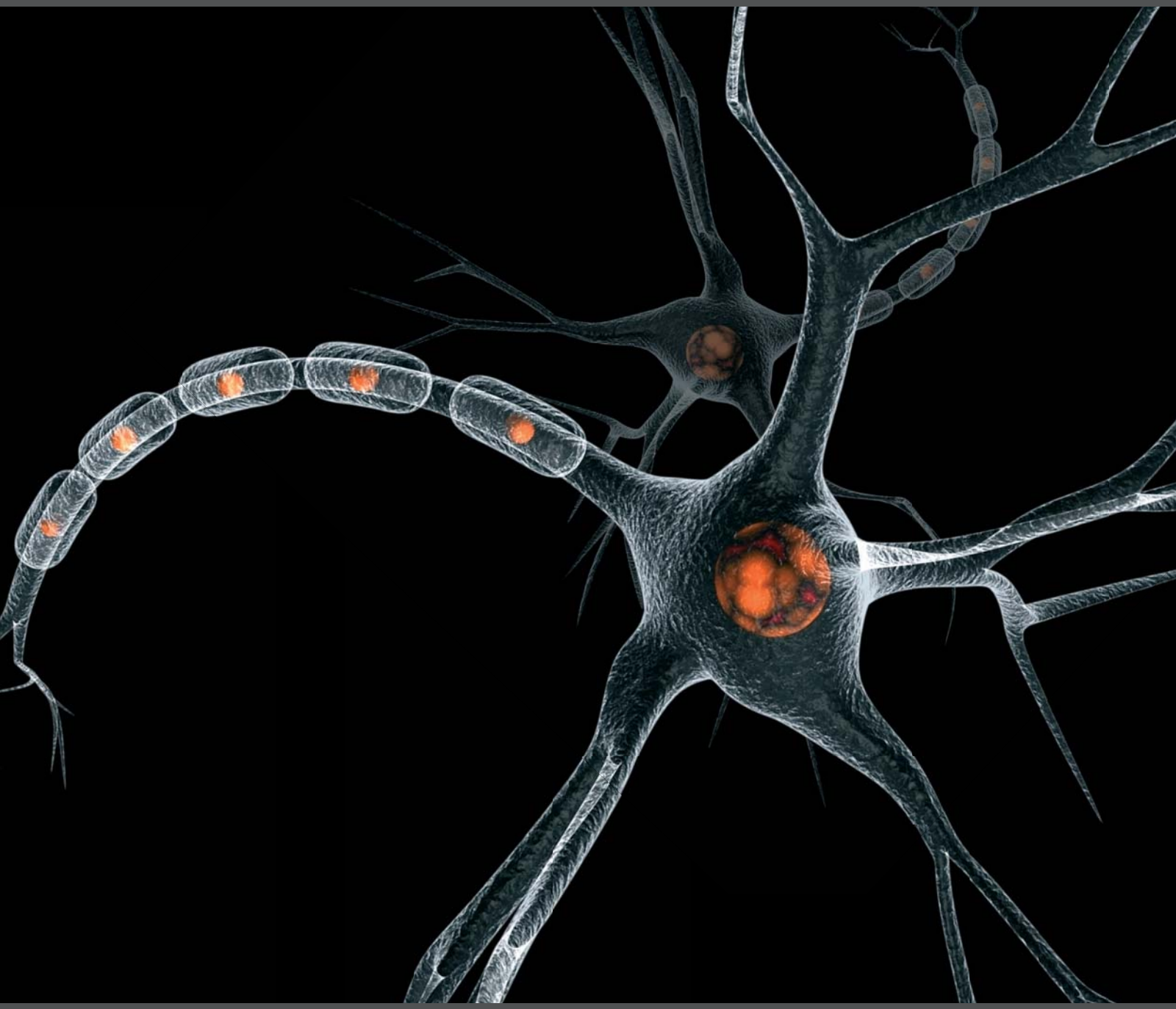
Neural Plasticity

GABAergic Circuit Development and Its Implication for CNS Disorders

Guest Editors: Graziella Di Cristo, Tommaso Pizzorusso, Laura Cancedda, and Evelyne Sernagor



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Editorial Board

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Editorial

GABAergic Circuit Development and Its Implication for CNS Disorders

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The function of the cerebral cortex requires the coordinated action of two major neuronal subtypes, the glutamatergic projection neurons and the GABAergic interneurons. Although, in terms of numbers, GABAergic interneurons represent a minor cell population compared to glutamatergic neurons in the neocortex, they play an important role in modulating network dynamics of neocortical circuits. Indeed, GABAergic interneurons have been shown to control neuronal excitability and integration, and they have been implicated in the generation of temporal synchrony and oscillatory behavior among networks of pyramidal neurons. Such oscillations within and across neural systems are believed to serve various complex functions, such as perception, movement initiation, and memory. Recently, the development of GABAergic inhibition has been shown to be a key determinant for critical period plasticity of cortical circuits. Critical periods represent heightened epochs of brain plasticity, during which experience can produce permanent, large-scale changes in neuronal circuits. Experience-dependent refinement of neural circuits has been described in many regions within the CNS, suggesting it is a fundamental mechanism for normal vertebrate CNS development. By regulating the onset and closure of critical periods, GABAergic interneurons may influence how experience shapes brain wiring during early life and adolescence.

Considering the multifaceted role played by GABAergic cells in the development, function, and plasticity of neural circuits, it is not surprising that alterations in the development of GABAergic circuits per se have been implicated in various neurodevelopmental and psychiatric disorders such as schizophrenia, autism, and epilepsy. However, how modification of GABAergic circuit development contributes to specific pathologies is largely unknown. Furthermore, GABA mimetic drugs, such as benzodiazepines and certain antiepileptic drugs, are widely used in clinical practice, but whether and to what extent these drugs cause deleterious effect on the developing brain is still not clear. A better comprehension of the mechanisms underlying the development and plasticity of GABAergic interneurons will likely indicate which cellular substrates might be affected in neurodevelopmental disorders. At the same time, identifying the genetics variants implicated in these disorders may generate major new insights into the normal and pathological function of GABAergic circuits.

Our understanding of GABAergic interneurons function is challenged by their startling heterogeneity; indeed, different subtypes of interneurons display distinct morphology, physiological properties, connectivity patterns, and biochemical constituents. Recent technical advances have significantly accelerated progress in this field. In particular,

the development of genetic strategies based on interneuron cell type-specific promoters and fluorescent protein reporters has allowed efficient high-resolution labelling of specific GABAergic interneuron classes in intact or semi-intact tissues, such as organotypic brain cultures.

Contributions to this special issue of provide an overview of recent discoveries in the field of GABAergic circuit development and related brain disorders. The genetic program for the construction of cortical GABAergic network is initiated early during brain development, and it orchestrates cell type specification, migration, and some aspects of synaptic connectivity. On the other hand, the establishment of mature patterns of GABAergic innervation and inhibitory transmission is not achieved until adolescence and is profoundly influenced by neuronal activity and experience. E. Rossignol describes the tightly controlled genetic cascades that determine the great diversity of cortical GABAergic interneurons and how dysfunctions in genes important for their generation, specification, and maturation might contribute to various neurodevelopmental disorders. B. Chattopadhyaya describes the molecular mechanisms underlying the activity-dependent maturation of GABAergic innervation in the postnatal brain.

Several articles in the special issue have investigated the evidence linking dysfunction in GABAergic signaling and plasticity to specific neurodevelopment disorders, such as autism (R. Pizzarelli and E. Cherubini, J. LeBlanc and M. Fagiolini, L. Baroncelli et al.), schizophrenia (G. Gonzales-Burgos et al.), and epilepsy (Griggs and Galanopoulou). The developmental role of GABAergic circuits is not limited either to the brain or to the developmental phase. A. E. Allain et al. discuss the role of GABA and GABAergic receptors in motoneuron development and in immature hypoglossal motoneurons of the spastic mouse, a model of human hyperekplexic syndrome. B. Imbrosci and T. Mittman describe the response of the GABAergic system to cortical injuries in the adult and how this response could be manipulated to help the functional recovery of patients.

In the last decades, cell-based therapies using GABAergic neuronal grafts have emerged as a promising treatment, since they may restore the lost equilibrium by cellular replacement of the missing/altered inhibitory neurons or modulating the hyperactive excitatory system. Advances in this field are reviewed by V. Broccoli and M. Dolado.

It is becoming increasingly clear that the strength of GABAergic synaptic transmission is dynamic. R. Wright et al. review some of the sophisticated ways in which GABA-A receptor driving force can vary within neuronal circuits. P. Méndez and A. Bacci discuss the plasticity and modulation of adult cortical and hippocampal GABAergic synaptic transmission, while P. Wenner describes new insight into the mechanisms of GABAergic homeostatis in developing motor networks. Finally, A. Ludwig et al. provide evidence that the trophin nurturin is implicated in the developmental regulation of the cotransporter KCC2, a key molecular player in the establishment of the chloride-gradient, which in turn regulates the strength of GABAergic transmission.

We hope that this special issue will serve to emphasize the new technical and conceptual advances in the field of GABAergic circuits development and to highlight the importance of this network for neurological disorders.

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Review Article

Genetics and Function of Neocortical GABAergic Interneurons in Neurodevelopmental Disorders

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A dysfunction of cortical and limbic GABAergic circuits has been postulated to contribute to multiple neurodevelopmental disorders in humans, including schizophrenia, autism, and epilepsy. In the current paper, I summarize the characteristics that underlie the great diversity of cortical GABAergic interneurons and explore how the multiple roles of these cells in developing and mature circuits might contribute to the aforementioned disorders. Furthermore, I review the tightly controlled genetic cascades that determine the fate of cortical interneurons and summarize how the dysfunction of genes important for the generation, specification, maturation, and function of cortical interneurons might contribute to these disorders.

1. Introduction

The exquisite complexity of cognitive functions stems from tightly regulated interactions between distributed cortical networks performing precise neural computations. GABAergic inhibitory interneurons (INs), which represent a minority of neocortical neurons (20% in rodents [1]), play a crucial role in these cortical circuits. GABAergic INs shape the responses of pyramidal cells to incoming inputs, prevent runaway excitation, refine cortical receptive fields, and are involved in the timing and synchronisation of population rhythms expressed as cortical oscillations [2–9]. Consequently, disruption of cortical GABAergic IN function has been linked to various neurodevelopmental disorders, including epilepsy, mental retardation, autism, and schizophrenia [10–15].

Cortical INs are diverse in terms of their anatomical laminar distribution, histochemical marker expression, intrinsic physiological properties, and connectivity (Figure 1) [5, 6, 9, 16–22]. This heterogeneity is characterized by the expression of specific combinations of ion channels, receptors, and membrane cell adhesion molecules [7]. These specific protein expression profiles are the result of tightly controlled genetic pathways that regulate cortical IN identity [8, 23–29]. Anomalies in these genetic pathways might therefore underlie some of the neurodevelopmental and neurocognitive disorders seen in humans. In the current paper, I will give an overview of cortical IN diversity, summarise the various roles of cortical INs in neuronal circuit development and function, review the genetic pathways involved in specifying cortical GABAergic IN diversity, and explore the pathological correlates of genetic anomalies leading to interneuron dysfunction in rodents and humans. As the current paper focuses on neocortical INs, readers are directed to other sources for a broader description of other GABAergic populations, including those of the amygdala, striatum, hippocampus, thalamus, and olfactory bulbs, which also participate in the corticolimbic and corticosubcortical circuits involved in cognition and emotional processing [7, 30–40].

1.1. Diversity of Cortical GABAergic Interneurons Subtypes and Roles. Neocortical GABAergic INs are heterogeneous, and different subtypes of INs have different spatial and temporal origins. As a group, neocortical INs are derived from transient ventral telencephalic structures referred to as the

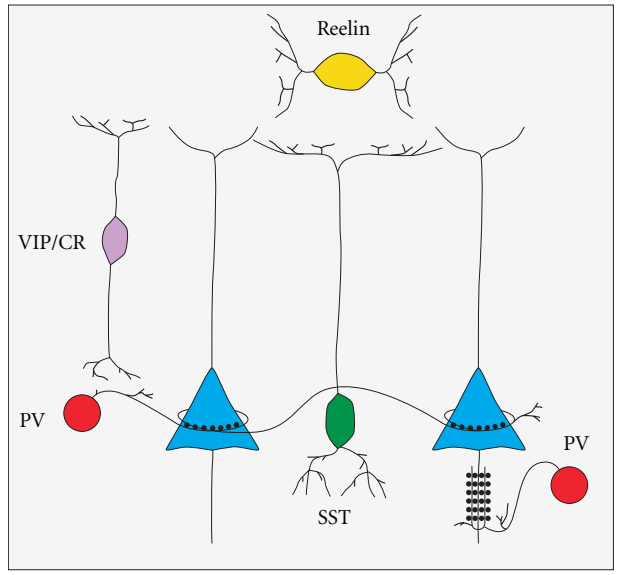

Figure 1: Interneuron diversity. Interneurons are diverse in terms of their histochemical profile, morphology, physiological properties, and connectivity. In this schematic representation, parvalbumin-positive (PV) interneurons (red) include basket cells forming perisomatic contacts on adjacent pyramidal cells (dark blue), as well as chandelier cells that target the pyramidal cell axon initial segment. Somatostatin-positive (SST) interneurons include Martinotti cells that contact pyramidal cell dendrites in layer I. Vasointestinal peptide (VIP) and calretinin (CR) double-positive bitufted interneurons target pyramidal cells and other interneurons. Neurogliaform cells, marked with reelin, are the most abundant interneurons in layer I and provide tonic GABAergic inhibition via volume transmission of GABA.

ganglionic eminences [27, 29, 41–46] as well as from the preoptic area [47]. The medial ganglionic eminence (MGE) produces approximately 70% of neocortical INs, including the parvalbumin-positive (PV) fast-spiking interneurons and the somatostatin-positive (SST) interneurons, which represent 40% and 30% of all neocortical INs, respectively [27, 46, 48]. By contrast, the caudal ganglionic eminence (CGE) gives rise to the remaining 30% of neocortical INs, a more heterogeneous group of cortical INs that share the unique expression of 5HT3A ionotropic serotoninergic receptors, rendering them highly responsive to the neuromodulatory effects of serotonin [9, 43, 46, 48, 49]. A majority of CGE-derived interneurons belong to either the reelin-positive multipolar population (including the late-spiking neurogliaform cells), the vasointestinal-peptide- (VIP-) positive bitufted population (including a calretinin- (CR)-positive population), or the VIP-positive, calretinin-negative bipolar population. Finally, the preoptic area contributes a small portion of neocortical INs (<3%) that are not labelled by the usual interneuron markers mentioned above [47]. The lateral ganglionic eminence (LGE) mainly produces olfactory bulb and amygdalar INs, as well as striatal and nucleus accumbens medium spiny neurons, but is generally thought not to give rise to cortical INs [23, 33, 38, 41, 50, 51]. Different subtypes of cortical INs are identified based on their immunohistochemical, morphological, physiological, and connectivity properties, and they mediate different functions in mature networks as detailed below.

1.2. Parvalbumin-Positive Basket Cells. PV-positive interneurons include the perisomatically targeting basket cells and the less abundant axon-initial segment-targeting chandelier cells. PV-positive basket cells can be further divided according to various morphological characteristics, including somatic diameter, firing properties, and extent of dendritic and axonal arborisation [5, 9, 52, 53]. As a group, PV-positive basket cells display many characteristics which render them one of the fastest and most reliable sources of inhibition in the cortex. They exhibit low input resistance, fast membrane kinetics, brief action potentials with large afterhyperpolarisation, and minimal spike adaptation and are able to sustain high frequency firing rates [5, 18, 19, 21, 44, 52, 54]. These fast kinetics are partly due to their expression of Kv3 voltage-gated potassium channels [52, 55–59], which ensure quick repolarisation and termination of action potentials. In addition, PV-positive fast-spiking cells mediate fast reliable neurotransmission, as they rely mainly on P/Q-type presynaptic Ca^{2+} channels for tight coupling between action potentials and neurotransmitter release [60–63]. Furthermore, these PV-positive basket cells might be able to buffer calcium more efficiently, as they express high levels of Ca^{2+}-binding proteins, including parvalbumin and calbindin. It is possible that this expression of Ca^{2+}-binding proteins renders these cells more resistant to Ca^{2+}-induced excitotoxicity in the face of high firing rates.

PV-positive INs are the main inhibitory target of thalamocortical projections in the cortex. In addition, PV-positive basket cells form intricate nests of synaptic contacts on the soma of adjacent pyramidal cells, giving them rapid control over the excitability of their pyramidal cell targets. These INs are therefore well positioned to provide strong and fast feedforward inhibition to adjacent pyramidal cells, limiting

the time window for temporal summation of excitatory inputs and spike generation within populations of pyramidal cells. This feature sharpens the cortical response and prevents runaway excitation following thalamocortical excitation [64–68].

In addition, PV-positive basket cells are highly interconnected with one another through both chemical and electric synapses (gap junctions), creating a vast web of synchronously active INs [69, 70]. This network of inhibitory INs triggers and maintains high-frequency gamma oscillations within ensembles of cortical pyramidal cells [69, 71–77]. In support of this, the loss of connexin32, which forms gap junction connections between PV-positive INs, results in the partial loss of task-induced gamma oscillations [78]. Gamma oscillations are important for the maintenance of attention, working memory, and the refinement of executive functions in humans and rodents [79–83]. Therefore, PV-positive interneuron dysfunction has been postulated to underlie the loss of gamma oscillations in schizophrenic patients displaying working memory and executive function abnormalities [14, 82, 84, 85].

1.3. Parvalbumin-Positive Chandelier Cells. Like PV-positive basket cells, PV-positive chandelier cells display brief non-adapting trains of action potentials upon stimulation and are able to sustain high frequency firing rates [17, 86, 87]. They are characterised morphologically by their cartridges of vertically oriented candlestick-like axonal arbors [88–90]) forming synapses on the axon initial segment (AIS) of pyramidal cells [89, 91]. Chandelier cells are unusual among interneurons in that their output has been postulated to be excitatory rather than inhibitory. Indeed, the stimulation of chandelier cells triggers depolarisations in target pyramidal cells in the cortex and dentate gyrus [92–95]. This has been attributed to the high concentration of chloride and elevated $GABA_A$ reversal potential at the AIS, due to efficient Cl^- import by the NKCC1 transporter in the absence of the KCC2 transporter (see below) [92, 93]. However, it is still unclear whether such depolarizing responses are obligatorily excitatory [95]. Furthermore, in other circuits, such as in the CA1 region of the hippocampus, chandelier cells appear to trigger hyperpolarising responses [96]. Overall, the net effect of chandelier cells might be dependent on the local state of network activity and on the particular ion channel composition of local pyramidal cells in different brain regions. In vivo, chandelier cells might be involved in the generation of specific oscillatory activities as they fire immediately before hippocampal pyramidal cells during sharp-wave-associated ripples [97].

1.4. Somatostatin-Positive (SST) Interneurons. Somatostatin-positive interneurons, including Martinotti cells and non-Martinotti cells, are heterogeneous in terms of their immunohistochemical profile (variable colabelling with calretinin and calbindin), morphology (multipolar, bipolar, or unipolar), axonal projections (most target pyramidal cell dendrites in layer I but some project locally within their cortical layer), and intrinsic electrophysiological properties [9, 45, 98–100]. A majority of SST interneurons (including the Martinotti cells) share some physiological characteristics, including a low spike threshold, prominent after-hyperpolarisation, and spike rate adaptation. However, these cells differ in their spiking pattern at threshold (regular versus bursting), especially when fired from hyperpolarized step currents [17, 98, 99]. In general, compared to fast-spiking basket cells, SST-positive interneurons tend to be more excitable: they display a lower spike threshold and have a higher resting membrane potential [101]. One exception to this rule is a population of non-Martinotti cells, which have a high firing threshold, higher firing rate, shorter spike half width, and lower input resistance [45, 99]. These cells have been mostly described in layer 4 of the cortex and are preferentially labelled in the X94 GAD67-GFP transgenic line [9, 99].

SST-positive Martinotti cells are found across cortical layers II-VI but are most abundant in cortical layer V. They project vertically towards layer I where they contact pyramidal cell dendrites and extend multiple axonal collaterals towards adjacent cortical columns [19, 102, 103]. Martinotti cells regulate pyramidal cell excitability by controlling the dendritic summation and integration of synaptic inputs and sharpening the coding of stimulus intensity [104]. Furthermore, as their connectivity is simultaneously divergent, convergent, and recurrent, they mediate disynaptic inhibition between interconnected pyramidal cells as well as recurrent feedback inhibition onto presynaptic pyramidal cells [105, 106]. They are therefore well suited to prevent excessive and recurrent excitation within cortical networks. Furthermore, they are increasingly recruited by sustained stimulation, owing to the fact that the synapses that they receive from pyramidal cells are in most cases facilitating [101, 107]. This renders them good candidates to dampen excitation during high activation states. Dysfunction of somatostatin cells has therefore been postulated to underlie some forms of experimental or poststatus epilepticus seizure disorders [108].

Because of their expression of low-threshold voltage-gated calcium channels and persistent sodium currents, about 40% of SST cells display intrinsic bursting abilities and might act as pacemaker cells, thereby triggering particular cortical oscillations [224]. Indeed, SST-positive cells, which are highly interconnected via gap junctions, tend to oscillate spontaneously in the theta range (3–9 Hz) when stimulated electrically or with cholinergic agonists in vitro [101]. They could therefore be involved in pacing cortical pyramidal cells in the theta range.

1.5. Vasoactive Intestinal Peptide- (VIP-) Positive Interneurons. CGE-derived interneurons tend to populate more superficial cortical layers than MGE-derived interneurons. Approximately 40% of CGE INs express VIP, and these cells tend to be enriched in layers II/III [19, 46, 48, 225, 226]. VIP-positive INs are diverse morphologically, histochemically, and physiologically [9]. The most abundant type are the bitufted VIP+ INs that tend to colabel with CR [46, 48], display an irregular-spiking firing pattern near threshold [18, 46, 48, 49, 227], and send a downward projecting axon towards deeper cortical layers. The second most abundant

type is the VIP+, CR− bipolar cells, which display a fast adapting firing pattern [44, 46, 48, 227] and send extensively branched projections both locally and towards deep cortical layers. Due to their high input resistance, VIP INs tend to be highly excitable [18, 46, 48]. They have been shown to target pyramidal cell dendrites and somata [17], but some subsets appear to target other interneurons more preferentially [228–230]. The precise function of VIP interneurons in cortical networks remains to be determined. However, their physiological characteristics and diverse synaptic targets render them well suited to rapidly modulate the interactions between pyramidal cells and MGE-derived interneurons. Furthermore, as they receive strong input from pyramidal cells in layers II-III [231], which also receive input from pyramidal cells in other functionally connected cortical areas, VIP interneurons might be important in regulating cross-cortical communication (i.e., sensorimotor modulation where inputs from the sensory cortex modulate the output of cortical motoneurons).

1.6. Neurogliaform Cells. Neocortical neurogliaform cells exist in all cortical layers but are the most abundant GABAergic population in superficial layer I [46, 48]. They express reelin (as well as alpha actinin 2 in the rat [103, 232]), but not VIP or SST [9, 46]. They are morphologically distinct as they have multiple radially oriented dendrites extending from a small round soma, as well as a finely branched dense axonal plexus typically extending well beyond the dendritic tree, giving them a spider web appearance [19, 233]. Neurogliaform cells display late-spiking firing patterns with spike accommodation during sustained depolarisations [19, 44, 45, 48, 234]. They have been shown to elicit slow long-lasting inhibitory events (IPSPs) in pyramidal cells and other interneurons by activating both $GABA_A$ and $GABA_B$ receptors after nonsynaptic volume release of GABA [233–235]. Some of this tonic inhibition is thought to be mediated through the activation of delta subunit-containing $GABA_A$ receptors, which are modulated by neurosteroids [235]. This effect might underlie the antiepileptic effect of steroids used to treat pharmacoresistant epilepsies [236, 237]. Furthermore, neurogliaform cells are extensively interconnected by electrical gap-junction synapses but also contact most other interneurons subtypes via similar electrical synapses [238–240]. They are therefore well suited to shape synchronous cortical oscillations. Finally, some neurogliaform cells release nitric oxide, a potent vasodilator, and may therefore play a role in the neurovascular adjustment of blood flow in the face of cerebral hypoperfusion (i.e., strokes, shock, etc.) [241, 242].

2. The Development of Cortical Interneurons Depends on Tightly Regulated Genetic Cascades

Cortical interneurons originate in the ventricular zone of the ventral telencephalic ganglionic eminences [41, 43, 243], migrate tangentially up to the cortex [218, 244], and reach their final destination after radial migration across cortical layers. This is quite distinct from cortical pyramidal cells, which originate from the cortical ventricular zone, migrate radially, and reach their final position after a brief bout of tangential migration [42, 245]. The ganglionic eminences are divided into three different subdomains, the medial (MGE), caudal (CGE), and lateral (LGE) ganglionic eminences, which produce distinct subtypes of interneurons in a temporally dynamic fashion [44–46, 246]. Cortical interneurons originate from the MGE and CGE [41, 43, 243], as well as from the preoptic area [47]. Although this has been debated, it is generally believed that the LGE does not give rise to cortical interneurons, instead generating medium spiny neurons of the striatum, nucleus accumbens, and olfactory tubercules, as well as olfactory bulb and amygdalar interneurons [23, 33, 38, 41, 50, 51].

The genetic code that governs the generation and specification of cortical interneurons has been extensively studied over the last decade (Figure 2). The *Dlx* homeobox genes, including *Dlx1/2* and *Dlx5/6,* encode a family of transcription factors crucial for the generation, specification, and migration of all interneurons. The proneural gene *mammalian achaete-scute homolog 1* (*Mash1*), which encodes a basic helix-loop-helix transcription factor, is also crucial for these processes. These genes are broadly expressed across the subpallial subventricular zone (SVZ) of the ganglionic eminences [218, 247–249]. In mice carrying compound *Dlx1* and *Dlx2* knock-out mutations, GABAergic interneurons fail to migrate out of the ganglionic eminences, resulting in striking reductions in cortical and olfactory bulb interneurons as well as abnormal striatal differentiation [23, 219]. Similar results are seen in mice lacking *Mash1* [250]. Interestingly, *Dlx1/2* gene dosage appears to be important, as interneurons in mice carrying a $Dlx1^{-/-}; Dlx2^{+/-}$ genotype displays normal tangential migration to the cortical plate, but shows altered laminar positioning and simplified morphology (long axons and dendrites with few branches) [220]. Furthermore, $Dlx1^{-/-}$ mutants display selective defects in the dendritic morphology of SST+/CR+ interneurons, with a progressive loss of these interneurons in the postnatal brain, resulting in spontaneous seizures [221].

The MGE and CGE give rise to distinct cortical interneuronal populations [25, 41]. The MGE generates the parvalbumin-positive (fast-spiking basket cells and chandelier cells) and somatostatin-positive interneurons (including Martinotti cells) [8, 9, 22, 25, 41, 44, 45, 251]. The specification of these interneurons relies on the expression of *NK2 homeobox 1* (*Nkx2-1*) [22, 222]. The loss of *Nkx2-1* as interneuron progenitors are exiting their last mitotic division in the ganglionic ventricular zone leads to respecification of these cells into CGE-type interneurons (of all major subtypes) and the consequent absence of cortical PV and SST interneurons [22]. Interestingly, PV interneurons originate mainly from the ventral MGE whereas SST cells are preferentially produced by the dorsal MGE [251, 252], a phenomenon likely mediated by the combinatorial expression of particular transcription factors within different subdomains of the MGE [252, 253], resulting in part from a gradient of SHH expression [254]. Furthermore, a portion of the dorsal MGE and the MGE-CGE sulcus region is delineated by the

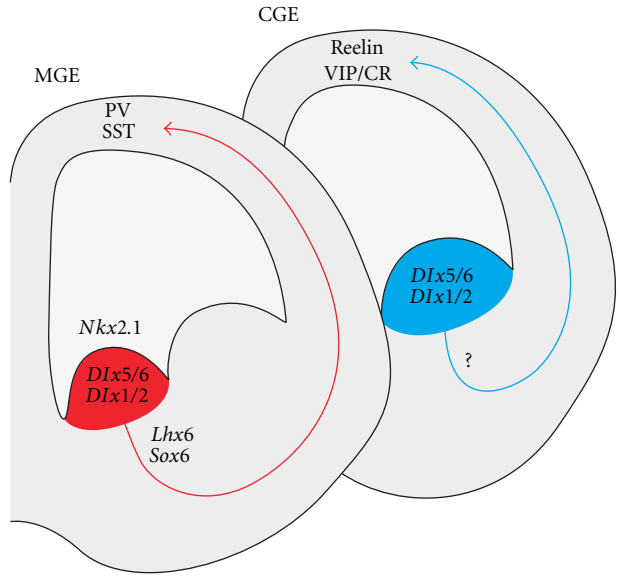

Figure 2: Genetic cascade governing cortical interneuron generation. Corticolimbic interneurons originate in the medial and caudal ganglionic eminences (MGE and CGE). The homeobox transcription factors *Dlx5/6*, *Dlx1/2* and the proneural gene *Mash1* (not shown) are expressed throughout the ganglionic eminences and are required for the generation of all GABAergic interneurons. The MGE generates parvalbumin-positive (PV) basket cells and chandelier cells, as well as somatostatin-positive (SST) cells (including Martinotti cells). These rely on the sequential expression of *Nkx2.1*, *Lhx6*, and *Sox6* for proper specification and maturation (see text). The genetic cascade governing the specification of CGE-derived interneurons has not been fully elucidated yet, but *Nkx6.2* and *Gsh2* are expressed in the CGE and might be important players (see text).

expression of the homeodomain transcription factor *Nkx6-2*, which partially overlaps with *Nkx2-1*. This area gives rise to the subgroup of somatostatin cells (about 30%) that coexpress somatostatin and calretinin and display a delayed nonfast spiking firing pattern [251, 255].

As they leave the ventricular zone, MGE-derived interneurons begin to express the transcription factor *LIM homeobox protein 6* (*Lhx6*), which is expressed into adulthood [26, 222, 251, 256, 257]. *Lhx6* is required for proper specification and migration of MGE-derived interneurons, and the loss of *Lhx6* results in misspecified hippocampal and cortical INs. These cells retain their GABAergic identity, but they fail to express PV or SST and are mislocalised within the neocortex [257]. Indeed, *Lhx6* loss disrupts the correct expression of downstream effectors known to be important for IN migration, including *v-erb-a erythroblastic leukemia viral oncogene homolog 4* (*ErbB4*), *C-X-C chemokine receptor type 4* (*CXCR4*) and *type 7* (*CXCR7*), and *aristaless-related homeobox* (*ARX*) [26, 258].

Downstream of *Lhx6* is *SRY-box 6* (*Sox6*), another transcription factor expressed by MGE-derived interneurons as they initiate their tangential migration. Sox6 is required for the proper laminar distribution and maturation of MGE-derived interneurons [28]. Its loss results in mislocalised MGE-derived INs that accumulate ectopically in layer I and deep layer VI, failing to adequately populate cortical layers II–V [28, 223]. Furthermore, these cells fail to express their mature markers, leading to a striking loss of cortical PV- and SST-expressing cells (PV being more severely affected). Although they remain correctly specified as MGE-INs, as evidenced by their morphology, electrophysiological properties, and expression of GABA, the resulting mutant cells fail to acquire mature intrinsic properties. For instance, PV-cells are unable to sustain the high frequency firing rates expected from these cells by P17-18 [28]. This results in a severe developmental epileptic encephalopathy with early lethality during the 3rd postnatal week [28].

As detailed above, the CGE produces a great variety of cortical interneurons, which populate the more superficial cortical layers. CGE-derived INs include all VIP- and reelin-positive cells, including the calretinin bipolar and neurogliaform cells, as well as multiple smaller subgroups of cortical interneurons, which are distinguishable by their morphological and physiological properties [25, 43, 44, 46]. The master regulatory genes for CGE cell-fate determination have yet to be fully determined. However, some transcription factors are expressed in both the CGE and dorsal MGE, including *Nkx6-2* and *CoupTF1/2*, and might play a role in the specification of CGE interneurons [255].

3. GABAergic Interneurons Play Fundamental Roles in Developing Circuits

GABA signalling is crucial during embryogenesis for both neural and nonneural populations of cells [259]. In fact, early GABAergic signalling has been shown to affect neurogenesis, differentiation, migration, and integration of developing neurons into neuronal circuits [260, 261]. Indeed, $GABA_A$ receptors are expressed early in newborn pyramidal neurons, which receive GABAergic inputs long before forming excitatory synapses [262, 263]. GABA is excitatory in immature

neurons due to the high level of *NKCC1* expression. *NKCC1* increases the intracellular concentration of Cl^-, shifting the GABA equilibrium potential (E_{GABA}) to more depolarised levels, thereby leading to an extrusion of negatively charged chloride anions upon activation of $GABA_A$ receptors and a depolarisation of the cell membrane [264]. With time, the progressive expression of another chloride transporter, KCC2, lowers the baseline intracellular concentration of Cl^- and underlies the developmental switch of E_{GABA} in favour of an inhibitory effect of GABA in mature neurons [265, 266].

This developmental switch is important in controlling the migration, final position, and morphological maturation of interneurons. Tangentially migrating interneurons have been shown to release GABA in a nonvesicular manner [267]. GABA then acts synergistically with AMPA/NMDA receptor-mediated currents to promote tangential migration of interneurons as long as it is depolarising. However, the gradual expression of KCC2 shifts the reversal potential of GABA and the resulting hyperpolarisation acts as a stop signal to arrest the migration of cortical interneurons [268]. Interestingly, interneurons derived from the MGE, which reach their final layer earlier than CGE-derived interneurons, also appear to express KCC2 earlier than CGE cells born simultaneously [246]. KCC2 might therefore regulate some of the differences observed in the laminar distribution of interneurons originating from different sources.

Furthermore, GABA-mediated depolarisations have recently been shown to promote excitatory synapse formation by facilitating NMDA receptor activation in cortical pyramidal neurons [269]. Blocking these GABA-mediated depolarisations, by *in utero* knock-down of NKCC1 or with the NKCC1 antagonist bumetanide, results in decreased numbers of functional excitatory synapses [269]. These manipulations also lead to altered cell morphologies, including thinner apical dendrites, simplified dendritic trees, and decreased dendritic spine densities [269]. These detrimental effects of bumetanide appear to be long-lasting, as they persist in the adult cortex and are associated with developmental delay and altered prepulse inhibition in adult mice [270]. Premature overexpression of KCC2 leads to similar dendritic anomalies in cortical pyramidal cells as those reported after blocking NKCC1 [271].

In summary, GABA plays fundamental roles at different stages of neuronal development, affecting migration, maturation, and synapse formation of both pyramidal cells and interneurons. Furthermore, the precise effect of GABA postsynaptically is dependent on the intracellular concentration of chloride, which is developmentally regulated via the expression of various chloride cotransporters and which varies depending on the age of the cells.

4. Interneurons and Early Network Activities

GABergic INs serve diverse functions in developing and in mature networks. As detailed above, they provide local circuit inhibition and participate in the genesis and organisation of specific mature neocortical and limbic oscillations, which in turn modify how local circuits respond to incoming signals. In addition, GABAergic interneurons are critical for the proper maturation and wiring of developing networks [8, 272], as well as for the regulation of critical period experience-dependent cortical plasticity [132, 273–276]. In particular, they have been involved in the generation of some of the early postnatal cortical and limbic oscillatory activities appearing during the first postnatal week in rodents. These synchronised network activities are thought to be important for the proper morphological maturation of excitatory and inhibitory neurons, including for the development of complex dendritic trees and synaptic contacts.

The first postnatal activities recorded are the synchronous plateau assemblies (SPAs), which are prolonged gap-junction-mediated calcium plateaus appearing between P0-P3 in the rat hippocampus [277] and neocortex [278]. The cellular substrates that drive these SPAs are still unknown, but it is interesting to note that some subsets of cortical interneurons are extensively interconnected through gap junctions [69, 70, 101] and could contribute to the generation of SPAs. In the cortex, SPAs progressively coexist with cortical early network oscillations (cENOs) between P0-P5. cENOs are infrequent (0.01 Hz) synaptically driven calcium events with slow kinetics that depend on glutamatergic AMPA- and NMDA-mediated synaptic activity and that cause sustained depolarisation of large groups of neurons.

These early network activities are then replaced by the giant depolarising potentials (GDPs) recorded in the hippocampus [279] and neocortex [278] between P6-P8. GDPs are much more frequent (mean 0.1 Hz), consist of fast calcium events, and are entirely dependent on GABAergic synaptic activity (as they are blocked by the $GABA_A$ antagonist bicuculline). In the hippocampus, GDPs have been shown to result from the spontaneous activity of a subset of highly connected GABAergic neurons, the hub neurons, that pace whole populations of pyramidal cells in a rhythmic fashion [280]. These hub cells receive more excitatory inputs (EPSPs), display a lower action potential threshold, and have a wider axonal arborisation than neighbouring local GABAergic interneurons [280]. These characteristics render them particularly well suited to generate waves of activity in wide sets of neurons upon stimulation by incoming inputs. GDPs coincide with a phase of active synaptogenesis within the developing neocortical and limbic circuits. It is therefore likely that a selective dysfunction of GABAergic interneurons in these early developmental steps might alter the process of synapse formation, either by decreasing these early network activities or by exerting more direct effects on the postsynaptic membrane.

In summary, interneurons participate in the genesis of early network activities which provide critical input for the normal maturation and plasticity of corticolimbic networks. In the mature brain, they provide local circuit inhibition and govern the onset and maintenance of some of the corticolimbic oscillations. These combined functions underlie the extensive impact of interneuronopathies on neurodevelopment and cognition.

5. Interneuronopathies and Neurodevelopmental Disorders

Interneuron anomalies have been suspected to underlie a variety of neurodevelopmental disorders in humans, including epilepsy, autism, and schizophrenia [14, 15]. This hypothesis stemmed from the observation of decreased GAD67 expression in postmortem brain tissue from affected individuals [110]. Later genetic studies also supported this hypothesis as variants in the GAD67 promoter area were discovered in patients with childhood-onset schizophrenia [113] or bipolar disorder [281]. Interestingly, many other genes linked to neuropsychiatric disease have since been shown to be preferentially expressed in developing cortical interneurons in mice [282]. It is therefore appealing to consider the possibility that genetic anomalies known to affect the development or function of interneurons in mice might be involved in neuropathologies in humans. Although genetic anomalies may manifest differently in mice and humans due to differences in expression patterns or compensation by other genes across species, alterations in highly conserved genetic pathways or disturbances in fundamental physiological processes might translate similarly in humans and mice. Furthermore, a host of environmental factors will likely modify the disease expression in these highly heterogeneous and likely polygenic pathologies. An exhaustive review of the genetic causes of schizophrenia, autism, and epilepsy is beyond the scope of this paper, but we will attempt to summarize some of the compelling evidence pointing to the roles of GABAergic neurons in these disorders.

5.1. Interneuron Development in Humans. Human GABAergic interneurons appear to be highly diverse as initially recognized by Ramon y Cajal [283, 284], with a similar array of PV-positive basket cells, PV-positive chandelier cells, SST-positive Martinotti cells, VIP-CR bitufted cells, VIP bipolar cells, and neurogliaform cells as that described in other species [284, 285]. However, the relative proportion of these various populations varies across species [286]. The superficial cortical layers II-III are considerably larger in the human cortex, presumably underlying some of the enhanced intercortical connectivity mediating higher brain functions in primates. Consequently, CR-positive double-bouquet INs appear to be considerably more numerous in the human cortex [284, 285]. Furthermore, although most cortical GABAergic interneurons develop in the ventral ganglionic eminences in humans, a proportion of cortical INs appear to originate from the cortical ventricular zone [284, 287–289]. Nonetheless, similarities do exist with regards to the molecular pathways involved in cortical interneuron genesis in humans and rodents, with preservation of some of the same fundamental genes including *Mash1*, *Dlx1/2*, *Nkx2-1*, and *Lhx6* [288, 290–293].

5.2. Interneurons and Schizophrenia. Schizophrenia is a chronic psychiatric condition that combines neurocognitive dysfunctions (i.e., delusions, hallucinations, and disorganisation of thought), negative symptoms (i.e., flat affect, avolition, and alogia), and social or occupational deterioration (i.e., altered social interactions, deterioration in personal hygiene, and inability to self-sustain) [294]. This is accompanied by more specific cognitive impairments such as abnormalities in perception, inferential thinking, volition, linguistic fluency, attention, executive functions (planning), and working memory [295, 296].

The involvement of interneurons in the pathophysiology of schizophrenia was suggested when the number of prefrontal cortical GAD67-expressing cells was found to be decreased in autopsy specimens from schizophrenic patients [109, 110]. There is no net loss of cortical PV-positive interneurons or calretinin-positive interneurons in schizophrenic cortices, as the total number of cells stained for either marker is preserved [111, 112]. However, there appears to be a selective downregulation of GAD67 in PV-positive interneurons in schizophrenic brains [112]. Furthermore, the level of parvalbumin expression in these cells is decreased [112]. As both parvalbumin and GAD67 expression are known to be regulated by cortical activity [297, 298], these findings could reflect secondary changes in response to altered levels of cortical activity in schizophrenic patients. Indeed, two schizophrenia susceptibility genes encoding the trophic factor neuregulin 1 (*NRG1*) and its receptor *ErbB4* (*ERB4*) [117–121] have been shown to facilitate activity-dependent GABA release from PV-positive basket cells in the mouse prefrontal cortex [299]. Selective loss of ErbB4 in PV cells causes a disinhibition of prefrontal pyramidal cells and results in a schizophrenia-like phenotype in mice [126]. In addition, the specific expression of ErbB4 in PV cells is required for neuregulin-1-dependent regulation of hippocampal long-term potentiation [127], which is altered in schizophrenic patients. Interestingly, hypostimulation of PV-positive basket cells via selective ablation of the NR1 subunit of the NMDA receptors in these cells resulted in schizophrenia-like behaviors (working memory deficits, impaired prepulse inhibition, locomotor hyperactivity, and anxiety) and decreased PV and GAD67 expression in PV basket cells in a mouse model of schizophrenia [128]. Therefore, hypofunction of prefrontal PV INs, either through a primary dysfunction of these cells or a decreased excitatory drive to these cells, appears to result in behavioural consequences in mice, which recapitulate aspects of the phenotype observed in schizophrenic patients.

Additionally, other genetic anomalies found in schizophrenic patients that are predicted to affect cortical maturation more broadly appear to impact interneuron maturation and GAD67 expression. For instance, brain-derived neurotrophic factor (BDNF) is normally released in an activity-dependent fashion from pyramidal cells and was shown to regulate the maturation of GABAergic INs [129]. Both BDNF and its receptor TrkB have been found to be downregulated in the prefrontal cortex of schizophrenic patients [122–124]. Knock-out mice for both BDNF and TrkB display behavioral anomalies and a decrease in the synaptic expression of GAD67/GABA [130, 131]. Similarly, the neural cell adhesion molecule NCAM, important for neuronal morphological maturation and synapse formation, requires the addition of a polysialic acid (PSA) moiety to function

properly. The activity-mediated expression of PSA has been shown to regulate PV-positive basket cell maturation and determine critical-period plasticity [132]. Interestingly, this PSA-NCAM coupling has been reported to be decreased in hippocampal specimens from schizophrenic patients [125], which would suggest abnormalities in interneuron maturation and cortical plasticity.

Another interesting hypothesis is that PV-positive chandelier cells might be affected in schizophrenic brains. Indeed, a specific loss of more than 40% of the axonal cartridges (the GAT-1 positive axonal branches from chandelier cells which contact the axon initial segments of pyramidal cells) has been demonstrated in the prefrontal cortex of schizophrenic patients [114, 115]. This is accompanied by enhanced expression of the alpha2 $GABA_A$ receptor subunit on the axon initial segment of pyramidal cells, likely as a compensatory mechanism for the decreased input from chandelier cells [300]. However, since chandelier cells are possibly excitatory [94, 95], the net effect of these structural changes on local cortical excitability is uncertain. More recently, the levels of SST, NPY, and CCK were shown to be decreased in a microarray analysis of prefrontal cortical samples from schizophrenic patients [116] (Table 1). Furthermore, there seems to be a specific decrease in SST-positive interneurons, as shown by *in situ* hybridisation staining, in these samples [116]. However, these results await replication.

Nonetheless, even if the numbers of various interneuron subtypes are preserved and if the morphological structure of these cells is intact in most cases, functional abnormalities in the connectivity of GABAergic circuits likely play a role in the pathogenesis of psychiatric disorders. Modifications of the specific $GABA_A$ receptor subunits expressed in the prefrontal cortex of schizophrenic patients have been described [116, 301]. Furthermore, cortical prefrontal gamma oscillations triggered by working memory tasks and selective attention in humans and primates [79–81] are decreased in schizophrenic patients with working memory deficits. These patients display a loss of gamma oscillation power and gamma oscillations are less tightly phase-locked to the task [82, 84, 85]. These changes might reflect functional alterations in the PV-positive basket cells, which contribute to the generation and regulation of the gamma oscillations that synchronise assemblies of pyramidal cells involved in a specific task [69, 72, 76–78, 83, 302]. In summary, multiple studies point to putative anomalies, either structural or functional, in PV-positive INs in the prefrontal cortex of schizophrenic patients.

5.3. Interneurons and Autism. Autism is a neurodevelopmental disorder combining impairments in socialization, communication, and restricted interests and/or stereotyped behaviors [294]. Autistic traits can be found in a variety of well-defined neurogenetic syndromes, including tuberous sclerosis [303, 304], fragile X syndrome [133, 134], and Rett syndrome [294]. In addition, nonsyndromic autism (re: without a clear underlying pathology, dysmorphic traits, or structural brain anomalies) has been associated with a variety of *de novo* copy number variants (CNVs) in large genome-wide association studies [141, 305–308], a finding which must be interpreted with caution [309]. However, the discovery of point mutations in genes encoding various synaptic scaffolding proteins in patients with nonsyndromic autism has begun to shed light on the pathophysiology of this disorder (recently reviewed in [309]). In particular, the discovery of mutations in postsynaptic neuroligins (*NRL4X*, *NRL3*) [135, 136], in other postsynaptic scaffolding proteins (*SHANK2*, *SHANK3*) [137–140, 310], in the presynaptic neurexins (*NRXN1*) [141, 142], and in fragile X mental retardation protein (*FMR1* gene) suggest that dysfunction in the maintenance of excitatory synapses, synaptic plasticity, and long-term depression participate in the neurobiology of autism and that this might be rescued by metabotropic glutamatergic antagonists [151–154, 156, 311, 312].

In parallel, a dysfunction in GABAergic signalling has been postulated to contribute to the emergence of autistic behaviours. In fact, epilepsy is a frequent comorbidity of autism. Interictal epileptic activity is recorded on scalp EEG in up to 85% of autistic children, although seizures occur in only ~30% of patients [313, 314] (Table 2). This, together with the finding of decreased cortical GAD67/GAD65 expression in autistic patients' brains [143], has suggested that inhibitory dysfunction might play a role in subsets of autistic patients. Furthermore, polymorphisms in the *Dlx1/2* genes have been associated with an increased susceptibility for autism [144] supporting the link between GABAergic anomalies and autism. In addition, nonsyndromic autism has been repeatedly associated with maternal chromosomal duplications in the 15q11-13 region [145, 146], which includes multiple genes encoding various $GABA_A$ receptor subunits (*GABRA5*, *GABRG3*, and *GABRB3*). Interestingly, MecP2, a transcription factor that broadly regulates gene expression by binding methylated CPG islands and which is responsible for the majority of cases of Rett syndrome (see next section), also exerts epigenetic control over this chromosomal region [157]. The loss of MecP2 results in dysregulation of multiple genes, including the downregulation of *GABRB3*. Furthermore, the loss of MecP2 is particularly detrimental to interneurons and a conditional MecP2 ablation in GABAergic neurons in mice was recently shown to recapitulate most of the behavioral anomalies associated with Rett syndrome, including autistic-like behavior [158].

Finally, another well-characterised mouse model of autism, the $uPAR^{-/-}$ mouse, displays a spatially selective defect in interneuron migration, such that the frontoparietal cortices of these mice show 50% less calbindin-positive interneurons (with a near absence of PV cells) whereas more caudal cortices are spared [11, 12]. These mice display autistic-like behaviors with increased anxiety and altered socialisation, as well as interictal epileptiform EEG activity and an increased susceptibility to seizures [11, 12]. *uPAR* encodes an urokinase plasminogen activator which is required for the proper processing of the hepatocyte growth factor (HGF). In turn, HGF, through its receptor MET, has been shown to be a critical motogen for interneuron migration and is able to rescue the interneuron migration defect and seizure susceptibility of $uPAR^{-/-}$ mice [159, 160]. Interestingly, polymorphisms in the MET promoter have

Table 1: Findings in schizophrenic patients and correlations in mice models.

Findings		References
	Humans	
GAD67	↓ GAD67 in prefrontal cortex	Volk et al. [109] Akbarian et al. [110]
	Preserved # number of PV cells, cortex	Woo et al. [111] Hashimoto et al. [112]
	↓ GAD67 level in PV cells, cortex	Hashimoto et al. [112]
	Association with polymorphisms in GAD67 promoter	Addington et al. [113]
Chandelier	Decrease in chandelier cells cartridges (GAT1+) in prefrontal cortex	Woo et al. [114] Volk et al. [115]
SST	↓ levels of SST in microarray analysis and ↓ number of SST cells, prefrontal cortex	Hashimoto et al. [116]
NPY/CCK	↓ levels of NPY and CCK in microarray analysis	Hashimoto et al. [116]
NRG1	Susceptibility locus in *NRG1*	Stefansson et al. [117, 118] Zhang et al. [119] Yang et al. [120]
ERB4	Susceptibility locus in *ERB4*	Silberberg et al. [121]
BDNF/Trkb	Downregulation of BDNF in prefrontal cortex	Weickert et al. [122] Wong et al. [123]
	Downregulation of BDNF and Trkb in prefrontal cortex	Takahashi et al. [124]
PSA/NCAM	↓ PSA-NCAM complexes in hippocampus	Barbeau et al. [125]
Gamma	Gamma oscillations are triggered by working memory tasks + selective attention	Tallon-Baudry et al. [79] Howard et al. [81]
	Decreased power of cortical gamma oscillations and phase locking to memory task	Spencer et al. [82, 84] Cho et al. [85]
	Mice	
Erb4	Selective interneuron loss of *Erb4*: "schizophrenia-like behaviors"	Wen et al. [126]
Erb4/Nrg1	Erb4 in PV cells is required for Nrg1-dependant regulation of LTP (hippocampus)	Chen et al. [127]
NR1	Selective loss of the NMDAr NR1 subunit in PV cells: decreased excitatory input to PV cells results in "schizophrenia-like behaviors" and ↓ expression of PV and GAD67	Belforte et al. [128]
BDNF	BDNF regulates activity-dependant maturation of PV cells $Bdnf^{-/-}$ and $Trkb^{-/-}$: ↓synaptic GAD67 and GABA and behavioral anomalies	Huang et al. [129] Cotrufo et al. [130] Hashimoto et al. [131]
PSA/NCAM	Activity-mediated expression of PSA regulates PV cells maturation and visual plasticity	Di Cristo et al. [132]
Gamma	Gamma oscillations are triggered by stimulating PV cells: enhanced performance	Cardin et al. [77] Sohal et al. [83]
	Gamma oscillations depend on PV cells-mediated fast-synaptic inhibition	Bartos et al. [72]

recently been described to confer an increased susceptibility to autism and this gene is included in one of the genomic sequences linked to autism susceptibility (7q31) [149, 150]. Autism is a complex disorder and alterations in other GABAergic circuits, including the striatocortical circuits, likely contribute to this behavioural phenotype. Indeed, an interneuron-selective ablation of MET results in decreased cortical PV cells, but massively increased dorsal striatal PV interneurons, leading to a disruption in striatal-mediated procedural and reversal learning [161]. Nonetheless, cortical and hippocampal GABAergic deficits certainly play a role in some of the cognitive-behavioral manifestations of autism, as well as in the associated susceptibility to seizures.

5.4. Interneurons and Epilepsy. Perhaps one of the most intuitive consequences of interneuron dysfunction is the development of epilepsy. Multiple mouse models carrying interneuronopathies have been shown to develop seizures [22, 28, 57, 63, 206, 207, 221]. In parallel, various reports point to probable GABAergic interneuron dysfunction in

Table 2: Findings in autistic children and correlations in mice models.

Findings		References
	Humans	
FMR1	Patients with fragile X syndrome often display autistic traits	Levitas et al. [133] Brown et al. [134]
NRL4X/NRL3	Point mutations in NRL4X and NRL3 associated with X-linked autism	Jamain et al. [135]
	Point mutations in NRL4X in nonsyndromic autism	Laumonnier et al. [136]
SHANK3	Mutations in *SHANK3* in nonsyndromic autism	Durand et al. [137] Gauthier et al. [138] Moessner et al. [139]
SHANK2	Mutations in SHANK2 in nonsyndromic autism	Berkel et al. [140]
NRXN1	Mutations in *NRXN1* nonsyndromic autism	Szatmari et al. [141] Kim et al. [142]
GAD65/67	↓ levels of GAD65/67 in cortex	Fatemi et al. [143]
Dlx1/2	Polymorphisms in *Dlx1/2* with increased susceptibility to autism	Liu et al. [144]
15q11-13	Maternal duplications in 15q11-13 in nonsyndromic autism	Baker et al. [145]
	including *GABRA5*, *GABRG3*, *GABRB3* ($GABA_AR$ subunits)	Hogart et al. [146]
MECP2	Mutations in *MECP2* explain the majority of Rett syndrome.	Amir et al. [147]
	Patients display autistic behaviors.	Buyse et al. [148]
MET	Polymorphisms in MET promoter associated with autism	Jackson et al. [149]
	Susceptibility locus for autism at 7q31 includes MET gene.	Campbell et al. [150]
	Mice	
Fmr1	*Fmr1* k/o: behavioral anomalies improve with glutamatergic antagonists	Dolen et al. [151] Bear et al. [152, 153]
Neuroligins/ neurexins	NRL1/2 expression in nonneuronal cells trigger synapse formation in presynaptic cells	Scheiffele et al. [154]
	NL-1 overexpression in hippocampal neurons promotes assembly of excitatory and inhibitory synapses and knock-down results in loss of inhibitory > excitatory synapses	Chih [155]
	Presynaptic β-neurexin induces GABA and glutamate synapse differentiation in postcell	Graf et al. [156]
	NRL1,3,4 localise at glutamatergic synapses, NRL2 at both excitatory and inhibitory	Graf et al. [156]
MecP2	Binds methylated CPG islands and exerts epigenetic control of *UBE3A* and *GABR3*	Samaco et al. [157]
	Interneuron selective loss of MecP2 recapitulates the Rett-like behavioral aN in mice	Chao et al. [158]
uPAR, HGF, MET	$uPAR^{-/-}$ displays 50% loss of IN in cortex and seizure susceptibility	Powell et al. [11]
	uPAR is required for the processing of HGF (an interneuron motogen),	Powell et al. [159]
	HGF, through its receptor MET, can rescue the phenotype of $uPAR^{-/-}$ mice	Bae et al. [160]
	Interneuron selective MET ablation: ↓ PV cortex, ↑ striatal PV cells, disrupts reversal learning	Martins et al. [161]

developmental and symptomatic (posttraumatic or poststatus epilepticus) epileptic disorders in humans [315–318]. In most situations, early developmental interneuron anomalies might contribute to seizure disorders both by altering the normal development of cortical circuits, as detailed above, and by failing to provide the acute inhibition required to control excessive excitation in the mature network. Paradoxically, in a state of chronic excitation, INs have been shown to contribute actively to ictogenesis when GABA becomes depolarizing due to the failure of chloride extrusion from damaged neurons [319, 320]. Therefore, both a primary dysfunction of GABAergic inhibitory transmission and a secondary switch to excitatory GABAergic transmission could contribute to the pathogenesis of epilepsy. Understanding the molecular mechanisms governing interneuron development, maturation, and normal function would therefore be very informative in our quest to comprehend human epileptic disorders.

Epilepsy is a heterogeneous disorder, and most cases are symptomatic of focal or widespread CNS lesions (e.g., malformations, tumors, infections, trauma, strokes, hypoxia, etc.). INs dysfunctions might contribute to seizure disorders

Table 3: Selected examples of genes causing epilepsy in humans and interneuron dysfunctions in mice.

Findings		References
	Humans	
SCN1A	*SCN1A* mutations explain the majority of Dravet syndrome	Claes et al. [162]; Ohmori et al. [163]
		Sugawara et al. [164]; Orrico et al. [165]
		Escayg and Goldin et al. [166]
	SCN1A mutations display phenotypic heterogeneity: GEFS, febrile seizures, cognitive impairment	Escayg et al. [167, 168]
		Fujiwara et al. [169]; Osaka et al. [170]
		Zucca et al. [171]; Orrico et al. [165]
	Variants in other channels modify the phenotype of *SCN1A: SCN8A*	Martin et al. [172]
	CACNB4	Ohmori et al. [173]
SCN1B	*SCN1B* mutations cause GEFS	Wallace et al. [174]
ARX	ARX mutations cause various phenotypes including infantile spasms	Shoubridge et al. [175]
		Kalscheuer et al. [176]; Weaving et al. 2004 [177]
CDKL5	*CDKL5* mutations cause early epileptic encephalopathies	Scala et al. [178]; Archer et al. [179]
		Cordova-Fletes et al. [180]; Mei et al. [181]
		Melani et al. [182]
MECP2	*MECP2* mutations explain most cases of Rett syndrome. These patients often display seizures.	Amir et al. [147]; Buyse et al. [148]
GABRG2	Mutations in the gamma2 subunit of the $GABA_AR$ cause childhood absence epilepsy ± febrile seizure	Wallace et al. [174]; Kananura et al. [183]
	Truncation of *GABRG2* causes generalised epilepsy with febrile seizure (GEFS)	Harkin et al. [184]
GABRA1	Mutations in the alpha1 subunit of the $GABA_AR$ cause juvenile myoclonic epilepsy	Cossette et al. [185]
	Mutations in the alpha1 subunit of the $GABA_AR$ can also cause childhood absence epilepsy	Maljevic et al. [186]
CACNA1A	Polymorphisms associated with generalised epilepsy syndromes	Chioza et al. [187]
	Mutations in *CACNA1A* can cause ataxia with generalized seizures	Jouvenceau et al. [188]; Imbrici et al. [189]
CACNB4	Mutations in *CACNB4* cause episodic ataxia with generalized seizures	Escayg et al. [190]
CACNA1H	Mutations in T-type calcium channel Cav3.2 cause childhood absence epilepsy	Khosravani et al. [191]
Nkx2.1	*Nkx2.1* haploinsufficiency leads to the "brain-lung-thyroid syndrome"	Carre et al. [192]
	variable phenotype: severe respiratory distress at birth, mild-moderate hypothyroidism, chorea	Guillot et al. [193]
	Some patients present benign hereditary chorea, occasionally with cognitive impairment and seizures	Kleiner-Fisman et al. [194, 195]
Dlx5/6	*Dlx5/6* mutations result in craniofacial and limb anomalies: ectodermal dysplasia	Morasso et al. [196]; Lo Lacono et al. [197]
Sox 6	1 patient described with heterozygote *Sox6* mutation: craniosynostosis and facial dysmorphisms.	Tagariello et al. [198]

Table 3: Continued.

Findings		References
	Mice	
Scn1a	*Scn1a* (Nav1.1) expressed in most neuronal populations	Yu et al. [199]
	Scn1a$^{+/-}$ and *Scn1a*$^{-/-}$ mice develop spontaneous seizures and die prematurely	Yu et al. [199]
	↓ sodium currents are specific to GABAergic interneurons in *Scn1a*$^{+/-}$ and *Scn1a*$^{-/-}$	Yu et al. [199]
	Selective loss of *Scn1a* in interneurons recapitulates seizure disorder	Martin et al. [200]
Arx	Role in neuronal proliferation and migration	Fricourt et al. [201, 202]
	Specific requirement of Arx for interneuron migration	Friocourt and Parnavelas [203]; Poirier et al. [204]
	Arx is a downstream target of *Dlx1*	Colasante et al. [205]
	Arx(GCG)10+7 mice display seizures including spasms and ↓ no. of CB and NPY interneurons	Price et al. [206]
	Selective loss of Arx in interneurons recapitulates the seizure disorder	Marsh et al. [207]
Cdkl5	Cdkl5 is coexpressed with Mecp2 in cortical neurons and can phosphorylate Mecp2	Mari et al. [208], Bertani et al. [209]
MecP2	Mecp2 broadly represses gene expression by binding methylated CPG islands	Nan et al. [210, 211]
Cacna1a	*Cacna1a*$^{tg/tg}$ tottering mutant displays ataxia and absence seizures	Noebels et al. [212]; Fletcher et al. [213]
	Gain of thalamic T-type currents cause enhanced rebound bursting of TC cells in *Cacna1a*$^{tg/tg}$, *Cacna1a*$^{ln/ln}$	Zhang et al. [214]; Tsakiridou et al. [215]
	Interneuron selective ablation of *Cacna1a* leads to multiple types of generalised seizures incl. absences	Rossignol et al. [63] (abstract)
Cacnb4	*Cacnb4*$^{lh/lh}$ loss-of-function mutants display spontaneous absence seizures and ataxia	Burgess et al. [216]
	Thalamic tonic $GABA_A$ currents enhance rebound bursting of TC cells in *Cacnb4*$^{lh/lh}$	Cope et al. [217]
Dlx1/2	*Dlx1*$^{-/-}$*Dlx2*$^{-/-}$ mice die perinatally and display a failure of IN migration to cortex and olfactory bulb	Anderson et al. [23, 218]; Bulfone et al. [219]
	Dlx1$^{-/-}$*Dlx2*$^{+/-}$ abnormal laminar distribution of IN and simplified morphology	Cobos et al. [220]
	Dlx1$^{-/-}$ morphological defect and postnatal loss of SST+/CR+ interneurons: spontaneous seizures	Cobos et al. [221]
Nkx2.1	*Nkx2.1*$^{-/-}$ die perinatally. Nkx2.1 is required for MGE interneuron generation.	Sussel et al. [222]
	Interneuron specific removal of *Nkx2.1* results in misspecification of MGE cells into CGE cells, and seizures	Butt et al. [22]
Sox6	*Sox6*$^{-/-}$ dies perinatally of craniofacial anomalies	
	Conditional loss of *Sox6* in interneurons results in misplaced/ectopic and immature basket cells (loss PV)	Batista-Brito et al. [28]; Azim et al. [223]
	Conditional loss of Sox6 in interneurons results in a severe epileptic encephalopathy	Batista-Brito et al. [28]

following such insults, as suggested by the finding of limbic interneuronal loss after brain trauma or prolonged seizures [321–324]. Hippocampal somatostatin-positive interneurons appear to be particularly sensitive to seizure-induced damage as demonstrated in animal models of drug-induced epilepsy [13, 108, 324, 325], as well as in patients with chronic temporal lobe epilepsy [326]. This might point to a more selective vulnerability of this cell type which could be amendable to neuroprotective therapies. A loss of hippocampal PV cells [327] and alterations in the axonal

projections of PV-positive chandelier cells have also been reported in patients with chronic epilepsy [316, 325, 328, 329]. Although it is not clear if these changes are the cause or the consequence of repeated seizures [330, 331], they probably contribute to the chronicity of the disease.

5.5. Interneurons in Genetic Developmental Epilepsies. Perhaps most interestingly, GABAergic interneuron dysfunction might contribute to a subset of genetic developmental epilepsies. In those cryptogenic epilepsies where no apparent etiology is found on examination or imaging (re: no dysmorphic traits or neurocutaneous stigma and normal brain CT/MRI), but where patients present clear neurological dysfunction as episodic seizures with or without interictal cognitive impairment, an underlying circuit dysfunction is postulated. These patients with severe developmental epilepsies (i.e., Ohtahara syndrome, West syndrome, Lennox-Gastaut syndrome, Dravet syndrome, etc.) are rarely amendable to surgical interventions, and only few reports of neuropathological examination of surgical or postmortem specimens are available. In most cases of West syndrome, the neuropathological evaluation reveals either focal cortical malformations or diffuse brain damage [332–335] but it is found to be "normal" in up to 45% of cases [336]. Nonetheless, functional inhibitory defects with disrupted $GABA_AR$ function or immature patterns of $GABA_AR$ subunit expression have been demonstrated in some cases of infantile spasms [318, 337]. Such inhibitory defects might arise as a consequence of genetic mutations that disrupt genes critical for proper interneuron generation or function. For instance, mutations in the alpha1 subunit of the voltage-gated sodium channel Na_V 1.1 (*SCN1A*), the *aristaless-related homeobox transcription factor* (*ARX*), the *cyclin-dependent kinase-like 5* (*CDKL5*), various $GABA_A$ receptor subunits and in the alpha 1 subunit of the voltage-dependent P/Q-type Ca^{2+} channel (*CACNA1A*) have been described in patients with a variety of epileptic disorders and similar mutations have been shown to impair GABAergic signalling in rodents (Table 3).

5.5.1. SCN1A. Mutations in *SCN1A*, which encodes the neuronal voltage-gated sodium channel Nav1.1, have been found to underlie a majority (75–85%) of cases of severe myoclonic epilepsy of infancy (Dravet syndrome) [162–166]. Interestingly, *SCN1A* mutations have also been found to cause generalised epilepsy with febrile seizures (GEFS) as well as a variety of disorders with neurocognitive impairment and variable seizure susceptibility [165, 167–171]. This extended phenotypic variability stems both from the nature of the mutations (nonsense mutations cause Dravet syndrome whereas missense mutations tend to cause different phenotypes depending on their location [166, 338–340]) and from the coexistence of genetic modifiers in other genes [172, 173]. Although Nav1.1 channels are found in most neuronal populations in the rodent brain, their loss was found to result in a more selective impairment of interneuronal transmission in mice [199, 200]. Nav1.1 channels tend to cluster predominantly at the level of the axon initial segment of PV-positive interneurons [341], and their loss results in failure of PV cells to maintain high frequency firing rates [341]. By contrast, pyramidal cell transmission is relatively well preserved in Nav1.1 mutants, presumably though compensation by other channels. Therefore, dysfunctions of INs might contribute significantly to the onset of epilepsy in *Scn1a* mutants.

5.5.2. ARX. In a similar fashion, mutations in the *ARX* gene are associated with a variety of neurological syndromes that combine epilepsy and various degrees of cognitive disabilities. The spectrum of phenotypes associated with *ARX* mutations extends from severe X-linked lissencephaly with ambiguous genitalia and severe myoclonic encephalopathies (Ohtahara syndrome, West syndrome), to isolated nonsyndromic mental retardation [175]. The *ARX* gene is necessary for proper neural proliferation, migration, and differentiation [201–203, 342]. In particular, *ARX* was shown to be essential for proper migration and laminar positioning of interneurons [203, 204], partly because it is a direct downstream target of *Dlx1* [205]. Interestingly, *ARX* knock-in mice carrying trinucleotide repeat insertion mutations recapitulating mutations found in IS cases, display decreased numbers of telencephalic NPY+ and calbindin+ interneurons, and present an epileptic phenotype with early epileptic spasms [206]. Furthermore, a conditional deletion of *ARX* in GABAergic interneurons leads to a similar loss of interneuron migration and is sufficient to cause a developmental epileptic phenotype including brief spasm-like seizures [207]. This supports the hypothesis that even if *ARX* mutations might have broader consequences for cortical development, the specific effect on IN migration is fundamental to the development of epilepsy.

5.5.3. CDKL5/MECP2. Other patients with early epileptic encephalopathies have been found to carry mutations in *CDKL5* [176–182], a protein kinase highly expressed in developing and mature neurons [343]. Interestingly, CDKL5 can directly bind and phosphorylate MecP2 and is coexpressed with MecP2 in cortical neurons [208, 209]. In turn, MecP2 is a transcription factor that broadly represses gene expression by binding methylated CPG islands [210, 211] and is therefore involved in the epigenetic control of gene expression. *MECP2* mutations explain a majority of cases of Rett syndrome [147, 148], a neurodevelopmental disorder manifested by progressive microcephaly, developmental regression, stereotypies, and epilepsy. Interestingly, an interneuron selective ablation of *MecP2* recapitulates most of the neurological and behavioral consequences of *MecP2* knock-out mutations in mice [158]. Since MecP2 is a direct downstream target of CDKL5, it is possible that interneuron dysfunction also contributes to the cognitive and epileptic phenotype seen in both *CDKL5* and *MecP2* mutants.

5.6. Voltage-Gated Ca^{2+} Channels. Finally, patients with idiopathic generalized epilepsy syndromes (IGE) have been shown to carry mutations in various $GABA_A$ receptor subunits [174, 183–186, 344], as well as mutations or polymorphisms in multiple subunits of voltage-gated calcium channels, including the *CACNA1A*, *CACNB4*, and *CACNA1H* genes [187–191, 345]. These patients present

various combinations of myoclonus, generalised tonic-clonic "grand-mal" seizures, and absence seizures "petit-mal." Mutant mice carrying loss-of-function mutations in *Cacna1a* or *Cacnb4* display similar generalised spike-wave absence seizures and have been instrumental in advancing our understanding of generalised epilepsies [212, 213, 216, 346]. In these models, an enhanced thalamocortical rebound bursting due to a gain in low-voltage activated Ca^{2+} currents and excessive thalamic $GABA_A$ signalling have been shown to result in hypersynchronisation of the thalamocortical circuitry and absence seizures [214, 215, 217, 347]. In addition, we recently demonstrated that selective loss of *Cacna1a* from cortical and limbic MGE-derived interneurons in mice is sufficient to create a severe epileptic encephalopathy with multiple types of generalised seizures [63]. We showed that *Cacna1a* loss resulted in unreliable neurotransmission from PV-positive interneurons. Furthermore, we demonstrated that concurrent loss of *Cacna1a* from both MGE-derived interneurons and cortical pyramidal cells results in a milder epileptic phenotype characterised by absence seizures [63]. These findings suggest that, in some cases, alterations in MGE-derived interneuron function might lead to a variety of generalised seizures and that the severity of the phenotype can be modulated by the involvement of other neuronal populations. Concurrent with these observations, various mouse models with either misspecified or immature MGE-derived interneurons have also been shown to develop severe epilepsies. For instance, *Nkx2-1*$^{-/-}$ and *Sox6*$^{-/-}$ null mutants die embryonically or perinatally due to a variety of craniofacial and lung anomalies [222, 348]. However, conditional mutants lacking either *Nkx2-1* or *Sox6* in an MGE-specific manner develop generalised seizures during the 2nd or 3rd postnatal week, leading to early lethality [22, 28]. In a similar fashion, *Dlx1*$^{-/-}$ mice also develop spontaneous seizures [221].

One of the limitations in extending some of the experimental findings from genetic models of interneuronopathy to human diseases is that most of the transcription factors important for interneuron development and specification are also involved in specification of other organs (bone, skin, cartilage, lung, and thyroid). Mutations in these genes therefore cause multisystemic disorders in which neurological involvement is often overlooked. For instance, human heterozygote mutations in *Nkx2-1* have been described in a variety of clinical disorders affecting the thyroid, the lungs, and the brain, the so-called "brain-lung-thyroid" syndrome [192]. In some cases, truncating mutations result in severe respiratory failure at birth, due to the lack of surfactant proteins, with mild congenital hypothyroidism and neurocognitive anomalies [193]. In other cases, *Nkx2-1* mutations have been described in patients with benign hereditary chorea, a movement disorder occasionally accompanied by intellectual impairment and seizures [194, 195]. In a similar fashion, heterozygous mutations in *Dlx5/6* genes cause craniofacial and limb anomalies (ectodermal dysplasias) [196, 197]. *Sox6* is known to be important for proper cartilage formation [348–350], and one child with craniosynostosis (premature fusion of the cranial sutures) and facial dysmorphisms has been shown to carry a heterozygous mutation in *SOX6* [198]. However, even when direct inferences cannot be made between mouse mutants and human patients, the study of these animal models is instrumental in clarifying the role of specific interneuron populations in preventing various types of seizures and is critical to our understanding of epileptogenesis.

6. Conclusions and Future Perspectives

In summary, GABAergic INs include diverse neuronal populations which present significant heterogeneity in terms of their biochemical, morphological, and physiological properties. The fate of these INs is governed by tightly regulated genetic cascades. Disruption of these genetic programs, or of genes important for the proper specification, migration, maturation, and/or function of these cells, leads to a variety of cognitive, behavioural, and neurological consequences including autistic behaviors and epilepsy in rodents and humans. For this reason, furthering our understanding of interneuron development across mammalian species might become the cornerstone for the subsequent development of improved diagnostic approaches, and hopefully new therapeutic strategies, for patients with a variety of neurodevelopmental disorders. A fascinating example of this is the development of stem cell transplantation in the treatment of epileptic disorders in rodents [351, 352]. Other such innovative therapeutic approaches will likely emerge as the exquisite complexity of cortical interneurons diversity unravels.

