Postnatal development of synaptic properties of the GABAergic projection from the inferior colliculus to the auditory thalamus

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Venkataraman Y, Bartlett EL. Postnatal development of synaptic properties of the GABAergic projection from the inferior colliculus to the auditory thalamus. J Neurophysiol 109: 2866–2882, 2013. First published March 27, 2013; doi:10.1152/jn.00021.2013.—The development of auditory temporal processing is important for processing complex sounds as well as for acquiring reading and language skills. Neuronal properties and sound processing change dramatically in auditory cortex neurons after the onset of hearing. However, the development of the auditory thalamus or medial geniculate body (MGB) has not been well studied over this critical time window. Since synaptic inhibition has been shown to be crucial for auditory temporal processing, this study examined the development of a feedforward, GABAergic connection to the MGB from the inferior colliculus (IC), which is also the source of sensory glutamatergic inputs to the MGB. IC-MGB inhibition was studied using whole cell patch-clamp recordings from rat brain slices in current-clamp and voltage-clamp modes at three age groups: a prehearing group [postnatal day (P)7–P9], an immediate posthearing group (P15–P17), and a juvenile group (P22–P32) whose neuronal properties are largely mature. Membrane properties matured substantially across the ages studied. GABA_A and GABA_B inhibitory post synaptic potentials were present at all ages and were similar in amplitude. Inhibitory postsynaptic potentials became faster to single shocks, showed less depression to train stimuli at 5 and 10 Hz, and were overall more efficacious in controlling excitability with age. Overall, IC-MGB inhibition becomes faster and more precise during a time period of rapid changes across the auditory system due to the codevelopment of membrane properties and synaptic properties.

Rapid changes in anatomy and physiology occur throughout the postnatal auditory pathway during development, with each auditory nucleus exhibiting a unique developmental profile. The developing auditory cortex (AC), in particular, has been the focus of several studies. Cortical sound-evoked response properties (Chang et al. 2005; de Villers-Sidani et al. 2007; Dorrn et al. 2010; Rosen et al. 2010; Sun et al. 2010; Zhang et al. 2003) as well as cellular intrinsic and synaptic properties (Oswald and Reyes 2011, 2008; Takesian et al. 2012, 2010) change sharply with age. The auditory thalamus is the primary sensory input to the AC. The importance of thalamic inputs in shaping AC development has been shown by a study that found that visual orientation maps develop in the AC after rerouting retinal inputs in place of collicular inputs to the auditory thalamus (Sharma et al. 2000). However, it is unclear whether the maturation of auditory cortical responses reflects the concurrent development of thalamic neurons, because development of the auditory thalamus has not been well studied.

Synaptic inhibition in the auditory system shapes spectral, temporal, and spatial selectivity properties of in vivo sound-evoked responses to simple and complex sounds (Chang et al. 2005; D’Angelo et al. 2005; LeBeau et al. 2001; Razak and Fuzessery 2009; Wang et al. 2002; Williams and Fuzessery 2011). Developmental studies in the superior olivary and lateral lemniscal nuclei have shown that inhibitory synaptic properties generally become faster and more precise with age, with each auditory synapse following a unique developmental timetable (Ammer et al. 2012; Awatramani et al. 2005; Chirila et al. 2007; Kapfer et al. 2002; Magnusson et al. 2005; Noh et al. 2010; Walcher et al. 2011). However, there is a strong inhibitory connection from the inferior colliculus (IC) to the auditory thalamus (Peruzzi et al. 1997; Winer et al. 1996), whose development has not been studied to date and is the focus of this study.

The connectivity of the auditory thalamus or medial geniculate body (MGB) is intriguing because it is both excited and inhibited by the IC. Excitatory information to MGB neurons is conveyed via glutamatergic inputs from the IC. However, the MGB also receives a unique, feedforward, monosynaptic, GABAergic input from the IC, which constitutes approximately one-third of the total projections from the IC to the rat and cat MGB (Bartlett and Smith 1999; Peruzzi et al. 1997; Winer et al. 1996). The IC-MGB inhibitory connection consists predominantly of large GABAergic neurons in the central nucleus of the IC, dorsal and lateral cortices on the input side (Geis and Borst 2012; Ito et al. 2009; Ito and Oliver 2012; Peruzzi et al. 1997), that project to thalamocortical relay neurons mainly in the MGB ventral (MGBv) and dorsal (MGBd) subdivisions (Bartlett and Smith 1999; Lee and Sherman 2010; Peruzzi et al. 1997). These are the two largest subdivisions in MGB and are considered to be lemniscal and nonlemniscal, respectively (Anderson and Linden 2011; Bartlett and Wang 2011; Bordi and LeDoux 1994; Calford 1983; Calford and Aitkin 1983; Edeline et al. 1999; Morel et al. 1987).

A feedforward inhibitory connection such as the IC-MGB input is not present in the visual or somatosensory thalamic nuclei, and its function in the auditory system is unknown. The IC-MGB GABAergic input is difficult to isolate in vivo, due to the presence of GABAergic inputs from the auditory sector of the thalamic reticular nucleus (TRN) (Bartlett and Smith 1999; Bartlett et al. 2000; Montero 1983; Zhang et al. 2008). However, the two inhibitory connections are spatially separated in a brain slice preparation. The overall aim of this study was to...
characterize the IC-MGB inhibitory synapse during development, when sound-evoked responses throughout the auditory system are known to become sharper and improve in fidelity. The overall hypothesis of this study was that IC-MGB inhibitory response properties will undergo developmental changes rapidly around hearing onset. Specifically, we expected age-related increases in the amplitude of inhibition, fidelity to repetitive stimulation, and overall potency in inhibiting action potentials, especially shortly after hearing onset, at approximately postnatal day (P)12 in rats (Blatchley et al. 1987). To test the above hypotheses, the physiology of the IC-MGB inhibitory synapse was studied at different ages using whole cell patch-clamp techniques in current-clamp and voltage-clamp modes, recording from neurons in brain slices containing MGB.

METHODS

Subjects

All protocols were approved by the Purdue Animal Care and Use Committee (no. 06-105). Pregnant Long-Evans hooded rats (Harlan) were monitored to determine the date of birth (designated as P0). Experiments were performed at three age groups: P7–P9, P15–P17, and P22–P32. Since hearing onset in rats can occur anytime between P11 and P13, the P7–P9 and P15–P17 ages in this study were chosen to represent pre- and posthearing onset groups, respectively. Rats were weaned at P21 and housed in small groups of two to four animals.

Surgery

Rats were overdosed with Beuthanasia and confirmed to be fully areflexive to a foot pinch. The brain was removed in ice-cold oxygenated sucrose solution and sectioned using a Leica VT-1000S microtome (Leica Microsystems). Horizontal sections were obtained at a thickness of 300–350 μm at P7–P9 and 400–450 μm at all other ages. Slices were transferred to oxygenated artificial cerebrospinal fluid (aCSF) at 35°C for 30–55 min and kept at room temperature thereafter (Moyer and Brown 1998). To improve slice health for the P22–P32 group, two additional measures were used: 1) a sucrose-saline solution (described below) was transcardially perfused through the left ventricle while the right auricle was cut (Aghajanian and Rasmussen 1989; Moyer and Brown 1998). To improve slice health for the P22–P32 group, two additional measures were used: 1) a sucrose-saline solution (described below) was transcardially perfused through the left ventricle while the right auricle was cut (Aghajanian and Rasmussen 1989; Moyer and Brown 1998) and 2) in some experiments, 2 mM kynurenic acid was added to the sucrose-saline solution to reduce glutamate-induced excitotoxicity (Li et al. 1999; Richardson et al. 2011).

Solutions

Concentrations of extracellular and intracellular solutions were adapted from Paul and Cox (2010). The intracellular solution consisted of (in mM) 120 K-glucuronate, 4 KCl, 1 MgCl₂, 0.07 CaCl₂, 0.1 EGTA, 10 HEPES, 2 disodium ATP, and 0.4 sodium GTP with 0.03–0.05% Neurobiotin (pH of 7.2 and osmolarity of 290 mosM). Recording aCSF consisted of (in mM) 2.5 KCl, 1.25 MgCl₂, 2 CaCl₂, 1.25 NaHPO₄, 26 NaHCO₃, 10 glucose, and 126 NaCl. Sucrose saline solution differed from aCSF in the concentrations of MgCl₂ (10 mM), CaCl₂ (0.5 mM), and glucose (11 mM) and contained 234 mM sucrose instead of NaCl. The chemicals used were purchased from Sigma-Aldrich. The following drugs were used: glutamatergic blockers 6.7-dinitroquinoxaline-2,3-dione (DNQX; 25–50 μM, Sigma-Aldrich) and dl-2-amino-5-phosphono-pentanoic acid (APV; 50 μM, Sigma-Aldrich and Santa Cruz Biotechnology), GABA_A receptor antagonist picROTOXIN (100 μM, Sigma-Aldrich), and GABA_B receptor antagonist CGP-52432 (1 μM, Tocris Bioscience).

