Refining the roles of GABAergic signaling during neural circuit formation

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Our understanding of the role of GABA signaling in circuit development is rapidly expanding. Here, we review three recent refinements in our understanding of the diverse roles that GABA plays at different stages of neural circuit formation. First, we discuss recent evidence that depolarizing GABA plays at least a permissive role in promoting both excitatory and inhibitory synaptogenesis in developing neurons (including newly generated neurons in the adult). Next, we discuss recent evidence that GABAergic circuits sculpt the temporal and spatial aspects of synaptic integration. Consequently, early developmental events affecting the establishment of GABAergic circuits will control subsequent activity-dependent refinements of information processing and circuit function. In the third section, we review recent evidence of molecular mechanisms by which GABAergic signaling plays a role in the regulation of the balance between GABAergic and glutamatergic transmission in developing circuits. Throughout the review, we concentrate on the effects of the signaling by GABA\(_A\) receptors, as told from the point of view of the GABA-responsive cells, and do not discuss mechanisms that govern GABA release or activity of GABAergic neurons per se.

Introduction

Fast GABAergic synaptic inhibition strictly regulates the spatial and temporal extent of neural activity throughout the brain. As such, phasic inhibitory inputs are essential for control information processing and transfer within and between brain regions [1]. Feedforward GABAergic circuits in the hippocampus and sensory cortex impose strict millisecond time limits during which excitatory synaptic inputs can summate to generate post-synaptic action potentials [2–4]. While marveling at the temporal precision that GABAergic transmission brings to the adult brain, it is just as impressive that the same neurotransmitter system is specialized to serve a distinct set of roles during the establishment and refinement of neural circuits. Recent advances in our understanding of how GABA influences growing neurons, how GABAergic neurons shape activity patterns in neural circuits and the mechanisms by which GABAergic synaptic activity is developmentally regulated are refining the way we view the function of this transmitter in CNS development.

During neural circuit development, simple, undifferentiated and unconnected cells grow into neurons, whose complex, three-dimensional morphologies provide the structural basis for the precise synaptic connections that characterize the adult brain. A wealth of experimental data supports the view that this process is regulated by neuronal activity and neurotransmission. Pharmacological, genetic and molecular manipulations indicate that GABAergic neurons and GABAergic signaling are particularly important for brain development [5,6]. The current challenge is to understand the cellular and subcellular mechanisms by which GABAergic neurons impact circuit development, neuronal growth and plasticity.

Since the discovery that GABA can depolarize the membrane potential of immature neurons [7–9], it has become clear that specialized features of GABAergic transmission enable it to serve multiple roles during development. Indeed, it appears that over the course of neural circuit formation, the maturational changes in GABAergic signaling parallel the changing requirements faced by developing neurons: first, they must take up the correct position in the tissue and begin to differentiate; second, they must establish their mature morphology and form synaptic contacts with other cells; and third, they must refine their connections to become part of a precise network. This is all achieved while balancing excitation and inhibition in such a way as to facilitate activity-dependent mechanisms, but not threaten the viability of the cell, for instance by excitotoxicity. The central role for GABA as a regulator of these multiple developmental processes has been affirmed by exciting recent observations that GABAergic signaling characteristic of developing circuits is recapitulated during adult neurogenesis, where newly born neurons must also grow processes and form new synapses to integrate into established neuronal networks [10–12]. In fact, these observations lead to the provocative idea that GABAergic responses could provide a signaling ‘toolkit’ that enables any immature nerve cell to be integrated into a circuit.
GABAergic transmission is characterized by several features that make it an ideal regulator of activity-dependent neuronal differentiation and circuit formation. First, GABA_A receptors are not permeable to potentially dangerous ions such as calcium (Ca^{2+}). Rather, GABA_A receptors are primarily permeable to chloride (Cl^−), a relatively inert ion, which is not believed to have a role in intracellular signaling events. Second, the resting intracellular Cl^− concentration is controlled by developmental expression and activity-dependent regulation of Cl^− transporter proteins [13]. This can have dramatic effects on the ionic driving force and, therefore, the reversal potential of the GABA_A receptor-mediated responses. This flexibility in how a neuron is affected by GABAergic inputs is not available to most other neurotransmitter systems. Third, canonical neural circuits, such as feedforward and feedback GABAergic circuits, impose specific temporal rules on the timing of glutamatergic and GABAergic inputs and, therefore, on patterns of activity within and between brain circuits.