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Review Article

Alterations of GABAergic Signaling in Autism Spectrum Disorders

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Autism spectrum disorders (ASDs) comprise a heterogeneous group of pathological conditions, mainly of genetic origin, characterized by stereotyped behavior, marked impairment in verbal and nonverbal communication, social skills, and cognition. Interestingly, in a small number of cases, ASDs are associated with single mutations in genes encoding for neuroligin-neurexin families. These are adhesion molecules which, by regulating transsynaptic signaling, contribute to maintain a proper excitatory/inhibitory (E/I) balance at the network level. Furthermore, GABA, the main inhibitory neurotransmitter in adult life, at late embryonic/early postnatal stages has been shown to depolarize and excite targeted cell through an outwardly directed flux of chloride. The depolarizing action of GABA and associated calcium influx regulate a variety of developmental processes from cell migration and differentiation to synapse formation. Here, we summarize recent data concerning the functional role of GABA in building up and refining neuronal circuits early in development and the molecular mechanisms regulating the E/I balance. A dysfunction of the GABAergic signaling early in development leads to a severe E/I unbalance in neuronal circuits, a condition that may account for some of the behavioral deficits observed in ASD patients.

1. Introduction

Autism spectrum disorders (ASDs) comprise a complex and heterogeneous group of pathological conditions including autism, Rett and Asperger syndromes, and pervasive developmental disorder-otherwise nonspecified, characterized by impaired social interactions, deficits in verbal and nonverbal communication, and a limited interest in the surrounding environment associated with stereotyped and repetitive behaviors [1]. The incidence of these disorders, which varies between 10 and 20 per 10000 children, has risen dramatically over the past two decades mainly because of the use of broader diagnostic criteria and the increased attention of the medical community [2]. Clinical signs are usually present at the age of 3 years, but prospective studies of infants at risk have demonstrated that deficits in social responsiveness, communication, and play could be present already at the age of 6–12 months.

ASDs are the most heritable neurodevelopmental disorders of early childhood. Genetic factors are thought to account for ~80% of autism cases, and since autism is a spectrum of disorders, it is conceivable that in most cases different genes act in combination in different individuals [3]. Genes, interacting with epigenetic factors, may influence neuronal migration, axon pathfinding, dendritic development, synaptogenesis, and pruning, thus contributing to alter neuronal connectivity and information processing [4].

Interestingly, a small percentage of ASDs patients carry single mutations in genes encoding for synaptic cell adhesion molecules of the neurexin (NRXN)-neuroligin (NLG) families [5]. These include mutations in genes encoding for NRXN1 [6, 7], for NLG3, NLG4 [8–10], and for Shank3 [11]. Although rare, these mutations provide crucial information on the synaptic abnormalities which possibly affect ASDs patients and point to synapses dysfunction as a possible site of autism origin. Synapses are specialized intercellular junctions which transfer information from a neuron to a target cell, usually another neuron.

Several lines of evidence suggest that an impairment of GABAergic transmission contributes to the development

of ASDs. GABA, the main inhibitory neurotransmitter in adulthood is released by interneurons which contain the GABA synthesizing enzymes glutamic acid decarboxylase (GAD)65 and GAD67. GABAergic interneurons, which constitute a heterogeneous group of cells, differently classified in virtue of their anatomical, physiological, and molecular features [12], represent only 10%–15% of the total neuronal population. Nevertheless, they provide the functional balance, complexity, and computational architecture of neuronal circuits [13]. They play a key role in regulating neuronal excitability *via* feedback and feed-forward inhibition. Axons of different inhibitory cells target different postsynaptic subcellular compartments, allowing them to selectively control the output of pyramidal cells [14], thus providing the temporal structure that orchestrates the activity of neuronal ensembles leading to coherent network oscillations [15].

While in the mature brain GABA acts as an inhibitory transmitter, during the embryonic and the perinatal period, this neurotransmitter depolarizes targeted cells and triggers calcium influx. GABA-mediated calcium signaling regulates a variety of different developmental processes from cell proliferation migration, differentiation, synapse maturation, and cell death [16]. Although the geometry and the cellular and subcellular selectivity of GABAergic axons are mainly genetically determined, axonal branching and arborization are regulated by activity and experience and often require brain-derived neurotropic factor (BDNF, [17]). Thus, sensory stimulation contributes to shape neuronal circuits, whereas sensory deprivation significantly retards their maturation [18–20].

Considering the multifacet of GABA activities particularly during development, it is not surprising that disturbance of GABAergic signaling can result in aberrant information processing, as found in neurodevelopmental disorders such as ASDs. In particular, it has been hypothesized that at least some forms of autism result from an imbalance between excitation and inhibition in local circuits involved in sensory, mnemonic, social, and emotional processes. The resulting hyperexcitability could disrupt the normal formation of cortical maps leading to a relatively unstable state [21]. The cortex is organized in vertical mini columns of functionally related glutamatergic and GABAergic neurons that process thalamic inputs. Local GABAergic circuits contribute to control the functional integrity of minicolumns *via* lateral inhibition. Interestingly, analysis of postmortem tissues from ASDs patients has revealed alterations in the number of mini columns, in the horizontal spacing separating cell columns, and in their internal structure [22]. The abnormal cytoarchitecture is often associated with an increased expression of calbindin-, calretinin- and parvalbumin-positive GABAergic interneurons [23]. In addition, changes in GAD65 and GAD67 [24], in the mRNA encoding for these enzymes [25–27], in $GABA_A$ [28, 29] and $GABA_B$ receptors [30] have been found in brain samples from ASDs patients. The altered GABAergic function may reduce the threshold for developing seizures as demonstrated by the high comorbidity of ASDs with epilepsy (one third of ASDs patients have seizures [31]). This further strengthens the hypothesis that an unbalance between excitation and inhibition contributes to these devastating neurological disorders.

This paper will focus on the functional role of GABA in regulating developmental processes, their experience-dependent refinement and, at the network level, the balance between excitation and inhibition. In addition, the implications that an altered GABAergic signaling may have in neurodevelopmental disorders such as ASDs will be discussed taking into account different animal models.

2. GABA, a Pioneer Neurotransmitter in Neuronal Circuits Formation

The construction of the brain relies on a series of well-defined genetically and environmentally driven factors whose disruption leads to pathological disorders including ASDs. During central nervous system development, a sequence of temporally related events during which neurons proliferate, migrate, differentiate, and establish proper synaptic connections occurs [16]. Further refinement of immature networks needs adaptive processes involving experience- or activity-dependent mechanisms, which lead to the formation of new synapses and elimination of others. Using imaging techniques and electrophysiological approaches, several patterns of coherent activity have been characterized early in development [32]. Uncorrelated spontaneous activity consisting of calcium action potentials has been suggested to play a crucial role in regulation of cortical neurogenesis at late embryonic stages [16, 33]. At birth, synchronous neuronal activity can be detected in the hippocampus and in the neocortex. This relies firstly on the activation of intrinsic conductances and gap junctions and later on synapse-driven events. Thus, small cell assemblies coupled to gap junctions generate nonsynaptic spontaneous plateau assemblies (SPAs, [32], Figure 1).

These involve small groups of neurons and are associated with sustained intrinsic membrane potential oscillations. SPAs are modulated by oxytocin, a maternal hormone essential for labour induction, which transiently converts GABA action from excitatory to inhibitory [34]. As the network matures and the density of functional synapses increases, synaptic-driven network oscillations replace SPAs. A downregulation in the expression of connexins *via* CREB signalling, following activation of NMDA receptors, may lead to SPAs silencing [35]. Two different patterns of network-driven synaptic oscillations have been described: the giant depolarizing potentials or GDPs [36] and early network oscillations or ENOs [37]. These are reminiscent of "long oscillations" and "spindle bursts", respectively, recorded from the rat somatosensory cortex *in vivo* [38] or of discontinue activity patterns observed in the EEG of preterm babies [39]. While ENOs (which usually precede GDPs) were initially thought to constitute the cortical counterpart of hippocampal GDPs, they have been shown to coexist with GDPs in the neocortex [32]. In the neocortex, ENOs critically depend on the activation of NMDA receptors [37]. In addition, evidence has been provided that extrasynaptic NMDA receptors activated by ambient glutamate generate a tonic

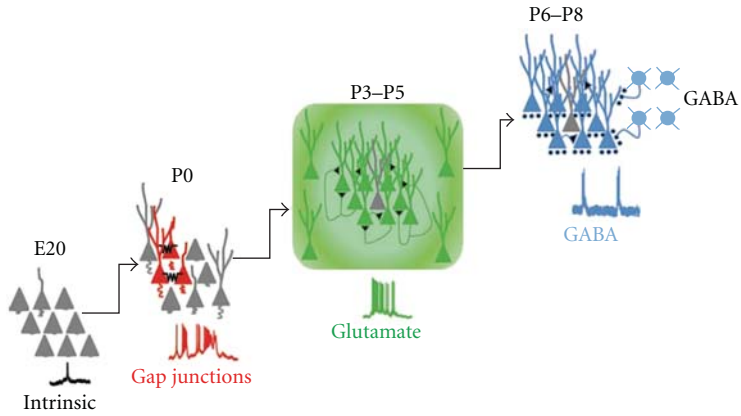

Figure 1: Patterns of electrical activity observed at late embryonic/early postnatal stages in the cortex. E20: uncorrelated calcium spikes; P0: Spontaneous Plateau Assemblies (SPAs) synchronized by gap junctions. P3–P5: early network oscillations (ENOs) mediated by glutamate. P6–P8: giant depolarizing potentials (GDPs) mediated by GABA and glutamate. (Modified from [32]).

current, which contributes to depolarize the membrane, to enhance cell excitability and to convert silent synapses into functional ones [40]. The activation of NMDA receptors by "ambient" glutamate would be facilitated by changes in subunits composition [41], in voltage dependence of the magnesium block [42] and in the high affinity for glutamate of extrasynaptic NMDA receptors.

In the hippocampus, GDPs are generated by the synergistic action of glutamate and GABA, which in the immediate postnatal period, orchestrates neuronal ensembles *via* its depolarizing and excitatory action [43]. Before synapses formation, GABA depolarizes targeted neurons *via* a paracrine type of action. GABA released in a calcium- and SNARE-independent way by nonconventional release sites such as growth cones and astrocytes diffuses away to activate extrasynaptic receptors [44]. The absence of an efficient uptake system will enable GABA to accumulate in the extracellular space and to reach a concentration sufficient to exert its distal action. The depolarizing action of GABA would activate voltage-dependent calcium channels and would facilitate the relief of the voltage-dependent magnesium block from NMDA receptors, thus allowing calcium entry and activation of second messengers.

Using network dynamics imaging, online reconstruction of functional connectivity and targeted whole-cell recordings, it has been recently demonstrated that, in immature hippocampal slices, functional hubs composed of subpopulations of GABAergic interneurons with large axonal arborizations are able to synchronize large neuronal ensembles [45]. The depolarizing action of GABA in immature neurons results from an outwardly directed flux of chloride. Chloride homeostasis is controlled by the Na-K-2Cl cotransporter NKCC1 and by the K-Cl cotransporter KCC2 that enhance and lower $[Cl^-]_i$, respectively [46]. Due to the low expression of the KCC2 extruder at birth, chloride accumulates inside the neuron *via* NKCC1. The progressive increase in the expression of KCC2 is responsible for the developmental shift of GABA from the depolarizing to the hyperpolarizing direction. KCC2 extrudes K^+ and Cl^- using the electrochemical gradient for K^+. Cl^- extrusion is weak in immature neurons and increases with neuronal maturation.

The functional role of the depolarizing action of GABA on early circuits development has been assessed by manipulating the expression levels of KCC2 and NKCC1, respectively. Thus, the premature expression of KCC2, has been shown to convert the action of GABA from excitatory to inhibitory and to impair the morphological maturation of cortical cells, without altering their radial migration [47]. This effect can be mimicked by overexpressing the inwardly rectifying K^+ channel which lowers the membrane potential and reduces cell excitability, strongly suggesting that membrane depolarization caused by the early GABA excitation is essential for the functional maturation of cortical circuits *in vivo*. On the other hand, knocking down the expression of NKCC1 to abolish $GABA_A$-mediated excitation, leads to a significant reduction in AMPA receptor-mediated synaptic transmission associated with a disruption of dendritic arborization and spines density further indicating that the depolarizing and excitatory action of GABA plays a permissive role in the formation of excitatory synapses [48]. Interestingly, these effects could be rescued by over expressing a mutant form of voltage-independent NMDA receptors, indicating that GABA depolarization cooperates with NMDA receptor to regulate the formation of excitatory synapses. It is worth noting that GDPs and associated calcium transients act as coincidence detectors for enhancing, in an associative type of manner, synaptic efficacy at emerging GABAergic [49], and glutamatergic synapses [50]. Using a "pairing" procedure, consisting of correlating GDPs-associated calcium rise with stimulation of mossy fibers or Schaffer collaterals, in the CA3 and CA1 region, respectively, we found that this procedure produced a strong and persistent potentiation of synaptic responses (Figure 2).

In the absence of pairing, no significant changes in synaptic efficacy could be detected. Similar results were obtained by progressively increasing the interval between GDPs and mossy fiber/Schaffer collateral stimulation. Pairing-induced potentiation was prevented when the cells were loaded with

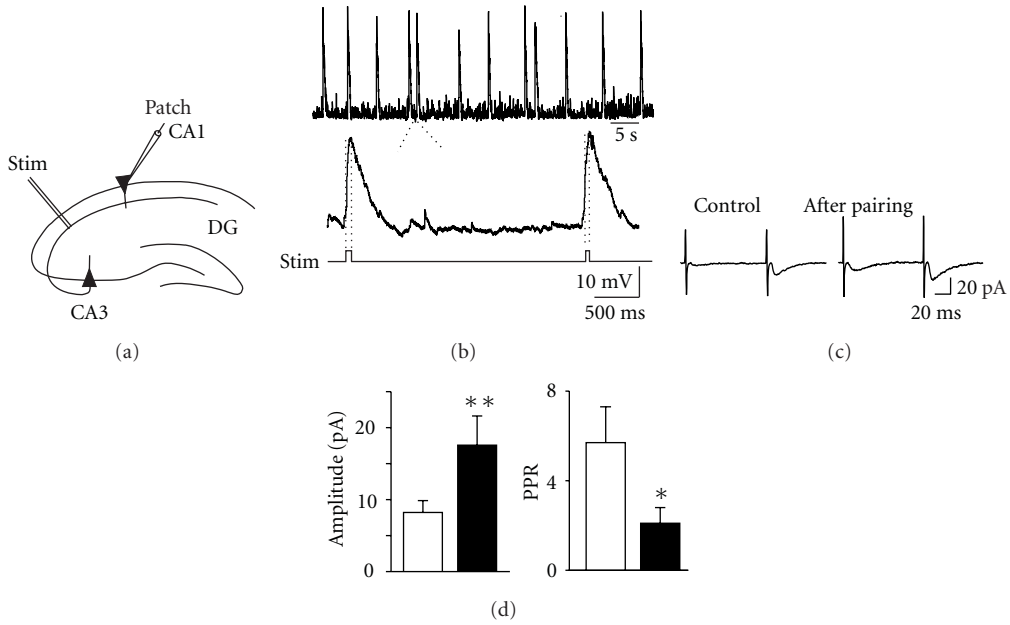

Figure 2: Pairing GABA-mediated GDPs with Schaffer collateral stimulation persistently enhances synaptic strength at glutamatergic CA3-CA1 connections. (a) Experimental paradigm. (b) The rising phase of GDPs (between the dashed lines) was used to trigger synaptic stimulation (stim) (c) EPSCs evoked in CA1 principal cells by minimal stimulation of Schaffer collateral, before and after pairing (average of 19 responses). (d) Each bar represents the mean peak amplitude of synaptic responses including failures ($n = 8$) and the paired pulse ratio (PPR; $n = 8$), obtained before (open) and after (closed) pairing. (Modified from [50]).

the calcium chelator BAPTA or when nifedipine (but not the NMDA receptor antagonist D-(-)-2-amino-5-phosphonopentanoic acid) was added to the extracellular medium, suggesting that activity-dependent changes in synaptic efficacy depend on calcium rise through voltage-dependent calcium channels and not *via* NMDA receptors.

Immature neurons are characterized by an elevated number of "silent" synapses [40]. These are synapses that do not conduct at rest either, because the neurotransmitter is not released when the presynaptic terminal is invaded by an action potential (presynaptically silent), or because they are unable to detect the release of the neurotransmitter due to the lack of the respective receptors on the subsynaptic membrane (postsynaptically silent). Silent synapses can be converted into active ones by activity-dependent processes and this represents the most common mechanism for LTP induction, not only during development but also in the mature brain [51]. Interestingly, the pairing procedure was able to convert silent synapses into active ones. In particular, in double pulse experiments, pairing caused the appearance of responses to the first stimulus and increased the number of successes to the second one, indicating that an increased probability of transmitter release accounts for long-term increase in synaptic strength. Therefore, calcium entry through voltage-dependent calcium channels, activated by the depolarizing action of GABA during GDPs, is instrumental in enhancing the number of functional GABAergic and glutamatergic synapses and/or the probability of GABA and glutamate release in a Hebbian way. This may contribute to refine neuronal connectivity before the establishment of the adult neuronal circuit.

3. Molecular Determinants of GABAergic Synapses Formation

In the adult brain, information processing relies on the integration of excitatory and inhibitory circuits which use glutamate and GABA/glycine as neurotransmitters, respectively. The so-called excitatory/inhibitory (E/I) balance represents a critical condition for the correct functioning of neuronal networks and it is essential for nearly all brain functions, including representation of sensory information and cognitive processes. The E/I balance is maintained *via* highly regulated homeostatic mechanisms [52]. Neurons are able to compensate for experimental perturbations by modulating ion channels, receptors, signaling pathways, and neurotransmitters. At the molecular level, these processes require chromatin remodeling, changes in gene expression and repression, changes in protein synthesis, turnover and cytoskeleton rearrangement [53]. A disruption of the homeostatic control, due to the lack of compensatory changes, leads to an imbalanced E/I ratio and to the developmental of neuropsychiatric disorders including mental retardation, epilepsy and ASDs [21].

During brain maturation, the development of a proper E/I balance is achieved with the shift of GABA action from the depolarizing to the hyperpolarizing direction, a process that in rodents starts appearing toward the end of the first, beginning of the second postnatal week [54]. Disturbances in the E/I balance may also occur when the formation or maintenance of one class of synapses prevails over the others. The selective loss of excitatory or inhibitory synapses can take place during the initial period of synapse formation

and consolidation or late in development during activity-dependent refinement of neuronal circuits and may involve mutations in genes encoding for ion channels or $GABA_A$ receptor subunits. These would lead to circuits with abnormal activity and prone to seizures [55]. For example, the disruption in mice of the *gabrb3* gene, which encodes for $\beta 3$ subunits of $GABA_A$ receptors, highly expressed during development, is sufficient to cause phenotypic traits which parallel those present in the Angelman syndrome [56]. Thus, mice lacking the $\beta 3$ subunits exhibit a major reduction of $GABA_A$ receptors, thalamic disinhibition and seizures associated with learning and memory deficits, poor motor skills on a repetitive task, hyperactivity, and a disturbed rest-activity cycle, all features characteristic of children affected by this neurological disorder. The cellular and molecular mechanisms underlying these phenomena are still poorly understood and their comprehension is further complicated by intrinsic differences among neuronal types, experimental conditions and the developmental stage of neurons [57].

During neuronal circuit assembly, GABA signaling precedes and promotes the formation of glutamatergic synapses [58]. The sequential development of GABA- and glutamate-mediated connections is independent on the arrival of afferent inputs but is related to the degree of maturation of targeted cells including changes in dendritic length, in somatic size and capacitance [58]. While functional GABAergic synapses require the presence of small apical dendrites in stratum radiatum of the hippocampus, glutamatergic connections require the presence of dendrites in stratum lacunosum moleculare.

The refinement of GABAergic connections and their translation into a potent inhibitory network is a protracted process which extends well beyond the first two postnatal weeks into the adolescent period and is regulated by neuronal activity and experience. In the visual cortex, for instance, experience-dependent regulation of the GABAergic innervation controls the onset of critical periods [59] during which neuronal circuits display a heightened sensitivity to environmental stimuli and are greatly shaped by sensory experience. Thus, a delayed and an accelerated onset in visual plasticity can be obtained by negatively or positively interfering with the GABAergic function, respectively [59]. GABA signaling itself would be responsible for the development of inhibitory connections as demonstrated by the observation that, knocking down GAD67 in basket interneurons severely impairs GABAergic innervation [20]. These effects may be attributed to the activity-dependent reduction in GABA synthesis and signaling following down regulation of GAD67 levels and/or enzyme activity [20].

To be highly efficient, synaptic transmission requires the presence of clustered postsynaptic receptors localized in precise apposition to presynaptic release sites. At inhibitory connections, this task is accomplished by gephyrin, a tubulin-binding protein which traps glycine and $GABA_A$ receptors in the right place anchoring them to the cytoskeleton [60].

Interestingly, a recent study has demonstrated that gephyrin directly interacts with adhesion molecules of the NLGs family [61] which in turn bind to their presynaptic partners NRXNs to regulate transmitter release (Figure 3).

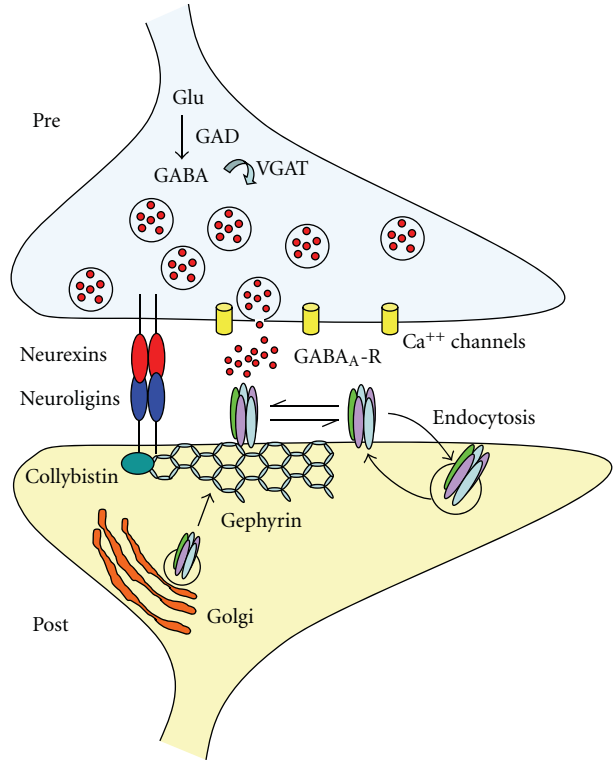

Figure 3: Structural organization of GABAergic synapses. The postsynaptic organization comprises a large number of proteins that allow the correct targeting, clustering and stabilization of $GABA_A$ receptors. Among them gephyrin forms hexagonal lattices that trap $GABA_A$ receptors in precise apposition to presynaptic release sites. Cell adhesion molecules of the neuroligin-neurexin families bridge the cleft and ensure transsynaptic signaling, essential for the maintenance of a proper E/I balance.

Therefore, gephyrin plays a key role not only in stabilizing $GABA_A$ receptors but also in regulating transsynaptic signaling and in maintaining an appropriate E/I balance. The NLG-NRXN complexes possess a potent "synaptogenic" or synapses organizing activities as demonstrated by their ability to induce presynaptic differentiation of contacting neuritis when expressed in heterologous nonneuronal cells. Postsynaptic NLGs promote the assembly of functional presynaptic specializations in axons while presynaptic NRXNs recruit postsynaptic scaffolding proteins and neurotransmitter receptors in dendrites *via* their interaction with NLGs [62]. By functionally coupling synaptic calcium channels with the release machinery, NRXNs are thought to play an essential role in calcium-triggered neurotransmitters release [63]. The NLGs family comprises five different genes (NLG1-NLG5 with various splice variants), which form homodimers through the extracellular domain. Among these, NLG2 is preferentially associated with GABAergic synapses, while NLG1 with glutamatergic synapses [64, 65]. The NRXN family includes α- and β-NRXN. Initially, β-NRXN was considered the main partner for NLG, but recently, also α-NRXN was found to bind NLG [66]. Unlike β-NRXN that participates in the formation of both glutamatergic and GABAergic synapses, α-NRXN seems to

be specific for GABAergic synapses [67]. Therefore, it is clear that within a neuronal network, the NLG-NRXN interaction controls the formation of both glutamatergic and GABAergic synapses [68]. At inhibitory synapses, $GABA_A$ receptors are firstly assembled in the endoplasmic reticulum from appropriate subunits and then delivered to the plasma membrane. Targeting and clustering $GABA_A$ receptors at synaptic and extrasynaptic sites is dynamically regulated by neuronal activity [69] and requires the precise interplay of various proteins and active transport processes along the cytoskeleton [60, 70].

Disrupting endogenous gephyrin with selective antibodies led to a reduction of $GABA_A$ receptor clusters [71], an effect that was associated with a decrease in the density and size of NLG2 clusters and with a loss of GABAergic innervation (Kasap, personal communication). Thus, pair recordings from interconnected cells demonstrated that, respect to controls, neurons transfected with recombinant antibodies against gephyrin exhibited a lower probability of GABA release. This reduction likely involves NLG2 which is preferentially concentrated at inhibitory synapses and directly binds gephyrin through a conserved cytoplasmatic domain [61]. Similarly, at glutamatergic synapses, the NLG-NRXN complex has been shown to act as a coordinator between postsynaptic and presynaptic sites [72]. Hence, overexpressing the glutamatergic scaffold protein PSD-95 on the postsynaptic site enhanced the probability of glutamate release *via* a retrograde modulation of neurotransmitter release which probably involves the NLG-NRXN complex. From the reported data, it is not surprising that single mutations in genes encoding for adhesion molecules belonging to the NLG-NRXN families, such as those found in few cases of ASDs [73], lead to defective architectural structuring of synaptic connections, molecular assembly of synapses and an E/I unbalance.

As outlined in the next section, the use of animal models of ASDs has enabled to investigate the mechanistic basis of the E/I imbalance for a range of neurodevelopmental disorders.

4. Altered GABAergic Signaling in Animal Models of ASDs

A dysfunction of GABAergic signaling mediates autism-like stereotypes in the majority of animal models of ASDs obtained by experimentally manipulating candidate genes for autism susceptibility or environmental risk factors. The characteristic ASDs phenotype is often associated with either a loss or a gain of the GABAergic function. Consistent with postmortem studies from brain tissues obtained from ASDs patients [74] alterations in GABA synthesising enzymes GAD65 and GAD67, in GABA release, in the expression of particular subtypes of $GABA_A$ receptors have been described.

A presynaptic reduction in glutamic acid decarboxylase 1 (*Gad1*) and glutamic acid decarboxylase 2 (*Gad2*) mRNA encoding for GAD67 and GAD65, respectively, has been recently found in mice lacking the *Mecp2* gene in GABA releasing neurons (*Viaat-Mecp2*$^{-/y}$, [75]). Mutations in the X-linked *Mecp2* gene, which encodes the transcriptional regulator methyl-CpG-binding protein 2 (MeCP2), cause the majority of Rett syndrome cases [76–78] which is characterized by an apparently normal early development followed by loss of language skill, motor abnormalities, cognitive deficits, stereotyped behavior, respiratory dysrhythmias, and seizures leading sometimes to premature death. *Viaat-Mecp2*$^{-/y}$ mice exhibit a significant reduction in amplitude (but not in frequency) of miniature inhibitory postsynaptic currents (mIPSCs) an effect which occurs in the absence of any alteration in amplitude or frequency of miniature excitatory postsynaptic currents (mEPSCs), indicating that MeCP2 deficiency in GABAergic neurons has a cell-autonomous impact on quantal release from glutamatergic neurons [75]. The reduction in GABA content and inhibitory neurotransmission affects synaptic plasticity processes as suggested by the impairment of long-term potentiation (LTP) induced by theta burst stimulation of Schaffer collateral [75]. Previous electrophysiological studies using *Mecp2* null mice, revealed a significant reduction in spontaneous firing associated with a decrease in amplitude of mEPSCs in layer 5 pyramidal neurons as compared to WT control animals at early presymptomatic and symptomatic stages [79]. In the hippocampus of *Mecp2* null mice, the diminished level of basal excitatory drive has been shown to contribute, at the network level, to slow down spontaneous rhythmic field potentials activity, generated by the interplay between excitation and inhibition [80]. This condition paradoxically makes the hippocampal network overresponsive to excitatory stimuli.

An imbalance between excitation and inhibition has been found also in individuals affected by Tuberous sclerosis, a genetic multisystem disorder characterized by widespread hamartomas in several organs, including the brain, heart, skin, eyes, kidney, lung, and liver [81]. Tuberous sclerosis patients exhibit a variety of neurological disorders including epilepsy and autism-like disorders. The affected genes are *Tsc1* and *Tsc2* encoding hamartin and tuberin, respectively. The hamartin-tuberin complex inhibits the mammalian-target-of-rapamycin pathway that controls cell growth and proliferation [81].

Interestingly, a loss of GABAergic function accounts for the hyper excitability observed in an animal model of fragile X syndrome (FXS), a common inherited cause of mental retardation with language deficits, hyperactivity, autistic behavior and seizures. FXS is caused by a trinucleotide expansion of fragile X mental retardation 1 (*fmr1*) gene which prevents the expression of the encoded protein called Fragile X mental retardation protein (FMRP, [82]). As the *Mecp2* gene, the *fmr1* gene is located in chromosome X (Xq27.3). The lack of FMRP in animal models of FXS (the *Fmr1*-null mouse) leads to an E/I imbalance in favor of excitation. Among the factors contributing to enhance cell excitability in *Fmr1* KO animals an impairment of GABAergic circuitry [83] and a decreased expression of $GABA_A$ receptor subunits have been reported [84–87]. In subicular neurons, for example, a down regulation of $GABA_A$-mediated tonic (but not phasic) inhibition associated with a reduced expression of $\alpha 5$ and δ $GABA_A$ receptors subunits

has been found [88]. These alterations may contribute to deficits in cognitive functions and to epileptic activity observed in FXS patients. In contrast, electrophysiological recordings from spiny neurons in the striatum, involved in motor control and in specific aspects of cognition and motivation, have revealed a selective increase in frequency of sIPSCs and mIPSCs, probably secondary to an enhanced probability of transmitter release from GABAergic terminals, suggesting that modifications in GABAergic function in *Fmr1* KO mice are region-specific [89].

Relevant inhibitory synaptic abnormalities (involving both phasic and tonic $GABA_A$-mediated inhibition), which may contribute to the abnormal social behavior of *Fmr1* null mice, are present in the basolateral nucleus of the amygdala [90], which regulates fear and anxiety behaviors.

Linkage and association studies have revealed that the chromosomal region 15q11-q13 is strongly implicated in ASDs [91]. Maternal duplications of this region remain one of the most common cytogenetic abnormalities found in cases of idiopathic ASDs, which account for 1-2% of cases. Deletion of this region results in either Angelman or Prader-Willi syndrome, depending from which parent the deletion has been inherited [92]. Interestingly, within this chromosomal region, exists a gene cluster of $GABA_A$ receptors, *G*abrb*3*, *G*abra*5*, and *G*abrg*3*, encoding for $\beta 3$, $\alpha 5$, and $\gamma 3$ subunits, respectively. $GABA_A$ receptors are hetero-oligomeric proteins spanning the membrane to form anion-permeable channels. Assembled from eight classes of subunits exhibiting different degrees of homology a large variety of functional receptors with different biophysical and pharmacological properties are expressed in mammalian brain. $GABA_A$ receptors play a crucial role in proliferation, migration, and differentiation of precursor cells thus contributing to the establishment of neuronal circuits [93]. A developmental deficit of $GABA_A$ receptors function would affect neurogenesis and maturation of neuronal network. Among different $GABA_A$ receptor genes, the targeted deletion of *Gabrb3* gene encoding for the $\beta 3$ subunit, which is highly expressed during brain development [94], leads to abnormalities in social behavior, cognitive deficits, motor stereotypes and seizures, reminiscent of the ASDs phenotype [56, 92, 95, 96].

Other mutations that affect the GABAergic system concern the homeobox genes *Dlx1* and *Dlx2*, involved in the development of most telencephalic GABAergic neurons [97]. Interestingly, the human locus with the highest LOD score for autism susceptibility (D2S2188 on chromosome 2q) maps very close to the gene encoding for the GABA synthesized enzyme GAD65 and to *Dlx1* and *Dlx2*. Furthermore, the autism susceptibility locus D7S477 on chromosome 7q maps within about six megabases of *Dlx5* and *Dlx6* which are implicated in the regulation of forebrain GABAergic neurons [98]. This region hosts the gene encoding for Reelin, a protein expressed in cortical GABAergic neurons [99]. Reelin is a signaling protein that plays a pivotal role in the migration of several neuronal cell types and in the development of neuronal connections [100, 101]. Reeler mice, devoid of Reelin, exhibit cytoarchitectonic alterations in their brain similar to those found in autistic patients [102] associated with decrease GABA turnover [103].

Interestingly, the removal of the homeobox containing transcription factors Engrailed-2 (*EN2*), known to be involved in the regionalization pattering and neuronal differentiation of the midbrain and hindbrain [104] in mice (*En2−/−mice)* leads to behavioral abnormalities similar to those observed in ASDs patients [105]. In addition, these mice exhibit a reduced expression of parvalbumin and somatostatin positive interneurons in the hippocampus, an effect associated with an increased susceptibility to seizures [105, Table 1].

While the majority of animal models so far examined exhibits a loss of GABAergic function, mice carrying the human R415C mutation in the *Nlgn3* gene display a gain of function. Neuroligins (NLGs) are specialized cell adhesion molecules that functionally couple the postsynaptic densities with the transmitter release machinery by forming transsynaptic complexes with their presynaptic-binding partners, neurexins [73]. NLG3 R451C KI mice bear a striking phenotype with mimics in many aspects that found in ASDs patients ([106] but see [107]). Functional characterization of these mice has revealed (in contrast with NLG3 KO mice) a loss of NLG3 in the forebrain associated with impaired social interactions and a 50% increase in the frequency of spontaneous inhibitory events with apparent no effects on excitatory synaptic transmission [106]. Interestingly, in NLG3 R451C KI mice, the gain of function is accompanied with a significant increase in the level of the vesicular transporter for GABA, VGAT, and gephyrin, a postsynaptic scaffolding protein, crucial for recruiting and maintaining neurotransmitter receptors in the right place and for ensuring a correct E/I balance. Whether the increased release of GABA selectively affects only a subset of GABAergic interneurons is still unclear. In addition, this animal model exhibits an asymmetric reduction of parvalbumin-positive basket cells across the two hemispheres [108]. However, immunocytochemical data from postmortem material obtained from ASDs patients have revealed an increased density of calbindin-, calretinin-, and parvalbumin-positive interneurons in the hippocampus [23], a condition that would alter neuronal signaling and synchronization leading to cognitive dysfunctions [109]. The enhanced GABAergic innervation may cause a compensatory downregulation of $GABA_A$ receptors. The reduction in benzodiazepine-binding sites on $GABA_A$ receptors observed in the hippocampus of autistic patients supports this hypothesis [110].

Among autism risk factors, prenatal or neonatal environmental challenges, including early exposure to valproic acid (VPA), a histone deacetylases inhibitor, are widely used as animal models of ASDs [111]. The VPA model has been developed on the basis of the observation that treatment of epilepsy or bipolar disorders in pregnant women (20–24 days after conception) with VPA leads to an increased incidence of ASDs in their children [112]. A unifying hypothesis where the core pathology of the autistic brain consists in hyper-functionality and excessive neuronal processing in local neuronal microcircuits in prefrontal, somatosensory cortex, and amygdala, leading to social and environmental withdrawal has been proposed [113, 114]. Interestingly, as the neuroligin-3 model, the VPA model of ASDs exhibits an

Table 1: Main alterations of GABAergic signaling present in different animal models of ASDs. For the Rett syndrome, different genotypes are expressed in brackets.

Mouse model	Alterations in GABAergic signaling	Ref.
Mecp 2-KO (Rett syndrome)	Reduced levels of GAD65 and GAD67 (*Viaat-Mecp2*$^{-/y}$)	[75]
	Reduced inhibitory quantal size in layer 2/3 pyramidal neurons of the somatosensory cortex	
	The E/I balance is shifted to favor inhibition over excitation in cortical networks (*Mecp2*$^{2lox/x}$, *Nestin-Cre*)	[79]
	Reduced frequency of IPSC-based spontaneous rhythmic field potentials in the hippocampus (*Mecp2*$^{tm1.1Bird}$)	[80]
Fmr 1-KO (X fragile)	Down regulation of GABAA-mediated tonic inhibition in the subiculum	[88]
	Reduced expression of α5 and δ GABAA receptor subunits in the subiculum	
	Increased frequency of sIPSCs and mIPSCs in the striatum	[89]
	Reduction in amplitude and frequency of sIPSCs and mIPSCs	[90]
	Reduced GABAA-mediated tonic inhibition	
	Reduced GABAergic innervation in the amygdala	[84–87]
	Reduced expression of GABAA receptor subunits	
Gabrb 3 KO	The E/I balance is shifted to favor excitation over inhibition in cortical networks (EEG recordings)	[56]
Dlx1/Dlx2 KO	Abnormal cell migration	
	Reduction in the number of GABAergic interneurons in the cortex, olfactory bulb and hippocampus	[97]
Reln-KO	Reduced level of GAD67	[103]
	Decreased GABA turnover	
En2-KO	Reduced expression of parvalbumin- and somatostatin- positive GABAergic interneurons in the hippocampus	[115]
	Increased susceptibility to seizures	
Nlg3 R451C KI	Increased frequency of mIPSC	
	Increased level of VGAT and gephyrin	[106]
	Asymmetric reduction of PV positive basket cells across cortical hemispheres	[108]
valproic acid	The E/I balance is shifted to favor excitation over inhibition in the lateral amygdala (multi electrode arrays)	[114]
	Asymmetric reduction of PV positive basket cells across cortical hemispheres	[108]

asymmetric reduction of parvalbumin-positive cells across the two hemispheres [108]. The disruption of inhibitory circuits may delay critical periods in specific ASDs brain regions [59], thus perturbing γ-oscillations implicated in high cognitive functions.

5. Future Perspectives

Although much more work is required to understand the cellular and molecular mechanisms regulating the E/I balance at synapses, it is clear from the reviewed data that GABAergic signaling plays a key role in the construction of neuronal networks and that disruption of GABAergic circuits accounts for several neurodevelopmental disorders including ASDs. A significant progress has been made in characterizing genes involved in synapses formation and maintenance but their role in the organization of neuronal circuits is still limited. From a clinical perspective, a challenged task will be to identify, in animal models of ASDs, the cellular substrates of microcircuits implicated in different cognitive and behavioral deficits associated with ASDs. This can be accomplished by using new optogenetic tools that would allow to selectively activate or silence specific interneuronal populations and to study their functional consequences [116]. With this technique, GFP fusions of channelrhodopsin-related proteins and halorhodopsin, can be delivered into the brain *via* viral infection. In response to different wavelengths of light, label cells and axons can be either depolarized (in the case of channelrhodopsin, [117]) or hyperpolarized (in the case of halorhodopsin), thus allowing to switch on and off selective groups of genetically targeted interneurons and to study the neural basis of different behaviors [118]. This will allow better understanding the mechanistic bases of ASDs and to develop new selectively targeted therapeutic tools for most effective interventions.

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Review Article

Autism: A "Critical Period" Disorder?

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Cortical circuits in the brain are refined by experience during critical periods early in postnatal life. Critical periods are regulated by the balance of excitatory and inhibitory (E/I) neurotransmission in the brain during development. There is now increasing evidence of E/I imbalance in autism, a complex genetic neurodevelopmental disorder diagnosed by abnormal socialization, impaired communication, and repetitive behaviors or restricted interests. The underlying cause is still largely unknown and there is no fully effective treatment or cure. We propose that alteration of the expression and/or timing of critical period circuit refinement in primary sensory brain areas may significantly contribute to autistic phenotypes, including cognitive and behavioral impairments. Dissection of the cellular and molecular mechanisms governing well-established critical periods represents a powerful tool to identify new potential therapeutic targets to restore normal plasticity and function in affected neuronal circuits.

1. Introduction

The developing brain is remarkably malleable, capable of restructuring synaptic connections in response to changing experiences. The basic layout of the brain is first established by genetic programs and intrinsic activity and is then actively refined by the surrounding environment in which the individual is immersed [1]. This experience-dependent sculpting of neuronal circuits occurs during distinct time windows called critical periods [2]. There are thought to be independent postnatal critical periods for different modalities, ranging from basic visual processing to language and social skills. They occur sequentially in a hierarchical manner, beginning in primary sensory areas. Critical periods close after a cascade of structural consolidation of neuronal circuits and their connectivity, preventing future plasticity as the brain reaches adulthood.

These sensitive periods of elevated plasticity are times of opportunity but also of great vulnerability for the developing brain. As many have experienced, it is easier to learn a new language, musical instrument, or sport as a child rather than in adulthood. On the other hand, early disruption of proper sensory or social experiences will result in miswired circuits that will respond suboptimally to normal experiences in the future. The devastating effects of early deprivation are scientifically documented [3, 4]. Studies of socially and emotionally deprived children raised in Romanian orphanages have demonstrated that the neglected children exhibit severe developmental delay, mental retardation, and neuropsychiatric symptoms [4]. Orphans need to be placed with caring foster families away from orphanages before two years of age in order to develop cognitive, social, and intellectual skills. Neglected children are not able to recover normal function even if they are later placed in similar foster homes. Comparable effects are seen for the development of the primary senses as well. Conductive hearing loss often associated with childhood ear infections can produce long-lasting deficits in auditory perceptual acuity if not treated before the age of seven [5–7]. Similarly, if a child's binocular vision is compromised by strabismus or cataract and is not corrected before the age of eight, loss of acuity in that eye, or amblyopia, is permanent and irreversible [8, 9]. If corrected promptly, restoration of normal binocular vision is possible.

Why the brain is able to recover function early in life, but loses this ability with maturity? What are the mechanisms underlying experience-dependent circuit refinement in early development? Can we recreate the plasticity of the immature brain later in life and eventually recover proper

function? It turns out that a very precise balance of cortical excitatory and inhibitory (E/I) neurotransmission is required for critical period plasticity [10, 11]. Studies in the rodent visual system have shown that, in particular, the level of the inhibitory neurotransmitter GABA and the maturation of specific inhibitory circuits are crucial [11, 12]. Since critical periods are so tightly regulated, this makes them vulnerable if the E/I balance is tipped in either direction without compensatory homeostatic correction. Recent research has indicated that neurodevelopmental disorders like autism may result from disruption of this balance early in life. This could be due to a combination of genetic or environmental insults that compromise excitatory or inhibitory components at the genetic, molecular, synaptic, or circuit level. Depending on the location and severity of imbalance, a spectrum of phenotypes could result, as is true for autism. Thus, it is attractive to hypothesize that autism may result from disruption of the expression and/or timing of critical periods across brain regions.

Autism is generally diagnosed within the first three years of life, during this time of intense experience-dependent circuit refinement. The diagnostic behavioral symptoms of autism are abnormal socialization and communication, and repetitive behaviors [13]. Many studies have focused on addressing how the autistic brain perceives relevant information, like face-processing, language, and theory of mind. These data have been valuable in beginning to understand how the higher-order processing centers of the brain differ in autism. However, it is important to realize that these areas rely on integration of inputs from lower cortical regions, building off a reliable and accurate representation of the world generated by primary sensory areas. Critical period disruption resulting in a slight degradation in the quality of any or all of these senses would compromise the ability to successfully execute behaviors relying on this information, creating severe deficits. Indeed, sensory deficits have been reported in autistic individuals, indicating possible improper primary sensory perception [14].

Autism is called a "spectrum disorder" because of the extraordinary heterogeneity of intellectual ability, associated symptoms, and possible etiology. Though there is clearly a genetic basis to autism, the majority of cases have unknown causes [15, 16] Autism is comorbid with a number of other diseases, including Rett, Fragile X, and Angelman Syndrome. These diseases have known genetic causes and have been well modeled via genetic modification of animals, thereby providing valuable tools to dissect the molecular changes underlying autism. Despite these advances, there is still no cure [17, 18].

A common emerging theme based on data from human patients and animal models is an imbalance in excitatory and inhibitory transmission. This review will summarize the research to date that supports this theory, focusing in particular on the disruption of inhibitory signaling and how this may compromise the expression of critical periods, ultimately leading to the characteristic behaviors of autism. With better understanding of the molecular changes in the autistic brain, we can begin to identify key experiments that will help guide therapeutic intervention.

2. Primary Sensory Function in Autism

The majority of autism research has focused on the higher cognitive symptoms of autism, for it is solely these features that comprise the diagnosis of autism. However, it must be considered that the development and proper execution of higher cognitive processes depends on normal primary processing [19]. The behaviors relevant to autism require concurrent information from many sensory areas. For example, communication and socialization involve parallel auditory, visual, and somatosensory information processing. It is interesting to consider a model in which defects in the development of primary sensory abilities are the original problem, which then results in a cascading effect on higher integrative areas of the brain [20].

A common feature of autistic individuals is atypical behavioral responses to sensory stimulation and reports of hyper- or hyposensitivity to sensory stimulation in multiple domains [14, 21]. There are many accounts of disruption of primary sensory processing in autism [22–25], and there is a growing body of evidence that tests these reports in a controlled laboratory setting. A recent meta-analysis of 14 parent-report studies on sensory-modulation suggests that autistic individuals exhibit significantly more sensory symptoms than control groups, particularly between the ages of six and nine [26]. Interestingly, most studies have concluded that several sensory processing are more commonly disrupted in autism than in other developmental disorders; these symptoms lessen with age; their severity correlates with the severity of social impairment [27]. We will touch on a few examples of altered sensory processing in the auditory, somatosensory, visual, and multisensory integration areas from the human autism literature.

Many studies of sensory phenotypes in autism have focused on the auditory system because of the language deficits characteristically observed in patients. There do appear to be lower-level cortical auditory processing abnormalities as measured by electroencephalograms (EEG) and magnoenetcephalography (MEG) in multiple studies, but the nature of these differences is variable and depends on the specifics of the individual studies [14]. For example, while some studies have found that autistic subjects have increased latency in cortical response to tones [28, 29], others observed a decreased latency in cortical response [30–32]. These contrasting results may reflect the wide spectrum of autism phenotypes, the limited number of tested subjects, their age, or different experimental paradigms used.

Abnormal somatosensory experiences are commonly reported in autistic individuals [33]. One psychophysical study by Tommerdahl et al. [34] tested the ability to spatially discriminate two vibrotactile stimuli applied to the skin of the hand in a small group of autistic subjects. After a priming stimulus, subsequent spatial discrimination in that same area of skin improved for controls but not for autistic subjects. The authors suggest that this may reflect a deficit in cortical inhibition of neighboring minicolumns, though this claim was not directly tested. Psychophysical studies rely on the behavioral report of the subject, and therefore may be complicated by behavioral impairments in autistic subjects.

Several somatosensory studies have measured brain activity instead as a means to evaluate sensory processing. Miyazaki et al. [35] found abnormal short-latency somatosensory evoked potentials (S-SEPs) in response to median nerve stimulation in about half of the autistic patients they tested. However, it must be noted that there were no concurrent controls tested in this study, and instead autistic S-SEPs were compared to S-SEPs from controls in a previous study. Another group used MEG to map the cortical representation of the hand and face regions of high-functioning autistic and control subjects [36]. Brain activity was recorded in response to physical stimulation of the skin. Interestingly, the autistic subjects had a spatially distorted cortical representation of the hand and face compared with controls. Overall, abnormal somatosensory processing may play a large role in the avoidance of affective contact that contributes to the social and communication abnormalities in autism [33].

Due to the social phenotype of autism, one of the better-studied visual impairments of autism is face processing [27]. However, an interesting hypothesis is that lower-order visual deficits would consequently impair higher-order visual processing of faces. A study by Vlamings et al. [37] recorded VEPs in autistic and control children while they looked at two types of stimuli—simple horizontal gratings or faces with neutral or fearful expressions. The gratings or faces were composed of either high or low spatial frequency lines. Autistic children, in contrast to controls, had an enhanced VEP response to high spatial frequencies and performed better at facial expression categorization when the faces were high-pass filtered. Nonautistic children generally use low spatial frequency information to categorize the emotions of facial expressions. This difference seen in autistic children is in agreement with previous findings that autistic perception is more detail-oriented [38–41]. This study suggests that abnormal primary visual processing could also contribute to social and communicative deficits in autism.

In addition to atypical unisensory experiences in autism, growing evidence points towards abnormalities in multisensory processing, which is the integration of information from different senses into one perceptual experience [14]. Deficits in multisensory integration (MSI) fit with a popular theory of the autistic brain, in which there is excessive local connectivity within one brain region but long-range hyperconnectivity between brain regions [19, 42, 43]. As for unisensory modality processing, MSI seems to be disrupted in a variety of ways depending on the study, including enhanced, decreased, or altered in some fashion. A recent study used high-density electrical mapping of the cortex with EEG to measure MSI in response to audio-somatosensory stimuli [44]. Vibrotactile stimulation and tones were presented to the passive subject either separately or in combination, and differences in event-related potentials (ERPs) between uni- and multimodal stimulation were measured. Overall, the autism group showed less MSI than controls. In contrast, a psychophysical study investigated audio-visual integration, as this has direct relevance to speech perception, and found an extended temporal window for MSI in the autism group [45].

Another way to evaluate audio-visual integration is with the McGurk effect [46]. In the McGurk effect paradigm, an individual hears the sound of one phoneme (/*ba*/) while watching a muted video of a person saying another phoneme (/*ga*/). Due to the multimodal quality of speech perception, the sound of the voice combines with the sight of the lips moving, and the individual reports hearing a third intermediate phoneme (e.g., /*da*/), the perceptual product of normal multimodal integration. This phenomenon was originally reported to occur less frequently for autistic individuals [47]. More recent studies confirmed some level of disruption in the McGurk paradigms in autism subjects mainly affecting the ability to read lips [48, 49] and the comprehension of speech in the presence of background noise compared to control subjects [49]. Audiovisual speech integration is already present in infants as young as 2 months old [50] and contributes to phonetic learning [51] and language development [52]. Combined deficits in audiovisual processing may then contribute to delays in language acquisition and speech comprehension during social interactions or school settings.

In order to better understand the role of sensory processing and perception in the pathogenesis of autism, a systematic developmental study must be conducted in autistic, high-risk infant siblings and control subjects. The development of primary senses, as well as their integration into meaningful behavior, requires experience-dependent plasticity. We propose that a disruption of neuronal circuit refinement during critical periods may represent the mechanistic link between these abnormal behaviors.

3. Critical Period Mechanisms

Critical periods have been demonstrated in a variety of contexts [2]. Critical or sensitive periods exist for complex phenomena such as filial imprinting [53], acquisition of courtship song in birds [54, 55], sound localization [56], and fear extinction [57–59]. They also exist for primary sensory modalities and such as tonotopic map refinement in auditory cortex [60] and barrel formation [61] and tuning to whisker stimulation [62, 63] in rodent somatosensory cortex. One of the most mechanistically well-characterized critical periods is for ocular dominance (OD) plasticity in the mammalian visual cortex. Here, we will focus our discussion on the OD critical period because its underlying molecular and cellular mechanisms have been extensively dissected, making it the best model system for testing our hypothesis that critical periods may be abnormal in autism.

Abnormal visual input to one eye during infancy results in permanent loss of visual acuity, amblyopia (*Greek for dull vision*), if not corrected during childhood. If perturbation of vision occurs in adulthood, the visual impairments are significantly milder or absent [64]. This observation in humans inspired the development of a simple laboratory paradigm to test the existence of a critical period in animal models. David Hubel and Torsten Wiesel began investigating OD plasticity in a series of Nobel Prize winning experiments in the 1960s [65, 66].

They found that the closure of one eye (monocular deprivation) of a kitten during a specific time window early in postnatal life results in an experience-dependent loss of visual acuity in the deprived eye despite no physical damage to the eye itself [67]. This is due to a competitive invasion by the nondeprived eye into cortical territory previously responsive to the deprived eye. A functional loss of responsiveness to the deprived eye and an increase of responsiveness to the open eye are followed first by pruning and then regrowth of dendritic spines on cortical pyramidal neurons [68, 69]. Further structural reorganization takes place in the form of shrinking thalamocortical projections (OD columns) serving the deprived eye and expansion of those serving the open eye [70].

The ocular dominance critical period is present in all mammals tested so far, from humans to mice, and the duration of plasticity is in direct correlation to lifespan and brain weight [71]. The identification of rodents as models of amblyopia has made possible a fine dissection of the mechanisms underlying critical period expression. In particular, by taking advantage of genetically modified mouse models, a specific inhibitory circuit has been identified that controls the timing of OD plasticity [11]. Fine manipulation of inhibitory transmission is difficult *in vivo,* because enhancing inhibition silences the brain, while reducing inhibition easily induces epilepsy. With the generation of a mouse lacking only one of the two enzymes that synthesizes GABA (GAD65), researchers were able to titrate down the level of inhibition and test its role in the OD critical period [12]. Strikingly, the visual cortex of *GAD65* knockout mice remains in an immature, precritical period state throughout life. At any age, functionally enhancing GABAergic transmission with benzodiazepine treatment triggers the opening of a normal-length critical period [72]. Historically, inhibitory neurotransmission was believed to develop postnatally to progressively restrict plasticity, but these key experiments proved GABA to actually be necessary for a normal OD critical period, prompting further investigation into the role of inhibition in brain plasticity.

Inhibitory interneurons account for nearly 20% of cortical neurons and exhibit heterogeneous morphological and physiological characteristics [73]. Included in this large variety of inhibitory interneurons is a specific subset of GABAergic neurons that expresses the calcium-binding protein parvalbumin. Fast-spiking parvalbumin-positive basket cells (PV-cells) regulate critical period timing and plasticity [11, 74]. PV-cells develop with a late postnatal time course in anticipation of critical period onset across brain regions [75, 76]. In the visual cortex, PV-cells mature in an experience-dependent manner, and dark-rearing delays their maturation as well as critical period expression [77, 78]. On the other hand, overexpression of brain-derived neurotrophic factor (BDNF) promotes the maturation of PV-cells and speeds up the onset of the OD critical period [77, 79]. Moreover, Di Cristo et al. [80] have shown that premature cortical removal of polysialic acid (PSA), a carbohydrate polymer presented by the neural cell adhesion molecule (NCAM), results in a precocious maturation of perisomatic innervation of pyramidal cells by PV-cells, enhanced inhibitory synaptic transmission, and an earlier onset of OD plasticity. Recent results indicate that PV-cell maturation is surprisingly regulated by the Otx2 homeoprotein, an essential morphogen for embryonic head formation [78]. Otx2 is stimulated by visual experience to pass from the retina to visual cortex and selectively into PV-cells, thereby promoting their maturation and consequently activating OD critical period onset in the visual cortex.

PV-cells receive direct thalamic input and also connect to each other in large networks across brain regions by chemical synapses and gap junctions [81, 82]. Moreover, PV-cells form numerous synapses onto the somata of pyramidal cells, which in turn enrich these sites with $GABA_A$ receptors containing the $\alpha 1$-subunit [11, 70, 74, 78, 83]. This makes PV-cells perfectly situated to detect changes in sensory input, to regulate the spiking of excitatory pyramidal cells, and to synchronize brain regions [84–86]. Manipulations that disrupt this specific circuit will disrupt the OD critical period [87]. Recent studies have made much progress regarding the origin and fate determination of cortical interneurons [88]. In particular, progenitors of PV-cells derive from the medial ganglionic eminence with a relatively late birth date, and their differentiation and migration into specific cortical layers can be regulated by homeoproteins like Lhx6 [88, 89], or excitatory projection neurons [90]. Although the closure of the OD critical period is tightly regulated, transplanting immature GABAergic cells into the visual cortex can reallow OD plasticity later in life [91]. This second sensitive period only emerges once the newly transplanted GABAergic cells reach a critical maturation stage of connectivity. This further supports a key role of inhibition in the timing of experience-dependent circuit refinement.

Once the critical period is initiated, plasticity is only possible for a set length of time, and then the critical period closes [92]. Several functional and structural brakes on plasticity have been identified in recent years [93]. Disruption of these brakes in the adult brain allows critical periods to reopen and neuronal circuits to be reshaped. In the case of OD plasticity, this means that monocular deprivation in adulthood would induce a shift in responsiveness to the nondeprived eye and cause a loss of acuity in the deprived hemisphere. Interestingly these brakes share a common theme of regulating E/I balance, and particularly the GABAergic system. Locally reducing inhibition in adulthood restores plasticity in visual cortical circuits [94, 95]. Treatment with the antidepressant drug fluoxetine also reopens plasticity, potentially by altering inhibitory transmission and increasing BDNF levels [96, 97]. Finally, knocking out lynx1, an endogenous prototoxin that promotes desensitization of the nicotinic acetylcholine receptor (nAchR), extends the critical period into adulthood [98]. Lynx1 likely modulates E/I balance because treatment with diazepam in lynx1 knockout mice abolishes adult plasticity by restoring this balance to normal adult levels.

Structural factors also restrict remodeling of circuits with the closure of critical periods. For example, PV-cells become increasingly enwrapped in perineuronal nets (PNN) of extracellular matrix with the progression of the critical period, and enzymatic removal of these nets or disruption of

their formation restores plasticity in adulthood [78, 99, 100]. In addition, the maturation of myelination throughout the layers of the visual cortex, as measured by myelin basic protein (MBP) levels, increases as the critical period closes [101]. Myelin signaling through Nogo receptors (NgRs) limits plasticity in adulthood, and genetic or pharmacological disruption of this receptor allows persistent OD plasticity later in life [101, 102].

In addition to reopening plasticity, disruption of these brakes also may allow recovery from early deprivation-induced loss of function, like amblyopia. In order to test this, animals are subjected to long-term monocular deprivation spanning the critical period. This results in permanent amblyopia, even if the deprived eye is reopened in adulthood and allowed to receive visual input. Significantly, some of the manipulations described above allow recovery of acuity, including enzymatic degradation of PNNs [103], disruption of NgR signaling [102], administration of fluoxetine [96], and enhanced cholinergic signaling by *lynx1* knockdown or treatment with acetylcholinesterase inhibitors [98]. Treatment with drugs like fluoxetine and acetylcholinesterase inhibitors offers particularly promising therapeutic potential because they are already FDA-approved for human use. As the mechanisms behind the closure of critical periods are explored, more light will be shed on potential interventions that could reopen plasticity or reset abnormal critical periods by restoring the brain to a more juvenile-like state.

How generally might these same mechanisms apply to critical periods in other parts of the brain? Interestingly, recent evidence has shown that similar mechanisms may exist in other brain regions. For example, the maturation of PV-cells in the barrel cortex peaks during the critical period for whisker tuning [75]. Furthermore, whisker trimming exclusively during this critical period in mice results in decreased PV expression and reduced inhibitory transmission *in vitro* [104]. In the zebra finch, brain regions dedicated to singing exhibit progressive PNN formation around PV-cells with a time course that parallels the critical period [105]. The maturity of the song correlates with the percentage of PV-cells that are enwrapped in PNNs, and this can be manipulated with experience by altering exposure to tutor song. In rodent auditory cortex, spectrally limited noise exposure prevents the closure of the critical period for regions of auditory cortex that selectively respond to those interrupted frequencies, and PV-cell number is also reduced in those regions [106]. In the rodent, conditioned fear can be eliminated during early life but is protected from erasure in adulthood [57]. A developmental progression of PNN formation around PV-cells coincides with this switch and enzymatic degradation of PNNs allows juvenile-like fear extinction in adulthood [58, 59], similar to the reopening of OD plasticity in the adult visual cortex [99].

While evidence that very distinct critical periods may share a common role for PV-cells and PNNs is promising, such findings are still largely correlative and will require further cellular and molecular dissection in the future. In light of these findings, it is interesting to note that at least nine different mouse models of autism share a common disruption of PV-cells [58, 59]. In relation to what we know about the importance of inhibitory transmission to critical period regulation, it is quite interesting to consider the evidence that inhibition, or E/I balance in general, is disrupted in neurodevelopmental disorders such as autism. A summary of the key evidence supporting the notion of E/I imbalance in autism is presented below.

4. GABAergic Inhibition in Autistic Patients

Autism is heritable, as evidenced by a very high concordance rate between monozygotic twins and a significant sibling risk [107]. However, it is difficult to sift through the many autism genetics studies, and many reports must be interpreted with caution. In any case, it is interesting that many genes that have been either directly or indirectly implicated are involved in establishing or maintaining E/I balance throughout life (see Table 1). An emerging trend from autism genetic studies is the disruption of synaptic components, like cell-adhesion molecules (CAMs) [108]. CAMs play a crucial role in synaptic development by initiating contact between pre- and postsynaptic cells, maintaining adhesion, and anchoring scaffolding proteins that assemble the essential components of a synapse. CAMs can determine the identity and function of synapses, thereby having a direct influence on E/I balance. This is exemplified by the pre- and postsynaptic pair of neurexins and neuroligins, for which different isoforms are expressed at inhibitory or excitatory synapses. Neuroligin-3 is a postsynaptic transmembrane molecule that is localized at both excitatory and inhibitory synapses, where it binds with presynaptic neurexins [109]. A point mutation (R451C) that replaces an arginine with a cysteine in the extracellular portion of *neuroligin-3* was identified in two brothers, one with severe autism and the other with Asperger syndrome [110]. In addition, a mutation in *neuroligin-4* has been discovered in another set of autistic brothers [110]. *Shank-3*, the causal gene for 22q13 deletion syndrome, has also been found to be disrupted in autism [108, 111–113]. Other CAMs or associated molecules have been implicated in autism as well, including neurexin-1, cadherin, protocadherin, contactins, and CASK [108].

Though there is clearly a genetic basis to nonsyndromic autism, there is no single gene or family of genes that is exclusively implicated. Rather, it is likely the inheritance of several risk factors, perhaps in combination with an environmental or epigenetic trigger, that ultimately cause autism. This would fit with the E/I imbalance theory, where the presence of one mutation that increases excitation may not alone be sufficient to disrupt the balance whereas coinheritance of this mutation with another that decreases inhibition would be enough to prevent homeostatic correction and result in a dramatic E/I imbalance [42]. There is substantial evidence of altered inhibition in autistic patients, suggesting a lack of homeostatic correction and a resulting E/I imbalance.

In support of inhibitory disruption, studies on autistic patients demonstrate broad alterations in the GABAergic system. The levels of GABA measured in the plasma of autistic children may be elevated [122, 123] while the enzymes that synthesize GABA (GAD65 and GAD67) are

Table 1: Excitatory/inhibitory balance-related genes implicated in autism.

Gene/chromosomal region	Type of disruption	Function	Reference
15q11–13 (*GABRB,GABRA5, GABRG3*)	Chromosomal abnormalities	$GABA_A\beta3$, $GABA_A\alpha5$, $GABA_A\gamma3$ subunits	[15, 114]
D2S2188 (2q) (*DLX1*, *DLX2*, *GAD65*)	High LOD score	Regulation of telencephalic GABAergic neuron development; GABA synthesizing enzyme	[19, 115]
D7S477 (7q) (*DLX5*, *DLX6*)	High LOD score	Regulation of forebrain GABAergic neuron development	[19, 115]
RELN	SNPs, CNVs, rare variants	Neuronal migration, lamination, minicolumn formation, neurotransmission regulation and synaptic plasticity	[15, 116]
Neuroligin-3	Point mutation	Postsynaptic cell adhesion molecule	[108, 110]
Neuroligin-4	Rare mutations, CNVs	Postsynaptic cell adhesion molecule	[108, 110, 117, 118]
Neurexin-1	Chromosomal abnormalities, CNVs	Presynaptic cell adhesion molecule	[108, 118–121]
Shank-3	Deletions, rare mutations, chromosomal abnormalities.	Postsynaptic scaffolding protein	[108, 111–113]

significantly reduced (~50%) in postmortem autistic parietal cortex and cerebellum [124, 125]. The relevance of GABA levels measured in the plasma to the actual levels in the brain is unfortunately unclear. Multiple studies have also found both $GABA_A$ and $GABA_B$ receptor disruption in autistic brains [126–129]. Altered modulation of $GABA_A$ receptors in the presence of GABA was suggested by a study that detected reduced radioactively labeled benzodiazepine binding to hippocampal $GABA_A$ receptors [130]. On a more structural level, autistic neocortical minicolumn number was increased and width decreased, indicating abnormal cortical organization regulated by inhibitory circuitry [131, 132]. In addition, cortical projection neurons exhibited increased dendritic spine densities, providing structural evidence for changes in connectivity in autism [133]. These combined data support the notion of changes in E/I balance at the level of cells, synapses, and circuits in autism.

4.1. Syndromic Autism. Perhaps the most striking indication of E/I imbalance is that approximately 30% of autistic patients also have epilepsy [134]. This predisposition to seizures suggests an increase in excitation and/or a decrease in inhibition, ultimately resulting in uncontrollable synchronous neuronal firing. Interestingly, Rett, Fragile X, and Angelman syndrome are not only associated with autism, but they all share a predisposition to epilepsy and other evidence of E/I imbalance. Each of these disorders has an identified and well-characterized genetic disruption (*MeCP2*, *Fmr1*, and 15q11-13/*Ube3a*, resp.). For each of these diseases, a certain percentage of the patients also fulfill the diagnostic requirements for autism. These are all disorders where a complex pattern of gene expression is disrupted, particularly affecting genes that regulate experience-dependent plasticity. Although these patients also exhibit other confounding symptoms not specific to autism, the advantage of studying these disorders as models of autism is the clear etiology and the relative homogeneity of patients in contrast to those with nonsyndromic autism.

4.1.1. Rett Syndrome. Rett syndrome is a rare X-linked disorder that affects 1 in 10,000 girls. Typically a girl with Rett Syndrome will develop normally until 6 to 18 months of age, and then undergo developmental regression, including hand wringing or clapping, loss of motor coordination, breathing abnormalities, seizures, shortened lifespan, and autism. Most cases are caused by *de novo* mutations in the gene *Methyl CpG binding protein 2* (*MeCP2*) [135]. *MeCP2* binds to methylated DNA and represses or activates gene transcription. Thus, the disruption of *MeCP2* leads to aberrant expression of a variety of genes. Studies in human Rett syndrome patients have identified clear signs of altered E/I balance, including abnormal cortical excitation in the form of altered somatosensory evoked potentials, abnormal EEG recordings [136], decreased cortical minicolumn size [132], reduced dendritic spine number [137, 138], and altered development of glutamate and GABA receptors in the basal ganglia [139]. Interestingly, *GABRB3* is a target of *MeCP2*, which could be a potential direct mechanism for abnormal $GABA_A$ receptor number found in Rett Syndrome [140]. *MeCP2* also regulates the expression of *BDNF* in an activity-dependent manner [141–143]. The expression of *BDNF* promotes GABAergic maturation, and manipulation of *BDNF* levels alters the timing of the ocular dominance critical period [77].

Experiments in a mouse model of Rett syndrome identified therapeutic potential in an FDA-approved drug, Insulin-like growth factor 1 (IGF-1) [144]. Systemic IGF-1 treatment of juvenile mice prevents many of the Rett syndrome symptoms, including shortened lifespan, locomotion and respiration, decreased brain weight, decreased cortical spine density, and abnormal ocular dominance plasticity. IGF-1 is known to stimulate synaptic maturation, function, and plasticity, though its exact mechanism of action is still unknown. IGF-1 is now in phase I and II clinical trials at Children's Hospital Boston to treat children with Rett syndrome (http://www.clinicaltrials.gov/, NCT01253317).

4.1.2. Fragile X Syndrome. Fragile X syndrome (FXS) is the most frequent cause of male mental retardation and the most common identified cause of autism, accounting for 2–5% of all known cases. FXS patients exhibit cognitive impairment, hyperactivity, anxiety, social deficits, repetitive motor behaviors, hypersensitivity to sensory stimuli, motor problems, and an increased incidence of epilepsy [19]. In addition, approximately 25% of FXS patients also have autism [15]. This disorder is most commonly caused by a trinucleotide repeat expansion in the promoter of the Fragile X mental retardation 1 (*Fmr1*) gene on the X chromosome, resulting in transcriptional silencing of the gene and reduction of Fragile X mental retardation protein (FMRP) [145]. FMRP is an mRNA binding protein that regulates the translation and transport of many synaptic proteins that are important for activity-dependent plasticity. Therefore, *Fmr1* mutations disrupt normal activity-dependent regulation of many different proteins. Postmortem analysis of FXS brains has revealed an increased number of long, thin dendritic spines on excitatory cortical neurons, a phenotype suggestive of immature synapses [146, 147].

The predominant mechanistic theory for FXS is the "metabotropic glutamate receptor (mGluR) theory" [148]. According to this model, reduction of FMRP releases negative regulation of mGlurR-dependent long-term depression (LTD), and ultimately causes exaggerated LTD at excitatory synapses onto other excitatory neurons. According to this hypothesis, a net loss of synapses would occur, potentially accounting for many of the symptoms of FXS, like developmental delay, cognitive impairment, and the preponderance of immature spines on excitatory neurons. In support of this theory, the FXS phenotype can be rescued by pharmacological treatment of mGluR inhibitors in drosophila, zebrafish, and mice, and by genetic manipulation of mGluR expression in mice. These animal model studies have paved the way for human clinical trials that are now in progress to test the efficacy of drugs that target mGluR5 function. These include several mGluR5 antagonists, such as AFQ056 from Novartis (http://www.clinicaltrials.gov/ [149]), RO4917523 from Hoffmann-La Roche (http://www.clinicaltrials.gov/), and STX107 from Seaside Therapeutics (http://www.seaside-therapeutics.com/). Arbaclofen, a $GABA_BR$ agonist that indirectly inhibits mGluR5 signaling, is also being tested (http:// www.sea-sidetherapeutics.com/).

Interestingly, mGluR5 is highly expressed at excitatory presynaptic terminals onto fast-spiking inhibitory neurons and regulates long-term potentiation (LTP) at this connection [150]. Alteration of mGluR5 activity, for example in FXS, could dramatically alter the dynamics of plasticity at this type of synapse, and ultimately affect the overall inhibitory output of fast-spiking cells. In fact, *in vitro* recordings from an FXS mouse model have shown a large reduction of excitatory drive onto fast-spiking cells [151]. The role of mGluR5-dependent LTP at this type of connection should be investigated in FXS to fully assess the impact of mGluR5 dysregulation on E/I balance. In relation to this, evidence of $GABA_AR$ disruption has also been documented in FXS, expanding the scope of E/I imbalance in syndromic autism [152]. $GABA_ARs$ are known to affect learning, memory, anxiety, depression, and epilepsy, all of which are disrupted in FXS.

4.1.3. Angelman Syndrome. Angelman syndrome (AS) is characterized by normal development during the first year of life followed by progressive mental retardation, motor dysfunction, speech impairment, and a high rate of autism [153]. AS is caused by maternal deletion of chromosome 15q11–13 and by more specific deletions of a gene found in this region, called *E3 ubiquitin ligase* (*Ube3a*). The transcription of *Ube3a* is normally regulated by synaptic activity and ultimately regulates excitatory synapse development. *Ube3a* regulates AMPA receptor internalization by controlling the degradation of Arc, an activity-regulated cytoskeleton-associated protein [154]. In the absence of *Ube3a*, Arc expression increases, more AMPA receptors are internalized, and excitatory synaptic transmission is reduced. *Ube3a* appears to be a key causal gene in AS, but the chromosomal segment 15q11–13 also contains other genes that likely contribute to the AS phenotype—most notably the $GABA_A$ receptor gene cluster.

Individuals with 15q11–13 deletions usually have more severe epilepsy than those with more specific *Ube3a* mutations that spare the $GABA_A$ receptor gene cluster [155]. The $\beta3$-$\alpha5$-$\gamma3$ $GABA_A$ subunit gene cluster encodes three of the ionotropic GABA receptor subunits. As would be expected, postmortem AS cortex shows abnormal subunit composition of these receptors, favoring other subunits that are not in this gene cluster (e.g., $\beta2$ and $\alpha1$). When these receptors were injected into *xenopus* oocytes, GABAergic transmission was altered, with a particular disruption of receptor pharmacology [156]. These results suggest that synaptic cortical GABAergic inhibition is intact or even augmented, but extrasynaptic inhibition is impaired. The authors suggest that this could account for the cognitive, behavioral, and epileptic symptoms of AS.

5. GABAergic Inhibition in Animal Models of Autism

For many human diseases, the generation and characterization of animal models is an essential bridge between understanding the molecular features of the disease and

the development of therapeutics. Unfortunately, the generation of mouse models of autism has been quite difficult and controversial. The reasons for this become apparent when considering the three ideal characteristics of an effective mouse model for neuropsychiatric diseases—face, construct, and predictive validity (similarity to human symptoms, cause of human disease, and response to treatment, resp.) [157, 158]. In the case of autism, face validity requires rigorous behavioral tests to examine socialization, communication, and repetitive behavior, which are rather difficult, though possible, to do in mice. In addition, the variability of human symptoms combined with the inherent variability of mouse behavior results in the need to test many mice with multiple different tasks to evaluate these three categories of behavior. Construct validity is also difficult because as discussed previously, the cause of the majority of autism cases is unknown. Finally, in the case of autism, no single treatment has been shown to have consistent positive results, thereby also making predictive validity complicated [18].

Over the years, mouse models of autism have been generated based on rare mutations identified in autism patients, environmental insults associated with autism, or mutations known to cause diseases that are comorbid with autism (reverse genetic approach). Existing mouse strains have also been screened for behaviors relevant to autism (forward genetic approach). In this section, several different mouse models of autism are reviewed, with their common disruption of E/I balance receiving special attention.

