Changing Properties of GABA\textsubscript{A} Receptor–Mediated Signaling During Early Neocortical Development

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Owens, David, F., Xiaolin Liu, and Arnold R. Kriegstein. Changing properties of GABA\textsubscript{A} receptor–mediated signaling during early neocortical development. \textit{J. Neurophysiol.} 82: 570–583, 1999. Evidence from several brain regions suggests \(\gamma\)-aminobutyric acid (GABA) can exert a trophic influence during development, expanding the role of this amino acid beyond its function as an inhibitory neurotransmitter. Proliferating precursor cells in the neocortical ventricular zone (VZ) express functional GABA\textsubscript{A} receptors as do immature postmigratory neurons in the developing cortical plate (CP); however, GABA\textsubscript{A} receptor properties in these distinct cell populations have not been compared. Using electrophysiological techniques in embryonic and early postnatal neocortex, we find that GABA\textsubscript{A} receptors expressed by VZ cells have a higher apparent affinity for GABA and are relatively insensitive to receptor desensitization compared with neurons in the CP. GABA-induced current magnitude increases with maturation with the smallest responses found in recordings from precursor cells in the VZ. No evidence was found that GABA\textsubscript{A} receptors on VZ cells are activated synaptically, consistent with previous data suggesting that these receptors are activated in a paracrine fashion by nonsynaptically released ligand. After neurons are born and migrate to the CP, they begin to demonstrate spontaneous synaptic activity, the majority of which is GABA\textsubscript{A} mediated. These spontaneous GABA\textsubscript{A} postsynaptic currents (sPSCs) first were detected at embryonic day 18 (E18). At birth, \(\sim 50\%\) of recordings from cortical neurons demonstrated GABA\textsubscript{A}-mediated sPSCs, and this value increased with age. GABA\textsubscript{A}-mediated sPSCs were action potential dependent and arose from local GABAergic interneurons. GABA application could evoke action potential–dependent sPSCs in neonatal cortical neurons, suggesting that during the first few postnatal days, GABA can act as an excitatory neurotransmitter. Finally, N-methyl-D-aspartate (NMDA)- but not non-NMDA-mediated sPSCs were also present in early postnatal neurons. These events were not observed in cells voltage clamped at negative holding potentials (\(-60\) to \(-70\) mV) but were evident when the holding potential was set at positive values (+30 to +60 mV). Together these results provide evidence for the early maturation of GABAergic communication in the neocortex and a functional change in GABA\textsubscript{A}–receptor properties between precursor cells and early postmitotic neurons. The change in GABA\textsubscript{A}–receptor properties may reflect the shift from paracrine to synaptic receptor activation.

\textbf{INTRODUCTION}

The amino acid GABA is considered the major inhibitory neurotransmitter in the adult cortex (Connors et al. 1988; Krnbjevic and Schwartz 1967). GABA exerts its influence in cortex by activation of GABA\textsubscript{A} and GABA\textsubscript{B} receptors, which primarily activate chloride (Cl\textsuperscript{-})- and potassium-dependent conductances, respectively (Bormann 1988; Connors et al. 1988; Kaila 1994). At early stages of cortical development, glutamatergic-mediated excitation is present, but there appears to be little functional inhibition (Agmon and O’Dowd 1992; Agmon et al. 1996; Burgard and Hablitz 1993b; Kim et al. 1995; Luhmann and Prince 1991). The paucity of synaptic inhibition in the immature neocortex has been interpreted as a developmental delay in the maturation of the GABAergic signaling system. However, evidence has accumulated that suggests that GABA-mediated signaling may develop quite early in the cortex. Messenger RNA (mRNA) and protein for GABA, the GABA biosynthetic enzyme glutamate decarboxylase, and various GABA\textsubscript{A}–receptor subunits have been localized in embryonic cortical tissue (Cobas et al. 1991; Lauder et al. 1986; Laurie et al. 1992; Ma and Barker 1995; Van Eden et al. 1989), and functional GABA\textsubscript{A} receptors have been shown to be present on both proliferative and early postmitotic cells (LoTurco et al. 1995; Owens et al. 1996). In developing neocortex, activation of GABA\textsubscript{A} receptors produces robust membrane depolarization (Agmon et al. 1996; LoTurco et al. 1995; Owens et al. 1996; Yuste and Katz 1991) due to a relatively high concentration of intracellular Cl\textsuperscript{-} (Cl\textsuperscript{-}) maintained in immature cells (Clayton et al. 1998; Owens et al. 1996; Rivera et al. 1999). Thus functional inhibition may be less effective in neonatal cortex in part due to the depolarizing actions of the principle inhibitory neurotransmitter GABA.

GABA can exert trophic influences during early CNS development, and many of the developmental actions of GABA are mediated through activation of GABA\textsubscript{A} receptors (Meier et al. 1991). For example, GABA\textsubscript{A}–receptor activation can influence DNA synthesis in proliferative cells (LoTurco et al. 1995) and cell motility and morphological development in early postmitotic neurons (Barbin et al. 1993; Behar et al. 1996, 1998; Marty et al. 1996). Whether the developmental effects of GABA are mediated exclusively by synaptic or nonsynaptic (e.g., paracrine) receptor activation is not completely understood. However, recent electrophysiological experiments and ultrastructural analysis have demonstrated that GABAergic synapses are present perinatally in the cortical plate (Agmon et al. 1996; De Felipe et al. 1997; Mischeva and Beaulieu 1996; Owens et al. 1996), suggesting that synaptic GABA–receptor activation, before the onset of mature inhibition, may play a role in cortical development. To further explore the maturation of the GABAergic system in neocortex, we have compared the functional properties of GABA\textsubscript{A} receptors in both proliferative and postmitotic neocortical cells in situ during the embryonic
and early postnatal period. In addition, we have investigated the development of spontaneous GABA_\text{A}-mediated synaptic transmission.

**METHODS**

**Tissue preparation**

Results were obtained using brain slices and slabs from embryonic (E15–20) and early postnatal (P0–5) Sprague-Dawley rats (Taconic, Germantown, NY). The day of birth was considered P0. For embryonic tissue, gravid rats were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg), and embryos were exposed by cesarean section. Embryos were decapitated, and heads were immediately placed in ice-cold artificial cerebrospinal fluid [ACSF; it contained (in mM) 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 1 MgSO_4, 2 CaCl_2, 26 NaHCO_3, and 10 glucose] oxygenated with 95% O_2–5% CO_2 (pH 7.4). Cortical slabs were removed and placed in ice-cold ACSF; however, conventional coronal slices were used in some experiments. For brain slices, whole embryonic brains were removed and embedded in warm (28–30°C) 3–4% low-melting point agarose (Fisher Scientific, Fair Lawn, NJ) in ACSF, hardened on ice, and cut into coronal sections (300–400 μm) with a vibratome. Neonatal rat pups were decapitated rapidly, and brains were removed and placed in ice-cold ACSF oxygenated with 95% O_2–5% CO_2. Coronal slices (300–400 μm), confined to the sensorimotor regions of the cortex, were made with a vibratome. Tissue was kept in oxygenated ACSF at room temperature (RT; 21–25°C) for ≥1 h before recording.

