedure as in the Social Approach-Avoidance Test were used, with a variant during the test session: an unfamiliar conspecific was introduced into each cylinder. Social partners were age/sex matched with experimental subjects, but one was a wild type and the other a knockout animal. This would allow evaluating attractiveness of mice belonging to the two homozygous lines. The position of wild type and knockout partners within the apparatus was balanced within each group and was assigned independently of exploration time in each compartment during habituation. Both 10 min sessions (habituation and test) were recorded by a video camera and the time the subject mouse spent in each chamber and in proximity to each cylinder (2 cm: time close) was measured by a video-tracking system (SMART 1.1). After each test, the entire apparatus was carefully cleaned with 10% ethanol. Habituation preference scores were measured to evaluate a priori discrimination between lateral compartments. Preference for different line partners was evaluated by three-way ANOVAs, the factors being genotype (μ-KO versus WT), rearing condition (L versus L+L), and sex (male versus female). Partner preference was measured as % or time (sec) spent close to the cylinder containing the mouse with different genotype compared to that of the subject (preference score for different genotype = 100 × time close different genotype/(time close different genotype + time close same genotype)).

3. Results

3.1. Body Weight. Table 1 reports data on μ-KO and WT body weights measured in concomitance with behavioral tests. The first evaluation was conducted immediately after the USVs test, on PND8. The 3-way ANOVA revealed a strong effect of the rearing condition, with pups reared in the presence of their biological mother plus the second lactating female showing higher body weight ($p < 0.001$), independently of the genotype and sex. No interaction reached a significant effect. At weaning (PND28–30), the effect of the rearing condition on body weight disappeared and no significant main and interaction effects emerged from the ANOVA. Finally, mice were weighted once more (PND75–80) after the emotionality evaluation in the plus maze test. A strong sex effect emerged from body weight data, confirming that males were heavier than females, but no other main and interaction effects reached statistically significant levels.

3.2. Ultrasonic Calls Emission (PND8). Isolated pups emitted ultrasonic calls as shown in Figure 1. According to our previous results [18], the 4-way ANOVA confirmed no significant main effect of the sex of the pup on USVs emission (male versus female: $F_{1/85} = 2.16$, ns). The successive three-way ANOVA indicated that the number of calls was strongly affected by rearing condition (L versus L+L: $F_{1/93} = 20.10$,

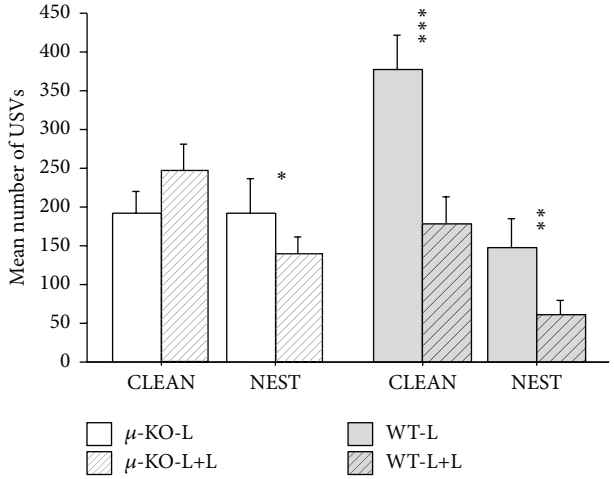


Figure 1: Mean number (+SEM) of ultrasonic vocalizations (USVs) emitted by 8-day-old pups of different experimental groups, when isolated in clean (CLEAN) or in their own home-cage (NEST) bedding for 5 min. *N*: 11–15 per group. μ-KO-L: *Oprm1*$^{-/-}$ pups reared by their mother [CLEAN: 9 males + 6 females; NEST: 8 males + 5 females]; μ-KO-L+L: *Oprm1*$^{-/-}$ pups reared by their mother plus a second lactating female [CLEAN: 4 males + 7 females; NEST: 6 males + 5 females]; WT-L: *Oprm1*$^{+/+}$ pups reared by their mother [CLEAN: 8 males + 6 females; NEST: 4 males + 8 females]; WT-L+L: *Oprm1*$^{+/+}$ pups reared by their mother plus a second lactating female [CLEAN: 7 males + 6 females; NEST: 6 males + 6 females]. Discrimination of clean versus nest bedding: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

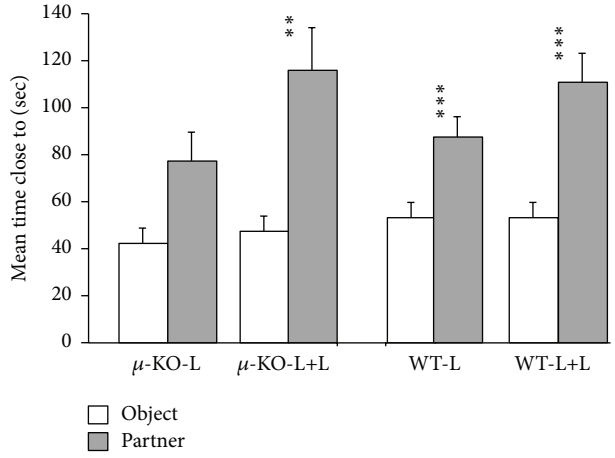


Figure 2: Mean time (+SEM) spent by juvenile male and female PND28 mice close to an object or to a same age/sex NMRI outbred partner in the Social Approach-Avoidance Test. *N*: 14–18 per group. μ-KO-L: *Oprm1*$^{-/-}$ pups reared by their mother [8 males + 6 females]; μ-KO-L+L: *Oprm1*$^{-/-}$ pups reared by their mother plus a second lactating female [8 males + 10 females]; WT-L: *Oprm1*$^{+/+}$ pups reared by their mother [8 males + 9 females]; WT-L+L: *Oprm1*$^{+/+}$ pups reared by their mother plus a second lactating female [8 males + 8 females]. Preference of partner versus object: $^{**}p < 0.01$, $^{***}p < 0.005$.