5.1. Cell-Adhesion Molecules. As mentioned previously, disruption of cell-adhesion molecules is a common theme emerging from autism genetic studies [108], including a point mutation in *neuroligin-3* (R451C) [110], and mutations in *Shank-3* [111–113]. Studies of the R451C mutation in *neuroligin-3* in cell culture have shown that 90% of the mutant protein is retained in the endoplasmic reticulum [159, 160]. The 10% of the protein that is transported to the cell surface exhibits reduced binding with its presynaptic partner, the neurexin molecule [159]. Interestingly, when this mutation is introduced in mice by homologous recombination, mice show upregulation of inhibitory markers per synapse, including vesicular GABA transporter (VGAT) and the postsynaptic scaffolding protein gephyrin [161]. However, the ratio of inhibitory to excitatory synapses is preserved. There is also a functional increase in inhibitory transmission in the somatosensory cortex evidenced by increased frequency of mIPSCs, increased amplitude of eIPSCs, and increased IPSC amplitude in response to GABA application. Mutant mice show some behaviors relevant to autism, including altered socialization and enhanced spatial learning (but also see [162]). None of these same molecular, physiological, and behavioral phenotypes were found in *neuroligin-3* knockout mice, suggesting that this particular mutation results in a gain-of-function, though the mechanism is still under investigation. A very recent study also found that introducing the R451C mutation in the motor neuron of *Aplysia* blocks intermediate-term and long-term facilitation that are necessary for memory storage, possibly having implications for social memory [163]. In addition, mice lacking *neuroligin-4* demonstrate deficits in reciprocal social interaction and reduced ultrasonic vocalization, providing further evidence that mutant *neuroligin* mouse models may be very useful to study autism [164].

Another recent autism mouse model based on cell-adhesion molecule disruption was generated by mutating the *Shank-3* gene [165]. These mice demonstrate anxious behavior, decreased social interaction, and impaired social novelty recognition. Most strikingly, they compulsively self-groom to the point of causing skin lesions. Compulsive grooming is generally considered to be the result of a corticostriatal abnormality. Hence, Peca et al. investigated the corticostriatal circuitry of these mice using a joint structure-function approach. They focused on excitatory synapses onto inhibitory medium spiny neurons (MSNs) of the striatum, because *Shank-3* is located in the excitatory postsynaptic density. They found altered postsynaptic density composition, abnormal morphology of MSNs, and reduced corticostriatal neurotransmission due to postsynaptic changes. In summary, *Shank-3* disruption results in striking autism-like behaviors and an E/I imbalance in the striatum. Based on this phenotype, the causal role of *Shank-3* in 22q13 syndrome [111, 166, 167], and an emerging association of *Shank-3* with autism [112, 113], this mouse model should be a valuable tool with which to further dissect E/I imbalance and circuit disruption in autism.

5.2. Prenatal Valproic Acid Insult. Other mouse models of autism incorporate the polygenetic complexity of the disease by mimicking an embryonic insult that has been linked to autism in humans. For example, human embryonic exposure to valproic acid (VPA) during a strict time window of 20–24 days post-conception is linked to a seven-fold increased likelihood of developing autism [168–171]. VPA is an anticonvulsant and mood stabilizer used to treat epilepsy and bipolar disorder, and is also a pharmacological histone deacetylase (HDAC) inhibitor. As such, VPA interferes with normal deacetylation of chromatin and causes aberrant expression of many genes, possibly including *Homeobox* (*Hox*) genes and *Wingless-Int* (*Wnt*) [172]. Rodent VPA models of autism have been generated by treating a pregnant female with a single dose of VPA at a time during embryonic development that is equivalent to the human susceptibility time window. Multiple studies from different groups have shown that the resulting offspring exhibit developmental, behavioral, molecular, and anatomical changes comparable to human autism symptoms [173]. Interestingly, a dramatic E/I imbalance is manifest in VPA-treated rats, with NMDAR-mediated synaptic currents, NR2A and NR2B subunit number, and postsynaptic LTP all showing enhancement in the somatosensory cortex [174]. Further, the medial prefrontal cortex, somatosensory cortex, and amygdala demonstrate local hyperconnectivity, hyperreactivity, and hyperplasticity in rats treated embryonically with VPA [175, 176].

5.3. BTBR T + tf/J Inbred Mouse Strain. One way to identify new mouse models of autism is to screen existing strains of

mice of varying genetic backgrounds for behaviors relevant to autism. This forward genetic strategy requires a high-throughput, reliable behavioral battery that can evaluate mice on a variety of behavioral tests, from general health to cognitive abilities. Using this strategy, Mc farlane [177] an inbred strain of mice was identified, BTBR T + tf/J (BTBR) that exhibits all three categories of autistic behaviors in a very specific manner. BTBR mice show reduced social approach, reciprocation, and play. They also exhibit communication deficits as evidenced by impaired transmission of food preference [177], and an unusual pattern of ultrasonic vocalizations [178]. Finally, BTBR mice are afflicted with extreme repetitive behavior in the form of high levels of self-grooming throughout life [177]. These autism-like behaviors are specific due to absence of anxiety or motor impairments that could complicate the interpretation of the affected behaviors. Interestingly, the high grooming behavior can be corrected by treatment with MPEP [179], an mGluR5 antagonist whereas the abnormal socialization can be corrected by treatment with fluoxetine [180], showing good predictive validity for this model. MPEP is effective in treating autism-related symptoms in the *Fmr1* mouse model of Fragile X syndrome [181–184], and fluoxetine is under evaluation to treat repetitive behavior and anxiety in autistic patients and is currently used to treat depression [180]. Interestingly, fluoxetine treatment in adult mice has been shown to reopen ocular dominance plasticity [96]. This effect may be mediated by inhibitory systems, as diazepam infusion into the cortex prevents this effect. Future studies should investigate GABAergic changes in the BTBR mouse.

5.4. Rett Syndrome. Deletion of part or all of *MeCP2*'s third exon results in mice that strikingly recapitulate many Rett syndrome symptoms [185–187]. These mice develop progressive symptoms, including clasping of the front paws, anxiety, tremors, respiratory problems, seizures, hypoactivity, and a shortened lifespan. They also demonstrate autistic behaviors, including altered ultrasonic vocalizations [188]. One mutation present in Rett syndrome patients that results in truncation of the MeCP2 protein causes disrupted social behavior [189, 190], altered home cage activity [189], and impaired learning and memory in mice [191]. A general feature of these Rett syndrome mouse models is disrupted excitation, shown by reduced dendritic spine number [137, 138], reduced spontaneous activity due to reduced mEPSC amplitude [192], and minor LTP deficits early in life due to reduced excitatory synaptic connectivity that progressively worsens with age [191, 193].

Deficits in inhibitory transmission have also been noted in Rett syndrome mouse models. For example, GABAergic synaptic transmission in the ventrolateral medulla is depressed at P7 in *MeCP2* knockout mice, a phenotype that likely stems from both reduced presynaptic GABA release (i.e., reduced VGAT) and reduced $GABA_A$ receptor subunit levels [194]. Based on the observation that wild-type GABAergic neurons express 50% more MeCP2 than wildtype non-GABAergic cells, a conditional mutant mouse was generated where *MeCP2* was exclusively disrupted in GABAergic cells using a *VGAT-Cre* mouse line [195]. These mice developed nearly all of the same symptoms as global *MeCP2* knockout mice, including limb clasping, self-injury from excessive grooming, motor deficiencies, increased prepulse inhibition, altered socialization, and decreased lifespan. GABAergic neurons exhibited reduced inhibitory quantal size, reduced GAD65 and GAD67 levels, and reduced GABA immunoreactivity. In addition, specific knockout of *MeCP2* in forebrain GABAergic neurons with the use of a *Dlx 5/6* promoter also recapitulated many of the symptoms seen in global *MeCP2* knockout mice [195]. This study suggests that disruption of *MeCP2* exclusively in inhibitory neurons is sufficient to cause Rett syndrome in mice.

Reactivation of endogenous *MeCP2*, *BDNF* overexpression, and pharmacological and environmental interventions can rescue some aspects of Rett-like pathology [196]. This suggests the possibility of rescuing Rett syndrome symptoms by directly acting on mechanisms which normally control plasticity in developing cortical circuits.

5.5. Fragile X Syndrome. The *Fmr1* knockout mouse generated by Bakker et al. [197], recapitulates many FXS phenotypes. These include abnormal socialization, learning and cognitive deficits, susceptibility to audiogenic seizures, and long, thin dendritic spines. Changes in glutamatergic and GABAergic systems have been reported in both mouse and fly models of FXS (see [198] for review). In particular, the cortex of mouse models of FXS show a decrease in LTP and an increase in glutamatergic cells, but a decrease in GAD and $GABA_AR$ subunit mRNA, decreased GABAergic cell number, and decreased excitatory drive onto inhibitory neurons. In the hippocampus, there is increased mGluR-dependent LTD, increased epileptiform discharges, as well as decreased $GABA_AR$ subunits and decreased tonic inhibition. Interestingly, both brain regions show elevated GAD protein levels despite decreased mRNA levels. Although results are variable due to differences in age, brain region, and method, the underlying theme appears to be an increased excitatory/inhibitory ratio.

5.6. Angelman Syndrome. Angelman syndrome has been successfully modeled in mice by inactivation of the maternal copy of *Ube3a* [199, 200]. This manipulation results in learning and hippocampal LTP deficits [199, 201], as well as deficient experience-dependent maturation of excitatory circuits [202]. Knocking out *GABRB3,* which is found on the 15q11-13 chromosomal segment, also produces a mouse model that seems very relevant to AS [203]. This mouse has problems with coordination and learning, is hyperactive, and has seizures and abnormal EEG patterns [203, 204]. In addition, the pharmacological function of $GABA_A$ receptors is altered, as binding of benzodiazepines is reduced [205].

6. Critical Period Disruption in Animal Models of Autism

Critical period plasticity has been reported to be altered in at least three animal models of syndromic autism

Yashoria et al. [202]. Examined OD plasticity in a maternal *Ube3a* knockout mouse model of Angelman syndrome. They recorded chronic visual evoked potentials (VEP) in response to low spatial frequency stimuli in order to evaluate the strength of input from each eye before and after monocular deprivation (MD) during the canonical critical period. Interestingly, these mutant mice did not exhibit any shift in favor of the open nondeprived eye. This was due to a lack of depression of the deprived contralateral eye response. *In vitro* analysis revealed that cortical synapses were still immature and unable to incorporate changes in sensory experience. Unfortunately they did not test before or after the normal critical period to see if the onset was accelerated or delayed. Similarly, Sato and Stryker [206] studied OD plasticity in *Ube3a*-deficient mice using optical imaging of intrinsic signals to evaluate the strength of input from each eye. Consistent with the Yashiro et al. study, they found that brief MD did not elicit plasticity in mutant mice during the normal critical period. However, when mice were deprived for a longer period (14 instead of 4 days), some degree of OD plasticity was revealed. Therefore, there is some capacity for plasticity during a restricted time window, but the strength is diminished.

Dölen et al. [207] tested OD plasticity in an *Fmr1* knockout mouse model of Fragile X syndrome. They recorded chronic VEPs before and after brief (3-day) MD during the canonical critical period. Knockout mice showed significant potentiation of the ipsilateral open eye response but no depression of contralateral deprived eye response as is seen in wildtype mice. The potentiation of the open eye is usually only seen after longer periods of MD, secondary to the depression of the closed eye. Therefore, the knockout mice do show an OD shift during the critical period, but the nature of this shift is unusual. Furthermore, visual acuity before and after MD was not measured, so it is unknown if the deprived eye ever became amblyopic and if critical period plasticity really took place. In addition excitatory thalamocortical synapses in somatosensory cortex during the perinatal critical period in Fmr1 knockout mice. FMRP ablation resulted in dysregulation of glutamatergic signaling maturation. The fraction of silent synapses persisting to later developmental times was increased; there was a temporal delay in the window for synaptic plasticity, while other forms of developmental plasticity were not altered in Fmr1 knockout mice. indicating that FMRP is required for the normal developmental progression of synaptic maturation impacting the timing of the critical period for layer IV synaptic plasticity [208].

Tropea et al. [144] tested OD plasticity in adult *MeCP2* heterozygous female mice that are considered an accurate model of Rett syndrome despite having less severe symptoms than *MeCP2* mutant male mice. Using optical imaging of intrinsic signals, they found adult OD plasticity after 4-day MD in mutant but not wildtype mice. Plasticity was not evaluated during the normal critical period or at any other age, so it is unknown if the critical period is delayed or extended in the absence of *MeCP2*. Interestingly, treatment with IGF-1 peptide abolishes this aberrant plasticity, and also alleviates other symptoms of Rett syndrome in these mice.

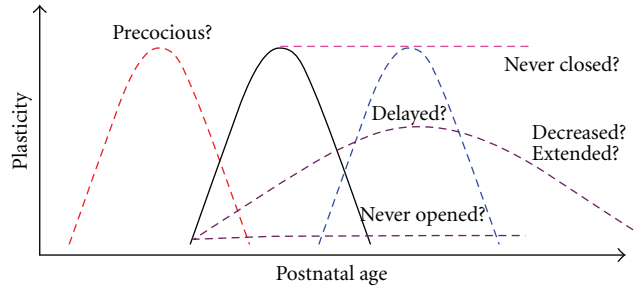

Figure 1: Possible critical period alterations in autism. The solid black curve represents the normal expression of a critical period, with a distinct onset and closure and characteristic duration. Onset could be precocious or delayed. Duration could be increased or decreased. Degree of plasticity could be increased or decreased. Finally, the critical period could fail to open or close.

Together these results show that critical period plasticity is abnormal in these mouse models of autism; however, the way in which critical periods are altered appears to differ depending on the particular etiology of autism. This would make sense in light of the heterogeneity that is so characteristic of autism. Therefore it is imperative to thoroughly test all aspects of critical periods to see if they are accelerated, delayed, extended, weaker, stronger, and so on (as portrayed in Figure 1). Unfortunately all of the above studies have only compared the ratio of responses between the two eyes and have not looked at the functional readout of acuity. In the future, we hope several lines of autism mouse models will be systematically analyzed, complete with an evaluation of normal visual system functional development before any sensory manipulation is performed. A detailed examination of single-cell excitability and visual spatial acuity will reveal whether the model's visual system suffers from perturbed sensory processing, which is indicative of abnormal circuit refinement in visual cortex during development. After the baseline visual function is determined, then plasticity can be tested by short- or long-term monocular deprivation performed at various ages between eye opening and adulthood. Given the possible common disruption of PV-circuits across brain regions [58, 59] and the importance of the GABAergic system to critical period regulation [70], a multilevel analysis of PV-cell maturation should also be performed. The recent identification of new pharmacological and environmental strategies to recreate the highly plastic state of GABAergic circuitry possessed by juvenile animals represents a promising therapeutic avenue for the restoration of normal function in affected neuronal circuits [93].

Finally, in addition to ocular dominance, testing other critical periods in somatosensory and auditory cortices would reveal whether any defects in critical periods in the visual system are representative of a more global phenotype.

7. Conclusion and Thoughts for the Future

The functional significance of critical periods is unclear, but the careful regulation of their timing indicates that their precise expression is crucial for normal development. There

is, perhaps, a tradeoff between adaptability and stability. The young brain must dynamically adapt to its environment in order to set up its circuits in the most efficient manner while the adult brain favors reliability instead.

The variable nature of E/I imbalance and altered plasticity in autism animal models suggests that the disruption of critical periods in autism is likely heterogeneous, in some cases resulting in excessive plasticity and in others, insufficient plasticity. This could be due to disruption of the mechanisms governing either the onset or closing of critical periods Figure 1, and both could be detrimental to functioning. A brain that is too plastic at the wrong times could result in noisy and unstable processing. On the other hand, a brain that lacks plasticity early in life might remain hyper- or hypoconnected and unresponsive to environmental changes early in life. A situation could also arise where plasticity is at an optimal level in some systems and an aberrant level in other systems, which could the case in Asperger and/or Savant syndrome.

Autism is diagnosed exclusively by cognitive behavioral symptoms, but there are likely underlying problems arising at lower-level stages of processing. By first understanding the development of primary senses in autism, a cumulative chain reaction of abnormalities could be prevented early on and save consequent behavior. In the long run, a collaborative multilevel analysis of different brain regions over development and in different animal models of autism is of paramount importance. Hypothesis-driven efforts may then have a wider implication for the diagnosis and treatment of neurodevelopmental disorders in general. We are now in the position to adopt a mouse model to human multilevel analysis approach to test well-defined, mechanistic hypothesis and to discover new therapeutic interventions to restore normal cortical function.

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Review Article

GABA Neuron Alterations, Cortical Circuit Dysfunction and Cognitive Deficits in Schizophrenia

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Schizophrenia is a brain disorder associated with cognitive deficits that severely affect the patients' capacity for daily functioning. Whereas our understanding of its pathophysiology is limited, postmortem studies suggest that schizophrenia is associated with deficits of GABA-mediated synaptic transmission. A major role of GABA-mediated transmission may be producing synchronized network oscillations which are currently hypothesized to be essential for normal cognitive function. Therefore, cognitive deficits in schizophrenia may result from a GABA synapse dysfunction that disturbs neural synchrony. Here, we highlight recent studies further suggesting alterations of GABA transmission and network oscillations in schizophrenia. We also review current models for the mechanisms of GABA-mediated synchronization of neural activity, focusing on parvalbumin-positive GABA neurons, which are altered in schizophrenia and whose function has been strongly linked to the production of neural synchrony. Alterations of GABA signaling that impair gamma oscillations and, as a result, cognitive function suggest paths for novel therapeutic interventions.

1. Introduction

Schizophrenia is a severe brain disorder that afflicts 0.5–1% of the world's population and that is typically first diagnosed in late adolescence or early adulthood. The illness is manifest as disturbances in perception, attention, volition, inferential thinking, fluency and production of language, and the recognition and expression of emotion that lead to substantial impairments in social and occupational functioning. Many affected individuals suffer from comorbid depression, an increased risk of cardiovascular disease, and excessive nicotine, alcohol, and cannabis use.

Three major domains of symptoms define schizophrenia. The first domain is positive or psychotic symptoms that include delusions, false beliefs firmly held in the face of contradictory evidence; perceptual disturbances and hallucinations, which may occur in any sensory modality but are most commonly auditory and experienced as hearing voices distinct from one's own thoughts; abnormalities in form of thought that are usually manifest as loose associations, over-inclusiveness, and/or neologisms; abnormal psychomotor activity that is usually manifest as grossly disorganized behavior, posturing, and/or catatonia. Negative symptoms include asociality (withdrawal or isolation from family and friends), avolition (impaired initiative, motivation, and decision-making), alogia (poverty in the amount or content of speech), and anhedonia (reduced capacity to experience pleasure). The third category of symptoms includes a number of cognitive abnormalities such as disturbances in selective attention, working memory, executive control, episodic memory, language comprehension, and social-emotional processing.

Although positive symptoms are usually the presenting and most striking clinical feature of schizophrenia, disturbances in cognition appear to be the core features of the illness as they are present before the onset of psychosis and are the best predictor of long-term functional outcome for schizophrenia patients [1]. Therefore, functional recovery (e.g., recovery of the capacity to maintain employment) is largely dependent on improving cognitive deficits.

Although schizophrenia was initially characterized over 100 years ago, we still haveonly a limited understanding

of its pathophysiology. Moreover, we lack efficient tools for its treatment or prevention. For example, the multicenter, NIMH-funded Clinical Antipsychotic Trials in Intervention Effectiveness project recently found that newer atypical antipsychotics are not significantly more effective for treating psychosis than older typical antipsychotic medications and showed little benefit for improving cognitive symptoms [2, 3]. These findings highlight the need to develop novel therapeutic interventions for schizophrenia [4, 5].

If functional recovery of patients with schizophrenia depends on improving cognitive deficits, then understanding the neural basis of the normal cognitive operations that are impaired in schizophrenia is crucial to develop new therapies. Interestingly, a number of findings from postmortem brain studies suggest that schizophrenia is associated with deficits of GABA-mediated synaptic transmission [6]. Furthermore, current hypotheses from cellular and systems neurophysiology suggest that a major role of GABA-mediated transmission is to produce synchronized neural network oscillations [7, 8] which by facilitating the processing and flow of information within and between brain regions may be essential for normal cognitive function [9]. Here we review convergent findings from schizophrenia research, cellular neurophysiology, and cognitive neuroscience that favor the hypothesis that deficits of cognitive function in schizophrenia result from a dysfunction in GABA-mediated synaptic inhibition that disturbs oscillatory neural synchrony.

This paper reviews recent evidence further indicating that in subjects with schizophrenia cognitive dysfunction is associated with alterations of oscillations in the gamma frequency band (30–80 Hz), which are normally induced during tasks that engage cognition [10]. In addition, it reviews the cellular and molecular machinery involved in GABA-mediated synaptic transmission and the mechanisms by which GABA-mediated inhibition may synchronize neural activity in cortical circuits, focusing on the role of parvalbumin- (PV-) positive GABA neurons, whose function has been increasingly linked to the production of synchronized gamma oscillations. Furthermore, data on the postnatal development of PV GABA neurons and their synaptic connections and the developmental trajectories of gene products involved in GABA-mediated synaptic inhibition is reviewed. Finally, recent studies further suggesting that schizophrenia is associated with alterations in GABA-mediated synaptic transmission, particularly, but not exclusively, from PV neurons are highlighted. Alterations of GABA signaling that impair gamma oscillations and therefore cognitive function in schizophrenia suggest potential paths for therapeutic interventions.

2. Altered Neural Synchrony and Cognitive Function in Schizophrenia

Schizophrenia is associated with deficits in behavioral tasks that assess perceptual and cognitive processes [10, 11]. Many such tasks normally increase synchronized oscillatory activity as measured in the electroencephalogram (EEG), and such increase in synchrony is altered in subjects with schizophrenia [11, 12]. For example, gamma-band synchrony during tasks that require visual gestalt perception is attenuated in schizophrenia patients [13]. One of the core cognitive deficits in schizophrenia is a dysfunction of working memory, a system to keep information in mind and to manipulate it while performing complex tasks [14]. Gamma oscillatory activity (30–80 Hz) may play an important role in normal working memory, given that gamma band synchrony increases with increasing working memory load [15, 16]. In patients with schizophrenia, working memory deficits are accompanied by altered patterns of cortical oscillatory activity [11], since schizophrenia patients actually fail to enhance gamma activity with increasing working memory load [16] and show overall increased gamma band power during working memory [16, 17]. Subjects with schizophrenia also have decreased oscillations in various frequency bands during specific phases of the working memory process, including encoding, maintenance, and retrieval [18].

Cognitive function, including working memory, implicates an interconnected network of brain regions, many of which show structural and functional abnormalities in schizophrenia [19]. The prefrontal cortex (PFC), which is extensively interconnected with cortical and subcortical regions, is thought to exert top-down control of the flow of neural activity between brain regions to provide cognitive control, coordinating incoming sensory and motor information with representations of internal goals and rules to select a context-appropriate behavioral response [20]. Subjects with schizophrenia have significant deficits in cognitive control [10] and attenuated gamma oscillations in PFC during cognitive control tasks [21]. Cognitive control-related gamma activity, but not theta activity, is reduced in the frontal cortex of first-episode schizophrenia patients independent of medication status, suggesting a deficit related to the disease process as opposed to medication side effects or the consequences of being chronically ill [22]. Interestingly, some studies reported a positive correlation between gamma oscillations and hallucination symptoms score in schizophrenia [23, 24], indicating that the propensity for auditory hallucinations correlates with an increased tendency to enter states of oscillatory synchrony [24]. It remains to be determined whether the decrease in gamma activity associated with cognitive deficits and the positive correlation between gamma activity and psychotic symptoms are due to different underlying mechanisms, manners of eliciting gamma, or cohorts of subjects.

If, as suggested by multiple lines of evidence, altered neural synchrony underlies impairment of cognition in schizophrenia, then understanding the neural mechanisms normally involved in production of synchronized oscillations in neocortical circuits is crucial to develop new therapeutic interventions. Whereas several mechanisms have been proposed, production of rhythms via GABA-mediated inhibition is currently a leading candidate mechanism, as reviewed in the following.

3. Basic Mechanisms of GABA-Mediated Fast Synaptic Transmission

By definition, GABA neurons have the capacity to synthesize GABA from glutamate via the enzymatic activity of glutamic acid decarboxylase (GAD), for which there are two gene products of different molecular weight, GAD65 and GAD67 [25]. Whereas the GAD isoforms differ in a number of properties, their specific functional roles are not fully understood [25]. Interestingly, GAD65 and GAD67 are differentially expressed in GABAergic terminals in a cell type-specific manner (see below).

GAD-mediated GABA synthesis occurs in the cytosol, and GABA is transported into synaptic vesicles by the vesicular GABA transporter vGAT (Figure 1). Shortly after an action potential arrives at the nerve terminal, vesicular GABA release is triggered with a certain probability and in a Ca^{2+}-dependent manner. In PV neuron terminals, PV may act as a Ca^{2+} buffer that binds residual Ca^{2+} after activation of the Ca^{2+} sensor that triggers vesicular GABA release. At all synaptic connections from cortical GABA neurons thus far studied, the effects of synaptically released GABA are mediated by $GABA_A$ receptors ($GABA_A$Rs), as the postsynaptic response is abolished by $GABA_A$R antagonists in hippocampus [26–33] and neocortex [34–41]. In contrast, $GABA_B$ receptors mediate the postsynaptic effects of GABA only at connections from GABA neurons of the neurogliaform cell subtype [42].

Postsynaptic $GABA_A$Rs are heteropentamers composed of subunits from 7 different families (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, θ, and ρ_{1-3}) typically combined following a $2\alpha:2\beta:\gamma$ stoichiometry to form a GABA-activated chloride channel [43, 44]. Importantly, the subunit composition of the $GABA_A$R complex determines many of its functional properties. For instance, GABA-activated chloride currents produced by α1 subunit-containing $GABA_A$Rs (α1-$GABA_A$Rs) have much faster decay kinetics than currents mediated by $GABA_A$Rs containing other α subunits [43].

The subunit composition also determines important pharmacological properties of the $GABA_A$Rs. Benzodiazepines bind to $GABA_A$Rs via a binding site localized at the interface between α and γ subunits [45, 46] and may modulate (potentiate or decrease) $GABA_A$R function in an α subunit-selective manner [47]. For example, zolpidem enhances GABA effects preferentially at α1-$GABA_A$Rs, whereas the α3IA and α5IA compounds are inverse agonists preferentially at α3-$GABA_A$Rs and α5-$GABA_A$Rs, respectively [47]. Other drugs, including TPA023 (also named MK0777), TPA023B, TPA123, and TPA003 have comparable binding affinity at α1-, α2-, α3-, and α5-$GABA_A$Rs but may lack pharmacological effects at certain $GABA_A$R subtypes. In particular, TPA023 is a partial agonist at α2- and α3-$GABA_A$Rs but has no agonist efficacy at α1- and α5-$GABA_A$Rs [48].

The magnitude and direction of the ionic current flowing through $GABA_A$Rs depends on its driving force or difference between its electrochemical equilibrium potential (E_{GABA_A}) and the resting membrane potential (V_{mr}) in the plasma membrane compartment where $GABA_A$Rs are located [49]. Because $GABA_A$R channels are largely permeable to chloride, E_{GABA_A} is close to the chloride equilibrium potential (E_{Cl}) and therefore E_{GABA_A} depends on the sodium-potassium-chloride cotransporter 1 (NKCC1) and the potassium-chloride co-transporter 2 (KCC2), which mediate chloride uptake and extrusion, respectively [43]. Importantly, since the active $GABA_A$R conductance tends to "clamp" the membrane potential at E_{GABA_A} [49], if E_{GABA_A} is negative relative to V_{mr} ($V_{mr} > E_{GABA_A}$), the $GABA_A$R currents are hyperpolarizing, whereas if E_{GABA_A} is positive to V_{mr} ($E_{GABA_A} > V_{mr}$), the $GABA_A$R currents produce depolarization. Commonly, E_{GABA_A} is negative to V_{mr}, however, in certain cell types or subcellular compartments (and in general, early in brain development) $E_{GABA_A} > V_{mr}$ [43].

Whether the $GABA_A$R conductance has inhibitory or excitatory effects depends on the relation between $E$$GABA_A$ and the voltage threshold for action potential firing (V_{th}), which is always depolarized relative to V_{mr} ($V_{th} > V_{mr}$). When $V_{th} > V_{mr} > E_{GABA_A}$, the chloride current is hyperpolarizing and clearly inhibitory because it shifts the membrane potential away from firing threshold, thus reducing the probability of firing. In contrast, if $E_{GABA_A} > V_{th} > V_{mr}$, the chloride current is excitatory because it tends to depolarize the membrane above V_{th}. However, if $V_{th} > E_{GABA_A} > V_{mr}$, the $GABA_A$R conductance is inhibitory because, even though $E_{GABA_A} > V_{mr}$, shunting by the active $GABA_A$R conductance keeps the membrane potential below firing threshold. Importantly, $GABA_A$R-mediated inputs that depolarize the membrane below V_{th} ($V_{th} > E_{GABA_A} > V_{mr}$) can have dual, time-dependent inhibitory/excitatory effects. Initially, when the $GABA_A$R conductance is active, the net effect of the synaptic input is inhibitory because of the shunting effect [50]. However, the depolarizing postsynaptic potential outlasts the duration of the $GABA_A$R conductance, thus increasing the firing probability once the $GABA_A$R conductance decays [50]. Importantly, the depolarizing $GABA_A$R-mediated post-synaptic potential may be amplified by voltage-dependent Na^+ currents localized perisomatically, possibly in the initial segment of the axon [51, 52], enhancing its excitatory effect. The inhibitory versus excitatory effect of the $GABA_A$R conductance may be dynamic, because V_{mr} and V_{th} are subject to modulation and change over time. In addition, E_{GABA_A} may vary between cell types and subcellular compartments, depending on the NKCC1/KCC2 activity ratio [43].

It has been commonly suggested that uptake of extracellular GABA by plasma membrane transporters could help terminate the synaptic effect of GABA and thus the duration of the inhibitory postsynaptic current (IPSC). In the CNS, GABA uptake is largely mediated by the plasma membrane GABA transporter 1 (GAT1) which translocates GABA through the neuronal and glial membrane (Figure 1). Interestingly, recent experiments indicate that GAT1 does not control the time course of $GABA_A$R-mediated IPSCs, since the duration of IPSCs produced at single synapses is not affected by pharmacological inhibition of GAT1, nor in GAT1 knockout mice [53–56]. The finding that GAT1 does not control IPSC duration may be explained by GAT1's predominantly extrasynaptic localization [57–62] and by

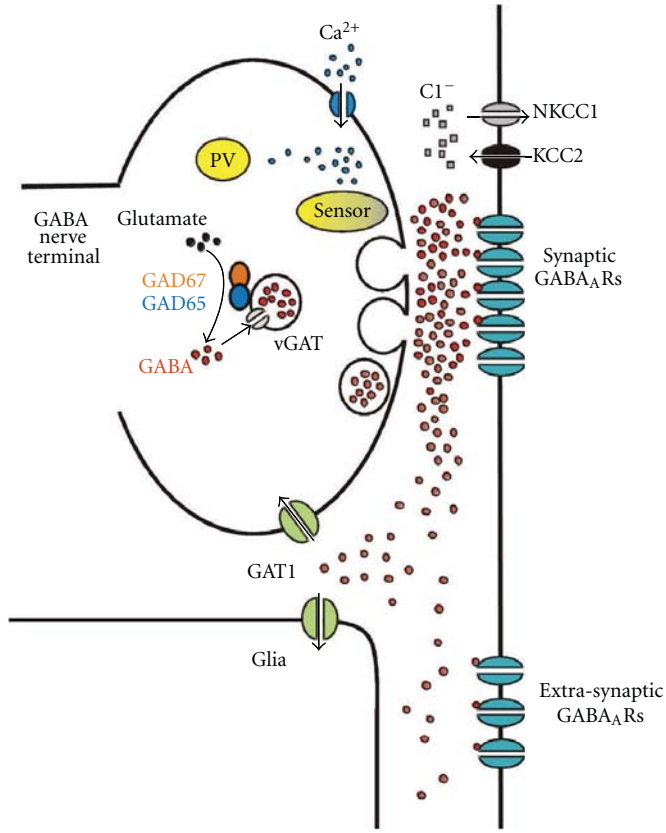

Figure 1: Scheme showing a nerve terminal from a parvalbumin- (PV-) positive GABA neuron shortly after an action potential triggered Ca^{2+}-dependent GABA release, highlighting components currently hypothesized to be altered in schizophrenia. In PV terminals, GABA release is tightly synchronized with Ca^{2+} influx, possibly due to the proximity between voltage-dependent Ca^{2+} channels and release sites. PV is a relatively slow buffer that probably is unable to bind Ca^{2+} before activation of the Ca^{2+} sensor promotes vesicle fusion. Ca^{2+} buffering by PV mainly accelerates the decay of the intraterminal Ca^{2+} transient (see text). GAD65 and GAD67, possibly acting as a dimer, drive GABA synthesis in the cytosol near synaptic vesicles. Vesicles uptake newly synthesized GABA via the vesicular GABA transporter vGAT. Vesicle fusion rapidly and transiently raises GABA concentration in the synaptic cleft, briefly exposing post-synaptic $GABA_A$ receptors ($GABA_A$Rs) to a high concentration of GABA. As GABA escapes from the synaptic cleft after $GABA_A$R activation, it may be taken up by the plasma membrane GABA transporter GAT1, apparently localized in the extrasynaptic neuronal membrane, as well as in glia. GAT1 therefore regulates the concentration of GABA reaching extrasynaptic $GABA_A$Rs and synaptic $GABA_A$Rs at other synapses (not shown in the scheme). The direction and magnitude of the chloride current produced by postsynaptic $GABA_A$R activation is regulated by the transporters KCC2 and NKCC1, which uptake and extrude chloride, respectively, setting the equilibrium potential for the $GABA_A$ current, E_{GABA_A}. Since PV accelerates the decay of the intraterminal Ca^{2+} transients, a decrease of PV in schizophrenia may facilitate repetitive GABA release, such as that observed during gamma oscillation episodes. A decrease of GAD67 levels in schizophrenia would reduce the cytosolic GABA concentration near synaptic vesicles. Because vGAT levels appear to be unaffected in schizophrenia, reduced GAD67 may lead to lower intravesicular GABA concentration, therefore decreasing the peak GABA concentration in the synaptic cleft and weakening the postsynaptic response. In schizophrenia, at some synapses postsynaptic $GABA_A$R density appears to be decreased, further weakening synaptic transmission, whereas at other synapses $GABA_A$R density is increased, possibly due to compensatory receptor upregulation. In schizophrenia, KCC2 and NKCC1 mRNA levels are normal, but two kinases that strongly regulate KCC2 and NKCC1 may be altered in ways that render an E_{GABA_A} value more depolarizing than normal. Finally, reduced GAT1 in schizophrenia may alter the effects of synaptically released GABA via an exaggerated activation of extrasynaptic and heterosynaptic $GABA_A$Rs. Alternatively, GAT1 activity may be reduced to compensate lower GABA levels due to GAD67 deficiency.

the slow GAT1-mediated GABA translocation rate [63, 64] compared with the rapid $GABA_A$R activation by synaptic GABA [43]. Other experiments suggest that GAT1's main role is preventing intersynaptic GABA spillover [53, 56], (e.g., the unintended activation of $GABA_A$Rs at a given synapse by GABA released at adjacent synapses). Four different GABA transporters have been cloned: GAT1, GAT2, GAT3, and BGT1 [65]; therefore, it is possible that some of the GABA transporters different from GAT1 have properties consistent with uptake-mediated control of IPSC duration. However, GAT2 is only found during very early brain development and BGT1 is not abundant in brain [65]. GAT3 is mostly localized in glia, and the effects of GAT3 blockade indicate that, similar to GAT1, GAT3's main role is reducing the effects of GABA spillover [66].

4. Mechanisms of $GABA_AR$-Mediated-Gamma-Band Synchronization

The mechanisms by which $GABA_AR$-mediated inhibition may synchronize postsynaptic cell activity have been reviewed in detail previously [7, 8, 67, 68]. Figure 2 illustrates a group of pyramidal neurons firing asynchronously in response to some excitatory inputs that receive common $GABA_AR$-mediated hyperpolarizing inhibition. If such GABA-mediated hyperpolarizing input is strong enough, then the postsynaptic neurons will be inhibited together during a certain time window and, as the $GABA_AR$ inhibitory effect decays, will escape from inhibition to resume firing nearly in synchrony (Figure 2). Such postinhibition synchronous spiking of pyramidal cells can be elicited by single-GABA neurons [28] and readily generates synchrony throughout large numbers of neurons in computational network models [7, 67]. Therefore, post-inhibition synchronous spiking is a strong candidate mechanism for production of neural synchrony. Alternative synchronization mechanisms, which are not reviewed here, involve gap junctions connecting pyramidal cell axons [8] or noisy but correlated inputs [69].

Importantly, neuronal synchrony is commonly observed during episodes of rhythmic/oscillatory network activity, especially in association with cognitive tasks [70, 71]. Therefore, the circuit mechanisms of synchronized oscillations via $GABA_AR$-mediated inhibition must involve rhythmic interneuron firing and trains of IPSCs in their postsynaptic target cells. As multiple subclasses of GABA neurons exist [72], a crucial issue is whether specific subtypes are involved in the mechanisms of synchronized oscillations. Synchronized oscillations occur at different frequency bands [70], including theta (~4–10 Hz), beta (~15–30 Hz), and gamma (~30–80 Hz). Whether oscillations of all frequency bands depend on $GABA_AR$-mediated inhibition and, if so, on particular GABA neuron subtypes is still a matter of investigation [73]. Here we focus on models for the mechanisms of gamma oscillations, which are commonly induced during cognitive tasks and seem to be impaired in the cortex of patients with schizophrenia.

Synchronization by the $GABA_AR$-mediated mechanism described in Figure 2 requires sufficiently strong GABA synapses activating a relatively large $GABA_A$ conductance via inhibitory inputs localized near the site of action potential initiation. In pyramidal cells, action potentials are commonly triggered near the axon initial segment (AIS), the axonal compartment that is closest to the soma [74]. Therefore, inhibitory inputs onto the perisomatic membrane compartment (soma, proximal dendrites, and AIS) produce stronger inhibition than inputs onto distal dendrites [75, 76], suggesting that perisomatic-targeting GABA neurons may be crucially involved in production of synchronized oscillations. Three main subtypes of perisomatic-targeting GABA neurons exist in neocortex and hippocampus, namely, the parvalbumin-positive and the cholecystokinin-positive basket cells (PVBCs and CCKBCs) and the PV-positive chandelier cells (PVChCs). Both PVBCs and CCKBCs innervate pyramidal cells at the soma and proximal dendrites (Figure 3), whereas PVChCs synapse exclusively onto the AIS (Figure 3). Because a synaptic $GABA_AR$ conductance has stronger inhibitory effect the closest it is localized to the site of spike initiation [50], PVChC inputs onto the AIS are predicted to have the strongest inhibitory power.

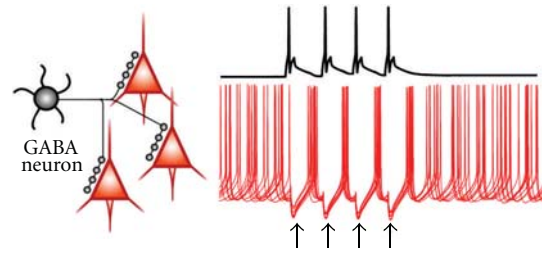

Figure 2: A model for $GABA_AR$-mediated synchronization mechanisms. The left panel shows a group of pyramidal cells that are common postsynaptic targets of an inhibitory GABA neuron. Perisomatic-targeting GABA neurons such as that in the scheme produce stronger inhibition than GABA neuron subtypes that target the dendrites. The right panel shows, above (black trace), the membrane potential of the GABA neuron which remains at rest before and after firing a sequence of four action potentials. The red traces below show the membrane potential simultaneously recorded from the postsynaptic pyramidal neurons, which are firing in response to a continuous excitatory input. Note that, before the GABA neuron starts firing, the pyramidal cells fire in an asynchronous manner. Shortly after the first GABA neuron spike, an inhibitory postsynaptic potential (IPSP) is produced (first black arrow) which simultaneously inhibits the firing of all pyramidal neurons. After the IPSP-mediated inhibition decays, the pyramidal neurons fire in nearly synchrony. Note that a similar postinhibition synchronization is observed with each of the IPSPs evoked by the interneuron spikes (each IPSP is denoted by a black arrow). Once the GABA neuron stops firing, pyramidal cell activity rapidly becomes asynchronous. Also note that a single action potential in a GABA neuron would synchronize the pyramidal cells only once, whereas production of a synchronized oscillation requires rhythmic GABA neuron firing. An oscillation episode composed of four cycles is shown in the figure. The production of synchronized oscillations via this mechanism may be impaired by various alterations of $GABA_AR$-mediated synaptic inhibition in schizophrenia (see Figure 1 and main text).

Surprisingly, some recent studies suggested that synaptic input from PVChCs is actually excitatory, since stimulation of PVChCs frequently initiates spikes in postsynaptic pyramidal cells via $GABA_AR$ activation [40]. In addition, electron microscopy studies support the idea that PVChC inputs are excitatory, as they show very low levels of KCC2 in the AIS compared to the soma or dendrites [40, 77]. Since KCC2 extrudes chloride, lower AIS levels of KCC2 should produce a more positive E_{GABA_A}, resulting in a depolarizing $GABA_AR$ current (Figure 3) [43]. In fact, in paired recordings using experimental conditions that preserve the physiological intracellular chloride concentration, PVChC inputs depolarize the postsynaptic pyramidal neurons [40, 78], whereas in identical experimental conditions PVBC inputs are hyperpolarizing [40, 78], consistent with higher levels of KCC2 transporters in the somatic membrane [40, 77]. Experiments with uncaging of GABA onto $GABA_ARs$ in

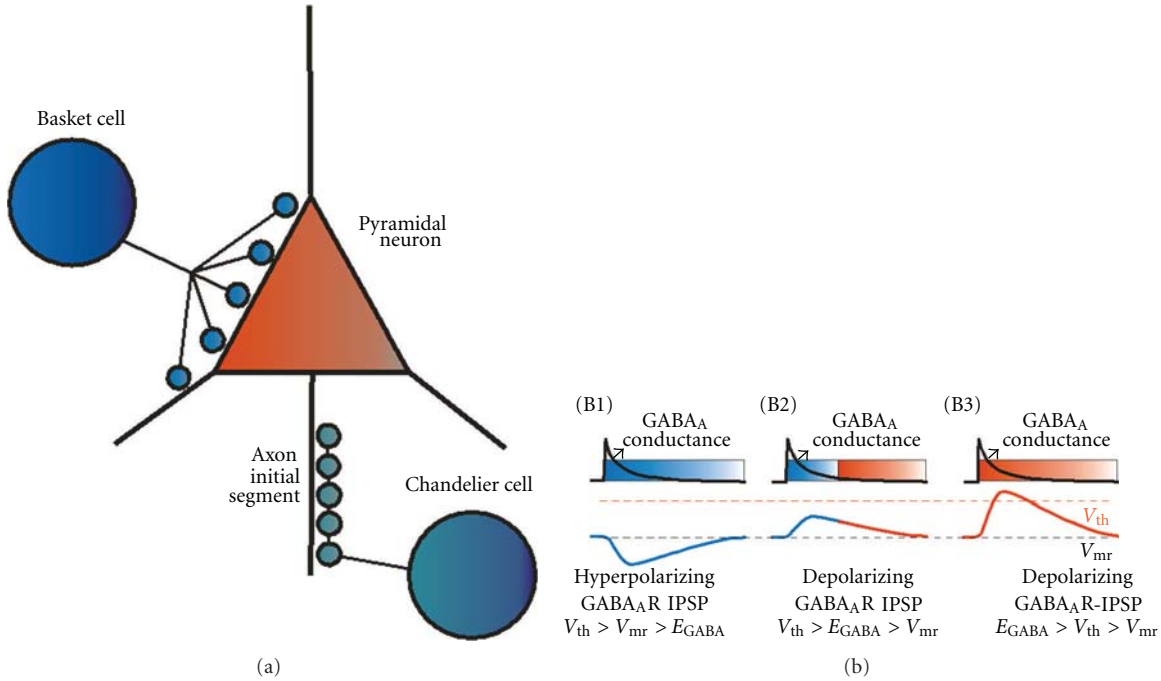

Figure 3: Diversity of perisomatic-targeting GABA neuron-mediated inhibition in cortical circuits may be due to differences in the reversal potential for the $GABA_A$-mediated current. (a) The scheme illustrates targeting by basket cells (either parvalbumin- or cholecystokinin-positive), which contact the soma and the proximal/perisomatic dendritic compartments, and by chandelier or axoaxonic neurons, which contact the axon initial segment. (b) The schemes illustrate differences in the postsynaptic effect of a $GABA_AR$ conductance according to the value of the reversal potential for the $GABA_A$ current (E_{GABA_A}) relative to the resting membrane potential (V_{mr}) and the threshold potential to fire spikes (V_{th}). In (B1) to (B3), the time course of the $GABA_AR$-mediated conductance, chosen to be identical in all panels, is shown by the black traces and the IPSPs are shown by the blue/red traces. Note that the IPSP time course is always slower than the $GABA_A$ conductance, although in the scheme the difference in time course is somewhat exaggerated for illustration purposes and does not match the actual time scales. (B1) Illustration of cases in which E_{GABA_A} is negative relative to V_{mr} ($V_{th} > V_{mr} > E_{GABA_A}$), and the $GABA_A$ conductance generates a hyperpolarizing IPSP (all blue IPSP trace). As the IPSP outlasts the $GABA_A$ conductance, the duration of the inhibitory effect of the synaptic input (shown by the shaded blue rectangle) is extended by the membrane hyperpolarization that remains after the $GABA_A$ conductance decays. (B2) Illustration of cases in which the IPSP is depolarizing because E_{GABA_A} is positive relative to V_{mr} ($V_{th} > E_{GABA_A} > V_{mr}$). Just as the hyperpolarizing IPSP, the depolarizing IPSP outlasts the $GABA_A$ conductance; however, in this case E_{GABA_A} is below V_{th}, and therefore the depolarizing IPSP could have a dual inhibitory/excitatory effect (blue/red IPSP trace), initially producing shunting inhibition which lasts approximately the same time as the $GABA_A$ conductance (shaded blue/red rectangle), followed by an enhanced excitability of the postsynaptic neuron due to the remaining phase of the depolarizing IPSP. (B3) Illustration of cases in which E_{GABA_A} is positive relative to V_{th} ($E_{GABA_A} > V_{th} > V_{mr}$). In this case, the depolarizing IPSP has a purely excitatory effect (shaded red rectangle). Basket cells are thought to produce hyperpolarizing $GABA_AR$-mediated inhibition of pyramidal cells such as that illustrated in (B1). In contrast, the effect of chandelier neuron inputs is currently debatable, some studies suggesting an excitatory as that in (B3), other studies suggesting a purely inhibitory effect. Here, we suggest that depolarizing chandelier cell inputs may have a dual inhibitory/excitatory effect, illustrated in (B2), which could synchronize postsynaptic cells as described in Figure 2, although the depolarizing nature of the IPSP may accelerate the timing of synchronous firing after the postsynaptic cells escape from inhibition.

specific membrane compartments similarly showed a more depolarized E_{GABA_A} at the AIS compared with the soma and dendrites [79].

An excitatory depolarizing $GABA_AR$-mediated input by PVChCs is inconsistent with their participation in inhibition-based synchronization. However, although E_{GABA_A} at the AIS is 10–20 mV depolarized before V_{mr}, it may be negative relative to V_{th} [40, 78, 79]. As highlighted above, when $V_{th} > E_{GABA_A} > V_{mr}$, the $GABA_A$ conductance has a dual inhibitory/excitatory effect (Figure 3), which in the case of PVChC inputs may be amplified by their proximity to the spike initiation site. Such inhibitory/excitatory effect may contribute to synchronization of postsynaptic cell activity at gamma band frequency [7, 80]. Specifically, since the postsynaptic potential outlasts the duration of the $GABA_AR$ conductance (Figure 3), once the conductance is deactivated, the depolarizing postsynaptic potential accelerates the post-inhibition synchronous spiking, facilitating synchronization at gamma frequency. Such a mechanism operates at $GABA_AR$-mediated synapses onto GABA neurons [7, 80] and could also apply at PVChC inputs onto pyramidal neurons.

The depolarizing effect of PVChCs described in neocortex [40, 78] contrasts with pioneer paired recording experiments showing that either PVBC or PVChC inputs hyperpolarize hippocampal pyramidal neurons [26].

A hyperpolarizing effect of hippocampal PVBCs and PVChCs was also suggested recently by experiments using conditions that preserve the intracellular chloride concentrations [81], but that are different from the intracellular chloride-preserving conditions employed in studies of neocortical PVChC inputs [40, 78]. Interestingly, the AIS membrane has low levels of KCC2 in neocortical [40] and hippocampal [77] pyramidal cells. The discrepancies between findings for hippocampal versus neocortical PVChCs highlight the need for further research to clarify the enigmatic role of this class of GABA neurons [82]. Whether excitatory or inhibitory, the unique properties of PVChC inputs suggest that the reported alterations of these cells must significantly contribute to cortical circuit dysfunction in schizophrenia [83].

In contrast to PVChCs, both PVBC and CCKBC inputs are hyperpolarizing ($V_{mr} > E_{GABA_A}$), although E_{GABA_A} is slightly, but significantly, different for PVBC versus CCKBC inputs [84]. Such differences in E_{GABA_A} were attributed to the activity of the voltage- and chloride-dependent chloride channel ClC-2, which helps maintaining a low internal chloride concentration (thus a hyperpolarized E_{GABA_A}) at inputs from PVBCs but not from CCKBCs [84]. The ClC-2-dependent regulation of internal chloride at PVBC inputs is dependent on the extent of $GABA_A$R activation [84]. These findings highlight a degree of functional diversity between BC subtypes, showing that PVBC inputs, but not CCKBC inputs, possess a mechanism to prevent internal chloride accumulation during high-frequency neuronal activity such as that observed in GABA neurons participating in gamma oscillations.

Functional diversity between PVBCs, PVChCs and CCK-BCs is also suggested by recent data demonstrating that the relative levels of GAD65 and GAD67 proteins in nerve terminals of these cells vary significantly in a cell-type-specific manner [85]. Using immunocytochemical labeling combined with a quantitative fluorescence microscopy methodology [86], the colocalization of GAD65 and GAD67 proteins in the same terminals was assessed for PVBCs, PVChCs and CCKBCs (Figure 4). The latter cells were identified using an antibody that detects the cannabinoid 1 receptors expressed by inhibitory terminals. Importantly, in the prefrontal cortex nearly all cannabinoid 1 receptor-expressing cells detected using this antibody are immunoreactive for CCK [87]. This assessment showed that the ratio of GAD67/GAD65 expression varied significantly across each type of terminal, with very high (15.42) and very low (0.18) ratios observed in terminals of PVChCs and CCKBCs, respectively, and a ratio of 1.49 for PVBC terminals [85]. These data reinforce the idea that synaptic connections from different perisomatic-targeting GABA neuron subtypes are functionally diverse. The impact of different GAD67/GAD65 ratio on the properties of the GABA synapses studied remains enigmatic because the differential functional roles of GAD67 and GAD65 are still poorly understood. In mice, GAD67 deficiency produces major alterations, as $GAD67^{-/-}$ animals are born with cleft palate (which is lethal) and with ~90% reduction of bulk GABA concentration in brain tissue [88, 89]. In contrast, $GAD65^{-/-}$ mice survive into adulthood, displaying ~20% reduction in total brain GABA concentration [90] together with increased susceptibility to seizures [90, 91], increased anxiety [92], and altered fear conditioning [93]. In synapses of $GAD65^{-/-}$ mice, $GABA_A$R-mediated synaptic transmission appears normal during low-frequency stimulation but is strongly impaired at stimulation frequency of 30 Hz or higher [94, 95]. In mice with cerebellum-specific GAD67 deficiency [96], $GABA_A$R-mediated transmission is markedly weaker even at low stimulus frequency [96], suggesting a crucial role of GAD67 in baseline synaptic transmission. Whether the effects of GAD67 deficiency on cerebellar synapses are observed in cortical synapses as well remains to be determined. Importantly, GAD67 deficiency markedly impairs the maturation of cortical GABAergic synaptic connections [97]. Therefore, testing the role of GAD67 in synaptic transmission independent of its role in synapse development requires a paradigm in which GAD67 is decreased once GABA synapse maturation has been completed. In addition, GAD65 and GAD67 both comprise ~50% of total GAD protein in mouse cortex, while GAD65 comprises ~70% in the rat cortex [98]. The GAD65 and GAD67 proportions of the total GAD protein in human cortex is unknown; however, the differences between mouse and rat strongly stress the need to study GAD function in molecularly relevant systems when trying to understand their roles in human disease.

$GABA_A$R-mediated inputs involved in gamma band synchronization must inhibit their postsynaptic neurons for a time compatible with the gamma oscillation period. For instance, long-lasting postsynaptic currents such as those activated by $GABA_B$ receptors (which may last hundreds of milliseconds) are inconsistent with gamma synchrony, as postsynaptic neuron inhibition would be longer than the typical gamma cycle period. Whereas all $GABA_A$R subtypes produce faster currents than $GABA_B$ receptors, the $GABA_A$current duration depends on the subunit composition [43, 99]. As mentioned, recombinant α1-$GABA_A$Rs produce currents with the fastest decay [100]. Therefore, PVBCs may produce IPSCs with faster decay, as α1-$GABA_A$Rs predominate at inputs from PVBCs [33, 41, 101, 102] whereas α2-$GABA_A$Rs predominate at both PVChC and CCKBC inputs [41, 101–103]. Interestingly, PVBCs, CCKBCs, and PVChCs elicit in pyramidal cells uIPSCs with nearly identical kinetics [39, 104–107]. Therefore, in addition to the $GABA_A$R subunit composition, the IPSC kinetics are determined by additional factors that may not affect recombinant $GABA_A$Rs. Such factors include $GABA_A$R subunit phosphorylation and other posttranslational modifications [108, 109], and the time course of the GABA concentration transient to which $GABA_A$Rs are exposed [41].

Although PVBCs and CCKBCs produce IPSCs with similar duration during individual GABA release events, CCK-BCs distinctively produce multiple asynchronous release events after single-action potentials [104]. Multiple GABA release events may prolong the postsynaptic inhibitory effect of each CCKBC spike [104]. In contrast, PVBC terminals produce a single synchronous GABA release event per presynaptic action potential [104, 110]. Asynchronous release from CCKBC terminals has been observed in multiple

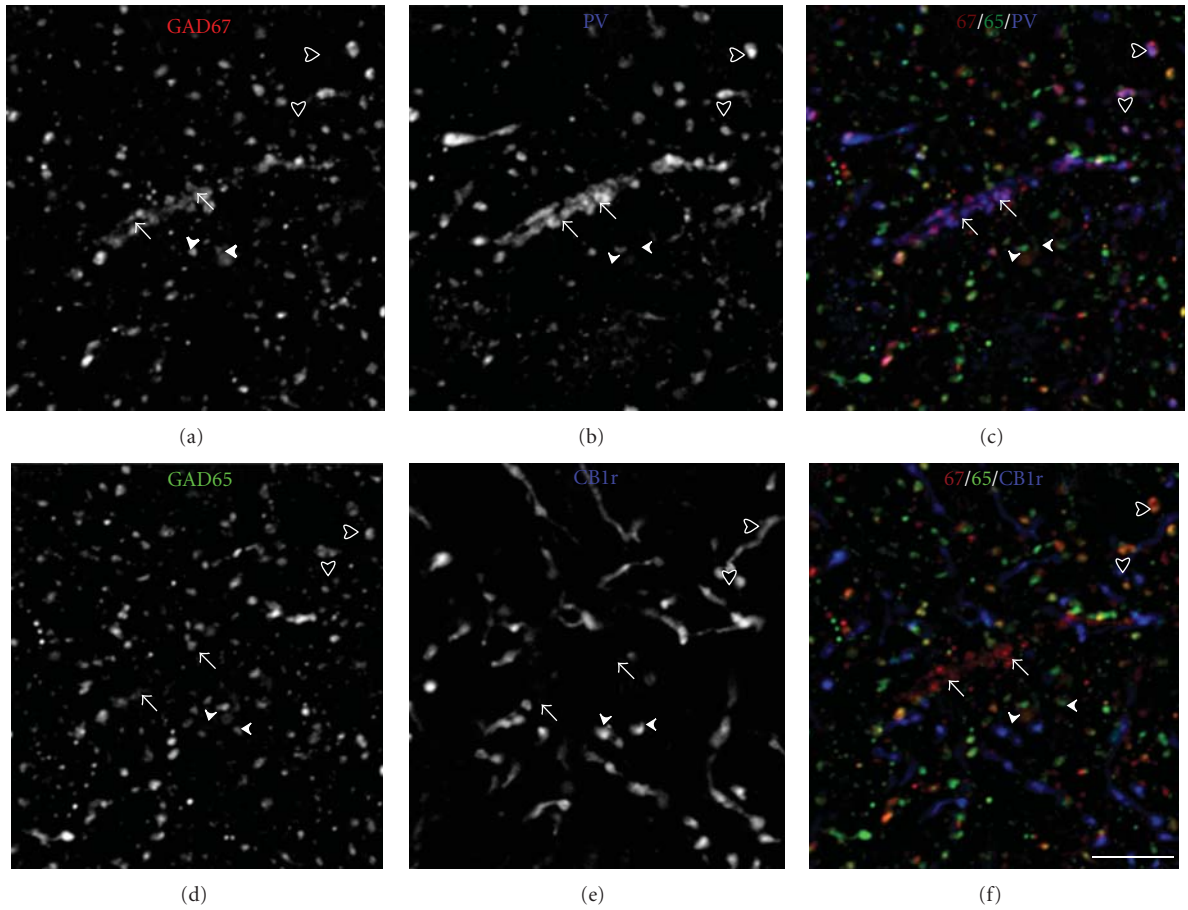

Figure 4: Relative levels of GAD65 and GAD67 in PVCh, PVBC, and CCKBC terminals. Cryostat sections of monkey PFC tissue (40 μm thick) were quadruple labeled for GAD65, GAD67, PV, and cannabinoid receptor 1 (CB1r). Among inhibitory synaptic boutons, CB1r is exclusively expressed in those CCK-positive neurons and is completely absent in terminals of PV-positive neurons. Presynaptic CB1rs are also present in excitatory synapses; however, the antibody used in the studies illustrated in this figure exclusively reveal CB1r expression in inhibitory boutons (see main text for details). Therefore, CB1r expression is a marker of CCK cell terminals. Single channel (a)-(b) and (d)-(e) and merged (c) and (f) projection images of deconvolved image stacks (2 z-planes 0.25 μm apart). Since four labels cannot be displayed together in a single image, they have been separated into two RGB images that contain GAD67 (red), GAD65 (green), and PV (blue (c)) or CB1r (blue (f)). Arrows: PV cartridge; solid arrowheads: $CB1r^+/GAD65^+$ and $GAD67^-$ terminals; open arrowheads: $GAD65^+/GAD67^+/PV^+$ terminals. Bar = 10 μm.

studies [104, 107, 111–115] suggesting that it is a fundamental property that prolongs the inhibitory effect of CCKBCs on postsynaptic neurons possibly linking their activity with synchronization at frequency bands lower than gamma.

The data reviewed above suggest that, relative to CCKBCs and PVChCs, PVBCs have unique properties consistent with a crucial role in the mechanisms of gamma band synchrony. However, such data do not directly assess involvement of any of these GABA neuron subtypes in the gamma oscillation mechanisms. Interestingly, some electrophysiological studies more directly indicate that among perisomatic-targeting GABA neuron subtypes, PVBCs are most likely to be involved in the production of gamma oscillations. For instance, whereas both BCs and ChCs are active during gamma oscillations in vivo [116–118] and in vitro [119–124], the firing of CCKBCs and PVChCs is more weakly coupled with the gamma oscillation cycle than PVBC firing [124], although CCKBC and PVChC firing is strongly coupled with theta oscillations [125–127]. Furthermore, gamma oscillations are significantly reduced or abolished by suppressing PV cell activity with optogenetic methods that do not directly affect CCKBCs [128] or by stimulation of presynaptic opioid receptors that suppress GABA release from PVBCs but not from CCKBCs or ChCs [124]. Therefore, perisomatic $GABA_AR$-mediated currents from PVBCs appear to be the main source of $GABA_AR$-mediated synchronization in the gamma frequency band.

Essential for modeling the circuit mechanisms of gamma synchronization is to understand how PVBCs are normally recruited to fire rhythmically at gamma frequency. Recruitment of PVBC firing depends on activation of not only excitatory but also inhibitory synaptic inputs onto them since PVBCs target other GABA neurons, including nearby PVBCs [129, 130], and are also inhibited by inputs from

different classes of GABA neurons, including the CCK-BCs [112]. Gamma oscillations are successfully generated in computational model networks that rely on reciprocal inhibition between GABA neurons and are thus called ING, for Interneuron Network Gamma [131–133]. In ING models, GABA neurons are recruited by a strong tonic excitation that drives them to fire at a frequency above gamma, and the reciprocal inhibition synchronizes GABA neuron firing at a frequency inversely related to the IPSC duration, falling within gamma range for durations typical of α1-$GABA_AR$-mediated IPSCs [132, 133]. Whereas gamma rhythms possibly induced by ING-like mechanisms have been observed experimentally [134], the actual source of the strong tonic excitation onto GABA neurons required by ING models is unclear. Metabotropic glutamate receptors [67, 134] or kainate receptors [135] could provide such a tonic drive, although this possibility is supported, only indirectly, by findings obtained with AMPA- and NMDA-mediated synaptic transmission blocked. A recent study employing genetically engineered mice with a deletion of $GABA_AR$ expression in PVBCs [136] directly tested whether inhibition onto PVBCs is necessary to generate gamma oscillations, as predicted by the ING models. In such mice, $GABA_AR$-mediated IPSCs were abolished exclusively in PVBCs [136] and theta oscillations and their coupling with gamma oscillations were severely disrupted [136]. However, in such mice hippocampal gamma oscillations in vivo were intact as compared with wild-type mice [136]. These data argue against the ING model for gamma band synchrony and suggest that inhibition onto PVBCs, potentially from CCKBCs [112], is crucial for coupling theta and gamma oscillations. Such theta-gamma coupling is thought to be important for cognitive function [137].

As the role of ING mechanisms in gamma oscillation production continues to be tested, some studies favor an alternative model, known as Pyramidal Interneuron Network Gamma (PING), which depends on recurrent excitatory-inhibitory synaptic interactions. In PING, PVBCs are recruited by phasic glutamate-mediated inputs from the pyramidal cells, and the PVBCs provide strong feedback inhibition that synchronizes pyramidal cell firing [67, 121, 138]. The PING model predicts that during the gamma oscillation cycle PVBCs fire after the pyramidal neurons, with timing consistent with monosynaptic recruitment by pyramidal cells [138]. The spike timing of pyramidal cells and putative BCs during gamma oscillations in awake behaving animals is actually consistent with the PING model, as BCs fire 2-3 ms later than pyramidal neurons [139, 140]. Similar findings were obtained for pyramidal and PVBC spikes during gamma oscillations in hippocampal and neocortical brain slices [119, 120, 122, 123, 141]. PING models also predict the presence of trains of gamma frequency IPSCs in pyramidal neurons and trains of gamma frequency EPSCs in PVBCs [67]. Evidence consistent with IPSC trains in pyramidal cells was obtained for gamma oscillations in vivo [117, 142] and in vitro [119–123, 141, 142]. In addition, during gamma oscillations in vitro, PVBCs display rhythmic EPSCs highly synchronized with the gamma rhythm [120–122]. Interestingly, optogenetics experiments show that driving PV neurons by nonrhythmic excitatory inputs is sufficient to generate gamma synchrony via feedback inhibition [128], a finding also consistent with the PING model of gamma.

PING mechanisms rely on recruitment of PVBCs by phasic excitatory input [138]; therefore, the properties of glutamate synapses onto PV neurons are extremely relevant for models of gamma oscillations. Interestingly, schizophrenia has been hypothesized to be associated with a deficit of glutamate transmission [143], more specifically with hypofunction of NMDA receptors, particularly in GABA neurons [143]. Moreover, some studies have suggested that NMDA hypofunction could especially affect PV cells [144–146]. Therefore, an important question is the following: what are the subtypes of glutamate receptors that mediate synaptic activation of PV GABA neurons? The answer to this question is relevant in the context of alterations of gamma synchrony in schizophrenia, because if NMDA receptors are important to recruit PV neurons in a PING mechanism of gamma, then NMDA hypofunction could be linked to deficits of gamma synchrony in schizophrenia. Data from recent studies showed that systemic administration of NMDA receptor antagonists increase the firing rate of putative pyramidal neurons and decrease the firing of putative inhibitory cells in the PFC in vivo [147], suggesting that NMDA receptors may be crucial to recruit inhibition. An important question not directly addressed by such studies [147] is whether the inhibitory neurons dependent on NMDA receptors belong to the PV-positive class of GABA neurons. Whereas several studies demonstrated that synaptic excitation of PV neurons is relatively NMDA receptor independent, until recently no studies directly compared the importance of NMDA receptors in synaptically evoked recruitment of PVBCs versus pyramidal neurons in neocortical circuits. Recent data from recordings in mouse PFC show that, compared with pyramidal cells, glutamate synapses onto PVBCs have EPSCs with faster decay and weaker NMDA receptor contribution [148], supporting the idea that the rapid activation of PVBCs [149] is largely dependent on fast AMPA receptor-mediated excitation. Moreover, in a computational model producing gamma oscillations via PING mechanisms, fast AMPA-mediated excitation of PVBCs was critical for gamma band synchronization because the slower decay time course of NMDA-mediated EPSCs disrupted gamma band synchrony [148]. Some studies indeed showed that gamma oscillations are not affected or are enhanced by NMDA receptor antagonists, whereas AMPA receptor antagonism completely abolished them [150–153]. Similarly, in mice with AMPA receptor deletion genetically engineered to occur exclusively in PV-positive neurons, gamma oscillations are strongly reduced [154]; however, NMDA receptor deletion selectively in PV-positive cells does not decrease and in fact increases gamma oscillation power [155]. The results of recent studies therefore suggest that, in mature cortical circuits, NMDA receptors only play a minor role in synaptically evoked excitation of PV-positive neurons and therefore on gamma oscillations produced via PING mechanisms. If indeed PV neuron excitation is normally weakly dependent on NMDA receptors, then such data suggest that excitatory synapses onto PV cells are an unlikely target of NMDA receptor

hypofunction mechanisms in schizophrenia. Importantly, although in most cases gamma synchrony is unaffected by NMDA receptor blockade, the effects of NMDA receptor antagonists on gamma oscillations may vary with cortical region or layer, in some cases ketamine producing a decrease, in others producing an increase in gamma power [152, 156]. In addition, some data show that whereas mature PVBCs display a relatively small NMDA receptor mediated component in synaptic responses [148, 157, 158], such NMDA component is substantially more prominent in immature PVBCs [157, 158]. Such findings suggest the interesting possibility that alterations of NMDA receptor-signaling during early brain development could alter PVBC function in ways that persist into adulthood, thus changing the role of PVBCs in mature local circuits. Interestingly, a recent study showed that a genetically engineered deletion of NMDA receptors from PV-positive cells does not have significant effects if the deletion is produced in the brain of adult mice [159]. However, a similar deletion produced during early brain development caused behavioral alterations in adult mice, some of which resemble behavioral dysfunction in patients with schizophrenia [159].

5. Postnatal Development of GABA-Mediated Synaptic Inhibition

Schizophrenia is hypothesized to be a neurodevelopmental disorder based on data linking the disease with adverse events during pre- and perinatal periods and the presence of cognitive and behavioral deficits in childhood many years prior to the onset of psychosis during late adolescence and early adulthood [160]. Adolescence, the developmental transition from parent-dependent childhood to independent adulthood, is associated with significant changes in behavior and with marked improvements in cognitive control [161]. Moreover, gamma band synchrony emerges during childhood and continues to mature until early adulthood [162, 163]. The postnatal developmental trajectory of GABA-synaptic function may therefore suggest critical periods of vulnerability during which the mechanisms producing neural synchrony could become dysfunctional in schizophrenia. In what follows we review developmental studies of the functional properties of PVBCs and their synaptic connections in rodents and of GABA-related gene products studied in the human and nonhuman primate brain.

Mature PV neurons have a unique fast-spiking (FS) firing pattern (Figure 5), which includes very narrow action potentials and high frequency firing without the spike-frequency adaptation typically observed in pyramidal cells and other GABA neuron subtypes [72]. Although the nonadapting properties of FS cells are revealed with artificial stimuli (rectangular currents steps lasting several hundred ms), they correlate strongly with the particular ability of FS cells to respond to oscillatory inputs at gamma frequency (Figure 5), which is likely due to the gamma frequency resonance of the FS cell membrane [164]. Immature FS neurons, in contrast, have significantly slower action potentials and stronger spike-frequency adaptation, fail to respond efficiently to gamma frequency oscillatory inputs, and show much slower conduction velocity of action potentials along their axon [33, 165–167]. In rodent hippocampus, as well as in auditory, somatosensory, and prefrontal cortices, maturation of FS neuron electrical properties is complete by postnatal day 25 (P25) after which FS neurons display adult-like electrical properties [33, 157, 165–167].

Functional properties of the inhibitory connections onto excitatory neurons also differ markedly between developing and mature PVBCs: unitary IPSCs (uIPSCs), from immature PVBCs, are weaker and have slower decay time than uIPSCs produced by mature PVBCs [33, 168]. Such acceleration of uIPSC decay is explained by an increase in the contribution of α1-$GABA_A$Rs with maturation [33]. A $GABA_A$R subunit switch may also contribute to the developmental acceleration of the uIPSP decay [167] although developmental changes in the pyramidal cell membrane time constant contribute as well [167]. Mature FSBCs produce highly synchronous GABA release, in contrast to asynchronous release from mature CCKBCs [33, 104, 110]. However, GABA release is less synchronous and less reliable in synapses from immature FSBCs [33]. Postnatal maturation of the FSBC connections takes place relatively rapidly, as uIPSCs acquire mature properties by P28 [33, 167].

Inhibition onto FS cells also undergoes significant developmental changes. For example, uIPSCs at FSBC-to-FSBC connections mature within 3-4 weeks postnatally [33], whereas miniature IPSCs (mIPSCs, which represent GABA release at single synapses) recorded from FSBCs acquire adult-like properties by P25 [165]. Unitary EPSPs (uEPSPs) at immature pyramidal cell-to-FSBC connections have slow decay time, which accelerates markedly during development, reaching adult-like values by ~P22 [167]. Similarly, miniature EPSCs (mEPSCs) recorded from immature FSBCs are slow and show a developmental acceleration of their decay to reach very fast mEPSC decay values in mature FS cells [148, 157, 158, 165]. Fast-decaying EPSCs and EPSPs in mature FSBCs are largely mediated by AMPA receptors [148, 157, 158, 167, 169], suggesting that the rapid EPSC decay in FSBCs is due, at least in part, to a weak contribution from the slow-decaying NMDA currents [148, 157]. Actually, the developmental acceleration of EPSC decay is accompanied by a marked decrease in the contribution of NMDA currents which is still ongoing at ~P40 to ~P96 [157], which in rodents corresponds to the transition from adolescence to adulthood [170]. In contrast to PFC, in hippocampus, auditory, and somatosensory cortices, NMDA receptor contribution in FSBCs decreases prior to adolescence [167, 169, 171, 172].

