Development of GABA$_A$ Receptor-Mediated Inhibitory Postsynaptic Currents in Hippocampus

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Development of GABA$_A$ receptor-mediated inhibitory postsynaptic currents in hippocampus. J Neurophysiol 88: 3097–3107, 2002; 10.1152/jn.00026.2002. Hippocampal CA1 pyramidal cells receive two kinetic classes of GABA$_A$ receptor-mediated inhibition: slow dendritic inhibitory postsynaptic currents (GABA$_{A,slow}$ IPSCs) and fast perisomatic (GABA$_{A,fast}$) IPSCs. These two classes of IPSCs are likely generated by two distinct groups of interneurons, and we have previously shown that the kinetics of the IPSCs have important functional consequences for generating synchronous firing patterns. Here, we studied developmental changes in the properties of GABA$_{A,fast}$ and GABA$_{A,slow}$ spontaneous, miniature, and evoked IPSCs (sIPSCs, mIPSCs, and eIPSCs, respectively) using whole cell voltage-clamp recordings in brain slices from animals aged P10–P35. We found that the rate of GABA$_{A,slow}$ sIPSCs increased by over 70-fold between P11 and P35 (from 0.0017 to 0.12 s$^{-1}$). Over this same age range, we observed a >3.5-fold increase in the maximal amplitude of GABA$_{A,slow}$ eIPSCs evoked by stratum lacunosum-moleculare (SL-M) stimuli. However, the rate and amplitude of GABA$_{A,slow}$ mIPSCs remained unchanged between P10 and P30, suggesting that the properties of GABA$_{A,slow}$ synapses remained stable over this age range, and that the increase in sIPSC rate and in eIPSC amplitude was due to increased excitability or excitation of GABA$_{A,slow}$ interneurons. This hypothesis was tested using bath application of norepinephrine (NE), which we found at low concentrations (1 μM) selectively increased the rate of GABA$_{A,slow}$ sIPSCs while leaving GABA$_{A,fast}$ sIPSCs unchanged. This effect was observed in animals as young as P13 and was blocked by coapplication of tetrodotoxin, suggesting that NE was acting to increase the spontaneous firing rate of GABA$_{A,slow}$ interneurons and consistent with our hypothesis that developmental changes in GABA$_{A,slow}$ IPSCs are due to changes in presynaptic excitability. In contrast to the changes we observed in GABA$_{A,slow}$ IPSCs, the properties of GABA$_{A,fast}$ sIPSCs remained largely constant between P11 and P35, whereas the rate, amplitude, and kinetics of GABA$_{A,fast}$ mIPSCs showed significant changes between P10 and P30, suggesting counterbalancing changes in action potential-dependent GABA$_{A,fast}$ sIPSCs. These observations suggest differential developmental regulation of the firing properties of GABA$_{A,fast}$ and GABA$_{A,slow}$ interneurons in CA1 between P10 and P35.

INTRODUCTION

Inhibitory circuitry in the hippocampus and neocortex undergoes several types of transitions during postnatal development. The polarity of GABA$_A$ receptor-mediated inhibition changes from depolarizing in neonates to hyperpolarizing in juveniles (>P7) and adult animals (Cherubini et al. 1991; Zhang et al. 1991). Changes in synaptic density accompany synaptogenesis and synaptic pruning (Ben-Ari et al. 1990; Blue and Parnavelas 1983). Receptor subunit composition changes (Kilisch et al. 1991; Laurie et al. 1992) with concomitant changes in receptor kinetics (Hutcheon et al. 2000), inhibitory postsynaptic current (IPSC) kinetics (Hollrigel and Soltesz 1997; Otis and Mody 1992; Taketo and Yoshioka 2000), and pharmacology (Kapur and Macdonald 1999; Rovira and Ben Ari 1993). These developmental transitions could result in changes in the functional capabilities of inhibitory networks. For example, modeling and experimental studies have demonstrated the importance of IPSC properties for generating synchronized network activity in cortical circuits and temporal integration in cortical pyramidal cells (Pouille and Scanziani 2001; Traub et al. 1996; Wang and Buzsaki 1996; White et al. 1998, 2000).

In the CA1 region of the rodent hippocampus, pyramidal cells receive input from multiple classes of GABAergic interneurons. We have shown previously that pyramidal cell dendrites are targeted by GABAergic synapses, generating IPSCs with kinetics and pharmacology that are distinct from perisomatic IPSCs. Dendritic GABA$_{A,slow}$ IPSCs have rise and decay times that are severalfold slower than perisomatic GABA$_{A,fast}$ IPSCs, but the interneurons generating these IPSCs have yet to be identified. We have also observed GABA$_{A,slow}$ spontaneous IPSCs (sIPSCs) in CA1 pyramidal cells, in contrast to several other studies in which sIPSC kinetics were reported to be uniformly fast (Mody et al. 1991; Ropert et al. 1990). Although one likely explanation for this discrepancy is that GABA$_{A,slow}$ sIPSCs occur infrequently, we investigated whether delayed development of GABA$_{A,slow}$ inhibitory circuitry may preclude the observation of these IPSCs in the young animals frequently used in patch-clamp studies of IPSCs in CA1. We show here that the frequency of spontaneous and the amplitude of GABA$_{A,slow}$ evoked IPSCs (eIPSCs) increases substantially between 11 and 35 days postnatal. Low concentrations of norepinephrine (NE) increased the rate of GABA$_{A,slow}$ eIPSCs even at age P13, suggesting that GABA$_{A,slow}$ interneurons become increasingly excitable over the first several postnatal weeks. The results underscore the importance of using developmentally mature animals in studies of dendritic inhibition in CA1.