Recordings

Slices were transferred to a RC 27 LD chamber (Warner Instruments) continuously perfused with oxygenated aCSF and maintained at 35 ± 2°C using a feedback controlled inline heater (Warner Instruments). A bipolar stimulating electrode (concentric at ages P7–P9 and P15–P17; parallel at ages P22–P32) was placed in the brachium of the IC to stimulate afferent axons from the IC, either in single shocks or repetitively. Bipolar stimulation electrodes were coupled to a Grass stimulator and a stimulus isolation unit (Grass Instruments).

Recording glass electrodes (World Precision Instruments) were pulled to have a resistance within the range of 1.5–4 MΩ (P97, Sutter Instruments) and guided using a micromanipulator (Siskiyou) to MGB neurons visualized with differential interference contrast optics (Olympus BX51-Wi). After a stable seal resistance >1 GΩ had been obtained, whole cell configuration was obtained through brief mouth suction.

Passive membrane properties were determined from a small hyperpolarizing current (−40 or −50 pA in amplitude and 200 or 250 ms in duration). Synaptic responses of the cell were first obtained in normal aCSF with single shocks (or repetitive shocks in a few cells). This was done to characterize the cell based on the presence of excitatory post-synaptic potentials (EPSPs) and/or inhibitory post-synaptic potentials (IPSPs). After initial characterization, the glutamatergic blockers APV and DNQX were added to record isolated IPSPs. In certain experiments, picrotoxin and/or CGP-52432 were added to block GABA_A and GABA_B receptors, respectively. In the voltage-clamp mode, only isolated GABA_A currents were recorded with APV + DNQX + CGP-52432. Recordings were made 10 min after drug addition.

To visualize IPSPs clearly, recordings were made from a base membrane potential (before synaptic stimulation) of approximately −50 mV for all protocols, adjusted by small current injection. At P7–P9, Cl⁻ reversal potentials were depolarized, resulting in reversal near −50 mV in two to three cells, for which base potentials were suitably adjusted. Recruitment of inhibitory fibers was evaluated with single shocks of 50–300 μs in duration. Stimulus duration was chosen for each cell such that antidiromic spikes were avoided at higher stimulation amplitudes and was unchanged throughout the recording session. To study the recruitment of fibers at different stimulation strengths, single shocks were applied at stimulation amplitudes from 10 V to a maximum voltage that did not evoke antidiromic spiking or to a maximum of 150 V, in steps of 10 V. Temporal properties were determined from IPSPs at least −3 mV in amplitude (2 mV in the case of depolarizing P7–P9 IPSPs). Responses to repetitive stimulation were recorded at three stimulation frequencies of 5, 10, and 50 Hz. Stimulation amplitude was adjusted to produce IPSPs at least 3 mV in amplitude when possible to clearly see effects of short-term plasticity. Reversal potential was determined by single shocks by polarizing the membrane around rest. The efficacy of inhibition was measured as the ability of a −5–mV IPSP to block action potentials generated by a 10-ms depolarizing current pulse delivered at various time windows relative to the IPSP (IPSP preceding the current pulse by 500, 200, 100, 50, 25, 10, 5, 3, and 1 ms; IPSP simultaneous with the current pulse, i.e., 0-ms delay). The amplitude of the depolarizing current pulse was set at 30 pA greater than the smallest current amplitude required to reliably generate action potentials across trials. Voltage-clamp protocols were similar to current-clamp protocols, and the clamp potential in voltage-clamp mode was maintained at −50 mV at P7–P9 and P15–P17 and P22–P32. At P7–P9, all cells obtained in voltage-clamp mode reversed near −50 mV (range: −43 to −56 mV), and hence the clamp potential was chosen as −70 mV at this age. Maximal inhibitory post-synaptic currents (IPSCs) differed considerably between age groups, and hence IPSC repetitive responses and temporal properties were determined at a stimulation voltage of 70 V (90 V for one P22–P32 cell) at all ages. The stimulation amplitude of 70 V was typically at (threshold + 40/50) V for most cells (10 of 12 cells across all ages).
Data Analysis

Whole cell recordings were made with a Multi Clamp 700B amplifier (Molecular Devices), filtered at 10 kHz, and digitized at 25 kHz. Voltage-clamp traces were further filtered at 2 kHz offline. Series resistance was monitored via the bridge balance in current-clamp mode using pClamp 10 software (Molecular Devices), and the recording was ceased when values increased above 20 MΩ. In voltage-clamp mode, pipette and cell capacitances were compensated, and 60–70% series resistance compensation was typically performed.

Cells were chosen for analysis if the following criteria were satisfied: 1) a minimum seal resistance of 1 GΩ, 2) series resistance at all times was <20 MΩ, 3) cells were capable of generating action potentials overshooting 0 mV, and 4) resting membrane potential ($V_{rest}$) for P22–P32 cells was more hyperpolarized than −50 mV.

IPSPs (and IPSCs) recorded at increasing stimulation amplitudes consisted of five trials that were averaged at each stimulation voltage. Averaged IPSPs were considered to be nonfailures if 1) the IPSP amplitude was greater than three times the SD of the membrane potential before stimulation and 2) the time of peak IPSP amplitude occurred between 2 and 40 ms poststimulation. Averaged IPSCs were considered to be nonfailures if 1) the IPSC amplitude was greater than three times the SD of the membrane current before stimulation and 2) the time of IPSC peak amplitude occurred between +5 and −5 ms of the time of peak at 70-V stimulation.

IPSP/IPSC temporal properties were determined from single shot data. IPSP latencies were measured from the onset of the stimulus artifact to where the PSP consistently changed the membrane potential (Bartlett and Smith 1999). Rise times were calculated as the 10–90% rise time of IPSPs. Decay times were calculated by a single-exponential fit from the peak to the offset of the IPSP after normalization to a peak amplitude of −1 mV. The fit was a good indicator of the presence of postsynaptic GABAA. In the absence of GABAA, a single exponential was sufficient to fit the decay phase. GABAA IPSC latency, rise, and decay times were similarly measured.

Responses to repetitive stimulation were measured from an average of three trials at each frequency, where the intertrial interval was typically 5 s. Paired-pulse (PP) ratios were measured using two different baseline measurements, referred to as the “individual baseline” and “common baseline” methods. In the individual baseline method, the peak amplitude of each IPSP in a train was calculated relative to the membrane potential 2 ms before the respective stimulus artifact. The PP ratio of the nth IPSP in the train was calculated as (amplitude of IPSP$n$/amplitude of IPSP$1$). In the common baseline method, the peak amplitude of each IPSP in a train was calculated relative to $V_{rest}$ before the first stimulus artifact. The PP ratio of the nth IPSP in the train was calculated as (amplitude of IPSP$n$/amplitude of IPSP$1$). The train recovery time was measured for 50-Hz trains as the total time required for the membrane potential to return to within 0.5 mV of $V_{rest}$. PP ratios of IPSC trains were measured similarly.

The efficacy of inhibition was determined in current-clamp mode, when IPSPs occurred relative to action potentials generated through depolarizing current injection. Three trials were recorded at each time window between the IPSP and the depolarizing current pulse, and the number of spiking failures was counted in each trial. The time window of 100% blockade was measured as the highest time window at which the IPSP blocked spiking across all three trials. The percentage of spikes blocked by inhibition was measured as the total number of spiking failures for windows between 0 and 50 ms divided by the total number of trials between 0 and 50 ms. Statistical comparisons were performed between each pair of age groups (P7–P9 vs. P15–P17, P7–P9 vs. P22–P32, and P15–P17 vs. P22–P32) using the Wilcoxon rank-sum test, and $P < 0.05$ was the minimum criterion for statistical significance. The Wilcoxon signed-rank test was used to compare data pairs in dual drug recordings. The Holm-Bonferroni stepwise correction for multiple comparisons was performed to determine significance [i.e., $P/(correction\ factor)]$, and $P$ values stated in the text are original $P$ values.