Although the mechanisms controlling postnatal development of PVBC firing properties and their synaptic inputs and outputs are important candidates as the substrate of alterations in schizophrenia, they are still poorly understood. Neuregulin-1 is a trophic factor crucial for brain development that is encoded by a schizophrenia susceptibility gene and is highly expressed during late developmental periods and in adulthood [173, 174]. Among various neuregulin-1 receptors, the ErbB4 receptor, whose gene also confers schizophrenia susceptibility [173, 174], is enriched in GABA neurons, particularly in PV-positive cells [175, 176]

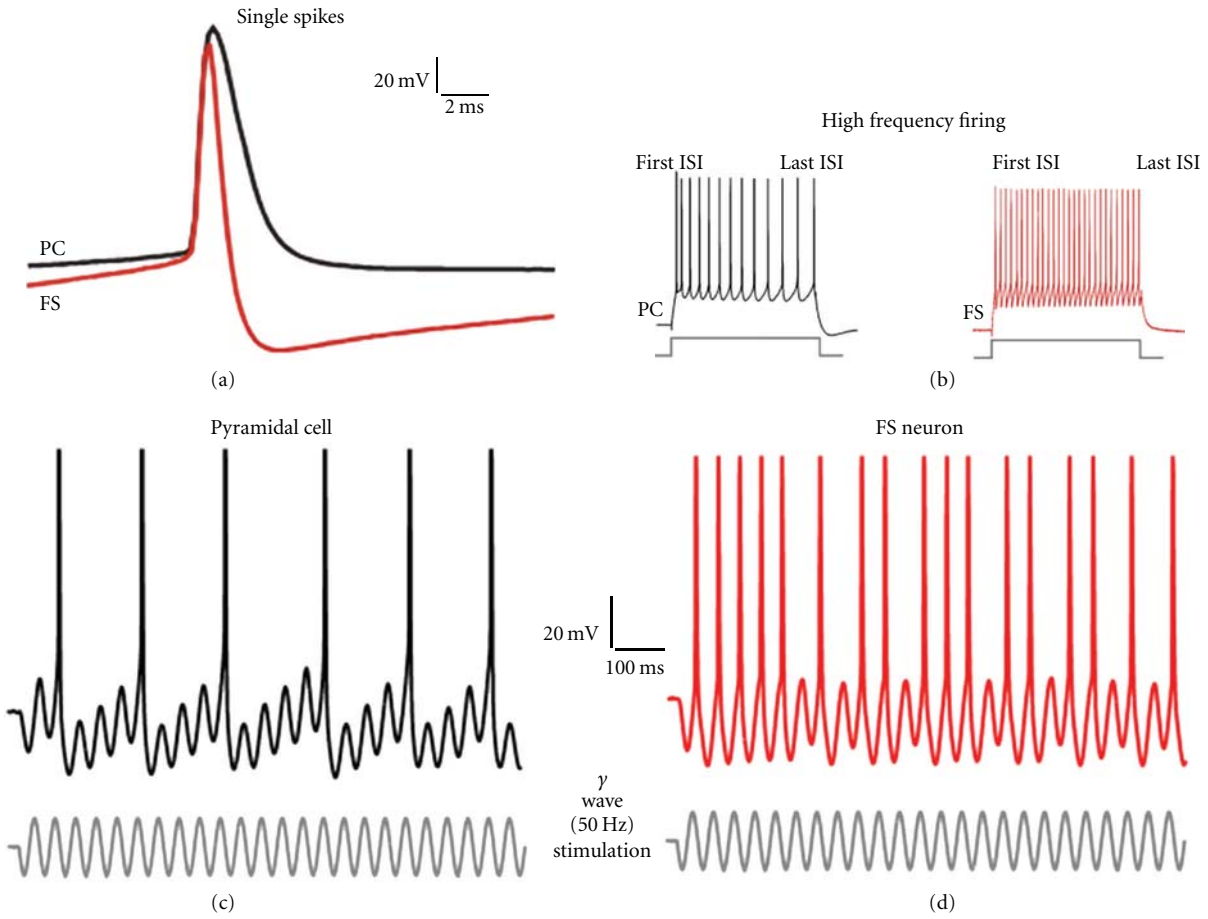

Figure 5: Intrinsic electrical properties of parvalbumin-positive GABA neurons. (a) Single action potentials in fast-spiking PV-positive neurons (FS) have significantly faster duration than pyramidal cell (PC) spikes or spikes in many other GABA neuron subtypes (not shown). (b) In response to sustained depolarizing current injection (500 ms rectangular current pulses shown below the traces), PCs produce high-frequency firing with significant spike-frequency adaptation as revealed by comparing the first and last interspike intervals (ISI). In contrast, adaptation of FS cell firing is much less significant or absent. (c) In response to gamma wave stimulation (sinusoidal current pulses shown below the traces), PCs show low capacity to respond with firing during each cycle of the gamma wave stimulus. (d) In response to gamma wave stimulation, FS cells show increased firing capacity, initiating spikes in the majority of gamma wave cycles. This property of the FS neuron membrane may contribute to the activation of FS neurons during gamma oscillations in vivo and is likely due to the resonance or frequency-preference properties (see text) that distinguish FS cells from PCs and also from other GABA neuron subtypes.

where it facilitates GABA release [176], possibly mediating neuregulin-1 enhancement of gamma oscillations [177]. Neuregulin-1 signaling appears to regulate early development of GABA synapses [178] and, via ErbB4 receptors, may control development of PV neuron synapses [179]. Interestingly, ErbB4-mediated neuregulin-1 effects are crucial for development of excitatory synapses onto PVBCs [179, 180].

In parallel to the maturation of their firing pattern and synaptic connections, PVBCs undergo significant developmental morphological changes. For instance, the total length of the dendritic and axonal trees of PVBCs increases significantly from P6 to P25 [33], the number of axonal branch points increasing five times during this developmental period [33]. The number of postsynaptic neurons innervated by individual PVBCs also increases markedly with postnatal development [97], resulting in a higher functional connectivity between mature PVBCs and excitatory neurons [33]. A crucial factor regulating development of innervation patterns by PVBCs is GAD67-mediated GABA synthesis [97]. For instance, GAD67 knockdown in single PVBCs dramatically decreases formation of axon branches and synapses, as well as the number of postsynaptic neurons innervated by each PVBC [97]. Such effects of GAD67 knock-down are observed in organotypic cell cultures and in the primary visual cortex in vivo with GAD67 knock-down induced at P13 and the patterns of innervation examined at P20 or P32 [97]. The role of GAD67-mediated GABA synthesis in formation and/or stability of PVBC synapses may involve neuroligin-neurexin interactions and modulation of GABA receptor trafficking [181].

Because detailed molecular and biophysical analysis of developmental changes in GABA neuron and GABA synapse function is feasible only using animal models, especially rodents, an important question is how developmental time

scales translate from animal to human brain [182]. Proper translation would require understanding if similar developmental stages are found in rodent and human brains, whether such developmental stages involve similar processes and underlying mechanisms, and whether developmental periods cover similar fractions of the total lifespan. Some developmental changes, for instance, excitatory synapse pruning, have similar proportional duration, with only the extent of synaptic pruning differing across mammalian species [183]. Also, functional maturation of glutamate synapses onto pyramidal cells occurs prior to adolescence in nonhuman primates [184], and in rodents [185–191].

Whereas some unique studies have assessed functional properties of PVBCs and PVChCs in the cortex of adult humans [40, 192, 193] and nonhuman primates [39, 184, 194–197], we lack information on the developmental trajectory of synaptic inhibition from primate PV neurons. Interestingly, functional properties of yet unidentified GABA synapses onto primate pyramidal neurons change during development through adolescence in a manner consistent with changes in the expression of gene products involved in GABA-mediated transmission [198]. Specifically, the decay time of $GABA_AR$-mediated synaptic potentials accelerates during adolescence in parallel to changes in the protein and/or mRNA levels for $\alpha 1$ and $\alpha 2$ $GABA_AR$ subunits that would predict such acceleration [198].

The synaptic connections from PVChCs onto the pyramidal cell AIS form vertical arrays of multiple synaptic boutons that are usually easy to distinguish and typically called cartridges. Developmental properties of inputs from PVChCs onto the AIS can be studied using immunocytochemistry to detect biochemical markers that are concentrated at the cartridges in the AIS. In monkey PFC, the density of chandelier neuron axon cartridges immunoreactive for either PV or GAT1 changes markedly during postnatal development [199]. Although the precise time course differs for the two markers, the density of labeled cartridges is low in the newborn, increases to reach a peak prior to the onset of puberty, and then declines markedly to adult levels. Because cartridges are readily visualized with the Golgi technique over this same time period [200], the changes in PV- and GAT1-immunoreactive cartridges may reflect developmental shifts in the concentration of these proteins, rather than in the number of axon terminals, but this remains to be experimentally assessed.

Substantial developmental changes also occur postsynaptically at the AIS. In the adult cortex, the majority of $\alpha 2$-$GABA_AR$s are found in pyramidal cell AIS [201]. The detectability of $GABA_A\alpha 2$ subunits in AIS is very high in the early postnatal period and then steadily declines through adolescence into adulthood [199]. Immunoreactivity for ankyrin-G, βIV spectrin, and gephyrin (a scaffolding protein that regulates the clustering of $GABA_AR$s containing $\alpha 2$ subunits at AIS) [202–204] also exhibit substantial changes during postnatal development [205]. The densities of ankyrin-G and βIV spectrin immunoreactive AISs are greatest at birth and then sharply decline to reach relatively stable values by one year of age. In contrast, the relative density of gephyrin-immunoreactive AIS did not appear to change through the two postnatal years but then sharply decline through adolescence and into adulthood.

The high density of AIS with detectable levels of ankyrin-G immunoreactivity in the first three postnatal months may reflect the recruitment to this location of a portion of the large number of GABA synapses that are formed in the monkey DLPFC during this developmental epoch [206]. Binding to ankyrin-G is also essential for the localization of many other membrane proteins to the AIS [207], including the voltage-gated Na^+ channels that are required for action potential generation [208]. Thus, the high levels of ankyrin-G immunoreactivity may also indicate an increased capacity of pyramidal neurons for repetitive firing that parallels their increase in excitatory inputs during early postnatal development [206, 209]. The parallel relative densities of ankyrin-G-IR and βIV spectrin-SD-IR AIS likely reflect that ankyrin-G is required for the recruitment of βIV spectrin to AIS [210]. Although βIV spectrin is not essential for the formation of the AIS, it does appear necessary for maintenance of membrane structure and molecular organization [211], and thus the stability [210], of the AIS. Given the general role of spectrins in maintaining membrane integrity and elasticity [207], high levels of βIV spectrin during early postnatal development might insure the stability of AIS structure while PFC thickness is increasing [212].

Interestingly, in human PFC the levels of GAD67 mRNA increase progressively during prenatal and postnatal development through childhood until around the peripubertal period, followed by a plateau or mild decline during aging [213]. A similar pattern was reported for GAD67 mRNA expression during mouse [213] and monkey [214] cortical development, suggesting a highly conserved developmental trajectory of GAD67 expression across mammals. Studying protein expression by immunoblotting, a recent study found that GAD67 protein levels did not change across the lifespan in human visual cortex [215]. In contrast, GAD65 showed a progressive 60% increase until teenage years and young adulthood, followed by slight decline in older adults [215]. Two other presynaptic proteins involved in GABA transmission, the cannabinoid receptor 1 and vGAT, showed higher levels in infants and young children, which declined to adult-like levels in preteenage years [215]. The levels of PV mRNA increase markedly in postnatal human PFC, from very low perinatal levels until adult-like levels are reached by 2–5 years of age [216], an early developmental trajectory which is similar to that reported for PV mRNA and protein in rodent neocortex [217, 218].

Interestingly, a comparative analysis using immunolocalization of the chloride transporters NKCC1 and KCC2 revealed a very similar developmental trajectory in rat and human cortex [219]. NKCC1 levels peaking during perinatal development and decaying rapidly thereafter reach adult-like levels during childhood; conversely, KCC2 is undetectable perinatally and increases until reaching adult levels during childhood [219]. Since the NKCC1/KCC2 activity ratio determines whether $GABA_AR$-mediated IPSCs depolarize or hyperpolarize the postsynaptic membrane, these data suggest that the very early developmental switch from excitatory to

inhibitory effects at most GABA synapses [220] is highly conserved between rodent and human neocortex.

Analysis of $GABA_AR$ subunit proteins during postnatal development in human visual cortex showed that $\alpha 1$ $GABA_AR$ subunit levels increase from <1 years until reaching adult levels at 13.5 years of age, whereas $\alpha 2$ $GABA_AR$ subunits decreased significantly with age to reach adult levels by ~ 10 years and $\alpha 3$ $GABA_AR$ subunit levels do not change significantly with age [215]. Consequently, the $\alpha 1/\alpha 2$ subunit protein ratio increased markedly with development attaining adult-like ratios at 4.5 years of age [215]. Remarkably, very similar developmental trajectories were found for the levels of $GABA_AR$ subunit mRNAs in postmortem samples of human PFC [221]. For example, $\alpha 1$ $GABA_AR$ subunit mRNA levels are very low perinatally and increase markedly until toddler ages, thereafter remaining consistently high through to adulthood [221]. In contrast, $\alpha 2$ subunit mRNA increased during the first postnatal months, decreasing subsequently until reaching mature levels at teenage years or young adulthood [221]. The mRNAs for $\alpha 4$ and $\alpha 5$ $GABA_A$ subunits in human PFC showed a developmental pattern similar to that of $\alpha 2$ mRNA, whereas $\alpha 3$ subunit mRNA did not change significantly with age [221]. Postnatal expression of mRNA for γ and β $GABA_AR$ subunits similarly shows significant age-dependent changes, with $\beta 1$ subunits showing a very early developmental decrease between neonate and infant ages, remaining constant thereafter, and $\beta 2$ increasing somewhat later, between toddler and teenage years [222]. On the other hand, $\gamma 1$ and $\gamma 3$ subunit mRNA levels decrease with age during childhood and teenage years, whereas $\gamma 2$ subunit mRNA levels decrease over the same period [222].

The developmental trajectories reviewed above suggest that similar processes underlie developmental changes in $GABA_AR$-mediated synapses across various areas of human and rodent cortex, although further studies are necessary to properly compare developmental trajectories across species. A major difference between species is that the maturation of GABA-related markers in humans involves progressive change over one to two decades, whereas in rodents GABA synapse maturation appears to be complete within 3-4 postnatal weeks. Such difference suggests that the absolute time window during which activity and experience may influence GABA synapse development is markedly expanded in primate versus rodent brains. The prolonged period that may be necessary for the normal developmental tuning of the more complex circuitry of the primate cortex probably also prolongs the time window during which environmental factors can produce subtle developmental alterations that may contribute to the pathophysiology of schizophrenia.

6. Evidence Suggesting Alterations of $GABA_AR$-Mediated Synaptic Transmission in the Cortex of Schizophrenia Patients

The hypothesis that a deficit in $GABA_AR$-mediated transmission underlies cortical circuit dysfunction in schizophrenia is supported by convergent lines of evidence from postmortem studies of the brain of subjects with schizophrenia [6]. Furthermore, such hypothesis is strengthened by the fact that one of the most reliably replicated findings in schizophrenia research is the decrease in GAD67 mRNA (for review, see [223]). Interestingly, equivalent measurements of GAD65 levels thus far failed to reveal alterations, suggesting that the role of GAD65 in GABA-mediated transmission maybe intact in schizophrenia.

A remarkable recent study found that in schizophrenia several GABA-related transcripts, including those for GAD67, PV, GAT1, somatostatin, and the $GABA_AR$ subunits $\alpha 1$ and δ, show decreased levels in dorsolateral PFC as well as in the anterior cingulate, primary motor, and primary visual cortices [224]. Such conserved regional pattern of GABA alterations suggests that dysfunctional GABA neurotransmission contributes to multiple clinical features of schizophrenia including perceptual and motor deficits that could contribute to impaired cognitive function [11]. The disruption of PV expression across cortical areas confirms the previous findings and, moreover, suggests that alterations of PV-positive cells are central to the schizophrenia disease process, although the consequences of such decrease in PV are not completely understood. PV is a Ca^{2+} buffer that is present in nerve terminals of PV-positive neurons (Figure 1). Due to its slow kinetics of Ca^{2+} binding, PV is unable to bind intracellular Ca^{2+} before Ca^{2+} influx activates the Ca^{2+} sensor that triggers GABA release, because in PV-positive terminals Ca^{2+} influx is tightly coupled with GABA release [110, 225]. Interestingly, GABA release by single stimuli does not differ between PV-deficient and wild-type mice, but PV deficiency facilitates repetitive GABA release [226–228]. In synapses from PV-deficient mice, the amplitude of intracellular Ca^{2+} transients in nerve terminals is not affected, but their decay is slowed, indicating that PV normally accelerates such decay [227, 228]. Therefore, one possibility is that the decrease of PV in schizophrenia, instead of contributing to deficits, is a compensatory response to enhance GABA release in the face of decreased GABA synthesis. Alternatively, reduced PV levels may produce synaptic dysfunction via loss of some asynchronous release normally produced when Ca^{2+} unbinds from PV, well after the presynaptic action potential ended [145]. A pathological loss of asynchronous GABA release by decreased PV levels, however, requires the existence of asynchronous release when PV is intact, a feature that is not observed in cortical PV-positive cells [104, 110], although it is found in PV-positive cerebellar interneurons [227].

Whereas schizophrenia may be associated with an increased density of $\alpha 2$-$GABA_ARs$ at the AIS synapses from PVChCs [6], one study failed to detect significant changes in total tissue levels of $\alpha 2$-$GABA_AR$ mRNA [221]. One possibility is that changes in $\alpha 2$-$GABA_ARs$ are synapse- or layer-specific and perhaps found exclusively at AIS synapses in superficial cortical layers, as initially reported [229]. Consistent with this interpretation, laminar analysis of mRNA levels for $GABA_AR$ subunits in the cortex of subjects with schizophrenia revealed significantly increased levels of mRNA for $\alpha 2$ $GABA_AR$ subunits exclusively in layer 2 [230]. Moreover, the same study revealed lower levels of $\alpha 1$ $GABA_AR$ subunit mRNA in layers 3 and 4 [230], which is consistent with a decrease in total tissue levels of $\alpha 1$ $GABA_AR$

subunit mRNA observed using quantitative PCR [224]. Given that α1 subunit-containing $GABA_A$Rs constitute about 60% of the total $GABA_A$Rs in adult brain [231], it is possible that α1 subunit mRNA is significantly more abundant than that for α2 subunits, thus increasing the chance of detecting changes of total tissue α1 mRNA levels in schizophrenia. Importantly, a decrease in α1 subunits in schizophrenia is consistent with weaker synaptic transmission from PVBCs, since α1 subunit-containing $GABA_A$Rs are predominant at synapses from mature PVBCs [33, 41, 101, 102].

In addition to $GABA_A$R levels, the strength of the postsynaptic response to GABA depends on the driving force for the $GABA_A$R current which is determined by its reversal potential E_{GABA_A}. As E_{GABA_A} depends on chloride extrusion by KCC2 and chloride uptake by NKCC1 [43], a recent study examined mRNA expression for both chloride transporters in the cortex of subjects with schizophrenia [232]. Interestingly, KCC2 and NKCC1 transcript levels were not altered in subjects with schizophrenia; however, transcripts for two kinases (OXSR1 and WNK3) that strongly regulate KCC2 and NKCC1 activity in opposite directions, are overexpressed in schizophrenia [232]. If increased levels of OXSR1 and WNK3 mRNA actually represent increased kinase activity, then the chloride gradient across the postsynaptic membrane may be decreased, resulting in an E_{GABA_A} significantly more depolarized than normal [232]. Since normally E_{GABA_A} varies with cell type and subcellular compartment, understanding the consequences of alterations in chloride transport requires a detailed quantitative analysis of protein localization and activity, a challenging task in this case, given that postmortem interval effects alter the integrity of some of these proteins [232].

Direct demonstration that GABA-mediated synaptic inhibition is decreased in the cortex of subjects with schizophrenia is challenging. Interestingly, magnetic resonance spectroscopy (MRS) was recently applied to noninvasively measured GABA concentration in human neocortex and determined whether a decrease of GABA is observed in schizophrenia. MRS does not distinguish extracellular GABA from transmitter stored in particular cellular compartments or cell types and also lacks adequate temporal resolution but nevertheless reveals activity-dependent changes in GABA levels. For example, acute psychological stress which elevates subjective anxiety produces a short-term decrease in GABA concentration in human dorsolateral PFC that can be detected with MRS [233]. Combining MRS and EEG in the same subjects, the relations between brain GABA content and oscillatory neural activity in schizophrenia may be explored. Interestingly, in normal human subjects, GABA concentration measured in visual cortex with MRS was positively correlated with the strength of gamma oscillations induced by visual stimulation [234]. Moreover, interindividual variation in GABA concentration determined by MRS in visual cortex was correlated with variability of performance in a visual stimulus orientation discrimination task that induces gamma oscillations [235].

Measurements of tissue GABA concentration with MRS may help in clarifying the relations between GAD67 levels, gamma oscillations, and cognitive performance in schizophrenia. One such study did not detect differences in GABA concentration in the anterior cingulate cortex of schizophrenia versus control subjects, whereas GABA concentration was apparently decreased by antipsychotic medications [236]. Another MRS study reported reduced GABA concentration in basal ganglia but not frontal cortex of schizophrenia patients [237]. In patients with relatively low antipsychotic exposure, MRS revealed a significant reduction of GABA concentration in visual cortex that did not covary with medication dosage but was correlated with behavioral abnormalities in a visual surround-suppression task thought to depend on GABA-mediated inhibition [238]. Moreover, a longitudinal study of early-stage first-episode schizophrenia patients showed that 6 months of treatment with atypical antipsychotics did not change GABA concentrations measured with MRS in frontal and parietal lobe cortices nor in basal ganglia [239]. In contrast, MRS revealed elevated GABA concentration in anterior cingulate and parietal cortex of subjects with chronically treated schizophrenia compared to control subjects [240]. The MRS findings suggesting that antipsychotics may change brain GABA concentration highlight the importance of addressing the effects of confounding factors such as medications [241]. Significantly, both postmortem studies in humans and experimental studies in animals have failed to show an effect of antipsychotic medications on GAD67 mRNA levels [224, 242, 243]. The data from MRS studies therefore underscore the importance of combining neurochemical, electrophysiological, and behavioral assessment, given the large interindividual variability in bulk GABA concentration, gamma activity levels, and behavioral performance. Instead of or in addition to medication effects, the large variability in cortical GABA content measured with MRS in human cortex may be explained by the effects of genetic variants in the *GAD1* gene that may differentially confer risk of schizophrenia [244].

7. Conclusions

The findings reviewed here suggest that alterations of GABA transmission produce cognitive deficits in schizophrenia by altering the circuit mechanisms of gamma oscillations. These observations suggest a molecular and cellular basis for the development of new therapeutic interventions [245]. Importantly, the proposal that GABA alterations are linked to altered gamma oscillations and cognition is supported, at least in part, by animal model studies showing that producing a functional loss of GABA-mediated inhibition diminishes gamma oscillations [246] and impairs cognitive function [247, 248]. Whereas work in animal models is essential, the difficulty of capturing in animals the complexity of behavioral alterations in a uniquely human disorder may explain the relative lack of success in developing drugs to treat schizophrenia compared with other disease areas [4, 5, 245]. Interpretation of studies in human subjects based on comparisons between healthy controls and patients is complicated as well, given that schizophrenia versus control differences may actually reflect pathogenesis but also could represent compensatory changes or effects of confounding factors [4, 241]. For example, the effects of producing

a transient deficit in $GABA_AR$-mediated signaling were tested recently in human subjects [249] using iomazenil, a compound that binds to the benzodiazepine site of $GABA_ARs$ and negatively modulates the effects of GABA (i.e., an inverse agonist). Consistent with dysfunctional $GABA_AR$ signaling in schizophrenia, iomazenil produced perceptual deficits and psychotic symptoms in schizophrenia patients at doses that did not affect healthy control subjects [249]. However, the schizophrenia patients in such study chronically received antipsychotics and anxiolytics, raising the question of whether an interaction between acute iomazenil and chronic medications influenced such findings.

Preliminary tests of the idea that enhancing $GABA_AR$ signaling improves behavioral and electrophysiological measures in subjects with schizophrenia were conducted in two recent studies evaluating the effects of MK-0777, an $\alpha2/\alpha3$ $GABA_AR$-preferring positive allosteric modulator [47]. In one study, randomized administration of MK-0777 or placebo in double-blind fashion improved performance of schizophrenia patients in a cognitive control task, simultaneously increasing gamma oscillation power in frontal cortex [250]. In contrast, the second study did not find significant effects of MK-0777 compared with placebo in the performance of patients in a battery of tests designed to assess cognitive function in schizophrenia [251]. The inconsistent beneficial effects of MK-0777 administration might be explained by the fact that MK-0777 is a partial agonist at $\alpha2/\alpha3$ subunit-containing $GABA_ARs$ with only about 10–20% potency compared to a full agonist [48]. Thus, one possibility is that more potent $\alpha2/\alpha3$ benzodiazepine site agonists need to be employed. Such drugs should also be more selective because, compared with placebo, MK-0777 had a tendency to produce sedation and somnolence [250, 251], which could mask improvements in cognitive performance. Importantly, the $\alpha2/\alpha3$ $GABA_AR$ modulator MK-0777 was selected based on the compelling rationale that inputs from PVChCs onto the AIS show important alterations in schizophrenia [83] and that, depending on cortical layer, such inputs involve $\alpha2$-$GABA_ARs$ or $\alpha3$-$GABA_ARs$ [101, 201, 252]. However, whether or not PVChCs play a role in production of gamma band synchrony remains unclear [82], and so it is possible that PVChC alterations in schizophrenia produce cognitive deficits unrelated to dysfunctional gamma band synchrony. Therefore, further information from both basic and clinical research studies is necessary to further assess the effectiveness of $\alpha2/\alpha3$ $GABA_AR$ modulators for treatment of gamma synchrony-related cognitive deficits in schizophrenia. Indeed, basic research studies continue to provide insight into the role of specific subtypes of GABA neurons on inhibition-mediated cortical network oscillations [127], and molecular pharmacology studies are identifying novel compounds acting at different sites within the $GABA_AR$ receptor complex [44, 47, 253].

Importantly, a potential role of GABA-mediated neural synchrony in cortical circuits is to enable spike-timing-dependent plasticity, indirectly modifying the strength and stability of excitatory synaptic connections [254]. Whether spike-timing-dependent plasticity at glutamate synapses is impaired in schizophrenia is not yet clear [12], but it is possible that the decrease of dendritic spine density in pyramidal neurons in schizophrenia is due to glutamate synapse loss produced by altered plasticity mechanisms [241]. If neural synchrony-dependent glutamate synaptic plasticity is dysfunctional in schizophrenia, then cognitive enhancement behavioral therapies that involve learning paradigms may help in preventing or reversing the consequences of altered circuitry on the induction of synaptic plasticity. Interestingly, cognitive enhancement behavioral therapy was recently shown to improve cognition and prevent gray matter loss in schizophrenia [255]. Potentially, combining cognitive therapies with pharmacological treatment that boosts otherwise weakened neural synchrony may constitute an effective treatment intervention in schizophrenia, as for other psychiatric disorders [256].

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Review Article

Maturation of the GABAergic Transmission in Normal and Pathologic Motoneurons

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γ-aminobutyric acid (GABA) acting on Cl^--permeable ionotropic type A ($GABA_A$) receptors ($GABA_AR$) is the major inhibitory neurotransmitter in the adult central nervous system of vertebrates. In immature brain structures, GABA exerts depolarizing effects mostly contributing to the expression of spontaneous activities that are instructive for the construction of neural networks but GABA also acts as a potent trophic factor. In the present paper, we concentrate on brainstem and spinal motoneurons that are largely targeted by GABAergic interneurons, and we bring together data on the switch from excitatory to inhibitory effects of GABA, on the maturation of the GABAergic system and $GABA_AR$ subunits. We finally discuss the role of GABA and its $GABA_AR$ in immature hypoglossal motoneurons of the spastic (SPA) mouse, a model of human hyperekplexic syndrome.

1. Introduction

γ-aminobutyric acid (GABA) is, with glycine, the major inhibitory neurotransmitter in the adult central nervous system (CNS) of vertebrates. GABA acts on Cl^--permeable ion-otropic bicuculline-sensitive type A ($GABA_A$) receptors ($GABA_AR$) and metabotropic baclofen-sensitive $GABA_BR$, these latter being coupled through G-proteins to K^+ and Ca^{2+} channels in neuronal membranes. More recently, it has been shown that GABA also activates Cl^--permeable bicuculline- and baclofen-insensitive $GABA_CR$, this receptor subtype being largely expressed in the retina and at lower level in other CNS area [1]. If all GABA receptors are present on the cell membrane, the common view is that $GABA_BR$ are presynaptically located, whereas $GABA_AR$ and $GABA_CR$ are postsynaptically. However, all GABA receptors seem to be located pre- and/or post-synaptically [2–5].

GABA is synthesized from the amino acid glutamate by the enzyme glutamic acid decarboxylase (GAD), this latter being present as two isoforms with different molecular weights of 65-kDa and 67-kDa [6]. The two GAD isoforms are product of two different genes. GAD65 gene (GAD2) is located on chromosome 10 (10p11.23) in human and on chromosome 2 (2 9.0 cM) in mouse, while GAD67 gene (GAD1) is located on chromosome 2 (2q31) in human and in chromosome 2 (2 43.0 cM) in mouse [7, 8]. In addition, during mouse and rat embryonic development, two alternatively splices forms are also synthesized from the GAD67 gene: the truncated 25-kDA leader (GAD25) and the enzymatically active protein GAD44 (for review, see [9]). GAD25 is a protein without GAD enzymatic activity. GAD25 and GAD44 are expressed during the development of the CNS, they are more abundant in proliferating progenitors [9–11], and they are downregulated during neuronal differentiation

concomitant with an upregulation of GAD67 expression [12–14]. The 67-kDa GAD form is diffusely distributed in the cytoplasm of the cells, while the 65-kDa GAD form is mainly found attached to synaptic vesicles [15].

During CNS development, GABA exhibits a large panel of activity ranging from the control of cell proliferation to the formation of synapses (for review, see [16–19]). In immature brain structures, most studies described GABA as operating through $GABA_AR$ subclass [18, 20], and it was first proposed that the other GABAR subclasses were not functional at early stage of life [21]. However, this hypothesis was invalidated by the observation of a pre- and post-synaptic $GABA_BR$ expression in the embryonic rat neocortex [22] and the modulation of cortical neuronal migration by $GABA_BR$ activation [23–25]. $GABA_BR$ activation triggers BDNF release and promotes the maturation of GABAergic synapses [26]. Finally, it has been shown that GABA can control the locomotor network in the rat neonatal spinal cord by acting on presynaptic $GABA_BR$ as well as on postsynaptic $GABA_AR$ [27]. In the brainstem, it has been recently shown that the interaural time difference detection circuit is differentially controlled by $GABA_BR$ during the second postnatal week [28]. An endogenous modulation of respiratory rhythm by $GABA_BR$ that increases after birth has also been reported [29]. Finally, functional $GABA_CR$ were detected in the spinal motoneurons (MNs) around birth, but a little is known about the function of these receptors in the immature spinal cord [1].

$GABA_AR$-related effects on immature neuronal cells are opposed to those observed on mature neurons in the sense that GABA exerts depolarizing effects during development, while it induces hyperpolarizing effects in most mature CNS regions [30]. Such depolarizing GABA-mediated effects, coupled with conventional excitatory effect of glutamate and other classical neurotransmitters such as acetylcholine, lead to Ca^{2+} influx and generate spontaneous electrical activities that are the features of almost all immature structures of the CNS [31, 32]. Numerous studies have demonstrated the permissive role of depolarizing GABA in the maturation of neurite outgrowth [33], in promoting both excitatory and inhibitory synaptogenesis [34] and in controlling its switch from depolarizing to hyperpolarizing [35, 36].

Brainstem and spinal motoneurons that are largely targeted by GABAergic interneurons require an appropriate maturation of their GABA receptors and GABA innervations. In the present paper, we will describe the ontogeny of the GABAergic system in spinal MNs in parallel to the establishment of an inhibitory transmission, and then we will present data about the maturation of GABA receptors in hypoglossal motoneurons (HMs, motoneurons innervating the tongue) of the spastic (SPA) mouse, a model of human hyperekplexic syndrome in which the impaired glycinergic neurotransmission [37] may be compensated, in certain strain lines, by an increased aggregation of $GABA_AR$ [38, 39]. The hyperekplexic syndrome, as well as the amyotrophic lateral sclerosis (ALS) pathology, highlights the plasticity of the GABAergic system that may temporally compensate genetic alteration of other inhibitory systems [40, 41].

2. Maturation of Chloride-Mediated Inhibition in MNs

GABA, when binding to $GABA_AR$, exerts effects that are mainly dependent upon the chloride equilibrium potential (E_{Cl}). In mature neurons, the intracellular Cl^- concentration $[Cl^-]_i$ is lower than extracellular Cl^- concentration $[Cl^-]_o$ and the activation of the chloride permeable channels by GABA induces a chloride influx. However, in immature neurons that express a higher $[Cl^-]_i$ compared to $[Cl^-]_o$, GABA acts as an excitatory neurotransmitter. Hence, during CNS development, a switch from excitatory to inhibitory effects of GABA occurs. In the mouse pre-Bötzinger complex (PBC), a brainstem respiratory structure that drives the rhythmic activity of the hypoglossal motoneurons, gramicidin perforated patch-clamp recordings that preserve the physiological $[Cl^-]_i$ indicate that the reversal potential of $GABA_AR$-mediated current ($EGABA_AR$ that corresponds to E_{Cl}) switches from depolarizing to hyperpolarizing within the first postnatal (P) week ($EGABA_AR$ drops from -44 mV at P2 to -71 mV at P4) [42]. Because the resting membrane potential (rmp) for all PBC neurons was -56 mV, a switch from excitatory to inhibitory effects of GABA is evidenced between P2 and P4. Results obtained from gramicidin perforate-patched HMs are in good agreement with those collected in PBC neurons, because E_{Cl} is measured as being -37 mV in neonates HMs (P2) and -73 mV in juveniles HMs (P15), but the exact time of the switch remains undetermined between P2 and P15 (rmp of HMs is -70 mV) [43]. However, two other studies [44, 45] reported that by birth, GABA induces a hyperpolarization of the membrane potential in respiratory medullary neurons and a suppression of respiratory frequency. These studies, which are based on gramicidin perforated-patch clamp recordings, rather indicate that the transition from excitatory to inhibitory effects occurs at approximately E19 but not during post-natal stages in respiratory networks. From a technical point of view, measures of the $GABA_AR$-related driving force may be considered with caution because invasive recordings (including perforated patch-clamp) combined with large input resistances of immature neurons may lead to inexact resting membrane potential values, true resting membrane potential values being more hyperpolarized (see [46]).

When does the switch from excitatory to inhibitory effects of GABA occur in spinal MNs? We have showed that there is a shift of $EGABA_AR$ toward negative values during the embryonic development of mouse lumbar spinal MNs [47]. Our data demonstrated that until E15.5, E_{Cl} is above the spike threshold, whereas after E16.5, it drops significantly below spike threshold. During the course of the embryonic development, rmp of mouse spinal MNs remains below the E_{Cl}. However, if $GABA_AR$ activation may trigger the firing of MNs until E15.5, after this embryonic developmental stage, such activation, although producing a depolarization, fails to trigger action potentials [47] (Figure 1). Our results indicate that GABA likely exerts a shunting action on mouse spinal MNs after E15.5, as demonstrated in the neonate rat spinal cord [48] and also described in current-clamp

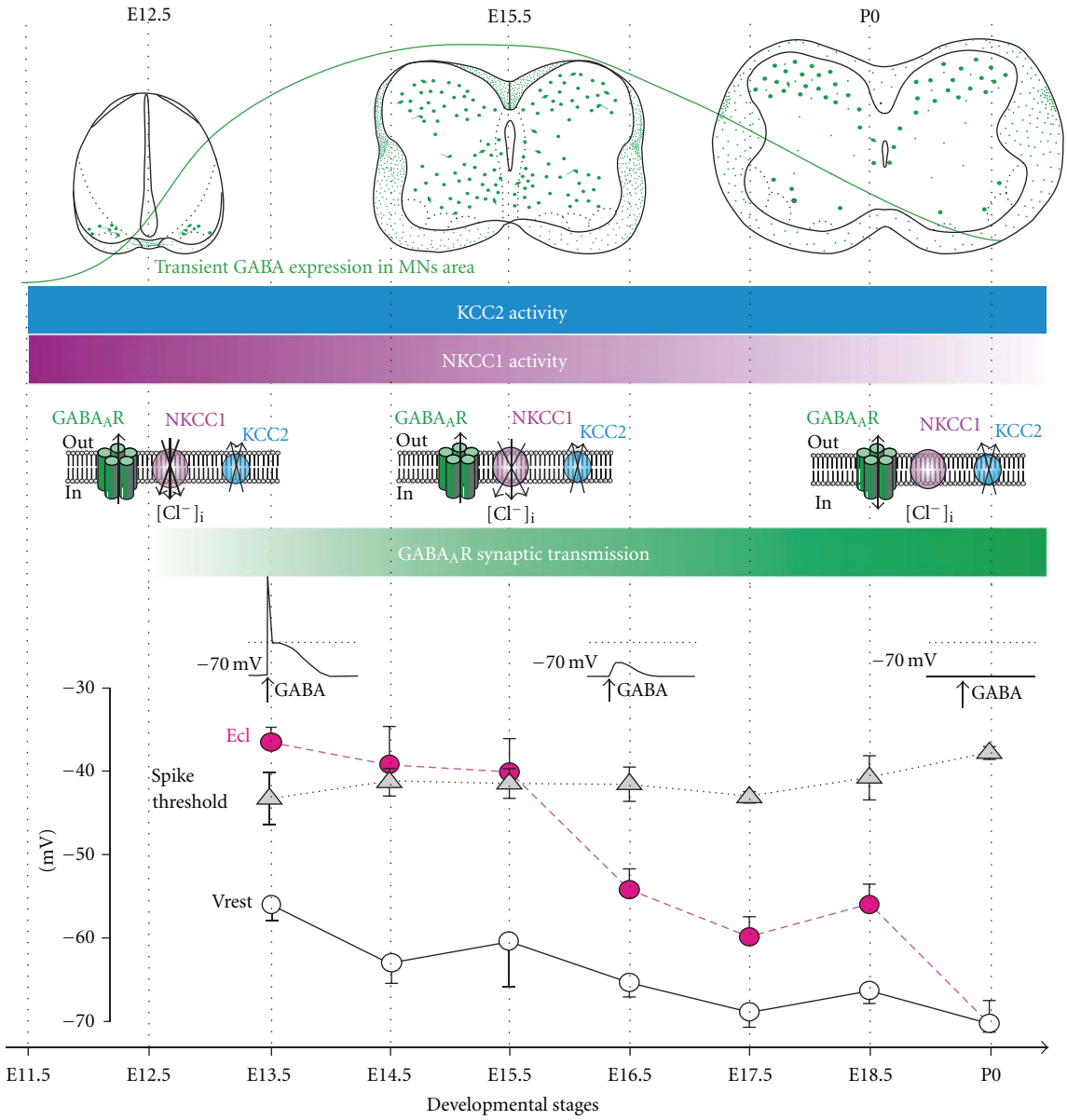

FIGURE 1: Development of the GABA$_A$R-mediated inhibitory transmission in mouse lumbar spinal MNs. From top to bottom: schematic drawings (frontal views) depict the transient expression of GABA in spinal ventral interneurons (in green), while horizontal bars indicate the permanent KCC2 (in blue) and transient NKCC1 activity (in violet). The color intensity encodes the level of activity. NKCC1 inactivation combined to KCC2 activity leads to a significant decrease in $[Cl^-]_i$ and a disappearance of GABA$_A$R-mediated excitatory effects. In parallel to the maturation of the chloride cotransporters KCC2 and NKCC1, the spinal cord starts to convey first synaptic activity at E12.5 that is GABAergic (green horizontal bar). Bottom: maturation of the chloride equilibrium potential (E_{Cl}), spike threshold and resting membrane potential (Vrest) across the embryonic stages of developmental. Note the drop of E_{Cl} at E16.5 that accounts for the "shunting" GABA$_A$R-mediated effect (modified from [47, 56, 66]).

experiments by Hubner and collaborators in E18.5 mouse spinal MNs [49]. This shunting depolarizing GABA effect likely persists during postnatal stages even though our experimental measurements indicate that E_{Cl} reaches MNs rmp at P0 [47]. A recent study based on conventional intracellular recordings clearly demonstrated that the shift from excitatory to inhibitory IPSPs occurs at P4-5 in rat spinal MNs [50]. This was in agreement with intracellular recordings performed by Wu and collaborators showing much smaller (but still) depolarizing effects of GABA at P0 compared to E16–E18 in rat spinal MNs [51]. Another study, based on gramicidin perforated patch-clamp recordings, indicates that EGABA$_A$R shifts between P5 and P10 in mouse spinal MNs, that is, at a later developmental stage compared to the rat [52]. Hence, further experiments would be needed to precisely determine whether the switch from excitatory to inhibitory effects of GABA really occurs in mouse spinal MNs, and it would be interesting to determine whether an

oxytocin-driven transient loss of chloride occurs at birth in spinal MNs as described in hippocampal neurons [53].

3. Transient Expression of GABA in Motoneuronal Region during the Embryonic Life

Analyzing the maturation of GABA effects in MNs implies that an endogenous GABAergic innervation is present. GABA effects are indeed often tested using local application of exogenous GABA or $GABA_AR$ agonist (i.e., muscimol or isoguvacine) [42, 47]. It is thus essential to examine the ontogeny of GABA and GABA receptors. The detailed mapping of the GABAergic system has been extensively described in the adult brainstem by *in situ* hybridization, immunohistochemistry using antibodies directed against GABA or the GAD protein, specifically the 67-kDa isoform (GAD67) [54] or by taking advantage of the GAD67-GFP knock-in mouse [55]. However, to our knowledge, the ontogeny of the GABAergic innervation of brainstem MNs has not been precisely mapped.

We have described the process of embryonic maturation of GABA immunostaining in the mouse spinal cord [56]. Our study indicated that GABA-ir somata are first detected at embryonic day 11.5 (E11.5), exclusively at brachial level, in the ventral horn. By E13.5, the number of GABAergic neurons sharply increases throughout the extent of the ventral horn both at brachial and lumbar level. At E15.5, stained perikarya decrease in number in the ventral gray matter, while GABA-ir fibers are detected contacting MNs. Such a transient expression of GABA immunoreactivity in the spinal ventral horn was also described in the developing rat [57, 58] and chick [59].

4. GABAergic Synaptic Activity: A Predominant Neurotransmission in MNs at Early Developmental Stages

From a functional point of view, GABA effects differ according to the developmental stage. At early stages, excitatory GABA effects contribute, with cholinergic inputs, to the genesis of spontaneous network activity in the chick [60], mouse [61, 62] and rat [63, 64] spinal cord. At these early stages, MNs are still growing to their peripheral targets and the GABA-mediated spontaneous activity is required for correct motor axon guidance [65]. We have recently showed that first synaptic activity occurs at E12.5 in mouse spinal MNs [66] when the GABAergic phenotype starts to be largely expressed by interneurons located in the ventral gray matter [56]. GABAergic synaptic activity then increases in frequency and coexists with a glycinergic synaptic transmission [66]. In most immature CNS regions, GABA signaling is established before glutamatergic transmission, suggesting that GABA is the principal excitatory transmitter during early development [30]. In the spinal cord, pharmacological approaches performed while recording spontaneous activity showed as well that GABA generates, with acetylcholine [67], the earliest spontaneous motor activity and then glutamate interfere [64]. Our analysis also revealed that the glutamatergic synaptic transmission mainly develops in the embryonic spinal cord after the GABAergic one at around E14.5 (personal observation). Hence, GABA appears as a sort of automated expressed first ubiquitous signal, and then and only then does the adult behavior resumes. Interestingly, it has been shown that the glutamatergic transmission regulates the strength of GABAergic synapses [68].

If the synaptic transmission develops during the embryonic life in spinal MNs, it maturates during postnatal stages and a developmental shift from primarily long-duration GABAergic mIPSCs to short-duration glycinergic mIPSCs occurs after birth in rat MNs [69].

At E15.5 in the rat, commissural GABAergic connections mediate synchronous excitatory action on rhythm-generating networks in the ventral spinal cord, while at E18.5, these GABAergic commissural connections are responsible for reciprocal inhibition during left and right alternation [70]. Interestingly, at E20.5 in rat embryo, these inhibitory commissural inputs become mediated by glycine and not anymore by GABA [70]. These results that take over the primordial role of $GABA_AR$ for ensuring spontaneous activity and then reciprocal inhibition between left and right sides of the ventral spinal cord may explain why such a huge expression of GABA is detected in ventral spinal networks at E15.5, in the mouse [56]. At postnatal developmental stages, when commissural connections are mostly mediated by glycine [71–74], GABAergic inhibition has been shown to regulate the onset and duration of neurochemically induced locomotor activity [75].

5. Ontogeny of KCC2 and NKCC1 Immunoreactivity

The switch from excitatory to inhibitory $GABA_AR$-related effects is closely related to the lowering of $[Cl^-]_i$ during the course of the development. This latter mainly relies on the differential ontogenic expression of the $Na^+/K^+/2Cl^-$ cotransporter isoform 1 (NKCC1), which uptakes chloride ions [76–78], and the neuronal K^+/Cl^-cotransporter type 2 (KCC2) [79], which extrudes chloride ions [49, 80]. However, other exchangers can control the chloride gradient as the anion (Cl^-–HCO_3^-) exchangers, either Na^+- independent (AE) or Na^+-driven (NDCBE also called NDAE) [81] (NCBE) [82]. AE mediates influx of Cl^- while exporting HCO_3^-, these exchanges being triggered by intracellular alkalinisation. NDCBE, known as an acid extruder (extrudes H^+), moves Cl^- out in exchange of HCO_3^-, driven by the Na^+ gradient [83, 84]. NCBE also lowers $[Cl^-]_i$ (and $[H^+]_i$) while importing Na^+ and HCO_3^- [82, 85].

It is generally accepted that early in development, NKCC1 is predominant and, therefore, maintains a high $[Cl^-]_i$, while at later stages, NKCC1 vanishes, and KCC2 develops, lowering intracellular chloride levels [86–88]. In spinal cord MNs, it was shown that the expression of KCC2 transcripts parallels neuronal differentiation during the embryonic life and preceded the decline of the $GABA_AR$ reversal potential ($EGABA_AR$) [52]. Thus, the relationship between KCC2,

NKCC1, and EGABA$_A$R during the course of the embryonic development remained an open question. We addressed this question in a previous study [47] and found that KCC2 immunoreactivity (KCC2-ir) can be detected in MNs area as early as E11.5, confirming the Stein's study [52], when NKCC1 is also largely expressed. At E14.5, KCC2 is largely present in the ventral gray matter and at later stages this protein keeps stable. At E11.5, a dense NKCC1 labelling is detected throughout the ventral grey matter. Thus, our data indicated that the main drop of E_{Cl} occurring at E16.5 is likely dependant on a reduction of the NKCC1 efficacy rather than a later expression of KCC2. In the rat, Stil and coworkers investigated the expression of KCC2 and NKCC1 in the ventral horn of the spinal cord from E17 to P20 and found that the expression of KCC2 increases significantly, while the expression of NKCC1 decreases during postnatal life when the shift from depolarizing to hyperpolarizing IPSPs occurs (at P4-P5) [50].

It must be mentioned that analyzing the shift from depolarizing to hyperpolarizing effects of GABA in spinal MNs by taking into account only KCC2 and NKCC1 may be simplistic, because the anion exchangers AE has been clearly demonstrated as accumulating chloride in immature chick MNs [89]. Hence, the expression of inhibitory GABA effects likely also relies on the reduction of AE in addition to NKCC1. Also, NCBE that is expressed as early as E14.5 in the mouse SC [90] may play an important role in lowering $[Cl^-]_i$.

On the whole, even though likely oversimplified, Figure 1, that is based on our data, illustrates the ontogeny of the GABAergic inhibitory synaptic transmission in parallel to the activity of the two main cotransporters KCC2 and NKCC1. It must be noted that the transient maximum expression of GABA in ventral motor network precedes the drop of E_{Cl}.

6. Ontogenic Changes of the GABAergic Receptors in MNs

GABA$_A$R and GABA$_C$R as glycine, nicotinic acetylcholine, and 5-hydroxytryptamine type 3 receptors belong to the cystein-loop receptor family. They are both pentameric assemblies of subunits, each subunits being characterized by extracellular N and C terminals and by four transmembrane domains (TM1–TM4), the domain TM2 forming the anionic channel pore [91]. GABA$_A$Rs are composed of a large variety of different subunits, sixteen GABA$_A$Rs subunits being cloned so far (α1–6, β1–3, γ1–3, δ, ε, θ, and π) and three (ρ1–3) for GABA$_C$R [92, 93]. The number of GABA$_A$R subunits is also theoretically increased by alternative splicing but only a dozen of subunit combinations have been detected so far [93]. The agonist binding site is carried mainly by α subunits, while γ subunits are responsible for linking GABA$_A$Rs to the postsynaptic cytoskeleton. The most abundantly expressed GABA$_A$R in the adult CNS has a stoichiometry of 2α, 2β, and 1γ2 subunit. In addition GABA$_A$R subunit combination varies according to the synaptic and extrasynaptic location of this receptor. For example, GABA$_A$Rs containing the δ subunit or the α5 subunit cannot accumulate at postsynaptic site, likely because they cannot anchor to postsynaptic scaffold protein complex [93–95]. Remarkably, the extrasynaptic GABA$_A$Rs containing the δ subunit ($\alpha\beta\delta$ GABA$_A$R) have a higher apparent affinity for GABA and desensitize more slowly and less extensively than postsynaptic GABA$_A$Rs containing the β and/or the γ subunits [96], while GABA$_A$Rs containing the α5 subunit display a reduction in their desensitization kinetics when compared with receptors containing other α subunits [97].

In the adult lumbar rat spinal cord, only α2, α3, β3, and γ2 mRNAs are expressed at significant levels, the α3, β3 and γ2 transcripts being present in many neurons throughout the Rexed laminae, whereas the α2 mRNA is restricted to motor neurons and adjacent cells [98]. A high expression level of the α1 and the α2 subunits is detected using immunohistochemistry in the adult rat oculomotor trochlear nuclei, the hypoglossal nucleus, and the dorsal nucleus of the vagus [99]. Interestingly, the motor trigeminal nucleus mainly expresses the α2 subunits, while α5 and β2/3 are poorly present in these CNS areas and the δ subunit is undetectable [99]. A recent immunohistochemical study, performed in human brainstem and cervical spinal cord, shows roughly similar results [100]. In this study, Waldvogel et al. did not analyze the expression of α4–α6 subunits and δ subunits, but they showed that α1, α2, α3, β2/3, and γ2 GABA$_A$R subunits are largely detected in the brainstem motoneuron nuclei and in the lamina IX as well as, in less extend, in the lamina X of the cervical spinal cord [100]. However, their data, collected from human brain, differ from Fritschy's group results obtained from rat tissue. Indeed, Waldvogel et al. find a high expression of α1, α2, α3, and β2/3 subunits in the motor trigeminal nucleus, while the γ2 subunit was poorly expressed [100]. This could reflect differences in GABA$_A$R subunit expression between species. However, because these two studies are based on a semi quantitative analysis of immunostaining, at a macroscopic level, discrepancies must be taken with caution. Effectively, it is well known that immunostaining, particularly for GABA$_A$R subunits, can strongly vary depending on the fixation procedure [101, 102].

From a developmental point of view, little is known about changes in GABA$_A$R subunit expression during spinal cord MNs development. In the rat cervical spinal cord, the α6 and δ subunits mRNAs are not detectable at all ages tested (from E12 to adult). During the ontogeny, as demonstrated for GABA [56, 57], subunits mRNA expression emerges along a ventrodorsal gradient. In fact, α2, α3, α5, β2, β3, γ2, and γ3 subunits emerge in presumptive MNs at E12–E13 and then can be detected in more dorsal regions [103]. A synchronized peak of α2, α3, β2, β3, γ2, and γ3 subunits mRNAs is detected at neonatal stages. In the adult rat cervical spinal cord, GABA$_A$R α1, α4, α5, β1-2, γ1, and γ3 subunit mRNAs are found only in relatively few cells scattered in the gray matter, whereas mature MNs exhibit α2β3γ2 transcripts [103]. Thus, contrary to that observed for glycine receptors [104], there is no obvious switch in GABA subunit expression during prenatal and postnatal development of MNs. Interestingly, the α3 mRNA level observed at early

developmental stage in the lateral motor column decreases around birth and was no longer detected in the adult [103]. In the hypoglossal nuclei, indirect proofs based on immunochemistry favor a switch from $\alpha 1$ to $\alpha 2$ subunits, during prenatal development [105]. As mentioned above, the $\alpha 1$ and $\alpha 2$ $GABA_AR$ subunits, together with the $\gamma 2$ $GABA_AR$ subunit, are the main $GABA_AR$ subunits expressed in the hypoglossal nucleus of the adult rat [99]. Assuming that $\gamma 2$ $GABA_AR$ clusters that do not colocalize with $\alpha 1$ $GABA_AR$ clusters reflect the presence of $GABA_AR$ containing $\alpha 2$ subunits, Muller and collaborators concluded for an increase in the proportion of $GABA_AR$ containing $\alpha 2$ $GABA_AR$ subunits [105]. However, this is in apparent contradiction to other studies showing that the $\alpha 2$ $GABA_AR$ subunits are expressed early in development and are progressively replaced by $\alpha 1$ $GABA_AR$ subunit in many brain areas [106]. A further quantitative immunohistochemical analysis of the developmental changes in the proportion of $\alpha 2$ and $\alpha 1$ $GABA_AR$ subunits in the hypoglossal nucleus is thus required in order to verify that developmental maturation processes of $GABA_ARs$ can vary between CNS areas.

If it is now clearly demonstrated that $GABA_AR$ subunits may evolve during development and vary according to brain areas, few data are available concerning the cellular location of these subunits on a single MNs. Using immunocytochemistry and confocal microscopy, Lorenzo et al. compared the subcellular patterns of expression of the main $GABA_AR$ subunits ($GABA_AR$ $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$) in the somatic versus dendritic compartments of rat abducens MNs [107] and revealed a differential organization of $GABA_AR$ subunits. They found that MNs somata contain only $GABA_AR$ $\alpha 1$, while both $GABA_AR$ $\alpha 1$ and $GABA_AR$ $\alpha 3$ are detected on dendrites [107].

7. Maturation of the GABAergic System on Motoneuron in Normal and Pathological Conditions: Mixed GABA/glycine Synapses and Mismatch between Pre- and Postsynaptic Elements

During the first 3 weeks of rodent postnatal development, inhibitory synaptic transmission changes in multiple ways that differ depending on brain areas. Electrophysiology and immunocytochemistry suggest that the respective contribution of the glycinergic and GABAergic transmission to the overall inhibitory message received by postsynaptic neurons may vary during the developmental period. For example, a developmental switch from a predominant GABAergic to main glycinergic neurotransmission occurs in the lumbar spinal cord [69] and in the lateral superior olive of young rodents [108, 109], while GABAergic neurotransmission dominates in developing collicular neurons [110] (Figure 2(a)).

As first demonstrated in neonatal spinal MNs, glycine and GABA can be coreleased from the same presynaptic vesicle resulting in a mixed glycinergic/GABAergic synaptic event [111]. Mixed inhibitory synapses have also been functionally identified in MNs of the hypoglossal nucleus [112, 113], but mixed synapses are not particular to inhibitory input on MNs, because they are also described on spinal interneurons [114, 115]. If mixed inhibitory synapses appear to reflect an intermediate stage of maturation of glycinergic synapses, it must be noted that although the proportion of mixed synapses decreases during development in Renshaw cells and other spinal cord interneurons [116], mixed inhibitory synapses remain functional in the adult [114, 116]. This is also the case in abducens MNs during rat postnatal development: before birth, only GABAergic axon terminals develop, whereas mixed GABA/glycine axon terminals appear at birth, and their number increases during the first postnatal week [117].

Functional mixed inhibitory synapses have also been described in rat HMs [112, 113]. However, a complete morphofunctional study of the development of inhibitory synapse on the mouse HMs, between P3–P5 and P15, revealed that the developmental shift from glycinergic/GABAergic to pure glycinergic neurotransmission depends mainly on the maturation of the presynaptic elements, while postsynaptic GlyRs and $GABA_ARs$ remain associated at the same postsynaptic density at all age tested. Effectively, although miniature inhibitory postsynaptic currents (mIPSCs) are mainly glycinergic and mixed glycinergic/GABAergic at P3–P5 and then predominantly glycinergic at P15 (Figures 2(b) and 2(c)), postsynaptic GlyRs and $GABA_ARs$ remain associated at the same postsynaptic density at all age tested [118]. In addition, because many GABAergic synapses are unlikely to contain postsynaptic $GABA_ARs$ yet, it was supposed that they represent newly formed "nonfunctional" GABAergic synaptic contacts, as previously observed in the cerebellum [119, 120]. It is, however, unclear whether such a discrepancy between the pre- and the postsynaptic element also occurs in other CNS area during development, but it must be noted that a similar maturation process of the inhibitory presynaptic terminals was also observed in neurons of the rat lateral superior olive [109]. Moreover, postsynaptic $GABA_ARs$ facing presynaptic terminals that do not release GABA have also been reported in the spinal cord and brain neuropil in culture [121–125]. Such a mismatch between the pre- and the postsynaptic element of inhibitory synapses was also observed in the adult Renshaw cells of the rat spinal cord [114]. In that case, it was proposed that GABAergic presynaptic terminals could face postsynaptic GlyR clusters [114]. Altogether, these data suggest that the maturation of inhibitory synapses rather results from a differential regulation of the GlyT2 and GAD65 expression at the level of a single synaptic terminal but not from a redistribution of GlyRs and $GABA_ARs$ at postsynaptic site.

Our data from the hypoglossal nucleus also suggest that pre- and postsynaptic elements mature independently [118]. However, a more recent study performed on spastic (SPA) mice, a model for hyperekplexia, argues against this hypothesis [126]. SPA mice display an insertion of an LINE-1 transposable element into the gene coding for the GlyR β subunit, which results in a truncated protein that impairs accumulation of GlyRs at postsynaptic sites and leads to a strong dysfunction of glycinergic synaptic transmission [127, 128]. In C57BL/6J strain, SPA mice which express a lower amount of GlyR β subunits die 2-3 weeks after birth

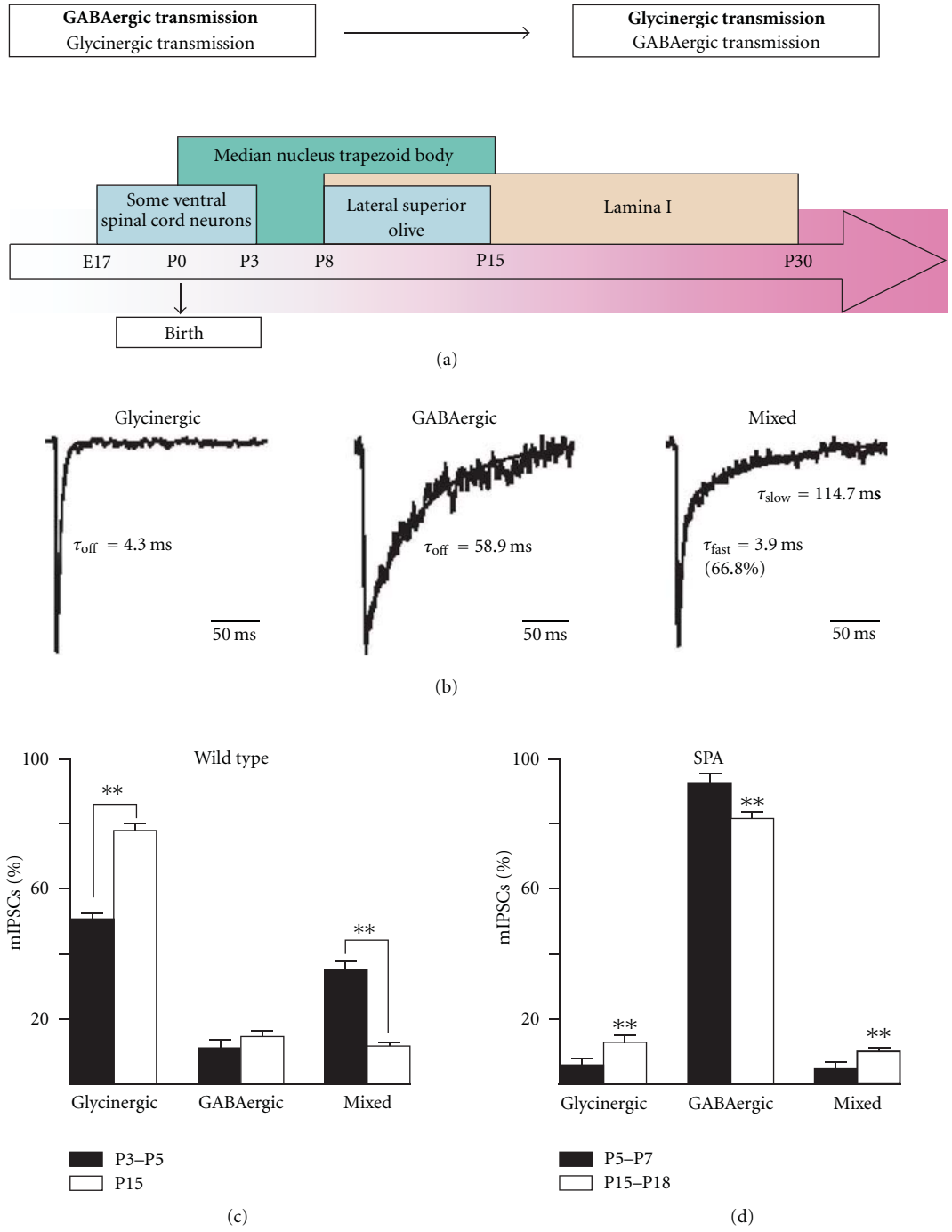

Figure 2: (a) Developmental changes in the proportions of GABAergic and glycinergic synaptic activity in various areas of the central nervous system. (b) Examples of individual glycinergic (left) GABAergic (middle) and mixed (right) miniature inhibitory postsynaptic currents (mIPSCs) recorded in a hypoglossal motoneuron at P15, in the presence of tetrodotoxin (a blocker of voltage-gated sodium channels). Note the slower decay phase of the GABAergic mIPSC compared to the glycinergic mIPSC. Decay phase of GABAergic and glycinergic events is better fitted with a single exponential function, while a double exponential function is required to fit the decay phase of mixed events. (c) Relative proportions of glycinergic, GABAergic and mixed mIPSCs at P3–P5 (black bars) and at P15 (white bars) in wild-type mice. (d) Relative proportions of glycinergic, GABAergic and mixed miniature postsynaptic events at P5–P7 (black bars) and at P15–P18 (white bars) in SPA mice. (Adapted from [118, 126]).

[129], suggesting that GABAergic compensation does not necessarily take place. It was first hypothesized that the progressive postnatal developmental lost of GABAergic presynaptic terminals that normally occurs in wild-type mice due to a switch to glycinergic terminals [118] could explain the progressive impairment of inhibitory synaptic activity and thus the lethality of this mutation. But surprisingly, in opposition to our observations made in wild-type animal, the

inhibitory synaptic activity is mainly GABAergic in SPA mice (Figure 2(d)): a developmental decrease in glycinergic presynaptic terminals occurs, while the density of GABAergic presynaptic terminals increases [126]. In addition, the proportion of inhibitory presynaptic terminals facing $GABA_A$Rs significantly increases during postnatal development in HMs of SPA mice. It must, however, be noted that many GABAergic synaptic boutons face diffuse $GABA_A$Rs staining, which contrasts to the situation observed in wild-type animal which most of the presynaptic terminals face aggregated $GABA_A$Rs. It is, thus, likely that GABAergic synapses are less efficient in SPA mice than in wild type [126]. Also, because SPA mice cannot survive, these results indicate that GABAergic neurotransmission does not compensate for defects in GlyR postsynaptic aggregation in this hyperekplexia model. They also suggest, contrary to that previously hypothesized [118], that a crosstalk exists between postsynaptic and presynaptic elements, leading to the developmental regulation of the presynaptic terminal neurotransmitter content that could be related to a downregulation of GlyT2 expression and an upregulation of GAD65 expression at inhibitory presynaptic terminals depending on the level of postsynaptic GlyR aggregation.

Alteration of $GABA_A$R and GlyR expression was also analyzed in MNs vulnerable and resistant to amyotrophic lateral sclerosis (ALS) [41]. Because a reduced level of expression of the $GABA_A$R α1 subunit mRNA has been shown in neurons of the motor cortex of patients with ALS [130], Lorenzo et al. investigated, using a quantitative immunohistochemical study, the possibility that $GABA_A$R and GlyR might be expressed differentially in ALS-vulnerable and ALS-resistant brainstem MNs in an ALS rat model [41]. Indeed, MNs controlling eye movements and bladder contraction are surprisingly unaffected (they are ALS-resistant) during terminal stages of ALS, while other MNs underlie an invariably fatal degeneration (they are ALS-vulnerable) [131]. Their main hypothesis was a reduction in $GABA_A$R and GlyR expression in vulnerable MNs, which could account for an alteration of the inhibition and hence for an amplification of the glutamatergic synaptic activity onto these MNs, an excessive excitatory transmission being known to be detrimental. Interestingly, Lorenzo et al. showed a differential expression of $GABA_A$R (and GlyR) in brainstem ALS-resistant oculomotor (III), trochlear (IV), abducens (VI) versus ALS-vulnerable MNs trigeminal (V), facial (VII), hypoglossal (XII) [41]. They demonstrated that $GABA_A$R in ALS-vulnerable MNs mostly express α2 subunits while $GABA_A$R in ALS-resistant MNs are α1 subunits enriched. They also showed that ALS-resistant MNs contain a larger proportion of extrasynaptic $GABA_A$R clusters than ALS-vulnerable MNs. Because extrasynaptic $GABA_A$R are activated by GABA spillover from synapses [132–134] and mediate a tonic inhibition that plays a crucial role in regulating neuronal excitability [135], the authors hypothesized that the presence of extrasynaptic $GABA_A$R in ALS-resistant MNs could protect these neurons from excessive depolarization by abnormal glutamate release. Their data demonstrated that the rate of occurrence of extrasynaptic $GABA_A$R clusters was approximately twice as high in ALS-resistant as in ALS-vulnerable MNs, but more experiments are necessary to determine to what extend this difference accounts for the vulnerability of MNs, as for example by manipulating extrasynaptic $GABA_A$R expression in specific MNs. On the contrary, recent reports show that glycinergic innervation but not GABAergic innervation of spinal MNs is deficient in the ALS mouse model expressing the mutant form of human superoxide dismutase-1 with G93A substitution ($SOD1^{G93A}$) [136, 137]. The authors examined, using whole-cell patch-clamp recordings, GlyR-mediated currents in cultured spinal MNs from this ALS mouse model. They found that glycine-evoked current density was significantly smaller in the SOD1 MNs compared to control. However, they did not find any change in GABAergic synaptic activity. This alteration in glycinergic synaptic activity is likely to be due to a lower GlyRα1 subunit mRNA expression in $SOD1^{G93A}$ MNs [137]. These results suggest that a selective alteration in GlyR expression can partly account for an alteration of inhibitory synapse efficacy in MNs early in the disease process of ALS, with $SOD1^{G93A}$ substitution at least. But these data obtained from GlyR expression in this ALS mouse model do not demonstrate, as data regarding $GABA_A$R expression, that a reduction of receptor subunit expression can effectively account for MNs vulnerability in ALS. Again, more experiment is necessary to resolve this issue.

Finally, these results on $GABA_A$R or GlyRs expression in ALS could be complementary rather than contradictory if one supposes that the expression of the different GlyR and $GABA_A$R subunits can be region specific. For example, $GABA_A$R α1 subunit is poorly expressed in the spinal cord compared to more central region [103], and it is important to note that glycinergic and GABAergic synapses control MNs development in a region-specific manner during programmed cell death as exemplified by data obtained in gephyrin-deficient mice that lack all postsynaptic GlyRs and some $GABA_A$R clusters [138]. In these gephyrin-deficient mice, there is a reduced respiratory MN survival and decreased innervation of the diaphragm, whereas limb-innervating MNs show increased survival and increased innervation of their target muscles [138].

8. Concluding Remarks

If GABAergic interneurons constitute only 17%–20% of the neurons in the brain [139], their primordial role in the maintenance of a good balance in neuronal connections is obvious. $GABA_A$R activation is likely to play an important role on spinal cord and brainstem MNs development as well as during pathological conditions, but it is unclear to what extend such a diversity leading to functionally different $GABA_A$Rs is important for a proper development of functional locomotor networks and to what extend a defect in a subunit expression can impact neuronal survival during development and in pathological condition as in ALS. For example, it will be of interest to determine to what extend the expression of α2 $GABA_A$Rs instead of α1 is important for neuronal development. This can be done using genetic tools as the knock in technique by substituting α2 expression

by α1. Another unknown mechanism that must be determined is the communication pathway between GABAergic/glycinergic pre-synaptic neurons and post-synaptic receptors. Thus, it would be worthy to examine changes in the presynaptic GABAergic and/or glycinergic phenotype, during development or in pathological conditions, when a post-synaptic receptor type is missing or altered.

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Review Article

Altered GABA Signaling in Early Life Epilepsies

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The incidence of seizures is particularly high in the early ages of life. The immaturity of inhibitory systems, such as GABA, during normal brain development and its further dysregulation under pathological conditions that predispose to seizures have been speculated to play a major role in facilitating seizures. Seizures can further impair or disrupt $GABA_A$ signaling by reshuffling the subunit composition of its receptors or causing aberrant reappearance of depolarizing or hyperpolarizing $GABA_A$ receptor currents. Such effects may not result in epileptogenesis as frequently as they do in adults. Given the central role of $GABA_A$ signaling in brain function and development, perturbation of its physiological role may interfere with neuronal morphology, differentiation, and connectivity, manifesting as cognitive or neurodevelopmental deficits. The current GABAergic antiepileptic drugs, while often effective for adults, are not always capable of stopping seizures and preventing their sequelae in neonates. Recent studies have explored the therapeutic potential of chloride cotransporter inhibitors, such as bumetanide, as adjunctive therapies of neonatal seizures. However, more needs to be known so as to develop therapies capable of stopping seizures while preserving the age- and sex-appropriate development of the brain.

1. Introduction

Epilepsy is a disease of recurrent seizures: that is, unprovoked episodes of aberrant synchronous excitation of brain regions that disrupt normal functioning [1, 2]. Epileptic seizures are thought to reflect a failure in the ability to maintain the balance between excitation and inhibition. The mechanisms underlying seizures are complex and not uniform across the numerous seizure types that exist [1]. Furthermore, our ability to study these mechanisms is often limited by the tools we can use: we can only see as far and as much as those tools allow. Consequently, many of the hypotheses describing the pathogenesis of seizures are biased by the dominant ictal phenomena, unbalanced excitation-inhibition and aberrant neuronal synchronization, which may not necessarily be the actual ictogenic mechanisms. Neurotransmitters involved in neuronal inhibition, such as GABA, have attracted the major focus of research aiming to decipher mechanisms involved in ictogenesis. Under certain conditions, and definitely not in the majority of cases, seizures may lead to epilepsy or neurodevelopmental deficits. The early periods of life, when brain development is still incomplete, susceptibility to seizures is increased [3, 4]. However, a combination of biological factors (genetic, age-related processes, epigenetic or environmental factors) protect neurons from seizure-induced injury, epileptogenesis, or mortality to a greater extent than the adult brain is protected [5]. It is increasingly recognized that seizures may leave their imprint on the developing brain by altering the way that neurons differentiate, connect, and communicate to each other, even if, in many cases, such changes may be ultimately compensated for. As extensively outlined in the reviews included within this special issue, GABA plays a central role in controlling neuronal development and communications. A major focus of research has therefore been thrown into efforts to elucidate its role not only in ictogenesis but also in the pathogenesis of the sequelae of early life seizures, whether this may be epilepsy, cognitive, or behavioral deficits [6].

There are three types of GABA receptors reported in the literature: $GABA_A$, $GABA_B$, and $GABA_C$, the latter classified more recently along with $GABA_A$ receptors, due to their functional similarities. Both $GABA_A$ and $GABA_C$ receptors

are ligand-gated ionotropic channels that allow primarily chloride but also bicarbonate to cross their pore in response to GABA binding. $GABA_B$ is a metabotropic receptor that signals through cascades that modify potassium and calcium current (reviewed in [7]), direct migration [8], and control gene transcription [9, 10]. In this review, we will focus primarily on $GABA_A$ receptors.

$GABA_A$ receptors are pentameric channels usually comprised of $2\,\alpha$ and $2\,\beta$ subunits, whereas the fifth is either a γ or a δ subunit. Less frequently, ε, θ, or π subunits are present [11–13]. There are 16 known mammalian $GABA_A$ receptor subunits ($\alpha 1-\alpha 6, \beta 1-\beta 3, \gamma 1-\gamma 3, \delta, \varepsilon, \theta, \pi$), which contribute towards the different pharmacokinetic, subcellular localization or affinity properties of each $GABA_A$ receptor complex. The presence of a ρ subunit defines the $GABA_C$ receptors. Unlike $GABA_A$ receptors, $GABA_C$ are insensitive to bicuculline. The expression of $GABA_A$ receptor subunits changes with development and as a result the responsiveness of immature and adult neurons to $GABA_A$ ergic modulators are significantly different.