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METHODS

Preparation of slices

Rats aged 10–35 days were decapitated under halothane anesthesia, and slices (400–500 μm) were obtained using standard techniques (Banks et al. 1998). Slices were held submerged at 35°C for 1 h before transfer to the recording chamber, which was perfused at 3 ml/min with artificial cerebrospinal fluid (ACSF; in mM): 127 NaCl, 1.2 KH₂PO₄, 1.9 KCl, 26 NaHCO₃, 2.2 CaCl₂, 1.4 MgSO₄, and 10 glucose, saturated with 95% O₂/5%CO₂.

Patch-clamp electrophysiology

Putative pyramidal cells in the stratum pyramidale of CA1 were visualized using a video camera (VE-1000, DAGE MTI, Michigan City, IN) connected to an upright microscope (BX-50WI, Olympus America, Melville, NY) equipped with an infrared band-pass filter (775 ± 75 nm), a long working-distance water-immersion objective (40×, NA 0.8), and differential interference contrast optics. The microscope and recording pipette were positioned using an integrated motorized control system (Luigs and Neumann, Ratingen, Germany).

Whole cell recordings were obtained at room temperature (24°C), using an Axopatch 200B (Axon Instruments, Union City, CA) patch-clamp amplifier. All data were collected using pClamp software (Axon Instruments). Data were filtered at 2–5 kHz, sampled at 5–10 kHz (Digidata 1200, Axon Instruments), and stored on a Pentium-based PC. Patch pipettes were fabricated from borosilicate glass (KB-33, 1.7 mm OD, 1.1 mm ID, Garnet Glass, Claremont, CA) using a Flaming-Brown two-stage puller (P-87, Sutter Instruments, Novato, CA), coated with Sylgard (Dow Corning) to reduce electrode capacitance, and fire polished. Tight-seal whole cell recordings were obtained using standard techniques (Edwards et al. 1989; Hamill et al. 1981). Patch pipettes had open-tip resistances of 2–4 MΩ when filled with the recording solution [composition (in mM): 140 CsCl, 10 Na-HEPES, 10 EGTA, 2 MgATP, and 5 QX-314, pH 7.3]. Access resistances were typically 10–20 MΩ and were then compensated 60–80%. Cells were held at −60 mV. Data collection commenced ≥5 and usually 10 min after obtaining whole cell access to ensure that the 6-cyano-7-nitroquinoline-2,3-dione (CNQX) and 2-amino-5-phosphonovaleric acid (APV) had sufficient time to have a maximal effect and that the cell was fully chloride loaded. Evoked and spontaneous GABA_A, fast IPSCs were isolated by bath application of 20 μM CNQX and 40 μM APV to block alpha-aminoadenosine-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-d-aspartate (NMDA)-mediated currents, and by the inclusion of CsCl and QX-314 in the patch pipette to block GABA_B-mediated currents. Although CNQX has been shown to increase the rate of sIPSCs in other studies, (Brickley et al. 2001; McBain et al. 1992), we did not observe a significant effect on the rate of sIPSCs under our experimental conditions for either GABA_A, fast or GABA_A, slow IPSCs: (fast: control 2.9 ± 0.7 s⁻¹, CNQX and APV 2.8 ± 0.6 s⁻¹; slow: control 0.02 ± 0.01 s⁻¹, CNQX and APV 0.02 ± 0.01 s⁻¹; n = 6 cells, age = 19 ± 4 days).

In each cell, sIPSCs and miniature IPSCs (mIPSCs) were recorded for variable periods of time. For sIPSCs, the recording periods lasted from approximately 20 to >1000 s (mean, >180 s), but in only four cells did the recordings last <60 s. In most cases, when the recordings were brief, it was because we were concentrating on recording eIPSCs, and not because the recordings, which typically lasted >30 min, were unstable. For mIPSCs, the recording periods lasted from 30 to >800 s; only one cell was recorded for <60 s and the average was >360 s. Miniature GABA_A, slow IPSCs were rare, with only about one such event recorded in each cell on average, and caution is warranted in quantitative analyses of such small numbers of events. However, the main purpose of recording mIPSCs was to determine whether we observed an age dependence of rate similar to what was observed for sIPSCs. Because the latter difference was >70-fold between P11 and P35, we are confident that even small numbers of events would enable us to observe such a large change in mIPSC rate.

Stimuli (10–100 μA) were applied to stratum lacunosum-molecular (SL-M) to evoke GABA_A, slow. A maximum stimulation rate of 0.05 Hz was used to minimize the previously observed rundown of GABA_A, slow over time (Pearce 1993). Glass patch pipettes filled with ACSF were used as stimulating electrodes and were consistently placed at approximately 50 μm on the SL-M side of the hippocampal fissure, approximately 100 μm deep in the tissue and at the same mediolateral position in CA1 as the apical dendrite of the cell being studied. In animals aged P30 or older, this stimulus evoked GABA_A, slow IPSCs in >90% of all cells tested. [Note, however, that only data from cells in which a full dose-response curve was obtained are included in Fig. 4; the number of cells in which we observed an SL-M-evoked GABA_A, slow IPSC was far greater (n > 100 cells).] In those cells in which no evoked GABA_A, slow IPSCs were observed, the electrode was repositioned within the SL-M until a response could be elicited or (more typically) a region of about 100 μm × 100 μm in the SL-M had been searched to no avail. In all cases, maximal currents were determined by varying stimulus intensity until the response amplitude no longer increased. NE was mixed up fresh daily and was protected from light and air to minimize degradation before its use. All drugs were bath applied and were obtained from Sigma-Aldrich Chemicals.

