

# IS THERE MORE TO GABA THAN SYNAPTIC INHIBITION?

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In the mature brain, GABA ( $\gamma$ -aminobutyric acid) functions primarily as an inhibitory neurotransmitter. But it can also act as a trophic factor during nervous system development to influence events such as proliferation, migration, differentiation, synapse maturation and cell death. GABA mediates these processes by the activation of traditional ionotropic and metabotropic receptors, and probably by both synaptic and non-synaptic mechanisms. However, the functional properties of GABA receptor signalling in the immature brain are significantly different from, and in some ways opposite to, those found in the adult brain. The unique features of the early-appearing GABA signalling systems might help to explain how GABA acts as a developmental signal.

The construction of the central nervous system (CNS) results from a series of events that begins with neural induction and cellular proliferation, and ends with synapse formation and circuit refinement<sup>1</sup>. Each step in the developmental sequence results from an interplay between cell-intrinsic and cell-extrinsic signalling mechanisms<sup>2</sup>. Over the past half-century, many secreted molecules have been identified and shown to significantly influence individual developmental programmes. Neurotransmitters are a class of secreted molecules that might be important signals during nervous system development. Although neurotransmitters are generally associated with neuronal communication in the mature brain, multiple transmitter systems have been shown to influence different aspects of neuronal maturation in various experimental systems<sup>3–6</sup>.

The amino acid GABA has long been considered to be the main inhibitory neurotransmitter in the adult mammalian brain. However, GABA-mediated signalling has also been implicated in the regulation of nearly all the key developmental steps, from cell proliferation to circuit refinement. Considering that nearly all organisms, ranging from bacteria to humans, can synthesize GABA, it would be surprising if multiple functions for GABA had not evolved<sup>7–9</sup>. Here, we address the role of GABA-mediated signalling during brain development. We first provide a brief

historical introduction to the establishment of GABA as a neurotransmitter substance, as well as an overview of the components of the GABA signalling system. Then we discuss ontogenetic changes in GABA signalling and potential developmental functions of GABA, focusing primarily on the mammalian neocortex, although other brain regions are also considered.

## GABA as a neurotransmitter

The discovery of direct synaptic inhibition, with GABA being the first clear example of an inhibitory neurotransmitter substance, markedly altered views of synaptic transmission, which had previously allowed for only excitatory signalling molecules<sup>10</sup>. GABA was first identified in the mammalian brain over half a century ago<sup>11,12</sup>. During the 1950s and 1960s, strong evidence accumulated that GABA was acting as an inhibitory neurotransmitter in both vertebrate and invertebrate nervous systems<sup>13</sup>. For example, extracts from the mammalian brain contained a substance, termed factor I, that blocked impulse generation in crayfish stretch receptor neurons<sup>14</sup>. Factor I was subsequently shown to be GABA<sup>15</sup>. In crustaceans, GABA was found to be approximately 100 times more concentrated in inhibitory axons than in excitatory ones<sup>16,17</sup>, and in response to nerve stimulation, inhibitory nerve terminals were found to secrete GABA<sup>18</sup>. Similar, although

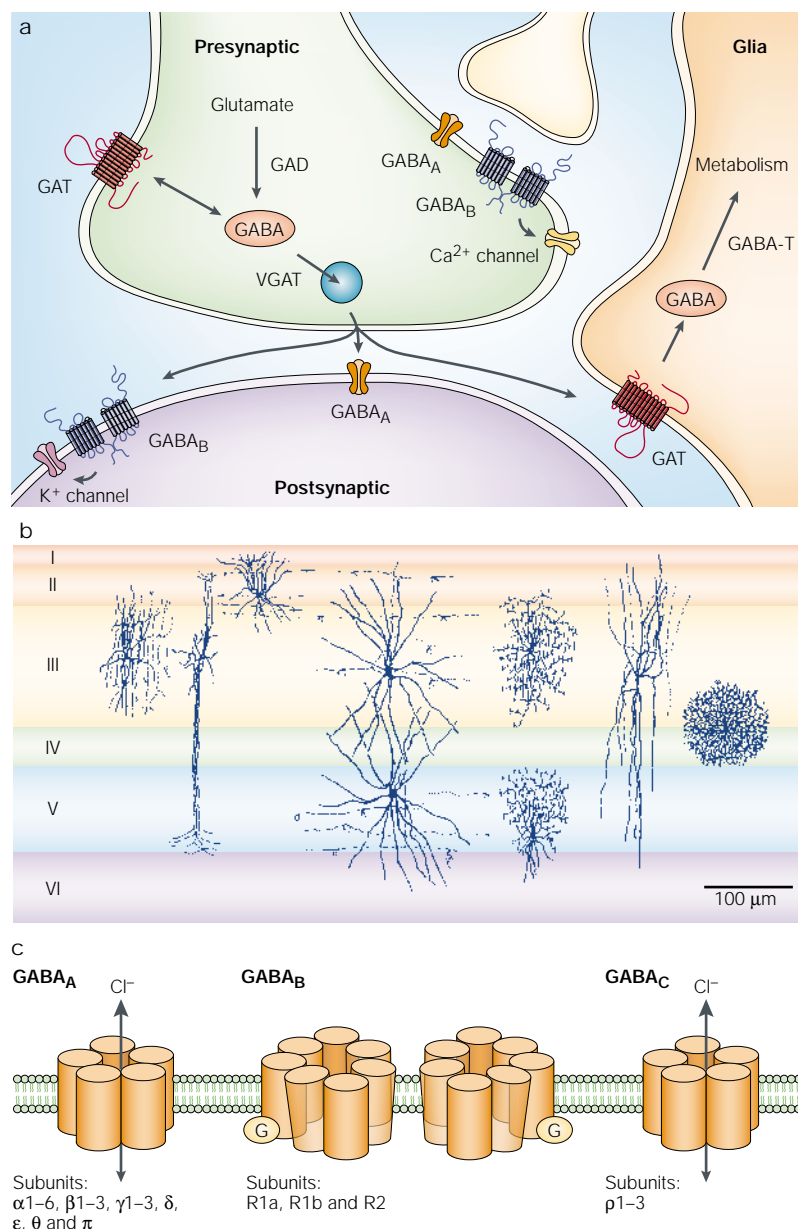
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**Figure 1 | Components of the GABA signalling pathway. a** | Schematic diagram of the synthesis and transport of GABA (γ-aminobutyric acid) at synapses. GABA is synthesized in inhibitory neurons from glutamate by the enzyme glutamic acid decarboxylase (GAD), and is transported into vesicles by a vesicular neurotransmitter transporter (VGAT). GABA can be released either vesicularly or non-vesicularly (by reverse transport). GABA receptors are located at pre- and postsynaptic sites. GABA<sub>B</sub> receptors are metabotropic receptors that cause presynaptic inhibition by suppressing calcium influx and reducing transmitter release, and achieve postsynaptic inhibition by activating potassium currents that hyperpolarize the cell. Reuptake of GABA by surrounding neurons and glia occurs through the activity of GABA transporters (GAT). Subsequently, GABA is metabolized by a transamination reaction that is catalysed by GABA transaminase (GABA-T). The metabolism of GABA occurs in both neurons (not shown) and glia. **b** | Cortical GABA interneurons are phenotypically diverse, as illustrated here by classification on the basis of morphological features. This figure illustrates (left to right) an interneuron with axonal arcades, a double bouquet cell, three types of basket cell, two chandelier cells, a cell spanning all cortical layers and a neurogliaform cell. **c** | GABA receptors differ in subunit composition and assembly. GABA<sub>A</sub> and GABA<sub>C</sub> receptors are closely related pentameric receptors that carry chloride; however, whereas GABA<sub>A</sub> receptors are composed of combinations of several subunit types, GABA<sub>C</sub> receptors are composed of only single or multiple ρ-subunits. GABA<sub>B</sub> receptors are metabotropic receptors that exist as R1a, R1b and R2 isoforms, and are associated with G proteins. Native GABA<sub>B</sub> receptors are dimers composed of one R1 subunit and the R2 subunit. Modified, with permission, from REF. 179 © 1987 The Institute of Mind and Behavior.

less compelling, findings of GABA being concentrated and released from inhibitory neurons were also made in the vertebrate brain<sup>19</sup>. The identification of biosynthetic and metabolic pathways for GABA showed that the production, release, reuptake and metabolism of this substance occurred in the nervous system<sup>13</sup>. When GABA was applied to nerve and muscle cells of both vertebrates and invertebrates, it was generally found to have inhibitory effects<sup>15,20–22</sup> and to produce conductance changes with ion sensitivities similar to those observed after the activation of inhibitory nerves<sup>21,23–27</sup>. Finally, in the 1970s, GABA was localized to mammalian nerve terminals<sup>28</sup>, and antibodies raised against GABA-biosynthetic enzymes were shown to be localized preferentially to known inhibitory neurons<sup>13</sup>.