Data Presentation

All results are expressed as means ± SE. In the subsequent text, combined GABA$_A$ + GABA$_B$ IPSPs recorded in the presence of aCSF + APV + DNQX are denoted as IPSPs. Isolated GABA$_A$ IPSPs recorded with aCSF + APV + DNQX + CGP-52432 are denoted as GABA$_A$ IPSPs. In voltage-clamp mode, isolated GABA$_A$ IPSCs were recorded (with aCSF + APV + DNQX + CGP-52432) and are denoted as GABA$_A$ IPSCs. In the text, figures, and tables, P8, P16, and P27 are used to denote the age groups P7–P9, P15–P17, and P22–P32, respectively.

Histology and Recording Locations

Typically, one cell was recorded per slice, and the slice was stored in 4% paraformaldehyde immediately after being recorded. Processing for histology was adapted from a protocol provided by Dr. Anne Takesian and Dr. Vibhakar C. Kotak (Takesian et al. 2010). Briefly, slices were washed in 0.12 M PBS, blocks with 1% hydrogen peroxide, and incubated overnight in ABC solution (Vectastain elite kit, Vector Labs) in 0.12 M PBS with 3% Triton X-100. Slices were thoroughly washed the following day, treated with diaminobenzidine, washed overnight, and then mounted on plastic slides with glycerol and PBS at a 1:1 ratio.

MGB subdivision information was identified when possible. Eighteen MGBv and eight MGBd neurons were confirmed across ages based on matching the recorded slice with that of the rat atlas of Paxinos and Watson (2006), so the two largest MGB subdivisions were represented in the recordings. At younger ages, dye spreading often obscured filled neurons. In addition to neurobiotin, the position of the recording electrode was also used to identify the recorded region of the MGB. The majority of recordings at all ages occurred in the caudolateral region closest to thickest projection of IC axons and hence most likely to maintain the IC-MGB synaptic connection.

RESULTS

Passive Membrane Properties

Clear trends emerged in the development of passive membrane properties of MGB neurons (statistics are shown in Table 1). $V_{rest}$ was most depolarized and membrane resistance ($R_{in}$) was highest at P8. $V_{rest}$ hyperpolarized (Fig. 1A) and $R_{in}$ decreased (Fig. 1B) between P8 and P16 and then again between P16 and P27. Paired comparisons between the three age groups revealed highly signif-

Table 1. Development of passive properties

<table>
<thead>
<tr>
<th>Property</th>
<th>P8</th>
<th>P16</th>
<th>P27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>$-34.8 \pm 1.8^{*\dagger} (n = 27)$</td>
<td>$-51.8 \pm 0.9^{\ddagger} (n = 25)$</td>
<td>$-61.2 \pm 0.9 (n = 39)$</td>
</tr>
<tr>
<td>Input resistance, MO</td>
<td>$642.2 \pm 32.7^{*} (n = 27)$</td>
<td>$350.2 \pm 15.2 (n = 25)$</td>
<td>$249.2 \pm 17.3 (n = 39)$</td>
</tr>
<tr>
<td>Membrane time constant, ms</td>
<td>$49.3 \pm 2.7 (n = 24)$</td>
<td>$56 \pm 2.1 (n = 26)$</td>
<td>$33 \pm 1.6 (n = 39)$</td>
</tr>
</tbody>
</table>

Values are shown as means ± SE; $n$, number of cells. $^{*}P < 0.001$, postnatal day (P8 vs. P16; $^{\dagger}P < 0.001$, P8 vs. P27; $^{\ddagger}P < 0.001$, P16 vs. P27.

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significant differences, indicating that each age group represented a distinct developmental time point with respect to $V_{\text{rest}}$ and $R_{\text{in}}$. The trend for the membrane time constant ($\tau$) was different from those of $V_{\text{rest}}$ and $R_{\text{in}}$, because $\tau$ of P8 and P16 neurons did not significantly differ. However, $\tau$ values at both these ages were significantly higher than those of P27 neurons (Fig. 1C). Thus, the membrane was slower to respond to changes in currents in both younger age groups compared with P27 neurons, which influences temporal processing.

The above findings have two main implications in our experimental design:

Implication 1. Postsynaptic currents may be differentially shaped by the membrane at different ages. For instance, a low-amplitude postsynaptic current at P8 in conjunction with the high $R_{\text{in}}$ at this age could still result in a large-amplitude postsynaptic potential that could significantly alter a cell’s spiking response or ability to spike. Moreover, a rapidly changing postsynaptic current at P8 could be significantly slowed by $\tau$, resulting in a slower postsynaptic potential, which, in turn, could affect responses to repetitive stimulation. To address this issue, we recorded both IPSPs in current-clamp mode and IPSCs in voltage-clamp mode.

Implication 2. $V_{\text{rest}}$ dramatically differed with age (Fig. 1A), whereas the Nernst potential for $\text{Cl}^-$ was fixed at $\sim 82$ mV with the solutions that were used and the recording temperature of $\sim 35^\circ$C. The reversal potential for GABA ($E_{\text{GABA}}$) was experimentally measured at each age group and is discussed below. All cells were maintained at a base membrane potential of approximately $-50$ mV while IPSPs and IPSCs were recorded, and the mean base membrane potential was confirmed to be $-51.96 \pm 0.23$ mV before single shocks ($n = 46$ across ages) and $-51.90 \pm 0.25$ mV before repetitive shocks ($n = 39$ across ages). A base membrane potential of approximately $-50$ mV was chosen so that the driving force for $\text{Cl}^-$ would be roughly similar at all ages and additionally to ensure that the $\text{Cl}^-$ driving force was high enough to clearly observe IPSPs. Passive membrane properties were also measured from a base membrane potential of $-50$ mV and showed identical statistical trends during development, as assessed by the $V_{\text{rest}}$ measurements (data not shown). All correlations with IPSP parameters in subsequent sections were made with respect to passive properties measured at $-50$ mV.

**Cell Types/Synaptic Responses**

IC axons were stimulated with single shocks, and postsynaptic potentials were recorded in normal aCSF from a base membrane potential near $-50$ mV (adjusted by current injection as needed). Similar to an earlier study in P21–P42 MGB neurons (Bartlett and Smith 1999), MGB cells were classified based on the presence and latencies of EPSPs and IPSPs, respectively. Purely excitatory (EX/0) cells were rarely encountered in this study. A purely inhibitory (IN/0) response with only an IPSP (Fig. 2A) was observed only in P16 and P27 neurons and not in P8 neurons. Mixed cells with both EPSPs and IPSPs were present at all ages. Mixed cells were classified as IN/EX cells if the IPSP preceded the EPSP (Fig. 2B) and as EX/IN cells in the opposite case (Fig. 2C). At all ages, the EPSP was glutamatergic and eliminated by the addition of APV and DNQX. Figure 2, A–D, bottom, shows the responses of the same cells after excitation was blocked. A variation of the mixed responses was observed in the P8 age group (and in one P15 cell), where the postsynaptic potential consisted of a large depolarizing component that could drive one or multiple action potentials, even at low stimulation amplitudes. An inhibitory component was not readily perceptible in this response type in normal aCSF from a base membrane potential of $-50$ mV (Fig. 2D). This response type was not EX/0 because blockade of glutamatergic receptors with APV and DNQX revealed an underlying IPSP in all cells tested ($n = 21$ of 21 cells). Hence, this response type, which consisted of a dominant excitatory component, was termed as EX$_{\text{DOM}}$. Spiking patterns of EX$_{\text{DOM}}$ cells were sensitive to $V_{\text{rest}}$ and single shocks produced two or more well-defined spikes near $-60$ mV (3 of 4 cells), whereas base membrane potentials near $-65$ mV prevented spiking (6 of 6 cells). In P8 neurons, even large hyperpolarizing IPSPs were masked and dominated by EPSPs.

Figure 2, E–G, shows the proportions of each cell type at the three age groups. Mixed cells predominated at P27 ($n = 22$; Fig. 2E) and P16 ($n = 23$; Fig. 2F), whereas IN/0 cells constituted the remainder. At P8 ($n = 21$; Fig. 2G), 52% were mixed cells and 48% were EX$_{\text{DOM}}$ cells.

IPSPs at all ages were found to be GABAergic. All cells contained a short-latency picrotoxin-sensitive GABA$_A$ component (confirmed in 11 of 11 cells across ages). Most cells (15 of 16 cells at P8, 19 of 19 cells at P16, and 20 of 21 cells at P27) also demonstrated a longer-latency hyperpolarizing component, which occurred in the decay phase of the initial GABA$_A$ component (see Fig. 2D, bottom). This component was sensitive to the GABA$_A$ blocker CGP-52432, as confirmed in 10 of 10 cells across ages. The addition of APV, DNQX, picrotoxin, and CGP-52432 eliminated all postsynaptic potentials to single shocks ($n = 3$ of 3 cells).