$p < 0.0001$) and by experimental condition during isolation (clean versus home-cage bedding: $F_{1/93} = 8.26$, $p < 0.01$). In addition, genotype × rearing condition ($F_{1/93} = 5.64$, $p < 0.05$), genotype × bedding ($F_{1/93} = 7.67$, $p < 0.01$), and genotype × rearing condition × bedding reached significant effects ($F_{1/93} = 4.64$, $p < 0.05$). These results confirmed previous data indicating no differences between home-cage and clean bedding exposure in μ-KO pups and confirmed that these KO pups emitted fewer calls, in comparison with WT animals, when in clean bedding. Splitting up the analysis by genotype to specifically assess the effects of L+L rearing on pups' USVs, it emerged that early enrichment was not able to modify the amount of calls in μ-KO mice, when isolated in a clean environment (μ-KO clean: $F_{1/24} = 0.01$, ns), but reduced USVs in all other groups (μ-KO nest: $F_{1/22} = 4.91$, $p < 0.05$; WT clean: $F_{1/25} = 14.89$, $p < 0.001$; WT nest: $F_{1/22} = 9.52$, $p < 0.01$).

3.3. Social Approach-Avoidance versus NMRI (PND28–30). Male and female adolescent mice did not differ for time spent close to the object or social stimulus contained in cylinders ($F_{1/57} = 0.53$, ns). The subsequent three-way ANOVA confirmed that all young mice spent more time close to conspecific rather than the object ($F_{1/61} = 31.58$, $p < 0.0001$). However, a significant effect of the rearing condition per se ($F_{1/61} = 5.21$, $p < 0.05$, Figure 2) emerged, together with a tendency towards a rearing condition × genotype ($F_{1/61} = 3.47$, $p = 0.07$) and rearing condition × stimulus effect ($F_{1/61} = 3.57$, $p = 0.06$). Considering together male and female performance, all experimental groups, but not μ-KO-L ($F_{1/13} = 2.27$, ns), spent more time close to the conspecific rather than the object (μ-KO-L+L: $F_{1/17} = 8.79$, $p < 0.01$; WT-L: $F_{1/16} = 11.5$, $p < 0.001$; WT-L+L: $F_{1/15} = 15.86$, $p < 0.01$).

3.4. Emotionality (PND75–90). Emotionality of adult WT and μ-KO mice was measured in the plus maze apparatus (Figure 3). No significant effect either of genotype, rearing condition, or sex was observed for the three parameters considered (TC + TO: $F_{1/58} = 1.98$, ns; rearing condition: $F_{1/58} = 2.59$, ns; sex: $F_{1/58} = 0.001$, ns; EO: genotype: $F_{1/58} = 0.37$, ns; rearing condition: $F_{1/58} = 3.35$, ns; sex: $F_{1/58} = 1.95$, ns; %TO: genotype: $F_{1/58} = 2.46$, ns; rearing condition: $F_{1/58} = 1.01$, ns; sex: $F_{1/58} = 2.58$, ns). In addition, no significant interaction effect was detected.

3.5. Social Recognition (PND90–110). Two relevant pieces of information emerge from data collected in this test: the first one refers to the interest in a social partner (total amount of social investigation) and the second one to the capability to recognize the same partner (decrease in investigation from session 1 to 4), from an unknown individual (session 4 versus 5). The general analysis indicated that all mice were able to recognize partners' familiarity, during repeated exposures, according to difference/reduction in time spent investigating it during different sessions ($F_{4/324} = 13.97$, $p < 0.0001$). However, significant genotype ($F_{1/81} = 8.78$, $p < 0.01$), sex ($F_{1/81} = 5.68$, $p < 0.01$), and genotype × rearing condition × session effects were detected ($F_{4/324} = 4.01$, $p < 0.01$).