Data analysis

Data were analyzed on a Pentium-based PC using ClampFit (Axon Instruments), Origin (OriginLab, Northampton, MA), and StatMost (Dataxiom Software, Los Angeles, CA). Spontaneous events were analyzed using an automated event detection algorithm (Banks et al. 1998). In this algorithm, two windows were moved along the data, a “peak” window and a “baseline” window. At each time point, the data within the each window was averaged and the baseline average subtracted from peak average. This yielded a “pseudo-differentiated” form of the data that was characterized by large, rapid peaks at the onset of GABA_A, fast IPSCs, and slower, smaller peaks at the onset of GABA_A, slow IPSCs. Threshold-level crossings were determined from this pseudo-differentiated data, with threshold set as 3σbaseline where σbaseline was measured during periods of no visually detectable events and was typically 2–4 pA. Because the baseline value was constantly updated during the analysis, slow changes in baseline had no effect on the algorithm’s accuracy. This algorithm successfully detected >99% of sIPSCs and mIPSCs.

Evoked GABA_A, slow IPSCs were well fit with the sum of two single rising and decaying exponential components. Although GABA_A, fast IPSCs decayed biexponentially (Banks et al. 1998), for simplicity, the decay was characterized using the weighted sum of these two exponential components (τdecay,sw). Spontaneous GABA_A, slow IPSCs were defined as those events having 10–90% rise times >4 ms and decay times >40 ms. Individual sIPSCs were selected for averaging and exponential curve fitting when no other detected events occurred within ±100 ms (GABA_A, fast) or ±250 ms (GABA_A, slow) of the peak.

Developmental changes in IPSC parameters were detected either by grouping data into age ranges and applying paired t-tests or by linear regression analysis of the unbinned data as a function of age. Correlation coefficients (r) and P values cited are from regression fits. All data are presented as mean ± SE.

RESULTS

sIPSCs

sIPSCs were recorded under whole cell voltage clamp from 76 putative CA1 pyramidal cells in slices taken from animals ranging in age from P11 to P35 (Fig. 1). These events represent a combination of action potential (AP)-dependent and AP-independent sIPSCs. For analysis purposes, the data were grouped into five age ranges: P11–P15, P16–P20, . . . , P31–
P26, no GABA \textsubscript{A,slow} sIPSCs were observed. Between P11 and P13, no GABA \textsubscript{A,slow} IPSCs were observed (n = 16 cells), and only 2 of 17 cells from animals aged P14 and P15 exhibited GABA \textsubscript{A,slow} sIPSCs. A linear regression of the unbinned data yielded a correlation of $r = 0.63$ ($P < 0.001$). Amplitude, rise, and decay kinetics of GABA \textsubscript{A,slow} sIPSCs were unchanged as a function of age (Table 1; Fig. 2, B–D; $r < 0.2$, $P > 0.25$ for all 3 parameters).

In contrast to the observed increase in the frequency of GABA \textsubscript{A,slow} sIPSCs, the rate of GABA \textsubscript{A,fast} sIPSCs exhibited no consistent change with age (Table 2; Fig. 3A; $r = 0.018$; $P > 0.8$). The amplitude of GABA \textsubscript{A,fast} sIPSCs exhibited a weak but significant correlation with age (Table 2; Fig. 3B; $r = 0.25$, $P < 0.05$). The faster component of the biexponential decay exhibited a significant decrease with age (Table 2; $r = −0.40$, $P < 0.0005$), as did the amplitude of this fast component (Table 2; $r = −0.50$, $P < 0.0001$), but the weighted decay time constant $\tau_W$ did not change with age (Table 2; Fig. 3C; $r = 0.17$, $P = 0.16$). There was no significant correlation between GABA \textsubscript{A,fast} and GABA \textsubscript{A,slow} sIPSC rate (data not shown), indicating that the change in GABA \textsubscript{A,slow} sIPSC rate as a function of age represented a delay in the development of the GABA \textsubscript{A,slow} circuit in CA1.

**SL-M–evoked IPSCs**

There are several possible explanations for the increase in the rate of GABA \textsubscript{A,slow} sIPSCs with age. One possibility is that the GABA \textsubscript{A,slow} synapses are not functional in younger animals, either because synaptic contacts are not established yet or because the postsynaptic receptors are not clustered in apposition to the synaptic contacts. A second possibility is that the synapses are present and functional, but the kinetics of the IPSCs are faster at birth and progressively become slower over the first postnatal month. The time course of IPSCs depends on receptor subunit composition (Tia et al. 1996), and it is known that the expression pattern of GABA \textsubscript{A} receptor subunits changes over the first few postnatal weeks (Kllisch et al. 1991; Laurie et al. 1992). Finally, it is possible that the synapses are functional and mature, but the interneurons generating GABA \textsubscript{A,slow} IPSCs in young animals exhibit less spontaneous activity. This scenario could arise if there is a developmental change in the expression of voltage-gated channels or in other membrane properties in the presynaptic cells.