**Properties of IC-MGB Inhibition: Reversal and Amplitude**

The reversal potential of the peak IPSP amplitude was calculated at all ages. IPSP reversal potential was significantly more depolarized at P8 ($-57.9 \pm 3.3$ mV, $n = 14$) compared with P16...
from a base membrane potential near Pstatistical significance (glutamate receptors were blocked with amino-5-phosphonopentanoic acid (APV) + 6,7-dinitroquinoxaline-2,3-dione (DNQX). A: a P29 cell with a purely inhibitory response type (IN/0 cell). B: a P16 mixed type cell where the excitatory postsynaptic potential (IPSP) preceded the excitatory postsynaptic potential (EPSP) (IN/EX cell). C: a P17 mixed type cell where the EPSP preceded the IPSP (EX/IN cell). D: a P9 cell with a dominant excitatory component (EXDOM cell). E–G: proportions of cell types at P27 (E), P16 (F), and P8 (G).

(−74.4 ± 2.1 mV, n = 15, P = 0.0004) and P27 (−75.2 ± 3.2 mV, n = 13, P = 0.0009). Further analysis of reversal potentials at P8 showed that EXDOM cells at P8 exhibited more depolarized values (−52.2 ± 5.5 mV, n = 6) compared with mixed cells (−64.1 ± 3.7 mV, n = 7), although this difference did not reach statistical significance (P = 0.07). Consequently, IPSP polarity from a base membrane potential near −50 mV in P8 EXDOM cells was variable, and depolarizing IPSPs were sometimes encountered at P8 (4 of 10 cells). The depolarizing IPSPs at P8 were still inhibitory in that they were unable to drive spiking on their own (in the presence of APV and DNQX), even at stimulation amplitudes equal or higher than those evoking multiple spiking responses in the absence of drugs (n = 4 of 4 cells). This shows that the IPSP reversal potential was below spiking threshold (shunting inhibition).

Only P8 cells that had a clearly hyperpolarizing IPSP from a base membrane potential of −50 mV were included in the analysis for IPSP amplitude (9 of 13 cells) to enable comparisons with the older ages. Increasing stimulation amplitude resulted in gradual increased IPSP amplitudes, denoting the recruitment of fibers at all ages (Fig. 3A). Peak IPSP amplitudes were compared between ages at threshold stimulation and at various suprathreshold levels. Peak IPSP amplitude at threshold or suprathreshold stimulation voltages [calculated at (threshold + 40) V, (threshold + 50) V, and (threshold + 60) V] were not different between any age groups (Table 2). This result indicates that the masking of IPSPs by EPSPs observed only at P8 was not necessarily due to low IPSP amplitudes at this age. Given this finding and the higher Rin values of P8 neurons, synaptic currents were estimated from IPSPs at (threshold + 40) V using cell-specific reversal potential and Rin values. Inhibitory currents estimated in this manner were lower at P8 compared with both older age groups (n = 7, 10, and 6 cells for P8, P16, and P27 respectively) and were significantly so between P8 and P16 (P = 0.01). This trend was also reflected in GABA A IPSCs from voltage-clamp measurements (Fig. 3B and Table 2). Figure 3B also shows the increase in IPSC amplitude with increasing stimulus strength. Overall, these results indicate that IPSC amplitude is lower at P8 but that IPSP amplitudes do not differ with age.

Temporal Properties of IC-MGB Inhibition

The temporal properties of IPSPs and IPSCs evoked by single shocks were analyzed. Voltage-clamp recordings were made in four neurons at each age and are shown as scatterplots that show all cells recorded, mainly to assist interpreting how synaptic currents interact with membrane properties to produce the observed IPSPs. Figure 4, A–C, shows one neuron from each age group from which both voltage-clamp and current-clamp recordings were made where GABA A IPSPs and IPSCs were evoked at the same stimulation parameters for each neuron. Clamp potential was −70 mV at P8 and −50 mV at P16 and P27 (see METHODS). Table 3 shows the temporal properties for the various recording conditions.
**Latency.** IPSP latencies remained long until P17 and decreased thereafter (Fig. 4D). IPSP latencies were similar for P8 neurons (3.0 ± 0.4 ms, n = 11) and P16 neurons (3.2 ± 0.3 ms, n = 17). At both these ages, latencies were significantly longer than those of P27 neurons (1.9 ± 0.1 ms, n = 16, P = 0.0203 between P8 and P27 and P = 0.001 between P16 and P27). Voltage-clamp recordings of isolated GABAA currents in four cells at each age showed similar trends as found in current-clamp recordings (Fig. 4G).

**Rise time.** IPSP rise time decreased between P9 and P15 and did not significantly change after that (Fig. 4E). The IPSP rise time of P8 neurons was 11.6 ± 1.2 ms (n = 11), which was significantly higher than values of P16 neurons (7.3 ± 0.7 ms, n = 17) and P27 neurons (5.8 ± 0.5 ms, n = 16, P = 0.003 between P8 and P16 and P = 0.0003 between P8 and P27). Voltage-clamp recordings of isolated GABAA currents in four cells did not show the same trends as observed in current-clamp recordings and instead showed a slight peaking at P16 (Fig. 4H). These differences may reflect, in part, the age-related changes in GABA receptors.

**Decay time.** The decay phase of the IPSP consisted of both GABAA and (postsynaptic) GABAB and varied greatly between cells depending on the extent of activation of GABAB during the decay phase of GABAA. Hence, decay time was calculated after blockade of GABAA with CGP-52432, and the decay phase of the isolated GABAA IPSP was fitted with an exponential fit. Single exponentials fit the decay phase with R² > 0.97 at all ages. GABAA IPSP decay time decreased after P9 and stabilized by P15 (Fig. 4F). The GABAA IPSP decay time decreased from 138.0 ± 25.7 ms at P8 (n = 4) to 67.6 ± 2.0 ms at P16 (n = 6) and remained stable at P27 (61.1 ± 6.4 ms, n = 6, P = 0.0095 between P8 and P16 and P = 0.019 between P8 and P27). GABAA IPSP rise and decay times were strongly correlated (R = 0.79, P = 0.0003). Voltage-clamp recordings did not reveal a similar clear development trend, with decay times that were similar at all ages and peaking slightly at P16 (Fig. 4I). Thus, the differences in GABAA IPSP decay time may reflect, in part, the age-related changes in τ. The correlation coefficient between τ and IPSP rise time was significant (R = 0.50, P < 0.001). IPSP rise time and amplitude were also correlated (R = 0.57, P < 0.001).

**Temporal Properties of IC-MGB Inhibition: Repetitive Stimulation**

The development of responses to repetitive shocks was examined at three stimulation frequencies of 5, 10, and 50 Hz. These frequencies correspond to those of thalamocortical oscillations.

<table>
<thead>
<tr>
<th>Property</th>
<th>Stimulation Amplitude, V</th>
<th>P8</th>
<th>P16</th>
<th>P27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold IPSP amplitude, mV</td>
<td>Threshold</td>
<td>−1.8 ± 0.4 (n = 9)</td>
<td>−0.8 ± 0.1 (n = 11)</td>
<td>−1.1 ± 0.3 (n = 12)</td>
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<tr>
<td>Suprathreshold IPSP amplitude, mV</td>
<td>Threshold + 40</td>
<td>−5.7 ± 1.2 (n = 9)</td>
<td>−4.3 ± 0.8 (n = 11)</td>
<td>−4.8 ± 0.6 (n = 10)</td>
</tr>
<tr>
<td>Threshold GABAA IPSC amplitude, pA</td>
<td>Threshold</td>
<td>−27.3 ± 5.9 (n = 4)</td>
<td>30.8 ± 10 (n = 4)</td>
<td>25.5 ± 8.7 (n = 4)</td>
</tr>
<tr>
<td>Suprathreshold GABAA IPSC amplitude, pA</td>
<td>Threshold + 40</td>
<td>−39.8 ± 6.4 (n = 4)</td>
<td>125.9 ± 26.9 (n = 5)</td>
<td>128.4 ± 28.1 (n = 4)</td>
</tr>
</tbody>
</table>

Values are shown as means ± SE; n, number of cells. IPSP, inhibitory postsynaptic potential; IPSC, inhibitory postsynaptic current.