The classical inhibitory $GABA_A$ signaling, as occurs in most adult neurons, is due to chloride influx through the channel pore, which hyperpolarizes the cells. This is achieved because the intracellular chloride concentration is maintained at a low level, allowing chloride to flow in along its electrochemical gradient, when $GABA_A$ receptors open (Figure 1). Multiple studies over the last few decades have confirmed that this electrochemical chloride gradient is developmentally regulated by changes in the expression of cation-chloride cotransporters (CCCs). CCCs are the electroneutral ion symporters that establish the chloride gradient between cells and their extracellular environment. There are 3 CCC classes. The chloride importing CCCs are either the sodium/potassium/chloride cotransporters (NKCCs), with known representatives the NKCC1 and NKCC2, or the sodium chloride cotransporters (NCCs). Chloride exporters are the potassium/chloride cotransporters (KCCs), with 4 known isoforms: KCC1-4 (reviewed in [11, 12, 14, 15]) (Figure 1). Immature neurons express predominantly chloride-importers, such as NKCC1 [16], which generate high intracellular Cl^- levels. This forces the open $GABA_A$ receptors to permit Cl^- efflux through their channel pore, giving rise to depolarizing $GABA_A$ responses [16–18]. During developmental maturation, the expression of chloride-extruding CCCs, like the potassium/chloride cotransporter 2 (KCC2), dominates over NKCCs [19–22], decreasing the intracellular chloride concentration [23]. As a result, when GABA opens $GABA_A$ receptors the ensuing influx of chloride results in hyperpolarizing currents [19] (Figure 1). However, cell type, sex, and species/strain differences occur in the timing of this developmental shift. KCC1, KCC3 and KCC4 are widely expressed, but KCC2 is specific to neurons. This makes KCC2 particularly interesting for the pathogenesis and therapy of neural diseases. NKCC2 expression is specific to the kidney, leaving NKCC1 as the most relevant chloride-importing cotransporter for the brain, though it is expressed ubiquitously. Bicarbonate, generated by carbonic anhydrase, is another negatively charged ion that can permeate the $GABA_A$ receptor, generating a depolarizing response [12, 24, 25]. The cytosolic carbonic anhydrase VII (CAVII) increases around postnatal day 12 (PN12) in the rat hippocampus [26], rendering bicarbonate-mediated $GABA_A$ depolarizations more prominent [25].

There is considerable evidence that alterations in GABA signaling can cause seizures, as well as that seizures can change GABAergic signaling. In this review, we will discuss the bidirectional relationship of seizures to $GABA_A$ signaling at the level of the neurons, $GABA_A$ receptors, and the ionic symporters that control chloride homeostasis and the efficiency of $GABA_A$ receptor mediated inhibition.

2. Correspondence of Developmental Stages between Rodents and Humans

To facilitate the translation of the experimental data into humans, it is worth reminding that the accepted correspondence of developmental stages between rodents and humans considers that the first week of life in rodents is equivalent to a premature newborn human, whereas the time of birth in rodents is considered to correspond to PN8-10. The rodent infantile stage is thought to extend till PN21, the onset of puberty is at PN32-35 in rodents, whereas PN60 rodents are considered young adults. However, it is important to emphasize that this is a very oversimplified translation, based mostly on correspondence of protein and DNA content in the brain. Each developmental process occurs at different tempos and is not always in synchrony with the above sequence of events. For example, by the end of the first postnatal week, rats are able to walk away from the nest, quite unlike the human newborns who cannot yet ambulate [27]. Direct demonstration of the time of shift of $GABA_A$ receptor responses to hyperpolarizing has not been demonstrated in humans, though it has been suggested to occur before or soon after birth, based on the developmental patterns of the relative expression of NKCC1 and KCC2 [21, 28].

3. The Immaturity of $GABA_A$ergic Systems as an Age and Sex-Specific Risk Factor for Early Life Seizures

Seizures are more common in the early periods of life and especially in males [3, 4]. The immaturity of GABAergic inhibitory systems has been implicated in the heightened susceptibility of neonates to seizures and may also underlie the increased vulnerability of males, in whom the maturation of these systems is delayed compared to females. GABA is depolarizing in the neonatal life and it stays depolarizing for longer developmental periods in the male brain than in females [17, 29–33]. Paradoxical exacerbation of seizures by GABA-acting drugs has been reported in newborns, especially in low weight premature babies [34]. GABA-acting drugs, such as benzodiazepines and barbiturates, however, still remain the mainstay of treatments for neonatal seizures, even if they may not always be as effective in newborn human babies as in older patients [21, 35–39]. This is thought to be due to shunting inhibition or inhibition via excitatory effects upon inhibitory interneurons [40]. The composition

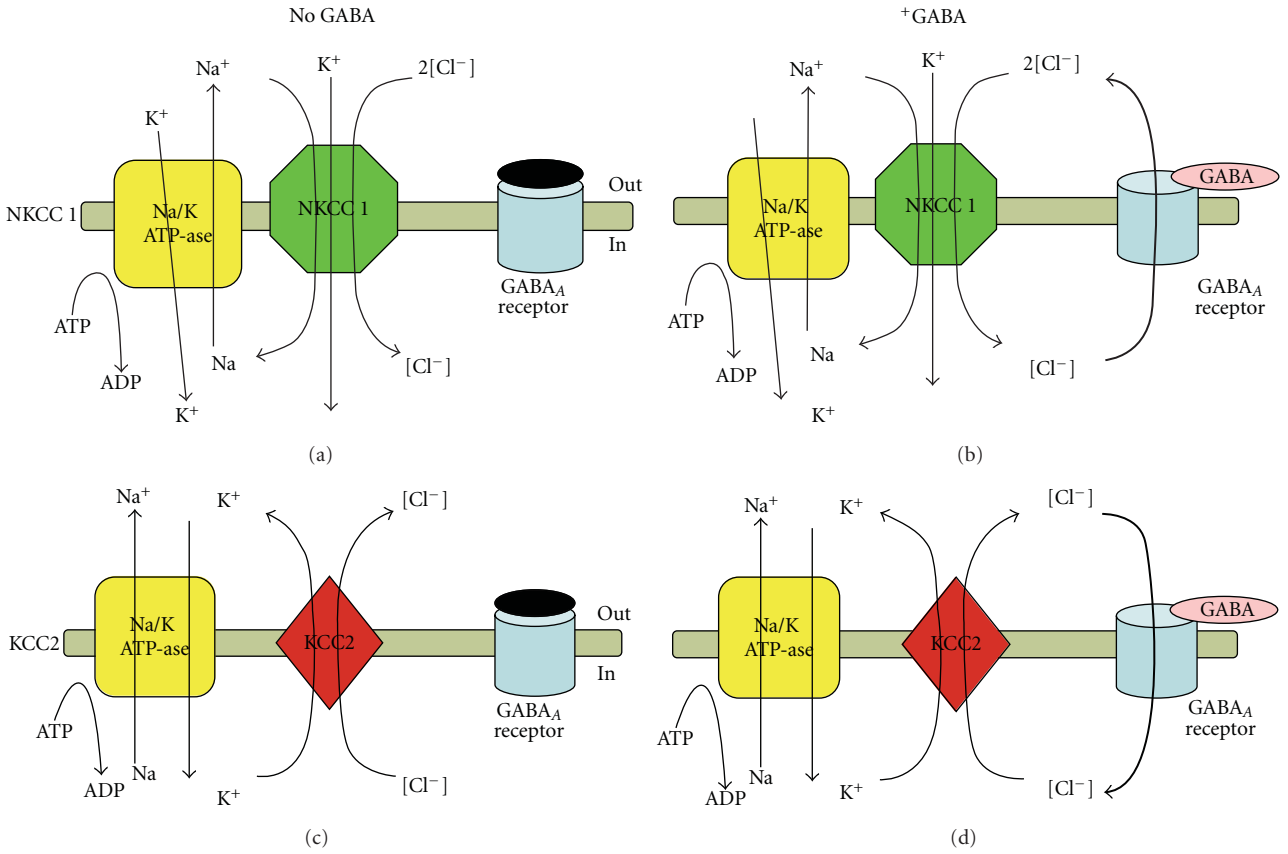

Figure 1: CCCs control $GABA_A$ receptor-mediated inhibition. Panels (a) and (b) show the effects of NKCC1 activity in the absence (panel (a)) or presence (panel (b)) of GABA. NKCC1 mediates the electroneutral cotransport of Na^+, K^+, and 2 Cl−, increasing the intracellular Cl^- concentration. As a result, upon binding of GABA upon the $GABA_A$ receptor, the channel pore opens and Cl leaves the neuron, causing a depolarization. Panels c and d show the effects of NKCC1 activity on $GABA_A$ receptor function in the absence (panel c) or presence (panel d) of GABA. KCC2 in contrast exports K^+ and Cl^- reducing intracellular Cl^-. Activation of $GABA_A$ receptors therefore results into influx of Cl and hyperpolarizing current. Their function is dependent upon the gradients of Na^+ and K^+, which are controlled by various factors, including background conductances, membrane voltage, and by the Na^+/K^+ ATPase.

of $GABA_A$ receptors is also different in newborns, with less $\alpha 1$ and more $\alpha 2/3$ subunits, rendering them less responsive to benzodiazepines [41, 42]. Furthermore, the subcortical GABAergic networks that control seizures, like the substantia nigra pars reticulata (SNR), have not fully developed [31, 42–46]. The excessive GABAergic stimulation of the SNR, as is thought to occur due to GABA release during seizures, has proconvulsant effects early in life and anticonvulsant in older animals and this switch occurs earlier in females [44, 45]. It is therefore important to investigate and clarify the exact molecular determinants that control $GABA_A$ inhibition in the young brain so as to optimize the treatment of seizures.

4. Aberrant $GABA_A$ Signaling Predisposes to Seizures

Clinical and experimental evidences indicate that an initial perturbation of $GABA_A$ signaling may facilitate seizures. A loss of inhibition could result in runaway excitatory circuits. Too much inhibition could also cause a seizure, either by disinhibiting epileptogenic networks or via promoting neuronal synchronization ([67] reviewed by [68]). Excessive inhibition has been implicated in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) ([69] reviewed in [70]) or absence seizures [71]. Moreover, as $GABA_A$ signaling is critical for brain development and early synaptogenesis [72–74], a disorder of $GABA_A$ signaling early in life may cause miswiring or malformations that predispose to seizures (Figure 2).

Many GABA-related mutations are known to cause early life epilepsy. These include loss of function mutations or deletions of $GABA_A$ receptor subunit genes that reduce their expression, or the duration, amplitude or agonist sensitivity of $GABA_A$ currents. $GABA_A$ receptor subunit mutations have been implicated in childhood absence epilepsy (CAE) [50, 51, 75], autosomal dominant epilepsy with febrile seizures plus ($ADEFS^+$) [76], and other epileptic syndromes (reviewed in Table 1 and [77, 78]). Conditional mutants indicate that the developmental period of exposure to insults that disrupt $GABA_A$ signaling may be critical in ictogenesis and epileptogenesis. Chiu et al. proposed that loss of function mutations of the $GABA_A$ receptor subunits may have

Table 1: GABA-related mutations linked with seizures.

GABA-related mutations	Species	Epilepsy type	Age at first observation	Ref.
		$GABA_A$ receptor mutations		
GABRA1	Human	ADJME, CAE	Childhood, Juvenile	[47, 48]
GABRA6	Human	CAE	Childhood	[49]
GABRB3	Human	CAE	Childhood	[50–52]
GABRD	Human	ADJME	Juvenile	[53]
GABRE	Human	Febrile, $ADEFS^+$ IGE	Infantile, childhood	[49]
GABRG2	Human, mouse	CAE^+ Febrile, $ADEFS^+$, SMEI $ADEFS^+$, SMEI, Febrile	Infantile, childhood	[54–59]
GABRP	Human	IGE, $ADEFS^+$, Febrile	?	[49]
		Other mutations		
GAD65 knockout	Mouse	Stress-induced, Limbic seizures	12 weeks	[60, 61]
ARX mutations	Human, mice	Early life epileptic encephalopathies (infantile spasms, Ohtahara)	Neonatal, Infantile	[62–66]

developmental effects in addition to their direct electrophysiological consequences [79]. Using a conditionally expressed loss of function mutation of the $\gamma 2$ $GABA_A$ receptor subunit in mice, the investigators expressed the mutant allele for different periods of time. Mice that were induced to express the mutant allele for longer developmental periods displayed higher seizure susceptibility to pentylenetetrazole (PTZ), a drug that acts as a $GABA_A$ receptor antagonist, compared to mice with late disruption of the $\gamma 2$ subunit expression.

Glutamic acid decarboxylase (GAD) isoforms GAD65 and GAD67 synthesize GABA in the brain. Knockout mice for the pyridoxal-5′-phosphate inducible GAD65 isoform, that generates the GABA reserve pools, have lower seizure threshold to picrotoxin, a $GABA_A$ receptor antagonist [61], or spontaneous seizures that can be precipitated by stress [60]. Although total GABA content in the brain may be normal or decreased in GAD65 knockout mice, depending upon the genetic substrate, it has been proposed that GAD65 loss of function may preferentially decrease the presynaptic reserve pool of GABA and decrease the tonic GABA inhibition, leading to increased seizure susceptibility [80–82]. Although no human GAD mutations have been found to consistently cause epilepsy [83], mutations in co-factors that are necessary for GAD65 function have been linked with early life seizures, as occurs in pyridoxine-de-pendency disorders [84, 85]. GAD65 or GAD67 loss suf-ficiently compensates for each other and does not appear to affect early brain development; albeit, cleft palate has been reported with GAD67 knockout mice [86]. Dual GAD65/67 knockout mice are not viable [87]. A small subset of patients manifests epilepsy secondary to an autoimmune response against GAD65/67, although these appear mostly in adults [88–91].

5. Disrupting CCC Function May Predispose to Seizures

Decreased expression or function of chloride extruders may change seizure susceptibility by not only diminishing the efficacy of $GABA_A$ inhibition and promoting cellular swelling and degeneration under hypotonic conditions, but also by exerting broader developmental effects. Human linkage studies or transgenic knockout animal studies document that, at least in certain cases, seizures and epilepsy may ensue. There is currently no known human mutation of KCC2 associated with epilepsy. This may rather reflect the indispensability of KCC2, as complete KCC2 knockout mice die postnatally from respiratory failure, due to the immaturity of the respiratory system [93]. KCC2 has two known isoforms, KCC2a and KCC2b, of which KCC2b is thought to contribute to the developmental shift to hyperpolarizing $GABA_A$ receptor currents [106]. KCC2b-knockout mice demonstrate hyperexcitability at PN10 to PN16 (equivalent to human infantile age) [94] (Table 2). Although the expected intracellular accumulation of chloride and depolarizing shift of $GABA_A$ responses could easily explain the hyperexcitability, application of the $GABA_A$ receptor antagonist picrotoxin paradoxically retains its excitatory responses [94]. Similarly, a different hypomorphic mutation in KCC2 causes a lower PTZ threshold for induction of clonic seizures in mice, despite the absence of gross morphological changes [95]. Such observations are

Table 2: Phenotype of CCC mutations.

CCC	Location	Mutation	Species	Neurological effect	Ref.
KCC1	Ubiquitous	Knockout	Mouse	None seen	[92]
KCC2	Brain	KCC2a and KCC2b knockout	Mouse	Death at birth	[93]
	Brain	KCC2b knockout	Mouse	Seizures, low weight, early mortality	[94]
	Brain	Hypomorph	Mouse	Increased seizure susceptibility and anxiety	[95]
	Brain	Heterozygote	Mouse	Hyperexcitability	[96]
KCC3	Ubiquitous	KCC3a-c knockout	Human, mouse	Peripheral neuropathy; seizures have been reported	[97–100]
KCC4	Kidney, heart, lungs, liver	Knockout	Mouse	Deafness	[101]
NKCC1	Ubiquitous	NKCC1a knockout	Mouse	Deafness, circling behavior	[102]
	Ubiquitous	NKCC1a and NKCC1b knockout	Mouse	Deafness, circling behavior, growth retardation, defective spermatogenesis, increased threshold to thermal stimulation	[103, 104]
NKCC2	Kidney	Knockout	Human	Bartter's syndrome	[105]

indicative of a residual inhibitory capacity of KCC2, either in the form of less potent hyperpolarizing $GABA_A$ receptor currents or shunting inhibition [107]. However, the function of KCC2 is more complex, due to interactions with dendritic cytoskeletal proteins [108] or with other modulators of neuronal activity (i.e., increasing extracellular potassium) [109] which need to be further analyzed as to their ability to influence the phenotype of these mice.

Loss of function mutations in KCC3, which is expressed in many tissues, have been reported in patients with hereditary motor sensory neuropathy, some of whom have seizures as well as developmental deficits, like agenesis of the corpus callosum [100].

Altered CCCs may also affect brain development in a more subtle fashion, which could predispose a brain to epilepsy even if it does not directly cause seizures. From various fronts evidence emerges that shifts in the timing of emergence of hyperpolarizing signaling may have significant impact on neuronal and brain development and connectivity. Precocious appearance of hyperpolarizing $GABA_A$ receptor signaling, either by KCC2 overexpression [72] or via loss of NKCC1 activity [110], disrupts cortical morphogenesis. Pharmacological inhibition of NKCC1 with bumetanide from embryonic day E15 to PN7 in otherwise normal mice disrupts cortical dendritic formation [74]. Abnormal cortical development and synaptic connectivity may predispose to seizures or cognitive impairment, which is both a predisposing factor and a common comorbidity of young patients with epilepsy [111].

6. Secondary Disruption of GABAergic Signaling in Risk Factors for Early Life Epilepsy

Conditions that predispose to epilepsy, genetic or acquired, may also create an imbalance in excitation/inhibition. Although their effects are not restricted to $GABA_A$ signaling, in certain cases they may show a predilection to preferentially impair GABAergic inhibition.

Mutations of the aristaless-related and X-linked homeobox gene ARX have attracted a lot of interest due to their linkage with early life catastrophic epileptic syndromes, such as infantile spasms, Ohtahara syndrome, X-linked myoclonic seizures, spasticity and intellectual disability, idiopathic infantile epileptic dyskinetic encephalopathy, X-linked mental retardation [63–66, 112–116] (reviewed in [117]). ARX is a transcription factor that regulates the proliferation and

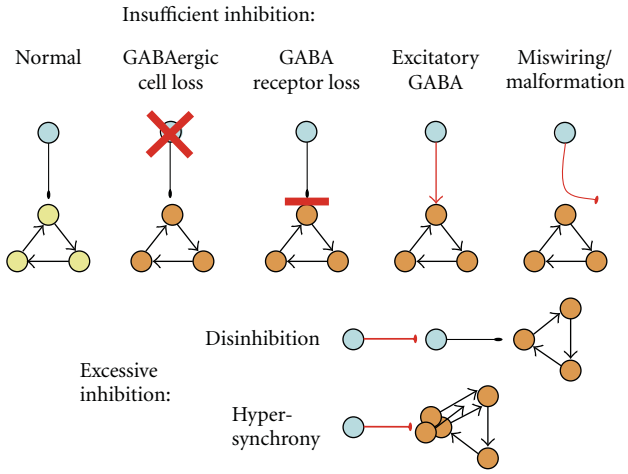

Figure 2: Schematic depiction of simple models through which dysregulation of $GABA_A$ receptor-mediated inhibition can increase the activity of neuronal networks, potentially generating seizures. GABA inhibition can fail when GABA or $GABA_A$ receptor expression is low, when GABA depolarizes neurons, or when miswiring and mistargeting of synapses occur. Excessive GABA inhibition may trigger seizures by disinhibiting target cells, or via excessive synchronization of the neurons in the epileptogenic focus. Please note that the effects of dysregulated GABA signaling in more complex neuronal networks, especially in the presence of abnormal circuitry or with specific pathologies, may differ. In such cases a combination of the above models may be applicable at different sites of the epileptogenic network rendering the pharmacological effect of a GABAergic agonist not completely predictable by a single model. Furthermore, shunting inhibition may explain situations where GABAergic drugs silence excessive excitatory network activity, in neurons with depolarizing GABAergic signaling.

migration of GABA, calbindin, or neuropeptide Y positive interneurons but also of striatal cholinergic neurons [64, 66, 117]. Two recently published mouse models of ARX loss of function mutations, one of which specifically disrupted it in GABAergic interneurons destined to migrate to the neocortex, have recapitulated several phenotypes of infantile spasms and associated phenotype (cognitive, behavioral deficits and epileptogenesis) emphasizing the importance of deficient GABA inhibition for their pathogenesis [64, 66].

Angelman syndrome, a rare chromosomal deletion, involves the loss of ubiquitin-protein ligase 3A (UBE3A), but in certain patients there is a more extensive deletion of the 15q11-13 chromosomal locus that contains three $GABA_A$ subunits, $\alpha 5$, $\beta 3$, and $\gamma 3$ $GABA_A$ receptor subunits [118]. Genotype-phenotype correlation suggested that deletion of the $GABA_A$ receptor subunits is associated with more severe seizures, including infantile spasms, atypical absences, and myoclonus whereas patients with UBE3A mutations had a milder phenotype [118]. The $\beta 3$ subunit knockout mouse strain also develops a similar epilepsy phenotype [119].

Loss of function mutations of the voltage-sensitive sodium channel SCN1A gene is found in not only the severe myoclonic epilepsy of infancy (Dravet syndrome) but also in ADEFS$^+$ syndrome [120–123]. SCN1A mutations have been proposed to preferentially impair the sodium channel activity of GABAergic interneurons, diminishing their activity [124]. Anti-NMDA autoantibodies detected in limbic encephalitis, a rare cause of refractory and frequent seizures [125], have been speculated to selectively target the NMDA receptors of presynaptic GABAergic terminals, reducing therefore GABA release [126].

Aberrant reappearance of depolarizing E_{GABA} and reduced $GABA_A$ergic responses have been proposed to underlie the pathogenesis of seizures from cortical malformations. Pathology and electrophysiological studies from human tissue specimens from patients with cortical dysplasias, that commonly predispose to early life seizures, have also suggested the presence of depolarizing GABA [20, 127, 128]. In the neonatal freeze lesion model, a shift to the immature pattern of high NKCC1/KCC2 ratio in the lesional site [129] as well as reduced $\gamma 2$ subunit expression and sensitivity to $\alpha 1$ subunit agonists in adulthood was described [130, 131]. In the rat model of cortical dysplasias induced by prenatal exposure to the 1-3-bis-chloroethyl-nitrosurea, reduced sensitivity to GABA was also seen in adulthood [132].

Traumatic brain injury in adults, such as in axotomized neurons, causes a reversal of $GABA_A$ signaling and CCC expression profile to the immature pattern (more depolarizing GABA and dominant NKCC1 over KCC2 activity) [133–135]. This appears to aid the survival and regeneration process, promoting the brain-derived neurotrophic factor- (BDNF-) dependent neuronal survival and may resolve with time, during recovery [135]. However, there is limited information as to the consequences of neuronal trauma upon the expression, physiology, and connectivity of GABAergic interneurons in developing animals. In the partially isolated undercut cortical model, reduced $GABA_A$ergic IPSCs and impaired chloride extrusion were found in juvenile rats, suggesting a possible correlation between impaired GABAergic inhibition and posttraumatic cortical excitability [136, 137]. Few studies have advocated against the use of GABA enhancing drugs and in favor of $GABA_A$ receptor inhibitors as interventions to improve cognitive outcomes [138]. More detailed studies are needed to determine the role of posttraumatic $GABA_A$ signaling changes for healing and regeneration in the developing brain as well as its impact on subsequent epileptogenesis and ensuing cognitive deficits.

7. Seizures Alter $GABA_A$ Signaling

Seizures can affect almost every neurotransmitter system in the brain. Seizures can have immediate effects on $GABA_A$ signaling, that is, during the ictal period, or delayed, appearing after the termination of seizures. In both scenarios, the observed changes are dynamic and evolving. Seizures may interfere with the expression, composition, and subcellular distribution of $GABA_A$ receptors and their regulatory factors, such as CCCs or regulatory kinases. Defining the timing of these events is crucial, not only to better understand the pathophysiological mechanisms investigating these changes but also to best interpret their pathophysiological relevance for epileptogenesis and brain function. The temporal

Table 3: Effects of early life seizures on $GABA_A$ receptors and currents in rats.

Seizure model	Age	Region	Effects on $GABA_A$ receptors	Ref.
		Ictal changes		
In vivo SE (Lithium-pilocarpine; continuous hippocampal stimulation)	PN30	Hippocampus	Reduced surface expression of $\beta 2/3$, $\gamma 2$ subunits but not of δ.	[139]
In vivo SE (lithium-pilocarpine)	4–7 week old	Hippocampus	Internalization of $\beta 2/3$, $\gamma 2$ subunits; reduced mIPSCs	[140]
		After seizures		
Recurrent flurothyl seizures	PN1-5	Hippocampus, somatosensory cortex	Decreased amplitude of GABAergic IPSCs	[141, 142]
Flurothyl seizures	PN6 or PN6-10	Hippocampus	Decreased numbers of $\alpha 1$-ir neurons	[143]
Kainic acid SE	PN9	Hippocampus	At 3 weeks postictally: $\alpha 1$, $\alpha 4$,$\gamma 2$ decrease; $\alpha 2$, $\alpha 3$ increase; $\alpha 5$ increase (CA3 only); $\beta 3$ increase compared to controls	[144]
Lithium-pilocarpine	PN10	Hippocampus (dentate gyrus)	In adulthood: increased $\alpha 1$ expression, larger GABA current, enhanced zolpidem sensitivity	[145]
Lithium-pilocarpine SE	PN20	Hippocampus	Decreased $\alpha 1$ and increased $\alpha 4$ expression in the hippocampus of epileptic versus non-epileptic rats	[146]

evolution of these events is also particularly important in developing rats, given the maturational changes that are ongoing. In addition, the age at first seizure, the type and severity of seizures, sex, epigenetic factors, medications, but also the cellular diversity of specific operant signaling systems further modify the final outcomes.

7.1. Ictal Attenuation of $GABA_A$ Receptor-Mediated Inhibition. The urgency in treating early SE has long been recognized in the clinical literature. GABA-acting drugs, like benzodiazepines or barbiturates, are more effective early at onset of seizures than later on, when SE has been established [147, 148]. The transience of the efficacy of GABAergic drugs has been attributed to either increase internalization of selective synaptic $GABA_A$ receptor subunits, such as of $\beta 2/3$ and $\gamma 2$, which mediate the effects of benzodiazepines and barbiturates [139, 140]. On the other hand, extrasynaptically located subunits that mediate tonic GABA inhibition, like the δ subunit, are not affected [139]. Failure of $GABA_A$ receptor-mediated inhibition during prolonged seizures may also occur due to a positive shift in E_{GABA} either because of buildup of intracellular Cl^- concentration, from intense $GABA_A$ receptor-mediated chloride inward pumping, or from impaired chloride extrusion mechanisms, due to increased NKCC1 activity or decreased KCC2-mediated Cl^- efflux [149–151].

7.2. Postictal Changes. Loss of GABAergic interneurons is a hallmark pathology of focal epilepsies, like mesial temporal sclerosis [152–157]. In experimental studies, prolonged seizures can lead to interneuronal loss but such effects are age-specific. In newborn rats, during the first week of life, even 3 episodes of status epilepticus (SE) do not injure GABAergic neurons [30]; yet cell death becomes a progressively more prominent feature as the age at exposure to SE increases [155, 158–160]. In contrast, early life seizures functionally disrupt the physiology of $GABA_A$ receptor system. Age at the time of seizures, etiology or model of seizures, biological factors such as sex, as well as cell type and region-specific features may determine the end effects upon $GABA_A$ receptor subunits or the direction of $GABA_A$ receptor-mediated responses (Tables 3 and 4). These changes may be either compensatory attempts to repair or restore normal function or, on the contrary, may contribute

Table 4: Effects of Seizures on CCCs.

Model	Species	Age at seizures	Region	Effects	Ref.
			Ictal changes		
Kainic acid	Rat	PN6-7	Hippocampus	Switch from hyperpolarizing to depolarizing E_{GABA}	[184]
Low Mg^{2+} seizures	Mice	PN5	Hippocampus	Bumetanide sensitive increase in $[Cl^-]_i$	[150]
			After seizures		
Kainic acid	Rat (male)	PN4-6	Hippocampus (at least 4 days postictally)	Increased KCC2; decreased NKCC1 activity; more hyperpolarizing E_{GABA}	[185]
Kainic acid	Rat (female)	PN4-6	Hippocampus (at least 4 days postictally)	No change in KCC2; increased NKCC1 activity; more depolarizing E_{GABA}	[185]
Kainic acid	Rat (male)	PN5-7	Hippocampus (immediate postictal period)	Increased surface expression of KCC2; hyperpolarizing shift of E_{GABA}	[171]

to the postictal dysfunction, comorbidities, or sequelae of seizures, such as cognitive dysfunction or epileptogenesis. Unlike the adults, in which the physiology of $GABA_A$ receptor-mediated signaling has reached a relative steady state, developmental research is further complicated by the evolving changes that normally occur during the period when brain matures[161]. There is no systematic research study taking us step-by-step through all the complexity of seizure-induced postictal alterations in $GABA_A$ receptor physiology and any extrapolations should be cautiously done pending confirmation by actual experimentations.

Seizures selectively interfere with the expression of certain, but not all, $GABA_A$ receptor subunits [141–146] (Table 3). Kainic acid SE at PN9 rats favors the preservation of the immature pattern of $GABA_A$ receptor complex (less $\alpha 1$, more $\alpha 2/\alpha 3$ subunits) on the third postictal week [144] that typically attributes slower IPSC kinetics and less sensitivity to benzodiazepines. Similarly, recurrent flurothyl-induced seizures, in the first 10 days of life, decrease $\alpha 1$ expression and the amplitude of $GABA_A$ receptor-mediated IPSCs [141–143]. Looking at longer-term outcomes of early life seizures, during adulthood, Brooks-Kayal's group has demonstrated that age at onset of SE is key at defining the final composition of $GABA_A$ receptors and that this, in turn, may contribute to epileptogenesis. Lithium-pilocarpine SE at PN10 increases $\alpha 1$ subunit expression in the dentate granule cells in adulthood; in contrast, if SE is induced at PN20, a decrease in $\alpha 1$ subunit is noted, but only in the epileptic animals [145, 146]. Interestingly, reconstitution of $\alpha 1$ subunit expression prevented the occurrence of spontaneous seizures [146, 162].

The reports of untimely appearance of depolarizing $GABA_A$ receptor signaling in a subpopulation of subicular neurons from adult human epileptic resected temporal lobes have attracted a lot of interest as a possible mechanism of epileptogenicity and potential refractoriness to GABA-acting antiepileptics [163, 164]. Depolarizing $GABA_A$ receptor signaling has been linked to a dominance of NKCC1 over KCC2 activity in certain neurons of the epileptic tissue. It may also occur because of effective replenishment of intracellular bicarbonate by carbonic anhydrase during intense $GABA_A$ receptor activation, which leads to a depolarization and to a consequent influx of Cl^-, that enhances KCC2-mediated K^+/Cl^- efflux [109]. The sequential interaction between carbonic anhydrase/$GABA_A$ receptors/KCC2 may therefore increase extracellular K^+, a factor that promotes the generation of ictal events. In support, carbonic anhydrase inhibitors have been used in certain cases as anticonvulsant therapies [109, 165].

Seizures in adult animals tend to increase the ratio of NKCC1 over KCC2 activity, reverting to a more immature pattern of CCC balance that favors depolarizing E_{GABA} [151, 166]. This is believed to occur in humans as well [127, 167–170]. But what happens, then, after early life seizures, when neurons are already in an immature state and how does this impact epileptogenesis and functional outcomes? In the immediate postictal period, following brief recurrent kainic acid seizures or an hour of kainic acid SE, KCC2 is reshuffled towards the plasma membrane, increasing its capacity to export Cl^- [171]. As a result E_{GABA} becomes more negative, contributing perhaps to the ability of the neurons to stop seizures.

In the longer run, further changes in E_{GABA} function occur, which are attributed to altered CCC expression or activity [30]. In our lab, we were interested in determining whether the original E_{GABA}, at the time seizures occur, may control the effects of seizures on CCCs and the direction of $GABA_A$ receptor-mediated signaling, in other words, whether seizures might have different effects upon $GABA_A$ receptor-mediated signaling in neurons with depolarizing or hyperpolarizing $GABA_A$ receptor mediated responses at the time of seizures. Taking advantage from the earlier appearance of $GABA_A$ receptor currents in females than in males, we compared the effects of 3 episodes of kainic acid SE elicited at PN4, 5, and 6 (3KA-SE) in CA1 pyramidal neurons with depolarizing E_{GABA} (i.e., male) or isoelectric/hyperpolarizing E_{GABA} (i.e., female) at the time of seizures [30]. We found that 3KA-SE caused only a transient appearance of depolarizing $GABA_A$ receptor mediated responses in neurons that had already started to shift to mature and more hyperpolarizing E_{GABA}, similar to what was previously described for the adult neurons. In contrast, in male neurons, with still depolarizing GABAergic responses, 3KA-SE caused a precocious emergence of mature, hyperpolarizing responses. These changes were attributed to

altered expression and/or activity of KCC2 and NKCC1. The precocious termination of depolarizing $GABA_A$ signaling would be expected to deprive brain from its neurotrophic effects that are important for normal development [72, 74]. Indeed, 3KA-SE-exposed pups develop learning and memory problems when they grow up (unpublished data). However, the inability of the immature neurons to persistently exhibit depolarizing $GABA_A$ receptor-mediated responses after seizures could be a protective feature against the development of subsequent epilepsy [30]. Our results indicate that age-specific factors, including the depolarizing GABA, may be important for this protection. Another dual regulator of CCCs and E_{GABA} through development is the brain-derived neurotrophic factor (BDNF) pathway, which is also activated in certain seizure models. BDNF increases KCC2 in developing neurons but decreases it in mature neurons [172, 173]. The opposite patterns of KCC2 regulation by BDNF in certain systems has been proposed to be due to trkB-mediated activation of different intracellular signaling cascades that regulate KCC2 expression [151].

The maturation of $GABA_A$ receptor system occurs asynchronously across different neuronal types and brain regions. As a result, since early life seizures change the direction and strength of $GABA_A$ receptor-mediated inhibition, their effects will be region and cell type specific, further confusing the interneuronal communication protocols. They may also disrupt the basic neural processes of learning and cognitive processing that depend upon GABA neurotransmission, such as long-term potentiation (LTP) [174–176], or social interactions [177–182]. The result will be a state of postictal confusion or more sustained cognitive or behavioral deficits [6]. Of interest, bumetanide treatment has shown benefit in five infants with autism [183]. However the exact mechanisms underlying this therapeutic effect are not yet known.

8. Implications for Early Life Seizures and Their Treatment

Human and experimental evidence indicates that similar to adults, aberrant preservation of depolarizing $GABA_A$ signaling may also be a feature of the medically refractory epileptogenic focus in early life epilepsies. At present we do not have any data to discuss the pathological features of the medically sensitive early life epilepsies. The idea of pharmacologically enhancing GABA inhibition to stop seizures by using NKCC1 inhibitors like bumetanide is under investigation as a rationally developed, smart intervention to overcome the barriers posed by the well-established molecular switch of $GABA_A$ receptor function [21]. Beneficial effects have been shown in few animal models [21, 186–189] and a human case report [190]. However, model-specific differences, as well as the timing of administration, can influence its efficacy in suppressing seizures [96, 191]. Moreover, concerns have been raised about potential adverse developmental effects on innocent bystander normal brain tissues, as may occur in chronic use in patients with focal epilepsies [74]. Undoubtedly, more studies need to be done to determine which seizure types are more likely to respond, when is the optimal time to administer, for how long, and how such interventions influence long-term outcomes in subjects who have already experienced seizures or have epilepsy. Similarly, by increasing our knowledge about the specific changes that occur in $GABA_A$ receptor composition and pharmacology, it may be possible to design more selective and specific $GABA_A$ receptor agonists for the very young or epileptic brain that is refractory to the existing medications. At the anatomical and electrophysiological level, it might be feasible, one day, to design such specific, very targeted, and individualized therapies to enhance GABA inhibition and stop seizures. The biggest challenge will be however to predict the functional state of $GABA_A$ receptor-mediated inhibition at the target areas, so as to implement such rational therapies. Emerging evidence suggests that GABA-acting drugs, hormones, and different stressors are among the factors that can alter $GABA_A$ receptor signaling, rendering it almost a moving target [11, 30, 31, 192–196]. The need for biomarkers of $GABA_A$ function is therefore a priority.

9. Conclusion

The study of GABA in seizure generation and consequences has become a very fruitful field not only by generating intriguing results but also by producing challenging new questions. We have learned a number of mechanisms that compromise $GABA_A$ inhibition in the very young or epileptic brain, predisposing to seizures and the associated cognitive and neurodevelopmental deficits. We still need to better understand and, most importantly, predict which is the normal balance between excitation and inhibition with sufficient age, sex, cell type, and regional, context, and function-related specificity, so as to preserve normal brain function and development.

Abbreviations

ADEFS$^+$: Autosomal dominant epilepsy with febrile seizures plus
ADJME: Autosomal dominant juvenile myoclonic epilepsy
ADNFLE: Autosomal dominant nocturnal frontal lobe epilepsy
ARX: Aristaless-related X-linked homeobox gene
BDNF: Brain-derived neurotrophic factor
CAE: Childhood absence epilepsy
GABA: Gamma aminobutyric acid
GABR: $GABA_A$ receptor
GAD: Glutamic acid decarboxylase
IGE: Idiopathic generalized epilepsy
IPSC: Inhibitory postsynaptic current
3KA-SE: 3 episodes of kainic acid SE at PN4,5,6
KCC: Potassium chloride cotransporter
LTP: Long-term potentiation
NKCC: Sodium potassium chloride cotransporter

PN: Postnatal day
PTZ: Pentylenetetrazole
SCN1A: Sodium channel 1A
SMEI: Severe myoclonic epilepsy of infancy
SE: Status epilepticus
TLE: Temporal lobe epilepsy
UBE3A: Ubiquitin-protein ligase 3A.

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Review Article

Molecular Mechanisms Underlying Activity-Dependent GABAergic Synapse Development and Plasticity and Its Implications for Neurodevelopmental Disorders

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GABAergic interneurons are critical for the normal function and development of neural circuits, and their dysfunction is implicated in a large number of neurodevelopmental disorders. Experience and activity-dependent mechanisms play an important role in GABAergic circuit development, also recent studies involve a number of molecular players involved in the process. Emphasizing the molecular mechanisms of GABAergic synapse formation, in particular basket cell perisomatic synapses, this paper draws attention to the links between critical period plasticity, GABAergic synapse maturation, and the consequences of its dysfunction on the development of the nervous system.

1. Introduction

More than four decades of research has demonstrated that although the brain remains plastic throughout life, continuously reorganizing its connections in the face of new experiences, childhood represents a specific phase in the development of the synaptic network that is characterized by overall remarkable plasticity. During this period of enhanced plasticity also called "critical period", experience can produce permanent, large-scale changes in neural circuits. Studies on mechanisms that underlie activation and regulation of critical periods in the central nervous system (CNS) are seminal in neuroscience, with the underlying motive being that manipulation of such mechanisms may potentially allow reactivation of neural circuit plasticity during times when the adult brain is less plastic, for example, to aid adaptive circuit rewiring following insult, such as stroke. Additionally, this line of inquiry may help us develop rational pharmacological approaches to correct alterations in the brain of children with neurodevelopmental disorders involving altered synapse formation and/or plasticity.

Critical periods have been observed across sensory, motor, auditory, and also higher cognitive areas; however much of our knowledge of the cellular and molecular mechanisms of onset, maintenance, and termination of these periods derive from seminal studies by Wiesel and Hubel [1] in the developing cat visual system. Electrophysiological recordings from neurons in the primary visual cortex show activation to different degrees by visual stimuli presented to one eye or the other, a property termed ocular dominance. Closing one eye during a specific postnatal time period starts a cascade of events leading to synaptic reorganization of neural circuits in visual cortex, resulting in lifelong, irreversible reduction of the ability of the deprived eye to drive neuronal responses in the cortex, and a dramatic increase in the number of neurons responsive to stimuli presented to the open eye. Such change in eye preference best able to elicit a response from cortical neurons in visual cortex following manipulation of visual inputs is called ocular dominance (OD) plasticity. In marked contrast to what happen in young animals, prolonged eye closure in adults elicits no change in visual cortical neuron responsiveness

[2]. Further, monocular deprivation during critical period causes loss of visual acuity in the deprived eye, which is not ameliorated by subsequent experience [3]. This is supported by human studies showing that treatment of amblyopia in children between 7 and 17 years of age was effective only in a fourth of the patients, and to a lesser degree than treatment in younger children [4]. To date, ocular dominance plasticity remains the best-studied experimental model for experience-dependent refinement of neuronal circuits because of the ease of manipulating visual experience independently in the two eyes.

An important question is which factors determine the timing of critical period plasticity. One of the main players implicated in the onset of critical period plasticity is the development of inhibitory circuitry [5, 6]. Cortical inhibitory neurons, or interneurons, comprise ~20–30% of all cortical neurons and predominantly use gamma-aminobutyric acid (GABA) as neurotransmitter. GABAergic interneurons control several aspects of neuronal circuit function from neuronal excitability [7] and integration [8], to the generation of temporal synchrony and oscillation among networks of excitatory neurons [9]. In addition, GABAergic interneurons also regulate key developmental steps, from cell migration and differentiation to experience-dependent refinement of neuronal connections [10, 11]. In the last years, many studies have started to elucidate the development and function of cortical GABAergic circuits.

In this paper the focus is on the molecular mechanisms regulating postnatal GABAergic circuit development and the onset of critical period plasticity, followed by a brief discussion on how aberrations in inhibitory circuit development and alteration in the timing of critical period plasticity could be implicated in neurodevelopmental diseases.

2. GABAergic Inhibition and the Onset of Critical Period

What dictates the time window of a heightened period of plasticity in the brain? Recent studies indicate that the development of inhibitory circuitry in the cortex plays a pivotal role in controlling the onset and time course of critical periods [5, 10, 12]. In particular, two elegant studies envisage a direct role of GABA in the onset of OD plasticity. In a first study, Hensch and collaborators [13] found that mice lacking the synaptic isoform of GABA-producing enzyme, Glutamic Acid Decarboxylase (GAD65), show no OD plasticity. This deficit can be rescued by cortical infusion of the GABAa receptor agonist diazepam, demonstrating that a decrease in inhibition effectively abolished critical period and impaired plasticity mechanisms. In the second study, Fagiolini and Hensch [5] showed that the early enhancement of GABA-mediated inhibition by diazepam application triggers the precocious onset of OD plasticity. Further, precocious development of inhibitory circuitry via action of the Brain Derived Neurotrophic Factor (BDNF) accelerates the onset of the critical period for visual plasticity [12].

Cortical GABAergic interneurons form a strikingly diverse and heterogenous group differing in morphology, physiological properties, and protein expression [14, 15]. The hypothesis that different interneuron subtypes play different roles in cortical development, function, and plasticity is therefore a tantalizing one. Fagiolini et al. [16] showed that GABA transmission mediated by the α1 subunit-containing GABAa receptors is required for the induction of critical period for OD plasticity. Because different classes of inhibitory synapses preferentially signal through GABAa receptors with different subunit composition [17], these results suggest that maturation of specific subclasses of GABA interneurons is crucial to initiate critical period plasticity. More recent data indicate that site-specific optimization of GABAa receptor numbers on the soma-proximal dendritic compartment of pyramidal cells triggers the onset of OD plasticity [18]. The soma proximal dendritic compartment of pyramidal cells is preferentially innervated by Parvalbumin (Pv) positive basket interneurons. Taken altogether, these data suggest a critical role for basket cell interneuron maturation in the onset of critical period plasticity.

A novel mechanism explaining how visual input is coupled to the onset of ocular dominance plasticity has been proposed by Sugiyama et al. [19]. Traditionally, the molecular signals linking visual experience to GABA interneuron maturation were thought to be recruited from within the cortex itself, such as the activity-dependent synthesis and release of BDNF by pyramidal neurons [12]. Instead, Sugiyama et al. [19] demonstrated that a retina-derived homeoprotein, Otx2, is first transferred into the primary visual cortex via a visual experience-dependent mechanism. Once in the cortex, Otx2 then nurtures GABAergic interneurons and promotes critical period plasticity. The investigation of the target genes and proteins of Otx2 will reveal further insights into the mechanisms linking experience, GABAergic circuit maturation, and critical period plasticity.

3. Molecular Mechanisms of GABAergic Circuit Development

The GABAergic network comprises of diverse interneuron subtypes that have different morphological and physiological characteristics and localize their synapses onto distinct subcellular locations on the postsynaptic targets. Precisely how activity and molecular-driven mechanisms conspire to achieve the remarkable specificity of GABAergic synapse localization and formation is unknown. The functional maturation of GABA-mediated inhibition is a prolonged process that extends well into adolescence, both in rodents and primates [20–23], and correlates with the time course of the critical period for OD plasticity [21, 23]. Moreover, the inhibitory maturation process strongly depends on sensory experience, since sensory deprivation, induced either by dark rearing or by intraocular tetradotoxin (TTX) injection, significantly retards the morphological and functional maturation of GABAergic synapses [21, 23]. This dependence of GABAergic synapse maturation on sensory experience is not limited to visual cortex, indeed similar results have been found in the somatosensory cortex [24].

What are the cellular and molecular mechanisms linking sensory experience to the maturation of GABAergic

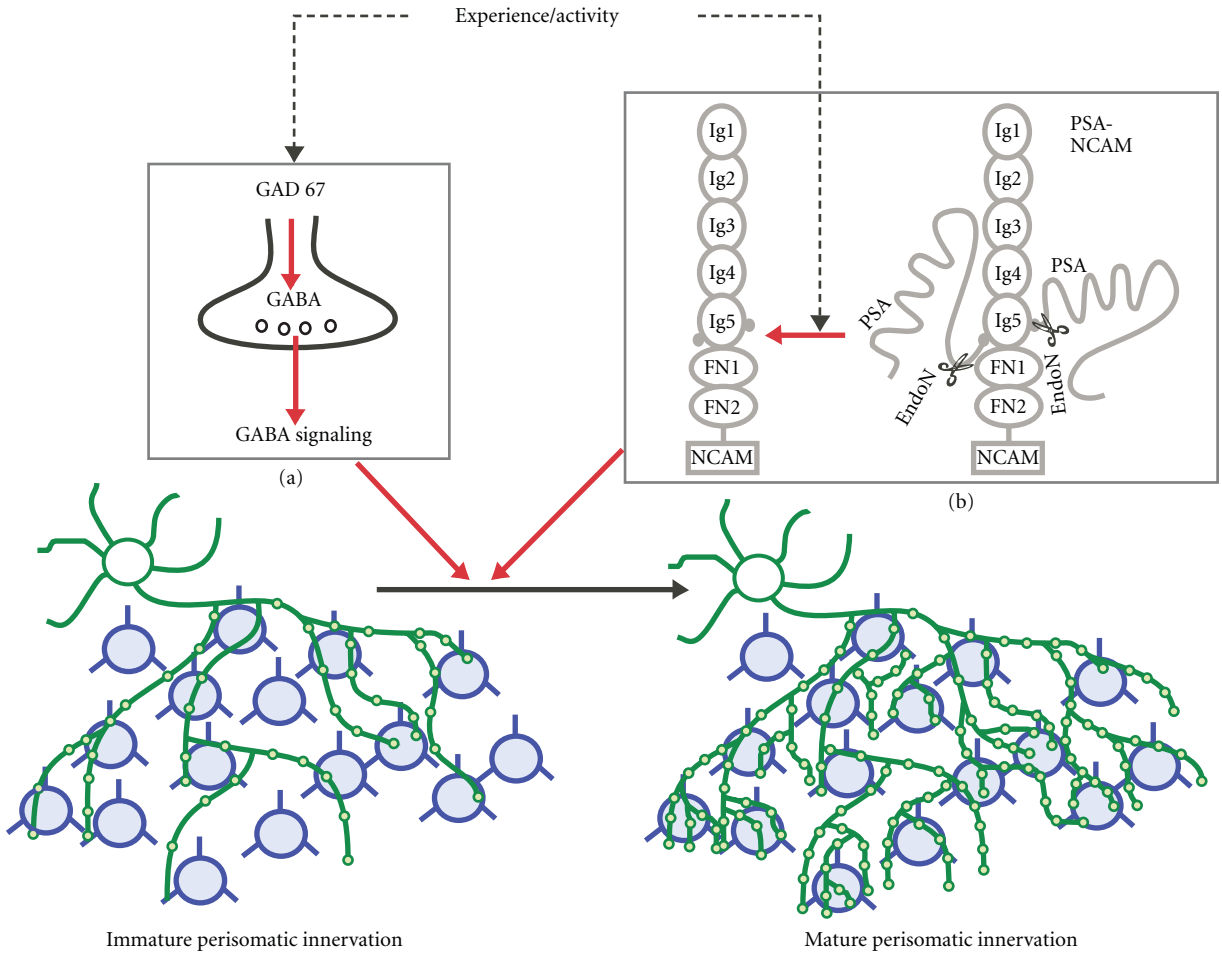

Figure 1: Sensory activity regulates perisomatic synapse maturation via multiple pathways. (a) Activity modulates GAD67 enzyme levels thereby ensuring normal GABA signaling for the appropriate downstream signaling events required for perisomatic synapse development. (b) Experience is also critical for removal of the PSA moiety from NCAM, allowing onset of perisomatic innervation at the right time.

synapses? Brain-Derived Neurotrophic Factor (BDNF), an activity-dependent molecule shown to be upregulated following light stimulation in the visual cortex [25, 26], is one of the first molecules implicated in the formation of GABAergic synapses in hippocampal and cortical cultures [27, 28]. Most importantly, in transgenic mice with precocious BDNF expression, a marked increase in perisomatic inhibitory innervation in the visual cortex is correlated with a premature onset and closure of ocular dominance plasticity, further supporting the link between GABAergic synapse maturation and onset of critical period plasticity [12, 29]. Since BDNF is produced only by pyramidal cells, it could work as an intercellular signaling factor that translates pyramidal cell activity to GABAergic synapse density.

Another factor that has been shown to positively regulate GABAergic synapse maturation is GABA itself. Early in development, GABA has been shown to be a trophic factor [30], involved in cell proliferation, neuronal migration, and neurite growth [31]. Since GAD67 is the main isoform of GABA synthesizing enzyme, its deletion reduces GABA levels by 90% [32]. Using transgenic mice to knockdown GAD67 in single basket interneurons during the period of their maturation, recent studies show that intact GABA signaling is critical for the maturation of GABAergic synapses [33] (Figure 1). Intriguingly, even a partial reduction of GAD67 was sufficient to cause aberrant perisomatic synapse maturation, underlying the importance of maintaining optimal GABA levels for normal synapse development [33]. Basket cell perisomatic synapses have an exuberant innervation pattern; a single basket interneuron connects to hundreds of pyramidal cells in its vicinity, making numerous synapses onto each individual pyramidal cell soma. It is therefore important to appreciate that reduced GABA levels compromise not only the number of synapses that are made onto each pyramidal soma, but also drastically reduce the number of pyramidal soma it connects to, causing a potential circuit-wide disruption in connectivity [33]. This study demonstrates that, in addition to mediating inhibitory transmission, GABA signaling also regulates interneuron axon arborization and synapse development in adolescent brain, which, in turn regulates critical period plasticity. Different aspects of this deficit were rescued by treatment with either GABAa or GABAb agonists, suggesting a receptor-specific effect of GABA-mediated signaling during

GABAergic synapse maturation [33]. Since GABAa and GABAb receptors are present on postsynaptic neurons, GABA terminal themselves, and surrounding glial processes, cell-autonomous activation of presynaptic GABAb receptors, which modulate Ca^{2+}channels and GABA release, could influence growth cone motility and bouton stability, or GABA signaling through postsynaptic or glia receptors could trigger the release of retrograde factors, which promote axon branching and synapse formation.

Modulation of GABA synthesis by the GAD67 enzyme plays a central role in regulating GABA-mediated signaling [34]. GAD67 itself is produced at a limiting level in the brain, since deletion of one copy of the *Gad1* gene results in a ~40% reduction of enzyme activity and GABA content in many brain regions [32]. Furthermore, the transcription of *Gad1*, the key step in the physiological control of GAD67 activity, is highly regulated during brain development [35], by neuronal activity [36], and experience [37, 38]. Activity-dependent production of GAD67 thus results in online adjustment of intracellular pool for GABA release. Since alterations in GAD67 and GABA levels profoundly influence interneuron axon growth, synapse formation and network connectivity during the establishment of inhibitory circuits, neuronal activity might regulate the strength and pattern of inhibitory synaptic innervation through GAD67-mediated GABA synthesis and signaling. Such activity-dependent and cell-wide regulation of a "transmitter resource" implies a novel logic for the maturation and plasticity of GABAergic synapses and innervation. Since subtle variations in GABA levels can cause such dramatic effects on inhibitory circuits, and therefore overall network connectivity, it is critical to understand its implications in neuropsychiatric disorders and strive to regulate optimal GABA levels for proper circuit function.

A recent study by Fiorentino et al. [39] proposes that the interaction between BDNF and GABA signaling influences GABAergic synapse maturation. The authors demonstrate that activation of metabotropic GABAb receptor triggers secretion of BDNF and promotes the development of GABAergic synapses, in particular, the perisomatic GABAergic synapses, onto CA3 pyramidal neurons in the hippocampus of newborn mice [39]. Whether a similar mechanism is at play in the visual cortex is still unknown; however, the picture so far indicates a positive interplay between sensory experience, BDNF, and GABA signaling, to induce GABAergic synapse maturation and in turn promote the onset of ocular dominance plasticity.

In addition to factors promoting GABAergic synapse maturation, recent studies have revealed inhibitory mechanisms that set the appropriate time course for establishment of mature GABAergic innervation patterns and the onset of critical period plasticity. In particular, polysialic acid (PSA), linked to the neural cell adhesion molecule (NCAM), acts as a negative signal to suppress the formation of inhibitory synapses and the onset of OD plasticity in the developing visual cortex [40]. In the mammalian brain, NCAM is a predominant carrier of the unusual long-chain, polyanionic carbohydrate, PSA, although outside the nervous system more carriers of PSA are known, including neuropilin-2 [41]. PSA is a long linear homopolymer of α-2,8-linked sialic acid that is synthesized in the Golgi by two polysialyltransferases, PST (also known as ST8SiaIV) and STX (also known as ST8SiaII), either of which is sufficient for the complete synthesis of PSA chain on a standard asparaginyl-linked core carbohydrate attached to NCAM [42, 43].

One of the most studied characteristics of PSA is its ability to act as a de-adhesive factor, causing steric hindrance, between cellular membranes. Cell surface expression of PSA constricts intercellular space between apposing cells [44], which in turn, decreases homophilic binding between NCAM and other cells adhesion molecules including Cadherins, L1 family, and Integrins [45], therefore acting as a permissive regulating factor rather than a specific instructive cue. PSA affects distinct developmental processes depending on the location and timing of its expression. For example, in the developing nervous system PSA creates conditions permissive for postmitotic migration of precursor cells. In the adult, migrating cells still retain PSA, such as progenitor cells migrating along rostral migratory stream from the subventricular zone to the olfactory bulb [46] and newborn granule cells in the hippocampus [47].

Recent studies show the ability of PSA to regulate ocular dominance plasticity [40]. Although PSA expression is highest in the embryonic stages, it is expressed in the postnatal brain at different levels depending on brain region and age. In the mouse visual cortex, PSA expression declines to almost undetectable levels shortly after eye opening, and this decline is attenuated by visual deprivation [40]. Indeed, PSA levels in visual cortex were higher in mice dark reared from birth compared to littermates reared in a normal light-dark cycle. This effect is echoed in the visual cortex contralateral to the eye that received daily intraocular injection of TTX compared to the ipsilateral cortex [40]. Since the developmental and activity-regulated expression of PSA inversely correlates with the maturation of GABAergic innervation [21], it is thus possible that PSA decline might be sufficient for GABAergic synapse maturation. Indeed, premature enzymatic removal of PSA in the developing visual cortex results in precocious maturation of perisomatic innervation by basket interneurons and enhanced inhibitory synaptic transmission. Most importantly, the same treatment causes an earlier onset of critical period plasticity in the visual cortex [40]. Since PSA removal promotes GABAergic synapse formation, and GABA signaling in turn further promotes the maturation of GABAergic innervation [33], together GABA signaling and PSA removal may constitute a positive feedback mechanism to accelerate GABAergic synapse formation once sensory experience begins, and consequently to induce the onset of critical period plasticity in the visual cortex. PSA also regulates glutamatergic synapse formation [48, 49] and affects neuron-glia interactions [50] thus the possibility of additional mechanisms by which PSA influences ocular dominance plasticity cannot be excluded.

What is the precise role of PSA in GABAergic circuit maturation? One possibility is that developmental and activity-dependent removal of PSA might coordinate the timing of axon and synapse morphogenesis during the maturation of GABAergic innervation; indeed precocious perisomatic synapse formation can be triggered by premature removal

of PSA. Excessive, premature synapse formation might constrain axon growth. Higher expression of PSA during the early postnatal weeks might attenuate interactions between basket cell axons and pyramidal neurons, thereby holding off synapse formation and promoting the elaboration of axon arbors. Subsequent activity-dependent removal of PSA might unmask mechanisms that are already in place along basket cell axon, allowing fast responses to local synaptogenic cues. A similar example of PSA regulating the timing of a biological process comes from studies of migrating neuronal precursor. When PSA is enzymatically removed from newly generated cells in the SVZ, they form neuronal processes and begin to express neuronal molecular markers. This premature developmental transition is dependent on cell contact and appears to involve signaling through NCAM and p59Fyn kinase [51].

Why is such a mechanism in place and what could be its purpose? Interestingly, long polymers of sialic acid are not found in invertebrates [43], where neural circuits are to a large extent genetically determined. This raises the possibility that PSA might have evolved to regulate vertebrate-specific developmental processes. An example is the role of PSA in cell migration and differentiation. In invertebrates, the differentiation of neuronal precursors occurs close to the region of their birth and involves interactions with its immediate neighbor cells. On the other hand, in vertebrates, newly generated precursors often migrate long distances before acquiring their fate, and thus need to delay their differentiation till they reach their destination. Here, PSA plays a dual role whereby it (a) promotes cell migration by reducing cell-cell adhesion and (b) blocks differentiation by interfering with contact-dependent signaling until the cells arrive at their final location.

Such multifaceted roles for PSA are well suited for the complex experience-dependent neural circuit fine-tuning that occurs in vertebrate CNS. It is interesting to note that vision-dependent critical period plasticity does not start at the onset of eye opening. Instead, it is hypothesized that the critical period cannot start until the input to the circuit has developed reliability and precision [52]. Thus, cellular mechanisms underlying critical period are not simply an activity-dependent process; instead, it is a sequence of timed events that appear to be important. PSA might then act as "brake" that holds off the onset of critical period plasticity until input information can be reliably relayed to the cortex. The challenge is to understand what happens if and when this timing is altered, whether onset of critical period before the appropriate time might lead to incorrect refinement of neural circuit based on unreliable, or nonoptimal inputs, and whether and how this would in turn affect behavior.

4. Implications for Neurodevelopmental Disorders

GABAergic circuit dysfunction has been implicated in various neurodevelopmental and psychiatric disorders such as autism and schizophrenia [22, 53, 54]. Therefore, our understanding of the mechanisms that control formation and plasticity of GABAergic circuits will likely yield molecular and cellular substrates that might be altered in neurodevelopmental disorders.

Efforts to explore molecular mechanisms linking sensory experience to GABAergic circuit maturation have revealed several players that include both GABAergic synapse promoting factors (BDNF, Otx2, and GABA itself) and GABAergic synapse inhibiting factors (PSA). It has become increasingly clear that mechanisms are in place to tightly time events leading to the onset of critical period plasticity. This raises the question as to what maybe the correct or most permissible sequence of events and whether the onset of critical period at a time when circuits are not "ready" could lead to an altered developmental trajectory.

GABA synthesis and signaling has been shown to regulate the maturation of GABAergic innervation in visual cortex and the onset of critical period plasticity [5, 33]. These findings suggest that alteration of GABA synthesis and signalling, either due to genetic or environmental causes, can potentially affect nearly all stages of cortical circuit formation, thereby leading to impaired brain development. For instance, SNPs in the 5′ regulatory region of the *Gad1* gene (coding for the GABA- synthesizing enzyme GAD67) are associated with childhood onset schizophrenia [55]. Moreover, allelic variations in *Gad1* have been shown to associate with schizophrenia and to influence multiple domains of cognition, including declarative memory, attention and working memory [56]. This is interesting because reduction in the expression levels of GAD67 in the dorsal lateral prefrontal cortex is one of the most consistent molecular pathological findings in individuals with schizophrenia [22]. However, whether and how these genetic variants are directly involved in the regulation of *Gad1* expression levels is still unknown.

In addition, the multifaceted role of GABA during cortical circuits development draws our attention to the possible deleterious effects of drugs acting on GABA receptors, notably benzodiazepines or certain antiepileptic agents, on brain development. Recent evidence from both clinical and animal studies suggests that certain antiepileptic drugs could interfere with normal cognitive development [57]. Further studies are required to understand if GABA-targeting drugs could have long-term consequences in young children by interfering, between other things, with critical period plasticity.

GABAergic circuit dysfunction has also been implicated in autism spectrum disorders, including Rett's syndrome [53, 54]. The homeodomain transcription factor Dlx5, which regulates the differentiation and maturation of forebrain GABAergic interneurons, has been identified as a direct target of MeCP2 [58], which is linked to Rett's syndrome. Critical period OD plasticity is altered in MeCP2 mutant mice, a well-recognized model for Rett's syndrome [59]. Recent studies using transgenic mice lacking MeCP2 selectively in GABAergic neurons show that these mice behaviorally recapitulate many features of Rett's syndrome, linking decreased Gad levels and compromised MeCP2 function in GABAergic neurons to the neuropsychiatric phenotype [60].

Altered PSA levels are associated with various neuropathological conditions including schizophrenia [61, 62]

and temporal lobe epilepsy [63]. In particular, a decrease in polysialylation of hippocampal neurons in schizophrenic brains correlates with early disease incidence [61, 64]. Recently, the chromosome where ST8SIA2, the human STX-encoding gene, is localized, 15q26, was reported as a common susceptibility region for both schizophrenia and bipolar disorder in a genome scan of Eastern Quebec families [65]. Convergent evidence from the Chinese Han and Japanese population [66, 67] strongly supports the possibility that developmental abnormalities associated with defective polysialylation may be involved in schizophrenia.

In summary, multiple lines of evidence concur that alterations in molecular mechanisms of GABAergic synapse development and regulation of critical period plasticity are associated with neurodevelopmental disorders. Aberrant development of GABAergic circuits has been implicated in various dysfunctions such as autism, schizophrenia, Rett syndrome, and epilepsy. Further research along these lines will help elucidate how and whether critical period plasticity is affected, which molecular pathway is critical, and whether therapeutic intervention is possible. Exciting recent evidence points to possible strategies to reopen plasticity in a mature brain [68–70]. Altogether, increasing knowledge of such molecular mechanisms will further our understanding of the regulation of developmental plasticity in the brain and aid in designing strategies aimed to increase adaptive circuit rewiring following insult, such as stroke, and in developing rational pharmacological approaches to correct alterations in the brain of children with neurodevelopmental disorders.

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Review Article

Spatial and Temporal Dynamics in the Ionic Driving Force for $GABA_A$ Receptors

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It is becoming increasingly apparent that the strength of GABAergic synaptic transmission is dynamic. One parameter that can establish differences in the actions of GABAergic synapses is the ionic driving force for the chloride-permeable $GABA_A$ receptor ($GABA_AR$). Here we review some of the sophisticated ways in which this ionic driving force can vary within neuronal circuits. This driving force for $GABA_ARs$ is subject to tight spatial control, with the distribution of Cl^- transporter proteins and channels generating regional variation in the strength of $GABA_AR$ signalling across a single neuron. $GABA_AR$ dynamics can result from short-term changes in their driving force, which involve the temporary accumulation or depletion of intracellular Cl^-. In addition, activity-dependent changes in the expression and function of Cl^- regulating proteins can result in long-term shifts in the driving force for $GABA_ARs$. The multifaceted regulation of the ionic driving force for $GABA_ARs$ has wide ranging implications for mature brain function, neural circuit development, and disease.

1. Introduction

$GABA_A$ receptors ($GABA_ARs$) are the principal mediators of fast synaptic inhibition in the brain. These receptors differ from most ligand-gated ion channels in that their reversal potential (E_{GABA}) is close to the resting membrane potential of neurons. Consequently, $GABA_ARs$ have the capacity to exhibit a different form of dynamics whereby small changes to the driving force of the underlying anionic currents can lead to significant changes in the nature and strength of $GABA_AR$-mediated transmission. For instance, if E_{GABA} is more negative than the resting membrane potential $GABA_AR$ activation will result in membrane hyperpolarisation and inhibition. If E_{GABA} is more positive than the resting membrane potential however, stimulating $GABA_ARs$ will result in a combination of membrane depolarization and shunting inhibition. $GABA_AR$ activation is only excitatory if E_{GABA} is positive enough to increase the probability of action potential generation.

The best described example of E_{GABA} modulation occurs during early development when neurons in the hippocampus and other brain structures have been shown to undergo a shift in the ionic driving force for $GABA_ARs$ from depolarising to hyperpolarising [1–4]. This change is the result of a developmental decrease in the levels of intracellular chloride ($[Cl^-]_i$), brought about by the increased contribution of the K^+-Cl^- cotransporter, KCC2, which extrudes Cl^-, compared to the Na^+-K^+-$2Cl^-$ cotransporter, NKCC1, which normally functions to raise $[Cl^-]_i$ [5]. Changes to $[Cl^-]_i$ and $GABA_AR$-mediated currents have also been described as a result of neural trauma [6–17]. Since the reports that the Cl^- driving force for $GABA_ARs$ is altered during development and in particular CNS disorders, there has been further careful examination of how neurons regulate $[Cl^-]_i$. This work confirms that $[Cl^-]_i$ and the associated ionic driving force for $GABA_ARs$ cannot be thought of as a fixed parameter. Rather, sophisticated mechanisms impact how Cl^- is regulated in space and time, such that $[Cl^-]_i$ can vary between cells, within different parts of the same cell, and as a function of the history of the cell and the network in which it resides. Appreciating these mechanisms is important for understanding GABAergic signalling, not only in the mature nervous system, but also during neural circuit formation and in the context of CNS disorders. The diagram in Figure 1 provides an outline for this review by illustrating three ways in which the ionic driving force for $GABA_ARs$ may exhibit differences. We will focus on recent

work that has examined how spatial properties of neurons have been linked to differences in $[Cl^-]_i$ and how activity-dependent mechanisms can generate both short- and long-term changes in $[Cl^-]_i$. In doing so, we will also discuss the potential functional consequences of spatial and temporal differences in driving force for $GABA_A$Rs.

2. Spatial Variations in E_{GABA}

Over recent years it has become increasingly apparent that the notion of universally hyperpolarising E_{GABA} in mature neurons of the CNS is a misleading one. E_{GABA} can vary across different types of neurons and this leads to different actions of $GABA_A$R postsynaptic potentials (GPSPs) depending on the cell type in question [18–20]. For example, fast spiking inhibitory interneurons in the cortex and amygdala exhibit a considerably more depolarised E_{GABA} than neighbouring pyramidal cells, which may contribute to differences in the excitability of these two cell types [18]. What has also come to be appreciated is the fact that as well as intercellular variability, E_{GABA} can show *intracellular* differences. One of the most prominent examples involves the axon initial segment (AIS). Here, the E_{GABA} of inputs from axoaxonic (or Chandelier) cells tend to be significantly more positive than the E_{GABA} of separate GABAergic inputs targeting the soma [21–23] (see Figure 1). Axonal E_{GABA}, as determined in three studies, was found to be between 6 and 22 mV more positive than somatic E_{GABA} [21–23]. Such within-cell variations in E_{GABA} have been linked to the differential distribution of Cl^- cotransporter proteins. Immunogold labelling of KCC2 in hippocampal pyramidal and dentate gyrus cells has shown that the levels of this transporter are severalfold higher in the soma compared to the AIS, with local KCC2 densities at the plasma membrane of the AIS at around 6% the level of somatic KCC2 [23, 24]. NKCC1-null cells, or cells treated with bumetanide, do not exhibit axosomatic $[Cl^-]_i$ gradients, which indicates that NKCC1 is key to maintaining the higher E_{GABA} values recorded at the AIS [22].

The degree of differences in E_{GABA} between axon and soma may vary across different cell types and whether the resultant effect of an axoaxonic GABAergic input to a neuron is depolarising, hyperpolarising, inhibitory, or even excitatory is still not clear [29, 30]. The location of the AIS is close to the proposed site of action potential initiation and thus one might predict that if axoaxonic inputs are indeed depolarising these could help promote action potential initiation [21]. However, despite numerous studies [21–23, 30, 31] there is limited evidence that $GABA_A$R synapses formed by axoaxonic cells at the AIS are able to trigger action potentials in the postsynaptic neuron. It is important to note that, even with depolarising driving forces, $GABA_A$R synapses may still exert strong inhibitory effects via their shunting action upon excitatory currents [32]. Consequently, whether AIS $GABA_A$R synapses are capable of evoking excitatory responses in pyramidal cells is still an open question and one that will be dependent on factors such as the number and relative timing of GABAergic and glutamatergic inputs, the magnitude of the $GABA_A$R conductance and whether or not the depolarising actions persist beyond the shunting effect [33].

Local $[Cl^-]_i$ differences can also be found between the soma and dendrites of several types of neurons [22, 34, 35]. For example, $[Cl^-]_i$ has been shown to be higher and more depolarising in the dendrites than in the soma of certain ON-type retinal bipolar cells, a difference that underlies the receptive field properties of these neurons [34]. Numerous other studies, utilising a wide array of different techniques and preparations, have reported considerable variation in the strength and direction of somatodendritic Cl^- gradients [22, 35–39]. These differences can typically be explained by compartment specific expression of Cl^- transporter proteins regulated as a function of development, cell type, and brain region [34, 40]. However, it is worth remembering that because the degree of phasic and tonic $GABA_A$R activity can itself influence $[Cl^-]_i$, and can also vary significantly between different experimental preparations, this may affect estimates of $[Cl^-]_i$ [41].

In a recent study, Földy et al. [42] discovered intracellular Cl^- regulation on an even more spatially refined scale and via a mechanism involving Cl^- regulators other than transport proteins. The authors examined the conductance and current-rectification properties of two types of GABAergic input onto the same perisomatic region of CA1 pyramidal neurons. Their recordings revealed that $GABA_A$R currents at synapses receiving presynaptic input from parvalbumin-expressing fast-spiking basket cells (PVBCs) are selectively modulated by the voltage-gated Cl^- channel ClC-2. ClC-2 is found in the soma of pyramidal neurons and is an inwardly rectifying channel, which is activated by neuronal hyperpolarisation and allows Cl^- to flow out of the cell more easily than into it [43, 44]. ClC-2 activity was found to be strongly associated with PVBC synapses, in contrast to neighbouring synapses formed by cholecystokinin-expressing basket cells (CCKBCs). As a consequence, rates of Cl^- extrusion following intense $GABA_A$R activity were found to be significantly faster at PVBC synapses. This is supported by Rinke et al. [45], who reported that neurons from mice lacking the ClC-2 channel show reduced rates of Cl^- removal and by the fact that the resting E_{GABA} at PVBC synapses is significantly lower than at CCKBC synapses [42]. The authors suggest that the presence of somatic CLC-2 and its contribution to Cl^- regulation could play an important role in preventing potentially detrimental increases in $[Cl^-]_i$ during periods of intense firing by soma targeting PVBCs [42]. As Földy et al. point out, their findings could be partly explained at a compartmental level, as the somatodendritic distribution of PVBC and CCKBC synapses does show some differences. Nevertheless, these recent studies have advanced our appreciation of Cl^- regulation by showing that, as well as being nonuniform across different neuronal compartments, E_{GABA} may vary between individual synapses within the same compartment. Thus, even assigning E_{GABA} to certain spatial regions of a cell may be an oversimplification and instead it could be more appropriate to consider E_{GABA} in terms of a particular input to a postsynaptic cell [46].