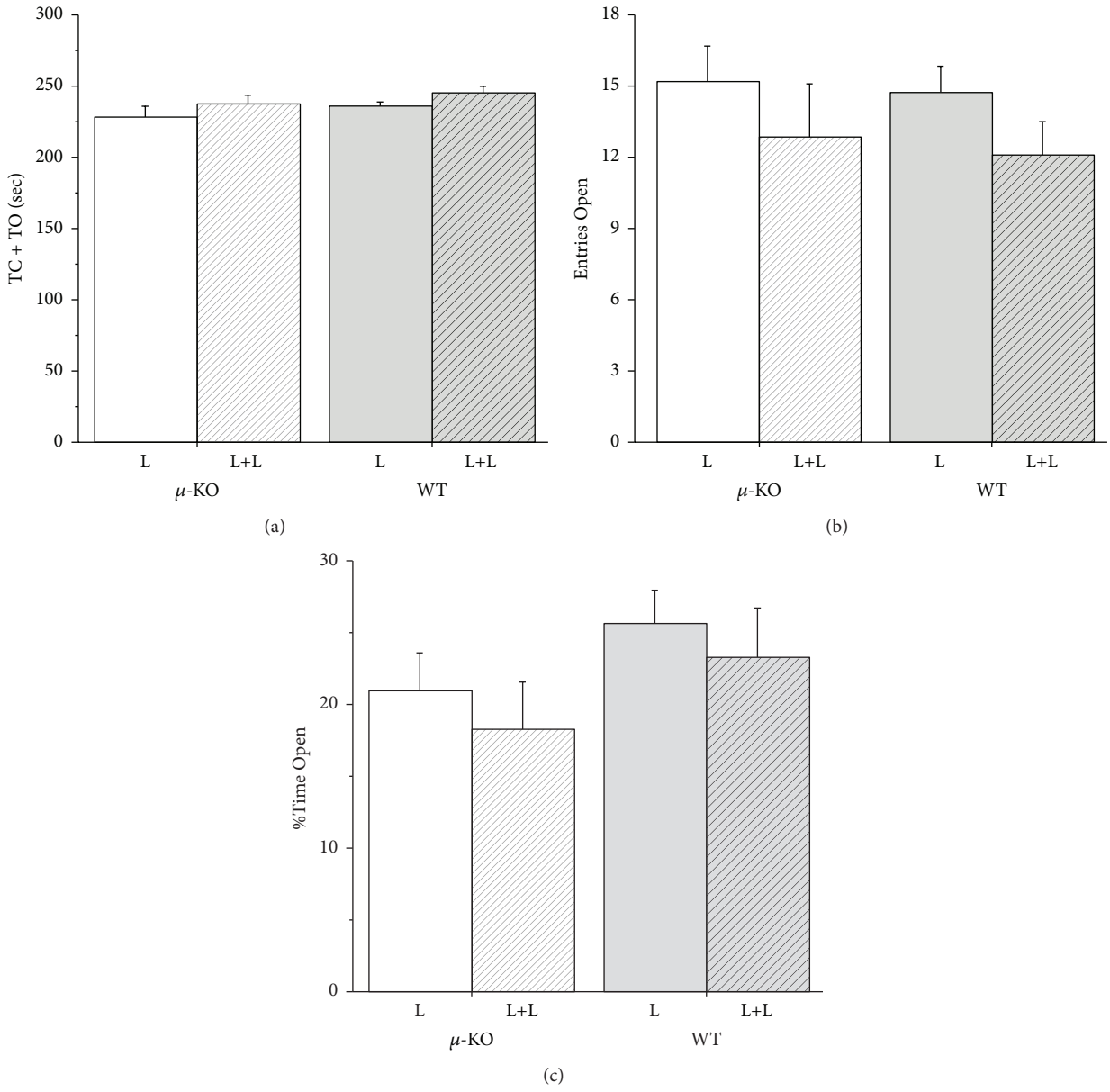


Figure 3: Adult mice emotionality measure in the plus maze apparatus: (a) Time Open + Time Closed, (b) number of entries in the open arms, and (c) percentage of time spent in the open arms. Data are presented as mean (+SEM). *N*: 11–20 per group. μ-KO-L: *Oprm1*$^{-/-}$ pups reared by their mother [9 males + 9 females]; μ-KO-L+L: *Oprm1*$^{-/-}$ pups reared by their mother plus a second lactating female [6 males + 5 females]; WT-L: *Oprm1*$^{+/+}$ pups reared by their mother [8 males + 12 females]; WT-L+L: *Oprm1*$^{+/+}$ pups reared by their mother plus a second lactating female [11 males + 6 females].

We analyzed separately these data in female and male mice, as shown in Figures 4(a) and 4(b), respectively. When considering the total amount of time spent investigating the partners in the 5 consecutive sessions (Figure 4(a), bar graph), no significant differences emerged for females according to the genotype ($F_{1/48} = 2.43$, ns), rearing conditions ($F_{1/48} = 0.01$, ns), and their interaction ($F_{1/48} = 0.38$, ns). Females (Figure 4(a), line graph) showed a significant time ($F_{4/192} = 7.21$, $p < 0.0001$) and a general interaction effect only (genotype × rearing condition × session: $F_{4/192} = 4.09$, $p < 0.01$), suggesting recognition of the unknown individual in the fifth session, an effect statistically significant in μ-KO-L and in WT-L and WT-L+L animals. As for males (Figure 4(b), bar graph), μ-KO spent less total time in social investigation in comparison with WT mice ($F_{1/33} = 6.57$, $p < 0.05$) and animals reared by double mothering scored higher than those reared in standard condition ($F_{1/33} = 5.10$, $p < 0.05$), with no significant interaction between factors ($F_{1/33} = 0.50$, ns). Investigating partner's recognition capability (Figure 4(b), line graph), significant genotype ($F_{1/132} = 5.38$, $p < 0.05$), rearing condition ($F_{1/33} = 4.10$, $p = 0.05$), and session effects emerged ($F_{4/132} = 6.54$, $p < 0.0001$), stressing very low levels