**GABAergic signaling during the establishment of neural circuits**

The earliest GABAergic signaling occurs before the formation of synaptic connections. At early stages of brain development, GABA is thought to be released from post-mitotic neuroblasts and to function through tonic activation of GABA_A receptors on neural progenitor cells to
control the transition from cell proliferation to neuronal differentiation [14–17]. Indeed GABA has recently been shown to have similar trophic effects on progenitors in the adult brain [12]. This mode of GABA signaling is non-synaptic, diffuse and can be mediated through non-vesicular release mechanisms [17]. Tonic GABA signaling, whether by vesicular release or not, is mediated by extrasynaptic GABA\(_A\) receptors and has less spatial or temporal precision than synaptic GABA signaling [1].

GABA\(_A\) receptor activation increases membrane permeability to Cl\(^-\) ions, which in mature neurons, typically leads to a net inward flow of Cl\(^-\) and hyperpolarization of the membrane potential. At early stages of development GABA\(_A\) receptor activation depolarizes the cell membrane as a result of the immature expression pattern of the Cl\(^-\) transporters NKCC1 and KCC2, which generates a positive Cl\(^-\) reversal potential relative to the resting membrane potential [6,15]. NKCC1, a sodium–potassium–chloride cotransporter, is expressed relatively early in neurons and leads to an accumulation of intracellular Cl\(^-\). KCC2 is a potassium–chloride cotransporter that is active in mature neurons and transports Cl\(^-\) out of the cell. Its expression is sufficient to generate hyperpolarizing responses to GABA, even in young neurons [18–20] (Figure 1). Although often referred to as a ‘switch’, the normal shift in GABA\(_A\) receptor reversal potential takes several weeks to reach mature hyperpolarizing levels. Because GABA depolarizes immature neurons throughout the period of their morphological development and synaptogenesis, membrane depolarization in response to GABA probably represents a fundamental signaling mechanism regulating neuronal development and circuit assembly.

In many brain regions, the first synaptic inputs of a neuron are GABAergic and early glutamatergic circuits can be relatively weak [18,21]. Again, recent studies in the context of adult neurogenesis suggest that this is a common principle for newly generated cells, as newly formed neurons in the adult hippocampus receive depolarizing synaptic GABAergic input before they receive synaptic glutamatergic input [10,11]. The establishment of GABAergic synapses on neurons, which had previously responded to ambient GABA released by non-synaptic mechanisms, marks a change in the spatial and temporal effects that GABA can have, both in terms of the magnitude of post-synaptic depolarization and how it interacts with other active conductances. Early synaptic GABAergic currents are typically large and slow [11,18,22,23] and the kinetics of the GABAergic currents are surprising similar to the slow currents mediated by N-methyl-D-aspartic acid (NMDA) receptors in young neurons [18], supporting the idea that depolarizing GABAergic inputs cooperate with NMDA receptor-dependent signaling during neuronal development [6,20].