The GABA neurotransmitter system  
FIGURE 1a provides a summary of the GABA neurotransmitter system. In the mammalian brain, GABA is synthesized primarily from glutamate in a reaction that is catalysed by two glutamic acid decarboxylase (GAD) enzymes, **GAD65** and **GAD67** (REF. 29). GABA is loaded into synaptic vesicles by a vesicular neurotransmitter transporter (**VGAT**)<sup>30</sup> and is liberated from nerve terminals by calcium-dependent exocytosis. However, non-vesicular forms of GABA secretion (for example, by reverse transporter action) have also been described and might be particularly important during development<sup>31,32</sup>. The effects of GABA can be mediated by the activation of either IONOTROPIC OR METABOTROPIC receptors, which can be localized either pre- or postsynaptically. GABA signals are terminated by reuptake of the neurotransmitter into nerve terminals and/or into surrounding glial cells by a class of plasma-membrane GABA transporters (**GATs**)<sup>33</sup>; thereafter, GABA is metabolized by a transamination reaction that is catalysed by GABA transaminase (**GABA-T**)<sup>13</sup>.

**GABA neurons.** There is a wide variety of GABA-producing neurons in the brain. In the neocortex, most GABA-containing neurons are local interneurons with few, if any, dendritic spines, and are therefore classified as sparsely spiny, aspiny or smooth cells<sup>34–36</sup>. These cells are further classified into one of several basic groups, such as basket cells, chandelier cells, double bouquet cells, local plexus neurons or neurogliaform cells (FIG. 1b). These subtypes differ in their morphology, neurochemical composition, somatic location and terminal arborization<sup>35,37</sup>. GABA-containing cells are distributed throughout the cortical lamina<sup>37–39</sup>. In addition, GABA interneurons can be classified by intrinsic membrane properties and synaptic connectivity<sup>40,41</sup>. Interestingly, experimental data have indicated that most, if not all, neocortical GABA neurons are generated and migrate not from cortical, but rather from subcortical locations<sup>42</sup>. However, this property might differ across species, as recent work in humans suggests that approximately 65% of neocortical GABA neurons arise from the neocortical proliferative zone<sup>43</sup>. Finally, in addition to local circuit neurons, direct GABA afferents project to the cortex from the basal forebrain and the ZONA INCERTA<sup>44–46</sup>.

**GABA synapses.** Initial electron-microscopic analyses of neocortical synapses resulted in the classification of two general synaptic types on the basis of differences in ultra-structure; these were termed type 1 and type 2 synapses<sup>47</sup>. Generally, type 1 synapses have an asymmetrical membrane density at the synaptic cleft and are considered to be excitatory, whereas type 2 synapses have a symmetrical appearance and are generally thought to be inhibitory<sup>48</sup>. Symmetrical synapses make up about 15% of adult cortical synapses and contain GABA<sup>38,39</sup>. GABA synapses are present in all neocortical layers, and are observed most frequently on cell somata, proximal dendrites and axon initial segments<sup>36–39</sup>. However, like asymmetrical synapses, GABA synapses can also be found on distal dendrites and dendritic spines<sup>36</sup>. Two general types of GABA-mediated postsynaptic potential have been described on the basis of distinctive pharmacological sensitivity, ionic selectivity and kinetic properties. Fast responses are mediated by ionotropic GABA<sub>A</sub> receptors and slow responses by metabotropic GABA<sub>B</sub> receptors<sup>49–51</sup>.

**GABA receptors.** GABA<sub>A</sub> receptors are members of the ligand-gated ion channel superfamily that includes nicotinic acetylcholine receptors (nAChRs), glycine receptors and the serotonin (5-hydroxytryptamine) 5-HT<sub>3</sub> receptor. For this class of receptors, ligand binding is followed by a conformational change in the channel protein that allows a net inward or outward flow of ions through the membrane-spanning pore of the channel, depending on the electrochemical gradient of the particular permeant ion. GABA<sub>A</sub> receptors carry primarily chloride (Cl<sup>−</sup>) ions; however, other anions, such as bicarbonate (HCO<sub>3</sub><sup>−</sup>), can also permeate the channel pore, although less efficiently<sup>51,52</sup>. Chloride-dependent GABA<sub>A</sub>-receptor-mediated synaptic inhibition can occur either pre- or postsynaptically (FIG. 1a). GABA<sub>A</sub> receptors are believed to be heteropentameric proteins that are constructed from subunits derived from several related genes or gene families<sup>53</sup>. At present, six  $\alpha$ -subunits, three  $\beta$ -subunits, three  $\gamma$ -subunits, one  $\delta$ -subunit, one  $\epsilon$ -subunit, one  $\pi$ -subunit and one  $\theta$ -subunit have been identified<sup>53–55</sup> (FIG. 1c). This multiplicity of subunits provides a daunting number of potential subunit combinations; however, it seems that certain subunit combinations are preferred<sup>56</sup>. Native receptors contain at least one  $\alpha$ -, one  $\beta$ - and one  $\gamma$ -subunit, with the  $\delta$ -,  $\epsilon$ -,  $\pi$ - and  $\theta$ -subunits able to substitute for the  $\gamma$ -subunit<sup>56</sup>. In addition, receptors with different subunit compositions seem to be distributed to different cellular locations, where they are positioned to mediate primarily synaptic or primarily extrasynaptic signalling<sup>57</sup>.

A related ionotropic GABA receptor, sometimes termed the GABA<sub>C</sub> receptor, has also been identified. This receptor is a chloride-selective ion channel, but is insensitive to the GABA<sub>A</sub> receptor antagonist bicuculline<sup>58</sup>. GABA<sub>C</sub> receptors are believed to be homo- or heteropentameric proteins that are composed of a single or multiple  $\rho$ -subunits (FIG. 1c). As the  $\rho$ -subunits share considerable sequence homology with the other identified GABA<sub>A</sub> receptor subunits, GABA<sub>C</sub> receptors can be considered as pharmacological variants of GABA<sub>A</sub> receptors<sup>55,59</sup>.

During the late 1970s and early 1980s, evidence was found for a bicuculline-insensitive, chloride-independent GABA response in the brain, mediated by a metabotropic receptor that was termed the GABA<sub>B</sub> receptor<sup>60–62</sup>. Metabotropic receptor signalling occurs by the activation of heterotrimeric G proteins<sup>63</sup>. G proteins transduce signals through the positive or negative regulation of primary effectors, second messengers and their associated enzymes, which can, in turn, modulate channel and receptor function<sup>62</sup>. GABA<sub>B</sub> receptors are localized both pre- and postsynaptically, and they use different mechanisms at these locations to regulate cell excitability (FIG. 1a). Presynaptic inhibition occurs through a GABA<sub>B</sub>-receptor-mediated reduction in calcium current at the nerve terminal and a subsequent reduction in transmitter release, whereas postsynaptic inhibition occurs by GABA<sub>B</sub>-receptor-mediated activation of potassium currents that hyperpolarize the neuron<sup>49</sup>.

The cloning of the GABA<sub>B</sub> receptor has revealed a putative seven-transmembrane G-protein-coupled receptor that exists as two isoforms, R1a and R1b<sup>64</sup>. This receptor shows sequence homology with the metabotropic glutamate receptors (mGluRs)<sup>64</sup>. Interestingly, functional native receptors seem to be heterodimers that are composed of the R1a or R1b subunit, and the more recently identified R2 subunit<sup>65</sup> (FIG. 1a,c). It has been suggested that the R1a and R1b isoforms localize preferentially to presynaptic and postsynaptic membranes, respectively<sup>66</sup>.

**Ontogeny of cortical GABA-mediated signalling**  
**Receptor expression.** After terminal cell division, newly 'born' neurons migrate away from proliferative regions and are deposited in the developing cortical plate (CP). Components of the GABA signalling system appear very early in corticogenesis and persist for the lifetime of the organism (see FIG. 2). Functional GABA<sub>A</sub> receptors are expressed by mitotically active precursor cells in the neocortical proliferative zone<sup>67–70</sup>. Recent data indicate that the GABA-responsive precursor cells are neurogenic radial glia<sup>71</sup>. **WHOLE-CELL RECORDINGS** made from embryonic and early postnatal cells have shown that the early-expressed GABA<sub>A</sub> receptors change significantly with development (FIG. 2). In the neocortex, GABA<sub>A</sub> receptors expressed by proliferating precursor cells (radial glia) have a higher apparent affinity for GABA and are relatively insensitive to receptor desensitization compared with receptors expressed by postmigratory neurons in the cortical plate<sup>70</sup>. Consistent with these findings are studies showing that dissociated neuroepithelial cells contain a population of GABA<sub>A</sub> receptors that have relatively long channel open times<sup>72</sup>.