**Electrophysiological recordings and data analysis**

Patch-clamp recordings were obtained at RT from cells in both slices and slabs of neocortex continuously superfused with 95% O_2–5% CO_2 oxygenated ACSF at a rate 1–2 ml/min (Blanton et al. 1989; Owens et al. 1996). For the majority of recordings, patch electrodes were filled with (in mM) 100 CsCl, 30 Cs gluconate, 10 HEPES (pH 7.3), 2 CaCl_2, and 11 EGTA. In some whole cell recordings, a KCl filling solution was used (containing (in mM) 130 KCl, 5 NaCl, 0.4 CaCl_2, 1 MgCl_2, 10 HEPES (pH 7.3), and 1.1 EGTA). In experiments calling for different concentrations of Cl\textsuperscript{−} in the pipette solution ([Cl\textsuperscript{−}]_p), glutamate substituted for Cl\textsuperscript{−} in the filling solution. The liquid junction potential for the modified solution was determined empirically (Ncher 1992) and data were corrected post hoc. Recordings were digitized and analyzed with pClamp6 software (Axon Instruments, Foster City, CA). Membrane resistance was calculated by measuring the steady-state current deflection during 200-ms voltage steps (±10–30 mV) from a holding potential of −60 mV. When using voltage ramps, the ramp protocol consisted of stepping the cell from a holding potential of −60 to +20 or +40 mV for 80 ms, then changing the voltage at a rate of 150 mV/s to −100 mV. I–V curves were plotted after subtracting the control response obtained in ACSF from the response obtained during agonist application. GABA and glutamate reversal potentials also were determined by applying agonists with the membrane held at a series of potentials. Peak current responses for each voltage were plotted, and the data were fitted using CA-Cricket Graph III software (Computer Associates International). The agonist-mediated reversal potentials were defined as the x-intercept value of the fit. Spontaneous PSC reversal potentials were estimated by holding the cell at a series of membrane potentials and determining the potential when sPSCs were nullified. This value then was compared with the reversal potential of the GABA- or muscimol-induced currents and the Cl\textsuperscript{−} equilibrium potential (E_\text{Cl}) that was determined by the Nernst equation of the form: E_\text{Cl} = −58 mV log10 ([Cl\textsuperscript{−}]_i/[Cl\textsuperscript{−}]_o), where [Cl\textsuperscript{−}]_i and [Cl\textsuperscript{−}]_o are the extracellular and intracellular Cl\textsuperscript{−} concentrations, respectively. A cell was considered not to have sPSCs if no synaptic potentials were observed after 5–10 min of recording. Dose-response data were fit with the Hill equation of the form: I/I_{max} = 1/[1+(EC_\text{50}/[GABA])^n], where I is the GABA-induced current, I_{max} is the maximal GABA-induced current, EC_\text{50} is GABA concentration producing a half-maximal response, [GABA] is the GABA concentration, and n is the Hill coefficient. GABA-induced current decays were expressed as: percentage of apparent rate of desensitization = (I_{peak} – I_20/I_{peak}) × 100. I_{20} was the level of current 20 s after the current peak (I_{peak}). The I_{20} value was selected to normalize data for comparisons. To isolate individual VZ cells, cortical slabs were maintained in 0 calcium (Ca\textsuperscript{2+}) ACSF for ≥30 min before recording and cell isolation. Using changes in membrane resistance and GABA-induced current as an indicator of successful cell isolation, we found that in ~40% of the attempts we were able to isolate cells. This is close to the reported value of 50% found in previous studies using this method (Mienvile et al. 1994). To uncouple VZ cell clusters pharmacologically, ACSF bubbled with 100% CO_2 was added to the bathing solution, and membrane resistance was monitored to assess the decrease in gap junction conductance (LoTurco and Kriegerstein 1991). In experiments examining the extent of bicuculline methiodide (BMI) antagonism of GABA-induced currents, the data were expressed as: percentage block = (I_{control} – I_{BMI}/I_{control}) × 100. Where I_{control} is the peak GABA-induced current and I_{BMI} is the peak current after the addition of BMI. All average values are expressed as means ± SE. Cells were accepted for analysis only if they maintained a stable access resistance throughout the recording. Unless otherwise noted, the holding potential for voltage clamp recordings was −60 mV.

**Filling Cells with Lucifer yellow**

In some recordings a saturating concentration of Lucifer yellow (LY; Sigma) was included in the pipette filling solution. After electrophysiological recording, the tissue was fixed in 4% paraformaldehyde for 30–60 min at RT or overnight at 4°C and then transferred to PBS. Tissue was dehydrated in ethanol, cleared with methyl salicylate (Sigma), and coverslipped. Filled cells then were viewed with scanning laser confocal microscopy using a Zeiss Axiosview microscope with illumination provided by a Zeiss argon crypton laser scanning confocal attachment or by conventional epifluorescence.

**GABA immunohistochemistry**

Whole animals were anesthetized with halothane and transcardially perfused with 4% paraformaldehyde. Heads were removed and post-fixed in 4% paraformaldehyde overnight at 4°C, then placed in PBS. Brains were removed and sectioned at 50–100 μm on a vibratome. Sections were treated in 0.3% H_2O_2 in PBS for 30 min at RT to remove endogenous peroxidase activity. Sections then were permeabilized and blocked with 0.5% Triton X-100 and 10% normal goat serum (NGS) in PBS for 1 h at RT. After washing, sections were incubated in polyclonal anti-GABA primary antibody (Sigma) (1:1,000 dilution) in 0.1% Triton X-100 and 3% NGS in PBS for 1–2 h at RT or overnight at 4°C. Tissue was washed in PBS and then incubated in biotinylated anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA) (1:200 dilution) in 0.1% Triton X-100 and 3% NGS in PBS for 1 h at RT. After washing, sections were incubated with VECTASTAIN elite ABC reagent (Vector Laboratories) for 30 min at RT, washed in PBS, and then reacted with diaminobenzidine tetrahydrochloride substrate (Vector Laboratories) for 10–15 min at RT. Sections were rinsed with H_2O, dehydrated in ethanol and xylene, and coverslipped. Tissue was viewed with a Zeiss Axioscope. In control experiments, no cellular staining was observed when the primary antibody was omitted (not shown).
Pharmacological agents and application