We tested whether GABA \textsubscript{A,slow} synapses were functional in young animals by investigating whether the properties of elec-

**TABLE 1. Properties of GABA \textsubscript{A,slow} sIPSCs**

<table>
<thead>
<tr>
<th>Age Range</th>
<th>No. Cells</th>
<th>No. Events*</th>
<th>$t_{\text{tot}}$ min</th>
<th>GABA \textsubscript{A,slow} sIPSC Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>P11–P15</td>
<td>33</td>
<td>3</td>
<td>42</td>
<td>$t_{\text{rise}}$ ms, $t_{\text{decay}}$ ms</td>
</tr>
<tr>
<td>P16–P20</td>
<td>10</td>
<td>55</td>
<td>18</td>
<td>0.0017 ± 0.0012, 78.1 ± 41.0, 9.4 ± 1.1, 70.8 ± 5.3</td>
</tr>
<tr>
<td>P21–P25</td>
<td>12</td>
<td>228</td>
<td>81</td>
<td>0.038 ± 0.014, 198.3 ± 36.9, 6.8 ± 1.1, 75.9 ± 5.6</td>
</tr>
<tr>
<td>P26–P30</td>
<td>13</td>
<td>229</td>
<td>48</td>
<td>0.064 ± 0.018, 119.4 ± 21.2, 5.8 ± 0.6, 74.5 ± 8.9</td>
</tr>
<tr>
<td>P31–P35</td>
<td>8</td>
<td>219</td>
<td>40</td>
<td>0.089 ± 0.021, 126.8 ± 16.5, 7.6 ± 0.6, 85.7 ± 9.4</td>
</tr>
<tr>
<td>P13–P15 (NE)</td>
<td>3</td>
<td>162</td>
<td>15</td>
<td>0.12 ± 0.04, 99.5 ± 22.1, 6.5 ± 0.7, 88.6 ± 7.8</td>
</tr>
</tbody>
</table>

Values are ± SE. $t_{\text{tot}}$ is the total observation time summed across cells. *In 31 of 33 cells from animals aged P11–P15 and in 1 cell from an animal aged P26, no GABA \textsubscript{A,slow} sIPSCs were observed. NE, recorded in the presence of 1 $\mu$M norepinephrine.
trically evoked GABA<sub>A</sub><sub>slow</sub> IPSCs changed with age. Synaptic currents were evoked in the presence of ionotropic glutamate receptor antagonists and thus represented monosynaptic GABA<sub>A</sub> receptor-mediated IPSCs. IPSCs evoked by stimulation in SL-M were recorded in 32 putative CA1 pyramidal cells (10 of which were also used for the spontaneous recordings described above).

We observed that even in P11 animals, SL-M stimuli could elicit a response with the kinetics of GABA<sub>A</sub><sub>slow</sub> IPSCs (Fig. 4). Similar to the change in sIPSC rate, however, we observed a significant increase in peak response amplitude as a function of age ($r = 0.54, P < 0.005$; Fig. 4C). Mean peak GABA<sub>A</sub><sub>slow</sub> IPSC amplitude was 202 ± 39 pA at P11–P15, and 718 ± 226 at P31–P35. These data indicate that at least some GABA<sub>A</sub><sub>slow</sub> synapses are present and functional at P11, and that the receptors at these synapses have kinetics similar to receptors at mature synapses. This suggests that the observed change in sIPSC rate is due to either a change in interneuron excitability or an increase in the number of GABA<sub>A</sub><sub>slow</sub> synapses as a function of age.

If the number of GABA<sub>A</sub><sub>slow</sub> synapses increases as a function of age, then it is likely that we would see this development manifested in the responses to spontaneously released vesicles at these synapses. Assuming that the spontaneous release properties of individual synapses are the same at different ages, as more synapses become functional the number of spontaneous vesicular fusions will increase and thus the number of AP-independent sIPSCs (mIPSCs) will increase as well. We recorded mIPSCs using 1 mM tetrodotoxin (TTX) to block all AP firing in the slice. Other than the TTX, recording conditions and data analysis were identical to those used while recording sIPSCs. Recordings were made from 57 CA1 pyramidal cells from animals between the ages of P10 and P30.

At all ages, GABA<sub>A</sub><sub>slow</sub> mIPSCs were very rare, occurring on average at rates of about 0.005 s<sup>−1</sup>. In contrast to the observed change in frequency of GABA<sub>A</sub><sub>slow</sub> sIPSCs with age, we found no change in the frequency of GABA<sub>A</sub><sub>slow</sub> mIPSCs as a function of age (Table 3; Fig. 5A). We also observed no