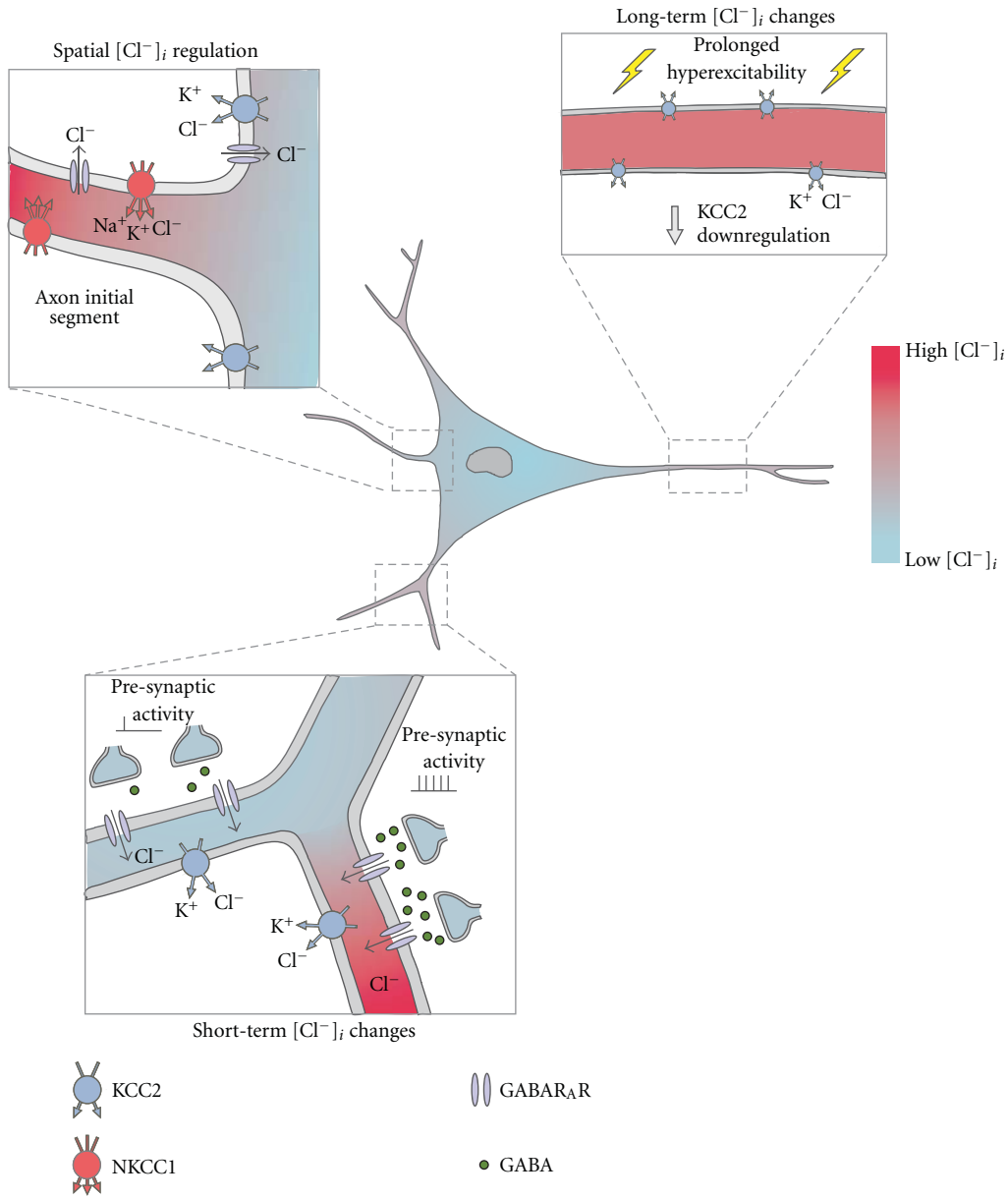

Figure 1: $[Cl^-]_i$ and the associated driving force for $GABA_A$Rs can be subject to spatial and activity-dependent temporal variations. The upper left panel shows an example of spatially regulated $[Cl^-]_i$. It has been reported that low levels of KCC2 expression within the axon initial segment enable NKCC1 to maintain relatively high levels of $[Cl^-]_i$ compared to the soma (indicated by the red colour inside the cell) [22–24]. This can generate a depolarising Cl^- driving force for $GABA_A$Rs within the axon [21–23]. The lower left panel shows an example of short-term $[Cl^-]_i$ loading within dendritic branches. Cl^- influx associated with low-level $GABA_A$R activity is dealt with by Cl^- regulation mechanisms (left-hand dendritic branch). However, during periods of intense $GABA_A$R activation, if E_{Cl^-} is hyperpolarised with respect to the membrane potential, high levels of Cl^- influx via $GABA_A$Rs can lead to localised increases in $[Cl^-]_i$ and consequently depolarising shifts in E_{GABA} (right-hand dendritic branch) [25, 26]. The upper right panel illustrates an example of long-term $[Cl^-]_i$ changes. Certain patterns of neural activity within mature neurons (e.g., repetitive coincidental pre- and postsynaptic spiking or prolonged postsynaptic spiking, interictal-like activity) can lead to a downregulation in KCC2 activity, resulting in long-term increases in $[Cl^-]_i$ [10, 27, 28].

3. Short-Term Temporal Changes in E_{GABA}

In addition to spatial variation, E_{GABA} can also show rapid temporal changes within individual cells (see Figure 1). It is well known that responses to intense $GABA_A$R activation can change from being hyperpolarising to depolarising in less than a second [36, 47, 48]. Such biphasic responses are now generally thought to represent a depolarising shift in E_{GABA}, caused by the differential collapse of the opposing concentration gradients of Cl^- and HCO_3^- [25, 26, 49]. $GABA_A$Rs are approximately five times more permeable to Cl^- than HCO_3^- [50]. Therefore at rest, E_{GABA} (typically -75 mV) is much closer to the very negative Cl^- reversal (E_{Cl^-}; typically -85 mV) than the considerably more positive

HCO_3^- reversal ($E_{HCO_3^-}$; typically −20 mV) [51]. During intense activation of $GABA_A$Rs however, rapid Cl^- influx exceeds Cl^- extrusion mechanisms and a breakdown in the Cl^- gradient occurs. An equivalent collapse of the HCO_3^- gradient is prevented by the activity of intra- and extracellular carbonic anhydrases, which use CO_2 as a substrate to rapidly regenerate intracellular HCO_3^-. As a result, with continued $GABA_A$R activation E_{GABA} shifts toward the more positive $E_{HCO_3^-}$, and this accounts for the depolarising phase of the biphasic response [25, 52]. Indeed, by blocking carbonic anhydrase with the drug acetazolamide, the depolarising response to strong $GABA_A$R activation is prevented [26]. Interestingly, a recent paper argues that this GABA elicited depolarisation is paradoxically accentuated by the activity of the electroneutral cotransporter KCC2 [53]. Following the $GABA_A$R—mediated accumulation of intracellular Cl^-, this leads to an accelerated extrusion of both Cl^- and K^+ by KCC2. Provided this extrusion of K^+ occurs within a large enough neuronal population, the increase in extracellular K^+ can result in inward K^+ currents that further depolarise the cell membrane [49, 53].

The shifts in E_{GABA} that are associated with intense $GABA_A$R activation are transient, such that once $GABA_A$R activity subsides $[Cl^-]_i$ returns to baseline levels within seconds or minutes [25, 54]. Any factor that affects the rate of Cl^- accumulation during $GABA_A$R activation will affect how rapidly and by how much E_{GABA} shifts. For instance, the volume of the neuronal compartment that receives the GABAergic input is one important parameter. For a given amount of synaptic $GABA_A$R stimulation and its accompanying Cl^- influx, smaller postsynaptic volumes will result in relatively larger increases in $[Cl^-]_i$. As a result, dendritic compartments are more susceptible to Cl^- accumulation (and hence depolarising shifts in E_{GABA}) than the soma [25, 41]. In a theoretical paper, Qian and Sejnowski [55] utilised this reasoning to suggest that $GABA_A$R-mediated inhibition is likely to be ineffective on dendritic spines. Due to their minute volume, even small amounts of Cl^- influx would result in a local increase in $[Cl^-]_i$ that would rapidly depolarise E_{GABA}. Consistent with this idea, it has since been confirmed that most GABAergic synapses are localised to dendritic shafts as opposed to spines [56, 57]. As described above, another important factor that affects Cl^- accumulation during $GABA_A$R activity is the presence, affinity and capacity of carbonic anhydrase. Given the significance of cell volume and carbonic anhydrase activity, it is perhaps not surprising therefore that different cell types might differ in their susceptibility to Cl^- accumulation. For example, Lamsa and Taira [54] found that 10–100 Hz stimulation trains produce depolarising switches in the E_{GABA} of interneurons of the CA3 stratum pyramidale and stratum oriens regions, but were unable to evoke similar shifts in CA3 pyramidal neurons.

In order to evoke the depolarising shifts in E_{GABA} described above, intense $GABA_A$R activation has been elicited either by exogenous application of $GABA_A$R agonists or high-frequency stimulation of GABAergic afferents. Evidence that such short-term changes in E_{GABA} could occur in vivo have come from studies of hyperactive network activity patterns, such as those generated in experimental models of epilepsy. It is believed that the intense activation of $GABA_A$Rs that occurs during seizures can cause rapid Cl^- accumulation [58–62]. Indeed, the resultant erosion of $GABA_A$R-mediated inhibition serves to initiate or exacerbate the hyperexcitability that is characteristic of epileptiform events [63]. Beyond seizure activity, it is currently an open question as to what range of physiologically relevant activity patterns could lead to short-term changes to E_{GABA}, and what the functional impact upon circuit function might be. Nevertheless it is interesting that levels of $[Cl^-]_i$ accumulation would appear to increase linearly with the intensity and number of stimulations, and even relatively weak stimulation can produce small changes in $[Cl^-]_i$ [62, 64].

Another area that has yet to be fully investigated concerns how short-term activity-dependent shifts in E_{GABA} might affect developing neurons. It has already been established that during the first two weeks of postnatal life, rat hippocampal neurons express low levels of intracellular carbonic anhydrase and therefore do not exhibit the HCO_3^- dependent $GABA_A$R depolarisation that mature neurons display following high-frequency synaptic activity [52, 65]. And it seems likely that other properties of immature neurons would contribute to a different susceptibility to activity-driven Cl^- accumulation or depletion. These include the higher resting $[Cl^-]_i$ observed in young neurons, plus different expression patterns of Cl^- transporter proteins [5, 66, 67] and Cl^- permeable channels [45, 68]. One area for future work will be to dissect the role that short-term activity-driven shifts in E_{GABA} play in both the normal and abnormal development of neural circuits.

4. Long-Term Temporal Changes in E_{GABA}

As we saw in the previous section, brief periods of high-intensity synaptic activity can give rise to short-term changes in the ionic driving force for $GABA_A$Rs. There are however, a growing number of examples whereby different forms of neural activity can give rise to more enduring changes in E_{GABA} and the underlying $[Cl^-]_i$ (see Figure 1). Many of these long-term changes in E_{GABA} are linked to hyperexcitability disorders, such as epilepsy [8–10, 15, 16, 69] and neuropathic pain [7, 17, 70, 71] and have also been observed in other cases of neuronal trauma such as neural axotomy [11], ischemia [12, 13], and in spasticity models following spinal cord injury [14]. Yet similar long-lasting changes to E_{GABA} have also been reported in healthy tissue following certain neural activity patterns [27, 28, 72–78]. In order to better understand these shifts in inhibitory plasticity and their roles in both healthy and pathological neural signalling, a number of studies have begun to investigate the underlying mechanisms behind long-term activity-dependent changes to E_{GABA}.

One of the first such investigations focused on the effects of epileptiform activity in hippocampal slices. Here, interictal activity, brought on with low Mg^{2+} conditions, switched the driving force of GPSPs from hyperpolarising to depolarising in CA1 pyramidal cells [10]. This depolarising shift in E_{GABA} corresponded to a significant reduction in

KCC2 mRNA and protein levels, as well as an increased rate of removal of the Cl^- cotransporter from the cell membrane [10]. Similar reductions in KCC2 mRNA and protein could also be observed following *in vivo* kindling [9], and in both cases the activity-led downregulation in KCC2 expression was found to be dependent on BDNF signaling. Scavenging endogenous BDNF with TrkB receptor bodies, or pharmacologically inhibiting TrkB, blocked the activity-induced downregulation of KCC2 and thus suggests that the mechanism involves a BDNF-TrkB signalling interaction [9, 10]. A similar role for BDNF-TrkB signalling has since been reported in the context of positive shifts in E_{GABA} and reductions in KCC2 levels within neuropathic and inflammatory pain models [7, 79, 80], suggesting that endogenous BDNF signalling may be a common mechanism by which KCC2 is downregulated during aberrant neural activity.

Aside from pathological models, changes to E_{GABA} and the resultant inhibitory plasticity have also been investigated in the context of more normal physiological signalling. For example, periods of paired pre- and postsynaptic spiking activity have been found to lead to a small but persistent depolarising shift in the postsynaptic E_{GABA}, of around 3-4 mV in mature rat hippocampal pyramidal neurons [27]. Such long-term depolarising shifts in E_{GABA} have also been observed following sustained periods of postsynaptic spiking at frequencies of 10–20 Hz, without presynaptic activity [28]. In both cases the reduction in GABAergic synaptic inhibition was linked to a sustained decrease in KCC2 transporter activity, which in turn was dependent upon Ca^{2+} signalling via L-type Ca^{2+} channels [27, 28]. Further investigation revealed that the activity-dependent downregulation in KCC2 activity requires protein kinase C (PKC) activity, although other studies have since shown that PKC can promote KCC2 activity by stabilising the cotransporter at the membrane surface [81]. Interestingly, Wang et al. [77] recorded from neurons of the subthalamic nucleus and showed for the first time that GABAergic plasticity could be induced in either direction, either generating hyperpolarising or depolarising shifts in GPSPs depending on the degree of rebound spiking activity. Based on further pharmacological experiments the authors proposed that the level of Ca^{2+} increases may be key to determining the nature of $GABA_AR$ plasticity, with large increases being associated with negative shifts in E_{GABA} and small rises in Ca^{2+} leading to positive shifts in E_{GABA} [77].

Developmental stage would appear to be critically important for determining the nature and mechanism underlying long-term changes in the ionic driving force for $GABA_ARs$. Within mature cells, such activity-driven changes appear to work by targeting KCC2 and reducing the activity and/or expression of this transporter. This raises the question of what happens within younger neurons when E_{GABA} is still depolarising and levels of KCC2 protein are typically low. Are immature neural networks subject to similar activity-dependent long-term $[Cl^-]_i$ alterations and if so, what are the downstream targets for such mechanisms? To date only a small number of studies have addressed this question directly but already an interesting dichotomy between mature and immature $GABA_AR$ plasticity regulation is beginning to emerge. For example, as already mentioned, in mature hippocampal slices when E_{GABA} is hyperpolarising, application of seizure models has been linked to a depolarising shift in E_{GABA} values coupled with a downregulation in KCC2 expression [9, 10]. By contrast, in neonatal hippocampal slices, seizure activity induced by kainic acid have been found to result in either a depolarising [8, 82] or a hyperpolarising shift in E_{GABA} [83, 84]. In the latter cases, more negative E_{GABA} values have been linked to an increase in KCC2 expression and activity [84, 85].

Such variations may be partially due to the type of seizure model used, yet similar age-dependent differences can also be found in other examples of activity-driven E_{GABA} changes. For example, a protocol of paired pre- and postsynaptic spiking at 5 Hz, which has been shown previously to elicit depolarising E_{GABA} shifts in the mature rat hippocampus [27], actually produces a long-term hyperpolarising shift when applied to the same neurons earlier in development [72, 78]. Rather than targeting KCC2, the hyperpolarising shift in E_{GABA} in immature neurons occurs via a down-regulation of the NKCC1 transporter, which results in a decrease in $[Cl^-]_i$ [72]. As in mature systems, the direction of such $GABA_AR$ shifts in developing neurons can change according to the nature of the stimulus. While paired pre- and postsynaptic spiking at 5 Hz hyperpolarised E_{GABA} at developing synapses, stimulation at higher frequencies (20–50 Hz) produces the opposite effect and results in E_{GABA} values that are more depolarising [78]. This long-term shift in driving force for $GABA_ARs$ induced by high-frequency paired spiking was again mediated through the regulation of NKCC1 activity and required increases in intracellular Ca^{2+}, either via L-type Ca^{2+} channels or from internal Ca^{2+} stores [78]. Thus, while spiking-induced activation of L-type Ca^{2+} channels can result in a similar increase in $[Cl^-]_i$ in both mature and immature hippocampal neurons, the frequency at which it occurs, and the Cl^- cotransporter that is targeted, varies according to developmental stage. A similar phenomenon has been observed following periods of experimentally induced postsynaptic spiking. Prolonged spiking at 20 Hz has been shown to lead to depolarising shifts in E_{GABA} within both the mature [28] and immature hippocampus [86]. Yet while the underlying mechanism has been linked to Ca^{2+} influx and KCC2 downregulation in mature cells [28], in younger neurons the change in E_{GABA} would appear to occur via a different mechanism. Here, postsynaptic spiking is believed to trigger increases in Na^+-K^+-ATPase activity, which alters the balance of Na^+ across the membrane. This shifts the thermodynamic equilibrium of NKCC1 and results in an increase in the rate at which Cl^- is transported into the cell [86]. Thus, just as spatial regulation of E_{GABA} can show age-specific variation, the mechanisms underlying long-term activity-dependent changes in E_{GABA} can also vary according to the developmental stage of the neuron.

What are the functional consequences of such long-term alterations to E_{GABA}? Changes to $[Cl^-]_i$ and the resultant Cl^- driving force for $GABA_ARs$ have been speculated to be involved in long-term potentiation (LTP)—the best

studied form of persistent change in synaptic efficacy. In a recent study, Ormond and Woodin [73] found that paired stimulation protocols designed to induce glutamatergic LTP in mature rat hippocampal slices also produced depolarising shifts in E_{GABA}. The resultant reduction in the strength of inhibitory synaptic input occurred in parallel to "classical" LTP at glutamatergic synapses, with both serving to potentiate synaptic transmission. As with classical LTP, this form of disinhibition-mediated potentiation was found to be dependent upon Ca^{2+} influx via NMDARs [73]. Indeed, other work has shown that activation of NMDARs can lead to a rapid and enduring decrease in KCC2-mediated Cl^- transport [87], while NMDAR signalling during LTP induction leads to a reduction in the total levels of KCC2 [88]. It has yet to be established whether or not this apparent GABAergic plasticity is relatively synapse specific, as has been reported for glutamatergic LTP, or whether GABAergic inputs are affected across larger parts of the dendrite or indeed across the entire cell. Nevertheless, these findings raise the possibility that the expression of NMDAR-mediated LTP might involve a component of GABAergic plasticity.

Amongst neurological disorders, neural trauma and hyperactivity have been shown to lead to long-term changes in the E_{GABA} of the affected neurons. Yet such changes to the ionic driving force for $GABA_A$Rs may in turn work to contribute to, or exacerbate, the abnormal activity patterns associated with these pathological states. In a landmark paper investigating the propagation of epileptic activity between two interconnected and intact hippocampi, Khalilov et al. [8] showed that seizure activity in one hippocampus could propagate to the naive hippocampus and eventually transform it into an epileptic structure capable of generating seizures. Subsequent investigation of the E_{Cl^-} of neurons in this secondary epileptic focus revealed that the cells had undergone an excitatory shift in the driving force of their $GABA_A$R synapses. Stimulating $GABA_A$Rs within the secondary focus resulted in bursts of action potentials in the absence of any glutamatergic signalling, leading the authors to conclude that such excitatory actions of GABA may generate seizures in the newly epileptic tissue [8]. Such shifts in the E_{GABA} do not need to be overtly excitatory in order to alter neural circuit activity. In rat dentate granule cells, induction of status epilepticus via *in vivo* pilocarpine injections can lead to depolarising E_{GABA} and impaired Cl^- extrusion capabilities [15]. The depolarising GPSPs increased the probability of action potential generation when paired with excitatory inputs and compromised the ability of the dentate gyrus to filter inputs from the entorhinal cortex [15].

The long-term and short-term changes to E_{GABA} observed in pathological states, or following pathological activity patterns, can be considered as relatively large changes, often switching the driving force of $GABA_A$Rs from hyperpolarising to depolarising and even excitatory [8, 10, 15, 60–62, 70]. By contrast, changes to $[Cl^-]_i$ following what could be considered more physiologically normal spiking activity typically result in much smaller modifications to the driving force for $GABA_A$Rs, usually within the range of approximately 3–10 millivolts. Given these relatively modest shifts an important question is to what extent such plasticity might alter subsequent activity in the affected cells. Artificially setting the E_{GABA} of a neuron is one way of exploring how changes to the ionic driving force for $GABA_A$Rs may impact activity. This has been achieved experimentally by either altering $[Cl^-]_i$ via intracellular dialysis of different Cl^- concentrations during whole-cell recordings, or by simulating GABAergic inputs with different E_{GABA} values using the dynamic-clamp recording configuration. In several studies that have adopted these approaches, shifting E_{GABA} to depolarising values led to increased neuronal excitability, resulting in enhanced spiking probability and reduced spike latencies in response to GABAergic inputs, as well as facilitation of voltage-sensitive NMDAR transmission [89–92]. Another approach which has made it possible to explore the functional impact of relatively small changes in $[Cl^-]_i$ and E_{GABA} has been computational modelling. These studies have shown that modest shifts in E_{GABA} can have a significant impact on neural signalling. For example, changing the E_{GABA} in a model of a mature CA1 pyramidal neuron from -75 mV to -70 mV (a similar level of long-term depolarising shift to that observed experimentally) results in an increase in action potential firing frequency by approximately 40% [92]. Likewise, positive shifts in inhibitory reversal potentials by as little as 10 mV can markedly shorten the duration of inhibitory inputs within the soma [33]. Changes to neural output resulting from modest shifts in E_{GABA} can be further exaggerated depending on other factors such as the frequency and location of GABAergic inputs [33, 93]. For instance, in neonatal spinal cord, GPSPs are depolarising but still mediate inhibitory effects via shunting actions. In computational models of these neurons, moving the E_{Cl^-} to more depolarised values reduces the time window over which GPSPs exert functional inhibition of excitatory activity within the soma, particularly when the shift in E_{Cl^-} occurs at distal inhibitory inputs so that shunting effects associated with the GABAergic conductance have less impact [33].

Modest changes to E_{GABA} are likely to be especially significant when the balance between $GABA_A$R inhibition and facilitation is a fine one. For example, in neocortical layer 5 pyramidal neurons E_{GABA} has been calculated to lie at values more depolarising than the resting membrane potential, but below the action potential threshold [94]. Depending on their timing in relation to glutamatergic inputs, somatic $GABA_A$R inputs can either shunt or facilitate excitatory inputs, which can impose a bidirectional modulation on neuronal firing rates [94]. By simulating different timing relationships between GABAergic and glutamatergic inputs in a model neocortical neuron, Morita et al. [95] showed that such bidirectional modulation of firing rates by $GABA_A$Rs was possible when the E_{GABA} lies within a narrow range of values close to the original E_{GABA} value calculated by Gulledge and Stuart [94]. Increasing E_{GABA} by only a few millivolts was enough to severely reduce the relative timing window in which $GABA_A$R inputs could have an inhibitory effect upon neuronal firing rate compared to a facilitating one. Moving E_{GABA} more negative by a few millivolts, such that it was equal to the resting membrane potential of the model cell, was sufficient to render $GABA_A$R inputs

completely inhibitory, regardless of their relative timing to glutamatergic inputs [95]. Similarly, it has been shown that when E_{GABA} falls within a specific voltage range, GPSPs can have a bidirectional effect on spike times in visual cortex—either delaying or advancing the time of spikes relative to oscillatory changes in membrane potential [96]. As precise spike timing has been widely implicated in neural processing [97–99] and synaptic plasticity [97, 98], the alterations in spiking activity brought on by small shifts in E_{GABA} may therefore have important consequences for information coding and brain development.

5. Summary

In summary, the driving force for $GABA_A$Rs should not be considered a fixed parameter underlying fast synaptic inhibition, but rather a dynamic parameter, that exhibits both spatial and activity-dependent modulation. The concept that E_{GABA} changes in the context of neural development and certain neuropathological conditions is well established. However, more recent studies in this area have revealed that neurons have a range of sophisticated mechanisms for regulating the ionic driving force for $GABA_A$Rs. E_{GABA} has been reported to vary between different cellular compartments and may even exhibit synapse-specific variation within a single neuron. In addition, the driving force for $GABA_A$Rs can be changed "on the fly" and is subject to both short- and long-term temporal changes via a range of activity-dependent mechanisms. These processes are further subject to developmental regulation, where changes in activity patterns can target different regulators of $[Cl^-]_i$ and drive E_{GABA} in different directions depending on the age of the neuron. Further dissecting the mechanisms that regulate such a fundamental aspect of GABAergic transmission should improve our understanding of synaptic integration mechanisms in both health and disease.

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Research Article

Neurturin Evokes MAPK-Dependent Upregulation of Egr4 and KCC2 in Developing Neurons

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The K-Cl cotransporter KCC2 plays a crucial role in the functional development of $GABA_A$-mediated responses rendering GABA hyperpolarizing in adult neurons. We have previously shown that BDNF upregulates KCC2 in immature neurons through the transcription factor Egr4. The effect of BDNF on Egr4 and KCC2 was shown to be dependent on the activation of ERK1/2. Here we demonstrate that the trophic factor neurturin can also trigger Egr4 expression and upregulate KCC2 in an ERK1/2-dependent manner. These results show that Egr4 is an important component in the mechanism for trophic factor-mediated upregulation of KCC2 in immature neurons involving the activation of specific intracellular pathways common to BDNF and Neurturin.

1. Introduction

The maturation of $GABA_A$ mediated neurotransmission encompasses long-term qualitative changes in postsynaptic responses. Particularly important is the developmental shift from depolarizing to hyperpolarizing $GABA_A$-evoked responses. This transition is attributed to the increase in expression of the K-Cl cotransporter KCC2. The functional expression of this cotransporter keeps the intraneuronal chloride concentration below that predicted from passive distribution rendering the $GABA_A$ reversal potential more negative than the resting membrane potential [1].

We have shown previously that BDNF-mediated TrkB activation can regulate the expression of KCC2 differentially in adult and in early postnatal neurons [2–4]. Activation of TrkB by BDNF triggers two major intracellular cascades [5–8], namely, the Shc and the PLCγ pathways. (i) The Shc pathway includes the activation of a number of adaptor proteins such as Shc, SOS, and Grb2 that results in the GTP-loading of Ras that subsequently leads to the activation of the MAP kinase cascade. This comprises the sequential phosphorylation of Raf, Mek, and Erk. The phosphorylated Erk is then translocated to the nucleus where it can activate and induce the expression of transcription factors. (ii) In the PLCγ pathway, direct activation of PLCγ by TrkB leads to the breakdown of PIP2 into DAG and IP_3. Activation of PKC by DAG promotes the release of intracellular Ca^{2+} and the activation of Ca^{2+}-dependent proteins. Interestingly, although BDNF induces under normal physiological conditions downregulation of KCC2 in adult neurons, it can induce KCC2 up-regulation if TrkB activation specifically evokes signaling through the Shc pathway only [2, 4]. Also in immature neurons BDNF can induce KCC2 up-regulation in an MAPK-dependent manner acting though the transcription factor Egr4 [3] suggesting that BDNF/TrkB-mediated

upregulation of KCC2 use intracellular pathways downstream of Shc.

Other neurotrophic factors, can also trigger Shc/MAPK signaling. Neurturin belongs to the GDNF family of neurotrophic factors and it specifically binds to the GPI-anchored receptor GRFα2. The ligand binding triggers association of GFRα2 with transmembrane tyrosine-kinase receptor RET that in turn can activate signaling pathways including the Shc/MAPK intercellular cascades [9].

In the present report, we study the effect of Neurturin on the regulation of KCC2 at the transcriptional level. We found that Neurturin can trigger the expression of Egr4. Accordingly, Neurturin induces the activation of the KCC2 proximal promoter region that results in a significant increase in KCC2 protein expression in immature neurons. Most importantly, this requires MEK-dependent ERK phosphorylation. We also show that Neurturin can evoke up-regulation of KCC2 *in vivo* after a single intrahippocampal application. Taking into account that BDNF, acting through similar intracellular signaling cascades, is able to up-regulate KCC2 in immature neurons, we propose a general mechanism for trophic factor-mediated KCC2 gene regulation in immature neurons, where Egr4 downstream of the MAPK/ERK signaling pathway plays a crucial role.

2. Methods

2.1. Dissociated Cultures. All animal experiments were approved by the local ethics committee for animal research at the University of Helsinki. Standard dissociated hippocampal cultures were prepared from embryonic day 17 (E17) mice as described in the original protocol [10], with slight modifications. Briefly, a pregnant mouse was anaesthetized in a CO_2 chamber and sacrificed by cervical dislocation, embryos were removed, and hippocampi were dissected. Cells were dissociated by enzymatic treatment (0.25% trypsin for 15 min at 37°C) and plated on poly-DL-ornithine-coated coverslips (50000 cells/cm^2) in neurobasal medium containing B27 supplement (Gibco, Life Technologies). Before plating, the medium was preincubated on astroglial culture for 24 hours. Neuronal cultures were fed once a week by changing half of the medium. Astroglial cultures were prepared according to Banker and Goslin, 1998, and maintained in DMEM supplemented with 10% of foetal calf serum, penicillin 100 units/mL, and streptomycin 100 μg/mL.

2.2. Organotypic Cultures. Hippocampal organotypic cultures were prepared according to the method of Stoppini [11]. Transverse slices (thickness 350 μm) were cut from the hippocampi from P8 mice using a McIlwain tissue chopper. They were immediately placed on sterile Millicell-CM membranes (Millipore) in 6-well culture trays with 1 mL of plating medium. The plating medium was neurobasal medium containing B27 supplement (Gibco, Life Technologies), penicillin 100 units/mL and streptomycin 100 μg/mL. One day after plating the medium was changed to the growth medium Neurobasal/B27 without antibiotics. The cultures were grown at 37°C under 5% CO_2 in air, and the medium was changed twice a week.

2.3. Application of Growth Factors. Treatments of organotypic cultures were performed from div (day *in vitro*) 2 until div5. Dissociated cultures were treated with Neurturin during div1–div4, div8–div11, and div15–18. Neurturin (PeproTech Inc.) was added from the frozen stock once on the first day of the treatment period. The final concentration of Neurturin is indicated in Section 3.

2.4. Western Blotting. Neuronal cultures were rinsed in PBS, scraped into ice-cold lysis buffer (NaCl 150 mM; TritonX-100 1%; Doc 0.5%; SDS 0.1%; TrisHCl 50 mM pH 8.0) and homogenized. Hippocampal slices were homogenized directly with lysis buffer. Protein concentrations were determined using D_C Protein Assay kit (Bio-Rad). Samples were separated using 7.5% SDS-PAGE and transferred onto Hybond ECL nitrocellulose membrane (Amersham, Pharmacia Biotech). Blots were probed with anti-KCC2 rabbit polyclonal antibody [12] at 1 : 5000 dilution and anti-β-tubulin rabbit polyclonal antibody (Covance, PRB-435P, 1 : 3000), developed with ECL-plus (Amersham, Pharmacia Biotech), and visualized with luminescent image analyzer LAS-3000 (Fujifilm). Optical densities of the bands were analyzed with AIDA imaging software (Raytest).

2.5. Semi-Quantitative RT-PCR. Neuronal dissociated cultures treated with growth factors were used for total RNA isolation and reverse transcription reaction. Total RNA was isolated using RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. Isolated RNA samples were reverse transcribed using random hexamer primers and Superscript II reverse transcriptase (Life Technologies). The samples after reverse transcription were diluted 1/10, 1/20, 1/50, 1/100, and amplified for 30–34 cycles using DyNAzyme EXT polymerase (Finnzymes) to keep the product amplification in the exponential range for every primer pair (see Table 1).

The PCR conditions for all primer pairs were the same: 2 min of the initial denaturation at 95°C followed by 30–34 cycles with 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min. Products were analyzed on 1.5% agarose gel and visualized with luminescent image analyzer LAS-3000 (Fujifilm). Optical densities of the bands were analyzed with AIDA imaging software (Raytest).

2.6. Quantitative RT-PCR Analysis. Total RNA was isolated with the RNeasy Micro (Qiagen) kit. Typically, about 1 μg of total RNA was reverse transcribed using the SuperScript III Reverse Transcriptase (Life Technologies) and random primers (at 37°C) according to the manufacturer's protocol. The cDNA samples were amplified using the SYBR Green PCR Master Mix (Applied Biosystems) and detected via the ABI Prism 7000 Sequence Detection System (Applied Biosystems). Primers for Egr4 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) quantification were designed with the Express v2.0 software (Applied Biosystems) and contained, when possible, intronic sequence in between (see Table 2).

Table 1

Product	Forward	Reverse
RET	5′-AGGACCACACATCACTTTGAG-3′	5′-ATGAAAGGGTACTGACCATGG-3′
GFRα2	5′-TATTGGAGCATCCATCTGGG-3′	5′-AGCAGTTGGGCTTCTCCTTG-3′
GAPDH	5′-GCAAAGTGGAGATTGTTGCCAT-3′	5′-CCTTGACTGTGCCGTTGAATTT-3′

Table 2

Product	Forward	Reverse
Egr4	5′-TCTCTCCAAGCCCACCGAAG-3′	5′-AACCGCCTGGATGAAGAAGC-3′
GAPDH	5′-GCAAAGTGGAGATTGTTGCCAT-3′	5′-CCTTGACTGTGCCGTTGAATTT-3′

2.7. Transfection and Luciferase Assay. The neurons were transfected with the luciferase reporter construct using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol at div5. To avoid cytotoxicity, we used relatively low amounts of the luciferase construct (0.5 μg per 1-cm-diameter well). Two days after transfection the neurons were briefly washed with phosphate-buffered saline (PBS) and lysed in Passive Lysis Buffer (Promega). Renilla and Firefly luciferase activities were measured with a Dual-Luciferase Reporter Assay System according to the manufacturer's protocol.

2.8. In Vivo Injections of Neurturin. Neurturin (1 μg) was injected in hippocampus of P5-P6 rats. Animals were hypothermically anesthetized and heads were fixed in a surgical mask to maintain the skull stable. A midline incision was made on the head, and the hole was drilled in the skull. The stereotactic coordinates for injection were anteroposterior-1.8 mm (relative to the bregma), mediolateral 2 mm (relative to the bregma), dorsoventral-2 mm from the cortical surface. Neurturin was dissolved in 4 μL of saline solution (123 mM NaCl, 5 mM KCl, 1,25 mM NaH_2PO_4, 2 mM $MgSO_4$, 10 mM glucose, 2 mM $CaCl_2$, 10 mM HEPES, and pH 7.2) and injected in right-side hippocampus at ~1 μL/min. Similarly left-side hippocampus was injected with 4 μL of pure saline solution. After injection, the needle was left in the tissue for 2 minutes. The incision was sutured and the rat was allowed to fully recover before being placed back with littermates.

2.9. Image Analysis. For each injected brain a series of coronal hippocampal sections (see immunohistochemistry) was made at P8-P9, three days after injection. The sections in the series were numbered in succession starting from most frontal part of hippocampus. Each section contained Neurturin-injected side (right) as well as control side (left). KCC2 levels were analyzed by immunostaining in sections number 45, 90, 132, 177, 222, and 273. The section thickness was 7 μM which gives a sample interval of approximately 315 μm. In each brain analyzed, the injection site (defined by a scar) was found between section number 60 and section number 90 and located in right and left hemisphere in CA1 area of hippocampus.

Confocal images of immunostained tissue were made. For each image nine consecutive optical slices (0.8 μm) were made and merged for quantification of KCC2 signal intensity. The region of interest was manually highlighted and total intensity of immunostaining in the region was divided by its area. KCC2 intensity in Neurturin-injected hemisphere was normalized to the KCC2 intensity in corresponding area of the same section control hemisphere.

2.10. Immunohistochemistry. Rats were deeply anaesthetized with pentobarbital and perfused with 4% PFA in PBS. Brains were removed and stored overnight in 4% PFA in PBS at 4°C. Tissues were paraffin embedded and cut into 7-μm-thick sagittal (*in situ*) or coronal (*in vivo* injections) sections. Deparaffinized sections were washed with 1% SDS in TBST (0.1% Tween in TBS), treated with 100 μg/mL saponin in TBST for 30 min at room temperature, and then treated with 5% bovine serum albumin (BSA) in TBST for two hours at room temperature. Next, tissues were incubated with anti-KCC2 rabbit polyclonal antibody [12] diluted at 1:5000 in 2% BSA, 0.2% Triton X-100, TBST overnight at 4°C. Species-specific secondary antibodies: donkey antirabbit Cy3 (Jackson Laboratories, 711-166-152), donkey anti-rabbit Alexa Fluor 488, and goat antiguinea pig Alexa Fluor 488 (Molecular Probes, Invitrogen, catalog number: A-21206, A-11073, resp.) were used at 1 : 400 dilution. Sections were visualized with Leica TCS SP2 AOBS confocal system.

2.11. In Situ Hybridization. *In situ* hybridization on paraffin-embedded sections was done as described [13]. Sagittal sections (thickness 7 μm) were hybridized using ^{35}S-labelled antisense and sense (control) cRNA probes: RET-specific probe (nucleotides 2595–3191, X67812) and GFRα2 (full length, AF003825) [14]. No labeling above background was observed in the sense controls.

2.12. Statistics. The data represents the mean ± SEM. Statistical analysis was performed using one sample t-test in GraphPad Prism statistical software. Statistical significance was defined as $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; n represents the number of independent experiments.

3. Results

3.1. Neurturin Upregulates KCC2 Expression in Developing Organotypic and Dissociated Hippocampal Cultures. Our previous results provided evidence that Egr4 and KCC2

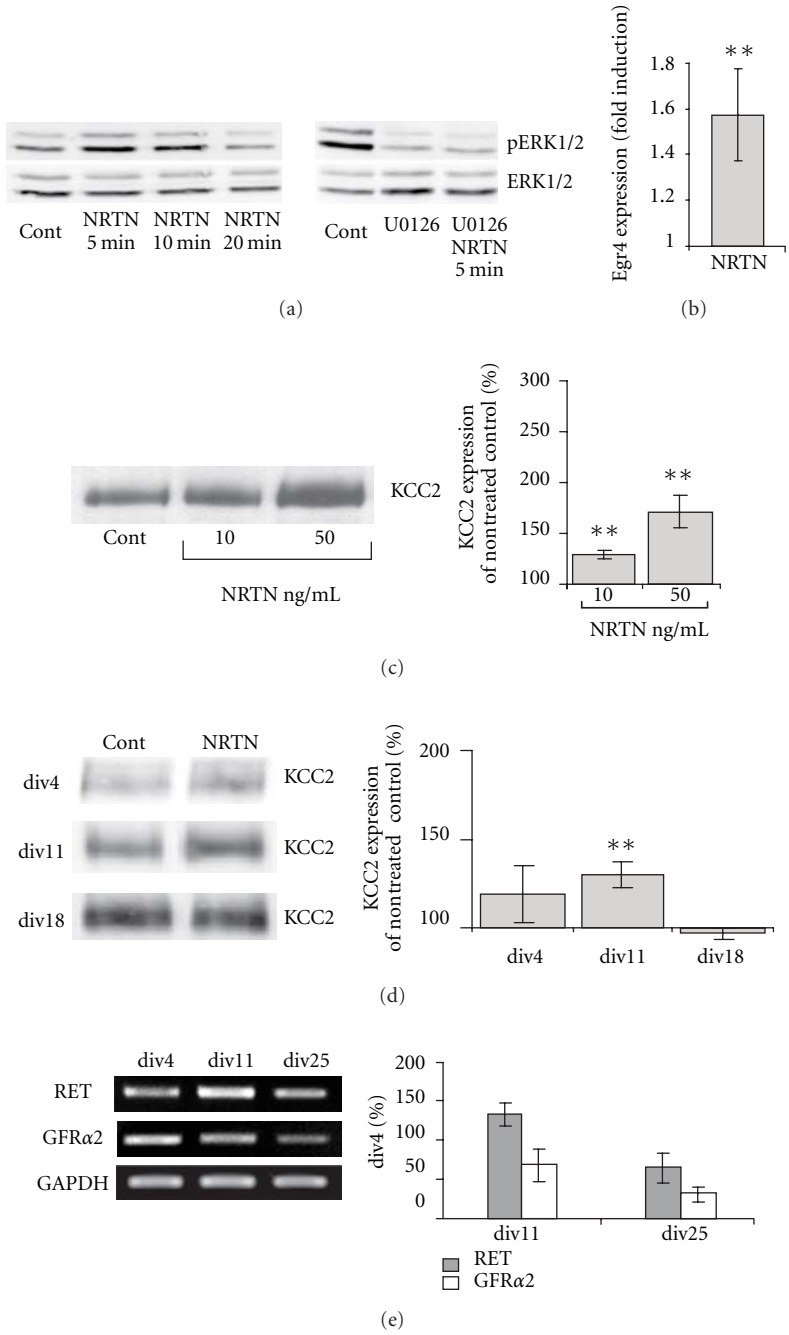

Figure 1: Regulation of KCC2 expression by Neurturin. (a) Representative Western blot analysis of Neurturin-induced ERK1/2 phosphorylation. Lysates of div5-dissociated cultures were collected 5–20 min after Neurturin (50 ng/mL) application. In some cases cultures were pretreated with MEK inhibitor U0126 (20 μM) 30 min before Neurturin application. (b) Egr4 mRNA level in div5–div10 dissociated cultures 1-2 hours after Neurturin (50 ng/mL) application as detected by real time PCR ($n = 5$). Nontreated control value was set to 1. ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Error bars represent SEM. (c) Representative Western blot analysis and quantification of KCC2 expression in organotypic hippocampal slice cultures treated with NTRN ($n = 4$–8). Organotypic cultures were treated with 10 ng/mL and 50 ng/mL Neurturin. Data are normalized to the value in nontreated controls ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Error bars represent SEM. (d) Representative Western blot analysis and quantification of KCC2 expression in dissociated hippocampal cultures treated with Neurturin (50 ng/mL) ($n = 3$–7). Dissociated cultures were treated with Neurturin at div1, div8 and div15 and analyzed 3 days after the treatment. Data are normalized to the value of non-treated controls of the corresponding age ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Error bars represent SEM. (e) Representative semiquantitative RT PCR from cDNA of different age cultures for RET, GFRα2, and GAPDH (used as internal standard) and summarized results of 3 similar PCRs. The data show that dissociated hippocampal neurons express detectable levels of growth factors receptors at all ages tested. Data are normalized to div4 value. Error bars represent SEM.

expression is regulated through ERK1/2-dependent mechanism [3]. This may imply that other means of MAPK activation might lead to the induction of KCC2 expression. Thus we analyzed the effect of Neurturin, another troph-ic factor that also induces MAPK activation [15]. In hippocam-pal primary cultures ERK1/2 phosphorylation was induced 5 minutes after Neurturin (50 ng/mL) application (Figure 1(a)). The effect was sensitive to MEK blocker U0126 (20 μM). Similarly to our previous results with BDNF [3], Neurturin induced Egr4 expression: Egr4 mRNA levels were 1.6 ± 0.2-fold higher than in control one hour after Neurturin application (Figure 1(b)).

Although acting through a very different type of tyrosine kinase receptor, Neurturin, similarly to BDNF, significantly upregulated KCC2 protein expression at 10 and 50 ng/mL (129 ± 4% and 171 ± 16%; 10 and 50 ng/ml resp. Figure 1(c)). Interestingly, the effect of Neurturin also had a ten-dency to decline with culture maturation. The strongest up-regulation of KCC2 was observed in two-weeks-old cultures (130 ± 7% of control; Figure 1(d)).

The developmental change in the effect of the neurotrophic factors could be caused by a difference in the expression level of corresponding receptors. Thus we monitored the expression of Neurturin coreceptors RET and GFRα2 in dissociated cultures at div4, div11, and div18 by semiquantitative RT-PCR (Figure 1(e)). The PCR results showed that mRNAs for these receptors were detectable at all ages investigated. When compared to div4, GFRα2 mRNA was down-regulated to 32 ± 16% at div25. This change in GFRα2 expression may have a role in the developmental differences in the effect of Neurturin on KCC2 expression.

These data provide evidence that Neurturin, similarly to BDNF, is able to enhance endogenous KCC2 expression *in vitro* during early postnatal period in a dose-dependent manner. The developmental change in trophic factor-mediated regulation of KCC2 expression may be a consequence of corresponding changes in trophic factor receptor expression.

3.2. Neurturin Activation of KCC2 Proximal Promoter Region is Dependent on MEK Phosphorylation. Using the luciferase (Luc) reporter construct *KCC2(−309/+42)* driven by the proximal promoter region of KCC2 (Figure 2(a)) we performed experiments on hippocampal primary cultures aiming to further study Egr4 involvement in the neurotrophic factor-induced KCC2 up-regulation (for detailed scheme of construct generation see [16]).

Dissociated neurons were transfected with *KCC2 (−309/+42)* at div4 and treated with 50 ng/mL Neurturin two days after transfection. Two to four hours after the trophic factor application, culture lysates were analyzed for Luc activity (Figure 2(b)). We observed substantial increase in KCC2 promoter activity in cultures treated with Neurturin (117 ± 5% of nontreated controls). The Neurturin-induced increase in KCC2 promoter activity was abolished by MEK1/2 inhibitor U0126 (20 μM).

3.3. Expression Pattern of the Neurturin Receptors GFRα2 and RET in the Early Postnatal Hippocampus. In contrast to TrkB receptor, in which expression is well characterized in early postnatal brain [17], detailed data on Neurturin coreceptors RET and GFRα2 expression in the hippocampus are scarce. Two studies addressed the question of GFRα2 and RET expression in developing hippocampus by RT PCR and *in situ* hybridization [18, 19]. Both studies showed that after birth GFRα2 mRNA expression reaches maximum at around P5 and it is downregulated during later development. The *in situ* hybridization study [18, 19] also demonstrated that at P4 a prominent RET and GFRα2 mRNA expression was localized to CA3 pyramidal layer while GFRα1 mRNA was only weakly abundant there.

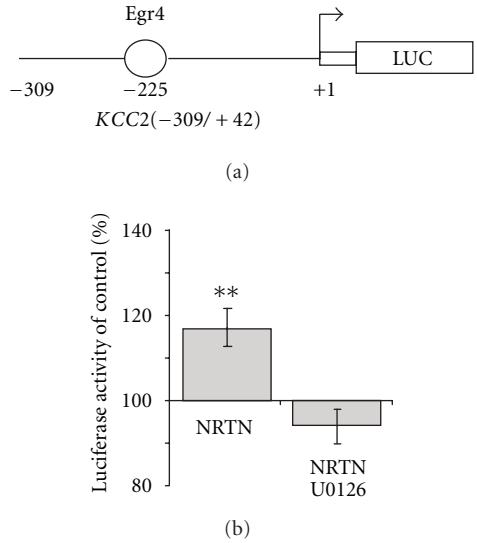

Figure 2: Neurturin-induced activation of KCC2 proximal promoter region. (a) Schematic drawing of the luciferase construct carrying the KCC2 proximal promoter region. The construct contains the luciferase reporter gene under control of a short (309 bp) upstream proximal part of the KCC2 promoter sequence. (b) Normalized luciferase activity 2–4 hours after application of 50 ng/mL Neurturin in div7 hippocampal neuronal cultures transfected with the KCC2 (−309/+42) construct ($n = 4$; $^{**}P < 0.01$). Error bars represent SEM.

We analyzed the temporal and spatial pattern of GFRα2 and RET mRNA expression during early postnatal development in our conditions in order to estimate the best optimal time point to test the effect of Neurturin *in vivo*. *In situ* hybridization of consecutive sagital rat brain sections at E17, P3, P5, and P9 (Figure 3(a)) showed that RET and GFRα2 signals were relatively low but most prominent at P3 and P5. RET mRNA expression showed a general dispersed pattern. Interestingly, GFRα2 mRNA was most prominent in the pyramidal layer of the CA3 region (white arrowheads).

3.4. Neurturin Increases KCC2 Expression in P5-P8 Mice Hippocampus In Vivo. We investigated the effect of intrahippocampal Neurturin injections on KCC2 expression. In accordance with receptor expression data, Neurturin was injected in hippocampi of P5 rats. In the initial series of experiments two doses of Neurturin were tested: 100 ng and 1 μg. The lower dose of Neurturin had no significant effect on

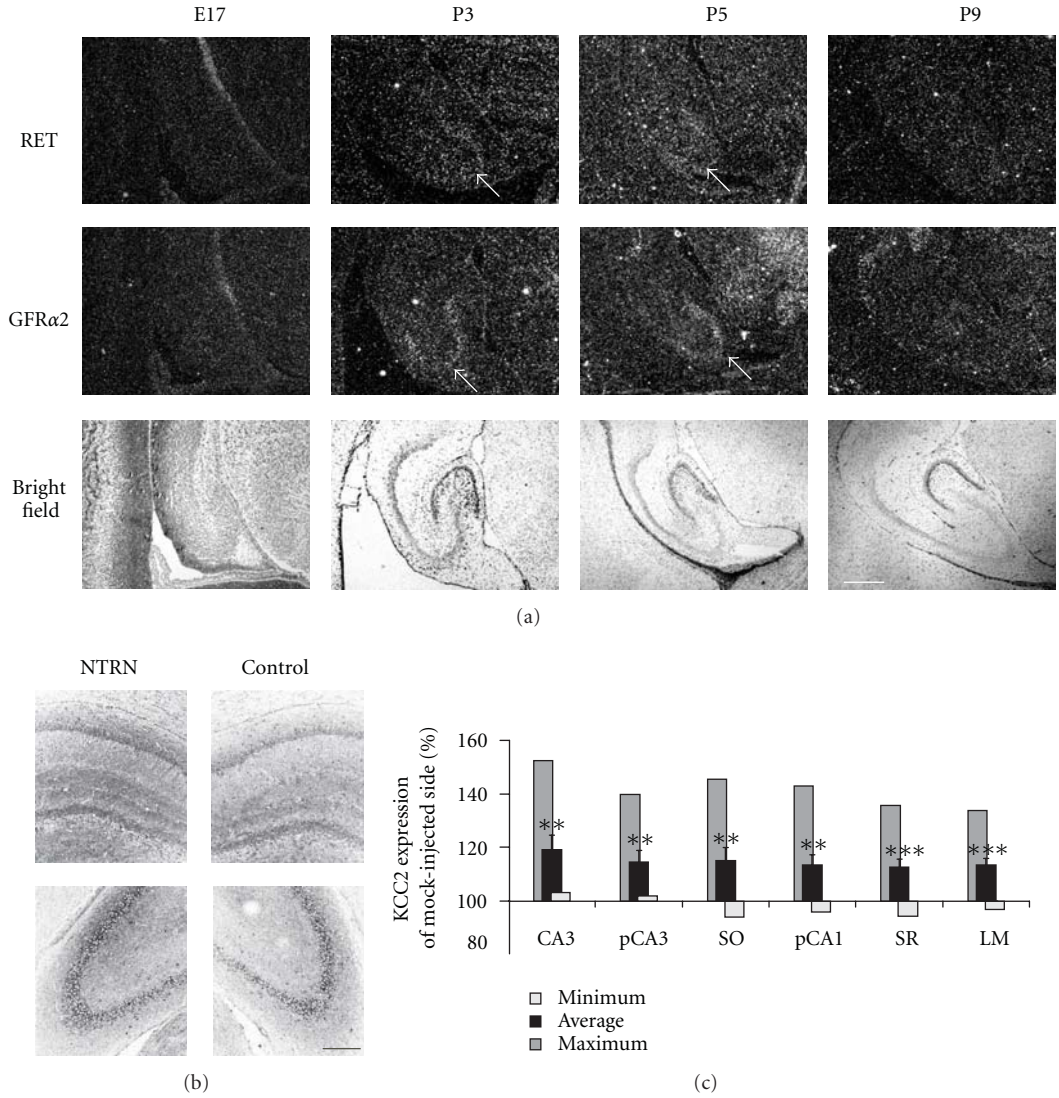

Figure 3: Regulation of KCC2 expression by Neurturin *in vivo*. (a) RET and GFRα2 mRNA expression was detected by *in situ* hybridization. Note the accumulation of RET and GFRα2 at P3 and P5 in the CA3 region of the hippocampus (marked with arrowheads). Scale bar = 500 μm. (b) Representative immunofluorescent stainings of KCC2 expression in Neurturin-treated and control hippocampi 3 days after injection. Neurturin (1 μg) was used for injection in right-side hippocampus of P5 rat while contralateral hippocampus was injected with saline solution. (c) Summary results of KCC2 immunostaining intensity for different layers of CA1 and CA3 in Neurturin-injected hemisphere (n = 16–21). KCC2 intensity was measured in 6 sections at various distances from injection site. The effect of Neurturin injection was uneven along the dorsal-ventral axis. Quantification was made for the following layers: CA3 is whole area of CA3; pCA3 and pCA1 are pyramidal layers of CA3 and CA1, respectively; SO is stratum oriens; SR is stratum radiatum and LM is stratum lacunosum-moleculare. In each section and each layer KCC2 intensity in Neurturin-injected hemisphere was normalized to the corresponding value in control hemisphere. Then for each layer the maximum, the minimum, and the average values of KCC2 intensity along the dorsal-ventral axis were calculated ($^{**}P < 0.01$, $^{***}P < 0.001$). Error bars represent SEM. Scale bar = 300 μm.

KCC2 expression (data not shown). All further experiments were performed using 1 μg of Neurturin.

Single injection of Neurturin produced a significant increase in KCC2 expression (Figures 3(b) and 3(c)). KCC2 levels were analyzed three days after the Neurturin injection by immunostaining. Representative KCC2 immunostaining of the areas with maximal Neurturin effect are presented in Figure 3(b). Summarized data for three independent experiments are shown in Figure 3(c). KCC2 up-regulation after Neurturin injection was significant in all hippocampal areas analyzed. The maximal effect of Neurturin (150% of control) was observed in CA3 area.

Taken together, the *in vivo* experiments indicate that within three days after a single application of Neurturin in developing hippocampus, there is a substantial increase in the level of KCC2 expression.

4. Discussion

Identification of the intracellular cascades involved in KCC2 regulation is crucial for understanding the mechanisms rendering long-term plastic changes in $GABA_A$ mediated transmission during development and trauma. Both BDNF and Neurturin can activate similar intracellular cascades that include Shc/Frs2 which in turn triggers the MAPK and AKT signaling [5, 15]. The MAPK pathway is known to be involved in differentiation and maturation whereas AKT is implicated in cell growth and survival. The results obtained in the present study clearly show that it is activation of the MAPK intracellular pathway that is crucial for both BDNF—[3] and Neurturin—induced expression of KCC2 in immature neurons. Suppression of MAPK signaling by the specific MEK inhibitor U0126 resulted in a significant block of BDNF-[3, 20] and Neurturin—induced KCC2 promoter activation as well as Neurturin-induced Egr4 expression. Our previously published results showed that in adult neurons activation of mutant TrkB receptor, in which only Shc/Frs2 docking site was preserved, resulted in KCC2 up-regulation [2] thus emphasizing the importance of this pathway not only during development but also under pathophysiological conditions.

Our previous results demonstrated that early growth response factor Egr4 induced KCC2 transcription [16] and mediated the BDNF-induced KCC2 up-regulation [3] in immature neurons. In the present work we show that similar to the effect of BDNF, Neurturin significantly up-regulates Egr4. This obviously leads to the question whether Egr4 is involved in the Neurturin-induced KCC2 up-regulation. The crucial role of Egr4 in this process is particularly indicated by the experiment where Neurturin displays a significant activation of the proximal promoter region of KCC2 that carries the binding site for Egr4. Consistent with the *in vitro* results, *in vivo* intrahippocampal injection of Neurturin produced a significant up-regulation of KCC2 protein in neonatal rat hippocampus.

The family of GDNF ligands are potent survival promoting trophic factors that act primarily through the interaction with GFRα coreceptors and signal through the activation of the receptor tyrosine kinase RET. Resent results suggest that, additionally, this family of trophic factors could signal through alternative signaling pathways [21]. The temporal and spatial effect of Neurturin correlated both *in vitro* and *in vivo* with the expression profile of RET and GFRα2, suggesting that this effect was mediated trough the specific activation of these receptors. More detailed analysis of the effect of Neurturin on KCC2 expression in GFRα2−/− mice in the future will give a more decisive answer to whether this effect is mediated trough GFRα2/RET.

In conclusion the present results are in agreement with a central role of the intracellular MAPK/ERK pathway as convergent point for different parallel extracellular cues. Activation of the MAPK pathway by these extracellular signals induces immediate early gene Egr4 expression that in turn stimulates KCC2 up-regulation. These may lead to in-creased Cl^- extrusion efficiency causing the maturation of $GABA_A$ mediated responses. These results suggest that there may be several extracellular signals able to induce KCC2 up-regulation in developing neurons. One question for the future is whether Neurturin and BDNF have synergistic action *in vivo* to regulate KCC2 during development. Another important question raised by the present study is whether parallel mechanisms regulating KCC2 expression are in place also under pathophysiological conditions.

Abbreviations

Shc:	Src homology 2 domain containing transforming protein
FRS2:	FGF receptor substrate 2
PLCγ:	Phospholipase Cγ
MAPK:	Ras/mitogen activated protein kinase
PI3K:	Phosphatidylinositol 3- kinase
AKT:	Cellular homolog of the viral oncogene v-Akt
TrkB:	Tyrosine receptor kinase B
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
BDNF:	Brain derived neurotrophic factor
NRTN:	Neurturin
Egr4:	Early growth response 4
div:	Days in vitro
GDNF:	Glial cell line-derived neurotrophic factor
RET:	Rearranged during transfection
GRFalpha2:	GDNF family receptor alpha 2
MEK:	MAPK/ERK kinase
SEM:	Standard error of the mean
SOS:	Son of sevenless
Grb:	Growth factor receptor-bound protein
MAPK:	Mitogen-activated protein kinase
Erk:	Extracellular signal-regulated kinase
PIP2:	Phosphatidylinositol 4,5-biphosphate
DAG:	Diacyl glycerol
PKC:	Protein kinase C.

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Review Article

Mechanisms of GABAergic Homeostatic Plasticity

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Homeostatic plasticity ensures that appropriate levels of activity are maintained through compensatory adjustments in synaptic strength and cellular excitability. For instance, excitatory glutamatergic synapses are strengthened following activity blockade and weakened following increases in spiking activity. This form of plasticity has been described in a wide array of networks at several different stages of development, but most work and reviews have focussed on the excitatory inputs of excitatory neurons. Here we review homeostatic plasticity of GABAergic neurons and their synaptic connections. We propose a simplistic model for homeostatic plasticity of GABAergic components of the circuitry (GABAergic synapses onto excitatory neurons, excitatory connections onto GABAergic neurons, cellular excitability of GABAergic neurons): following chronic activity blockade there is a weakening of GABAergic inhibition, and following chronic increases in network activity there is a strengthening of GABAergic inhibition. Previous work on GABAergic homeostatic plasticity supports certain aspects of the model, but it is clear that the model cannot fully account for some results which do not appear to fit any simplistic rule. We consider potential reasons for these discrepancies.

1. Introduction

Alterations in the influence of inhibitory GABAergic circuits can have a profound impact on the excitability of neural network function, and have been associated with hyperexcitable conditions such as epilepsy [1, 2]. Recent work has identified what may be one of the most important processes in ensuring that networks maintain appropriate activity levels; homeostatic plasticity is thought to maintain network spiking activity levels within a physiologically relevant range through compensatory adjustments in intrinsic cellular excitability, as well as excitatory and inhibitory synaptic strength [3–8]. These changes are induced following perturbations in spiking activity levels for many hours. This phenomenon has been identified in several systems, at different developmental stages, *in vitro* and to a lesser extent *in vivo*. When activity levels of cultured neuronal networks (cortical, hippocampal, spinal) are altered for days, cellular excitability and synaptic strength within the network are adjusted in a direction that appears to oppose the alteration in activity [9–14]. For instance, when spiking activity is blocked for 2 days, AMPAergic synaptic strength increases and GABAergic synaptic strength decreases in excitatory neurons. When network spiking activity is increased, AMPAergic synaptic strength decreases. In each case the amplitude of miniature postsynaptic currents (mPSCs) changed in a direction to compensate for the perturbation.

Several reviews have examined homeostatic plasticity, typically focusing on excitatory components within networks [3–6]. In this paper we will instead concentrate on the findings of homeostatic plasticity within GABAergic neurons and at GABAergic synapses onto excitatory neurons. Based on previous work studying homeostatic plasticity in the glutamatergic system, we make the simplistic prediction that inhibition would be reduced following network activity blockade and increased following increases in network activity. Therefore, following chronic reductions in network activity (Figure 1 left), we would expect compensatory weakening of both GABAergic synapses on excitatory neurons and glutamatergic synapses on inhibitory neurons and to see reductions in the intrinsic cellular excitability of inhibitory neurons. If network activity is increased for days (Figure 1 right), we would expect compensatory strengthening of both GABAergic synapses on excitatory neurons and glutamatergic synapses on inhibitory neurons and to observe increases

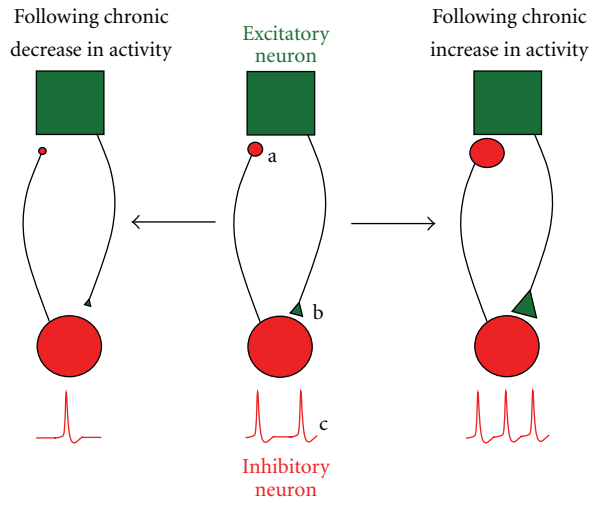

Figure 1

in the intrinsic cellular excitability of inhibitory neurons. The review focuses on compensatory changes in cellular excitability and mPSC amplitude.

2. Homeostatic Synaptic Plasticity of GABAergic Inputs to Excitatory Neurons

The most studied aspect of inhibition in homeostatic plasticity has examined the inhibitory GABAergic inputs to excitatory neurons (Figure 1(a)). Immunocytochemical studies gave the first indication that GABAergic circuits experienced homeostatic plasticity, as reduced visual input led to decreased cortical expression of $GABA_A$ receptors, GABA, and GAD [15, 16]. Compensatory changes in the amplitude of GABAergic mPSCs have now been demonstrated in excitatory neurons following network activity perturbations in several different studies [10, 17–20]. These changes in GABAergic mPSC amplitude are often mediated by changes in the number of synaptic $GABA_A$ receptors, and this is typically shown by quantitative immunocytochemistry [10, 20, 21]. In addition, compensatory changes in the vesicular inhibitory amino acid transporter, VIAAT, have been observed suggesting there are coordinated presynaptic contributions to homeostatic changes in mIPSC amplitude [18, 20, 21]. While these studies have shown that mIPSC amplitude is reduced following chronic activity blockade or increased following increased network activity, two studies suggest the opposite can occur. One study demonstrates that a subset of GABAergic inputs to hippocampal pyramidal cells are strengthened following activity block; however, the overall population of mIPSCs homeostatically scale downward [19]. Another study shows that *in vivo* application of TTX for 2 days resulted in an increase in mIPSC amplitude in pyramidal cells recorded from cortical slices [22], which also does not fit the simple homeostatic model. These studies highlight the need to carry out more homeostatic studies *in vivo*, as perturbations in living networks are likely to be more complicated in terms of network homeostasis, but crucial in elucidating the goals of homeostatic plasticity.

In a separate study where spiking activity was blocked *in vivo* for 2 days in the embryonic spinal cord, the result appeared to obey our homeostatic model. Following activity blockade, GABAergic mPSCs in excitatory motoneurons increased in amplitude [17]. These changes in GABAergic currents were compensatory because GABA was depolarizing and excitatory at this developmental stage. This is due to chloride accumulation through transporters expressed at these stages [23]. Interestingly, homeostatic increases in GABAergic mPSCs occurred through increased chloride accumulation, thus depolarizing the GABAergic reversal potential (E_{GABA}) and enhancing the driving force for these currents [24]. Similarly, another study indirectly demonstrated that homeostatic changes in GABAergic currents could be produced by a shift in E_{GABA} [25]. In this study, activity was perturbed in hippocampal organotypic cultures and compensatory changes in GABAergic currents were observed in pyramidal cells at a stage when GABA was no longer excitatory. These findings are important for understanding the maturation of GABAergic synaptic strength but also may have implications for neuronal injury in mature circuits where the same depolarizing shifts in chloride reversal potential are observed following spinal cord injury, peripheral nerve injury, and traumatic brain injury [26–38]. It is tempting to speculate that following injury, homeostatic mechanisms may be engaged that produce the maladaptive increases in excitability associated with neuronal injury [30]. Consistent with this idea, work in a model of febrile seizure suggests the possibility that compensatory increases in GABAergic strength appear to promote hyperexcitability by triggering the hyperpolarization-activated current, I_h [39, 40].

Other studies in cultured networks demonstrated that homeostatic changes in mIPSC amplitude were not due to changes in E_{GABA} [10, 41]. However, these studies used whole-cell recordings to measure $E_{GABA,}$which may dialyze intracellular Cl^- and mask the experimenter's ability to observe changes in GABAergic driving force. Future studies assessing homeostatic changes in mIPSCs could use perforated patch recordings or chloride indicators to resolve this issue.

Although not as common as homeostatic changes in mIPSC amplitude, homeostatic changes in mIPSC frequency have been reported. Increases or decreases in network activity have been shown to increase and decrease mIPSC frequency, respectively, in excitatory neurons [10, 20]. This appears to be mediated by changes in the number of GABAergic inputs to excitatory pyramidal cells. To a large extent, GABAergic mPSC amplitude and frequency in excitatory neurons follow the homeostatic model, strengthening after chronic increases in activity and weakening after activity blockade.

3. Homeostatic Plasticity in GABAergic Interneurons

Our homeostatic model predicts that AMPAergic synaptic inputs to GABAergic neurons will strengthen following increases in activity and weaken following activity blockade (Figure 1(b)). Using hippocampal cultures, it was shown that

parvalbumin-expressing inhibitory interneurons (PV INs) increased mEPSC amplitude following chronic enhancement of activity levels and reduced mEPSC amplitude following activity blockade [42]. The changes in mEPSC amplitude were mediated by changes in the number of an AMPA receptor subunit, GLUA4, which was regulated homeostatically by neuronal activity-regulated pentraxin (NARP). Similarly, chronic increases in activity induced a strengthening of excitatory inputs to inhibitory interneurons in neocortical cultures, expressed presynaptically as an increase in the vesicular glutamate transporter, VGLUT2 [43]. Consistent with these findings, another study demonstrated that increasing BDNF levels, as occurs with increased network activity, led to an increase in mEPSC amplitude in inhibitory bipolar interneurons [41]. In this study, the increase in mEPSC amplitude was mediated by an increase in the sensitivity of the postsynaptic cell to glutamate, consistent with an increase in synaptic glutamate receptors. However, when spiking activity was blocked for days in several different cultured cortical networks, mEPSC amplitude was unaltered in multiple classes of inhibitory interneuron [8, 41, 44]. Thus far, the results are consistent with the idea that increased network activity levels triggered homeostatic increases in mEPSC amplitude in interneurons, but that mEPSC amplitude was typically unaltered by reductions in network activity. In none of these studies were changes in mEPSC frequency observed. Finally, we know of no homeostatic studies examining mIPSCs in inhibitory interneurons following activity perturbations.

Changes in interneuronal intrinsic cellular excitability (Figure 1(c)) following activity block have been described in 2 different cortical cultures. In both studies, intrinsic cellular excitability was increased following activity blockade in 3 different classes of inhibitory interneuron [44, 45]. One of the studies suggested that the increased excitability was the result of an increase in input resistance [44]. From a simplistic network perspective, increasing the excitability of an inhibitory neuron in an activity-blocked network is not what our homeostatic model would predict (Figure 1(c) left). The enhanced inhibition may be offset by the observation that pyramidal cells also have increased intrinsic excitability following activity blockade, but the finding underlines the complexity of the homeostatic process [22, 25, 44, 45]. It is possible that activity perturbations result in changes in synaptic strength that are homeostatic for the network, while changes in intrinsic cellular excitability are homeostatic from the perspective of the individual cell.

4. Evoked Responses between Inhibitory and Excitatory Neurons

We have focused on mPSCs because they provide a nice measure of a standard unit of synaptic strength. However, another potentially useful measure of synaptic strength is provided by looking at the functional connections between 2 components of the circuitry. The strength of the connections between inhibitory and excitatory neurons can be assessed through paired recordings, stimulating an inhibitory or excitatory neuron and recording a response in the other. The results of these studies have been somewhat mixed. When retinal activity is reduced *in vivo* by TTX infusion or lid suture, input to pyramidal cells in the visual cortex from inhibitory interneurons was homeostatically reduced in certain cases [46, 47]. In other cases reductions of visual input to cortical neurons resulted in a strengthening of both inhibitory inputs to pyramidal cells and pyramidal input to inhibitory neurons [46, 48]; from a network perspective, these results are opposite to that predicted by our model of homeostatic plasticity. One complication in these studies is that when visual input is perturbed *in vivo*, it is not always clear how this affects the activity of the visual cortical circuitry that is being studied; for instance, different results have been described when retinal activity is altered by lid suture versus TTX infusion [47]. However, in one study in neocortical organotypic cultures, where network activity was clearly blocked, changes in the strength of connections between excitatory and inhibitory neurons were not simplistically homeostatic [44].

5. BDNF

Brain-derived neurotrophic factor (BDNF) has been implicated in the signaling pathway for homeostatic plasticity of both glutamatergic and GABAergic systems. BDNF exerts its influence through changes in intrinsic cellular excitability, mEPSC and mIPSC amplitude and frequency. From these studies a pattern is beginning to emerge; when BDNF signaling is reduced, as occurs during activity blockade, there is an increase in the influence of excitatory neurons; when BDNF signaling is increased, as occurs during chronic increases in network activity, there is an increase in the influence of inhibitory neurons (Figure 2). When activity is blocked in cortical cultures using TTX, pyramidal cells become more excitable through increases in mEPSC amplitude [41], decreases in mIPSC or spontaneous IPSC amplitude [21, 49], and increases in the intrinsic cellular excitability of these cells [45]. All three of these compensatory changes appear to be mediated by reduced BDNF signaling because they are prevented by coapplication of BDNF and TTX and recapitulated by blocking BDNF signaling through its receptor, TrkB. On the other hand, increases in BDNF signaling that would be associated with overly active networks enhanced the influence of inhibitory interneurons through increases in interneuronal projections (increased mIPSC frequency), or through increased mEPSC amplitude onto inhibitory interneurons [20, 41]. While the model shown in Figure 2 is well supported by most of the experimental evidence, one exception to the homeostatic model is the observation that activity block triggers a BDNF-dependent increase in inhibitory interneuron intrinsic excitability in cortical cultures [45].

6. Sensors for Activity Perturbations

The sensors of activity that trigger homeostatic plasticity changes are a major focus in the field but are poorly understood. Activity sensors triggering changes in inhibitory circuitry are even less well understood. In the vast majority

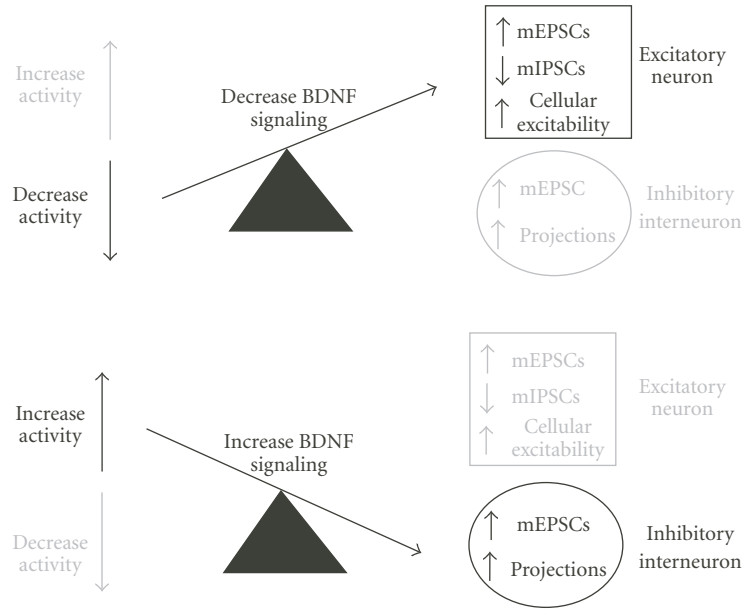

Figure 2

of homeostatic studies, network activity is reduced by TTX or glutamate receptor blockers or increased by $GABA_A$ receptor antagonists. All of these treatments alter activity levels, as well as neurotransmission, throughout the network. Therefore, it is possible that changes in network spiking activity, cellular spiking activity, or synaptic transmission trigger homeostatic changes in mIPSCs. Very few studies exist that allow us to independently test these different triggers. One recent study increased spiking activity in an individual cell in an otherwise unperturbed network. The activity-increased cell exhibited increases in mIPSC amplitude and frequency [20]. The finding is consistent with the idea that increases in individual cellular spiking activity trigger homeostatic compensations of GABAergic inputs. However, when activity was blocked in individual hippocampal pyramidal cells by transfecting them with a K^+ channel or a mutant voltage-gated Na^+ channel, no change in mIPSC amplitude was observed [18]. The finding indicated that reductions in the activity of individual excitatory neurons did not trigger homeostatic changes in mIPSC amplitude, but suggested that reductions in network-wide activity or neurotransmission may be required to induce this plasticity. Consistent with the possibility that sensors assess neurotransmission, we have determined that *in vivo* blockade of depolarizing $GABA_A$ transmission in the embryonic spinal network triggered compensatory increases in excitatory GABAergic mPSC amplitude and cellular excitability in motoneurons [50, 51]; these forms of compensatory plasticity were not dependent on alterations in spiking activity, suggesting that the network could sense reduced spiking activity levels through reduced $GABA_A$ transmission, essentially using GABA as a proxy for activity levels. A better understanding of the sensors that trigger compensatory changes in inhibitory neurotransmission will require more extensive work than the current studies, but it will be important to consider the possibility that neurotransmission is involved in the process.