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and to frequencies that can demonstrate differences in short-term plasticity. Figure 5 shows examples of repetitive responses under different recording conditions. Figure 5, A–C, shows IPSP trains at 10 Hz at the three age groups. Figure 5, D–F, shows three pairs of isolated GABAA IPSPs/IPSCs at 10 Hz at the three age groups. Each pair shown in Fig. 5 consists of current-clamp and voltage-clamp recordings from the same cell at the same stimulation parameters. Two types of PP measurements were performed on repetitive responses, as detailed in METHODS and as performed in an earlier study (Bartlett and Smith 2002). In

<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>GABAA IPSP Latency (ms)</th>
<th>GABAA IPSP Rise Time (ms)</th>
<th>GABAA IPSP Decay Time (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P8</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>P16</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>P27</td>
<td>2</td>
<td>3</td>
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</table>

<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>GABAA IPSC Latency (ms)</th>
<th>GABAA IPSC Rise Time (ms)</th>
<th>GABAA IPSC Decay Time (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P8</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>P16</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>P27</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 4. Temporal properties of IC-MGB inhibition to single shocks. GABAA and ionotropic glutamate receptors were blocked to isolate GABAA components. A: P9 neuron GABAA IPSC (gray trace, $V_{\text{clamp}} = -70$ mV) and GABAA IPSP (black trace, membrane potential ($V_{\text{mem}}$) = $-72.4$ mV). B: P15 neuron GABAA IPSC (gray trace, $V_{\text{clamp}} = -50$ mV) and GABAA IPSP (black trace). C: P28 neuron GABAA IPSC (gray trace, $V_{\text{clamp}} = -50$ mV) and GABAA IPSP (black trace). In D–F, open bars indicate P8, gray bars indicate P16, and filled bars indicate P27. Error bars are SEs. D: mean IPSP latency at the ages shown. E: mean IPSP rise time. F: mean GABAA IPSP decay time. In G–I, each symbol represents a measurement from an individual neuron. G: GABAA IPSC latency. H: GABAA IPSC rise time. I: GABAA IPSC decay time.
the text below, stimulation number refers to the position of the IPSP in a train of 10 pulses.

**PP ratios using the individual baseline method.** In the first type of measurement, referred to as the individual baseline method, IPSP (IPSC) amplitudes were measured from a baseline membrane potential (current in voltage clamp) that immediately preceded the stimulus artifact for that IPSP (IPSC) (see Fig. 6A). PP ratios calculated using the individual baseline method are a typical measure of synaptic short-term plasticity and showed clear developmental trends, which are shown in Table 4. Even at a low stimulation frequency of 5 Hz, IPSPs of P8 neurons depressed rapidly, as shown in Fig. 6B. PP ratios for all IPSPs in the train (relative to IPSP) at P8 were significantly lower than those of P16 (P < 0.001). PP ratios at P8 were comparable with those of P27 for the first 3 pulses but were significantly lower than those of P27 from the 4th through 10th IPSP in the train (P < 0.01). One surprising result at 5 Hz was that P16 neurons showed significantly lesser depression than P27 neurons at several IPSPs in the train.

At a stimulation frequency of 10 Hz, P8 MGB neurons were again significantly more depressed than P16 and P27 MGB neurons for most IPSPs in the train (P < 0.05; Fig. 6C). At 10 Hz, the ratios between P16 and P27 neurons were quite similar.

At 50 Hz, rapid depression was observed at all ages. PP ratios were slightly higher (i.e., depression was lower) at P27 than other ages (Fig. 6D), but significant differences were generally not observed.

When repetitive stimulation was performed after blockade of GABAB, the developmental trends in GABAA IPSP PP ratios from an individual baseline were similar to those observed with the IPSP PP ratios described above (compare Fig. 6, E–G with B–D). In voltage-clamp mode, GABAA IPSC PP ratios (Fig. 6, H–J) at 5 and 50 Hz showed similar trends as observed in current-clamp mode (n = 4, 4, and 3 for P8, P16, and P27, respectively). At 10 Hz, trends were not clear due to greater variability at the youngest age (Fig. 6f). Additionally, IPSCs/C IPSC at 10 Hz showed facilitation or was close to 1 at P8 alone. Overall, P8 neurons showed greater depression to repetitive stimulation at 5 and 10 Hz, while all ages showed depression at 50 Hz.

**PP ratios using the common baseline method.** In the second type of PP ratio measurement, which we refer to as the common

### Table 3. Development of IPSP and IPSC amplitudes

<table>
<thead>
<tr>
<th>Latency</th>
<th>P8</th>
<th>P16</th>
<th>P27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined IPSP latency, ms</td>
<td>3.0 ± 0.4(^{*}) (n = 11)</td>
<td>3.2 ± 0.3(^{*}) (n = 17)</td>
<td>1.9 ± 0.1 (n = 16)</td>
</tr>
<tr>
<td>GABAA IPSP latency, ms</td>
<td>2.9 ± 0.7 (n = 4)</td>
<td>3.3 ± 0.6 (n = 6)</td>
<td>2.0 ± 0.2 (n = 6)</td>
</tr>
<tr>
<td>Latency of GABAA IPSC, ms</td>
<td>3.2 ± 0.2 (n = 4)</td>
<td>3.0 ± 0.3 (n = 4)</td>
<td>2.2 ± 0.4 (n = 4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rise time</th>
<th>P8</th>
<th>P16</th>
<th>P27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined IPSP rise time, ms</td>
<td>11.6 ± 1.2(^{*}) (n = 11)</td>
<td>7.3 ± 0.7 (n = 17)</td>
<td>5.8 ± 0.5 (n = 16)</td>
</tr>
<tr>
<td>GABAA IPSP rise time, ms</td>
<td>14.5 ± 2.5 (n = 4)</td>
<td>9.2 ± 1.8 (n = 6)</td>
<td>7.1 ± 0.6 (n = 6)</td>
</tr>
<tr>
<td>GABAA IPSC rise time, ms</td>
<td>1.7 ± 0.3 (n = 4)</td>
<td>3.4 ± 0.2 (n = 4)</td>
<td>2.9 ± 0.5 (n = 3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decay time</th>
<th>P8</th>
<th>P16</th>
<th>P27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decay time of GABAA IPSP from all cells, ms</td>
<td>138.0 ± 25.7(^{*}) (n = 4)</td>
<td>67.6 ± 2 (n = 6)</td>
<td>61.1 ± 6.4 (n = 6)</td>
</tr>
<tr>
<td>Decay time of GABAA IPSC, ms</td>
<td>8.9 ± 1.1 (n = 4)</td>
<td>12 ± 1.4 (n = 4)</td>
<td>10.8 ± 1.7 (n = 4)</td>
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</table>

Values are shown as means ± SE; n, number of cells. \(^{a}\)P < 0.01, P8 vs. P16; \(^{b}\)P < 0.05, \(^{c}\)P < 0.01, and \(^{d}\)P < 0.001, P8 vs. P27; \(^{*}\)P < 0.01, P16 vs. P27.

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Fig. 5. Examples of inhibitory responses to repetitive shocks of IC axons. A–C. IPSP trains (recorded with ACSF + APV + DNQX) at P8 (A), P16 (B), and P32 (C). D–F. GABA\(_B\) and ionotopic glutamate receptors were blocked to isolate GABA\(_A\) components. Three pairs of GABA\(_A\) IPSC trains (top traces) and GABA\(_A\) IPSP trains (bottom traces) recorded at P9 (D; \(V_{mem} = -71.9\) mV and \(V_{clamp} = -70\) mV), P15 (E; \(V_{clamp} = -50\) mV), and P28 (F; \(V_{clamp} = -50\) mV). The same stimulation parameters were used for each pair in current-clamp and voltage-clamp modes.
baseline method, a common baseline [determined from the membrane potential (current) preceding the first IPSP (IPSC)] was used to measure IPSP (IPSC) amplitudes in a train (see Fig. 7A). Inhibitory PP ratios calculated using the common baseline method provide a measure of the net membrane hyperpolarization resulting from summation during a train. Common baseline PP ratios did not reveal developmental trends, and hence data were pooled across ages. Overall, there appeared to be two populations of responses from this analysis depending on the extent of hyperpolarization observed at the end of the train, which can be obtained from the IPSP_{10} to IPSP_{1} ratio. A histogram of common baseline IPSP_{10} to IPSP_{1} ratios (Fig. 8A, pooled across ages) showed a wide range of values. In contrast, individual baseline IPSP_{10} to IPSP_{1} ratios (Fig. 8D) generally clustered around 0.5.