### Table 2. Properties of GABA<sub>A</sub><sub>fast</sub> sIPSCs

<table>
<thead>
<tr>
<th>Age Range</th>
<th>No. Cells</th>
<th>No. Events</th>
<th>GABA&lt;sub&gt;A&lt;/sub&gt; Rate, s&lt;sup&gt;−1&lt;/sup&gt;</th>
<th>Amplitude</th>
<th>$\tau_{Dec1}$</th>
<th>$A_1$</th>
<th>$\tau_{Dec2}$</th>
<th>$\tau_{WT}$, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>P11–P15</td>
<td>33</td>
<td>19929</td>
<td>8.0 ± 1.4</td>
<td>49.1 ± 5.4</td>
<td>12.3 ± 0.7</td>
<td>0.67 ± 0.04</td>
<td>34.8 ± 2.3</td>
<td>19.1 ± 0.7</td>
</tr>
<tr>
<td>P16–P20</td>
<td>10</td>
<td>7446</td>
<td>7.1 ± 0.7</td>
<td>63.4 ± 4.5</td>
<td>10.6 ± 1.1</td>
<td>0.55 ± 0.04</td>
<td>36.6 ± 3.8</td>
<td>21.4 ± 1.0</td>
</tr>
<tr>
<td>P21–P25</td>
<td>12</td>
<td>61955</td>
<td>12.4 ± 1.6</td>
<td>75.6 ± 9.4</td>
<td>8.4 ± 0.7</td>
<td>0.41 ± 0.03</td>
<td>29.9 ± 3.7</td>
<td>20.7 ± 1.8</td>
</tr>
<tr>
<td>P26–P30</td>
<td>13</td>
<td>21240</td>
<td>7.4 ± 1.0</td>
<td>63.6 ± 7.1</td>
<td>9.9 ± 1.0</td>
<td>0.46 ± 0.05</td>
<td>31.9 ± 1.6</td>
<td>21.9 ± 1.2</td>
</tr>
<tr>
<td>P31–P35</td>
<td>8</td>
<td>21393</td>
<td>7.0 ± 2.1</td>
<td>55.9 ± 10.4</td>
<td>7.0 ± 0.9</td>
<td>0.41 ± 0.07</td>
<td>28.4 ± 1.8</td>
<td>19.7 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Total observation time is the same as for Table 1.
change in amplitude or kinetics as a function of age (Table 3; Fig. 5, B–D). These data are consistent with the hypothesis that GABA \textsubscript{A,slow} synapses are fully mature even at P10, and that the developmental changes observed in spontaneous and evoked GABA \textsubscript{A,slow} IPSCs are due to changes in the membrane properties of the interneurons mediating GABA \textsubscript{A,slow}. However, we cannot exclude a contribution from a developmental increase in the number of functional GABA \textsubscript{A,slow} synapses on CA1 pyramidal cells, if this increase is accompanied by a concomitant decrease in the rate of spontaneous vesicular fusion averaged across the population of GABA \textsubscript{A,slow} synapses.

Interestingly, GABA \textsubscript{A,fast} synapses did manifest evidence of developmental changes with age: the rate of GABA \textsubscript{A,fast} mIPSCs increased 175% (Table 4; Fig. 6A; \( r = -0.36, P < 0.01 \)), and the weighted decay time constant decreased by 33% (Table 4; Fig. 6C; \( r = -0.49, P < 0.0005 \)). The decrease in decay time constant was driven by decreases in both time constants of the biexponential fit to the decay (Table 4; \( \tau_1; r = -0.31, P < 0.05; \tau_2; r = -0.42, P < 0.005 \)). No change was observed in the amplitudes of either component.

**Selective effects of NE on GABA \textsubscript{A,slow} sIPSCs**

Our data suggest that changes in the excitability of GABA \textsubscript{A,slow} interneurons contribute to the developmental changes we observed in GABA \textsubscript{A,slow} IPSCs. In this scenario, synapses are mature and the absence of GABA \textsubscript{A,slow} sIPSCs is due to limited spontaneous spiking activity in the presynaptic cells. To test this hypothesis, we induced spontaneous activity in interneurons and recorded sIPSCs in pyramidal cells. If GABA \textsubscript{A,slow} interneurons and synapses are mature but silent under control conditions, then under conditions of increased...
spontaneous activity we would expect to see an increase in 
GABA_{A,slow} sIPSCs recorded in pyramidal cells. When bath 
applied at 10 μM, NE has been shown to depolarize and trigger 
spontaneous action potentials in interneurons throughout CA1, 
causing a dramatic increase in the frequency and amplitude of 
sIPSCs recorded in pyramidal cells (Bergles et al. 1996). 
Similar to these previous reports, we found that 10 μM NE 
increased the frequency and amplitude of both GABA_{A,fast} and 
GABA_{A,slow} IPSCs (n = 2; data not shown). Unexpectedly, we 
found that a lower concentration of NE (1 μM) selectively 
increased the rate of GABA_{A,slow} sIPSCs, with little effect on 
GABA_{A,fast} sIPSCs, thus allowing us to study GABA_{A,slow} in 
isolation (n = 11 cells). Even in slices from young animals 
lacking GABA_{A,slow} sIPSCs under control conditions (P13– 
P15, n = 3), NE caused an increase in GABA_{A,slow} sIPSCs 
recorded in pyramidal cells (Fig. 7). The amplitude and kinetics 
of GABA_{A,slow} sIPSCs observed in these young animals in 
the presence of NE were comparable to those observed in 
mature animals under control conditions (Table 1).