Muscimol, bicuculline methiodide (BMI), glutamate, and tetrodotoxin (TTX) were obtained from Sigma (St. Louis). GABA, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 6,7-dinitroquinoxaline-2,3-dione (DNQX), 2-amino-5-phosphonopentanoic (AP-5), N-methyl-D-aspartate (NMDA), and cis-4-aminoenorotic acid (CACA) were obtained from RBI (Natick, MA). Drugs were applied with a D-12 Superfusion System (ALA Scientific Instruments, Westbury, NY). This application system permitted more rapid drug exchange than the bath-perfusion system used previously (LoTurco et al. 1995). Generally, a drug-free ACSF wash was applied immediately before and after drug. In some experiments CNQX and TTX were bath applied. Drugs were kept as concentrated stock solutions at −20°C (Muscimol, BMI, CNQX, DNQX, AP-5, NMDA, CACA, and GABA) or 4°C (TTX) and diluted to the desired concentration on the day of the experiment.

RESULTS

A summary of neocortical development is shown schematically in Fig. 1A (see legend for details). The cell populations investigated are in areas marked with bold type in Fig. 1A and are displayed after filling of cells with LY in Fig. 1, B–D. These include proliferating precursor cells in the VZ (VZ cells), immature neurons in the embryonic CP (CP cells), and further differentiated postnatal cortical neurons (P0–5, collectively termed PN cells). A hallmark of proliferating precursor cells in the VZ is that they are coupled into discrete radial cell clusters by gap junction channels (LoTurco and Kriegstein 1991). These clusters contain both proliferating precursor cells and one or two radial glia (Bittman et al. 1997; LoTurco et al. 1995). Figure 1B shows an example of a gap junction coupled cell cluster at E17. Due to coupling, electrophysiological recordings from cells in clusters display lower membrane resistances (LoTurco and Kriegstein 1991) than would be expected of single small cells (~5–10 μm in diameter). The probability of encountering coupled cell clusters in the VZ decreases with age so that by E19–20 one can obtain recordings from both coupled precursor cells as well as from postmitotic neurons (LoTurco and Kriegstein 1991; LoTurco et al. 1991). For this reason, we performed the majority of VZ recordings at E15–17. Recordings were identified as deriving from gap junction coupled clusters by their low membrane resistances (47–333 MΩ), and in all such recordings when including LY in the recording pipette, we filled multicellular clusters (n = 11). After exit from the cell cycle, newly born neurons migrate to the CP. Embryonic CP cells have higher membrane resistances (1–3 GΩ) than coupled VZ cells, and intracellular filling with LY (Fig. 1C) or biocytin (Bittman et al. 1997) reveals cells with relatively immature neuronal morphology (n = 12). Because the CP contains both migratory and postmitatory neurons, CP recordings could derive from either of these immature neuronal types. During the early postnatal period, electrodes were targeted to the upper cortical layers (i.e., presumptive layers II–IV). The recorded cells displayed a relatively large range of membrane resistances (range 0.2–5 GΩ) and, when filled with LY, had pyramidal-like morphology (n = 15, Fig. 1D).

Evidence suggests that postmitotic cortical neurons are uncoupled during migration through the IZ (Bittman et al. 1997). Once settled in the CP, neurons become coupled again into progressively larger clusters that peak in size during the first two postnatal weeks (Kandler and Katz 1998; Peinado et al. 1993; Rorig et al. 1996). However, the sites of contact and degree of junctional dye spread differ between VZ and CP cells. For example, LY stains coupled VZ cells better than coupled neonatal neurons (Fig. 1, B–D), while biocytin and neurobiotin stain coupled cells in both regions well (Bittman et al. 1997; Peinado et al. 1993). Additionally, coupling in cortical neurons is thought to occur at distal dendritic sites (Peinado et al. 1993), whereas coupling in VZ cells most occurs proximally because VZ cells have a relatively simple bipolar morphology (Nadarajah et al. 1997).

FIG. 1. A: schematic of neocortical development. Most neocortical neurons are generated from precursor cells in the ventricular zone (VZ). Migration of cells out of the VZ generally follows on exit from the cell cycle. Earliest born neurons form the preplate layer (PPL). Subsequently born neurons migrate through the intermediate zone (IZ) to form the cortical plate (CP), which splits the PPL into the marginal zone (MZ) and subplate (SP). By birth neurogenesis is complete. By P5 most neurons have completed migration, and the characteristic 6-layered neocortex is more evident. Subventricular zone (SVZ); white matter (WM); ependymal layer (E). B–D: Lucifer yellow staining of recorded cells. B: VZ-coupled cell cluster at E17. C: CP neuron at E19. D: layer II/III pyramidal neuron at P3. Insets: currents induced by application of GABA (20–50 μM) in the illustrated cell types. V_m = −60 mV in all cells, scale bars: 125 pA and 5 s (B), 250 pA and 5 s (C), and 300 pA and 5 s (D).
Changes in the functional properties of GABA<sub>A</sub> receptors between precursor cells and neurons

In whole cell recordings ([Cl<sup>-</sup>]<sub>i</sub> = 104 mM), we found that at ages ≥ E15, application of GABA produced inward currents in all VZ cells tested (Fig. 1B, inset). GABA-induced inward currents also were observed in all recordings tested from CP and PN neurons (Fig. 1, C and D, insets). These findings are consistent with previous studies that have shown functional GABA<sub>A</sub> receptors to be present in these cell populations (LoTurco and Kriegstein 1991; LoTurco et al. 1995; Owens et al. 1996).

Dose-response properties. We first compared the dose-response properties of GABA currents in recordings made from embryonic VZ (E16–17), embryonic CP (E19), and early PN (P4–5) cells. In all cells GABA produced dose-dependent currents (Fig. 2A); however, the properties were different between the distinct cell populations (Fig. 2B). In VZ cells GABA produced a half-maximal response at a concentration of 5.1 μM with a Hill coefficient of 1.9 (n = 16); this value is consistent with previous findings (LoTurco et al. 1995). In contrast, recordings from CP and PN cortical neurons demonstrated that GABA was approximately six- and eightfold less potent, respectively. GABA produced a half-maximal response at a concentration of 28.2 μM with a Hill coefficient of 1.0 in CP cells (n = 5) and 40.1 μM with a Hill coefficient of 1.3 in PN cells (n = 7). Also, consistent with receptors that have a higher apparent affinity for GABA, VZ cell responses took longer to recover after removal of GABA than CP and PN cell responses (see Fig. 3A). This effect was observed most readily after longer drug applications, and to be sure this was not simply due to less effective drug clearance from the VZ environment, we compared the recovery after glutamate application. Consistent with effective drug clearance from the VZ, glutamate (300 μM)-induced currents in VZ cells, mediated by AMPA/kainate-type receptors with an EC<sub>50</sub> of ~75 μM (LoTurco et al. 1995), displayed relatively rapid recovery rates (Fig. 2C).