In the majority of responses (called type I), the common baseline IPSP_{10} to IPSP_{1} ratio was >0.5 because IPSPs summed, resulting in a hyperpolarization lasting until the last pulse in the train. Figure 7B shows a type I response at P26 at 5-Hz stimulation, where membrane hyperpolarization at the 10th pulse measured from a common baseline (dotted line) was similar to that at the beginning of the train, although the amplitude of IPSP_{10} measured from an individual baseline was clearly smaller. In type II responses, membrane hyperpolarization was reduced in the latter part of the train, and IPSP_{10} to IPSP_{1} ratios were <0.5. Figure 7C shows a type II response at P25 at 5-Hz stimulation, where the membrane potential returned near the prestimulus baseline by the end of the IPSP train. Figure 7, D–F, shows mean IPSP PP ratios calculated from a common baseline at 5, 10, and 50 Hz, respectively. The filled circles show type I PP ratios that remained steady across the train, and the gray circles show the contrasting type II PP ratios that depressed across the train. Similarly, Fig. 7, G–I, shows mean GABA\textsubscript{A} IPSC PP ratios of type I responses (filled circles) and type II responses (gray circles) in voltage-clamp mode.

<table>
<thead>
<tr>
<th>Stimulation frequency</th>
<th>5 Hz</th>
<th>10 Hz</th>
<th>50 Hz</th>
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<tr>
<td><strong>Stimulation frequency</strong></td>
<td>5 Hz</td>
<td>10 Hz</td>
<td>50 Hz</td>
</tr>
<tr>
<td>IPSP/IPSP\textsubscript{1}</td>
<td>0.53 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78 ± 0.03&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.66 ± 0.03</td>
</tr>
<tr>
<td>IPSP\textsubscript{10}/IPSP\textsubscript{1}</td>
<td>0.31 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.68 ± 0.05&lt;sup&gt;r&lt;/sup&gt;</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>IPSP\textsubscript{10}/IPSP\textsubscript{1}</td>
<td>0.23 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.63 ± 0.04&lt;sup&gt;r&lt;/sup&gt;</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td><strong>Stimulation frequency</strong></td>
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<td>50 Hz</td>
<td></td>
</tr>
<tr>
<td>IPSP/IPSP\textsubscript{1}</td>
<td>0.52 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.60 ± 0.02</td>
<td>0.64 ± 0.03</td>
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<tr>
<td>IPSP\textsubscript{10}/IPSP\textsubscript{1}</td>
<td>0.34 ± 0.05</td>
<td>0.45 ± 0.02</td>
<td>0.45 ± 0.05</td>
</tr>
<tr>
<td>IPSP\textsubscript{10}/IPSP\textsubscript{1}</td>
<td>0.23 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.41 ± 0.03</td>
<td>0.38 ± 0.03</td>
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<tr>
<td><strong>Stimulation frequency</strong></td>
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<td></td>
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<tr>
<td>IPSP/IPSP\textsubscript{1}</td>
<td>0.33 ± 0.07</td>
<td>0.4 ± 0.06</td>
<td>0.32 ± 0.04</td>
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<tr>
<td>IPSP\textsubscript{10}/IPSP\textsubscript{1}</td>
<td>0.06 ± 0.03</td>
<td>0.09 ± 0.04</td>
<td>0.13 ± 0.05</td>
</tr>
<tr>
<td>IPSP\textsubscript{10}/IPSP\textsubscript{1}</td>
<td>0.07 ± 0.03</td>
<td>0.09 ± 0.04</td>
<td>0.11 ± 0.03</td>
</tr>
</tbody>
</table>

Values are shown as means ± SE; n, number of cells. *P < 0.01 and *P < 0.001, P8 vs. P16; †P < 0.05 and †P < 0.01, P8 vs. P27; ‡P < 0.05 and ‡P < 0.01, P16 vs. P27.
Based on IPSP_{10}\text{-to-}IPSP_{1} values, type I responses were the dominant response type observed (5 Hz: 5 of 9 cells at P8, 10 of 11 cells at P16, and 9 of 11 cells at P27; 10 Hz: 5 of 9 cells at P8, 9 of 10 cells at P16, and 8 of 11 cells at P27). A developmental trend was observed with our sample at 5 and 10 Hz, with similar numbers of type I and type II responses only at P8, in contrast to the older ages, where type I numbers were much greater, although differences were not significant. At 50 Hz, only one cell at P16 (gray circles in Fig. 7F) showed a type II IPSP response, and the remainder (n = 30 across ages) were type I responses, indicating the greater summation of synaptic potentials at increasing stimulation frequencies.

**Modulation of GABA_A by GABA_B**

Dual drug changes were made in a subset of cells to determine the effect of GABA_B blockade on IPSP properties. Responses were first obtained with aCSF + APV + DNQX and then with aCSF + APV + DNQX + CGP-52432 to block GABA_B receptors, using the same stimulation parameters. Results were similar across ages, and hence data were pooled. GABA_B can modulate GABA_A inhibitory responses via presynaptic receptors through presynaptic Ca^{2+} regulation (Takahashi et al. 1998). GABA_B can also modulate inhibitory responses via postsynaptic receptors, which are visible in the cell’s synaptic potential.
response as a long-lasting postsynaptic GABA_B IPSP. CGP-52432 added to aCSF can block both pre- and postsynaptic GABA_B receptors. However, the two GABA_B receptor mechanisms may be roughly differentiated based on the latency of activation of the postsynaptic GABA_B response. Single shocks activated the postsynaptic GABA_B IPSP with a latency of \( \approx 50 \) ms (data recorded with aCSF + APV + DNQX + picrotoxin; data not shown). Hence, modulation of the GABA_A IPSP peak amplitude that occurs before 50 ms may be attributed to presynaptic GABA_A, whereas blockade of late hyperpolarization may be attributed to postsynaptic GABA_B.

**Modulation of IPSP amplitude by GABA_B**. Blockade of GABA_B significantly increased the peak GABA_A IPSP amplitude compared with the IPSP amplitude observed without CGP-52432 \((P = 0.04, n = 10\) pooled across ages and at a stimulation amplitude of 40–50 V above threshold). The ratio of peak GABA_A IPSP amplitude (with CGP-52432) to peak IPSP amplitude (without CGP-52432) was \( 1.5 \pm 0.2 \). Figure 9A, **left**, shows IPSPs evoked at (threshold + 90) V stimulation from a P28 neuron. The black trace shows responses in aCSF + APV + DNQX, whereas the gray trace shows the increase in amplitude after CGP-52432 was added. Figure 9A, **right**, shows the responses of the same neuron shown in Fig. 9A, **left**, at different stimulation levels relative to threshold in the absence (filled circles) and presence (gray stars) of CGP-52432. The effect of CGP-52432 was greater at increasing stimulation amplitudes.

**Modulation of IPSP temporal parameters by GABA_B**. GABA_B blockade with CGP-52432 did not significantly affect IPSP latency or rise time \((n = 11\) pooled across ages). Removal of the postsynaptic GABA_B component significantly reduced the overall IPSP duration \((P = 0.0039, n = 9\) pooled across ages). The reduction in duration was variable between cells and depended on the extent of activation of postsynaptic GABA_B but was typically several hundreds of milliseconds (7 of 9 cells). In the only P23 cell that did not have a postsynaptic GABA_B component in this study, IPSP durations before \((102.2\) ms) and after \((113.2\) ms) CGP-52432 did not differ. Figure 9B shows the effects of GABA_B blockade on the IPSP of a P17 neuron, with the black and gray traces showing recordings in the absence and presence of CGP-52432, respectively. Figure 9C shows the waveform obtained by subtracting the gray trace in Fig. 9B from the black trace in Fig. 9B. The positive component of the difference waveform shows the increase in peak IPSP amplitude after GABA_B blockade, whereas the negative longer-lasting component shows the reduction in IPSP duration after GABA_B blockade.

**Modulation of repetitive responses by GABA_B**. Although presynaptic GABA_A receptors can potentially modulate inhibitory PP ratios (measured by the individual baseline method) (Takesian et al., 2010), no consistent effect of GABA_B blockade was seen in current-clamp mode for paired pulse ratio 2 (PP2) at 5, 10, or 50 Hz.