NE can act to alter synaptic release indirectly by depolariz-
ing the presynaptic cell beyond threshold for action potentials, 
or by acting directly on the synaptic release machinery. We 
distinguished between these two possibilities by blocking APs 
with TTX, and then reapplying NE in four cells. In all cases, 
the effect of NE was blocked by coapplication of TTX, sug-
gest that NE was acting to increase spontaneous action 
potential activity in GABA_{A,slow} interneurons (Fig. 8). These 
data are consistent with a developmental change in the excit-
bility of GABA_{A,slow} interneurons.

**DISCUSSION**

**Summary**

In this study, we investigated the properties of GABA_{A} 
receptor-mediated IPSCs recorded in CA1 pyramidal cells as a 
function of animal age between P10 and P35. We observed that 
the rate of spontaneous GABA_{A,slow} IPSCs increased by 70-
fold over the age range studied, and we observed a parallel but 
smaller change in the amplitude of SL-M–evoked GABA_{A,slow} 
IPSCs. We found no evidence for changes in the strength of 
individual synapses underlying GABA_{A,slow} IPSCs, as minia-

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### Table 3. Properties of GABA_{A,slow} mIPSCs

<table>
<thead>
<tr>
<th>Age Range</th>
<th>No. Cells</th>
<th>No. Events</th>
<th>t_{tot}, min</th>
<th>GABA_{A,slow} mIPSC Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>P11–P15</td>
<td>21</td>
<td>29</td>
<td>160</td>
<td>Rate, s^{-1}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>t_{Rise}, ms</td>
</tr>
<tr>
<td>P16–P20</td>
<td>20</td>
<td>28</td>
<td>116</td>
<td>0.0053 ± 0.0020</td>
</tr>
<tr>
<td>P21–P25</td>
<td>14</td>
<td>15</td>
<td>55</td>
<td>0.0044 ± 0.0027</td>
</tr>
<tr>
<td>P26–P30</td>
<td>5</td>
<td>5</td>
<td>15</td>
<td>0.0059 ± 0.0038</td>
</tr>
</tbody>
</table>

Values are means ± SE. t_{tot} is the total observation time summed across cells.
tured GABA<sub>A</sub><sub>,slow</sub> IPSCs exhibited no detectable change in their amplitude. We also observed no change in the frequency of GABA<sub>A</sub><sub>,slow</sub> mIPSCs, consistent with the idea that the number of functional GABA<sub>A</sub><sub>,slow</sub> synapses does not change between P10 and P30. We additionally found no evidence of maturation of the kinetic properties of either spontaneous or miniature GABA<sub>A</sub><sub>,slow</sub> IPSCs. Even in P13–P15 animals, bath application of NE selectively activated GABA<sub>A</sub><sub>,slow</sub> interneurons, triggering an increase in GABA<sub>A</sub><sub>,slow</sub> sIPSCs recorded in pyramidal cells with kinetics and amplitude similar to that observed in mature animals. These observations suggest that the observed increase in the rate of GABA<sub>A</sub><sub>,slow</sub> IPSCs and amplitude of evoked GABA<sub>A</sub><sub>,slow</sub> IPSCs are due primarily to changes in the excitability of the interneurons underlying GABA<sub>A</sub><sub>,slow</sub> IPSCs, for example, due to developmental regulation of the expression of voltage-gated ion channels. However, we cannot exclude the possibility that an increase in the number of GABA<sub>A</sub><sub>,slow</sub> synapses also contributes to the increase in GABA<sub>A</sub><sub>,slow</sub> sIPSC rate and sIPSC amplitude.

An unexpected finding of this study is that changes in the properties of GABA<sub>A</sub><sub>,fast</sub> mIPSCs were not mirrored in the properties of GABA<sub>A</sub><sub>,fast</sub> sIPSCs. If we assume that different populations of interneurons contribute equally to the generation of mIPSCs and sIPSCs, then since sIPSCs represent a combination of AP-dependent and AP-independent sIPSCs, these data suggest counterbalancing changes in pre- or postsynaptic properties. For example, although the rate of mIPSCs increased (from 2.4 to 4.2 s<sup>−1</sup>) between P10–P30, there was no change in the frequency of GABA<sub>A</sub><sub>,fast</sub> sIPSCs over this age range. This observation suggests that the interneurons underlying GABA<sub>A</sub><sub>,fast</sub> IPSCs undergo a collective decrease in spontaneous AP activity over this age range, from about 5.6 to 3.2 spikes/s (i.e., total spontaneous activity observed on average in a pyramidal cell), an opposite developmental change compared with GABA<sub>A</sub><sub>,slow</sub> interneurons. In contrast to the small decrease in mean amplitude observed for GABA<sub>A</sub><sub>,fast</sub> mIPSCs, GABA<sub>A</sub><sub>,fast</sub> sIPSCs exhibited a small increase in mean amplitude, suggesting that postsynaptic reductions in receptor density are counterbalanced by increases in synaptic number and release probability. Finally, in contrast to the decreases observed in weighted decay time constant of GABA<sub>A</sub><sub>,fast</sub> mIPSCs, the weighted decay time constants of GABA<sub>A</sub><sub>,fast</sub> sIPSCs were unchanged with age. This suggests that AP-dependent sIPSCs actually became slower to decay with age, exactly the opposite of mIPSCs. This result is difficult to interpret, but may reflect differences in which microscopic transition is rate-limiting when multiple synapses are activated synchronously (as is the case for AP-dependent sIPSCs) versus asynchronously (as is the case for mIPSCs). Alternatively, the observation that changes in GABA<sub>A</sub><sub>,fast</sub> mIPSCs are not mirrored in changes in GABA<sub>A</sub><sub>,fast</sub> sIPSCs may result from selective developmental changes in different populations of GABA<sub>A</sub><sub>,fast</sub> interneurons (Freund and Buzsaki 1996; Nusser et