7. Concluding Remarks

Certain rules of our simplistic homeostatic plasticity model appear to be generally followed. GABAergic inputs to excitatory neurons in several different networks are strengthened following increases in activity and weakened following activity block; increases in activity lead to increased mEPSC amplitude in inhibitory neurons; increases in BDNF signaling (associated with increases in activity) increase the excitability of inhibitory interneurons, while decreases in BDNF signaling (associated with decreased activity) increase the excitability of excitatory neurons. However, there are several clear examples that do not fit into any simplistic homeostatic model (interneuron intrinsic excitability, mEPSC amplitude in interneurons following activity block, evoked responses between excitatory and inhibitory neurons). These apparent exceptions to the homeostatic model could arise for several reasons. It will be important to identify common mechanisms of homeostatic plasticity, but it is likely that different preparations (e.g., *in vitro* versus *in vivo*) and different neural circuits use different homeostatic mechanisms. Further, compensatory mechanisms will be experienced in different elements of the circuitry at different developmental stages [52]. In addition, the methods of altering network activity are likely to trigger different homeostatic mechanisms, for instance, increasing versus decreasing activity. In some cases, particularly *in vivo* studies, assumptions are made about alterations in cellular or network activity, but are not directly tested, leaving open the possibility that apparent antihomeostatic responses are actually homeostatic, or vice versa. It is also possible that in some cases absolute levels of spiking activity are not the homeostatic goal, but rather some more sophisticated pattern of activity, for instance, synchrony of the output neurons, which could be achieved through more complicated changes in GABAergic interneurons [53, 54]. In the end, it is

important to recognize that changes in GABAergic synaptic strength or cellular excitability in inhibitory neurons are being tested in isolation, but they occur within complex networks where it is difficult to know the functional consequences of these changes. As the field matures it will be important to take these complexities into consideration. Because network-wide activity is clearly maintained across many neural circuits, there are likely to be strong homeostatic mechanisms maintaining global network activity; it will be important to differentiate these homeostatic mechanisms from those that maintain individual cellular activity or individual synaptic activity, each potentially being triggered by different sensors.

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Review Article

Assortment of GABAergic Plasticity in the Cortical Interneuron Melting Pot

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Cortical structures of the adult mammalian brain are characterized by a spectacular diversity of inhibitory interneurons, which use GABA as neurotransmitter. GABAergic neurotransmission is fundamental for integrating and filtering incoming information and dictating postsynaptic neuronal spike timing, therefore providing a tight temporal code used by each neuron, or ensemble of neurons, to perform sophisticated computational operations. However, the heterogeneity of cortical GABAergic cells is associated to equally diverse properties governing intrinsic excitability as well as strength, dynamic range, spatial extent, anatomical localization, and molecular components of inhibitory synaptic connections that they form with pyramidal neurons. Recent studies showed that similarly to their excitatory (glutamatergic) counterparts, also inhibitory synapses can undergo activity-dependent changes in their strength. Here, some aspects related to plasticity and modulation of adult cortical and hippocampal GABAergic synaptic transmission will be reviewed, aiming at providing a fresh perspective towards the elucidation of the role played by specific cellular elements of cortical microcircuits during both physiological and pathological operations.

1. Introduction

The cerebral cortex (which includes the hippocampus, the entorhinal cortex, the piriform cortex, and the neocortex) is the origin of the most sophisticated cognitive functions and complex behaviors. Indeed, the constant computation of incoming sensory information is dynamically integrated to provide a coherent representation of the world, elaborate the past, predict the future, and ultimately develop a consciousness and the self. In particular, the specific activity states of intricate cortical networks often produce a wide range of rhythmic activities, believed to provide the computational substrate for different aspects of cognition and various behaviors [1, 2]. Cortical oscillations range from slow-wave activity (<1 Hz) to ultrafast oscillations (>100 Hz), with several intermediate rhythms (e.g., theta, beta gamma), each of which is considered to underlie specific cognitive aspects, such as non-REM sleep (slow-waves), sensory integration (gamma), working memory (theta), and motor planning (beta) [1]. Importantly, inhibitory neurons were proposed to play a fundamental role in the genesis of most of these rhythms [3–13] through the specialized activity of their GABAergic synapses [7–10]. In fact, it is noteworthy that malfunctioning of specific GABAergic circuits is often indicated as a leading pathophysiological mechanism (among others) of psychiatric diseases, such as schizophrenia and autism [14–18].

Synapses are very specialized structures responsible for the propagation of information between neurons. One of the hallmarks of synaptic transmission is its ability to be modified by certain activities or specific modulators. Modifications of synaptic strength can occur in a short- (seconds) or long-term (from hours to days) fashion. In the last decades, the plasticity of excitatory glutamatergic synapses was extensively studied as it has been proposed to be the synaptic correlate of learning and memory [19–21]. In contrast, plasticity of GABAergic synapses received less attention until recently, when it became clear that also inhibitory synapses can undergo short- and long-term plasticity [22]. However, the underlying mechanisms for GABAergic plasticity are not completely understood, given also the staggering diversity of

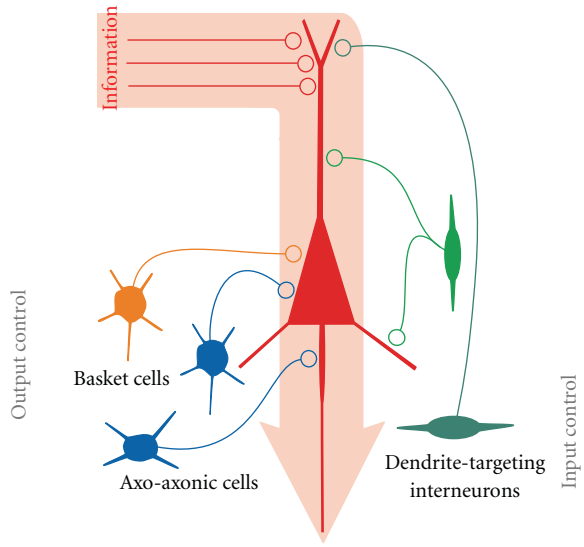

Figure 1: *Oversimplified scheme of the inhibitory control of cortical pyramidal neurons by several general classes of GABAergic interneurons*. Information (pink wide arrow) is transferred from excitatory glutamatergic synapses (red axon terminals) to the pyramidal neuron (red cell) dendrite. Excitation (information) travels along the dendritic tree to the soma and axon initial segment, where it could generate an action potential. Along this dendro-somatic-axonal axis, information can be differently filtered by GABAergic synapses possessing specific basic and plasticity properties. On the left-hand side, interneurons controlling the output are illustrated as different classes of basket and axo-axonic cells. Different GABAergic interneurons controlling the input into pyramidal neurons are shown on the right, as impinging the dendrite(s) at different distances from the soma. Details in the text.

inhibitory neurons embedded in cortical circuits and their equal heterogeneity of synaptic properties [3, 9, 23–38].

Here, we review some aspects of GABAergic synaptic plasticity in the context of the great disparity of GABAergic interneuron classes and the putative roles of specific changes of GABAergic synaptic strength during cortical operations. Notably, a recent review by Castillo et al. [39] covered several aspects of GABAergic synaptic plasticity, focusing on the pre- versus postsynaptic induction and expression mechanisms (see in Table 1 in [39]).

2. Interneuron Diversity

In the mammalian cerebral cortex, the stereotyped interactions of multiple neuron types arranged in layers result in complex networks composed by excitatory (glutamatergic) and inhibitory (GABAergic) neurons. Although some heterogeneity of cortical excitatory neurons exists in terms of anatomy, electrophysiology, and connectivity patterns [40–46], the morphological and physiological properties of excitatory neurons are relatively homogeneous. In contrast, inhibitory neurons of cortical structures encompass a vast number of different cell types [3, 23, 34–38]. For example, in CA1 region of the hippocampus, 16 different types of interneurons have been identified so far [3]. Inhibitory neurons release GABA and are locally projecting cells, hence their "interneuron" denomination, indicating that cell body, dendrites, and axonal projections, are confined within the same anatomical area. The vast majority of interneurons show aspiny dendrites, or a relatively small spine density [47], and, unlike glutamatergic cells, they can be contacted by both glutamatergic and GABAergic synapses at the soma [48]. The classification of interneurons is based on the expression of certain calcium binding proteins and/or neuropeptides, specific electrophysiological signatures (action potential waveform and dynamic range), and functional characteristics of synapses that they form and receive, as well as specific anatomical and morphological properties [7, 25, 27, 34–38, 49, 50]. Overall, interneurons provide inhibition to neuronal networks and dictate the temporal pattern of activity of principal pyramidal and other inhibitory neurons. In this context, the rich diversity of GABAergic cells operates a division of labor during cortical activities (oversimplified in Figure 1) [11, 13], and the specific roles played by each interneuron subtype in the functional organization of cortical networks has only recently begun to be elucidated [3].

Whereas interneuron dendritic morphology is highly variable, the axonal arborization can reveal specific functional features (Figures 2(a) and 2(b)). Indeed GABAergic interneurons are specialized in targeting specific domains of excitatory principal cells, and specific patterns of axonal projection result in one of the most relevant functional classifications of interneurons. For instance, oriens to lacunosum-moleculare (OL-M) neurons in the hippocampus and their neocortical counterpart, the Martinotti cells, represent a prominent type of dendrite-targeting interneurons [28, 37, 52, 53]. Other dendrite-targeting interneurons include the neurogliaform cells [35, 54–57], the bi-stratified and tri-stratified interneurons [3, 38, 58], and ivy cells [59] in the hippocampus. All these cell types target the dendrites of pyramidal neurons (at different distances) and are thus optimally predisposed to filter synaptic glutamatergic inputs that are exclusively present on pyramidal cell dendrites (Figure 1) [41, 60]. On the other hand, basket cells (BCs, representing ~50% of all inhibitory neurons) are specialized in targeting the soma and proximal dendrites of pyramidal cells [10]. By setting the timing of action potentials of many pyramidal neurons, BCs crucially regulate the neuronal output and promote synchronous discharge of a large population of principal cells (Figure 1) [5, 6, 10]. Moving along the dendro-soma-axon line of pyramidal neurons, another type of interneuron is specialized in targeting the axonal initial segment of principal cells: the axo-axonic or chandelier cells [23, 35, 37, 61]. GABAergic synapses formed by these cells on axons of pyramidal neuron suggest a powerful role as controllers of their output (Figure 1). A clear functional distinction of the division of tasks between axo-axonic and perisomatic targeting interneurons is still unclear, as both cell types target the output region of pyramidal neurons. Interestingly, GABAergic synapses from neocortical axo-axonic cells were recently found to exert a paradoxical excitatory role, promoting action-potential generation in pyramidal neurons [62–64], although this is still matter of debate [64, 65].

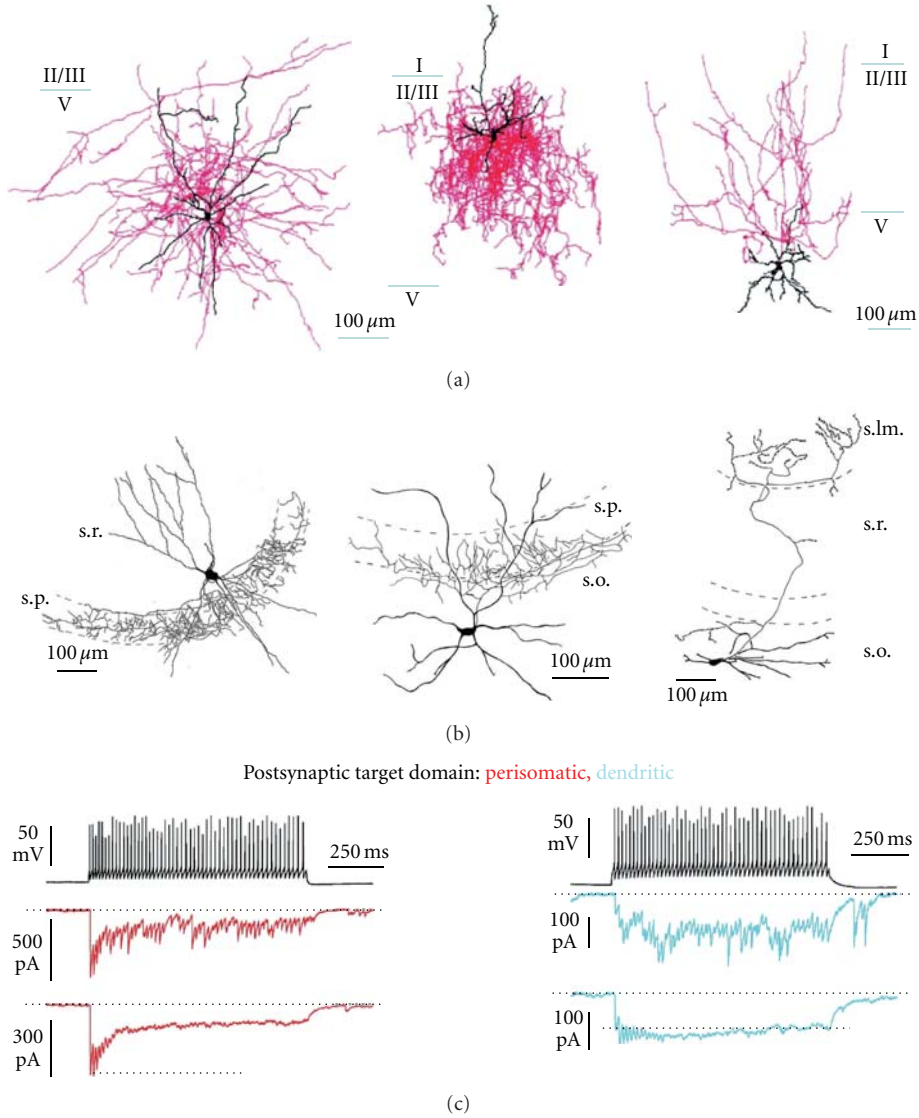

Figure 2: Example of diverse functional classes of inhibitory interneurons. (a) Single-neuron reconstructions of three different interneuron types of the neocortex: a basket cell (left), an axo-axonic cell (middle), and a dendrite-targeting Martinotti cell (right). Axons and somatodendritic compartments are shown in red and black respectively. Modified with permission from [51]. (b) Exampled of similar classes of GABAergic interneurons as in a, but in the hippocampus. modified with permission from [11]. (c) Different hippocampal interneuron classes show distinct properties of synaptic transmission. Examples of depressing (red traces) and facilitating (blue traces) unitary GABAergic responses originating from perisomatic and dendrite-targeting interneurons respectively. The upper red and blue traces are single-trial responses, whereas the bottom traces are averaged of multiple trials. Modified with permission from [52].

In the hippocampus and cortex, BCs can be subdivided in two major, nonoverlapping subtypes with different physiological properties. Parvalbumin (PV) expressing basket cells can sustain high-frequency firing (hence their fast-spiking or FS denomination) and receive strong and fast glutamatergic input that relies mainly on AMPA receptors and efficiently recruits them during cortical activity [7, 25, 66, 67]. PV+ BCs are selectively surrounded by polyanionic chondroitin sulfate-rich perineuronal nets [68], which seem to play an important role in controlling ocular dominant plasticity in the neocortex [69, 70] and protect erasure of fear memories in the amygdala [71]. FS BCs release GABA very reliably due to the tight coupling between Ca^{2+} channels and Ca^{2+} sensors at their terminals [72, 73] and are extensively interconnected through chemical and electrical synapses [49, 74–78]. In particular, in the neocortex, FS BCs make a large number of synaptic contacts with themselves (autapses) [79–82] that modulate their own spike frequency and greatly contribute to improve precise spike-timing [83]. All these features allow PV+ BCs to synchronize a large population of principal cells and are thus believed to be the clockwork of cortical networks as they entrain oscillations that underlie several complex cognitive functions, including sensory integration, attention, exploratory behavior, sleep, and several forms of memory [1]. Remarkably, FS interneurons might promote network desynchronization in response to certain pattern of intense

activity. This effect is mediated by massive asynchronous release of GABA from FS interneurons both at autapses and synapses with pyramidal cells resulting in reduced spike-timing precision [82].

In contrast, interneurons belonging to another perisomatic targeting interneuron subclass express cannabinoid receptor type 1 (CB1Rs) and the neuropeptide cholecystokinin (CCK), cannot sustain high-frequency firing, are contacted by less glutamatergic synapses, and their soma-targeted synapses tend to release GABA asynchronously and unreliably, often resulting in prolonged inhibition of target cells [30, 31]. Remarkably, GABAergic synapses formed by CCK+ BCs are negatively modulated by endocannabinoids yielding to both short- and long-term synaptic plasticity [84–86] (see below).

Importantly, alterations of cortical inhibition were implicated in several neuropsychiatric (e.g., schizophrenia, autism, mood disorders) [14, 16–18, 87–89] and neurological (e.g., epilepsy, and Rett syndrome) diseases [90, 91]. Several lines of evidence indicate that the pathological mechanisms leading to the development of these diseases do not affect inhibitory circuits globally, but they seem to be restricted to specific interneurons types. Indeed, animal model of these diseases [92] and postmortem analysis of human tissue [93, 94] indicate a decreased number and function of PV+ BCs. In line with these anatomical results, abnormal oscillatory activity was associated to schizophrenia, autism, and epilepsy [95, 96]. Conversely, the prominent subcortical aminergic input to CCK basket cell [97, 98] has prompted the hypothesis that this particular BC subtype is the substrate of plastic changes that control mood and its disorders [10]. However, an increasing amount of evidence suggests that PV+ basket cells are indeed the target of several neuromodulators such as CCK, opioids, and serotonin [99–101] and could be affected by hormones and stress that has a facilitating role towards the development of depressive disorders [102, 103].

3. Plasticity of Adult GABAergic Synapses: Cellular Mechanisms

Since the discovery of activity-dependent potentiation of synaptic strength in the hippocampus [104], considerable effort has been done to elucidate the mechanisms underlying the plasticity of glutamatergic transmission as it is supposed to rule the functional and structural refinement of synaptic contacts and be the neuronal correlate of learning and memory [20]. Conversely, plasticity of GABAergic synaptic transmission has received much less attention, but an increasing effort made during the last two decades is starting to give us some cues about the mechanisms and roles of inhibitory plasticity. Today, there are examples of GABAergic plasticity in many different brain areas such as cerebellum, brain stem, deep cerebellar nuclei, VTA, thalamus, lateral superior olive, and amygdala [22]. In the cortex and hippocampus, both long- and short-term changes in GABA transmission were described [22].

3.1. Retrograde Synaptic Signaling and GABAergic Plasticity. Retrograde synaptic signaling has emerged as one of the major mechanisms for GABAergic synaptic plasticity. Indeed, postsynaptic depolarization- or activity-dependent short-term suppression of presynaptic GABA release was described in the early 90s in the hippocampus and cerebellum and termed depolarization-induced suppression of inhibition (DSI) [108, 109]. In 2001, it was shown that endogenous cannabinoids (or endocannabinoids; eCBs) are the actual retrograde messengers mediating this post- to presynaptic communication (Figure 3(a)) [105, 106, 110–113]. eCBs are ubiquitous signaling molecules through the CNS. In the cortex and hippocampus, 2AG and anadamide, the two major endogenously produced cannabinoids [106, 114–116], are responsible for different forms of plasticity of GABAergic neurotransmission, including short- and long-term modification of synaptic strength and homo- and heterosynaptic forms of plasticity [85, 107, 111]. eCBs can be synthesized on demand, in response to many stimuli such as postsynaptic depolarizations, increased Ca^{2+} concentrations, action potential trains and metabotropic glutamate (mGlu), dopamine, and acetylcholine receptor activation [106]. After their synthesis, eCBs travel backwards from the postsynaptic cell—where they are produced—to presynaptic terminals and generate a short-term (seconds to minutes) and/or long-term (minutes to hours) suppression of GABA release through activation of CB1 receptors, G-protein coupled receptors, located mainly on presynaptic terminals [85, 106, 114]. Distinct stimuli set the duration of CB1R-mediated plasticity by activating different downstream signaling mechanism. Short-term postsynaptic depolarization results in short-term GABAergic transmission inhibition, (DSI, Figure 3(a)) that occurs through inhibition of voltage-dependent calcium channels by CB1Rs [106, 107]. Intense high-frequency synaptic stimulations of afferent fibers induce a long-term disinhibition of pyramidal cells in CA1 area of the hippocampus (Figure 3(b)) [86, 107, 111]. This form of long-lasting plasticity of GABAergic transmission, termed eCB-dependent long-term depression (eCB-LTD), depends on CB1R-mediated regulation of presynaptic protein kinase A (PKA) and the phosphatase calcineurin [117, 118]. These two signaling proteins control a cascade that results in long-term inhibition of the presynaptic release machinery.

Another form of eCB-independent retrograde signaling has been described in cortical GABAergic synapses formed by nonaccommodating FS cells and pyramidal cells in layer 2/3 of the cortex. Zilberter showed that increase of postsynaptic pyramidal-cell Ca^{2+} concentrations induced by trains of action potentials results in a short-term decrease of GABAergic transmission between these two cell types [120]. Pair-pulse ratio analysis indicated a presynaptic locus for this phenomenon and suggested the involvement a retrograde signal. Although increases in pyramidal neuron dendritic Ca^{2+} levels are a triggering signal for the synthesis of eCBs, FS cells in L2/3 of the cortex do not express detectable CB1Rs, therefore ruling out the participation of eCBs in this form of plasticity [119]. Further investigations have shown that this form of disinhibition is likely mediated by somatodendritic release of glutamate-filled vesicles expressing the vesicular glutamate transporter vGLUT3 with consequent activation

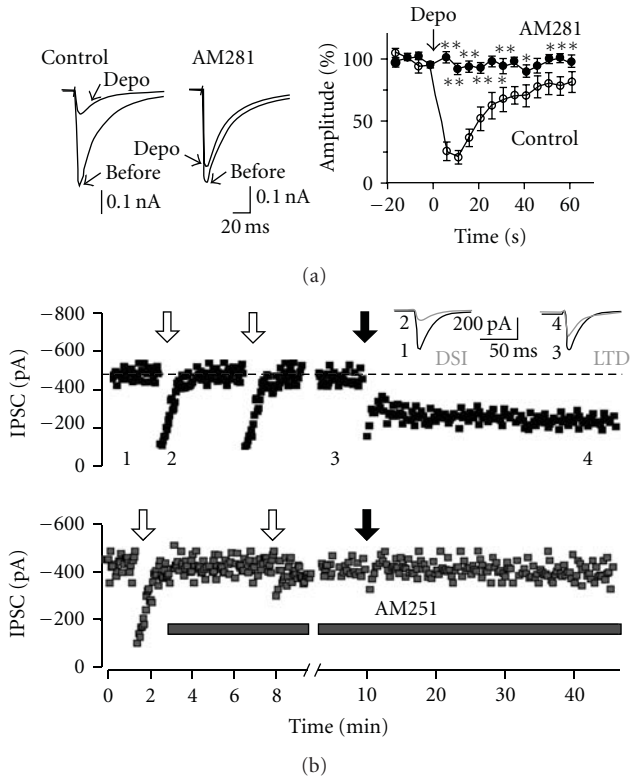

Figure 3: *Endocannabinoid-dependent plasticity of GABAergic synapses*. (a) In cultured hippocampal neurons, eCBs mediate a form of short-term retrograde signaling strongly reducing GABAergic responses. This can be observed by the reduction of unitary inhibitory postsynaptic currents (IPSCs) evoked after a 5 sec-long depolarization (depo) of the postsynaptic neuron. The CB1R antagonist AM281 blocked the depolarization-induced suppression of inhibition (DSI). Time course of DSI is indicated in the right panel. Modified from [105]. For details, see reference [105, 106]. (b) Time course of extracellularly evoked IPSC amplitude in the CA1 area of the hippocampus. Brief depolarizations (white arrows) of the recorded pyramidal cell induce DSI (see Figure 3), whereas high-frequency stimulation (black arrow) of afferent fibers induces LTD of GABAergic responses. Traces correspond to the time points indicated by numbers in the upper graph. Both DSI and LTD induction are blocked by the selective CB1R antagonist AM251 (gray bar, lower graph). Modified with permission from [107].

of presynaptic metabotropic glutamate receptors (Figures 4(a) and 4(b)) [119, 120].

3.2. Spike Timing-Dependent Plasticity of GABAergic Synapses. Spike timing-dependent plasticity (STDP) is a form of synaptic plasticity that requires both pre- and postsynaptic firing, inducing changes in synaptic strength whose polarity (potentiation or depression) depends on the temporal order of pre- and postsynaptic spiking. Glutamatergic STDP has been shown to follow precise general rules: long-term potentiation (LTP) of synaptic transmission is produced when presynaptic spiking precedes (in a millisecond time window) postsynaptic action potential, whereas LTD is induced when postsynaptic spikes precede presynaptic action potentials [122–124]. STDP of GABAergic synapses (and of glutamatergic synapses onto inhibitory cells [125]) has only recently been investigated and seems a bit more complex than glutamatergic STDP. Indeed, in the hippocampus, a symmetric dependency was found: LTP of GABAergic connections was induced when pre- and post-stimuli where paired at ±20 milliseconds whereas longer intervals led to LTD [126]. Conversely, in the entorhinal cortex, GABAergic STDP follows the same temporal dependency as glutamatergic STDP [127]. Both hippocampal and entorhinal cortex spike-timing LTPs depend on postsynaptic Ca^{2+} rises induced by back-propagating action potentials and were proposed to have a postsynaptic origin [126, 127]. Interestingly, in hippocampal neurons (both cultured and in slices), it has been shown that coincident pre- and postsynaptic firing that results in LTP of GABAergic transmission produced a shift of the reversal potential for GABA-mediated (E_{GABA}) responses at this particular synapse. Indeed, the coincident activity resulted in the inhibition of the Cl^- cotransporter KCC2 resulting in a more depolarized E_{GABA} [126].

Given the rich heterogeneity of GABAergic interneuron subtypes, one key question is whether plasticity of GABAergic neurotransmission follows some general rules regardless of the GABAergic cell subtype or if specific inhibitory cell subclasses are more susceptible to develop certain forms of plasticity. Remarkably, Holmgren and Zilberter demonstrated that in neocortical layer 2/3 unitary connections between FS interneurons and pyramidal neurons are substrate for long-term modification of synaptic strength induced by pairing pre- and postsynaptic action potentials [121]. Indeed, this study showed that LTP of GABAergic responses was induced when the presynaptic FS cell fires at least 400 ms after the postsynaptic pyramidal did. Interestingly, the plasticity of this particular GABAergic synapse is bidirectional and LTD was induced if presynaptic FS fires during or shortly after a train of action potentials in a pyramidal cell (Figures 4(c) and 4(d)) [121]. In contrast with the results observed in hippocampal cells, STDP of FS to pyramidal neurons did not alter the reversal potential for synaptic responses, suggesting an alternative mechanism for this form of plasticity [121]. Although the exact mechanism leading to STDP of FS to pyramidal cell GABAergic transmission is still unknown, the dependency on intact calcium signaling and unchanged pair-pulse ratio of unitary postsynaptic responses after conditioning does not favor a presynaptic origin [121]. In line with a postsynaptic expression of GABA-mediated synaptic plasticity onto neocortical pyramidal neurons, recent evidence indicated the role of postsynaptic L- and R-type Ca^{2+} channels in activity state-dependent LTD and LTP of GABAergic inhibition in layer 5 pyramidal neurons [128].

3.3. Other Types of Plasticity of GABAergic Synaptic Transmission. Activity-dependent plasticity of GABAergic synapses has been demonstrated in adult cortex and hippocampus. Both LTP and LTD of GABAergic transmission can be triggered by different forms of stimuli that consist mostly in high-frequency afferent stimulations [86, 129–132]. Several forms of heterosynaptic long-term changes of GABAergic

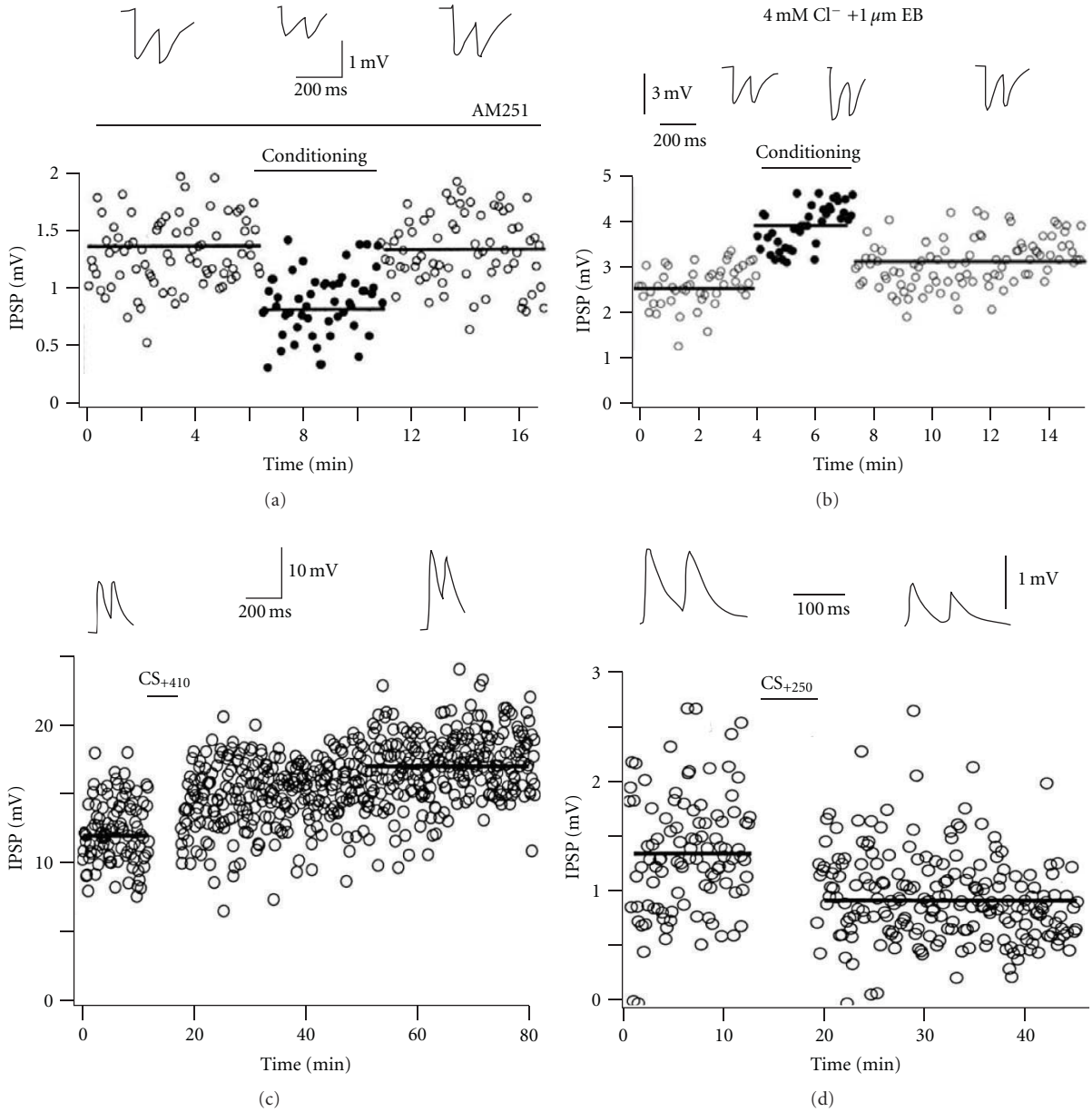

Figure 4: *Endocannabinoid-independent plasticity of GABAergic synapses*. (a) Brief train of action potentials (conditioning) in cortical pyramidal cells depresses unitary inhibitory postsynaptic potentials (uIPSPs) evoked by synaptically connected FS interneurons (top traces, from left to right: before, during and after conditioning). This form of short-term retrograde depression is indicated by the black dots during the conditioning paradigm (conditioned response is measured 250 ms after the conditioning stimulus), and it is not blocked by the selective CB1R antagonist AM-251, ruling out the involvement of eCB signaling. (b) Conditioning mediated depression of uIPSPs from FS interneurons (top traces, from left to right: before, during, and after conditioning) is prevented by the nonselective vesicular glutamate transporter Evans Blue (EB) suggesting a critical role for dendritically released glutamate in this form of plasticity. Modified with permission from [119]. For details see [119, 120]. (c, d) Spike timing-dependent plasticity (STDP) results in potentiation (c) and depression (d) of uIPSPs (top) elicited by FS interneurons onto cortical pyramidal cells. Long-term potentiation (LTP) of uIPSP amplitudes (top traces) is obtained when presynaptic FS cell fires 410 ms after the beginning of a brief train of action potentials (10 action potentials at 50 Hz) in the postsynaptic pyramidal cell (c). Conversely, long-term depression (LTD) of uIPSPs is observed when the presynaptic FS cell fired 250 ms after the start of an identical train (d). (c and d): Modified with permission from [121].

responses were shown in adult hippocampus and have the activation of glutamatergic fibers as a common origin [86, 129]. Although induction is invariably postsynaptic, the expression locus can be either pre- or postsynaptic. In CA1 region of the hippocampus, glutamate released by Schaffer-collaterals activates mGluRs, triggering the synthesis of eCBs that act presynaptically to reduce GABA release (see above) [86, 107, 111]. Notably, a different study reported that glutamate induces postsynaptic Ca^{2+} increases through NMDA receptors that, in turn, activate postsynaptic calcineurin [129]. Importantly, this calcium-sensitive phosphatase has been involved in the negative regulation of $GABA_A$ receptors

activity resulting in a postsynaptic locus of expression for this form of GABAergic LTD [129]. As a common theme, it seems that the induction of all these forms of GABAergic plasticity requires the sustained firing of the GABAergic cell that produced GABAergic LTD. This suggests a dual role of GABAergic interneurons: promoting synaptic plasticity and conferring synapse specificity [117, 133–136].

Another form of activity-dependent potentiation of inhibitory synaptic transmission is mediated by astrocytic calcium signaling in the hippocampus. In synaptically coupled pairs of interneurons and pyramidal cells, a train of high-frequency action potentials in the presynaptic inhibitory cell produces an increase in the probability of GABA release that lasted for 15–20 minutes [137]. Strikingly, neighboring astrocytes were shown to be critical mediators of this effect. Indeed, interneuron firing and consequent release of GABA triggered $GABA_B$-mediated calcium signaling in astrocytes adjacently located to the inhibitory neuron. Upon $GABA_B$ receptor activation and through a mechanism dependent on AMPA and NMDA receptors, astrocytes induced potentiation of inhibitory transmission between interneuron and pyramidal cells [137].

Another form of GABAergic synaptic potentiation has been described in FS to stellate cells connections in layer 4 of mouse visual cortex [138]. At this synapse, paring of presynaptic FS spikes with subthreshold depolarization of postsynaptic stellate cells resulted in a significant potentiation of the GABAergic synapses that lasted for at least 30 minutes. In this study, no changes in the PPR were detected and the reversal potential of synaptic responses remained unaltered [138]. Interestingly, this form of plasticity is prevented by coupling pre- and postsynaptic spikes suggesting that STDP at neocortical FS to principal cell connections is layer dependent.

4. Functional Role of GABAergic Plasticity

Many examples of GABAergic synaptic plasticity come from studies focused on the development of cortical inhibitory circuits. Indeed, in the developing mouse neocortex, GABA levels are modulated by neuronal activity and sensory experience through the regulation of the *Gad1* gene [139, 140], which codes for GAD67, a glutamic acid decarboxylase that is the rate-limiting enzyme responsible for GABA synthesis [141]. In turn, modified GABA transmission increases the number of synaptic contacts, axon branching, and innervation field of single perisomatic interneurons [142, 143]. In the dentate gyrus, both pre- and postsynaptic changes occur during development of GABAergic synapses originating from PV+ BCs, including increased amplitude, decreased failure rate, and decay constant of unitary inhibitory responses [144]. These changes reflect a developmentally regulated plasticity of FS cell-mediated GABAergic transmission transforming this cellular element into the well-known precise synaptic metronome and fast signaling unit.

Despite the growing evidence in favor of GABAergic transmission as a pivotal mechanism for several functions of neuronal circuits, little is know about the actual role of activity-dependent modifications of inhibitory synapses in altering network activities that are strongly dependent on specific GABAergic circuits. In fact, functional consequences of changes in inhibitory synapse strength can vary dramatically depending on the interneurons subtype involved. Indeed, different interneuron subclasses possess different mechanisms underlying basic GABAergic transmission, such as, for example, different expression of presynaptic voltage-gated Ca^{2+} channels and/or metabotropic receptors that modulate GABA release [10, 52]. Since these differences result in specific modes of GABAergic transmission, it is likely that specific GABAergic synapses originating from specific interneuron types will generate different forms of plasticity in response to similar activity patterns. To complicate things even further, different classes of inhibitory interneurons are activated by glutamatergic synapses exhibiting peculiar properties, including short- and long-term plasticity and expression of specific ionotropic and metabotropic glutamate receptors [26–29, 33, 37, 145]. This diversity of excitatory properties onto different interneuron classes was shown to underlie differential temporal recruitment of different GABAergic cell types during cortical activities [146], therefore limiting or promoting induction of downstream GABAergic plasticity in selective cell types. Some indirect evidences for plasticity of GABAergic transmission arising from specific interneuron types were found in development when sensory activity is a critical regulator of GABAergic plasticity. For example, FS cell-mediated transmission in visual cortex was shown to develop an LTP at these inhibitory synapses in mice that were visually deprived (see above) [138]. In neocortical low-threshold spiking interneurons (including dendrite targeting Martinotti cells) similar sensory deprivation (whisker trimming) induced a change in the pattern of inhibitory transmission, with increased amplitude and decay kinetics [147]. On this line, sensory deprivation induces a decrease in the number of dendrite targeting GABAergic synapses in L4 [148] and somatic targeting interneurons [149].

The induction of plastic changes in GABAergic synapses may have different outcomes depending not only on the polarity or duration of the change, but also on the location and origin of these GABAergic synapses. In the hippocampus, stimuli that induce LTP of glutamatergic transmission also induce eCB-dependent LTD of GABAergic synapses. This form of LTD is likely restricted to interneuron types expressing CB1 receptors that include CCK-positive basket cells and Schaffer collateral-associated (SCA) interneurons [85, 150]. The plasticity of this GABAergic input has been shown to be responsible for the increased excitability of pyramidal cells after eCB signaling activation and for the EPSP-to-spike (E-S) coupling, that is, an important component of LTP of glutamatergic transmission [129].

The increased strength of GABAergic transmission between PV+ BCs and pyramidal neurons would decrease the excitation-to-inhibition ratio in the somatic compartment of principal cells and limit their time window for spike generation. Since each PV+ BC contact a large number of pyramidal neurons, the plasticity of its GABAergic

connections will influence a large portion of the network, and therefore change some global properties of network activities. This applies if plasticity of GABAergic synapses results from a broad change of presynaptic neurotransmitter release, regardless of postsynaptic activity. On the other hand, combined presynaptic and single pyramidal neuron firing might induce STDP modifying a small portion of GABAergic synapses. This can happen during theta and gamma activities, when the firing of pyramidal neurons and FS cells are temporally displaced as they are locked to different components of the oscillation phase [3, 13]. Another form of fine regulation of few components of a network is represented by eCB-dependent synaptic plasticity. Indeed, eCB-mediated decrease of perisomatic inhibition arising from CCK+ interneurons will likely disinhibit and thus increase excitability of those single pyramidal cells that retrogradely delivered eCBs. This mechanism will therefore provide a self-induced fine tuning of inhibition. In addition, since these signaling molecules are produced by highly active principal cells, eCBs are ideally placed to organize cell assemblies that fire in close relation during certain behavioral states, although the role of eCB-mediated retrograde signaling onto CCK+ cells during oscillations and network activities is far from being clear [151, 152]. In this scenario, it is possible that sustained firing activities of pyramidal cells will induce an eCB-dependent overall depression of GABAergic transmission originating from CCK+ interneurons. This will likely shift the balance of perisomatic inhibition towards the fast, precise, and reliable inhibition from PV+ basket cells, which are insensitive to eCBs. Since these two types of interneurons differentially contribute to feed forward and feed back inhibition onto CA1 cells, retrograde eCB signaling has the potential of changing the integration properties of principal cells by narrowing the time window for spike generation and allowing increased temporal resolution [10, 146]. As detailed above, neocortical pyramidal cells use different mechanisms to selectively modulate specific sources of perisomatic GABAergic transmission in a retrograde fashion (eCBs in CCK+ basket cellls versus glutamate in FS interneurons). It is still unclear, however, if these two modulation mechanisms can be uncoupled, thus leading to a change in the perisomatic inhibition balance originating from PV+ and CCK+ basket cells.

Synaptic plasticity of GABAergic synapses can be target specific. It has been shown that eCB-mediated suppression of GABA transmission is present at GABAergic synapses on pyramidal neurons but not on interneurons in layer 2/3 of the mouse neocortex [153, 154]. In the hippocampus, however, both GABAergic synapses on interneurons and pyramidal cells can be modulated by retrograde eCB signaling [150]. In addition, GABAergic inputs to layer 5 pyramidal cells in the neocortex is cannabinoid-insensitive, whereas GABAergic synapses onto layer 2/3 principal cells are strongly modulated by retrograde eCB signaling [155, 156]. These observations raise the possibility that certain forms of eCB-mediated plasticity may rely on the identity and location of both pre- and postsynaptic neurons. Therefore, specific activities can differentially suppress inhibition in distinct cortical layers and specific cell types (glutamatergic versus GABAergic).

There is little (if any) direct evidence for plasticity of GABAergic transmission at distal dendritic sites, such as that provided by O-LM interneurons and Martinotti cells in the hippocampus and neocortex, respectively. Importantly, Martinotti cells mediate a prominent disynaptic dendritic inhibition triggered by high-frequency firing of pyramidal neurons [157–159]. Plasticity of these GABAergic connections will, therefore, be crucial for information filtering by these dendrite-targeting interneurons [160].

Interestingly, the polarity of STDP of glutamatergic synapses depends on the location of the synapses within the dendritic arbor. The same timing of pre- and postspiking gives rise to LTD at most distal synapses, but LTP at more proximal dendritic synapses [161]. It will be interesting to investigate whether interneurons targeting different compartments of principal cells, for example, dendritic versus somatic, have different plasticity rules and whether specific patterns of network activation have differential effects on inhibition arising from specific sources.

5. Conclusions and Future Directions

In this paper we emphasized how the great diversity of interneuron types gives rise to an even greater diversity of GABAergic transmission and plasticity. Indeed, the specific key role of each GABAergic circuit in sculpting different forms of cortical activity has only recently begun to be elucidated [3]. Since it has been shown that GABAergic synapses exhibit plasticity, it will be fundamental to reveal the governing rules of GABAergic transmission originating from different neuron subclasses.

In addition to the interneuron type-specific forms of synaptic plasticity, several open questions remain, such as, for example: (i) what are the physiological activities (single neuron and/or network activities) necessary to induce plasticity of GABAergic synapses? (ii) Is there a heterogeneity or bidirectional plasticity of GABAergic synapses in different cortical areas? (iii) What is the functional role of GABAergic transmission during different cortical activities? (iv) What other neuromodulators, in addition to endocannabinoids and glutamate, can induce activity-dependent changes of GABAergic synaptic strength? (v) Could GABAergic plasticity lead to complex Cl^- gradients inside a principal neuron [126], such that the direction (inhibition versus excitation) of GABA-mediated responses might, in some cases, contribute to some forms of hyperexcitability? (vi) Is plasticity of inhibitory synapses altered in pathological situations? Addressing these questions will help define the fundamental molecular, cellular, and synaptic mechanisms governing several core functions of cortical activities, therefore advancing our knowledge on the basic rules underlying complex cognitive and behavioral functions, with likely important implications for neurological and psychiatric diseases.

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Review Article

Functional Consequences of the Disturbances in the GABA-Mediated Inhibition Induced by Injuries in the Cerebral Cortex

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Cortical injuries are often reported to induce a suppression of the intracortical GABAergic inhibition in the surviving, neighbouring neuronal networks. Since GABAergic transmission provides the main source of inhibition in the mammalian brain, this condition may lead to hyperexcitability and epileptiform activity of cortical networks. However, inhibition plays also a crucial role in limiting the plastic properties of neuronal circuits, and as a consequence, interventions aiming to reestablish a normal level of inhibition might constrain the plastic capacity of the cortical tissue. A promising strategy to minimize the deleterious consequences of a modified inhibitory transmission without preventing the potential beneficial effects on cortical plasticity may be to unravel distinct GABAergic signaling pathways separately mediating these positive and negative events. Here, gathering data from several recent studies, we provide new insights to better face with this "double coin" condition in the attempt to optimize the functional recovery of patients.

1. Introduction

Cortical injuries are one major cause of death and permanent disabilities worldwide. In the attempt to ameliorate the survival rate and the postlesion rehabilitation of patients, researchers have developed several animal models of cortical injury to reproduce different aspects of this pathological condition.

In particular, a great effort has been dedicated in the investigation of the physiological disturbances spreading in the surrounding uninjured tissue and sometimes even in remote brain areas [1].

Even though these lesion-induced functional alterations might notably differ depending on many factors, such as the nature of the insult (cerebrovascular rather than traumatic), the extent of the damage and the cortical structures affected, some pathophysiological events have been systematically reported following many different experimental models of cortical lesion.

Interestingly, one of the most frequently observed functional change postlesion is a reduction in the GABA-mediated inhibition which, therefore, seems to be (with some degrees of variability) a general phenomenon taking place as a consequence of a massive neuronal death.

Because a deficit in the GABAergic transmission might easily compromise the delicate balance between excitatory and inhibitory neurotransmission [2] this lesion-induced phenomenon has been strongly implicated in the generation of hyperexcitable cortical networks [3] and in the genesis of epileptic events often observed after brain injuries [4, 5].

However, the inhibitory action of GABA is going far beyond the control of the excitability of neuronal networks. The temporal and spatial precise release of GABA can also guarantee high specific responses of cortical neurons [6, 7]. Moreover, the GABAergic transmission has a fundamental role in controlling the plastic capacity of cortical networks. On this concern, different studies indicate that if the strength of the GABA-mediated inhibition is falling below a certain

threshold, the plastic properties of the cortical networks will be augmented, sometimes even to levels similar to those observed during the critical period for plasticity [8–10].

In light of these findings, the impaired inhibitory transmission observed postlesion might not be only a deleterious process but, by enhancing the plastic capacity of the cortex, could also promote the functional reorganization of the surrounding uninjured cortical tissue contributing to the functional recovery from the lesion-induced neurological deficits.

The injury-induced reduction of inhibition may, therefore, share both detrimental and beneficial effects.

Unraveling distinct GABAergic signaling pathways separately mediating these positive and negative events could be extremely helpful in the design of a more effective postlesion rehabilitation therapy.

In the attempt to provide new insights to better face with this "double coin" condition, in this paper we will discuss several studies which documented a reduced and/or an altered GABAergic transmission as a consequence of a lesion in the cerebral cortex, and most importantly, we will try to explain how and through which cellular mechanisms the altered GABAergic transmission could influence functions, excitability, and plasticity of cortical networks.

2. Physiology of GABAergic Signaling

The GABA receptors are divided into 2 classes: $GABA_A$ receptors ($GABA_A$Rs) and $GABA_B$ receptors ($GABA_B$Rs) (previously $GABA_C$Rs were considered to form a third separated class; however, because of their strong structural and functional similarity to $GABA_A$Rs, they are today classified as a subfamily of $GABA_A$Rs).

$GABA_A$Rs. $GABA_A$Rs belong to the cys-loop superfamily of ligand-gated ion channels and mediate fast synaptic inhibition in the central nervous system (CNS).

$GABA_A$Rs are heteropentameric structure composed by distinct types of subunit. In the mammalian brain, the majority of synaptic $GABA_A$Rs are formed by two α, two β and one γ subunit.

Although many different α, β, and γ subunits have been identified (α 1–6, β 1–3, γ 1–3), in the CNS defined combinations of subunits are more frequently found (the most abundant combinations are $\alpha1$, $\beta2$, $\gamma2$; $\alpha2$, $\beta3$, $\gamma2$; $\alpha3$, $\beta3$, $\gamma2$) [11].

Importantly, the combination of these subunits can determine the localization and the functional properties of the receptors. To mention a peculiar example, $GABA_A$Rs in which the γ subunit has been replaced with the δ subunit are exclusively found extrasynaptically [12], are activated by low concentrations of GABA and they display a reduced desensitization [13, 14]. Thanks to these properties δ subunit-containing $GABA_A$Rs are ideally suited to mediate tonic inhibition [15].

$GABA_A$Rs are selectively permeable to Cl^- and to a less extent to HCO_3^- [16].

In the mature CNS, the asymmetrical distribution of Cl^- across the membrane (the Cl^- inside the cells is maintained relatively low in comparison with the Cl^- concentration in the extracellular space, mainly through the action of the potassium-chloride cotransporter 2, KCC2) strongly contribute in defining the reverse potential of $GABA_A$-mediated currents (E_{GABA}), and it is, therefore, of fundamental importance to guarantee the inhibitory actions of GABA. This also explains why in immature neuronal networks, where the Cl^- intracellular concentration is relatively high, GABA can exert excitatory actions [17].

$GABA_B$Rs. On contrary, $GABA_B$Rs are metabotropic, G protein-coupled receptors. They exert their inhibitory action either by decreasing Ca^{2+} currents or by increasing K^+ conductance [18]. $GABA_B$Rs are also expressed at the presynaptic site where by reducing the probability of neurotransmitter release seem to offer a negative feedback mechanism to limit synaptic transmission within a certain physiological range [19].

3. Interneuronal Diversity

In the mammalian neocortex, approximately 20%–30% of neurons use GABA as neurotransmitter [20, 21].

In contrast to pyramidal cells, GABAergic neurons are an extremely heterogeneous population of cells. Different criteria have emerged in the attempt to classify interneurons based on their different morphological, physiological, and neurochemical features [22], but nonetheless, a universal categorization is still missing. Furthermore, it is extremely difficult to attribute a potentially singular functional role to each subclass [23].

A detailed description of the anatomical and functional properties of different interneurons subclasses is beyond the purpose of this paper; however, it is noteworthy to mention one important structural-functional relation emerging from recent studies: interneurons targeting different domains of principal cells seem to subserve specific functional roles [24].

For instance, interneurons preferentially innervating dendrites of principal cells are particularly suited to modulate excitatory postsynaptic potentials (EPSPs) occurring at nearby synapses, thereby limiting the spatiotemporal summation of excitatory inputs and potentially preventing hyperexcitability. Interneurons predominantly sending axons onto the soma and the proximal dendrites (as basket cells) of principal cells are strategically located to control the output of the target neurons, and by operating as a precise clockwork, they can synchronize the firing of large population of principal cells contributing to the generation of cortical oscillatory patterns [25, 26].

4. The Effect of Cortical Lesions on GABAergic Transmission

Injuries in the cerebral cortex often lead to an abnormal excitability of the surrounding neuronal networks.

An increased spontaneous and evoked neuronal firing has been reported following different experimental models of brain injury [27, 28]. In addition, different functional magnetic resonance imaging (fMRI) studies reported an abnormal activation of commonly silent brain areas in patients suffering from a stroke [29, 30].

An increased susceptibility to epileptiform discharges have been observed to progressively develop in lesion models of partially isolated cortex [31–33], in photothrombotic cortical lesion models [34, 35] as well as in patient suffering from brain injuries [5].

In parallel, data from many *in vitro* studies revealed a postlesion reduction of the GABAergic intracortical inhibition. This reduced inhibitory transmission was, therefore, considered primarily responsible for the lesion-induced hyperexcitability and for the increased propensity to epileptogenesis.

A reduced strength in the GABAergic intracortical inhibition was reported following a photochemically induced cortical infarct [36, 37] in an experimental model of middle cerebral artery occlusion [38, 39] as well as in the vicinity of a local cortical thermolesion [40] performed in rodents. In these studies, electrophysiological recordings from the surviving neighboring cortical tissue disclosed an impairment of the recurrent intracortical inhibition.

Beyond this functional evidence, quantitative receptor autoradiography studies have reported a downregulation of radiolabeled muscimol binding to $GABA_A$Rs in the surround of a cerebral photothrombosis [28] as well as after unilateral permanent focal cerebral ischemia in the rat brain [41]. The decreased binding of radiolabeled muscimol was interpreted as a reduced density of $GABA_A$Rs.

All together, these studies indicate that cortical injuries, independent of their etiology, can similarly lead to a reduced strength of the inhibitory neurotransmission.

Time Window. The lesion-induced suppression of inhibition developed relatively fast, since the effect was already visible one day after the lesion induction [36, 40], it seems to reach a peak in the first week postlesion, and afterwards, it slowly and only partially recovers to a subnormal level two months after the lesion induction
[28].

Unfortunately, due to the few chronic investigations, it is still not clear if these relative long-lasting effects are typical of some lesion models and if they depends on the size and location of the cortical damage. Nonetheless, in the subacute phase postlesion (first week postlesion), the impaired inhibition seems to be a phenomenon systematically observed.

Cellular Mechanisms. Several cellular mechanisms have been proposed to underlie the lesion-induced suppression of inhibition.

The degeneration of particular vulnerable interneurons subtypes could constitute one plausible mechanism, especially when brain injuries are followed by extensive secondary brain damage. Some studies, performed in models of ischemic and traumatic brain lesions, indeed reported signs of selective suffering and death of interneurons at the border of the injury [42, 43].

GABAergic interneurons could also survive but enter a functional suppress status.

A large body of evidence demonstrates the existence of a series of modulatory (or homeostatic) mechanisms in the CNS trying to maintain the firing rate of neurons within a certain physiological range in face of dynamic changes in synaptic drive [44–46]. The observed downregulation of the inhibitory strength could be, therefore, seen as a homeostatic mechanism in response to the lesion-induced loss of some excitatory synaptic inputs in the attempt to restore the initial level of neuronal activity.

Consistent with this hypothesis, a reduction in the number of functional GABAergic synapses has been suggested by several studies. In a lesion model of partially isolated cortex, the structural reconstruction of fast spiking interneurons in the vicinity of the "undercut cortex" revealed a significant reduction in their axonal length and a reduced number of large axonal boutons [47]. At the postsynaptic site, a significant downregulation of the $\alpha 1$ and a slight reduction of the $\alpha 2$ subunit of $GABA_A$Rs were found in the surround of a photochemically induced cortical lesion in rats [43]. Although, based on this finding one cannot rule out a compensatory increase in the expression of others subunits, the parallel decreased binding of radiolabeled muscimol to $GABA_A$Rs, observed in another study performed with the same lesion model (see above) suggests an overall reduction in the expression of postsynaptic receptors [28].

Furthermore, since the combination of the subunits determines the cellular localization and the functional properties of the $GABA_A$Rs [11], even only a shift in the subunits composition, with no change in the expression of the receptors, might profoundly influence GABA-mediated neurotransmission.

Changes in the physiological properties of GABAergic signaling have been also reported. Intracellular recordings from pyramidal cells in the vicinity of an experimentally induced focal cortical infarct [48], in the surrounding of a phototrombotic cortical lesion in rats [3], as well as in a lesion model of partially isolated cortex [49] revealed a positive shift in E_{GABA}.

This shift in E_{GABA} toward more depolarized potentials has been primarily attributed to a downregulation of the specific K^+-Cl^- cotransporter 2 (KCC2) with a consequent impaired extrusion of Cl^-. In support of this hypothesis, some studies performed in traumatic models of axotomized neurons, both *in vitro* and *in vivo,* reported a reduction in KCC2 expression at mRNA and protein level [50, 51].

Interestingly, some of the alterations in the GABA-mediated inhibition (e.g., the likely reduced number of GABAergic synapses and the positive shift in E_{GABA}) seem to describe a developmental juvenile status when the GABAergic system is still not fully mature.

5. Consequences of the Altered Inhibitory Transmission on Cortical Networks Excitability and Functions

Although the association of the reduced GABA-mediated inhibition observed *in vitro* with the hyperexcitability of cortical networks often observed *in vivo* following cortical injuries might seem relatively straightforward, the complexity of the GABAergic signaling and the diversification of interneuronal classes with potential distinct functional roles [22, 23] makes the identification of the underlying cellular mechanisms and the functional consequences on neuronal networks an arduous task.

Here, bringing together many outstanding studies on neuronal networks function, we provide new elements which will be hopefully helpful in the comprehension of how the altered inhibitory transmission induced by cortical injuries could affect excitability and function of neuronal circuits.

5.1. Brain Injury Induced Disturbances in the Excitation-Inhibition (E-I) Balance. In the CNS, the fine-tuned balance between excitatory and inhibitory neurotransmission is essential to guarantee a proper function of neuronal circuits.

At first glance, such a statement might suggest neuronal transmission to be prone to instability, especially in light of the fact that the excitation-inhibition (E-I) balance is continuously challenged by peripheral stimuli constantly bombarding the CNS.

However, accumulating lines of evidence indicate that in sensory cortices, an increase in excitatory conductance is normally counterbalanced by a similar augmentation of inhibitory conductance [52]. Furthermore, this parallel increase in the level of excitation and inhibition can be maintained over a wide dynamic range conferring to the CNS the capability to respond to a large variation of stimulation intensity without becoming overexcited.

Despite the substantial flexibility, this dynamic equilibrium can be relative easily compromised by different pathological conditions, such as a brain damage. Different studies performed in animal models of ischemic and traumatic brain injuries indeed reported an important shift in the E-I balance in favour of excitation. Morphological and functional analyses of the rat hippocampus performed few months following a global ischemic episode revealed a dramatic loss of GABAergic presynaptic terminals accompanied by an increase in glutamatergic synapses [53]. Furthermore, in a traumatic brain injury model, recordings from the chronically injured rat sensory-motor cortex did also disclose changes in the efficacy of excitatory and inhibitory neurotransmission in favour of excitation [54]. These anatomical and physiological changes were found to be associated with the onset of epileptic activity indicating a potential important contribution of the shifted E-I balance in the generation of these events.

5.2. Recurrent Inhibitory Networks and Potential Consequences of Their Dysfunction. Since the recruitment of recurrent inhibitory circuits plays a key role in the maintenance of the E-I balance, the lesion-induced impairment of intracortical inhibition function [36–39] is most likely one major cause for the development of hyperexcitable neuronal networks.

However, as already mentioned above, the GABAergic system is extremely heterogeneous, being composed by diverse interneuron cell types with potential specific functional properties [22]. As a consequence of that, the impairment of distinct subpopulations of GABAergic cells will likely have different effects on the excitability and function of cortical networks.

Interestingly, a subclass of dendritic projecting GABAergic neurons expressing somatostatin (SOM neurons) seems to be particularly efficient to counteract increasing levels of cortical excitation. The excitatory synapses impinging on this category of interneurons generate EPSPs which are initially small in amplitude but that progressively increase with the number of subsequent stimuli (facilitating excitatory synapses) eventually leading to the generation of action potentials. This suggests that these cells have the capability to "buffer" a wide range of excitatory inputs before becoming saturated [55], thereby preventing hyperexcitability to occur.

A selective loss of dendritic-projecting SOM containing interneurons has been also reported in human patients [56] and in experimental animal models of temporal lobe epilepsy [57] suggesting a potential involvement of these interneurons in the generation of epileptic seizures.

Another interneuron subtype which seems to strongly contribute in dampening excessive cortical excitability is constituted by chandelier cells. This category of interneurons, by selectively forming GABAergic synapses onto the axon initial segment of principal cells, can strongly control the generation of action potentials in pyramidal neurons, and therefore, they might have the capability to prevent excessive firing [58]. In line with this assumption, *in vivo* electrophysiological recordings from the somatosensory cortex of rats strongly indicate that chandelier cells do not seem particularly suited to encode incoming ascending information, but they seem indeed strongly involved in preventing hyperexcitability of cortical networks [59]. Furthermore, the selective loss of chandelier cells (or of their axonal terminals) at epileptic foci, reported by different studies, indicates that this cell type might be involved in the generation of epileptic activity [60, 61].

Parvalbumin-containing (PV) basket cells constitute another important class of interneurons strongly participating in the cortical recurrent inhibitory circuits. In distinction to the above-described subtypes of interneurons, PV basket cells seem to strongly participate in the cortical information processing.

The fast spiking phenotype [62–64], the strong glutamatergic inputs and the short membrane time constant [24] attribute to these interneurons the capability to encode presynaptic inputs with high temporal precision [65]. Furthermore, PV basket cells exhibit strong electrical coupling with each other through gap junctions [66–68] and can finely control the output of pyramidal cells by predominately innervating their perisomatic region [69].

Together, these electrophysiological and anatomical properties define the fundamental role of PV basket cells in

synchronizing action potential discharges of large numbers of principal cells promoting the emergence of network oscillations in the gamma frequency band (30–80 Hz) [64, 70, 71].

Oscillatory activity in the gamma range has been reported to play a crucial role in the perception and processing of sensory stimuli [72], in focusing the attention toward relevant stimuli [73], and in the performance of complex motor actions requiring sensorimotor integration [74]. These findings suggest that the performance of different cognitive tasks requires a physiological function of PV basket cells.

Nowadays, it is still unknown whether the observed reduction of inhibitory transmission following cortical injuries is the result of a lesion-induced effect on a specific subpopulation of inhibitory cells or if all classes of interneurons are equally affected. Potentially, a lesion-induced reduction in the activity of SOM neurons or chandelier cells might critically compromise the E-I balance especially during high level of excitation, while a lesion-induced change in the activity of PV basket cells could have a profound impact on the cortical information processing.

The recent availability of transgenic mice expressing fluorescent proteins (such as the green fluorescent protein GFP) in defined classes of interneurons [75–77] offers nowadays the possibility to easily investigate how different categories of neurons respond to a cortical lesion, and we are, therefore, confident that in the next years, many of the still open questions will be answered.

5.3. Influence of E_{GABA} on the E-I Balance. The reduced strength of the inhibitory transmission, often observed following cortical injuries, does not seem to be simply the result of a lesion-induced degeneration or reduced activity of GABAergic interneurons. The situation is far more complicated, since functional modifications of the GABAergic signaling have been reported after cortical lesions as well.

An important phenomenon, described following different cortical lesion models, which could potentially compromise the E-I balance, is the positive shift in E_{GABA} [3, 48–51].

Generally, GABA is known to exert its inhibitory action by leading to a hyperpolarization of the postsynaptic neuron, thereby driving the membrane potential away from the threshold for the generation of spikes (spike threshold). The positive shift of E_{GABA} at values above the resting membrane potential (V_m) could lead to the straightforward conclusion of an increase in the neuronal excitability due to a GABA-mediated depolarization of the postsynaptic membrane.

However, depolarization is not always synonymous of excitation [11].

$GABA_A$-mediated depolarizing responses can still exert an inhibitory action (on conditions that E_{GABA} remains more negative than the spike threshold) by increasing the membrane conductance of the postsynaptic neurons, and thereby shunting excitatory inputs "just" generated at nearby synapses. This inhibitory mechanism, known as "shunting inhibition", has been shown to operate even under physiological conditions at many cortical and hippocampal synapses, where E_{GABA} was found between the resting V_m and the spike threshold [78–81].

From this evidence, one can predict that the pathological positive shift in E_{GABA} might convert many hyperpolarizing GABAergic synapses into shunting ones.

Nonetheless to estimate the consequences of such a phenomenon on the E-I balance is not an easy task. Shunting inhibition can have in some instances a stronger inhibitory effect than hyperpolarization. This is because at depolarized membrane potentials $GABA_A$Rs exhibit a higher ionic conductance (or outward rectification) [16, 82]. Moreover, shunting inhibition cannot lead to the opening of hyperpolarization-activated cation channels and does not favour the deinactivation of voltage sensitive sodium and low threshold calcium channels as hyperpolarizing postsynaptic potentials do [83]. For these reasons, shunting inhibition can prevent the generation of "rebound excitation" in some neurons [11].

However, on the other side, the efficacy of shunting inhibition is strictly dependent on how the excitatory and inhibitory inputs are spatially and temporally related on the membrane of the postsynaptic cell [84]. Temporally, excitatory glutamatergic inputs can be maximally attenuated when shortly preceding the activation of neighbouring shunting inhibitory postsynaptic potentials (IPSPs). Spatially, shunting IPSPs close to the soma of the cells can better control the integration of excitatory depolarizing inputs coming from distal dendrites.

In contrast, IPSPs temporally and spatially isolated from EPSPs need to be hyperpolarizing to provide an inhibitory action; otherwise, they will generate depolarizing waves propagating toward the soma of the cell which will sum to depolarizing EPSPs [85]. A pathological shift in E_{GABA} at these synapses could, therefore, critically favour hyperexcitability of cortical networks.

Finally, if the depolarized E_{GABA} is due to an impaired Cl^- extrusion, as suggested by Jin and colleagues, repetitive synaptic $GABA_A$Rs activation, normally occurring *in vivo*, could promote a transient additional intracellular Cl^- accumulation which will depolarize E_{GABA} further until the action of GABA will be fully excitatory [49]. This hypothetical transient depolarized shift in E_{GABA} could facilitate recurrent excitation between pyramidal cells potentially leading to the generation of epileptic discharges.

All these considerations explain why it is difficult to predict the consequences of the reported lesion-induced depolarized E_{GABA} on the excitability of neuronal networks. Nonetheless, if E_{GABA} is equally affected at all synapses, a general shift in favour of excitation should be expected.

5.4. Increase in Tonic Inhibition Postlesion. Despite the large number of studies strongly indicating a postlesion impairment in GABA-mediated inhibition, a recent study performed in a photothrombotic model of stroke in the motor cortex of mice, revealed a lesion-induced enhancement of tonic inhibition due to an increased activity of $GABA_A$Rs containing the subunit $\alpha 5$ and δ in the peri-infarct

cortex [86]. $GABA_A$ receptors containing these subunits are normally located extrasynaptically [12, 87], where they can be tonically activated by low concentration of GABA in the extracellular space (ambient GABA) leading to the generation of a tonic conductance in the postsynaptic neuron [15]. The authors reported that the enhanced GABA-mediated tonic inhibition was due to an increased ambient GABA as a consequence of an impaired GABA uptake from the astrocytic GABA transporters, GAT-3/GAT-4.

Interesting, in a study performed in the hippocampus of guinea pigs tonic inhibition was found to be most prominently expressed at interneurons [88]. Assuming a similar scenario in the neocortex, the excessive tonic inhibition postlesion might strongly suppress interneurons activity leading to a decrease in the GABAergic synaptic transmission.

A cortical lesion may, therefore, produce a shift from a phasic to a tonic GABAergic transmission with profound consequences on neuronal network functions [86].

Tonic inhibition lacks the spatial and temporal precision of synaptic transmission, and by producing a "long-term" reduction in the resistance of the postsynaptic neurons, it could prevent an appropriate neurotransmission, potentially constraining plastic processes to occur. Moreover, the enhanced tonic inhibition might also contribute to the above-mentioned depolarizing shift in E_{GABA} by promoting intracellular Cl^- accumulation (especially if the rate of Cl^- influx, through tonically active extrasynaptic $GABA_A$Rs, overcomes the function of the Cl^- extruder KCC2).

5.5. Changes in the GABAergic Transmission in Brain Areas Remote to the Injury. The functional consequences of neocortical injuries are often not limited to the neuronal circuits surrounding the primary lesion but can be observed in remote projection cortical areas as well as in some subcortical structures [89]. These remote alterations in brain function following a focal brain damage are known as "diaschisis" and were firstly described by von Monakov as early as in the 1914. He suggested that these remote effects must be likely attributed to the deafferentiation of damaged fibers from the injured area. Nowadays, the term "diaschisis" is used by many authors to describe acute and chronic changes in cerebral blood flow, metabolism, and electrical activity in remote areas following brain lesions. Particularly interesting is the frequently observed "transhemispheric diaschisis" following unilateral lesions in the cerebral cortex likely due to the deafferentiation of transcallosal connections from the damaged area [1].

In different clinical studies [29, 30] and experimental animal models of stroke [90], this "transhemispheric diaschisis" has been described as an abnormal increase in the activity of the cortical hemisphere contralateral to the lesion.

In parallel, *in vitro* extracellular recordings performed in photothrombotic and ischemic unilateral cortical lesion models [37–39] revealed a reduced strength in the GABAergic transmission widespread throughout the intact contralateral hemisphere.

It is, therefore, conceivable that the reduced inhibitory tone may be responsible for the described abnormal activation of the hemisphere contralateral to the lesion.

As a consequence of these findings, the hypothesis emerged that a lesion-induced dysinhibition of the contralateral cortex could potentially contribute to the functional recovery postinjury by compensating or at least partially taking over the function of the damaged brain area.

In this regard, longitudinal studies, comparing the extent of the hyperexcitability of the unaffected cortex with the degree of recovery from neurological deficits, suggested that the abnormal activity of the contralateral hemisphere during the acute/subacute phase postlesion could indicate a sort of bihemispheric cooperation which might be indispensible for performing even simple tasks involving the affected side of the body. However, the contribution of the contralateral cortex, in the recovery of function, seems to diminish over time, since the better final outcomes are observed when the brain regions, normally executing a function, are reintegrated into the active network [91].

It is, therefore, plausible that shortly after a focal cortical injury, the dysinhibition of anatomically connected remote areas might constitute a compensatory mechanism to temporary relieve the neurological deficits before a consistent functional reorganization will gradually guaranty a permanent, at least partial, functional recovery. However, it is also not possible to rule out a potential involvement of these hyperexcitable remote neuronal networks in promoting the generation of epileptic events after a brain injury.

5.6. Lesion-Induced Alterations of Thalamocortical Activity as Potential Source of Hyperexcitability. Since the brain areas most likely affected by a cortical damage are the one anatomically connected to the lesion site, the dense corticothalamic thalamocortical connections strongly suggest a likely lesion-induced physiological alteration at the level of the thalamus. One study performed in a phototrombotic model of cortical infarct in the somatosensory cortex of rats indeed reported a strong reduction in the excitability of interneurons located in the reticular thalamic nucleus [92]. The reticular thalamic nucleus is constituted by GABAergic interneurons which, by receiving the main excitatory drive from the cortex and providing inhibition onto thalamocortical relay cells, can strongly modulate the thalamocortical flow of information [93]. The authors suggested that the dysfunction of this inhibitory thalamic nucleus might produce a powerful dysinhibition of thalamocortical activity which could be potentially involved in the generation of postlesion epileptiform activity. Consistently, dysfunctions of the thalamocortical circuitry have been already implicated in the genesis of generalized epilepsy [94–96].

It is, therefore, important, when searching for the cellular mechanisms responsible for epilepsy after cortical injuries, to do not underestimate potential alterations in the physiological properties of thalamic neurons.

6. The Influence of GABAergic Transmission on Neuronal Network Plasticity, the Other Side of the Coin

From the considerations drawn so far, it seems pretty evident that the pathological alterations of GABAergic inhibition following a cortical lesion can lead to severe negative consequences on the excitability and function of neuronal circuits. One might, therefore, conclude that a simple pharmacological enhancement of the GABAegic synaptic transmission could be the best approach to restore a normal brain activity after a lesion.

However, the reduced inhibition could be also viewed as an evolutionary conserved mechanism initiated in front of a dramatic alteration of cortical activity, as in the case of cortical injuries, with potential beneficial effects.

In line with this assumption, different studies indicated that the level of intracortical inhibition is important to define the plastic properties of neuronal circuits.

6.1. The Influence of the Level of Cortical Inhibition on Neuroplasticity. During the development of the mammal CNS, the slowly increasing strength of inhibitory transmission is suggested to modulate cortical plasticity by crossing two thresholds: crossing the first threshold defines the onset of a period, known as critical period, characterized by high experience-dependent plasticity of neuronal networks, while passing the second threshold closes the time window of high plasticity and open a period of restricted plasticity which is protracting throughout the life of an animal [97, 98].

The relation between the level of intracortical inhibition and the critical period for plasticity has been extensively studied in the visual cortex of rodents, where the critical period is normally determined by the successful induction of ocular dominance (OD) plasticity [99]. In this system, a developmental modulation of the GABAergic strength, achieved by combining pharmacological and genetic tools, has been shown to be effective in shifting the onset and closure of the OD plasticity [100, 101]. Furthermore, in a recent study, Harauzov and colleagues could demonstrate that the pharmacological reduction of a mature GABAergic inhibition was sufficient to trigger the reactivation of the OD plasticity in the visual cortex of adult rats [10]. This finding suggests that even a simple functional modification of inhibition could be enough to modulate the plastic properties of neuronal networks.

Based on these observations, if the reduced strength of inhibition observed after a cortical lesion matches a level similar to that found during the critical period, the remodeling capacity of the surrounding cortical networks could be strongly enhanced.

6.2. GABA-Mediated Inhibition as a "Filter" for Plasticity at Excitatory Inputs. In order to take advantage of the described findings, it is fundamental to elucidate the cellular and physiological mechanisms mediating the influence of the strength of inhibition on cortical plasticity.

As early as in 1987, Artola and Singer proposed that strong inhibitory synapses, by reducing EPSPs, could prevent the activation of NMDARs, indispensible for many forms of synaptic plasticity [102].

Shortly afterwards, Kirkwood and Bear also suggested that a mature inhibitory circuitry in layer 4 of sensory cortices might act as a kind of filter by limiting the activity pattern able to gain access from subcortical structures to the supragranular layers of the cortex [8].

This observation indicates that inhibition may control plasticity of neuronal networks by selectively permitting or preventing plasticity at excitatory synapses.

A reduced/immature GABAergic transmission might, therefore, act as a permissive substrate allowing sensory experience to remodel structure and functions of cortical networks. However, a too drastic impairment of synaptic GABAergic transmission might be deleterious by preventing accurate cortical information processing and by promoting epileptic activity. On this concern, Feldman proposed the existence of an ideal level of inhibition, on the one hand low enough to permit the potentiation or the depression of excitatory connections but on the other hand sufficient to guarantee an appropriate temporal encoding of relevant inputs [97].