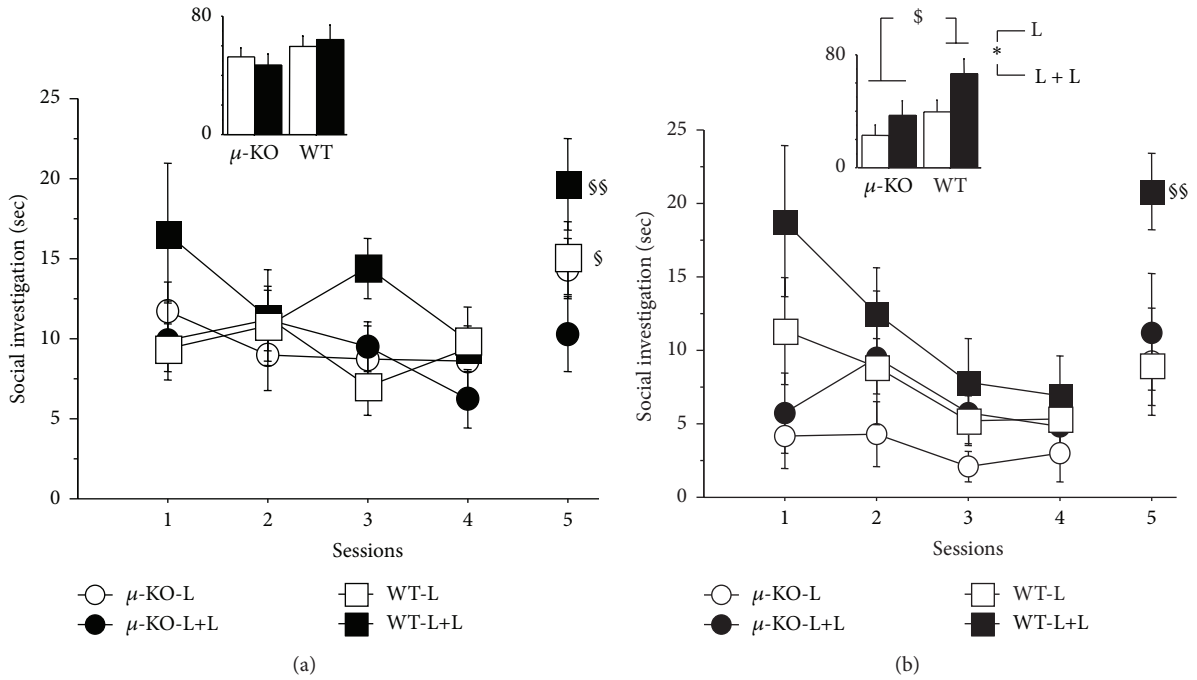


Figure 4: Mean time (+SEM) spent by female (a) and male (b) mice in social investigation of a younger same sex/genotype/standard reared intruder mouse during 5 consecutive 1 min social interaction sessions (line graph). The same intruder was used during the first 4 sessions, while an unknown one (same characteristics) was introduced in the fifth session. Histograms represent the total amount of investigation shown during the five sessions by different experimental groups. N: 8–15 per group. μ-KO-L: $Oprm1^{-/-}$ pups reared by their mother [10 males + 15 females]; μ-KO-L+L: $Oprm1^{-/-}$ pups reared by their mother plus a second lactating female [9 males + 11 females]; WT-L: $Oprm1^{+/+}$ pups reared by their mother [10 males + 15 females]; WT-L+L: $Oprm1^{+/+}$ pups reared by their mother plus a second lactating female [8 males + 11 females]. Genotype difference: $^{\$}p < 0.05$; rearing condition difference: $^{*}p < 0.05$; recognition of new partner (session 4 versus 5 and paired t-test): $^{\$}p < 0.05$ (μ-KO-L and WT-L females) and $^{\$\$}p < 0.01$ (μ-KO-L+L males and females).

of social investigation in μ-KO mice, and rearing condition (L+L versus L) increasing this behavior in both genotypes. Only WT males reared by two lactating females were able to discriminate an unknown from a familiar mouse (session 4 versus 5). Only 7 out of 89 residents showed some agonistic behavior (total 5-session score between 3.3 and 18.3 sec).

3.6. Partner Preference (PND90–110). Adult μ-KO and WT mice were tested in a modified version of the Approach-Avoidance Test where the subject was simultaneously exposed to mice with different genotypes. First of all, the total time spent close to partners (data not shown) was not affected by genotype ($F_{1/110} = 0.15$, ns) and sex of the subject ($F_{1/110} = 2.36$, ns) but was slightly reduced in animals reared by two females ($F_{1/110} = 4.31$, $p < 0.05$). We then tested whether there was a difference in partner preference (same line versus different line) according to the genotype and early experience of the subject. In Figures 5(a) and 5(b), the %preferences for WT in μ-KO and for μ-KO in WT female and male subjects are reported, respectively. All female groups, independently of genotype ($F_{1/52} = 0.51$, ns) and rearing condition ($F_{1/52} = 0.25$, ns), showed similar interest towards their female partners, whether the latter were μ-KO or WT. Males behaved differently. μ-KO showed preference, whereas WT males showed avoidance of males of the alien line ($F_{1/58} = 7.17$, $p < 0.01$), indicating a generalized avoidance of all subjects towards μ-KO male partners. No other significant effects emerged.