Several recent studies have provided convincing evidence that GABA\(_A\) receptor-mediated membrane depolarization is a key regulator of several features of early circuit development. The significance of early depolarizing GABAergic synaptic activity was investigated by manipulating the expression of the Cl\(^-\) transporters NKCC1 and KCC2. Early expression of KCC2 in the Xenopus tadpole retinotectal system in vivo caused a premature decrease in intracellular Cl\(^-\) levels [18]. The resulting premature conversion of GABA from a depolarizing to a hyperpolarizing transmitter prevented the normal maturation of glutamatergic synapses and increased the development of inhibitory GABAergic inputs (Figure 1). Similarly, premature KCC2 expression in cultured neurons disrupted synaptic development, by selectively augmenting GABAergic synapses [19]. Ge and colleagues [10] used a different strategy to shift GABA responses from depolarizing to hyperpolarizing by knocking down the expression of NKCC1 in newly generated neurons in the adult hippocampus. This severely impaired the synaptic and morphological development of the immature neurons and their ability to integrate into the adult hippocampal circuit. Previous studies demonstrate that maturation of glutamatergic synapses is required for the normal development of dendritic arbor structure [24]. This suggests that early depolarizing responses to GABA could be required for dendritic arbor development, and might influence dendritic development indirectly by interfering with glutamatergic synaptic maturation. Studies with KCC2 knockout mice also suggest that failure to establish a hyperpolarizing response to GABA impairs the development of circuit function, and can affect structural aspects of brain development [25]. In addition, tonic GABA\(_A\) receptor activation could represent a paracrine signal that promotes the extension of dendrites [26]. In summary, the manipulation of Cl\(^-\) transporter proteins supports the conclusion that early depolarizing GABAergic synaptic transmission has widespread effects on circuit development. The exact consequences of a shift in intracellular Cl\(^-\) could reflect the timing of the manipulation, the extent to which the Cl\(^-\) reversal potential is shifted relative to voltage-sensitive conductances, and the possible involvement of other Cl\(^-\) permeable channels such as glycine receptors. In the future, it will be important to probe the extent to which depolarizing GABAergic transmission controls different aspects of neuronal circuit development.

GABA depolarization might not necessarily increase excitation of neurons or the network, because the reversal potential of the GABA\(_A\) receptor could be below action potential threshold and GABA\(_A\) receptor activation can shunt other conductances [27]. However, depolarization generated by GABA\(_A\) receptors is well placed to facilitate local Ca\(^{2+}\) influx, either through activation of voltage-gated Ca\(^{2+}\) channels (VGCCs) or through cooperation with immature glutamatergic inputs where GABAergic depolarization can facilitate relief of the voltage-dependent Mg\(^{2+}\) block of NMDA receptors. Cooperation with NMDA receptor activation represents an early form of coincidence detection between GABAergic and glutamatergic inputs. Indeed, the gradual developmental decrease in the Cl\(^-\) reversal potential results in a systematic and significant decrease in the facilitation of the NMDA receptor conductance by GABAergic inputs [18]. NMDA receptor activation is known to be crucial for proper dendritic development and synapse formation [28–30], and the degree of Ca\(^{2+}\) influx through the NMDA receptor has been shown to dictate whether synapses are strengthened or weakened [31,32]. The fact that a depolarized GABA reversal potential can
limit excitation by shunting conductances, at the same time as facilitating NMDA receptor transmission, could be key to understanding how GABA can be permissive for synapse formation without threatening the developing brain with dangerous excitability levels. Indeed, this ‘dual role’ for GABA might also explain why circuit development can be disrupted by pharmacological manipulations that either block or enhance GABAergic synaptic transmission during this period [33].

**GABAergic signaling during the refinement of developing neural circuits**

Recent work has highlighted how feedforward inhibition in the mature brain tightly regulates synaptic integration windows and spike timing [2,3,34,35]. The observations that feedforward GABAergic inhibition can suppress the induction of long term potentiation (LTP) in the mature brain [36], and that developmental maturation of the GABAergic system raises the threshold for inducing LTP...
at excitatory synaptic connections [37], are consistent with the idea that inhibitory GABAergic inputs narrow the integration window for synaptic plasticity. Local feedforward and feedback GABAergic connections become established during the early period of synaptogenesis [18,21,33]. Because these inputs impose temporal structure on activity patterns, whether GABAergic transmission is depolarizing or hyperpolarizing and when GABA conductances are active relative to other conductances could have important consequences for circuit activity and synaptic plasticity. These observations suggest that the maturation of hyperpolarizing GABAergic activity could be required for developmental plasticity and for establishing circuits whose development depends on mechanisms such as spike-timing-dependent plasticity.