The postsynaptic GABA_A component is strongly activated in response to high-frequency stimulation. Removal of the postsynaptic GABA_A component can reduce the time required for the membrane potential to return to \( V_{rest} \) after the cessation of stimulation, which significantly influences the firing properties of thalamocortical neurons. Paired comparisons between pre- and post-CGP-52432 recovery times showed a significant reduction in recovery time after GABA_A receptors were blocked \((P = 0.01, n = 9\) ). The extent of reduction depended on the extent of GABA_A activation and was >250 ms in 7 of 9 neurons. Figure 9D shows the reduction in recovery time at 50-Hz stimulation in a P16 neuron, after GABA_A receptors were blocked. Since postsynaptic GABA_B had a strong effect on IPSP recovery time after high-frequency stimulation, we examined the recovery times of isolated GABA_B IPSPs (recorded in the presence of APV + DNQX + picrotoxin; see Fig. 9E) in P16 neurons. Mean recovery time was 1,083.9 ± 198.7 ms at 50-Hz stimulation \((n = 3\) ) and 872.6 ± 55 ms at 100-Hz stimulation \((n = 4\) ). Additionally, GABA_A recovery time was strongly correlated with peak GABA_B amplitude \((R = 0.86, P = 0.01\) ).

**Efficacy of Inhibition**

**Inhibition and intrinsic spikes**. Using injected currents as a proxy for excitatory inputs, the efficacy of inhibition was tested by presenting IPSPs simultaneously or at a fixed time window preceding the onset of suprathreshold depolarizing current pulses of 10 ms in width. These durations are comparable with IC-evoked excitatory postsynaptic currents (EPSCs) (Y. Venkataraman, C. F. Rabang, and E. L. Bartlett, unpublished observations). The base membrane potential before the start of the protocol was held at approximately \(-50\) mV for all cells (mean membrane potential for this protocol was \(-51.8 \pm 0.9\) mV at P8, \(-52.5 \pm 0.8\) mV at P16, and \(-52.3 \pm 1.0\) mV at P27, means ± SD). The synaptic stimulation amplitude was adjusted to produce an IPSP of approximately \(-5\) to \(-6\) mV in amplitude at all ages \((-5.0 \pm 0.8\) at P8, \(-6.3 \pm 0.6\) at P16, and \(-5.8 \pm 1.6\) at P27). The amplitude of the injected current pulse was 30 pA greater than the spiking threshold for the recorded neuron, and its ability to produce spikes was confirmed to be 100% across trials at the beginning and end of the protocol.

**Fig. 8.** Histograms of PP ratios at the end of a stimulus train using the common baseline and individual baseline methods. **A**. histogram of cells (pooled across ages) based on IPSP_10/IPSP_1 (IPSP_0/IPSP_1) ratios measured using the common baseline method. An IPSP_0/IPSP_1 ratio of 0.5 was used to differentiate a type 1 response (IPSP_0/IPSP_1 ≥ 0.5) and a type II response (IPSP_0/IPSP_1 < 0.5). **B**. histogram of cells (pooled across ages) based on IPSP_10/IPSP_1 ratios measured using the individual baseline method.
At all ages, simultaneous presentation of action potentials and IPSPs resulted in the suppression of action potentials (n = 3, 4, and 4 at P8, P16, and P27, respectively). When the IPSP preceded the current pulse at windows ranging from 500 to 1 ms, the maximum time window at which spikes were completely blocked across trials was calculated (Fig. 10A). This time window was higher at P8 (28.3 ± 11.7 ms, n = 3) and P16 (26.2 ± 9.2 ms, n = 4) versus P27 (10.3 ± 5.3 ms, n = 4). The total percentage of spikes blocked across all trials from time windows of 50 to 0 ms was calculated at each age group (Fig. 10B). This was also higher at P8 and P16 than at P27. Taken together from these data, IPSPs at P8 and P16 are equally or more efficacious than P27 neurons in blocking single action potentials evoked by near-threshold current injection that simulate EPSCs.

Inhibition and synaptic spikes. The ability of IPSPs to block action potentials of synaptic origin was tested in mixed type cells with excitatory and inhibitory responses by recording synaptic responses in the absence of any drugs from a base membrane potential near −50 mV. In 22 mixed P8 neurons, 5 of 22 cells did not spike, although they did produce depolarizing postsynaptic potentials. Of the remaining mixed P8 neurons, 5 of 22 cells did not spike, although they did produce depolarizing postsynaptic potentials. Of the remaining mixed P8 neurons, 5 of 22 cells did not spike, although they did produce depolarizing postsynaptic potentials. Of the remaining mixed P8 neurons, 5 of 22 cells did not spike, although they did produce depolarizing postsynaptic potentials. Of the remaining mixed P8 neurons, 5 of 22 cells did not spike, although they did produce depolarizing postsynaptic potentials.
neurons, 17 of 22 neurons produced one spike \((n = 3)\), two spikes \((n = 6)\), or multiple spikes \((n = 8)\). At \(-50\) mV, many of the late spikes observed in P8 neurons were broad. The black traces in Fig. 10, C and D, show a P9 neuron that produced multiple action potentials from a base membrane potential of \(-50\) mV (Fig. 10C) and also at \(-60\) mV (Fig. 10D) to a single shock. The gray traces in Fig. 10, C and D, show the underlying IPSP uncovered with the addition of APV and DNQX to aCSF. These responses can be contrasted to the single spiking response of a P17 neuron in aCSF (black trace, top) and the underlying IPSP in aCSF + APV + DNQX (gray trace, bottom).

**DISCUSSION**

Here, we studied the postnatal development of MGB membrane properties in conjunction with the development of the IC-MGB inhibitory connection. We found that 1) \(R_m\) and \(\tau\) decreased with age, whereas \(V_{rest}\) became more hyperpolarized with age; 2) IC-MGB IPSPs were present from the earliest age recorded (P7) and surprisingly did not differ in amplitude with age; 3) IPSP latency, rise time, and decay time decreased with age.
and McCormick 1994), and rat ventrobasal (VB) nucleus (Lee potentials during development have also been reported in the oldest age used in that study). The decrease in the rat MGB, Tennigkeit et al. (1998) also reported a decrease in \( R_{\text{in}} \) and \( \tau \) during the first 2 wk (although P21 was the oldest age used in that study). The decrease in \( \tau \) with age may be attributable in part to the decrease in \( R_{\text{in}} \) (\( R = 0.53, P < 0.001, n = 87 \) cells across ages). Depolarized membrane potentials during development have also been reported in the rat MGB (Tennigkeit et al. 1998), ferret and mouse dorsal lateral geniculate nucleus (LGN) (MacLeod et al. 1996; Ramoa and McCormick 1994), and rat ventrobasal (VB) nucleus (Lee et al. 2010; Perez Velazquez and Carlen 1996). Changes in \( V_{\text{rest}} \), with age may be attributed to ion gradients maintained between hyperpolarization-activated cation channels and K\(^+\) leak channels (Meuth et al. 2006) as well as Cl\(^-\) extrusion via the K\(^+\)-Cl\(^-\) cotransporter, which has been found in other developing auditory structures (Blaesse et al. 2006). One possibility is that dialyzing the cytosol during whole cell patch clamp with the same intracellular solution at all ages eliminates age-dependent differences in cytoplasmic concentrations. However, trends in \( V_{\text{rest}} \) were similar to those obtained with nondialyzing intracellular recording techniques (MacLeod et al. 1996). The persistence of differences with age despite the use of consistent intracellular solutions argues for differences in the number or function of ion channels. Such changes have been observed in the thalamus and other auditory nuclei, in hyperpolarization-activated cation channels (Kanyshkova et al. 2009), KCC2 (Blaesse et al. 2006), and the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter (Yan et al. 2001), to name a few.

**Development of MGB Passive Properties**

Declines in \( R_{\text{in}} \) and \( \tau \) are a typical occurrence during the first 2–4 wk of development and have been reported across brain regions and across species (Ammer et al. 2012; Lee et al. 2010; Oswald and Reyes 2008; Perez Velazquez and Carlen 1996; Ramoa and McCormick 1994; Yamada et al. 2012). An earlier study on the rat MGB, Tennigkeit et al. (1998) also reported a decrease in \( R_{\text{in}} \) and \( \tau \) during the first 2 wk (although P21 was the oldest age used in that study). The decrease in \( \tau \) with age may be attributable in part to the decrease in \( R_{\text{in}} \) (\( R = 0.53, P < 0.001, n = 87 \) cells across ages). Depolarized membrane potentials during development have also been reported in the rat MGB (Tennigkeit et al. 1998), ferret and mouse dorsal lateral geniculate nucleus (LGN) (MacLeod et al. 1996; Ramoa and McCormick 1994), and rat ventrobasal (VB) nucleus (Lee et al. 2010; Perez Velazquez and Carlen 1996). Changes in \( V_{\text{rest}} \), with age may be attributed to ion gradients maintained between hyperpolarization-activated cation channels and K\(^+\) leak channels (Meuth et al. 2006) as well as Cl\(^-\) extrusion via the K\(^+\)-Cl\(^-\) cotransporter, which has been found in other developing auditory structures (Blaesse et al. 2006). One possibility is that dialyzing the cytosol during whole cell patch clamp with the same intracellular solution at all ages eliminates age-dependent differences in cytoplasmic concentrations. However, trends in \( V_{\text{rest}} \) were similar to those obtained with nondialyzing intracellular recording techniques (MacLeod et al. 1996). The persistence of \( V_{\text{rest}} \) differences with age despite the use of consistent intracellular solutions argues for differences in the number or function of ion channels. Such changes have been observed in the thalamus and other auditory nuclei, in hyperpolarization-activated cation channels (Kanyshkova et al. 2009), KCC2 (Blaesse et al. 2006), and the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter (Yan et al. 2001), to name a few.