4. Discussion

Many studies have shown that environmental enrichment, once applied to animals previously reared in standard husbandry, is able to revert and/or prevent pathological conditions resulting from genetic, environmental, and pharmacological insults [26]. This experimental condition, highly variable across studies, includes generally sensory-motor stimulation that provides the animal with increased opportunities for physical exercise, learning experiences, and social interactions. Whether this early environmental enrichment (EEE) condition represents enrichment or rather, a reduction of deprivation condition, is not an issue considered here. Juvenile animals may utterly benefit from these additional stimulations especially when provided during the early postnatal life, a period of development characterized by neural plasticity. In this case, EEE may represent a possible alternative inexpensive treatment.

The mother represents the main component of the environment of an infant laboratory mouse and, in order to enhance stimulation during early life, we manipulated mother-infant interactions. We have already shown that this form of early environmental enrichment, consisting in

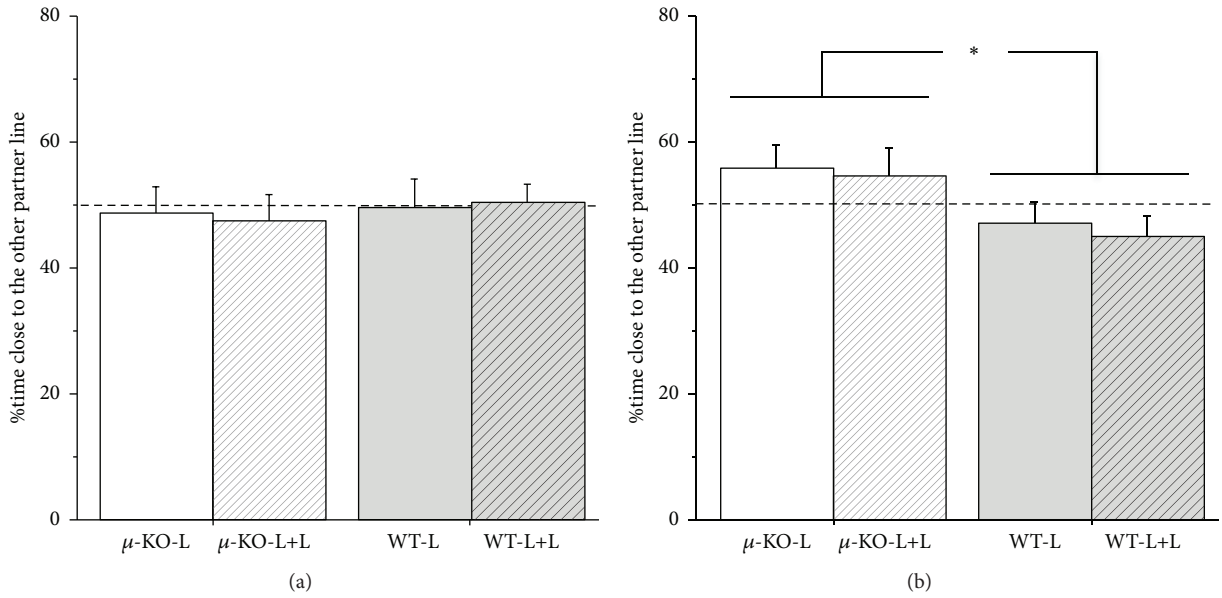


Figure 5: Mean (+SEM) percentage of time spent by adult females (a) and males (b) close to a same sex partner of the other homozygous line in a modified version of the Approach-Avoidance Test, where a μ-KO and a WT partner mouse were simultaneously presented to the experimental subject. The dotted line represents chance level (50%). *N*: 12–16 per group. μ-KO-L: *Oprm1*$^{-/-}$ pups reared by their mother [16 males + 16 females]; μ-KO-L+L: *Oprm1*$^{-/-}$ pups reared by their mother plus a second lactating female [15 males + 15 females]; WT-L: *Oprm1*$^{+/+}$ pups reared by their mother [16 males + 13 females]; WT-L+L: *Oprm1*$^{+/+}$ pups reared by their mother plus a second lactating female [15 males + 12 females]. Difference due to genotype $^{*}p < 0.05$.

housing pups with an additional lactating or nonlactating female from birth until weaning, exerts long-lasting beneficial effects on brain and behavior in outbred mice [16]. A similar approach has also been used in the Fmr1-KO mice and significant long-term beneficial effects on the pathological fragile X syndrome (FXS) phenotype have been detected [17]. Specifically, this rearing condition rescued Fmr1-KO adult mice deficits, namely, hyperactivity, social interactions, and cognitive deficits. In addition, early social enrichment also eliminated the abnormalities shown by adult Fmr1-KO mice in the morphology of hippocampal and amygdala dendritic spines. Importantly, this rearing condition did not induce neurobehavioral changes in WT mice, thus supporting specific effects on FXS-like pathology.

The aim of the present study was to evaluate whether being reared from birth in a socially enriched environment could also affect social motivation and behavior in a mouse model of social deficit: the μ-opioid knockout mice. These knockout animals have already been characterized for their deficits in social behavior from infancy onward and for this reason they seem to be the ideal candidates for this study. Firstly, we will discuss the results observed in WT animals to ascertain the impact of this early social enrichment on the social phenotype; then, we will assess whether this rearing condition is able to rescue the social deficits in this monogenic mouse model of autism [18].