One hypothesis that emerges from these ideas is that during circuit development, the relatively depolarized reversal potential, combined with other aspects of the immature GABAergic system, such as slower decay kinetics [22,23] and preferential recruitment by bursts of afferent activity [18], sets a less stringent temporal rule for synaptic integration of excitatory synaptic inputs. This would provide a period during circuit development that would be more permissive for recognizing and strengthening convergent synaptic inputs, which could be essential for a cell to establish excitatory synaptic connections and become part of the network. By extension, the developmental shift towards hyperpolarized GABA_A receptor reversal potentials and faster GABA_A receptor conductances would be expected to narrow integration windows. Under these conditions, only strictly coincident synaptic inputs would fall within the integration window and have the opportunity to be strengthened by spike-timing-dependent plasticity mechanisms. This model predicts that depolarizing GABAergic synaptic activity is required for the development of neuronal circuits and that manipulations that interfere with immature GABAergic signaling would result in abnormal circuit function. Such a deficit in circuit development could account for the respiratory failure that kills KCC2 knockout mice [25] and further suggests that these animals could have more widespread defects in circuit structure and function.

Support for the hypothesized importance of depolarizing GABAergic transmission in circuit formation is provided by an exciting recent study from Kanold and Shatz [38], which illustrated how a manipulation that prevents the maturation of hyperpolarizing GABAergic activity can dramatically alter the rules for developmental plasticity (Figure 2). In this case, ablation of subplate neurons in visual cortex interferes with the development of several aspects of GABA signaling in cortical layer IV neurons. Subplate ablation prevents the normal increase in layer IV KCC2 expression, the shift towards hyperpolarizing GABA responses and a developmental increase in GABA_A receptor subunits. Modeling experiments support the conclusion that, under conditions in which subplate was ablated and the plasticity of the system was challenged by monocular deprivation, spike-timing-dependent plasticity rules in fact favor thalamic inputs from the deprived eye, which are less active and less temporally coherent. Furthermore, in vivo experimentation reveals that precise neural circuits, such as those that underlie orientation selectivity, fail to form [38,39]. A more thorough understanding of how GABAergic transmission influences neural circuit refinement will be gained from manipulations that offer spatial and temporal control of GABAergic synaptic transmission and of the response of the post-synaptic cell to GABA.

A further specialization of the GABAergic system that could be significant during circuit refinement is that the Cl^- reversal potential, and its developmental shift, are not fixed parameters. Evidence from several groups has indicated that the activity of chloride transporters can be modulated [13,40–43]. Periods of high frequency activity and/or brain-derived neurotrophic factor release result in downregulation of KCC2 function and a return towards relatively depolarized Cl^- reversal potentials [40,43]. Recently, it has been shown that the level of nicotinic cholinergic activity in developing networks modulates the Cl^- reversal shift and the effects of GABAergic signaling, apparently through the coordinated control of both KCC2 and NKCC1 expression [41]. Thus activity-dependent post-synaptic Cl^- regulation could represent a sliding threshold during circuit refinement that would modulate the temporary stringency of the rules for coincidence detection. The functional significance of reverting to a depolarizing GABA (or reducing inhibitory strength), or how this would change circuit refinement, is not yet clear.

**Balancing excitatory and inhibitory synaptic inputs during neural circuit development**

An important requirement of circuit development is to maintain the balance of excitation and inhibition within a functional range, for instance to permit activity-dependent plasticity while avoiding excitotoxicity. Intracellular Cl^- is a key component in the delicate balance of excitation–inhibition during development, and alterations in Cl^- transporter function have been shown to increase the susceptibility of the immature brain to unstable network activity and neonatal epilepsy [44,45]. Both hyperpolarizing GABAergic inhibition and glutamatergic excitation are relatively weak during early development, and both increase as development progresses [22,23,46]. Studies in cultured neurons show that the balance of excitatory and inhibitory synaptic inputs can be homeostatically regulated in an activity-dependent manner [46,47]. One explanation for this coordinated development is that inhibition is regulated to match the level of excitation. In other words, the developmental increase in hyperpolarizing GABA transmission could be a response to changes in the glutamatergic excitatory drive, and not a director of circuit development. It is interesting, therefore, that several recent studies indicate that early depolarizing GABAergic transmission might be crucial for the coordination of subsequent levels of excitatory and inhibitory inputs. A premature hyperpolarizing shift in the Cl^- reversal potential results in an increase in the ratio of inhibitory to excitatory inputs under three independent experimental conditions [10,18,19]. This is consistent with the general idea that depolarizing GABAergic transmission is required for the formation of glutamatergic synapses, which in turn would regulate the development of inhibitory GABAergic inputs (although see Ref. [19]). A breakdown in homeo-
static regulation of the two types of input could reflect the fact that depolarizing GABA is most crucial at early stages of synaptogenesis, perhaps when different types of homeostatic mechanisms are in place. Alternatively, dynamic regulation of GABAergic depolarization–hyperpolarization, potentially through activity-dependent control of Cl⁻ transporters [13], could itself be part of the mechanism that normally matches excitation and inhibition.