<table>
<thead>
<tr>
<th>Age Range</th>
<th>No. Cells</th>
<th>No. Events</th>
<th>Rate, s&lt;sup&gt;−1&lt;/sup&gt;</th>
<th>Amplitude, pA</th>
<th>τ&lt;sub&gt;Dec1&lt;/sub&gt;, ms</th>
<th>A&lt;sub&gt;1&lt;/sub&gt;</th>
<th>τ&lt;sub&gt;Dec2&lt;/sub&gt;, ms</th>
<th>τ&lt;sub&gt;w&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P10–P15</td>
<td>33</td>
<td>23397</td>
<td>2.4 ± 0.3</td>
<td>49.2 ± 5.2</td>
<td>11.6 ± 0.7</td>
<td>0.5 ± 0.0</td>
<td>38.7 ± 1.7</td>
<td>23.7 ± 0.8</td>
</tr>
<tr>
<td>P16–P20</td>
<td>10</td>
<td>22644</td>
<td>3.7 ± 0.3</td>
<td>42.0 ± 2.6</td>
<td>11.9 ± 0.5</td>
<td>0.54 ± 0.03</td>
<td>40.7 ± 2.3</td>
<td>24.4 ± 0.7</td>
</tr>
<tr>
<td>P21–P25</td>
<td>12</td>
<td>15191</td>
<td>5.1 ± 0.8</td>
<td>38.7 ± 2.6</td>
<td>10.5 ± 0.8</td>
<td>0.52 ± 0.05</td>
<td>32.6 ± 1.5</td>
<td>21.0 ± 0.8</td>
</tr>
<tr>
<td>P26–P30</td>
<td>13</td>
<td>4145</td>
<td>4.2 ± 0.5</td>
<td>31.3 ± 2.8</td>
<td>7.5 ± 2.0</td>
<td>0.47 ± 0.07</td>
<td>23.8 ± 3.9</td>
<td>15.8 ± 2.5</td>
</tr>
</tbody>
</table>

Values are ± SE. Total observation time is the same as for Table 3.

**FIG. 6.** Development of GABA<sub>A</sub><sub>,fast</sub> mIPSCs. Plots of mean rate (A), mean peak amplitude (B), and mean weighted decay time constant (τ<sub>w</sub>) (C) for all GABA<sub>A</sub><sub>,fast</sub> mIPSCs recorded in each cell as a function of the age of the animal. Each open square represents the data from 1 cell. Each closed square is the mean of means binned according to age (P10–P15, P16–P20, . . . , P26–P30). Dashed lines are the linear regression fits to the unbinned data.
al. 1996; Wilson et al. 2001) that do not contribute equally to the generation of mIPSCs versus sIPSCs.

Comparison to previous results

Recently, Cohen et al. (2000) published the first systematic study of the postnatal development of IPSC properties in CA1, focusing on fast rising and decaying (i.e., GABA<sub>A</sub>,fast) mIPSCs. The authors found effects of age similar to what we observed, including an increase in frequency, decrease in amplitude, and decrease in decay time between P0 and adulthood. In addition, there have been several developmental studies of IPSCs in dentate gyrus and CA3. Interestingly, the latter studies reported divergent effects of age depending on whether miniature, spontaneous, or evoked IPSCs were investigated, similar to the divergence we report here. In CA3, for example, Taketo and Yoshioka (2000) found that decay time constants of sIPSCs decreased and mean sIPSC amplitude increased between P2–P4 and P18–P38, whereas Rovira and Ben-Ari (1999) found no difference in mIPSC kinetics or amplitude between P4–P8 and P26–P35. In dentate gyrus, effects of age on IPSC kinetics and amplitude were also different depending on whether mIPSCs, sIPSCs, or eIPSCs were studied (Cooper et al. 1999; Draguhn and Heinemann 1996; Otis and Mody 1992), although part of the difference in these results could be explained by differences in the age ranges compared. Contradictory effects of age on mIPSC kinetics have also been reported (Hollrigel and Soltesz 1997; Rovira and Ben-Ari 1999). These studies indicate that it is difficult to predict developmental changes in AP-dependent IPSC properties based on studies of mIPSCs, and vice versa.

Interestingly, Nurse and Lacaille (1999) reported a developmental increase in GABA<sub>B</sub> receptor-mediated IPSCs in CA1 pyramidal cells over a time window nearly identical to what we have observed for GABA<sub>A</sub>,slow (i.e., absent at P12–P14 and maximal by P35–P45). Baclofen responses developed over the same time window, suggesting that the increase in GABA<sub>B</sub> IPSCs with age was mediated by changes in postsynaptic receptor density. This is in contrast to our data, which are consistent with presynaptic changes mediating the develop-
mental increase in GABA_A,slow IPSC amplitude and spontaneous rate. However, because it is difficult to assay the presence and activity of presynaptic boutons in the absence of functional postsynaptic receptors, a coordinated change in pre- and postsynaptic properties of GABA_B inhibition may occur but remain unobserved. As discussed in the following text, this scenario may apply to GABA_A,slow IPSCs as well.