We did not compare our dams' molecular and behavioral profile with that of females housed in EEE [27], but the presence of a second female in the breeding cage could represent a condition of environmental enrichment for the mother, as well as for the pups. As for pups, as a result of higher parental care, they might weigh more during development (as reported here) and at adulthood [16, 17]. In addition, tactile stimulation as well as olfactory and gustatory information from the adoptive female should expand the range of stimuli pups were exposed to from birth. Data presented here indicate that control pups reared by two lactating females (WT-L+L) vocalized less than standard reared wild type pups (WT-L) (Figure 1) but did not differ from them in their interest in conspecifics (Figure 2), emotionality (Figure 3), and partner preference (Figure 5). Double mothering increased social investigation in adult male WT mice, improving social competence (social recognition, Figure 4), an effect already reported in communal reared outbred mice [15].

In the present study, all animals in the breeding cages shared the same genotype: μ-KO and WT mice were maintained in homolines and the allomother had the same genotype as the mother-infant dyad. This could have restricted the variability of new stimuli supplied by the allomother to developing pups, since females with the same genotype may share similar physical and behavioral characteristics but should nevertheless have increased maternal cues, adding together stimuli from the two mothers. This strategy was selected to provide a quantitatively more significant stimulation to μ-KO pups to facilitate the development of infant attachment bond to this "super-mother," possibly by recruiting alternative neurobiological systems participating in the reward circuit. High levels of pup grooming, for example, should result in an increase of OTR expression in the MPOA [28, 29] and stroking behavior in adult rats activates hypothalamic

oxytocin neurons [30]. The higher amount of oxytocin released as a consequence of higher amount of dams' stimulation may activate the dopaminergic reward pathways in response to maternal cues, also in the absence of a functional μ-opioid system.

Data presented here partially support our hypothesis: being reared in a socially enriched environment seems to be able to rescue social motivational deficits shown by μ-opioid receptor knockout mice [20]. In fact, μ-KO pups reared by two lactating females showed (1) differential USVs emission, according to test context, and (2) preference for a social stimulus versus an object at weaning, as WT mice.

Isolated pups usually emit ultrasonic vocalizations, and the presence of familiar nest odors leads to a reduction of these calls [21, 31–34]. The low and similar number of USVs emitted by μ-KO pups in clean and nest condition suggested the following: (1) no calming effect of familiar cues and (2) lack of attachment behavior in these mutant pups [21]. Data presented here indicate that the emotional response to separation/isolation can be modified in μ-KO pups by rearing condition. While the effects of strongly aversive rearing conditions on pups' emotional responses were already known, the effects of an enriched/positive environment have more rarely been reported.

Double mothering could have quantitatively and qualitatively increased the amount of olfactory/tactile/gustatory and nursing stimulation, accelerating maturation processes and/or increasing stress thresholds. This hypothesis seems acceptable for WT animals, which showed a generalized tendency to vocalize less. The same occurred in μ-KO-L+L pups, which behaved as their WT-L+L controls, reducing isolation-induced USV emission in the presence of their home-cage odor. These results suggest that double mothering in μ-KO mice, by accelerating maturation processes and/or by providing additional maternal stimulation, restored the differential emotional response to mothers' cues presence. Home-cage bedding had a calming effect on μ-KO-L+L, whatever the contribution of the mother(s) and/or littermates in this process. The rescue of social motivation by early social enrichment emerged also from the social performance in juveniles (Figure 2). μ-KO-L+L mice behaved as WT animals, showing preference for the social versus the inanimate stimulus. It should be stressed that in this case the partner was an NMRI mouse, an albino conspecific with characteristics completely different and new for both WT and μ-KO subjects.

Contrary to what was expected on the basis of data from the literature that linked the amount of maternal care received with the HPA axis activity [35], rearing conditions did not modify emotionality in our mice, at least when measured in the plus maze apparatus. Similar results have already been reported in our previous studies [16], supporting the idea that it is not simply the amount of licking and grooming that epigenetically modulates gene transcription.

Social skills in adult animals were assessed in the social recognition test. This test allows evaluating, not only the amount of social interaction with unknown conspecifics, but also the mouse's ability to discriminate between known and unknown intruders. Females did not differ either for genotype or for early social environment in the total amount of social investigation towards the female intruder and generally recognized the new partner in the fifth/last social session, when the unknown subject was presented (but not the μ-KO-L+L group). As for males, the presence of the two lactating females increased social investigation in WT as well as in μ-KO mice, the latter showing very low interest in conspecifics in standard condition, confirming a reduced social motivation to interact with peers [20].

Finally, we wondered whether the reduced sociability of μ-KO animals might depend on their partners' characteristics. The results of the social preference test indicated that both WT and μ-KO mice preferred WT partners. This suggests that social performance of μ-KO mice could have been affected by the characteristics of their μ-KO partners in social tests (but not in the Approach-Avoidance Test, where an NMRI neutral animal was presented). The relevance of this result lays on the fact that partner's characteristics, and whether these match the experimental subject's preferences, are usually not considered. Sex, strain, food eaten, social rank, reproductive condition, and others are some of the characteristics that can affect the attractiveness of a partner for one particular subject [36]. It is possible that, in the presence of a different partner, we could improve social performance of "antisocial" animals as well, or we could improve sociability in these individuals through repeated interactions with preferred partners. This aspect has been investigated by Crawley's group in BTBR mice, a mouse strain characterized as an ASD model because of its poor sociability and repetitive social behaviors. Interestingly, they found no deficit in sensory inputs in the BTBR mouse but an improving effect of cohousing after weaning with the "social" C57/B6 mice [37].