The differences in GABA<sub>A</sub> receptor properties in precursor cells and postmitotic neurons might be due to differences in subunit composition. Immunohistochemical and *in situ* hybridization methods have revealed a changing pattern of GABA<sub>A</sub> receptor subunit expression in the developing neocortex<sup>73–78</sup>. In the neocortical proliferative zone,  $\alpha 4$ -,  $\beta 1$ - and  $\gamma 1$ -subunits seem to be expressed most prominently<sup>73,75–78</sup>; however, messenger RNA for other GABA<sub>A</sub> receptor subunits has also been localized to

#### IONOTROPIC

A term that describes a receptor that exerts its effects through the modulation of ion channel activity.

#### METABOTROPIC

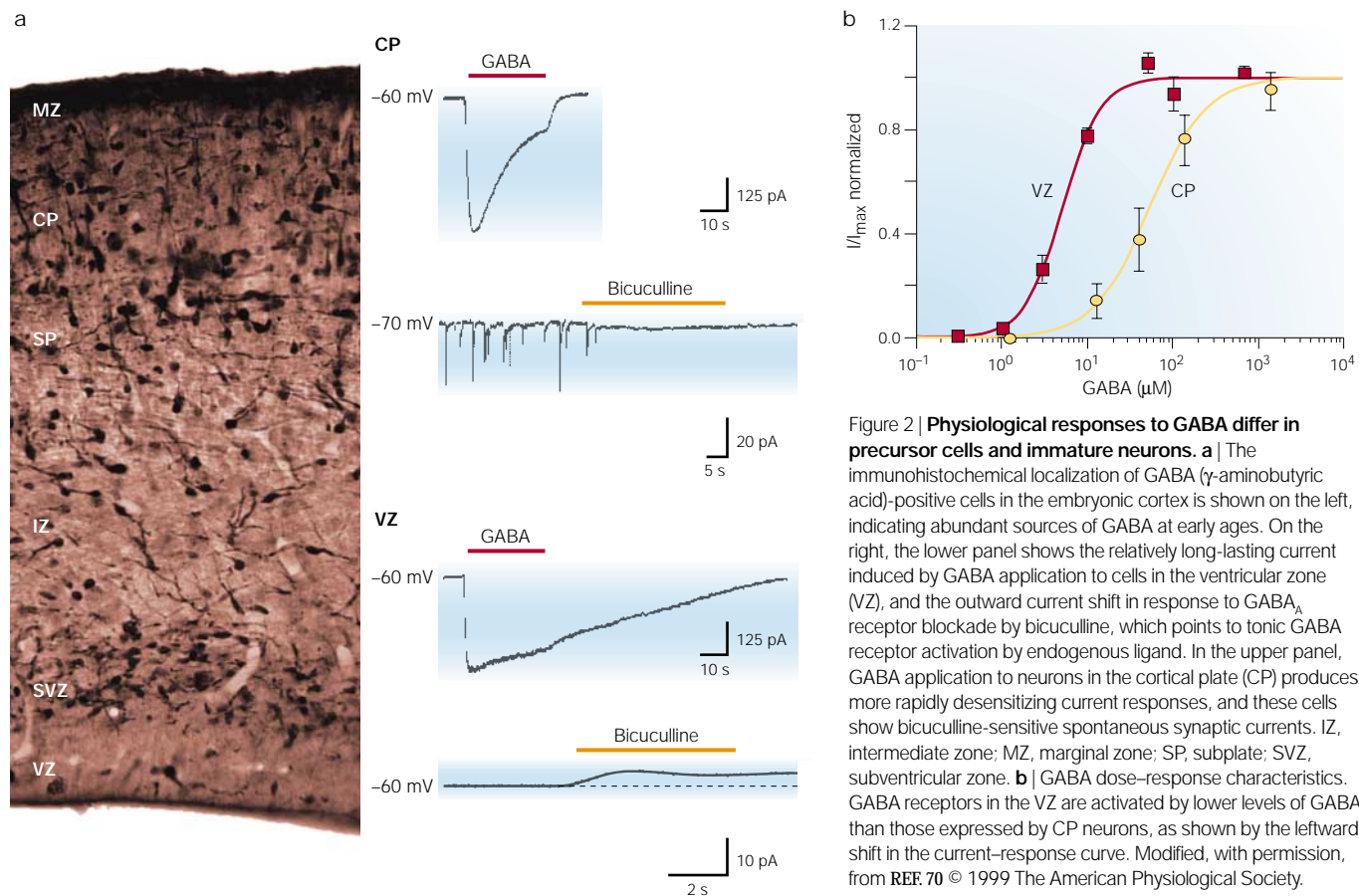
A term that describes a receptor that exerts its effects through enzyme activation.

#### ZONA INCERTA

A thin layer of grey matter that is situated in the dorsal region of the subthalamus.

#### WHOLE-CELL RECORDING

A high-resolution electrophysiological recording technique in which a very small electrode tip is sealed onto a patch of cell membrane and, with suction, the membrane patch is ruptured to allow low-resistance electrical access to the cell interior. Electrical currents flowing across the cell membrane can then be recorded, but the ion composition of the cell interior is altered to that of the electrode-filling solution. By contrast, in gramicidin-perforated-patch recordings, suction is not applied to rupture the patch. Instead, gramicidin in the electrode-filling solution creates tiny pores in the membrane patch. The pores allow low-resistance electrical access for whole-cell recording, but do not allow the passage of anions, and so leave [Cl<sup>−</sup>] unchanged.



**Figure 2 | Physiological responses to GABA differ in precursor cells and immature neurons. a** | The immunohistochemical localization of GABA ( $\gamma$ -aminobutyric acid)-positive cells in the embryonic cortex is shown on the left, indicating abundant sources of GABA at early ages. On the right, the lower panel shows the relatively long-lasting current induced by GABA application to cells in the ventricular zone (VZ), and the outward current shift in response to GABA<sub>A</sub> receptor blockade by bicuculline, which points to tonic GABA receptor activation by endogenous ligand. In the upper panel, GABA application to neurons in the cortical plate (CP) produces more rapidly desensitizing current responses, and these cells show bicuculline-sensitive spontaneous synaptic currents. IZ, intermediate zone; MZ, marginal zone; SP, subplate; SVZ, subventricular zone. **b** | GABA dose-response characteristics. GABA receptors in the VZ are activated by lower levels of GABA than those expressed by CP neurons, as shown by the leftward shift in the current-response curve. Modified, with permission, from REF. 70 © 1999 The American Physiological Society.

the ventricular zone (VZ)<sup>75,78</sup>. At present, it is uncertain which of the detected subunits form native channels, and whether there is a differential expression of specific subunits in proliferative and newly born postmitotic neurons. Nevertheless, there is a clear shift in subunit expression between proliferative and postmitotic zones. In the embryonic CP, which contains almost exclusively postmitotic neurons,  $\alpha 3$ ,  $\beta 2/3$  and  $\gamma 3$  seem to be the predominant subunits<sup>76</sup>. Other  $\alpha$ -subunits ( $\alpha 1$ ,  $\alpha 2$  and  $\alpha 5$ ), the  $\gamma 2$ -subunit<sup>75</sup> and the  $\delta$ -subunit<sup>78</sup> have also been localized to the perinatal CP, but are expressed at lower levels. Interestingly, expression studies have shown that addition of the  $\gamma 2$ -subunit can increase the desensitization rate of recombinant receptors<sup>79</sup>. In addition, the  $\gamma 2$ -subunit is crucial for the postsynaptic clustering of GABA<sub>A</sub> receptors and the formation of functional synapses in cultured cortical neurons<sup>80</sup>. It seems, then, that most GABA<sub>A</sub> receptors in young postnatal neurons are composed of combinations of  $\alpha 2/3/5$ -,  $\beta 2/3$ - and  $\gamma 2/3$ -subunits<sup>75</sup>.

As is the case for cells of the proliferative zone, it is uncertain which subunits form native channels in developing neurons. However, one consistent theme is that receptors expressed by more immature cells tend to have synaptic currents with slower decay kinetics<sup>81,82</sup>, and this property correlates with the presence or absence of certain subunits. For example,  $\alpha 1$ -subunit expression is low in the cortex perinatally, but increases significantly with postnatal development<sup>74</sup>. Experiments with cultured

cortical neurons have shown that the increase in  $\alpha 1$ -subunit expression correlates with an increase in the decay kinetics of GABA<sub>A</sub>-receptor-mediated synaptic currents<sup>82</sup>. Moreover, developmental changes in the channel kinetics seem to be a general phenomenon for ligand-gated ion channels. In addition to GABA<sub>A</sub> receptors, nAChRs<sup>83</sup>, ionotropic glutamate receptors<sup>84</sup> and glycine receptors<sup>85</sup> have all been shown to have slower synaptic decay kinetics and/or longer channel open times in immature cells. The relatively prolonged synaptic actions of transmitters in young neurons probably increase the efficacy of transmission in cells with immature membrane properties.