Reducing the strength of inhibition or adjusting an impaired inhibition postlesion to an ideal level could, therefore, constitute a promising tool to restore and/or enhanced experience-dependent plastic processes in the adult CNS.

Among the different interneuron subtypes, PV basket cells have been suggested to contribute, more than others, in the expression of the critical period for cortical plasticity [103, 104].

Different functional properties of PV basket cells can indeed support their role in modulating plastic processes. Their fast somatic inhibition could filter the action potentials able to access the dendritic arbor by back propagation, thereby allowing postsynaptic spikes to meet presynaptic inputs within specific temporal windows appropriate for synaptic plasticity induction [105]. Furthermore, PV basket cells, being electrically coupled through gap junctions (see above) are able to detect strong synchronous activity arriving in the cortex, which normally carries relevant information from the periphery [106]. These interneurons are, therefore, well suited to produce competitive outcome by reinforcing relevant and favouring the elimination of irrelevant connections based on the sensory experience [98].

6.3. Plasticity of Inhibitory Circuits. Beside permitting or preventing structural and functional modifications of excitatory connections, inhibitory networks can themselves undergo plastic processes.

Firstly, the activity of cortical interneurons is highly sensitive to global changes in the activity of cortical circuits. A reduced cortical activity leads normally to an impaired GABAergic innervation [107, 108] and to a decreased GABAergic neurotransmission [109–111]. This activity-dependent modulation of inhibitory strength seems to be an

important homeostatic mechanism playing a crucial role in the maintenance of a proper E-I balance [46].

Not only homeostatic but also Hebbian plastic mechanisms have been observed at inhibitory synapses. Several *in vitro* studies could demonstrate the efficacy of different stimulation protocols in the induction of long-term modifications at cortical GABAergic synapses [112–114].

Moreover, some *in vivo* studies performed in different sensory systems provided evidence of inhibitory-plasticity-dependent changes in cortical maps.

In these studies, a shift in cortical maps was obtained by exposing the animals to an abnormal sensory experience for a define period of time. This condition produced a receptive field shift away from deprived/inappropriate inputs towards new behavioral relevant inputs. Interestingly, this receptive field plasticity could be reversed by the application of a $GABA_A$Rs blocker indicating that the suppression of responses to irrelevant inputs was likely due to a potentiation of GABAergic synapses [115, 116].

6.4. Influence of Intracortical Inhibition on Cortical Map Plasticity. Intracortical GABA-mediated inhibition also strongly contributes in shaping the receptive fields of cortical neurons.

This important function of GABA was first appreciated in a series of electrophysiological studies mainly performed in primary sensory systems of mammals. In these studies, the application of a $GABA_A$Rs antagonist produced an enlargement of the receptive field's size of single neurons [117, 118] as well as profound alterations in the receptive field properties such as the loss of orientation and direction selectivity in neurons of the visual cortex [6, 119] and a dramatic expansion of tuning curves in the auditory cortex [120].

Remarkably, increased and/or altered receptive fields were observed following cortical lesions in the surrounding brain areas [121–123].

The lesion-induced reduction of inhibition might enlarge the receptive fields by bringing suprathreshold and thereby unmasking previously silent (subthreshold) inputs [124].

Converting silent connections into functional ones is *per se* a mechanism of functional reorganization, but most importantly, as already outlined above, this process may allow new functional excitatory inputs to enter in competition with others and to undergo potentiation or depression following Hebbian-based learning rules.

In this way, a reduced level of inhibition could strongly contribute in the plasticity of cortical maps.

6.5. A Cellular Model of Functional Reorganization Following Cortical Injuries. The above-mentioned studies provide several lines of evidence for an important role of inhibition in influencing the plasticity of cortical networks.

Here, we briefly discuss how the plasticity of neuronal networks surrounding a cortical lesion could mediate the recovery of function and how the lesion-induced reduction in inhibition could contribute to this process.

First of all, to achieve a functional recovery after a cortical lesion the information previously processed by the injured cortex needs somehow to be rerepresented by the remaining cortical areas.

In the neocortex, due to the dense and exuberant cortical connectivity [125], some of the normally silent connections projecting onto surviving neurons could be anatomically capable of transmitting information previously process by the damaged tissue (Figure 1(a)). Remarkably, these silent connections can be converted into functional ones by the extensively described lesion-induced reduction of inhibitory neurotransmission (Figure 1(b)).

The initial depression of GABAergic inputs, by unmasking subthreshold excitatory connections, plays therefore a crucial role in the initiation of cortical map plasticity.

This is of fundamental importance since cortical map plasticity is largely responsible for the "long-term" functional recovery postlesion.

Subsequently, to guarantee a stable rewiring of neuronal circuits, experience-dependent plastic processes will likely lead to the reinforcement of some of these new functional inputs, which turn out to be behaviorally relevant, and eventually lead to the suppression of inputs which became irrelevant after the lesion (Figure 1(c)).

The reinforcement of the relevant inputs most likely involves long-term potentiation (LTP) of excitatory connections [126–128], while improper inputs could be masked by potentiated inhibitory connections [115, 116] or may directly undergo long-term depression (LTD) [129].

Finally, structural modifications might stabilize the new connectivity patterns.

This process can therefore induce at least a partial functional recovery postlesion, because it promotes the cortical area surrounding the damage to gradually take over the functions before belonging to the death cortical tissue.

7. Strategies toward a Better Functional Recovery Following Cortical Injuries

As we intensively discussed, the altered GABA-mediated inhibition often observed following cortical injuries can have both detrimental consequences by modifying excitability and functions of cortical networks as well as beneficial effects by promoting cortical plasticity.

Intuitively, in order to optimize the functional recovery of patients suffering from a cortical injury, a therapy should aim to minimize the deleterious consequences of a modified inhibitory transmission without preventing the potential beneficial effects on cortical plasticity.

This scope can be achieved if we could distinguish that the positive and negative effects of the altered GABAergic transmission differ somehow in the cellular mechanisms of their induction, in the GABAergic networks that they affect and/or in the temporal window postlesion of their expression.

Although much more needs to be done to give a final answer to these questions, a consistent amount of information can already be found in many studies investigating the role of GABA-mediated inhibition on cortical function and plasticity.

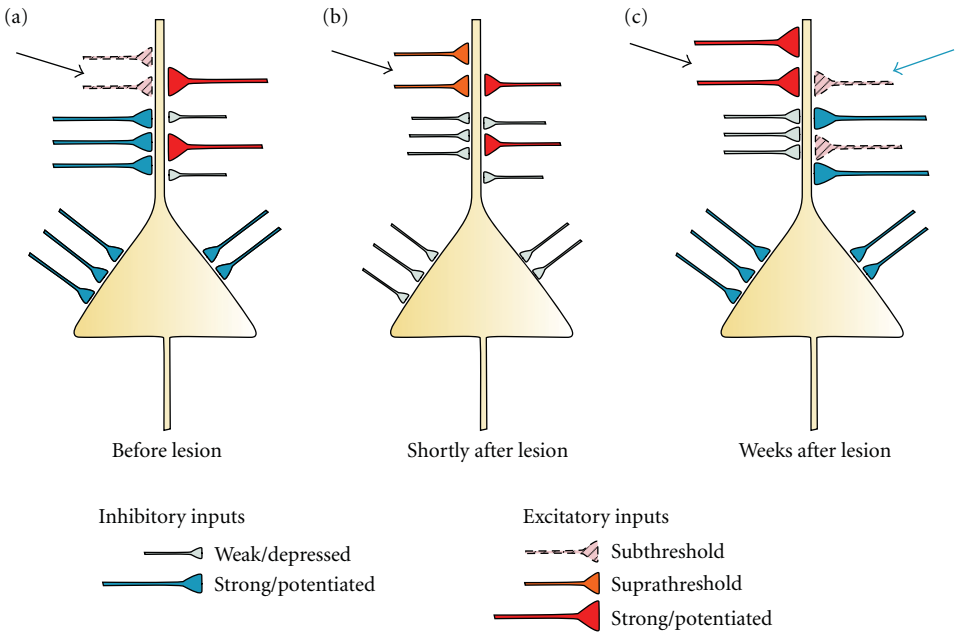

Figure 1: Schematic illustration representing a model pyramidal neuron in the cortex surrounding the lesion with its excitatory and inhibitory inputs before, shortly after and some weeks after the lesion occurrence. This model shows the potential cellular mechanisms responsible for the functional rewiring of neuronal networks following cortical injuries. (a) Before the occurrence of a cortical lesion, some excitatory inputs are subthreshold (arrow) being masked by strong inhibitory inputs; (b) early after the cortical lesion occurrence (first week postlesion), subthreshold connections can be converted into functional (suprathreshold) ones (arrow) by the lesion-induced weakening of inhibitory inputs; (c) some weeks after the lesion, experience-dependent plastic processes will likely lead to the reinforcement of some of the new functional inputs, which turn out to be behavioral relevant after the lesion (black arrow) and to the suppression of excitatory inputs which became irrelevant (blue arrow). For clarity, many cellular and subcellular elements have been omitted; this draw represents, therefore, an oversimplification of a real scenario.

For instance, in the above-mentioned study of Clarkson and colleagues it has been proposed that the stroke-induced increase in the tonic GABAergic transmission is one of the constraining factors for cortical plasticity. The authors were indeed able to demonstrate that dampening the excessive tonic inhibition, by selectively antagonize the function of extrasynaptic $GABA_A$Rs, produced a significant improvement in the motor recovery of the animals [86].

The availability of antagonists for specific $GABA_A$Rs subunits exclusively or predominately contained in extrasynaptic $GABA_A$Rs [130] makes the selective reduction of tonic inhibition a plausible tool to improve the functional recovery of patients suffering from a cortical lesion.

The identification of specific networks of GABAergic neurons primarily involved in the reorganization of cortical circuits postlesion could also promote the development of a better targeted therapy to improve the functional recovery of patients. On this regard, although it is still not possible to attribute an exclusive function to each subtype of cortical interneurons, compelling evidence indicates that some classes of GABAergic cells might be more relevant than others in mediating cortical plastic processes.

In particular, through the lines of this paper different points stress the importance of the fast-spiking PV basket cells in the regulation of cortical network functions as well as in the modulation of experience-dependent plastic processes. A drastic impairment in the function of these interneurons should be, therefore, avoided although a moderate reduction in their activity might facilitate cortical plastic processes.

On contrary, other subtypes of interneurons seem to contribute to a lesser extent in the induction of cortical plasticity and to be more closely involved in controlling the excitability of cortical networks. On the basis of recent findings, these subpopulations might include dendritic projecting SOM interneurons and chandelier cells [55, 59, 104]. Preventing a drop in the activity of these interneuron subtypes could constitute a neuroprotective tool against the development of postlesion epileptic discharges, while a lesion-induced moderate reduction in the activity of PV basket cells might be better tolerate and could even offer enhanced plastic properties to the surviving cortical tissue.

Interestingly, PV basket cells form predominately perisomatic synapses enriched in α1 subunit-containing $GABA_A$Rs [131], while other interneurons subtypes, as in particular chandelier cells, formed synapses enriched in α2-containing $GABA_A$Rs [132].

The development of pharmacological agents showing specific-subunit sensitivity might, therefore, provide a strategic tool able to modulate the function of a particular class of interneurons and might be more effective in reducing postlesion cortical hyperexcitability without constraining cortical plasticity.

Since experience-dependent changes in synaptic plasticity likely contribute to the functional rewiring of cortical

networks, a physical rehabilitation accompanying a pharmacological approach will remain essential.

Finally, the identification of an optimal therapeutical time window for pharmacological and rehabilitative interventions could also be extremely helpful.

In this regard, results from clinical studies indicate that pharmacological therapies following cortical injuries showed a moderate efficacy and only if administered very early after the lesion (few hours postlesion) [133]. This might be due to the fact that so far the largest effort has been dedicated in the development of a neuroprotective tool to prevent or reduce the secondary brain damage.

Now, it seems that the attention is shifting toward the development of a therapy aiming to amplify endogenous mechanisms of repair [134]. This might produce better functional outcomes and could offer a prolonged temporal window of intervention potentially extending into the subacute and chronic phase postlesion.

As extensively described in this paper this time window postlesion seems to be characterized by a profound alterations in the GABAergic transmission which might strongly influence cellular mechanism of neuroplasticity. A therapeutical approach able to precisely target the GABAergic signaling involved in the modulation of neuronal plastic processes may, therefore, constitute a powerful instrument to improve the rehabilitation of patients suffering from traumatic brain injuries and stroke.

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Review Article

GABAergic Neuronal Precursor Grafting: Implications in Brain Regeneration and Plasticity

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Numerous neurological disorders are caused by a dysfunction of the GABAergic system that impairs or either stimulates its inhibitory action over its neuronal targets. Pharmacological drugs have generally been proved very effective in restoring its normal function, but their lack of any sort of spatial or cell type specificity has created some limitations in their use. In the last decades, cell-based therapies using GABAergic neuronal grafts have emerged as a promising treatment, since they may restore the lost equilibrium by cellular replacement of the missing/altered inhibitory neurons or modulating the hyperactive excitatory system. In particular, the discovery that embryonic ganglionic eminence-derived GABAergic precursors are able to disperse and integrate in large areas of the host tissue after grafting has provided a strong rationale for exploiting their use for the treatment of diseased brains. GABAergic neuronal transplantation not only is efficacious to restore normal GABAergic activities but can also trigger or sustain high neuronal plasticity by promoting the general reorganization of local neuronal circuits adding new synaptic connections. These results cast new light on dynamics and plasticity of adult neuronal assemblies and their associated functions disclosing new therapeutic opportunities for the near future.

1. Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS), playing a key role in the balance between inhibitory and excitatory circuits [1, 2]. Therefore, it is not surprising that dysfunctions in the GABAergic system lead to pathological conditions including hypokinetic diseases such as Parkinson's disease (PD), and hyperkinetic diseases, such as Huntington's disease (HD), when disruption of the GABAergic system occurs in the basal ganglia [3–5]. Epilepsy, a pathology characterized by uncontrolled hyperactivity, is also tightly linked to deficits in GABA levels, as well as alterations in its synthesis, secretion, and reuptake, or reductions in the number of GABAergic interneurons [6–8].

Almost 25 years ago, it was already postulated that controlling GABA delivery to specific brain areas should benefit each of these diseases [9, 10]. Cell transplantation is a powerful tool to introduce a new source of GABA and may allow reconstitution of neural circuits in the diseased brain [11, 12]. To be successful, grafted cells should possess the ability to disperse through affected areas and differentiate into fully mature neurons expressing appropriate neurotransmitters, in this case GABA. Ideally, these cells should also functionally integrate and modulate circuitry activity in the damaged host brain; for instance, affecting its plasticity. Since the pioneer works from Lindvall and Björklund [9] and Isacson et al. [10], several transplantation assays with different GABA-producing cell types have been performed with disparate success in animal models of diseases. Many cell types were partially successful in reverting some of the pathological anomalies observed in the grafted models. However, some of them presented important drawbacks, such as their poor tissue distribution, transient effect, maybe

due to decreased GABA release over time [13–15], or in the case of ES cells, the lack of safety due to potential generation of teratocarcinomas [16, 17].

In the last decade, a better comprehension of how and where the cortical and hippocampal interneurons originate has led to use their neuronal precursors in transplantation [18, 19]. We currently know that most of the GABAergic interneurons in the cortex and hippocampus are mainly generated in two regions of the subcortical telencephalon, known as the caudal and medial ganglionic eminence (CGE and MGE), from where they migrate tangentially to their final destination in the cerebral cortex [19–22]. In the last years, several groups have reported regenerative works using these MGE-derived GABAergic precursors, with striking results [23–28]. At present, they represent the most promising cell-based therapeutic alternative for GABA-related diseases.

In this paper, we will summarize the main regenerative approaches using GABAergic grafts for the treatment of epilepsy and neurodegenerative disorders. These include the use of different sources of GABAergic precursors, with a special emphasis in the MGE-derived cells, and their transplant in several model organisms of disease. In addition, we will also describe the implications of the GABAergic grafts on the modulation of synaptic activity and circuitry plasticity of the host.

2. GABAergic Cell Therapy for Epilepsy

Epileptic seizures reflect a hyperexcitation in the brain, which is attributed to an imbalance between inhibitory and excitatory networks [6]. Given the close relationship between GABA and epilepsy [6, 8], antiepileptic drugs (AED) targeting the GABAergic system are traditionally the preferred treatment, presenting an acceptable efficacy [29, 30]. However, up to a third of patients continue to experience seizures on maximal tolerated drug therapy [31, 32]. Refractory epilepsy remains a large clinical problem, since surgical resection is only appropriate for a minority of patients [33, 34]. In the last decades, cell-based therapies using GABAergic grafts have emerged as an alternative treatment for epilepsy, since they may restore the lost equilibrium by cellular replacement of the missing/altered inhibitory neurons or modulating the hyperactive excitatory system [35–37]. The therapeutic strategies are multiple: general secretion of GABA, by the grafted cells to increase the seizure threshold, or specifically located in the focus of epilepsy and/or the areas responsible for seizure transmission to block it; direct replacement of malfunctioning or lost inhibitory interneurons; interaction of the transplanted GABAergic cells with activating system to modulate its plasticity, and levels of activity; finally, rewiring of aberrant excitatory fibers, such as mossy fiber in temporal lobe epilepsy (TLE), towards inhibitory GABAergic cells.

According to these strategies, several GABAergic cell types, with different origins and characteristics, have been assayed in animal models of epilepsy to evaluate their therapeutic potential. In the following sections, we describe in detail the most representative cell types and assays.

2.1. GABA-Producing Cells. A first therapeutic approach for epilepsy includes transplantation of fetal precursors from different brain regions, and cells genetically modified to produce and secrete GABA were transplanted into the hippocampus or in regions implicated in seizure generalization [9, 13–15, 38–41]. More than 20 years ago, Stevens et al. transplanted embryonic cerebellar and cortical tissue, rich in GABA or norepinephrine neurons, in the amygdala-kindled rat model of epilepsy [41]. Transplantation into the deep prepiriform area transiently raised seizure thresholds, showing for the first time that cell transplantation could be valuable for epilepsy treatment. Previously, Isacson et al. had already shown that transplantation of GABAergic cells from striatal primordia significantly ameliorates the lesion-induced locomotor hyperactivity in the ibotenic acid rat model of HD [10]. These seminal works strongly suggested that intracerebral grafting of inhibitory neurons may be an adequate strategy for seizure suppression [9]. Following this strategy, several groups isolated cells from the late striatal primordial (E14-15 in rats), to transplant them in regions thought to be critically involved in seizure propagation, such as the substantia nigra, as an effective means of permanently blocking seizure generalization in different models of epilepsy, mainly kindled rats [13, 14, 38–40]. All the kindling studies reported significant increase in afterdischarge thresholds and marked reduction in seizure severity compared with pretransplantation values [13, 17, 42]. In drug-induced models of epilepsy, these cells also suppressed the development of motor-limbic seizures and reduced the mortality rate [38–40]. However, the seizure-suppressing effect of GABAergic grafts was transient, likely due to progressive reductions in GABA levels. For this reason, it was attempted the development of immortalized glial and neuronal cell lines genetically engineered to produce GABA [37–39, 43, 44]. The ability to generate self-renewing clonal populations of transplantable GABA-producing cells provides an unlimited cell source and a good level of control on GABA production. GABA-producing cell lines demonstrated the ability to retard the development of seizures and block the expression of established seizures in kindling, kainic acid, and pilocarpine models of epilepsy [37–39, 43, 44]. However, these cell lines presented serious limitations that diminish their clinical potential [37]. The use of the SV40 large T oncogene for their immortalization raises concerns about tumorigenicity [38, 39]. In addition, in the host brain, a strong tissue reaction was reported including graft rejection, massive infiltration of inflammatory immune cells, and gliosis. Besides the concerns of tumorigenicity and immunogenicity, a major problem was the inability to sustain long-term effects due to the lack of survival or integration of the graft-derived cells [37]. To date, there is no report of engineered neuronal cells becoming fully differentiated and integrated into the seizure circuit of the host. This lack of integration may limit access to trophic

factors and thus reduce the survival potential of these cells, and as a consequence, their mediated effects are transient.

2.2. Neuronal and Embryonic Stem Cells. The establishment of techniques that allow the isolation and culture of embryonic stem cells (ESCs), and neuronal stem cells (NSCs) from fetal and adult brain tissue, provided new sources of GABAergic cells for treating epilepsy to the scientific community [45, 46]. ESCs are isolated from the inner cell mass of the developing blastocyst and retain the ability to generate every cell type present in the body, including neurons [47, 48]. NSCs show a more restricted ability to generate only those cell types that constitute the nervous system; neurons, astrocytes, and oligodendrocytes [49]. NSCs can be expanded in culture using mitogens, mainly bFGF and EGF, which keep them in an undifferentiated state, forming floating cell aggregates, named neurospheres [50, 51]. Both cell types, ESCs and NSCs, are very promising in terms of providing an infinite supply of donor cells for neuropathological condition treatments. An additional advantage is the possibility to direct their differentiation toward specific cell types, in this case GABAergic neurons. In fact, default differentiation pathway for many SC lines in culture seems to be the GABAergic lineage [52, 53].

Despite these interesting properties, few studies report on the use of ESC and NSC in animal models of epilepsy [45, 46]. Rüschenschmidt et al. [54] reported that ESC-derived neuronal precursors (ESNPs) transplanted into the hippocampi of both control and pilocarpine-treated rats were able to generate action potentials and expressed voltage-gated Na^+ and K^+ currents, as well as hyperpolarization-activated currents. Anyway, electrophysiological activity and action potentials were lower than those in host neurons, typical of immature cells and suggesting an incomplete maturation process. Indeed, the grafted cells formed big clusters, and there was no evidence of cell type-specific differentiation one month after the transplant. In addition, no obvious difference was found between the functional properties of the transplanted cells in sham control and in pilocarpine-treated rats, and no improvement was described in the symptoms or electrophysiological activity of the epileptic rats after the transplants.

In contrast, Carpentino et al. [55] reported that grafted ESNP into the normal and kainic acid-treated mice partially migrated and differentiated towards neuroblasts and dentate granule neurons, or oligodendrocytes and astrocytes, depending on the brain area where they were finally located. However, some cells grafted in mice not subjected to seizures displayed a marked tendency to form tumors, and this effect was more pronounced in the dentate gyrus than in the fimbria. This suggested that seizures induce molecular changes that promote region-specific neural differentiation and suppress tumor formation. Finally, effects on the epileptic condition of the mice after ESNP transplantation were not reported, as well.

More recently, Shindo et al. [56] optimized a method to induce differentiation of GABAergic neurons from ESNP, and transplanted them into kindled epileptic mice to analyze a possible morphological and functional recovery. Two weeks after transplant, they observed a partial recovery of seizures. This was likely due to GABA production of transplanted cells, since histological analysis showed a high percentage of cells expressing GAD67. However, the morphology and cluster formation of the grafted cells suggest a lack of integration in the host circuitry.

From these reports, it is evident that the use of ESC in epilepsy treatment needs to be improved. Safer conditions to avoid tumorigenicity are necessary, and percentage and quality of differentiation toward GABAergic neurons should be increased. Improving the differentiation protocols from ESC and generating cell lines that are strongly committed to specific neural lineages in culture prior to grafting might be helpful. Several groups are working with this idea and have reported advances in the generation of GABAergic interneurons from ESC with high efficiency *in vitro* [57] and a good degree of functionality *in vivo* [58].

NSCs partially overcome some of the problems presented by ESC. They can be isolated from fetal or adult brain regions already committed in the generation of certain types of neurons; moreover, they prevent ethical issues and do not form tumors; and they could potentially be harvested in culture for prolonged periods, as neurospheres, to be used as a source of donor tissue for grafting [49–51]. Shetty et al. have isolated and cultured NSC from two fetal regions of the rat and grafted them in epileptic models. In a succession of reports using E19 hippocampal grafts or cultured NSC from this region [59–65], they reported the ability of this precursors to give rise to both hippocampal pyramidal-like neurons and interneurons in the host brain. However, barely more than 50% of transplanted neurospheres became differentiated cells, showing mainly an astrocyte phenotype and only in a small proportion a neuronal one. Integration of the transplanted cells was also poor; they form big clusters interfering with the normal hippocampal morphology. However, grafts located in or near the degenerated CA3 cell layer established commissural projections with the contralateral hippocampus. In addition, they revealed the capability of these grafts to restore disrupted hippocampal mossy fiber circuitry by attracting host mossy fibers sufficient to suppress the development of aberrant circuitry in hippocampus. The graft-induced long-term suppression of aberrant sprouting may provide a new avenue for amelioration of hyperexcitability [62].

Similarly to the previous reports, the Shetty's group transplanted striatal NSCs in the hippocampus of adult rats after status epilepticus induced by kainic acid [66]. The cells, pretreated with fibroblast growth factor-2 and caspase inhibitors, presented a good survival rate but limited ability to migrate, remaining close to the injection site. Nonetheless, a small percentage of these cells differentiated into GABAergic neurons and were able to reduce the seizure frequency in the kainic acid model of TLE.

Human NSC have been also tested in the pilocarpine-induced rat model of TLE [67]. They differentiated into cells that were positive for GABAergic (26%), glutamatergic (2%), or astrocytic (21%) markers. Grafted cells reduced the amplitude of extracellular field excitatory postsynaptic

potentials in the hippocampal CA1, decreased the percentage of pilocarpine rats that developed spontaneous seizures, and declined both seizure frequency and severity.

2.3. MGE-Derived Neuronal Precursors. As mentioned in the introduction, in the last decade, the origin of cortical and hippocampal GABAergic interneurons has been elucidated [19–22]. Located in a restricted region of the ventral telencephalon known as the MGE and CGE, these precursors migrate long distances to cover the neocortex and hippocampal primordial where they complete their differentiation. In theory, these precursors should be good candidates for treating GABA-related diseases, since they are already committed to interneurons and migrate naturally long distance covering the brain parenchyma. They should overcome the difficulties presented by other sources of cells and achieve higher levels of inhibition or modulate the excitatory activity in the host. To verify this possibility, our group grafted fresh isolated, with no other manipulation, MGE-derived precursors into the neonatal normal brain [18]. MGE-derived cells gave rise to neurons that migrated, embracing wide areas of the cortical plate, striatum, and the hippocampus. More than 70% of the grafted cells differentiated into fully mature GABAergic interneurons, demonstrated by the expression of molecular markers such as calcium binding proteins. More importantly, electrophysiological analysis demonstrated these cells were able to integrate into the local circuits and make functional synapses with existing neurons, influencing the level of GABA-mediated synaptic inhibition. This was the first time that full mature electrophysiological activity and modulation of the host activity by GABAergic grafts was demonstrated. These observations strongly suggested the complete maturation of the grafted cells and its suitability for cell-based antiepileptic therapies.

In the following years, several groups tested these MGE-derived cells in different animal models of epilepsy. As proof of principle for a cell replacement therapy after lost or reductions in GABAergic neurons, our group grafted MGE-derived cells into a mouse model with a disinhibited brain environment caused by specific ablation of interneurons [28]. This was achieved by intrahippocampal microinjection of the neurotoxic Saporin conjugated with an analog of substance P (SSP-Sap), that selectively targets and eliminates the GABAergic interneurons expressing the substance P receptor, neurokinin-1 (NK-1) [68]. This experimental approach helped to address whether MGE-derived interneurons can integrate under neuropathological conditions and not only increase but also restore deficits in the inhibitory synaptic function as consequence of reductions in the number of GABAergic neurons. The specific GABAergic ablation leads to reductions in GABA-mediated synaptic inhibition, hyperexcitability, and increased susceptibility to pentylenetetrazol-induced seizures (PTZ), similarly to other models with reductions in interneurons [68–70]. MGE-derived cells in SSP-Sap-treated mice repopulate the hippocampal ablated zone with cells expressing molecular markers of mature interneurons. Similar to transplants in normal neonatal brain [18], the grafted MGE-derived cells migrated long distance covering the whole ablation area and acquired a fully mature morphology two months after transplantation with good survival rates (~25%). Immunohistochemical analysis revealed that more than 60% of graft-derived cells expressed GABA and specific molecular markers for mature interneuron subpopulations. Interestingly, electrophysiological analysis showed a restoration of the postsynaptic inhibitory current kinetics on CA1 pyramidal cells of ablated hippocampus after transplant, and more importantly, this was associated with reduction in seizure severity and decrease in postseizure mortality induced by PTZ [28] consistent with an enhancement of GABAergic inhibition after cell transplantation. In addition, these effects were stable over time. We performed a followup to 6 months after the transplant with similar results. This is logical, since we have verified MGE-derived cell survival more than 1 year after the transplant and, importantly, tumor formation was never detected.

MGE-derived cells are able not only to replace a loss of GABAergic neurons and reduce the mortality to PTZ-induced seizures, but also they show an intrinsic antiepileptogenic activity. Baraban et al. [23] grafted MGE-derived cells into neonatal Kv1.1 mutant mice, characterized by the lack of voltage-activated K^+ channel, Kv1.1. These mice exhibit a high frequency of behavioural and electrographic seizures few weeks after birth. GABA-mediated synaptic and extrasynaptic inhibition onto host brain pyramidal neurons was significantly increased after bilateral transplant, and significant reductions in the total number, duration, and frequency of spontaneous electrographic seizures were observed. These findings suggest that MGE-derived interneurons could prevent and ameliorate abnormal excitability in infants. This is an interesting possibility, since MGE grafts may block generalization of seizures and improve life conditions in the patients. We have confirmed the anticonvulsant ability of these cells by maximum electroconvulsive shock (MES) assay after grafting in neonatal mice [24]. MES has remained one of the gold standards for AED screening [71]. The test evokes a single seizure applying a high-intensity current. Two months after transplantation in postnatal day 3 mice, MGE-grafted cells were able to protect against clonic seizures induced by MES, and a 5-fold reduction in the mortality rate was observed. This data strongly suggests that MGE grafts block the generalization of the seizures and allow a better control of the transition between tonic and clonic seizures. If we consider the MGE-grafts as a new AED, they perform better in MES assay than many AEDs already commercially available in the clinic.

However, before thinking of a clinical application of this cell type, some technical problems should be eliminated for instance, the limiting number of cells available for transplantation. One possibility is the amplification of MGE-derived cells in culture. MGE cells, cultured as neurospheres, have also been tested in the kainic acid model of TLE [27]. However, the interaction of MGE precursors with mitogens in culture seems to modify importantly their behaviour and neuronal commitment. MGE neurospheres gave rise mainly to astrocytes and only in a small proportion to

GABAergic cells after transplantation. Nonetheless, these cells grafted into the hippocampi of adult rats restrained spontaneous recurrent motor seizures, with no improvement of the cognitive function. Authors suggested that expression of GDNF by more than 50% of the grafted cells may underlie the therapeutic effect of MGE-NSC grafts, given the role in seizure suppression of this neurotrophic factor [72].

Taken together all these works, we have a scenario where MGE-derived cells are the most promising source of GABAergic neurons for cell-based therapies. However, before clinical application, we should continue studying the possible implication of the interaction of new grafted cells with those from the host; their modulation of synaptic activity, maybe by modifying neuronal plasticity; the possible consequences on behaviour. We will review these implications in the following section. However, we should have in mind that epilepsy etiology is multiple, and in consequence not all of the epilepsies should response equally to MGE-derived interneuron grafts. In addition, we should be cautious. Certain types of GABAergic interneurons together with aberrantly behaving excitatory pyramidal neurons in the subicular region of the hippocampus can precipitate epileptic seizures instead to stop them [73]. In keeping with this idea, it has been also reported a role of GABA-mediated signaling in ictogenesis, contributing to epileptiform synchronization that lead to the generation of electrographic ictal events in the cingulated cortex and limbic areas of the brain [74, 75]. Therefore, grafting of certain subclass of GABA-producing cells in a wrong location in some epilepsy types may lead to seizure exacerbation.

Before clinical application, we should continue exploring the effects of the grafts on several animal models of epilepsy with different etiologies; study the possible implication of the interaction of new grafted cells with those from the host; their modulation of synaptic activity, maybe by modifying neuronal plasticity and the possible consequences on behaviour. We will review these implications in the following sections.

3. GABAergic Grafts for Parkinson's Disease (PD) and Stroke

PD is triggered by the loss of mesencephalic dopaminergic neurons localized in the substantia nigra pars compacta (SNpc). This cellular loss eliminates dopaminergic projections to the striatum and their supply of dopamine which modulates striatal-dependent extrapyramidal motor behaviour. Therefore, PD patients experience motor dysfunctions including tremor, rigidity, bradykinesia, and postural instability. Alleviation of motor symptoms is obtained by the administration of the dopamine precursor L-DOPA; however, its prolonged use over years induces the development of severe side effects known as dyskinesia (abnormal involuntary movements) that only in part are mitigated by different regimens of pharmacological coadiuvants.

In addition, many attempts of gene and cell-based therapies are in progress to establish treatments that can be complementary and additive to the standard pharmacological approach. In particular, a gene therapy approach has been developed to deliver the glutamic acid decarboxylase (*GAD*) gene, catalysing the synthesis of GABA, directly into neurons of the subthalamic nucleus [76]. In PD, activity of the subthalamic nucleus (STN) is increased mainly because of reduced GABAergic input from the globus pallidus. Interestingly, the focal delivery of GAD in the STN contributed to reducing its overactivity leading to an amelioration of the clinical neurological symptoms. Safety and tolerability of this gene therapy protocol has been demonstrated in a phase I trial although with a reduced number of patients and over a limited period of time (1 year) [77]. Thus, promoting GABAergic neuronal activity in specific nuclei can contribute in restoring a balance in the basal ganglia neuronal outflow controlling the extrapyramidal motor system.

In addition to these approaches, the clinical impact of cell replacement has been evaluated in animals and humans over the last two decades. These approaches are aimed to reconstitute a local dopaminergic network capable of a feedback controlled dopamine release upon delivering of dopaminergic neurons in the affected striatal compartment. A similar procedure has been pioneered in humans using cellular grafts of fetal nigral tissues [78–80]. This approach led to some important symptomatic improvements, however, often associated with the development of extremely severe dyskinesia [81]. These side effects are probably due to the high heterogeneity of the grafted tissue containing only a minimal fraction of dopaminergic precursors (5%) in an overall population of different cell types including serotonergic and GABAergic neurons [82, 83]. Recently, an alternative strategy of cell transplantation has been validated in a PD animal model. This is based on transplanting GABAergic precursors isolated from the embryonic MGE/CGE into the adult striatum [84]. At first glance, this approach introducing inhibitory neurons in a tissue already deprived of dopamine might seem counterintuitive. However, thinking of PD as an activity outflow unbalance among different striatal neuronal networks, this methodology finds a strong rationale [85]. Noteworthy, E14.5 MGE/CGE cells injected into a single site were able to migrate throughout the striatal tissue, but not beyond it. The extent of migration is similar to that described for these cells upon transplantation into the adult cortex (see above). Therefore, MGE/CGE GABAergic neuronal precursors have a tendency to actively disperse within different adult brain tissues, and this represents a strong attractive feature for an efficient cell-based therapy. However, this should not be considered of general rule, since the same cells grafted in the subthalamic nucleus are unable to migrate from the injection site [84]. Possibly, this is the case since cortex and striatum, contrary to thalamic areas, are the forebrain regions which are normally colonized by these cells during embryogenesis and might retain some molecular or structural cues that allow this behaviour even in adulthood. Are these cells able subsequently to survive, mature, and integrate in the host striatal tissue? Martínez-Cerdeño and colleagues found that despite the great majority of the MGE/CGE-transplanted cells which were lost after 1 year from transplantation, 1% of them survived

and presented morphological and functional features of mature interneurons of the three major subtypes, namely, calretinin+, parvalbumin+, and somatostatin+ cells [84]. Therefore, only a minimal fraction of MGE-transplanted cells are able to survive for long time in the striatal tissues and this probably reflects the need for establishing stable and functional connections with the host neuronal network for promoting their survival. Remarkably, even though the transplanted interneurons accounted for only about 5% of the total endogenous GABAergic neuronal population, they were sufficient to elicit a significant motor and behavioural recovery in the 6-hydroxydopamine-lesioned rats. How might this occur? The authors revealed the integration of the grafted interneurons by showing the formation of de novo synapses with the host neurons, and hence, they suggest that it is the graft-mediated reorganization of the basal ganglia network that fosters the functional recovery observed [84]. In fact, the striatum is the key centre of the extrapyramidal tract which controls thalamic efferents to the motor cortical regions. This circuitry is organized in two main neuronal assemblies known as the direct and the indirect pathways. The first connects striatum-internal globus pallidus and thalamus and activates thalamic activity. The second restrains thalamic activity and is connecting striatum-internal and -external globus pallidus and thalamus. Hence, these two pathways converge to the thalamus as their final target centre and regulate its activity by playing reciprocal opposing functions. In PD, dopamine depletion in the striatum produces two concomitant effects. First, it reduces the activity of the direct pathway while promoting the indirect pathway creating an upraised inhibitory outflow to the thalamus.

One plausible mechanism by which MGE-transplanted cells promote a symptomatic relief in PD animals is to restore a balance in the total output of these two pathways over the thalamus by preferentially inhibiting the indirect pathway [85]. Although this explanation needs more experimental evidences, nonetheless these studies reveal how a small transplanted population of interneurons has the capability to modulate the plasticity of long-ranging and complex neuronal circuitry and restore a functional unbalance between related neuronal systems.

Recently, similar cell transplantations of embryonic MGE GABAergic precursor cells have been carried out in a mouse model of stroke [86]. Focal ischemia in cortical and nearby striatal areas was produced by middle cerebral artery occlusion, and embryonic MGE cells were transplanted in multiple sites in adjoining regions. Noteworthy, MGE-transplanted animals improved in their locomotion and motor coordination with a significant improvement in both tests respect to sham-injected controls [86]. Similar to previous studies, embryonic MGE cells developed in fully mature neurons featuring spontaneous action potentials and connecting to host neurons. However, the amount of MGE grafted cells that differentiated into mature neurons after 4 weeks from transplantation were only a limited fraction accounting for 20% of the total. Surprisingly, the rest of the cells resulted negatively for astrocyte or oligodendrocyte markers indicating that the transplanted cells remain blocked to a progenitor state unable to complete the differentiation in any cell lineage [86]. This is in striking contrast with the differentiation behavior of grafted MGE cells in the other disease murine models previously described [82].

Nonetheless, the authors noted that the MGE cell grafting stimulates axonal reorganization of the host tissue [86]. In fact, the axonal sprouting and neurite reorganization in the injured site was strongly increased after cell transplantation. These results suggest that grafted MGE neurons might stimulate endogenous repairing mechanisms or formation of alternative neuronal assemblies to support the functions of the lost tissue. To which extent exogenous MGE cells can trigger neuronal rewiring and plasticity of the host tissue remains to be better exemplified. To be noted, also MGE cell graftings in PD animal models induced some changes in the host tissue as for instance the re-expression of the calcium-binding proteins calretinin and calbindin by host striatal cells nearby the transplantation site [84]. The changes might also be promoted by all sorts of trophic factors released by the grafted GABA neuronal precursors that can stimulate neuritogenesis or synaptic connections.

Although many questions remain unanswered, transplantation of embryonic MGE GABAergic cells has resulted surprisingly effectively in promoting clinical improvements in animal models for different chronic or acute neurological disorders. These results call for a better understanding of the cellular and molecular mechanisms by which the MGE grafts can promote this positive outcome. One of them may be the increased delivery of GABA neurotransmitter. To date, direct biochemical measure of GABA concentrations after MGE-derived grafting has not been reported. However, patch clamp analysis of spontaneous postsynaptic inhibitory currents in projection neurons of the grafted area strongly suggests a direct effect on their frequency and amplitude, mediated specifically by presynaptic GABA delivery from the transplanted cells [18, 28]. One other mechanism may be secondary to release of trophic factors by the grafted cells. GDNF has been reported to be secreted by the astrocytes cotransplanted with the MGE cells [27], what improve epileptic condition. We cannot discard the delivery of some other neurotrophic factors that stimulate endogenous repairing mechanisms, or even neurogenesis, as mentioned above. Further efforts should be devoted to decipher the multiple mechanisms implicated in the functional outcomes mediated by GABAergic cell transplants, including modulation of endogenous plasticity.

4. MGE GABAergic Cellular Grafts Induce Cortical Plasticity

Cortical circuits are sensitive to experience during well-defined intervals of early postnatal development called critical periods [87, 88]. After the critical period, plasticity is reduced or absent. Monocular deprivation (MD) is a classic model of experience-dependent plasticity. In the mammalian binocular visual cortex, neurons are activated to different degrees by visual stimuli presented to one eye or the other, a property called ocular dominance (OD). If vision is normal

for both eyes during development, the majority of visual cortical neurons are binocular. If one eye is occluded during development, visual cortical neurons become dominated by the nondeprived eye. This change in OD is taken as a sensitive index of plasticity of visual connections. OD plasticity is particularly high during a critical period of postnatal development and declines with age [89, 90].

Accumulating evidence supports a pivotal role for late-developing excitation versus inhibition circuit balance in the initiation of sensitive periods. For example, the onset of visual cortical plasticity is delayed by genetic disruption of GABA synthesis [91, 92]. Conversely, the application of benzodiazepines or other treatments that accelerate GABA circuit function triggers premature plasticity [93]. Therefore, the onset of OD plasticity is triggered by the establishment of a functional network of inhibitory synaptic transmission. Southwell and colleagues asked whether transplantation of MGE GABAergic precursors could be sufficient to trigger a plasticity respond in the host cortex [94]. In mice, OD plasticity reaches a peak in the fourth postnatal week, when cortical inhibitory neurons are 33–35 days old. Thus, the authors transplanted E13.5 mouse MGE GABAergic precursors in perinatal or early-postnatal brains and ascertained the induction of visual plasticity in the host brains [94]. Grafted MGE cells differentiated into GABAergic interneurons with a great efficiency and only 0.2% of them developed morphology of astrocytes. Remarkably, the cellular graftings were able to successfully trigger visual plasticity in the host [94]. However, this induction was achieved only for a short period of time which correlated with the age of the transplanted cells. In fact, transplantation was effective when the cells were 33–35 days while soon later at 43–46 days old the same cells failed to trigger the same effect. Therefore, the cellular age of the transplanted population strictly determines the effects on cortical plasticity.

The introduction of a supplemental amount of inhibitory interneurons would suggest that an increased inhibitory tone is the trigger for such neural rejuvenation. However, this is not the case since pharmacological enhancement of inhibition does not induce similar effects [95]. The answer is rooted probably in the nature of the synaptic contacts established by grafted interneurons with the host neuronal circuitry. Indeed, transplanted inhibitory neurons form weak but numerous synaptic connections with neighbouring excitatory neurons in the host brain. Thus, these new connections are believed not to simply raise the general inhibitory tone, but rather to promote an overall reorganization of the cortical circuitry by introducing a new set of weak inhibitory synapses. This pattern of newly established synaptic contacts represent an ideal biological substrate capable of enhancing the Hebbian plasticity mechanisms during the critical period [96].

It is noteworthy that the grafted interneurons promote plasticity only when they reach a cellular age comparable to that of the endogenous counterpart during the critical period. This data strongly suggest that plasticity is successfully initiated by a cell-autonomous program endowed in interneuron progenitors which is minimally influenced by the age of the host tissue. These findings open a new scenario where cell transplantation might be effective in reprogramming neural activity up to triggering plasticity processes. Nonetheless, a number of questions need to be answered to understand the safety and efficacy of this procedure. For instance, (i) whether transplantations of interneurons are able to induce visual plasticity even in the adulthood, (ii) if the grafted animals display any neuronal misbehaviour at later stages triggered by the action of the transplanted cells, (iii) if plasticity is promoted by a specific class of interneurons, and (iv) the assessment of the minimal number of cells to be grafted for inducing brain plasticity.

Although the transplanted-induced plasticity lasts for few days, this might be sufficient to trigger long-lasting neural circuitry reorganization. On this view, this procedure opens the exciting opportunity to induce or facilitate the restoration of normal function in injured or degenerative disorders. Future studies are warranted to assess the regenerative potential of this approach in the developing and adult-diseased brains.

5. New Sources for MGE GABAergic Neurons

Considering the findings described above, MGE GABAergic neurons exhibit properties well suited for therapeutic applications in seizures and other neuropsychiatric and neurodegenerative diseases. However, to explore such possibility, it is necessary to identify a renewable source for these cells compatible with their preclinical exploitation. An interesting possibility is generating these cells from *in vitro* differentiation of embryonic or somatic neural stem cells (ESCs and NSCs, resp.). NSCs can be isolated from mouse and human neural tissues and can be propagated for long time in cultures as neurospheres or in adhesive conditions [97–100]. Upon differentiation, NSCs generate a mixed population of GABAergic and glutamatergic neurons, whose ratio is dependent on specific growth culture and differentiation conditions [97, 101]. However, these cells show generally poor developmental plasticity. In fact, after prolonged time in culture, they retain only in part the molecular regional code identity of the area from which they originate and result generally resistant to be coaxed to other neuronal subtypes [102–104]. In contrast, numerous results have shown how ESCs can be converted efficiently in various neuronal subtypes. In particular, some procedures have been recently proposed for directing ESC differentiation into cortical GABAergic interneurons. In an elegant set of experiments, Danjo and colleagues refined the timing and concentrations of Sonic Hedgehog (Shh) stimulation for inducing ESCs neural ventralization and generating either LGE or MGE progenitors [105]. In the latter case, ESC-derived MGE progenitors displayed the ability to migrate and distribute into the developing cortex generating GABAergic interneurons. Interestingly, the authors further showed how a diverse source of FGF signalling can alternatively select for an MGE- or CGE-derived GABAergic cell fate. These results set the experimental conditions to generate different subtypes of cortical GABAergic interneurons with specific electrophysiological and connectivity properties. Further, a

different study showed the ability of ESC-derived MGE progenitors to complete their maturation once transplanted *in vivo* generating functional cells with physiological and neurochemical characteristic of GABAergic cortical interneurons [106]. These findings lay the ground for testing the potential of ESC-derived GABAergic interneurons to treat preclinical model of neurological disorders upon direct cell transplantation.

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Review Article

Brain Plasticity and Disease: A Matter of Inhibition

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One major goal in Neuroscience is the development of strategies promoting neural plasticity in the adult central nervous system, when functional recovery from brain disease and injury is limited. New evidence has underscored a pivotal role for cortical inhibitory circuitries in regulating plasticity both during development and in adulthood. This paper summarizes recent findings showing that the inhibition-excitation balance controls adult brain plasticity and is at the core of the pathogenesis of neurodevelopmental disorders like autism, Down syndrome, and Rett syndrome.

1. Introduction

The term "plasticity" refers to the ability of the nervous system to reorganize its connections functionally and structurally in response to changes in environmental experience, underlying the adaptive development of neuronal circuitry. The existence of time windows in early postnatal life (critical periods) during which neural circuits display a heightened plasticity in response to external stimuli has been established for various brain regions subserving major behavioural functions (for review, see [1, 2]). After the end of the critical period, neural plasticity dramatically wanes. Since the pioneering work by Wiesel and Hubel, the visual system stands as the prime model for studying experience-dependent plasticity. These authors reported that occluding one eye early in development (a treatment usually referred to as monocular deprivation) leads to an ocular dominance shift of cortical neurons, that is, a reduction in the number of cortical cells responding to that eye and a robust increment in the number of neurons activated by the open eye [3]. The imbalance of activity between the two eyes eventually results in the loss of synaptic inputs from the thalamic regions representing the closed eye and in the expansion of those driven by the open eye [4–7], accompanied by a remodelling of cortical horizontal connections [8].

In the last 50 years, great effort has been made to elucidate cellular and molecular mechanisms underlying the activation and regulation of critical periods in the brain. Unravelling these processes may potentially enable researchers to enhance plasticity in the adult brain. Moreover, a detailed knowledge of the events involved in the maturation and plasticity of neuronal circuitry would be a determinant in improving our understanding of the aetiology of developmental brain disorders.

Although a complete picture in the field is still lacking, a large body of evidence has been accumulated (see, [9, 10]). In this paper, we will focus our discussion on intracortical inhibitory circuitry which convincingly emerges as a key factor not only for defining the boundaries of cortical plasticity but also in developing of pathological states characterized by severe intellectual disabilities (see also [11, 12]).

2. GABAergic Inhibition and Ocular Dominance Plasticity in the Adult Visual Cortex

By sculpting the pattern and timing of neuronal electrical activity, inhibitory GABAergic circuits are an ideal candidate for regulating the processes of experience-dependent

synaptic modifications. Taking advantage of gene-targeting technology, this hypothesis has been directly tested by abolishing the expression of the 65 kD isoform of GABA-synthetic enzyme, hence reducing activity-dependent GABA synthesis and release at synaptic terminals. Mice that carry such a disruption of the GAD65 gene do not exhibit ocular dominance plasticity in response to monocular deprivation; only an enhancement of inhibition achieved with local delivery of diazepam enables a full rescue of ocular dominance plasticity in these mice [13].

Converging results obtained with different experimental approaches have subsequently confirmed the key role of GABAergic inhibition in brain development and plasticity (e.g., [14–17]). It is noteworthy that BDNF-overexpressing mice show an accelerated maturation of GABAergic cortical inhibition paralleled by a faster time course of critical period for ocular dominance plasticity [13], strongly suggesting that the progressive development of the inhibitory tone not only enables the onset of the critical period but subsequently underlies the closure of neural plasticity gates.

One of the major challenges in neuroscience is the development of strategies aimed at promoting nervous system plasticity in adulthood, when recovery from injury and functional rehabilitation are severely hampered. Recently, new evidence has challenged the classic dogma that ocular dominance plasticity is a physiological phenomenon exclusively restricted to the early postnatal development and pointed to a reduction of intracortical inhibition levels as a crucial step for the restoration of plasticity processes in the adult brain. The most direct demonstration that GABAergic inhibition limits plasticity in the adult visual cortex derives from a recent study reporting that pharmacological reduction of intracortical inhibition obtained through the infusion of either MPA (an inhibitor of GABA synthesis) or picrotoxin (a $GABA_A$ antagonist) directly into the visual cortex reactivates ocular dominance plasticity in response to monocular deprivation in adult rats [18]. Moreover, this treatment leads to a full rescue of long-term potentiation (LTP) of layer II-III field potentials induced by theta-burst stimulation from the white matter, an activity-dependent form of synaptic plasticity which is normally occluded in visual cortical slices from adult animals due to the maturation of inhibitory transmission [18, 19]. The reduction of intracortical inhibition is accompanied by processes of structural plasticity. The visual cortex of MPA or PTX-treated animals, indeed, shows a decrease in the density of chondroitin sulfate proteoglycans (CSPGs), indicating the activation of endogenous mechanisms of extracellular matrix remodelling which are known to be crucially involved in the expression of neural plasticity [20–22]. It is also possible that other molecular components of the extracellular milieu regulating synaptic plasticity in the adult brain, such as myelin proteins [23] and adhesion molecules [24], may undergo changes in their expression levels in response to a reduction of intracortical inhibition.

These results show that a brief reduction of GABAergic inhibition is sufficient to reopen a window of plasticity in the visual cortex well after the normal closure of the critical period. Similar conclusions have been drawn from recent evidence showing that the inhibitory tone is a central hub for the restoration of plasticity in the adult visual cortex and that a decrease of intracortical inhibition levels is required for the reinstatement of neural plasticity triggered by different experimental approaches. We demonstrated that environmental enrichment, a condition of increased sensory-motor and cognitive stimulation, reactivates juvenile-like ocular dominance plasticity in the visual cortex of adult rats, with a shift in ocular dominance of cortical neurons following monocular deprivation clearly detectable using both visual evoked potentials and single-unit recordings [25]. Recovery of plasticity in enriched animals is paralleled by a marked reduction of the inhibitory tone in the visual cortex. Importantly, the decrease of inhibitory neurotransmission is a crucial molecular mechanism underlying the enhancement of visual cortex plasticity induced by environmental enrichment: preventing the reduction of GABAergic inhibition during the period of exposure to environmental enrichment (via micro-osmotic pumps infusing the GABA agonist diazepam into the visual cortex), indeed, completely blocks the ocular dominance shift of cortical neurons in response to monocular deprivation [25]. The enhanced environmental stimulation provided by environmental enrichment also leads to a twofold enhancement of serotoninergic transmission and to an increase in the number of BDNF-expressing neurons in the visual cortex. Interestingly, infusion of a serotonin synthesis inhibitor not only blocks plasticity in response to monocular deprivation but also fully counteracts the effects produced by environmental enrichment on inhibition and BDNF levels. We suggested a model in which serotonin is the first trigger in the molecular chain set in motion by environmental enrichment, eliciting the decrease of GABA-mediated intracortical inhibition and, in parallel or in series, the enhancement of BDNF levels [25].

It is interesting to point out that while, during development, environmental enrichment increases BDNF and accelerates the maturation of inhibition in the visual cortex [15], in adult animals reared in an enriched environment increased levels of BDNF are associated with reduced GABAergic inhibition. One possible explanation for these apparently contrasting results is that the influence exerted by the environment on these molecular factors may follow a temporarily distinct sequence in the adult compared to the developing brain. Specifically, we propose that the very early (postnatal day 7, see [15]) increase in BDNF detected in mice reared from birth in an enriched environment may be the prime factor that directly drives the development of inhibitory circuitry in the immature brain; on the contrary, the enhancement of BDNF expression in animals exposed to environmental enrichment in adulthood may occur downstream to the decrease of intracortical inhibition, which could promote the expression of many activity-dependent genes involved in neural plasticity.

Given the central role of serotonin in promoting adult visual cortex plasticity, one might expect that the effects induced by environmental enrichment should be reproducible through an artificial modulation of cerebral levels of this neurotransmitter. This possibility has been addressed in a study by Maya Vetencourt et al. [26], showing

that the administration of fluoxetine, a selective serotonin reuptake inhibitor (SSRI) widely prescribed in the treatment of depression for its capability to enhance extracellular serotonin levels, reinstates plasticity in the visual cortex of adult animals, with treated rats exhibiting a marked shift of ocular dominance in favour of the open eye after one week of monocular deprivation. Also in this case, a pronounced reduction of intracortical inhibition has been detected in the visual cortex, and the osmotic infusion of the GABA agonist diazepam fully prevents the ocular dominance shift induced by monocular deprivation, thus impeding plasticity in fluoxetine-treated animals. Further support to the notion that diffuse projecting systems of the brainstem affect plasticity in adulthood has been very recently provided by the demonstration that a genetic enhancement of nicotinic cholinergic transmission restores ocular dominance plasticity well after the end of the critical period. This effect is abolished by diazepam treatment, suggesting that the cholinergic signalling mechanisms may adjust excitatory-inhibitory balance [27].

Using an approach quite different from environmental enrichment, a study by He and colleagues reported that exposing adult animals to complete darkness can also promote plasticity in the visual cortex [28]. These authors provided indirect evidence that the enhanced cortical plasticity might be related to a shift in the balance between inhibition and excitation towards levels more similar to those found in the immature cortex, caused by a reduced expression of $GABA_A$ receptors relative to AMPA receptors. This suggestion has been confirmed in a very recent study [29] showing that dark exposure decreases inhibitory synaptic density and paired-pulse depression and reinstates in the visual cortex the expression of endocannabinoid-dependent inhibitory long-term depression, a form of synaptic plasticity normally restricted to the juvenile age [30].

Two different hypotheses, not mutually exclusive, could be formulated for explaining how the reduction of the inhibitory tone to juvenile-like levels leads to a recovery of cerebral plasticity in the adult brain. According to one hypothesis, the maturation of GABAergic intracortical transmission sets the point after which the editing activity of visual cortex pyramidal neurons enables ocular dominance plasticity; as development proceeds further, the inhibitory tone surpasses a threshold, and this causes the closure of the critical period. A reduction of inhibition levels may reinstate in the adult visual cortex the capability of binocular neurons to detect the imbalance in retinal inputs induced by the closure of one eye. According to an alternative hypothesis, the overall increase of cortical activity due to the shift in excitation-inhibition balance is the key factor favoring plasticity recovery. Activity-dependent regulation of gene expression could induce a genetic transcriptional program critical for promoting plasticity.

3. Beyond the Visual Cortex

The critical role of GABAergic inhibition in regulating experience-dependent plasticity is not restricted to the visual cortex.

In the barn owl, the optic tectum contains a map of space consisting of bimodal neurons whose auditory and visual receptive fields are mutually aligned. In juvenile owls, alternative maps of interaural time difference can be acquired as a result of abnormal experience. The group of Knudsen and colleagues has demonstrated the existence of a sensitive period for plasticity in the optic tectum by exposing owls at different ages to prismatic spectacles that cause a large horizontal shift of the visual field [31]. Owls bearing these spectacles experience a modification of the visual locations to which the interaural time difference values correspond, eliciting the adjustment of auditory receptive fields according to the optical displacement [31, 32]. Very interestingly, the environmental rearing conditions can have a dramatic impact on this form of plasticity. Indeed, the period during which owls respond adaptively to prismatic displacement of the visual field ends at about 70 days of age when owls are housed in individual cages, while it does not end until 200 days of age when owls are housed in groups and in larger enriched rooms [31]. At the same manner, also the ability to recover after restoration of normal visual experience is strongly affected by the environment, because it ends at 200 days of age when prism-reared owls are housed in small cages but extends throughout life when they are housed in group flight rooms. Soon after the characterization of the sensitive period for visual calibration of the auditory space map, Zheng and Knudsen demonstrated that when a new learned map is expressed in the external nucleus of the owl optic tectum, the neural circuitry underlying the old map is not structurally inactivated but becomes silent due to a functional suppression operated by inhibitory connections and involving GABAA receptors [33].

In the mammalian auditory system, a well-defined critical period exists for tone-specific enlargement in the primary auditory cortex (A1) representation resulting from transient exposure to sound stimuli [34]. Strikingly, the Merzenich's group has recently demonstrated that while in adult control rats this exposure produces no measurable alteration of A1 tonotopy, rats transferred to an environment of continuous moderate-level noise exhibit a re-establishment of a period of sound exposure-driven plasticity [35]. This effect, which is reminiscent of the reopening of critical period plasticity triggered in the visual system by dark exposure, is paralleled by a decrease in the expression level of GABAA $\alpha 1$ and $\beta 2/3$ subunits in A1.

Thus, reduction of GABAergic inhibition may emerge as a common feature of the strategies that successfully reopen a period of stimulus exposure-based plasticity in the adult brain [18, 25, 26, 28, 35].

4. Pathological Inhibition of Cerebral Function: The Case of Amblyopia

During the critical period, the high susceptibility of neuronal connections to experience-dependent changes is essential for a proper maturation of normal sensory functions. This high potential for plasticity, however, may also favour the emergence of developmental pathological states when an

anomalous perturbation of sensory-driven activity takes place. A paradigmatic case is that of amblyopia, a widely diffused and still untreatable pathology of the visual system affecting 2–4% of the total world population [36]. Amblyopia derives from conditions of early abnormal visual experience in which a functional imbalance between the two eyes is predominant owing to anisometropia (unequal refractive power in the two eyes), strabismus (abnormal alignment of one or both eyes), or congenital cataract, resulting in a dramatic loss of visual acuity and a broad range of other perceptual abnormalities, including deficits in stereopsis and contrast sensitivity [37, 38]. It is worth stressing that in amblyopic patients the visual impairment is caused by an abnormal processing of visual information at the central level; thus, the use of corrective lenses is completely ineffective [39–41].

It is currently accepted that, due to a lack of sufficient residual plasticity within the brain, the reinstatement of visual functions in amblyopic subjects is possible only if corrective treatment is started early in development. The classic amblyopia therapy consists in patching or penalizing the preferred eye, thus forcing the brain to use the visual input carried by the weaker amblyopic eye [42]. However, an increasing number of clinical and animal studies are now challenging these traditional beliefs, reporting that repetitive visual training based on sensory enrichment procedures may represent a very useful approach for the treatment of amblyopia (for a comprehensive review, see [38, 43]).

The mechanisms underlying vision improvements in adult amblyopic patients remain to be elucidated, since the activation of cortical plasticity may occur at several different levels of the visual system and through a variety of neural processes. A number of studies, however, suggested that an impairment of the balance between excitation and inhibition could affect visual cortex development and that cortical overinhibition could underlie the degradation of spatial vision abilities [44–48]. Accordingly, recent advances in our understanding of the cellular and molecular brakes that limit amblyopia recovery to a critical period underscored intracortical inhibition as a main obstacle for reinstatement of normal visual functions after a period of early abnormal visual experience. In animal models, amblyopia can be induced by imposing a long-term reduction of inputs from one eye by lid suture (i.e., with a protocol of long-term monocular deprivation). Similarly to that observed in humans, animals rendered amblyopic by long-term monocular deprivation display a permanent loss of visual acuity in the affected eye and a pronounced ocular dominance shift of visual cortical neurons in favour of the normal eye (e.g., [49–51]).

Early studies in animal models of amblyopia reported that the administration of anti-inhibitory compounds (e.g., bicuculline) leads to a substantial restoration of binocularity in the visual cortex [52, 53]. Recently, it has been shown that the same experimental paradigms discussed in Section 2 and associated with a reduced inhibition-excitation balance in the adult cerebral cortex are also able to recover sight from amblyopia (for review [54, 55]). Among these treatments, environmental enrichment emerges as a totally non-invasive approach [56]. We reported that a brief exposure (two-three weeks) of adult amblyopic rats to environmental enrichment promotes a complete recovery of both visual acuity and ocular dominance, as demonstrated both with electrophysiological recordings of visual evoked potentials from the primary visual cortex and with a standard visual acuity behavioural test (visual water-box task). The environmental enrichment-induced recovery of visual acuity is long-lasting, persisting for a minimum of two weeks [56]. A reduced intracortical inhibition is a crucial mechanism underlying the enhancement of visual cortex plasticity in environmental enrichment: preventing the reduction of GABAergic inhibition during the period of environmental enrichment, indeed, completely blocks the recovery of binocularity and visual acuity. These findings draw attention to the environmental enrichment procedure as a prospective, injury-free, intervention strategy for amblyopia and further substantiate a major role for GABAergic transmission in the control of plasticity windows in the sensory cortices.

5. Inhibition and Neurodevelopmental Disorders

While the physiological maturation of GABAergic connections is essential for a tight control of developmental cortical plasticity and for promoting the acquisition of mature sensory abilities, it is currently accepted that abnormal levels of inhibition achieved during development can cause pathological states of severe brain disability [11, 57, 58]. On this regard, Rett syndrome, Down syndrome, and autism disorder stand as the most informative cases (the role of inhibition in schizophrenia is discussed in another review published in this issue).

5.1. Rett Syndrome. Rett syndrome is a progressive developmental disorder characterised by mental retardation and severe dysfunction in motor coordination skills [59], predominantly affecting the female population in early childhood. Using a systematic gene screening approach, loss-of-function mutations in the X-linked gene encoding the methyl-CpG binding protein (MeCP2) have been identified as the cause of Rett syndrome [60]. MeCP2 is involved in the regulation of expression of a wide range of genes [61] and in RNA splicing [62]. Transgenic mice carrying conditional deletion or neuron specific expression of mutated MeCP2 forms exhibit abnormalities in motor coordination, social interaction, and cognitive abilities, providing a useful model for analysing the behavioural and molecular phenotype of the Rett syndrome [63–66].

Detailed electrophysiological analysis of these animal models showed a reduction of neuronal activity in cortical and hippocampal neurons due to a shift in the balance between cortical excitation and inhibition in favour of inhibition [67, 68] and an attenuation of LTP expression in the hippocampus and in the motor and somatosensory cortex [68, 69]. These results led to the hypothesis that an anomalous increase in the inhibition/excitation ratio could be responsible for the motor, behavioural, and cognitive defects associated with Rett syndrome [11]. This interpretation

is supported by autoradiographic labelling studies on human postmortem brain samples, showing a significant increase in the density of GABA receptors that may correlates with cognitive and motor symptoms of Rett syndrome [70]. A very recent work by Chao and colleagues further demonstrated that a dysregulation of GABAergic system has a role in modulating the pathogenesis of Rett syndrome: mice lacking MeCP2 selectively in GABA-releasing neurons, indeed, recapitulate most of the behavioural features of Rett syndrome [71]. Surprisingly, these mice display a reduced inhibitory tone, while no data were presented concerning levels of excitation. Therefore, while these results confirm that a dysfunction of GABAergic neurons can contribute to the Rett phenotype, they also outline a more complex framework for the involvement of inhibitory transmission in Rett syndrome.

Since the gene encoding BDNF is under MeCP2 regulation [72] and the severity of behavioural symptoms in MeCP2 deficient mice correlate with levels of circulating BDNF [73], attempts have been made to rescue the Rett syndrome phenotype by delivering BDNF. It has been shown that exogenous BDNF in MeCP2 mutant mice is able to compensate for deficits at the behavioural, anatomical, and electrophysiological level [73, 74]. Pre-weaning environmental enrichment, which results in augmented cerebral BDNF levels, ameliorates motor and cognitive impairment and reverses cortical LTP deficits [75]. Very interestingly, environmental enrichment increases the number of cortical excitatory synapses with no changes found in inhibitory synaptic density, thus resulting in overall reduction of the cortical inhibitory tone [75].

5.2. Down Syndrome. Down syndrome is caused by triplication of chromosome 21 (Chr21) and is the most common genetic cause of mental retardation [76]. People with Down syndrome have moderate to severe cognitive impairment, with various disturbances in learning and memory abilities [77, 78]. In search of possible molecular and cellular processes involved in the pathogenesis of the syndrome, several murine models have been generated, carrying triplications of different segments of Chr16, which has a large degree of synteny with human Chr21 [79, 80]. Currently, the prime model is the Ts65Dn transgenic mouse [81, 82], which recapitulates all main hallmarks of the Down syndrome phenotype, including characteristic craniofacial abnormalities, impaired spatial and nonspatial learning abilities, and attention and visual function deficits (e.g., [83–85]). Anatomical studies indicated that Ts65Dn mice have a reduced number of cerebellar and hippocampal neurons [86–88], impaired neurogenesis in the dentate gyrus of the hippocampus (see [86, 89]; see also [90] for similar evidence in human foetuses), and simplified dendritic branching in several brain regions, associated with alterations in spine size and shape [91–93]. Moreover, dysfunctions in the mechanisms driving nerve-growth factor (NGF) retrograde transport from the hippocampus to the basal forebrain [94, 95] are responsible for a prominent degeneration of basal forebrain cholinergic neurons in adult Ts65Dn mice [96], which is also a hallmark of the Alzheimer's disease. Accordingly, nearly one hundred per cent of persons born with Down syndrome develop Alzheimer's disease if they live into their fourth decade of life [96, 97].

A large number of studies have shown that the cognitive impairment displayed by Ts65Dn mice is mainly related to excessive levels of inhibition in temporal lobe circuitry, causing a failure of long-term synaptic plasticity in the hippocampus [98–100]. The deficit of synaptic plasticity is linked to marked morphological changes in the structure of synapses, with a selective enlargement of the active zones of symmetric synapses and increased immunostaining for synaptic proteins localized at inhibitory synapses in cortex and hippocampus [101, 102]. The central role of overinhibition in Down syndrome pathogenesis has been recently confirmed by the demonstration that administration of non-competitive antagonists of GABAA receptors reverses spatial learning disabilities and LTP deficits in Ts65Dn mice [100].

One of the major challenging tasks in the field of Down syndrome therapy is unravelling dosage-sensitive genes whose dysfunction, due to the presence of an extra copy of chromosome 21, might be responsible for the main functional and morphological defects. A recent study by Chakrabarti et al. [103] has shown that two genes, Olig1 and Olig2, are essentially involved in the syndrome. The authors first reported that, very early in development, Ts65Dn mice have a marked increase in the number of forebrain GABAergic neurons generated in the medial ganglionic eminence (one of two regions in the ventral telencephalon where most inhibitory neurons proliferate and differentiate). More specifically, an overproduction of two specific classes of inhibitory neurons (i.e., parvalbumin- and somatostatin-positive neurons) has been detected. This anatomical phenotype is directly related to increased levels of inhibitory transmission in the forebrain of Ts65Dn mice, as assessed with electrophysiological methods [103]. Remarkably, a genetic reinstatement of dysomia at the level of Olig1 and Olig2 genes (obtained by breeding Ts65Dn mice with a line having only one copy of each of these genes) was sufficient to rescue the Ts65Dn phenotype, correcting the interneuron overproduction and restoring synaptic transmission to euploid levels [103]. Even if a behavioural assessment of the cognitive performance in Ts65Dn mice after re-establishment of dysomia was not reported, these results suggest that a few dosage-sensitive genes might eventually be responsible for many of the deficits displayed by people with Down syndrome and further support a causal link between aberrant inhibition in cortical and hippocampal circuitries and cognitive impairment due to Down syndrome.

Despite the increasing knowledge concerning the molecular mechanisms underlying Down syndrome, a suitable treatment for this disorder is still lacking. Since environmental enrichment is particularly effective in reducing GABAergic inhibition [104], it may have a great potential for therapeutic application to Down syndrome. Martínez-Cué et al. have reported increased exploratory behaviour and enhanced spatial learning in enriched Ts65Dn mice, albeit the effect was gender-specific [105]. Despite these results, a detailed investigation of the environmental enrichment effects on Down syndrome pathogenesis is still lacking.

5.3. Autism. Autism is a heterogeneous developmental disorder characterised by significant impairments in the social, communicative, and cognitive domain and by the presence of repetitive patterns of stereotyped activities [106, 107], mostly affecting males in early childhood [108]. The advent of magnetic resonance imaging enabled the *in vivo* investigation of structural brain morphology in people with autism. Several regions have been reported to be enlarged or reduced relative to controls, but a large consensus on these results is currently missing (for a review, [109]).

The aetiological mechanisms of autism are at present poorly defined. Despite a likely contribution of environmental causes, genes play a crucial role in the onset of this pathology with concordance between monozygotic twins reaching 90%, as compared with less than 10% for dizygotic twins and siblings [110, 111]. Only recently, considerable efforts have been focused on understanding the genetic basis of autism and led to the identification of multiple chromosomal loci and epigenetic factors associated with autism heritability (for a review, [112]). Given the complex repertoire of symptoms characterising autistic syndrome, it has been proposed that defects in the development and functioning of multiple and relatively independent neural systems work together to generate the pathological phenotype. In particular, neural circuits underlying social and emotional behaviour, language processing, and higher-order cognition are considered natural candidates [113].

Converging results have pointed to an increased excitation/inhibition ratio in sensory, mnemonic, social, and emotional systems as a core mechanism underlying neurological and behavioural deficits observed in autistic patients [58]. Consistently, clinical studies showed that epilepsy displays a good percentage of comorbidity with autism [114]. An imbalance of neural circuits leading to a disproportionate high level of excitation could be due to increased glutamatergic transmission or suppressed GABAergic inhibition. The hypothesis that a reduction of inhibitory neurotransmission shared in common between many systems could be a key factor in the pathogenesis of autism is consistent with a large body of evidence [115]. Indeed, a significant reduction in protein levels of both isoforms of glutamic acid decarboxylase [116, 117] and GABA receptors [118–120] has been reported in autistic cerebral cortex. Linkage genetic studies uncovered that polymorphism, copy number, and epigenetic alterations in chromosomal regions containing GABA receptor subunit genes are associated with autistic phenotype [121–123].

On the cellular level, it has been shown that in a valproic acid rat model of autism, the amygdala is hyperreactive to electrical stimulation and displays enhanced synaptic plasticity as well as defective inhibitory transmission [124]. Moreover, a direct demonstration that inhibitory circuitries are activated atypically and are less synchronized in the brain of autistic people has been provided by studies of functional magnetic resonance imaging [125, 126].

Since autism is a developmental disorder, the imbalance in the ratio of excitation versus inhibition could result from abnormal processes during neural circuit maturation. Indeed, defects in synaptogenesis and synaptic refinement have been suggested to be a leading cause of autism, and mutations of genes that normally control the patterning of synaptic maturation of specific neuronal subpopulations have been shown to segregate with the pathological phenotype [127, 128]. Among these genes, Dlx1 and Dlx2 encode transcription factors exerting a crucial role in the generation of GABAergic cortical interneurons and lie in a chromosomal region associated with autism susceptibility [129]. In accordance with the excitation/inhibition model, it has been proposed that pharmacological agents that reduce neural excitation, such as anticonvulsivants and benzodiazepines, could represent a suitable therapeutic treatment for autism [58]. At present, some evidence that anticonvulsivants could be effective in ameliorating autistic symptoms is available (e.g., [130–132]).

It should be pointed out, however, that the exact role of excitation/inhibition balance in autism is still debated. Indeed, an increased inhibitory synaptic transmission and a decreased glutamatergic excitation have been also reported in different transgenic mouse models of autism [133, 134].

6. Concluding Remarks

Altogether the results reviewed here show how dramatic can be the influence exerted by inhibitory transmission on brain plasticity. Not only are these findings crucial to our knowledge about the molecular mechanisms underlying the expression and regulation of plasticity processes, but they also have strong implications for the treatment of neurological disorders related to an aberrant development of GABAergic circuits. The possibility of rescuing a normal phenotype in animal models of these pathologies by manipulating levels of intracortical inhibition draws attention on the GABAergic system as an eligible candidate for the development of new therapeutic strategies.

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