Wild type and μ-KO mice that were used for the present experiments were derived from homozygous breeding pairs. If we consider the copresence of two μ-KO mothers to be equivalent to the caring work done by one wild type mother, if μ-KO mothers are to show any maternal care deficit, then we might not exclude the hypothesis that the early social enrichment has cured the phenotype of the homozygous *Oprm1*-deficient mother rather than that of the pup and juvenile −/− mice. However, we did not find differences in maternal care behavior between μ-KO and WT mothers in our previous study [20]. Therefore, we are prone to consider the effects of the social enrichment acting directly on the pups, although we do not exclude that also the mothers might have benefited from it. In future experiments it remains to be determined whether the present results can be replicated with pups derived from heterozygous mothers.

5. Conclusions

This experiment suggests that social environmental enrichment during early postnatal life can reduce stress during development and improve sociability in social defective subjects. We are not aware whether pups benefit directly from additional warmth, olfactory, tactile, or nutritional stimulation provided by the second dam in the cage or whether they received indirect benefits through their mother's more

relaxed state. To this purpose, particular attention has been devoted in this study to reducing conflict between the dams, housing them together, from mating onward. Whatever the causal mechanisms, μ-KO mice reared by their mother plus an additional lactating female showed increased social motivation from early age to adulthood. These results may encourage the investigation of the causal mechanisms underlying the rescue of social behavior we reported, in order to promote useful therapeutic interventions for all those developmental psychopathologies characterized by social malfunctioning.

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 Ben Spruit for collaborating in the collection of experimental data in this study. This study has been partially supported by funds from Regione Lazio for "Sviluppo della Ricerca sul Cervello."

References

[1] American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders*, American Psychiatric Publishing, Arlington, Va, USA, 5th edition, 2013.

[2] R. L. Findling, "Pharmacologic treatment of behavioral symptoms in autism and pervasive developmental disorders," *Journal of Clinical Psychiatry*, vol. 66, supplement 10, pp. 26–31, 2005.

[3] S. Levine, A. Kodesh, Y. Goldberg et al., "Initial severity and efficacy of risperidone in autism: results from the RUPP trial," *European Psychiatry*, vol. 32, pp. 16–20, 2016.

[4] O. Peñagarikano, B. S. Abrahams, E. I. Herman et al., "Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits," *Cell*, vol. 147, no. 1, pp. 235–246, 2011.

[5] F. Cirulli, A. Berry, L. T. Bonsignore et al., "Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels," *Neuroscience and Biobehavioral Reviews*, vol. 34, no. 6, pp. 808–820, 2010.

[6] V. Doulames, S. Lee, and T. B. Shea, "Environmental enrichment and social interaction improve cognitive function and decrease reactive oxidative species in normal adult mice," *International Journal of Neuroscience*, vol. 124, no. 5, pp. 369–376, 2014.

[7] G. Laviola, M. Rea, S. Morley-Fletcher et al., "Beneficial effects of enriched environment on adolescent rats from stressed pregnancies," *European Journal of Neuroscience*, vol. 20, no. 6, pp. 1655–1664, 2004.

[8] E. M. Marco, S. MacRì, and G. Laviola, "Critical age windows for neurodevelopmental psychiatric disorders: evidence from animal models," *Neurotoxicity Research*, vol. 19, no. 2, pp. 286–307, 2011.

[9] A. Bartolomucci, L. Gioiosa, A. Chirieleison, G. Ceresini, S. Parmigiani, and P. Palanza, "Cross fostering in mice: behavioral and physiological carry-over effects in adulthood," *Genes, Brain and Behavior*, vol. 3, no. 2, pp. 115–122, 2004.

[10] L. Lu, T. Mamiya, P. Lu et al., "The long-lasting effects of cross-fostering on the emotional behavior in ICR mice," *Behavioural Brain Research*, vol. 198, no. 1, pp. 172–178, 2009.

[11] O. Malkesman, Y. Lavi-Avnon, R. Maayan, and A. Weizman, "A cross-fostering study in a genetic animal model of depression: maternal behavior and depression-like symptoms," *Pharmacology Biochemistry and Behavior*, vol. 91, no. 1, pp. 1–8, 2008.

[12] P. A. Matthews, A.-M. Samuelsson, P. Seed et al., "Fostering in mice induces cardiovascular and metabolic dysfunction in adulthood," *Journal of Physiology*, vol. 589, no. 16, pp. 3969–3981, 2011.

[13] I. Branchi and E. Alleva, "Communal nesting, an early social enrichment, increases the adult anxiety-like response and shapes the role of social context in modulating the emotional behavior," *Behavioural Brain Research*, vol. 172, no. 2, pp. 299–306, 2006.