### Developmental changes in GABA_A,slow interneurons

The observations that the rate of GABA_A,slow sIPSCs and the amplitude of GABA_A,slow eIPSCs increase with age, whereas GABA_A,slow mIPSC frequency and amplitude remain unchanged suggest that either the membrane properties of GABA_A,slow interneurons that contribute to spontaneous activity observed in pyramidal cells change between the ages of P10 and P35, or that they are subject to greater excitatory influences from metabotropic glutamatergic or nonglutamatergic sources at older ages. The net result is that in older animals, interneurons generating GABA_A,slow IPSCs in pyramidal cells fire more spontaneous APs and have lower thresholds for activation by exogenous electrical stimuli. There have been no systematic studies of the changes in spontaneous activity or membrane excitability in interneurons as a function of age in CA1. However, developmental regulation of voltage- and calcium-activated K channels (Aoki and Baraban 2000; Du et al. 1996) has been reported. Developmental changes in these or other K channels would be expected to alter the firing properties of interneurons, and may account for the changes in excitability suggested by our data.

The observation that the rate of GABA_A,slow mIPSCs remains stable between P10 and P30 is consistent with the hypothesis that the observed increase in the rate of GABA_A,slow sIPSCs and the amplitude of GABA_A,slow eIPSCs as a function of age is not due to an increase in the number of GABA_A,slow synapses on CA1 pyramidal cells. However, we are cautious about this interpretation because it relies on the assumption that the spontaneous release properties of individual synapses do not change with age. Indeed, it would not be surprising if changes in the activity of the presynaptic interneurons were accompanied by changes in synaptic density, as has been reported for several other systems (Seil and Drake-Baumann 2000). In this scenario, part of the increase in sIPSC frequency and eIPSC amplitude observed with age would be attributed to the properties of the presynaptic cells, and part to the development of additional synapses.

### Selective activation of GABA_A,slow interneurons by NE

Norepinephrine acts via G-protein–coupled receptors to alter membrane excitability and synaptic release in a wide variety of cortical cells, consistent with the widespread forebrain projections of the locus coeruleus (Morrison et al. 1979). In the

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**FIG. 8.** Norepinephrine has no effect on GABA_A,slow mIPSCs. A and B: left: continuous recordings of GABA_A sIPSCs obtained under whole cell voltage clamp from a CA1 pyramidal cell in a slice taken from an animal aged P13 in the presence of 1 μM TTX (A) and in the presence of 1 μM TTX + 1 μM norepinephrine (NE) (B). Right: plots of 63% decay time (τ_{dec}) vs. 10–90% rise time (τ_{rise}) for approximately 500 events recorded from the cell on left. Note that in TTX, there are no GABA_A,slow mIPSCs with or without NE present. Scale bars: 100 ms, 250 pA.
CA1 region of hippocampus, NE has a net inhibitory effect on pyramidal cell activity, presumably via depolarization of GABAAergic interneurons via α2 adrenergic receptors (Bergles et al. 1996). Although 10 μM NE depolarizes interneurons throughout CA1 (Bergles et al. 1996), we have shown here that 1 μM NE selectively increases the rate of GABA_A,slow sIPSCs in pyramidal cells, suggesting that GABA_A,slow interneurons are particularly sensitive to activation by noradrenergic inputs. In CA1, noradrenergic innervation is not uniform, with SL-M receiving the highest innervation and other layers weaker innervation (Oleskevich et al. 1989). The observation that SL-M stimulation selectively activates GABA_A,slow IPSCs suggests that GABA_A,slow interneurons may be preferentially targeted by noradrenergic terminals, or alternatively may express a receptor subtype with higher affinity for NE.

**Functional implications**

The relative paucity of GABA_A,slow IPSCs even in older animals does not imply that GABA_A,slow interneurons are unimportant for CA1 network activity. Indeed, when activated by excitatory stimuli applied to SL-M, GABA_A,slow interneurons generate large amplitude and long-lasting inhibition in CA1 pyramidal cells and in other interneurons (Banks et al. 1998, 2000; Pearce 1993), and can suppress all AP-dependent spontaneous activity in GABA_A,fast interneurons (Banks et al. 2000). Thus although GABA_A,slow interneurons do not exhibit high levels of spontaneous activity, they mediate IPSCs that can profoundly influence the state of excitability in large numbers of CA1 neurons.

We have previously shown that GABA_A,slow IPSCs have the appropriate kinetics to participate in interneuron-based theta frequency oscillations (White et al. 2000). In this computational study, we proposed a model in which theta frequency input from entorhinal cortex to the SL-M region of CA1 activates GABA_A,slow interneurons, and by virtue of these cells’ connections to GABA_A,fast interneurons, organizes and amplifies phase-dispersed, heterogeneous theta frequency excitatory inputs. The developmental changes in GABA_A,slow interneurons suggested by the data presented here predict that this mechanism would be far less effective in young animals than in adults. Although there have been no studies to date concerning the development of theta oscillations in CA1, the results presented here provide a convenient means for testing the functional role of GABA_A,slow in regulating synchronous activity in CA1.

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**REFERENCES**


Nurse S and Lacaille JC. Late maturation of GABA_A synaptic transmission in area CA1 of the rat hippocampus. Neuropharmacology 38: 1733–1742, 1999.