Response magnitude. The dose-response experiments indicate that GABA-induced current increases in magnitude with development. In recordings from VZ (E15–17) cells, the peak current induced by a saturating GABA concentration (50 μM) was −324 ± 36 pA (n = 19), whereas in CP (E19) and PN (P4–5) neurons saturating GABA concentrations (500 μM) produced peak currents of −792 ± 161 pA (n = 8) and −1604 ± 229 pA (n = 13), respectively. This could reflect a developmental increase in the GABA<sub>A</sub> channel conductance or an increase in the number of GABA<sub>A</sub> channels per cell. Considering that the main conductance state of GABA<sub>A</sub> receptor channels in VZ cells is similar to that reported for neurons (LoTurco and Kriegstein 1991; Rabow et al. 1995; Xiang et al. 1998), these results suggest that the number of GABA receptors per cell increases with maturation. Furthermore the number of receptors is likely to be particularly low in VZ cells because GABA-induced currents in VZ recordings most likely reflect activation of receptors on multiple cells within a gap-junction-coupled cell cluster (see following text).

Receptor desensitization. In addition to differences in peak current and apparent affinity for GABA between VZ cells and cortical neurons, GABA application to VZ cells produced currents that persisted in the continued presence of agonist, suggesting differences in receptor desensitization (Fig. 3A). To investigate this further, we applied 50 μM GABA to VZ, CP, and PN cells for 20–30 s and measured the level of desensitization in each cell population. The percentage apparent rate of desensitization (see METHODS) in VZ cells was 15.8 ± 2.4% (n = 9), whereas in CP and PN cells, these values were 65.3 ± 3.1% (n = 10) and 64.1 ± 3.8% (n = 9), respectively (Fig. 3B). This difference could not be accounted for by differences in response magnitude because peak currents with similar amplitudes displayed differences in receptor desensitization in VZ and CP/PN cells. In VZ recordings with a mean peak current of −418 ± 35 pA, the percentage apparent rate of desensitization was 14.9 ± 4.2% (n = 4), whereas in CP/PN
recordings with a mean peak current of $-415 \pm 53\ pA$, the percentage apparent rate of desensitization was $61.9 \pm 3.1\%$ ($n = 3$).

We were concerned that poor voltage control in gap-junction-coupled VZ cells could contribute to the large differences in apparent receptor desensitization. For example, GABA$_A$-receptor desensitization in cortical neurons is voltage dependent with less pronounced current relaxation at more positive membrane potentials (Frosch et al. 1992). If voltage-dependent desensitization of GABA$_A$ receptors occurs in proliferative cells, then because of poor space-clamp conditions, depolarizing responses in VZ cells distal to the recording site might obscure the actual level of receptor desensitization. Ideally, single-channel recording could resolve this issue. However, attempts to obtain single-channel recordings from VZ cells were unsuccessful, presumably because of the low number of receptors per VZ cell. We therefore tried a different approach based on the observation that removing Ca$^{2+}$ from the bathing medium allows individual cells from VZ clusters to be pulled away from clusters during whole cell recording (Mienville et al. 1994). This technique should allow recording of agonist responses in isolated cells under good voltage control.

We obtained whole cell recordings from coupled VZ cells in 0Ca$^{2+}$ ACSF and monitored the membrane resistance to verify

**FIG. 3.** Receptor desensitization of GABA$_A$ responses in VZ (E15–17), CP (E16–20), and PN (P0–4) cells. A: examples of GABA (50 \(\mu M\)) current in VZ and PN cells demonstrating the distinct rates of desensitization. B: mean percentage apparent receptor desensitization values for VZ ($n = 9$), CP ($n = 10$), and PN ($n = 9$) cells. Inset: scaled responses of the traces shown in A. C: GABA (50 \(\mu M\))-induced current from an isolated VZ cell obtained in 0Ca$^{2+}$ artificial cerebrospinal fluid (ACSF). Note the lack of current relaxation. D: GABA (50 \(\mu M\))-induced current from an outside-out patch from a PN neuron. Note the pronounced current decay. $V_{\text{hold}} = -60\ mV$ for all cells.

**FIG. 4.** Pharmacology of GABA$_A$ receptors in VZ (E16–17), CP (E19), and PN (P4) cells. A: example of bicuculline methiodide (BMI; 100 \(\mu M\)) antagonism of GABA (20 \(\mu M\))-induced current in a VZ cell. B: example of BMI (100 \(\mu M\)) antagonism of GABA (100 \(\mu M\))-induced current in a PN cell. C: example of BMI (100 \(\mu M\)) antagonism of cis-4-aminocrotonic acid (CACA; 100 \(\mu M\))-induced current in a PN cell. D: compiled data of the percentage block of GABA- or CACA-induced currents in the presence of BMI. $V_{\text{hold}} = -60\ mV$ for all cells.
that cells were initially members of gap-junction-coupled clusters. Before isolating single VZ cells, recordings displayed low membrane resistances (50–300 MΩ) and after cell separation membrane resistances increased to $1 GΩ$. For example, in the recording shown in Fig. 3C before isolation the membrane resistance was 137 MΩ; this value increased to 1.4 GΩ after removal consistent with previous reports using this technique (Mienville et al. 1994). Furthermore we found that before isolation peak GABA (50 μM)-induced currents were substantially larger than after isolation. On average before and after isolation the peak GABA-induced current was $2 \pm 191 \pm 24$ pA ($n = 19$) and $-4.4 \pm 1$ pA ($n = 7$), respectively. As with recordings made from intact VZ cell clusters, we found that currents induced by 50 μM GABA showed little receptor desensitization with prolonged agonist application (Fig. 3C). Additionally, in a second series of experiments, we uncoupled VZ gap junctions pharmacologically by acidifying the bath (LoTurco and Kriegstein 1991). We found that after this manipulation muscimol (30 μM), a GABA_A-receptor agonist, produced currents that showed little desensitization ($n = 3$; not shown). These results support the idea that GABA_A receptors on VZ cells are less sensitive to receptor desensitization compared with postmitotic neurons and that space-clamp artifacts cannot entirely account for this difference.