It should also be noted that differences in the physiological state of the cell, and not simply GABA receptor subunit composition, could also contribute to differences in GABA<sub>A</sub> receptor function in different cell types. For example, intracellular calcium concentration ( $[\text{Ca}^{2+}]_i$ ), degree of receptor phosphorylation and association with cytoskeletal anchoring proteins have all been shown to alter ligand affinity, peak current size and the rate of desensitization of GABA<sub>A</sub> receptors<sup>86–88</sup>. Interestingly, when receptors with the same subunit composition are unclustered, they have higher ligand affinity and slower off kinetics than when they are clustered<sup>86</sup>, and these properties match those of VZ GABA<sub>A</sub> receptors. Possibly, then, VZ GABA<sub>A</sub> receptors might simply be unclustered receptors.

**PARACRINE**

A mechanism of signalling between cells that relies on the diffusion of signalling molecules through the intercellular spaces.

**INTERMEDIATE ZONE**

A transient layer in the developing cortex through which neurons migrate on their way from the proliferative zone to the cortical plate. With maturation, this zone is replaced by the subcortical white matter.

**SUBPLATE**

A transient layer of cells in the fetal brain that lies beneath the cortical plate.

**MARGINAL ZONE**

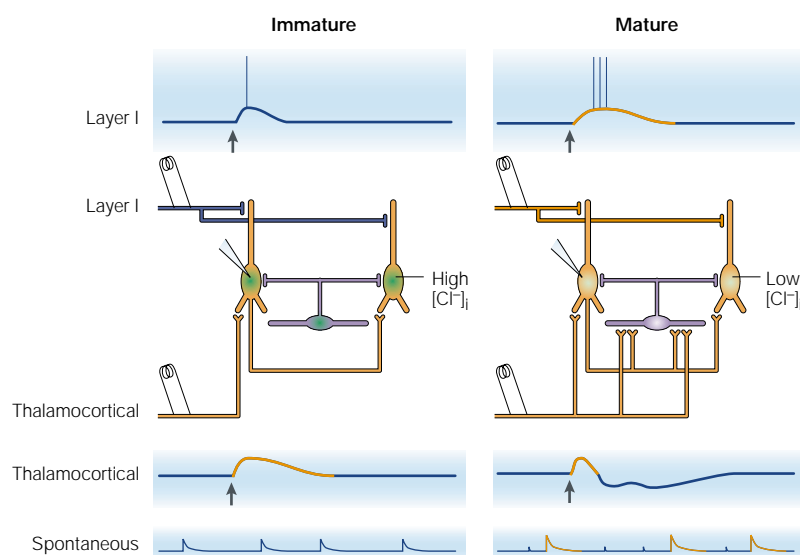
The embryonic equivalent of layer I. This is the most superficial layer of the developing cortex.

**Receptor activation.** In addition to GABA<sub>A</sub> receptors, other components of the GABA signalling system, including GABA-producing neurons (FIG. 2) and GABA transporters, are expressed early in development<sup>89–92</sup>. However, it is unlikely that synaptic activation of GABA<sub>A</sub> receptors occurs in cells of the proliferative zone. Anatomically defined synaptic contacts have not been detected in the cortical proliferative zone, in contrast to the CP, where they have been observed as early as embryonic day (E) 16 (REF. 93). Consistent with anatomical studies, physiological studies have also failed to detect synaptic potentials in VZ cells<sup>70,94</sup>. Nevertheless, evidence has indicated that endogenous GABA<sub>A</sub> receptor activation does occur in the proliferative zone<sup>68,95</sup>. WHOLE-CELL RECORDINGS of VZ cells show outward current shifts in response to the application of a GABA<sub>A</sub> receptor antagonist, indicating the presence of a tonically released ligand<sup>68</sup> (FIG. 2). This finding implies that GABA<sub>A</sub> receptors on VZ cells might be activated by a PARACRINE mechanism<sup>70</sup>. In addition, the physiological properties of GABA<sub>A</sub> receptors in VZ cells — namely, the relatively high apparent affinity for GABA and the relative lack of desensitization — would increase the likelihood of receptor activation by low levels of non-synaptically released ligand. Immunostaining has shown that GABA-positive cells are localized directly above as well as in the VZ<sup>89–91</sup> (FIG. 2). Growth cones that arise from these cells could be the source of endogenous GABA release<sup>32</sup>.

Systematic physiological studies have not been carried out on radially migrating neurons in the INTERMEDIATE ZONE (IZ); however, recordings from tangentially migrating IZ neurons show the expression of functional GABA<sub>A</sub> receptors<sup>96</sup>. Furthermore, calcium-imaging studies indicate that GABA<sub>A</sub> receptors are expressed on both radially and tangentially migrating IZ cells<sup>97</sup>. The synaptic activation of GABA<sub>A</sub> receptors is unlikely to occur during neuronal migration, as no synapses have been detected in the IZ at an ultrastructural level<sup>93</sup>. However, as in the case of VZ cells, non-synaptically released ligand could activate GABA receptors that are expressed by migrating neurons, and several studies have indicated a possible role for GABA in neuronal migration (see below). There are several potential cellular sources of GABA as an IZ signal. Immunohistochemical studies have revealed a differential distribution of GABA-positive cells throughout the embryonic cortical wall. For example, there are many GABA-positive cells at the top of the VZ, and in the SUBPLATE and MARGINAL ZONE, at E17 in the rat<sup>89–91</sup>, as well as a GABA-containing axon plexus in the marginal zone<sup>44</sup> that is contributed, at least in part, by cells of the zona incerta<sup>46</sup>. These cells could secrete GABA and establish GABA gradients in the cortical environment that a cell might sense as it migrates from the VZ to the CP.

Not until cells have completed migration is synaptic transmission likely to have a role in GABA<sub>A</sub> receptor activation. GABA<sub>A</sub>-receptor-mediated spontaneous postsynaptic currents have been observed in embryonic CP neurons<sup>70</sup> (FIG. 2). In fact, the first physiologically detectable cortical synapses are GABA-mediated and arise from spontaneously active local GABA neurons<sup>70,98,99</sup>. Moreover, ultrastructural analyses of the developing somatosensory cortex of mice and rats have identified GABA-containing synaptic contacts at the earliest ages studied (postnatal day 4), confirming that these synapses are anatomically as well as physiologically developed in the neonate<sup>38,39</sup>. The analysis of ultrastructural features, combined with immunohistochemistry, has indicated that GABA-releasing synapses might comprise close to 50% of the synapses in the early postnatal cortex, a number that declines to 15% in the adult<sup>39</sup>.

Although immature cortical GABA neurons are spontaneously active, they are not easily activated by afferent stimulation. Little or no GABA<sub>A</sub>-receptor-dependent synaptic potentials are evoked with stimulation of cortical afferents in the perinatal cortex<sup>100–103</sup> (FIG. 3). During the first few postnatal days, the activation of cortical afferents can produce polysynaptic responses that include GABA<sub>A</sub>-receptor-mediated components, but only after high-intensity stimulation<sup>104</sup>. However, GABA<sub>A</sub>-receptor-mediated polysynaptic responses in the perinatal period fatigue easily with repeated stimulation, indicating that afferent drive of cortical GABA interneurons is weak. So, although GABA-containing interneurons form functional synapses and are spontaneously active in the early neocortex, afferent activation of these cells by excitatory feedback and feedforward pathways is poorly developed (FIG. 3). By contrast, a GABA axon plexus is present in layer I at birth, and stimulation of this plexus can reliably



**Figure 3 | Developmentally regulated changes in GABA actions in the cortical circuitry.** Presumed changes in cortical circuitry, shown diagrammatically in the centre panel, are reflected by developmental changes in the pyramidal cell response to stimulation of cortical afferents in layer I (shown above) and of the thalamocortical pathway (shown below). In the immature brain, stimulation of layer I elicits a pure GABA ( $\gamma$ -aminobutyric acid)-mediated synaptic response that is depolarizing and can activate action potential firing. In the mature cortex, layer I stimulation evokes a largely glutamate-mediated synaptic response (orange), triggering multiple action potentials. Thalamocortical activation in the immature cortex produces a glutamate-mediated synaptic potential (orange), whereas similar stimulation in the mature cortex elicits early glutamate excitation (orange) followed by early GABA<sub>A</sub>-receptor-mediated and later GABA<sub>B</sub>-receptor-mediated inhibitory potentials (blue, shown here for a cell with the membrane potential depolarized above rest). In the immature cortex, spontaneous synaptic activity consists entirely of depolarizing GABA-mediated events (blue); by contrast, in the mature cortex, spontaneous GABA activity is less depolarizing, and frequent excitatory glutamate-mediated synaptic potentials (orange) are present.

activate synaptic GABA<sub>A</sub> receptors on the distal dendrites of pyramidal neurons<sup>44</sup> (FIG. 3). In addition, functional GABA<sub>A</sub>-receptor-mediated synaptic responses have been found in cells of the marginal zone (that is, layer I) and the subplate<sup>105,106</sup>. These cortical regions contain transient cell types that might provide regulatory signals that are used by migrating cells and fibres to navigate the developing cortex<sup>107,108</sup>. Some of these signals are possibly mediated by fleeting synaptic connections with marginal zone and subplate neurons<sup>107</sup>.