The processes by which a transient period of GABA receptor-mediated depolarization leads to changes in synapse formation and the maturation of glutamatergic and GABAergic synapses, remain to be determined. However, a recent series of exciting studies have identified candidate molecules that appear to regulate the balance of glutamatergic and GABAergic synapses [48–51]. Trans-synaptic signaling involving pre-synaptic β-neurexins and post-synaptic neuroligins promotes synapse formation [52] and importantly, different neuroligin isoforms in combination with the scaffolding protein PSD-95 preferentially affect the formation of either excitatory or inhibitory synapses by coordinating the assembly of the relevant pre- and post-synaptic machinery (Figure 3). Although PSD95 accumulates only at glutamatergic synapses [53,54], it associates with all isoforms of neuroligins and therefore appears well placed to change the strength and spatial distribution of β-neurexin–neuroligin signaling along the dendrites, thereby controlling the relative balance of glutamatergic and GABAergic synapse formation [48–50]. Furthermore, independent work has shown that the levels of PSD-95 protein are controlled by neural activity [55], and perhaps more intriguingly, that physiologically relevant stimuli regulate how PSD-95 is mobilized into the dendrites and synapses of developing neurons [56]. In this regard, it will be interesting to establish how developmental changes in Cl⁻ transporters and GABA receptors mediated transmission impact the activity-dependent regulation of PSD-95 and, in turn, β-neurexin–neuroligin signaling (Figure 3). Pre-synaptic activity might also be relevant, as mutation of two predicted Ca²⁺-binding residues in neurexin-1β has now been shown to disrupt the interaction with post-synaptic neuroligins and the clustering of synaptic receptors [57]. These trans-synaptic signaling systems might therefore be equipped to translate developmental patterns of synaptic activity into circuit formation. Additional molecular players are likely to provide greater complexity to the coordinated development of excitatory and inhibitory inputs [51,58], and the broader effects of these mechanisms on the morphological development of neurons in addition to circuit function will be crucial areas of future investigations.

In conclusion, the neurotransmitter GABA serves multiple roles during nervous system development. Recent work has demonstrated the effects of these diverse roles with respect to cell proliferation, differentiation, synaptogenesis, circuit development and circuit function, such as the processing of sensory information [38,59]. It is interesting to note that the different roles GABA plays seem to depend both on cell intrinsic properties, such as the ‘age’ of the cell, and on extrinsic factors, such as the pattern and type of activity of the neuron. Given the growing interest in the regulatory function of GABAergic transmission during brain development and function, we are likely to see further transformations in our understanding of GABAergic function over the next decade.

Acknowledgements
This work was supported by the NIH (EY011261 and DP OD000458) and Dart Neuroscience Inc. (H.T.C.) and the Wellcome Trust and a Research Councils UK Fellowship (C.J.A.). We thank members of the Cline laboratory for fruitful discussions.

References

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8 Ben-Ari, Y. et al. (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurons. J. Physiol. 416, 303–325
12 Tozuka, Y. et al. (2005) GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. Neuron 47, 803–815
21 Tyzio, R. et al. (1999) The establishment of GABAergic and glutamatergic synapses on CA1 pyramidal neurons is sequential and correlates with the development of the apical dendrite. J. Neurosci. 19, 10372–10382
31 Cummings, J.A. et al. (1996) Ca<sup>2+</sup> signaling requirements for long-term depression in the hippocampus. Neuron 16, 825–833
44 Dahala, V.I. et al. (2005) NKCC1 transporter facilitates seizures in the developing brain. Nat. Med. 11, 1205–1213
47 Kilman, V. et al. (2002) Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA<sub>B</sub> receptors clustered at neocortical synapses. J. Neurosci. 22, 1328–1337
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