Physical isolation of VZ cells also allowed us to estimate the GABA_A-receptor number on individual VZ cells. Assuming equal channel density for all cells in a VZ cluster, a peak current of approximately $-200$ pA in the 0 Ca^2+ conditions and a cluster size of 30–45 cells (LoTurco and Kriegstein 1991), we would predict that with maximal receptor activation each cell would contribute $; 4 –7 pA of current to the response. In isolated VZ cell recordings, we found that cells gave an average current of $-4.4 \pm 1$ pA of current ($n = 7$ cells). This is close to the predicted value and suggests that the density of GABA_A channels in individual VZ cells is quite low compared with postmigratory cortical neurons. Considering that the VZ cell GABA_A-receptor unitary current is approximately $2 p A$ at a holding potential of $-60$ mV (LoTurco and Kriegstein 1991), then each VZ cell would contain a very small number of functional channels.

Finally, to rule out the possibility that increased buffering or reuptake of GABA in the CP might influence the observed differences in receptor desensitization between proliferative and postmitotic cells, we obtained outside-out patches from PN (P3–4) neurons ($n = 7$). Consistent with whole cell recordings, GABA-induced currents in outside-out patches desensitized significantly with prolonged drug application (Fig. 3D). This result, in combination with experiments with isolated VZ cells, rules out the possibility that environmental changes between the VZ and CP are responsible for the observed differences in receptor desensitization. In addition, we found that 50 μM GABA produced $-87.9 \pm 14.4$ pA of current in outside-out
patches (n = 7), which is equivalent in magnitude to peak current responses in some intact VZ cluster responses. Considering the small area of membrane in an outside-out patch, this provides further confirmation that receptor number increases with maturation.

RECEPTOR PHARMACOLOGY. The properties of the GABA-induced current in VZ cells (i.e., a relatively high affinity for GABA and relative lack of receptor desensitization) are similar to those of the GABA<sub>C</sub> subtype GABA receptor, a Cl<sup>-</sup>-selective ion channel distinguished pharmacologically from GABA<sub>A</sub> receptors by being insensitive to BMI (Bormann and Feigenspan 1995). However, previous results have demonstrated that GABA-induced currents in VZ cells are sensitive to BMI (LoTurco and Kriegstein 1991; LoTurco et al. 1995), suggesting that these are not GABA<sub>C</sub> receptors. In agreement with these studies, we found that in eight of eight VZ (E16–17) recordings currents induced by application of 20 μM GABA were blocked entirely by 100 μM BMI (Fig. 4, A and D). In all cases, the antagonism produced by BMI was reversible (not shown). In addition, CACA (100 μM), an agonist with some specificity for GABA<sub>C</sub> receptors, produced only small currents (28.6 ± 1 pA) in four of six cells. In three of three of the CACA-responding cells, 100 μM BMI blocked all of the current (Fig. 4 D). These results strongly suggest that all of the GABA-induced current in VZ cells are mediated by GABA<sub>A</sub> receptors.

The possible presence of GABA<sub>C</sub> receptors in CP (E19) and PN (P4) cells also was explored. BMI (100 μM) substantially blocked the GABA (20–30 μM)-induced current in CP and PN neurons; however, in two of five CP and three of four PN recordings, a small residual GABA-induced current remained (Fig. 4, B and D). Currents also could be induced by 100 μM CACA; however, they were smaller than with 30 μM GABA (−346 ± 108 pA for GABA and −42 ± 17 pA for CACA) and...
were blocked entirely by 100 μM BMI (Fig. 4, C and D). Furthermore the CACA-induced currents still were blocked by BMI even when the CACA concentration was increased to 300 μM and BMI decreased to 50 μM (Fig. 4D). As with VZ cells, these results suggest that most if not all of the current induced by GABA application is mediated by GABAA receptors. Furthermore these data confirm reports that CACA may not be an entirely specific ligand for GABAC receptors (Qian and Dowling 1996). The small residual current that remained in the presence of BMI in CP and PN cells could be induced by another GABA-receptor subtype (Strata and Cherubini 1994) that is relatively insensitive to 300 μM CACA or could simply result from the larger number of GABAA receptors in CP and PN neurons limiting the ability of BMI to compete at all the receptor sites. This idea is supported by the observation that increasing the BMI concentration from 50 to 100 μM produces a larger percentage block of the GABA-induced current (91 ± 2.1% and 96 ± 1.9% block for 50 μM and 100 μM BMI, respectively; Fig. 4D).

**GABAergic synaptic transmission**

**DEVELOPMENTAL ONSET OF SPONTANEOUS GABA<sub>A</sub>-MEDIATED SYNAPTIC TRANSMISSION.** Spontaneous sPSCs were observed in recordings from early neocortical neurons consistent with previous reports (Luhmann and Prince 1991; Owens et al. 1996). These sPSCs were mediated by GABAA receptors because they were reversibly blocked by BMI (n = 21; Figs. 5, A and B; 6, A and C; 7A; and 8, A and C3), but not by the glutamate-receptor antagonists CNQX, DNQX, or AP-5 (n = 6; Fig. 5B). Glutamate-receptor antagonists could never eliminate the sPSCs; however, modulation of GABAA-mediated sPSCs by glutamate-receptor blockade (Salin and Prince 1996) was not examined. The properties of the sPSCs varied from cell to cell (Fig. 6, A and B). The frequency ranged from 0.02 to 1.4 Hz, and in many of the cells, sPSCs occurred in relatively high-frequency bursts (Fig. 6B). Also, consistent with the sPSCs being GABAA mediated, sPSCs reversed near ECl and matched the reversal potential found for exogenously applied GABA and muscimol but not glutamate or NMDA (Fig. 6C). Considering that [Cl<sup>-</sup>], is relatively high in immature cortical neurons (Ben-Ari et al. 1989; Owens et al. 1996) and the resting membrane potential is generally more negative than ECl (Burgard and Hablitz 1993b; Luhmann and Prince 1991; Owens et al. 1996), these synaptic currents would serve to depolarize the membrane.

Using these sPSCs, we characterized the onset of GABAergic synaptic transmission in the developing neocortex (Fig. 6D). We never observed sPSCs in VZ cells at any age examined (E15–19). In recordings from late embryonic CP (E18–20) cells, we observed GABAA-mediated sPSCs in 14% of the recordings. This number increased by the day of birth (Fig. 6D). In P0–2 recordings, the number of cells with BMI-sensitive sPSCs increased to 51%. In recordings from P3–5 neurons, ~73% of cells displayed GABAA-mediated sPSCs. Additionally, the frequency of the sPSCs also increased with age (Fig. 6D). At E18–20, the mean frequency was 0.15 ± 0.04 Hz; at P0–2, the mean frequency was 0.32 ± 0.07 Hz; and at P3–5, the mean frequency was 0.50 ± 0.08 Hz.