**GABA<sub>B</sub> receptors.** Functional GABA<sub>B</sub>-receptor-mediated postsynaptic responses do not occur in the neocortex until after the second postnatal week<sup>101</sup>, although pre-synaptic receptor activation occurs by the first week<sup>109</sup>. A similar situation occurs in the hippocampus, where pre-synaptic GABA<sub>B</sub> receptor function is present perinatally, but postsynaptic receptor function is delayed until about one week later<sup>110,111</sup>. These findings are consistent with GABA<sub>B</sub> receptor expression patterns, which show that the presynaptically localized R1a subunit is expressed earlier than the R1b subunit<sup>112</sup>. However, immunohistochemical studies have shown that both R1 and R2 subunits are present in the embryonic cortex, and GABA<sub>B</sub> receptor activation can influence the movement or migration of immature cortical neurons (see below), indicating that expression and activation of GABA<sub>B</sub> receptors occur during the embryonic period<sup>91,113–115</sup>.

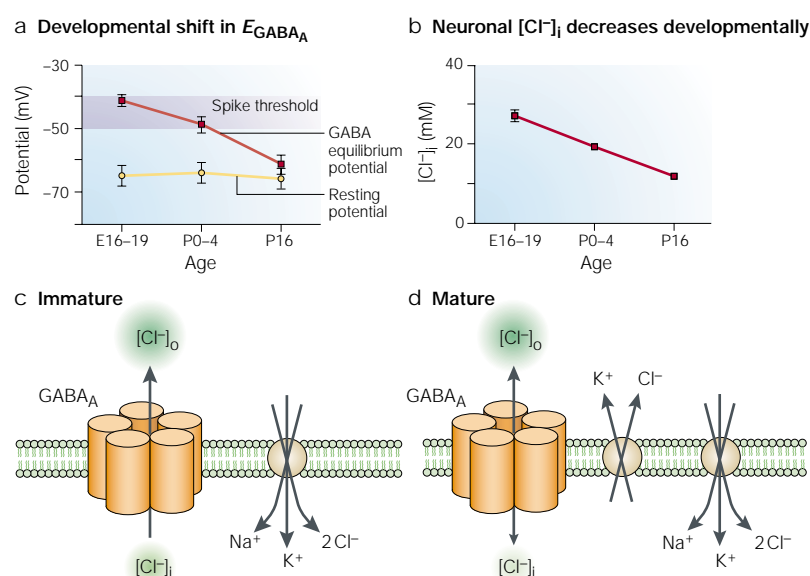
Functional GABA-mediated signalling is thus in place early in neocortical development, but there are maturational changes in the physiological and pharmacological properties of GABA receptors and in the mode of endogenous receptor activation. The effect of GABA<sub>A</sub> receptor activation on membrane potential also changes during development (see below). By around the third postnatal week, thalamocortical afferent stimulation will produce the stereotypical adult postsynaptic response of a fast glutamatergic excitatory postsynaptic potential (EPSP), followed by a fast and a slow inhibitory postsynaptic potential (IPSP), mediated by GABA<sub>A</sub> and GABA<sub>B</sub> receptors, respectively<sup>50,101</sup> (FIG. 3).

**Excitation and inhibition.** In the adult mammalian brain, GABA has been associated primarily with the mediation of synaptic inhibition. However, there are several ways in which synaptic inhibition can be produced, and this has resulted in some confusion in the classification of GABA receptor actions in immature neurons. The first intracellular recordings in the vertebrate CNS showed that activation of inhibitory neurons resulted in membrane hyperpolarization, leading to the suggestion that inhibition resulted by driving the membrane potential further from the action potential threshold<sup>116–118</sup>. On the other hand, experiments in the crustacean nervous system showed that inhibition could be produced by an increase in membrane conductance that was associated with either no change in membrane potential or even depolarization<sup>22,27,119</sup>. Recordings from adult cortical neurons have shown that activation of GABA<sub>A</sub> receptors, either synaptically or by the application of exogenous GABA<sub>A</sub> receptor agonists, can result in hyperpolarization,

depolarization or no change in membrane potential, depending on the experimental conditions<sup>25,26,50,120,121</sup>. However, in nearly all cases, GABA-mediated signalling in adult cortical cells produces inhibition. Collectively, these observations indicate that the direction of membrane potential change might not be the most important factor in the inhibitory process. As Stephen Kuffler stated in 1960, “Since the inhibitory potentials may be repolarizing [that is, hyperpolarizing] or depolarizing or may be absent if the cell is at the inhibitory equilibrium level, it follows that the electrical potential changes themselves are secondary and are not an essential part of inhibition”<sup>22</sup>. The key element in synaptic inhibition is the increase in membrane conductance, as this will act to shunt the ability of excitatory potentials to depolarize the membrane to spike threshold, provided that the inhibitory equilibrium potential is below this value. This formulation generally applies to fast chloride-dependent GABA<sub>A</sub>-mediated synaptic inhibition. In adult cells, the equilibrium potential (or reversal potential) for chloride ions ( $E_{Cl}$ ) is  $-60$  to  $-70$  mV, usually well below the threshold for action potential generation ( $-40$  to  $-50$  mV). By contrast, GABA<sub>B</sub>-receptor-mediated postsynaptic potentials are potassium dependent, and they generally hyperpolarize the membrane towards the equilibrium potential for potassium ions (below  $-70$  mV). These potentials typically produce less change in membrane conductance than GABA<sub>A</sub> potentials, but are strongly inhibitory because they keep the membrane potential further from the spike threshold<sup>121</sup>.

It should therefore be stressed that, in adult cortical neurons, GABA<sub>A</sub>-receptor-mediated synaptic inhibition can be produced effectively by membrane depolarization. However, in immature neurons, the situation can be different. In developing neurons, activation of GABA<sub>A</sub> receptors produces robust membrane depolarization that can, in some cases, directly evoke action potential discharge<sup>44,69,70,122–126</sup> (FIG. 4). The more intense depolarizing actions of early GABA<sub>A</sub> receptor activation are due to the relatively high intracellular chloride concentration ( $[Cl^-]_i$ ) of immature neurons and resting membrane potentials that are significantly more negative than  $E_{Cl}$  (REFS 69,122–124,127) (FIG. 4).

As development proceeds, neuronal  $[Cl^-]_i$  decreases and the GABA<sub>A</sub> reversal potential ( $E_{GABA_A}$ ) becomes more negative<sup>69</sup> (FIG. 4), allowing the effect of GABA to become progressively inhibitory. This is reflected in the ability of the GABA<sub>A</sub> receptor antagonist bicuculline to induce epileptiform activity by blocking inhibition, an effect that develops during the latter part of the first postnatal week<sup>128,129</sup>. In the neocortex, the most depolarized values of  $E_{GABA_A}$  are found in cortical precursor cells in the embryonic VZ<sup>68,69</sup>. Among cortical neurons,  $E_{GABA_A}$  was found to shift negatively by more than 20 mV over a three-week developmental period. This shift corresponds to an approximately 20-mM drop in  $[Cl^-]_i$  (REF. 69) (FIG. 4). These data indicate that, during development, cells might go from a stage of chloride accumulation to one of chloride extrusion. Consistent with this idea, cation-chloride co-transporters are expressed differentially in the cortex at different stages of development<sup>130,131</sup>. The inwardly



**Figure 4 | A developmental shift in GABA actions occurs as a result of changing intracellular chloride concentration.** **a** | The GABA ( $\gamma$ -aminobutyric acid) type A receptor equilibrium potential ( $E_{\text{GABA}_A}$ ) decreases during development. In the perinatal period,  $E_{\text{GABA}_A}$  can be above spike threshold and trigger action potential discharge in some neurons. E, embryonic day; P, postnatal day. **b** | The developmental shift in  $E_{\text{GABA}_A}$  is due to a developmental decrease in the intracellular chloride concentration ( $[\text{Cl}^-]_i$ ). **c** | In immature neurons, an inwardly directed  $\text{Na}^+/\text{K}^+/\text{Cl}^-$  co-transporter (NKCC1) acts to maintain relatively high intracellular chloride concentrations.  $[\text{Cl}^-]_o$ , extracellular chloride concentration (approximately 130 mM). **d** | In mature neurons, intracellular chloride concentration is decreased by the expression of an outwardly directed  $\text{K}^+/\text{Cl}^-$  co-transporter (KCC2), thereby diminishing the driving force for chloride flux in response to GABA $_A$  receptor activation. Part **a** and **b** modified, with permission, from REF. 69 © 1996 Society for Neuroscience.

directed co-transporter **NKCC1** (for  $\text{Na}^+/\text{K}^+/\text{Cl}^-$  co-transporter 1), a chloride accumulator, is first expressed in the embryonic CP. By contrast, the outwardly directed co-transporter **KCC2** (for  $\text{K}^+/\text{Cl}^-$  co-transporter 2), a chloride extruder, begins to be expressed perinatally and shows a marked increase in expression after the first postnatal week (FIG. 4). Antisense knockdown and genetic deletion of the KCC2 protein have confirmed the link between transporter function and the shift in GABA membrane effects, as both manipulations retard or prevent the negative shift in  $E_{\text{GABA}_A}$  (REFS 131, 132). These data support earlier suggestions that poorly developed chloride extrusion could account for higher  $[\text{Cl}^-]_i$  in young neocortical neurons<sup>101</sup>, and provide a mechanistic explanation for the developmental shift in  $[\text{Cl}^-]_i$ .