[14] I. Branchi and F. Cirulli, "Early experiences: building up the tools to face the challenges of adult life," *Developmental Psychobiology*, vol. 56, no. 8, pp. 1661–1674, 2014.

[15] I. D'Andrea, E. Alleva, and I. Branchi, "Communal nesting, an early social enrichment, affects social competences but not learning and memory abilities at adulthood," *Behavioural Brain Research*, vol. 183, no. 1, pp. 60–66, 2007.

[16] F. R. D'Amato, C. Zanettini, C. Sgobio et al., "Intensification of maternal care by double-mothering boosts cognitive function and hippocampal morphology in the adult offspring," *Hippocampus*, vol. 21, no. 3, pp. 298–308, 2011.

[17] D. Oddi, E. Subashi, S. Middei et al., "Early social enrichment rescues adult behavioral and brain abnormalities in a mouse model of fragile X syndrome," *Neuropsychopharmacology*, vol. 40, pp. 1113–1122, 2015.

[18] D. Oddi, W. E. Crusio, F. R. D'Amato, and S. Pietropaolo, "Monogenic mouse models of social dysfunction: implications for autism," *Behavioural Brain Research*, vol. 251, pp. 75–84, 2013.

[19] J. A. J. Becker, D. Clesse, C. Spiegelhalter, Y. Schwab, J. Le Merrer, and B. L. Kieffer, "Autistic-like syndrome in mu opioid receptor null mice is relieved by facilitated mGluR4 activity," *Neuropsychopharmacology*, vol. 39, no. 9, pp. 2049–2060, 2014.

[20] C. Cinque, S. Pondiki, D. Oddi et al., "Modeling socially anhedonic syndromes: genetic and pharmacological manipulation of opioid neurotransmission in mice," *Translational Psychiatry*, vol. 2, article e155, 2012.

[21] A. Moles, B. L. Kieffer, and F. R. D'Amato, "Deficit in attachment behavior in mice lacking the μ-opioid receptor gene," *Science*, vol. 304, no. 5679, pp. 1983–1986, 2004.

[22] H. W. D. Matthes, R. Maldonado, F. Simonin et al., "Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene," *Nature*, vol. 383, no. 6603, pp. 822–823, 1996.

[23] J. F. Cryan and A. Holmes, "The ascent of mouse: advances in modelling human depression and anxiety," *Nature Reviews Drug Discovery*, vol. 4, no. 9, pp. 775–790, 2005.

[24] J. N. Ferguson, L. J. Young, E. F. Hearn, M. M. Matzuk, T. R. Insel, and J. T. Winslow, "Social amnesia in mice lacking the oxytocin gene," *Nature Genetics*, vol. 25, no. 3, pp. 284–288, 2000.

[25] J. T. Winslow and F. Camacho, "Cholinergic modulation of a decrement in social investigation following repeated contacts between mice," *Psychopharmacology*, vol. 121, no. 2, pp. 164–172, 1995.

[26] G. Laviola, A. J. Hannan, S. Macrì, M. Solinas, and M. Jaber, "Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders," *Neurobiology of Disease*, vol. 31, no. 2, pp. 159–168, 2008.

[27] A. J. Hannan, "Review: environmental enrichment and brain repair: harnessing the therapeutic effects of cognitive stimulation and physical activity to enhance experience-dependent plasticity," *Neuropathology and Applied Neurobiology*, vol. 40, no. 1, pp. 13–25, 2014.

[28] F. Champagne, J. Diorio, S. Sharma, and M. J. Meaney, "Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 22, pp. 12736–12741, 2001.

[29] 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.

[30] S. Okabe, M. Yoshida, Y. Takayanagi, and T. Onaka, "Activation of hypothalamic oxytocin neurons following tactile stimuli in rats," *Neuroscience Letters*, vol. 600, pp. 22–27, 2015.

[31] I. Branchi, D. Santucci, A. Vitale, and E. Alleva, "Ultrasonic vocalizations by infant laboratory mice: a preliminary spectrographic characterization under different conditions," *Developmental Psychobiology*, vol. 33, no. 3, pp. 249–256, 1998.

[32] A. Marchlewska-Koj, J. Kapusta, and P. Olejniczak, "Ultrasonic response of CBA newborn mice to bedding odour," *Behaviour*, vol. 136, no. 3, pp. 269–278, 1999.

[33] J. Kapusta and H. Szentgyörgyi, "Ultrasonic responses of CBA pups to the odour of genetically different mice," *Behaviour*, vol. 141, no. 2, pp. 157–167, 2004.

[34] C. Zanettini, V. Carola, L. Lo Iacono, A. Moles, C. Gross, and F. R. D'Amato, "Postnatal handling reverses social anxiety in serotonin receptor 1A knockout mice," *Genes, Brain and Behavior*, vol. 9, no. 1, pp. 26–32, 2010.

[35] 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. 6, p. 1140, 2013.

[36] A. Moles and F. R. D'Amato, "Ultrasonic vocalization by female mice in the presence of a conspecific carrying food cues," *Animal Behaviour*, vol. 60, no. 5, pp. 689–694, 2000.

[37] M. Yang, K. Perry, M. D. Weber, A. M. Katz, and J. N. Crawley, "Social peers rescue autism-relevant sociability deficits in adolescent mice," *Autism Research*, vol. 4, no. 1, pp. 17–27, 2011.