In many instances, application of BMI could produce an outward current shift (Fig. 6A, inset), suggesting the presence of endogenous ligand in the cortical environment. This may result from the spill over of GABA from immature synapses (Brickley et al. 1996), action potential independent tonic release (Valeyev et al. 1993), or a combination of both. Outward shifts in currents during BMI application also have been observed in VZ recordings (LoTurco et al. 1995). Because VZ cells do not appear to have synapses, this suggests that GABA may be tonically released from GABA positive cells adjacent to or within the VZ (Behar et al. 1996; Cobas et al. 1991).

**NMDA-MEDIATED sPSCs.** Our results indicate that in early postnatal cortical neurons the majority of sPSCs in cells voltage clamped at negative holding potentials (~60 to ~70 mV) are mediated by activation of GABAA receptors. Glutamatergic sPSCs were not observed; however, previous studies have demonstrated that glutamate-receptor–mediated synaptic currents can be evoked by at least P0 (Kim et al. 1995) and are detected spontaneously by at least P3 in neocortical cells (Burgard and Hablitz 1993a). Because nearly all immature cortical neurons express NMDA receptors (LoTurco et al. 1991), it is possible that glutamate-receptor–mediated sPSCs...
are dominated by NMDA receptors, making them functionally silent at negative membrane potentials (Isaac et al. 1997). To test this, we applied BMI to recordings held at positive potentials (+30 to +60 mV) and monitored the sPSCs. In these recordings, we found that the sPSCs were not always eliminated with BMI (Fig. 7A), and consistent with the BMI-insensitive events being mediated by NMDA receptors, they were blocked by AP-5 (n = 4; Fig. 7B).

**SOURCE OF GABAERGIC sPSCs.** Most BMI-sensitive sPSCs could be abolished by application of TTX (n = 6; Fig. 8A), indicating that the majority of GABA_A-mediated sPSCs resulted from action potential dependent activity of GABAergic neurons. Consistent with previously published reports (Cobas et al. 1991; Lauder et al. 1986; Van Eden et al. 1989), we observed GABA immunopositive cells in the developing cortical layers that could be the presynaptic source of the sPSCs (Fig. 8B). To investigate whether activity of local GABAergic interneurons could generate PSCs in neighboring neurons, we used focal applications of glutamate to locally excite neurons while recording from nearby pyramidal cells. E_Cl was set at −45 mV ([Cl^−]_o = 22 mM) and cells were voltage clamped at −0 mV, close to the glutamate reversal potential. Pulses of glutamate (100 μM) induced BMI-sensitive PSCs (n = 5; Fig. 8C2). This result suggests that local activation of GABA-containing cells can evoke GABA_A-mediated PSCs. Additionally, we isolated small pieces of neocortex by making two complete radial cuts 2–3 mm apart and a horizontal cut above the white matter (n = 3; Fig. 8C3). GABA_A-mediated sPSCs were still present after this microdissection. Collectively these results suggest that the sPSCs are mediated by local spontaneously active GABAergic neurons that reside in the cortex and that cortical afferents are not required to drive these cells.

**GABA_A RECEPTOR ACTIVATION CAN BE EXCITATORY.** Previous studies have demonstrated that during the early postnatal period GABA_A-receptor activation is depolarizing when [Cl^−]_i is kept intact (Owens et al. 1996). It is possible that GABA_A-receptor activation is excitatory and may depolarize cells sufficiently to induce action potential activity. To test this idea, we
Functional properties of GABA<sub>A</sub> receptors in proliferative and postmitotic cells

The differences in GABA<sub>A</sub>-receptor properties in precursor cells and postmitotic neurons are likely due to differences in subunit composition. GABA<sub>A</sub> receptors are thought to be heteropentameric proteins constructed of subunits derived from five related gene families (Macdonald and Olsen 1994). Presently, six α, three β, three γ, one δ, and one ε subunit subtypes have been identified (Davies et al. 1997; Macdonald and Olsen 1994; Whiting et al. 1997), providing an enormous potential number of subunit combinations. However, it has been suggested that the actual number of different subunit combinations in native channels is more restricted (McKernan and Whiting 1996). Immunohistochemical and in situ hybridization methods have been used to localize a variety of GABA<sub>A</sub>-receptor subunits in developing neocortex (Araki et al. 1992; Fritschy et al. 1994; Laurie et al. 1992; Ma and Barker 1995; Poulter et al. 1992, 1993). In the neocortical proliferative zone, the most prominently expressed subunits appear to be α<sub>4</sub>, β<sub>1</sub>, and γ<sub>1</sub> (Araki et al. 1992; Laurie et al. 1992; Ma and Barker 1995; Poulter et al. 1992, 1993), however mRNA for other GABA<sub>A</sub>-receptor subunits also has been localized to the VZ (Laurie et al. 1992; Poulter et al. 1993, 1993). Presently, 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, comparison of the physiological properties of GABA<sub>A</sub>-receptors examined in expression systems to the properties of the native receptors may permit tentative conclusions concerning possible subunit combinations. For example, expression studies have demonstrated that addition of the δ subunit to α<sub>1</sub>/β<sub>1</sub> and α<sub>1</sub>/β<sub>1</sub>/γ<sub>2</sub>L combinations produces receptors that have relatively high affinity for GABA, little receptor desensitization, and slow recovery (Saxena and Macdonald 1994, 1996). These properties resemble those described here for GABA<sub>A</sub>-receptors in VZ cells. The δ subunit may contribute to the distinct properties of native GABA<sub>A</sub>-receptors in proliferative cells because δ subunit mRNA has been detected in the neocortical VZ (Poulter et al. 1993), and recent evidence has shown that the δ subunit selectively associates with the α<sub>4</sub> subunit, which is expressed at high levels in the VZ (Homanics et al. 1998; Ma and Barker 1995).