It is often stated that, during cortical development, GABA-mediated synaptic inhibition lags behind the development of glutamate-mediated excitation<sup>102,103,133</sup>. Although this might be the case, it does not apply to GABA-mediated synaptic transmission *per se*. Local spontaneous GABA $_A$ -receptor-mediated synaptic transmission seems to dominate over glutamate-mediated synaptic activity during the first postnatal week<sup>70,122,134</sup>, and GABA-containing synapses might actually be the first to form<sup>70,98</sup>. So, the lack of functional inhibition during the first few postnatal days is not due to a delay in the formation of GABA synapses (see above), but is probably attributable to a lack of synaptic drive or to

recruitment of GABA-containing interneurons<sup>100,101,104</sup>. In addition, as early GABA $_A$ -receptor-mediated synaptic potentials invariably depolarize postsynaptic cells, it has been suggested that, in the immature brain, fast excitatory synaptic transmission is mediated by GABA $_A$  receptors<sup>135,136</sup>. However, as stated above, depolarization and excitation are not necessarily equivalent. Even when GABA $_A$  receptor activation can depolarize the membrane potential above spike threshold and excite a cell, it is still able to shunt and inhibit other inputs<sup>137,138</sup>. Therefore, in the embryonic and early postnatal brain, when GABA $_A$  receptor activation can, by itself, be excitatory, the resulting change in conductance can modulate other excitatory inputs as well, either inhibiting or facilitating them depending on their timing<sup>137</sup>.

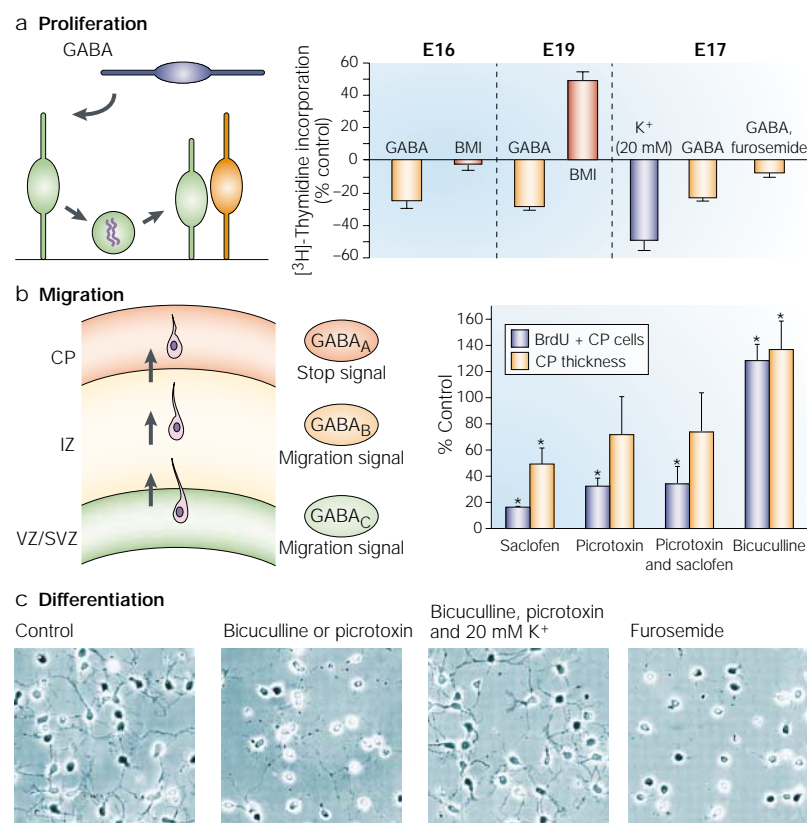
GABA as a developmental signal

**Early studies.** One of the first indications that GABA might act as a trophic substance during nervous system development came from studies by Wolff *et al.*<sup>139</sup> in the rat superior cervical ganglia (SCG). Here, it was shown that the continuous application of GABA could promote dendritic growth *in vivo*, influence ganglion cell sensitivity to acetylcholine and alter the development of pre-synaptic specializations<sup>139,140</sup>. Furthermore, only in the presence of GABA could an ectopic nerve innervate the SCG. From these studies, it was concluded that GABA acts to promote synaptogenesis or the synaptogenic capacity of the SCG<sup>140</sup>. During the 1980s, several studies showed that the application of GABA could influence aspects of neuronal differentiation *in vitro*<sup>5,141</sup>. For example, in cultured cerebellar granule cells, GABA treatment increased the number of neurite-extending cells and the density of cytoplasmic organelles<sup>142</sup>. In addition, GABA application was found to result in a change in expression of the GABA receptor itself<sup>143</sup>. Similar results were found in chick cortical and retinal neurons<sup>144</sup> and in NEUROBLASTOMA CELLS<sup>145</sup>, leading to the conclusion that GABA could act as a general neurodevelopmental factor<sup>5,141</sup>. Although the mechanisms of GABA's trophic actions were not elucidated, they seemed to be mediated by the activation of GABA receptors, as GABA $_A$  receptor antagonists could block the effects. It was further suggested that GABA-mediated membrane hyperpolarization was an important step<sup>5</sup>. This contrasts with more recent studies, which indicate that the trophic actions of GABA probably result from the depolarizing effects of GABA $_A$  receptor activation in immature cells.

**Functional consequences of early depolarizing actions.**

GABA $_A$ -receptor-mediated membrane depolarization has been observed in developing cells from many brain regions, indicating a general role for GABA-mediated depolarization during development<sup>146</sup>. This depolarization is sufficient to increase  $[\text{Ca}^{2+}]_i$  by the activation of voltage-gated calcium channels (VGCCs)<sup>68,69,147–149</sup>. These results indicate that one potential downstream consequence of early GABA $_A$  receptor activation is the activation of calcium-dependent second-messenger pathways<sup>135</sup>. These findings, coupled with evidence that endogenous GABA receptor activation occurs early in

**NEUROBLASTOMA CELLS**  
An immortalized cell line derived from tumours that arise from the neural crest.



**Figure 5 | Non-synaptic actions of GABA might include modulation of proliferation, migration and differentiation.** **a** | Proliferation. DNA synthesis can be modulated by activating GABA ( $\gamma$ -aminobutyric acid) type A receptors. GABA reduces [ $^3$ H]-thymidine incorporation at both embryonic day (E) 16 and E19. Blocking GABA<sub>A</sub> receptors with bicuculline (BMI) has little effect at E16, but leads to an increase in [ $^3$ H]-thymidine incorporation at E19. This indicates that an endogenous agonist is acting to inhibit DNA synthesis in the developing cortex, and that this process develops at some point after E16. The GABA effect on DNA synthesis seems to be mediated by membrane depolarization, as it can be mimicked by depolarization with potassium and blocked by altering the chloride ion gradient with furosemide. Modified, with permission, from REF. 68 © 1995 Elsevier Science. **b** | Migration. Specifically blocking GABA<sub>A</sub>, GABA<sub>B</sub> or GABA<sub>C</sub> receptors can inhibit specific stages of neuronal migration in an *in vitro* neuronal migration assay. The bar graph indicates the thickness of the cortical plate (CP) and the number of 5-bromodeoxyuridine (BrdU)-labelled cells that migrated to the CP in an *in vitro* migration assay. Blocking GABA<sub>A</sub> receptors (bicuculline) led to more neurons in the CP, whereas blocking GABA<sub>B</sub> or GABA<sub>C</sub> receptors (with saclofen or picrotoxin, respectively) decreased the number of neurons reaching the CP. IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. Modified, with permission, from REF. 113 © 2000 Oxford University Press. **c** | Differentiation. The growth of neurites in cultured cortical neurons can be inhibited by GABA<sub>A</sub> receptor blockade with bicuculline or picrotoxin, an effect that might be mediated by membrane depolarization, as it can be overridden by potassium depolarization and mimicked when the chloride gradient is attenuated with furosemide. Modified, with permission, from REF. 115 © 2001 Society for Neuroscience.