**Development of IC-MGB Inhibition Versus Inhibition in the Auditory Brain Stem and AC**

\( E_{\text{GABA}} \) was significantly more depolarized in P8 MGB neurons, similar to auditory brain stem nuclei (Kandler and Friauf 1995; Kullmann and Kandler 2001; Milenković et al. 2007), but not in the AC (Kotak et al. 2007; Oswald and Reyes 2011). The \( E_{\text{GABA}} \) of MGB neurons at P8 (−57.9 ± 3.3 mV) and P16 (−74.4 ± 2.1 mV) with an extracellular Cl\(^-\) concentration of 135 mM was comparable with the \( E_{\text{GABA}} \) of rat cochlear nucleus cells from perforated-patch recordings when a similar extracellular Cl\(^-\) concentration was used (Song et al. 2012). The hyperpolarization of \( E_{\text{GABA}} \) with age has been attributed to improved Cl\(^-\) extrusion via KCC2. In the diencephalon, as in the lateral superior olive, KCC2 is present at P4, but in an inactive monomeric form that undergoes oligomerization during development (Blaesse et al. 2006).

**Development of IC-MGB Inhibition Versus Other Sources of Inhibition in the MGB**

One stereotypic inhibitory connection across sensory systems is the GABAergic input from the sensory sectors of the TRN to the respective sensory thalamus. While the development of TRN-MGB synaptic properties has not been studied to date, GABA immunoreactivity has been reported in all sensory sectors of the TRN from P0 (De Biasi et al. 1997). In the VB nucleus of rodents, TRN-VB IPSPs are composed of GABA\(_A\) and GABA\(_B\) components, both present from P0 (Evrard and Roport 2009; Warren et al. 1997; Warren and Jones 1997).

An additional source of inhibition is from GABAergic interneurons that form distinctive triadic synapses with afferent inputs on relay neurons, which are found in the visual thalamus across species and in the cat and monkey MGB (Majorosy and Kiss 1976; Winer and Larue 1996). These neurons are rare in the rat MGB (Winer and Larue 1996). Interneuron-mediated GABA\(_A\) inhibition develops in dorsal LGN neurons within the first postnatal week, and GABA\(_B\) responses develop subsequently (Perreault et al. 2003; Ziburkus et al. 2003). We observed IC-mediated GABA\(_A\) and GABA\(_B\) responses at all ages in the MGB.

**IC-MGB IPSPs Become Faster With Age**

IC-MGB IPSP latencies decreased by a factor of 1.7 in the third postnatal week (and a similar trend was observed in voltage clamp), similar to what has been observed in several auditory nuclei (Kandler and Friauf 1995; Oswald and Reyes 2011; Wu and Oertel 1987). The reduced latencies are likely due mainly to increased myelination of IC axons, which can be seen in the brain slices, possibly with additional potential contributions from faster presynaptic action potentials (Oswald and Reyes 2011; Tennigkeit et al. 1998) or increased vesicle exocytosis (Johnsson et al. 2005).

IPSP rise time and GABA\(_A\) decay time were significantly higher at P8 compared with both other age groups and decreased with age. This result partly reflected concurrent changes in \( \tau \). Rise and decay times of P16 neurons showed deviations from the fitted lines to \( \tau \), and the correlation between rise/decay time and \( \tau \) became even stronger when only P8 and P27 data were used (\( R = 0.65, P < 0.001 \) for rise time vs. \( \tau \); \( R = 0.75, P < 0.05 \) for decay time vs. \( \tau \)). This deviation at P16 may reflect the presence of multiple GABA R subunits (\( \alpha_1, \alpha_2, \text{and } \alpha_3 \)) at this intermediate age that promote high kinetic variability (Eyre et al. 2012). Thalamic nuclei undergo a sharp development shift from \( \alpha_2 \) [and to a lesser extent from \( \alpha_3 \) (Laurie et al. 1992)] to \( \alpha_1 \) between P10 and P20 (Fritschy et al. 1994; Peden et al. 2008). Overall, evoked GABA\(_A\) IPSC temporal properties did not show much change with age [similar to TRN-VB IPSCs between ages P8-P12 and P21-P30 (Huntsman and Huguenard 2000)], but further study is needed to characterize developmental changes in receptor subunit composition.

**Reduced Synaptic Depression With Age**

Similar to the data shown in Fig. 6, in other auditory nuclei, reduced depression to repetitive stimulation during development has also been observed in both excitatory synapses (Crins et al. 2011; Joshi and Wang 2002; Oswald and Reyes 2011,
mechanisms other than GABAB at P8 that may contribute to underlying the development of inhibitory plasticity in the AC frequencies (Fig. 7, all ages, whose steady-state amplitude increases for higher inhibitory inputs will produce a sustained hyperpolarization at for feedback TRN inhibition. Sustained activation of the IC shape the onset and sustained tuning of MGB neurons rather than being restricted to sustained tuning, as would be expected in the time course of maturation of MGBv single tone tuning precision, which is known to be shaped by inhibition (Bartlett et al. 2011; Bartlett and Wang 2011)).

A second type of measurement of PP ratios was made with respect to a common prestimulation baseline. As reported in an earlier study (Bartlett and Smith 2002), most IPSPs maintained a PP ratio close to 1 from a common baseline, implying that the extent of membrane hyperpolarization does not change or increases across a train stimulus when summation occurs. Therefore, sustained spiking of IC inhibitory inputs will produce a sustained hyperpolarization that will dampen MGB neuron excitability. A small number of cells showed strong depression of common baseline PP ratios (called type II responses; see Fig. 7, D–I). Further work needs to be done to determine whether, for example, postsynaptic subunit composition is different in neurons with type II responses.

Significance and Functional Implications of IC-MGB Inhibition Overall, we found that IC-MGB inhibition is present from P7 and becomes faster and exhibits reduced depression with age. IPSPs also become more potent in controlling the rising phase of excitation.

Age-related changes in the properties of IC inhibition mostly occurred between the youngest (P8) and intermediate (P16) ages, but some, such as latency, continued through the first postnatal month. In vivo recordings (Barkat et al. 2011) found that P11–P15 was a period of plasticity in the mouse MGBv postsynaptic GABA	extsubscript{B} ratio and IPSP	extsubscript{2}/IPSP	extsubscript{1} at 25 Hz were in agreement with measures obtained by Luo et al. (2011) at 20 Hz. Our findings suggest the involvement of mechanisms other than GABA	extsubscript{B} at P8 that may contribute to the increased depression. This can include reduced presynaptic vesicle depletion, less effective GABA reuptake (Vitellaro-Zuccarello et al. 2003), or GABA receptor desensitization (Jones and Westbroek 1995).

A recent in vivo study of GABAergic neurons in the dorsal cortex of the IC (projecting to the MGBv and MGBd) found short spike latencies in response to tones (Geis and Borst 2012), demonstrating that the IC-MGB inhibitory pathway is active during sensory stimulation. This early IC inhibition may control the latency of MGB neurons or it may selectively gate sound-evoked responses, such as in shaping selectivity of awake marmoset MGB neurons to tone or noise stimuli (Bartlett and Wang 2011). Bartlett and Wang (2011) reported that 25% MGBd neurons were inhibited by tones and noise, whereas 30% were inhibited by tones and excited by noise. IC inhibition may also play a role in more complex stimulus paradigms, such as stimulus specific adaptation, which has been shown to be prominent in the nonlemniscal MGB (Anthony et al. 2010) and independent of cortical feedback (Anthony and Malmierca 2011). Moreover, GABA	extsubscript{A} inhibition has recently been shown to contribute to stimulus-specific adaptation in the IC (Pérez-González et al. 2012).

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