In the embryonic CP α<sub>3</sub>, β<sub>2</sub>/3, and γ<sub>3</sub> appear to be the predominant subunits expressed (Ma and Barker 1995); however, other α subunits (α<sub>1</sub>, α<sub>2</sub>, and α<sub>5</sub>), the δ subunit (Poulter et al. 1993), and the γ<sub>2</sub> subunit (Laurie et al. 1992) also have been localized to the CP. Interestingly, expression studies have shown that addition of the γ<sub>2</sub> subunit can increase the desensitization rate of recombinant receptors (Saxena and Macdonald 1994). Expression of the γ<sub>2</sub> subunit by neurons in the CP could account for some of the differences in receptor properties observed between cells in the VZ and CP. Furthermore the γ<sub>2</sub> subunit has been shown to be critical for the postsynaptic clustering of GABA<sub>A</sub> receptors and synaptogenesis in cultured cortical neurons (Essrich et al. 1998).

Although differences in subunit composition may underlie the differences in receptor properties, we cannot dismiss the possibility that the physiological state of the cell can influence receptor function. Levels of intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) and degree of receptor phosphorylation have been shown to change the peak current and rate of desensitization of GABA<sub>A</sub> receptors (Moss and Smart 1996; Mozzymas and Cherubini 1998). Thus differences in intrinsic cell properties and not simply subunit composition may contribute to the observed differences between GABA<sub>A</sub> receptors in proliferative and postmitotic cells.


Endogenous mode of GABA$_A$-receptor activation

It is unlikely that synaptic activation of GABA$_A$ receptors occurs in VZ cells. 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 E16 (Balslev et al. 1996). Consistent with the anatomic studies, physiological studies have failed to detect synaptic potentials in either gap junction coupled (Fig. 6) (LoTurco et al. 1995) or uncoupled, presumably postmitotic, VZ cells (LoTurco et al. 1991). Nevertheless, evidence has suggested that endogenous GABA$_A$-receptor activation does occur in the proliferative zone (LoTurco et al. 1995). Whole cell recordings of VZ cells display outward current shifts on application of BMI, suggesting the presence of a tonically released endogenous ligand (LoTurco et al. 1995). The physiological properties of GABA$_A$ receptors in VZ cells, namely the relatively high apparent affinity for GABA and the relative lack of desensitization, would increase the likelihood of tonic receptor activation from low levels of nonsynaptically released ligand. Immunostaining has demonstrated that GABA positive cells are localized directly above as well as within the VZ (Behar et al. 1996; Cobas et al. 1991; Lauder et al. 1986; Van Eden et al. 1989). Growth cones arising from these cells could be the source of endogenous GABA release (Taylor et al. 1990).

Synaptically mediated GABA$_A$-receptor activation is also unlikely to occur during neuronal migration because no synapses have been detected in the intermediate zone (Balslev et al. 1996; Bourgeois and Rakic 1993). However, GABA$_A$ receptors expressed by migrating neurons could be activated by nonsynaptically released agonist. Studies of neocortical cells in culture have shown that immature neurons migrate toward a localized source of GABA (Behar et al. 1996). In addition, the migratory promoting effects of GABA occur at lower concentrations for VZ-derived cells than for CP-derived cells (Behar et al. 1998). Our data concerning the greater sensitivity of VZ cell GABA$_A$ receptors are consistent with this observation.

There are several potential cellular sources for GABA as a migration signal in the developing cortex. Immunohistochemical studies have demonstrated a differential distribution of GABA-positive cells throughout the embryonic cortical wall. For example, there are a large number of GABA-positive cells at the top of the VZ and in the SP and MZ at E17 in rat (Behar et al. 1996; Cobas et al. 1991). These cells may provide GABA gradients in the cortical environment that a cell may sense as it migrates from the VZ to the CP.

Not until cells settle in the CP would synaptic transmission play a role in GABA$_A$-receptor activation. As shown here, sPSCs first are detected at E18. Additionally, recent immunocytochemical analyses of the developing somatosensory cortex of mice and rats have identified GABAergic synaptic contacts at the earliest ages studied (P4 in mouse and P5 in rat), demonstrating that these synapses are anatomically as well as physiologically developed in the neonate (De Felipe et al. 1997; Micheva and Beaulieu 1996). Our results indicate that during early cortical development, the majority of spontaneous synaptic activity is mediated by activation of GABA$_A$ receptors. This contrasts with a previous study in which GABA$_A$-mediated sPSCs were not observed in recordings from P3 to 8 layer II/III pyramidal neurons (Burgard and Hablitz 1993b). However, as emphasized by these authors, the recordings were preformed with $E_{Cl}^{-}$ set near the resting membrane potential, which would diminish the driving force for $Cl^{-}$ through the GABA$_A$ channel, making the detection of these events difficult (Burgard and Hablitz 1993b). These early sPSCs most likely arise from intrinsic GABAergic neurons in the developing cortex (Fig. 8A). GABAergic cells in marginal zone (MZ) and subplate (SP) cannot be ruled out as contributing to the sPSCs recorded in CP and PN cells; however, the majority of subplate projections to CP are glutamatergic (Finney et al. 1998). Additionally, while GABAergic cells are present in the MZ and SP at E16, the earliest sPSCs are detected at E18, the same age as GABA-positive cells appear in the CP (Cobas et al. 1991). Although immature GABAergic cortical neurons are spontaneously active, they are not easily activated by afferent stimulation. Little or no GABA$_A$-dependent synaptic potentials are evoked with stimulation of cortical afferents in the perinatal cortex (Agmon et al. 1996; Burgard and Hablitz 1993a; Kim et al. 1995; Luhmann and Prince 1991). Using high-intensity stimulation, it was found that activation of cortical afferents during the first few postnatal days can produce polysynaptic responses that contain a GABA$_A$-mediated component (Agmon et al. 1996). However, the GABA$_A$-mediated polysynaptic responses fatigue easily with repeated stimulation, suggesting that afferent drive of cortical GABAergic interneurons is weak. Therefore although GABAergic interneurons form functional synapses and are spontaneously active in early neocortex, afferent activation of these cells, both by feedback and feedforward pathways, is poorly developed. During the second postnatal week, activation of cortical inputs can reliably evoke GABAergic synaptic potentials (Agmon and O’Dowd 1992; Luhmann and Prince 1991).