**TRITIATED-THYMIDINE INCORPORATION**  
An assay in which a radiolabelled form of thymidine is incorporated into the DNA of dividing cells. These cells can then be detected by autoradiography.

**5-BROMODEOXYURIDINE**  
An analogue of thymidine that can be incorporated into replicating DNA. It is used to label dividing cells, which can then be detected with an antibody.

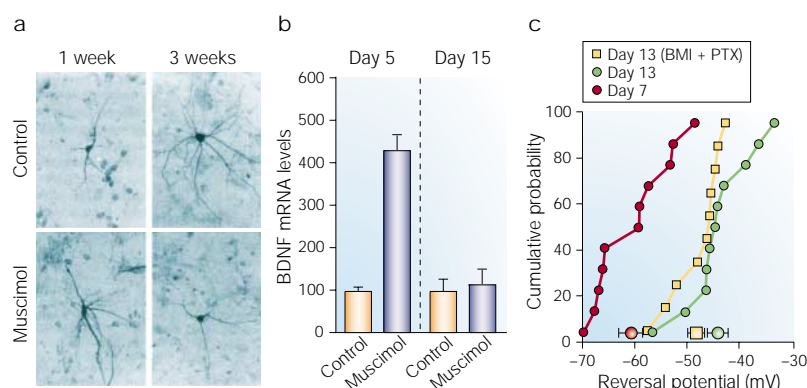
development (in some cases before synapse formation) have provided a signalling framework in which GABA-mediated cell communication can influence many processes in brain development, from cell proliferation to synaptogenesis and circuit formation (FIG. 4).

In precursor cells in the neocortical proliferative zone, activation of GABA<sub>A</sub> receptors has been shown to influence DNA synthesis<sup>68,95</sup> (FIG. 5). Activating GABA<sub>A</sub> receptors in intact rat neocortical explants led to a significant decrease in DNA synthesis, as assessed by TRITIATED-THYMIDINE INCORPORATION, and a reduction in the number of 5-BROMODEOXYURIDINE (BrdU)-labelled cells<sup>68</sup>. Depolarizing cells by exposing cortical explants to elevated potassium

was sufficient to inhibit DNA synthesis, and the effect of GABA was abolished when the chloride gradient was altered so that GABA was no longer depolarizing.

More compelling evidence that GABA<sub>A</sub> receptor activation regulates DNA synthesis during cortical development came from experiments in which explants were exposed to only the GABA<sub>A</sub> receptor antagonist bicuculline. In the presence of bicuculline, there was a significant increase in DNA synthesis in cortical precursor cells, indicating that GABA is released endogenously and influences the rate of DNA synthesis<sup>68</sup>. The bicuculline-induced increase in DNA synthesis was seen readily at E19, but was absent in younger (E14–E16) cortical explants<sup>68</sup>. This implies that endogenous GABA<sub>A</sub> receptor activation affects only late-stage neurogenesis and/or gliogenesis. However, GABA<sub>A</sub>-receptor-mediated reduction of cell proliferation might occur indirectly by interactions with other factors. Experiments in dissociated cell culture have shown that activation of GABA<sub>A</sub> receptors can inhibit the proliferative effect that basic fibroblast growth factor (bFGF) exerts on neo-cortical precursor cells, but has no effect when applied alone<sup>150</sup>. So, it is possible that the observed effects in intact tissue represent an indirect action of GABA<sub>A</sub> receptor activation — namely, the regulation of bFGF responsiveness. More recent studies point to a more complicated situation. In experiments with embryonic mouse cortical slices maintained under tissue-culture conditions, GABA<sub>A</sub> receptor activation led to an increase in the number of BrdU-labelled cells in the VZ, but to a decrease in the number of BrdU-labelled cells in the subventricular zone<sup>95</sup>. So, depending on the experimental conditions, GABA might have differential effects on cortical cell proliferation. A somewhat analogous situation has been observed in cerebellar granule cells, in which potassium-induced depolarization could either increase or decrease cell proliferation depending on the experimental conditions<sup>151,152</sup>. Although these experiments all support a role for GABA signalling during cell proliferation, ultimately, it will be important to determine whether GABA does indeed influence the cellular composition of the cortex (see below).

GABA receptor activation has also been proposed to regulate neuronal migration. In a series of studies carried out in both dissociated-cell and slice cultures, GABA receptor activation was shown to influence the movement and migration of immature cortical neurons<sup>91,113,114</sup>. In cultured slices of rat brain, it was determined that GABA<sub>C</sub> and GABA<sub>B</sub> receptor activation promoted migration out of the VZ and the IZ, respectively, whereas GABA<sub>A</sub> receptor activation provided a stop signal once cells reached the CP<sup>113</sup> (FIG. 5). Selectively blocking GABA<sub>C</sub> and GABA<sub>B</sub> receptors for two days could retard migration, but this effect could be overcome with longer culture periods. These results indicate that, although GABA-mediated signalling might influence neuronal migration, it is not essential for this process, or can be compensated for if absent. Interestingly, in similar studies with cultured slices of mouse brain, GABA seemed to have little, if any, role in neuronal migration<sup>153</sup>. Rather, NMDA (*N*-methyl-D-aspartate)-type glutamate



**Figure 6 | Effects of GABA on neuronal differentiation depend on GABA-induced membrane depolarization.** **a** | GABA ( $\gamma$ -aminobutyric acid) type A receptor activation promotes interneuron development in a time-dependent fashion. Facilitation of neuron growth after the application of the GABA<sub>A</sub> receptor agonist muscimol is evident in cells treated during the first week of culture, but is absent, and possibly reversed, for cells treated during the third week. Modified, with permission, from REF. 155 © 1996 Elsevier Science. **b** | The neurite-promoting effect is thought to result from the ability of GABA to stimulate brain-derived neurotrophic factor (BDNF) expression, an ability that depends on membrane depolarization and increases in intracellular calcium (not shown), and declines with time in culture. Modified, with permission, from REF. 156 © 1995 The Company of Biologists Ltd. **c** | The shift in the chloride gradient might itself be under GABA regulation, as the developmental shift can be blocked through chronic GABA<sub>A</sub> receptor blockade by bicuculline (BMI) or picrotoxin (PTX). Modified, with permission, from REF. 161 © 2001 Elsevier Science.

receptors appeared to promote migration in this system. The reason for the differences between these two rodent species is unclear, but these studies indicate, once again, that the results and conclusions of a study might depend on the experimental conditions.

In addition to proliferation and migration, aspects of neuronal differentiation might be regulated by early GABA-mediated signalling. In cultured embryonic hippocampal and neocortical neurons, GABA<sub>A</sub> receptor activation has been shown to promote neurite outgrowth and maturation of GABA interneurons<sup>115,154,155</sup> (FIGS 5,6). These effects depend on GABA<sub>A</sub>-receptor-mediated membrane depolarization and increases in  $[Ca^{2+}]_i$  (REFS 115,156), as do GABA's effects on the survival of rat embryonic striatal neurons *in vitro*<sup>157</sup>. In the case of interneuron development, GABA might exert its trophic effects by stimulating an increase in brain-derived neurotrophic factor (BDNF) expression and release from target neurons, an effect that diminishes with development<sup>155,158</sup> (FIG. 6). The observation that the enhancement by GABA of interneuron growth is diminished when using cells derived from BDNF-knockout mice supports the link between GABA growth effects and BDNF<sup>155,156</sup>.

Early GABA signalling might also interact with NMDA receptor activation to regulate synapse maturation<sup>136</sup>. NMDA receptors are believed to be involved in synaptic plasticity and development; however, in developing neurons, NMDA receptors are often functionally silent at negative membrane potentials due to blockage of the channel by magnesium<sup>159</sup>. So, an endogenous depolarizing influence must be present at immature synapses to relieve the magnesium block of the NMDA receptor, a role that has been attributed to non-NMDA receptor activation in the more mature brain. In the developing hippocampus, GABA<sub>A</sub>-receptor-mediated synaptic

activity has been shown to depolarize cells and relieve the magnesium block of NMDA channels, allowing calcium to flow into the cell<sup>160</sup>. The resulting increase in  $[Ca^{2+}]_i$  might activate downstream signalling pathways that are crucial for neuronal maturation and synaptogenesis<sup>136</sup>.

