Protracted Postnatal Development of Inhibitory Synaptic Transmission in Rat Hippocampal Area CA1 Neurons

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Cohen, Akiva S., Dean D. Lin, and Douglas A. Coulter. Protracted postnatal development of inhibitory synaptic transmission in rat hippocampal area CA1 neurons. J Neurophysiol 84: 2465–2476, 2000. In the CNS, inhibitory synaptic function undergoes profound transformation during early postnatal development. This is due to variations in the subunit composition of GABA_{A} receptors (GABA_{A}Rs) at differing developmental stages as well as other factors. These include changes in the driving force for chloride-mediated conductances as well as the quantity and/or cleft lifetime of released neurotransmitter. The present study was undertaken to investigate the nature and time course of developmental maturation of GABAergic synaptic function in hippocampal CA1 pyramidal neurons. In neonatal [postnatal day (P) 1–7] and immature (P8–14) CA1 neurons, miniature inhibitory postsynaptic currents (mIPSCs) were significantly larger, were less frequent, and had slower kinetics compared with mIPSCs recorded in more mature neurons. Adult mIPSC kinetics were achieved by the third postnatal week in CA1 neurons. However, despite this apparent maturation of mIPSC kinetics, significant differences in modulation of mIPSCs by anesthetics in adolescent (P15–21) neurons were still evident. Diazepam (1–300 nM) and zolpidem (200 nM) increased the amplitude of mIPSCs in adolescent but not adult neurons. Both drugs increased mIPSC decay times equally at both ages. These differential agonist effects on mIPSC amplitude suggest that in adolescent CA1 neurons, inhibitory synapses operate differently than adult synapses and function as if sub-synaptic receptors are not fully occupied by quantal release of GABA. Rapid agonist application experiments on perisomatic patches pulled from adolescent neurons provided additional support for this hypothesis. In GABA_{A}R currents recorded in these patches, benzodiazepine amplitude augmentation effects were evident only when nonsaturating GABA concentrations were applied. Furthermore nonstationary noise analysis of mIPSCs in P15–21 neurons revealed that zolpidem-induced mIPSC augmentation was not due to an increase in single-channel conductance of subsynaptic GABA_{A}R subunits but rather to an increase in the number of open channels responding to a single GABA quantum, further supporting the hypothesis that synaptic receptors may not be saturated during synaptic function in adolescent neurons. These data demonstrate that inhibitory synaptic transmission undergoes a markedly protracted postnatal maturation in rat CA1 pyramidal neurons. In the first two postnatal weeks, mIPSCs are large in amplitude, are slow, and occur infrequently. By the third postnatal week, mIPSCs have matured kinetically but retain distinct responses to modulatory drugs, possibly reflecting continued immaturity in synaptic structure and function persisting through adolescence.

INTRODUCTION

During postnatal development in central neurons, there is a regionally distinct progression in cellular expression patterns of GABA_{A} receptor (GABA_{A}R) subunits mRNAs (Brooks-Kayal et al. 1998a; Killisch et al. 1991; Laurie et al. 1992). Furthermore significant changes in the kinetic properties and pharmacology of GABA_{A}-mediated currents are also evident (Oh et al. 1995; Rovira and Ben-Ari 1993). All of these shifts, either individually or in concert, may contribute to alterations in inhibitory synaptic function evident during maturation of the nervous system (Hollrigel and Soltesz 1997). For example, in hippocampal dentate granule cells (DGCs), the frequency of spontaneous inhibitory synaptic currents increases, while rise and decay times decrease until adult values are achieved. In addition, spontaneous inhibitory synaptic currents in DGCs from developing animals exhibit an increased sensitivity to zinc and reduced or absent benzodiazepine (BDZ) sensitivity (Hollrigel and Soltesz 1997). A lack of BDZ sensitivity is also evident in developing CA3 pyramidal neurons (Rovira and Ben-Ari 1993). These functional changes contrast dramatically with GABA_{A}R properties in adult animals and are most probably attributable to changes in GABA_{A}R subunit composition. Thus a developmental, cell-specific shift in the expression of GABA_{A}R subunits may play an essential role in transitions in the function of inhibitory circuitry in the brain (Brooks-Kayal et al. 1998a; Killisch et al. 1991; Laurie et al. 1992). However, the impact that different levels of expression of postsynaptic receptor subunits may have on synaptic function in the developing brain is difficult to infer. Synaptic currents are shaped in part by postsynaptic channel kinetics, which are determined in turn by receptor subunit composition. However, in addition to channel-gating properties, other factors play a prominent role in shaping inhibitory receptor currents, including the time course of transmitter release and the concentration and lifetime of neurotransmitter in the cleft. These latter properties are determined by variations in specific physiologic and pharmacologic properties of the CNS, inhibitory synaptic function undergoes profound transformation during early postnatal development. This is due to variations in the subunit composition of GABA_{A} receptors (GABA_{A}Rs) at differing developmental stages as well as other factors. These include changes in the driving force for chloride-mediated conductances as well as the quantity and/or cleft lifetime of released neurotransmitter. The present study was undertaken to investigate the nature and time course of developmental maturation of GABAergic synaptic function in hippocampal CA1 pyramidal neurons. In neonatal [postnatal day (P) 1–7] and immature (P8–14) CA1 neurons, miniature inhibitory postsynaptic currents (mIPSCs) were significantly larger, were less frequent, and had slower kinetics compared with mIPSCs recorded in more mature neurons. Adult mIPSC kinetics were achieved by the third postnatal week in CA1 neurons. However, despite this apparent maturation of mIPSC kinetics, significant differences in modulation of mIPSCs by anesthetics in adolescent (P15–21) neurons were still evident. Diazepam (1–300 nM) and zolpidem (200 nM) increased the amplitude of mIPSCs in adolescent but not adult neurons. Both drugs increased mIPSC decay times equally at both ages. These differential agonist effects on mIPSC amplitude suggest that in adolescent CA1 neurons, inhibitory synapses operate differently than adult synapses and function as if sub-synaptic receptors are not fully occupied by quantal release of GABA. Rapid agonist application experiments on perisomatic patches pulled from adolescent neurons provided additional support for this hypothesis. In GABA_{A}R currents recorded in these patches, benzodiazepine amplitude augmentation effects were evident only when nonsaturating GABA concentrations were applied. Furthermore nonstationary noise analysis of mIPSCs in P15–21 neurons revealed that zolpidem-induced mIPSC augmentation was not due to an increase in single-channel conductance of subsynaptic GABA_{A}R subunits but rather to an increase in the number of open channels responding to a single GABA quantum, further supporting the hypothesis that synaptic receptors may not be saturated during synaptic function in adolescent neurons. These data demonstrate that inhibitory synaptic transmission undergoes a markedly protracted postnatal maturation in rat CA1 pyramidal neurons. In the first two postnatal weeks, mIPSCs are large in amplitude, are slow, and occur infrequently. By the third postnatal week, mIPSCs have matured kinetically but retain distinct responses to modulatory drugs, possibly reflecting continued immaturity in synaptic structure and function persisting through adolescence.