Downstream effects of GABA$_A$-receptor activation during development

In addition to serving an inhibitory function in more mature cells, GABA$_A$-receptor activation may play a maturation role. Many of the developmental effects of GABA are thought to occur during the embryonic and early postnatal periods when GABA$_A$-receptor activation produces membrane depolarization (Berninger et al. 1995; Cherubini et al. 1991). In proliferative cells in the VZ, activation of GABA$_A$ receptors has been shown to downregulate DNA synthesis measured by thymidine or 5-bromo-2'-deoxyuridine incorporation assays (LoTurco et al. 1995). The GABA effect on DNA synthesis was abolished by furosemide, which negatively shifted $E_{Cl}^{-}$ suggesting that GABA-induced depolarization is the signal that influences DNA synthesis. Likewise, activation of GABA$_A$ receptors inhibits the proliferative effects of basic fibroblast growth factor in cortical progenitor cells in culture (Antonopoulos et al. 1997). GABA$_A$-receptor activation also has been shown to influence the morphology and motility of young hippocampal and neocortical neurons in cell culture (Barbin et al. 1993; Behar et al. 1996, 1998; Marty et al. 1996). Additionally, activation of GABA$_A$ receptors has effects on cell survival (Ikeda et al. 1997) and gene expression (Marty et al. 1997). The depolarizing action of GABA$_A$-receptor activation has been shown to increase $[Ca^{2+}]_i$ through activation of voltage-gated Ca$^{2+}$ channels (LoTurco et al. 1995; Yuste and Katz 1991), suggesting Ca$^{2+}$-dependent second-messenger pathways may mediate the developmental effects of GABA.
For example, GABA-induced depolarization has been shown to upregulate brain-derived neurotrophic factor expression (Berninger et al. 1995). The mechanism may involve downstream activation of the transcription factor CREB because a pathway involving depolarization, voltage-dependent Ca\(^{2+}\) increase, and activation of CREB has been shown to induce BDNF expression (Shieh et al. 1998; Tao et al. 1998).

Early GABAergic communication also may function in concert with NMDA-receptor activation to regulate synaptogenesis and/or synaptic consolidation. It is thought that NMDA receptors underlie the robust synaptic and developmental plasticity seen in immature animals; however, in many cases NMDA receptors are silent at negative holding potentials in developing cortical neurons (Fig. 7) (Durand et al. 1996; Isaac et al. 1997). Therefore an endogenous depolarizing influence must be present at immature synapses to relieve the Mg\(^{2+}\)-block of the NMDA receptor, a role attributed to AMPA/kainate-receptor activation in more mature cortex. Possibly GABA could play this role because GABAergic synapses develop early in cortex and have depolarizing effects (Agmon et al. 1996; Owens et al. 1996). Support for this idea has come from studies in the developing hippocampus in which GABA-mediated synaptic activity has been shown to have synergistic actions with NMDA-receptor activation by providing the depolarization necessary to relieve the Mg\(^{2+}\)-block of the NMDA channel (Ben-Ari et al. 1997). Furthermore activation of NMDA receptors may be a signal for synapse stabilization of non-NMDA receptors (Durand et al. 1996; Isaac et al. 1997).

Excitation versus inhibition

It often is stated that during cortical development GABA-mediated synaptic inhibition lags behind the development of glutamate-mediated excitation (Burgard and Hablitz 1993a; Kim et al. 1995). The present results demonstrate that GABA\(_A\)-mediated synaptic transmission is present in the perinatal cortex. During early development, spontaneous or evoked GABA\(_A\)-mediated synaptic potentials depolarize postsynaptic cells (Agmon et al. 1996; Owens et al. 1996). Depolarizing effects of GABA have been reported in developing neurons from a number of brain regions including the hippocampus (Ben-Ari et al. 1989; Cherubini et al. 1990), spinal cord (Reichling et al. 1994; Rohrbaugh and Spitzer 1996; Wang et al. 1994; Wu et al. 1992), cerebellum (Brickley et al. 1996; Connor et al. 1987), olfactory bulb (Serafini et al. 1995), hypothalamus (Chen et al. 1996), and retina (Yamashita and Fukuda 1993), strongly suggesting a general role for GABA-mediated depolarization during development. This has led to the suggestion that, in the immature brain, fast synaptic transmission is mediated by GABA\(_A\)-receptors (Cherubini et al. 1991). However, it should be emphasized that depolarization and excitation are not necessarily equivalent. Data presented here suggest that in the developing neocortex GABA-induced depolarization can excite, that is, trigger action potential discharge, in presynaptic cells because GABA-evoked PSCs can be blocked by TTX. The excitatory effect of GABA\(_A\)-receptor activation is likely due to E\(_{Cl}\), which can be above spike threshold in some immature neurons (Owens et al. 1996). Whether GABA\(_A\)-receptor activation inhibits postsynaptic cells depends on E\(_{Cl}\) and the resting membrane potential. In cases where E\(_{Cl}\) is more positive than the resting potential but more negative than action potential threshold, depolarizing GABA\(_A\)-mediated responses can produce inhibition by shunting other conductances (Kaila 1994; Mody et al. 1994). However, even when GABA\(_A\) currents produce shunting inhibition in immature neurons, the resulting membrane depolarization can facilitate action potential discharge to a subsequent excitatory input that occurs during the falling phase of the GABA\(_A\) response (Chen et al. 1996; Gao et al. 1998). As |Cl\(^-|\), decreases perinatally, the excitatory effects of GABA diminish, and the net effect of synaptic GABA\(_A\)-receptor activation becomes inhibitory even though GABA may still depolarize the cell membrane. This is reflected by the ability of BMI to induce epileptiform activity, which develops during the latter part of the first postnatal week (Burgard and Hablitz 1993a; Kriegstein et al. 1987). Therefore in the early postnatal cortex, GABA\(_A\)-receptor activation may excite cells, but it is also likely to inhibit or facilitate other excitatory inputs depending on their temporal pattern.

Concluding remarks

The current report adds to a growing body of literature that suggests GABA-mediated signaling plays a role in the development of neural structures. This role of GABA may be unrelated to its importance as a mediator of fast synaptic inhibition in the mature nervous system. Initially, the trophic effects of GABA were thought to be mediated by membrane hyperpolarization or inhibitory action (Meier et al. 1991). It now appears that GABA-mediated developmental effects are dependent on membrane depolarization (Berninger et al. 1995; LoTurco et al. 1995). A role for GABA in nervous system development is supported by observations in cortex that show that GABAergic signaling occurs well before the onset of synaptic inhibition. Many of the growth-related effects may rely on nonsynaptic or paracrine receptor activation. Interestingly, GABA signaling systems are present in invertebrates including flatworms and snails (Bargmann 1998; Morse et al. 1980), demonstrating that a signaling role for GABA has been conserved through evolution. Moreover, in mollusks GABA exerts its effects through membrane depolarization (Trapido-Rosenthal and Morse 1986). It is thus possible that a GABA signaling pathway arose in ancient organisms to serve a trophic role the effects of which on growth or gene expression were dependent on depolarizing membrane effects. As neural assemblies evolved, GABA may have acquired a new role as an inhibitory synaptic transmitter possibly as a consequence of a change in neuronal Cl\(^-\) homeostasis.

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