Finally, a recent study in cultured hippocampal neurons provides intriguing evidence that GABA<sub>A</sub>-receptor-mediated signalling itself seems to produce the developmental  $[Cl^-]_i$  switch<sup>161</sup>. In this study, the authors found that application of GABA produced increases in  $[Ca^{2+}]_i$  through GABA<sub>A</sub>-receptor-mediated membrane depolarization and activation of VGCCs in young neurons. When GABA<sub>A</sub> receptors were chronically blocked with specific receptor antagonists, the authors found that the GABA-induced  $[Ca^{2+}]_i$  increases persisted and that  $E_{GABA_A}$  remained at a relatively depolarized value in older neurons (FIG. 6). Evidence was provided that GABA<sub>A</sub>-receptor-mediated miniature synaptic potentials were the endogenous source of GABA<sub>A</sub> receptor activation. KCC2 levels were reduced in cells that had been chronically treated with GABA<sub>A</sub> receptor antagonists, indicating that the activation of GABA<sub>A</sub> receptors is required to upregulate the expression of the KCC2 co-transporter and decrease  $[Cl^-]_i$ . Moreover, the GABA-mediated upregulation of KCC2 expression was dependent on the activation of VGCCs and calcium influx.

**Genetic studies.** It seems that early GABA-mediated signalling is involved in many aspects of brain development. But surprisingly, examination of mice with null mutations in key genes of the GABA pathway has revealed relatively few developmental abnormalities in the CNS. For example, the fetal brains of mice lacking both GABA-synthesizing enzymes, GAD65 and GAD67, have 0.02% of the normal content of GABA, and these mice die at birth, yet they have no obvious structural brain abnormalities<sup>162</sup>. However, no quantitative measurement of cell density or detailed analysis of cellular ultrastructure was carried out in this study. Considering that the endogenous influence of GABA<sub>A</sub> receptor activation on DNA synthesis or cell-cycle progression occurs relatively late in cortical cell proliferation, the effects of GAD65/67 deletion might be subtle, and a more rigorous analysis of these mice might uncover defects. It is noteworthy that a study examining the effect of GABA<sub>A</sub> receptor  $\beta 3$ -subunit deletion found no gross morphological deformities, although several more subtle development differences were found on more detailed inspection<sup>163</sup>.

Many GABA-deficient mutant mice have a lower seizure threshold<sup>164</sup> or spontaneous seizures<sup>165</sup>, as do mice with targeted deletion of the GABA<sub>A</sub> receptor  $\beta 3$ -subunit<sup>166</sup>, reflecting the importance of GABA-mediated inhibition in regulating excitability in mature cortical circuits. In addition, although there are no major structural abnormalities involving the CNS, these mice do have significant craniofacial deformities. GABA seems to be important for normal palate development, as most mutant animals with impaired GABA function develop CLEFT PALATE<sup>162,164,167,168</sup>. Most of these animals die at birth, and although the cleft palate is often blamed, this might not be the cause of death, as 30% of mice with severely

#### MINIATURE SYNAPTIC POTENTIALS

Synaptic potentials observed in the absence of presynaptic action potentials; they are thought to correspond to the response elicited by a single vesicle of transmitter.

#### CLEFT PALATE

A congenital craniofacial defect in which the palatal shelves fail to fuse, leaving an opening in the roof of the mouth.

impaired GABA<sub>A</sub> receptor function that die at birth do not have cleft palate<sup>166</sup>.

One caveat of all the knockout experiments is that they involve germ-line mutations and are subject to developmental compensation that could reduce the severity of the brain phenotype. For example, glutamate, glycine and taurine can depolarize embryonic cortical neurons in a manner similar to that of GABA at early ages<sup>68,169</sup>, and pathways involving one or more of these transmitters could potentially reduce the severity of the GABA loss-of-function mutations. However, the brains of mice that are null for two isoforms of the synaptic vesicle protein Munc13 are devoid of both GABA- and glutamate-mediated vesicular release, but brain histogenesis and synapse development also appear to be normal<sup>170</sup>. Similar results have been reported for Munc18-knockout animals<sup>171</sup>. So, if transmitters have developmental effects, they are probably mediated by non-vesicular release mechanisms<sup>32,169</sup>.

As described above, expression of the potassium-chloride co-transporter KCC2 normally lowers [Cl<sup>-</sup>]<sub>i</sub> and underlies the developmental shift towards less depolarizing effects of GABA<sup>131</sup>. Mice with targeted disruption of the KCC2 gene have no obvious structural CNS abnormalities, but they have increased muscle tone and die perinatally of respiratory failure due to hyperactive brainstem motor neurons<sup>132</sup>. These animals also show frequent spontaneous seizures, presumably due to a relative lack of functional GABA inhibition in the cortex and hippocampus<sup>172</sup>.

Recently, mice with non-functional GABA<sub>B</sub> receptors have been generated<sup>173,174</sup>. They develop spontaneous epilepsy and behavioural abnormalities, including hyperalgesia and impaired performance on memory tasks, but their brain morphology appears to be grossly normal<sup>173,174</sup>.

Nearly all the genetic experiments completed so far have shown few, if any, developmental defects when perturbing the GABA system. The most common CNS problem might be diminished inhibition and seizure generation, which can be observed after birth. Therefore, GABA signalling specifically, and vesicular neurotransmitter release in general, seem to have little effect on early structural brain development. A more rigorous analysis involving stereological approaches, as well as an evaluation of cellular morphology and circuit formation, might uncover developmental defects in some of these knockout mice. In addition, to demonstrate a role for early GABA signalling, it might be necessary to generate CONDITIONAL MUTANT LINES that allow disruption of the temporal pattern of GABA signalling to circumvent compensatory mechanisms that might arise in non-conditional mutants.

Developmental neurotransmitters might provide tonic, non-vesicular depolarization that keeps cells healthy or responsive to other environmental signals. It might be that developing cells are promiscuous in their use of transmitter signals, and that any signalling system that induces membrane depolarization can be used to influence developmental programmes. Moreover, modulation of proliferation and migration

by transmitter signalling might be exclusively mediated by non-vesicular release. It would therefore be interesting in future work to examine the effect of membrane depolarization and/or downstream signal transduction on early developmental events, as this approach might reveal more pronounced effects than those produced by manipulations of an individual transmitter system.

#### Conclusions

Early GABA-mediated signalling might be a general feature of brain development that has been evolutionarily conserved. Components of the GABA signalling system, both receptor and ligand, have been observed in brain development in many vertebrate species, and GABA-containing neurons are present in or near the cortical proliferative, migratory and differentiation zones during embryonic development in species ranging from reptiles to primates<sup>90,175–178</sup>. These observations are complemented by evidence from various *in vitro* model systems indicating that GABA can influence proliferation, migration and neuronal differentiation.

The early developmental roles of GABA are unrelated to its importance as a mediator of adult fast synaptic inhibition; instead, they probably reflect GABA-mediated membrane depolarization that results from a relatively high [Cl<sup>-</sup>]<sub>i</sub> in immature cells. Interestingly, in invertebrates, GABA exerts a growth-promoting effect through membrane depolarization<sup>8</sup>. It is therefore possible that a GABA signalling pathway arose in ancient organisms to serve a trophic role, the effects of which on growth or gene expression were dependent on depolarizing membrane actions. Many of these growth-related effects seem to rely on non-synaptic or paracrine receptor activation. In addition, early-appearing GABA receptors favour tonic activation by low levels of agonist because of higher agonist affinity and longer-lasting activation kinetics. After GABA synapses form, receptor activation produces faster, shorter-acting currents, and results in little or no membrane depolarization owing to a relatively low [Cl<sup>-</sup>]<sub>i</sub>. As neural assemblies evolved, GABA might have acquired a new role as a synaptic transmitter with inhibitory circuit effects as a consequence of a change in neuronal chloride homeostasis.

A challenge for future study will be to reconcile the proposed roles of GABA signalling in brain development with the relatively normal outcomes that have been observed in GABA-deficient mutant mice. One approach might be to investigate the mechanism by which membrane depolarization by various transmitters can influence developmental events. Such studies could reveal redundant roles for early-appearing transmitter systems and help to define the true contribution of GABA to brain development. Advances in our understanding of how GABA signalling exerts its effects on neuronal precursor cells and immature neurons have shifted our attention to questions of why GABA pathways are established early and why they undergo such marked developmental changes. These questions will probably drive the next wave of discovery in exploring the role of GABA in brain development.

CONDITIONAL MUTANT LINES  
Mutant mouse lines in which a gene is inactivated in a temporally and/or spatially restricted fashion.

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#### Online links

##### DATABASES

The following terms in this article are linked online to: **LocusLink**: <http://www.ncbi.nlm.nih.gov/LocusLink/> BDNF | GABA<sub>A</sub> receptors | GABA<sub>B</sub> receptors | GABA<sub>C</sub> receptor | GABA-T | GAD65 | GAD67 | GATs | KCC2 | NKCC1 | VGAT

##### FURTHER INFORMATION

**Encyclopedia of Life Sciences**: <http://www.els.net/> amino acid neurotransmitters | amino acid transporters | chloride channels | GABA<sub>A</sub> receptors | GABA<sub>B</sub> receptors  
Access to this interactive links box is free online.