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anatomic features of synapses, including the amount of neurotransmitter released, possible cooperation between release sites, and the size and shape of the synaptic cleft. These properties may also exhibit systematic fluctuations during development of the nervous system and therefore could also result in ontogenetic alterations in inhibitory synaptic function. For example, in developing neurons, the synaptic area (measured as postsynaptic density length) appears to be larger (Blue and Parnavelas 1983; Markus and Petit 1989). Furthermore synaptic cleft width may be greater in immature inhibitory synapses. Both of these anatomical changes could increase the volume of the synaptic cleft during early development. Accompanying synaptic cleft volume changes are possible changes in subsynaptic receptor density. If CNS synapse development mirrors that present in the peripheral nervous system, then postsynaptic receptor density may gradually increase during synaptogenesis as receptors coalesce and become concentrated directly under the presynaptic terminal (Frank and Fischbach 1979). In addition, vesicle number and/or vesicular content could be different in developing synapses. These factors, individually or in concert, could decrease the level of postsynaptic receptor occupancy during development, in contrast to full receptor occupancy of subsynaptic inhibitory receptors during synaptic transmission present in the adult CNS (see Mody et al. 1994 for review).

The functional relationship between ligand-gated receptors and their agonists has most often been studied using isolated cells and steady-state concentration-response kinetics (cf., Oh et al. 1995; Rovira and Ben Ari 1993). Extrapolating the significance of these data to synapses, where agonist concentration rises and falls rapidly, is difficult. The present study combining synaptic recording and rapid agonist application to perisomatic patches was undertaken to examine how the aforementioned developmental transitions in GABA_A R function and subunit composition interact to determine inhibitory synaptic function in hippocampal CA1 neurons from developing and adult brain. Preliminary reports of this work have appeared (Cohen and Coulter 1998; Lin et al. 1998).

METHODS

Tissue preparation

Male Sprague-Dawley rats were used in all experiments. Recordings were obtained from visually identified pyramidal neurons in stratum pyramidale of area CA1 of the rat hippocampus. For the purpose of this study, animals were divided into four groups: neonatal postnatal day [(P) 1–7], immature (P8–14), adolescent (P15–21), and adult (P56+) days). Brain slices were prepared using previously reported methods (Rafiq et al. 1993). In brief, rats were anesthetized with halothane and decapitated, and the brain was quickly removed and chilled for 1–2 min in a modified sucrose-based artificial cerebrospinal fluid (aCSF) composed of (in mM) 201 sucrose, 3.2 KCl, 1.25 NaHPO_4, 2 MgCl_2, 2 CaCl_2, 26 NaHCO_3, and 10 glucose (equalized with 95% O_2-5% CO_2 at 32.5°C). The brain was glued, frontal side down, to a glass platform with cyanoacylate cement, and coronal whole brain slices (350 μM for neonatal, immature and adolescent animals, 225 μM for adult animals) were sectioned using a Vibratome (Lancer 1000, St. Louis, MO). Brain slices were subsequently hemisected, transferred to a holding chamber, and incubated in warm (35°C) normal aCSF containing 126 mM NaCl substituted for sucrose and allowed to equilibrate for at least 2 h before being transferred to the recording chamber.

Patch recording in slices

Whole cell voltage-clamp recordings were conducted at room temperature from visually identified CA1 pyramidal neurons using infrared differential interference contrast or Hoffman modulation contrast video microscopy (cf. Stuart et al. 1993). Cells were voltage clamped at −60 mV, and signals were recorded and amplified with an Axopatch 1D (Axon Instruments, Foster City, CA), filtered at 2 kHz, digitized, sampled at 44 kHz with a PCl digitizer (Neuro-Corder DR-890, Neurodata Instruments, NY), and stored on videotape for off-line analysis. Electrodes were fabricated from thick-wall borosilicate glass (World Precision Instruments, Sarasota, FL) and pulled to a resistance between 2 and 6 MΩ when filled with an internal solution composed of (in mM) 135 CsCl, 10 HEPES, 2 MgCl_2, and 4 MgATP, pH 7.25 (CsOH) on a two-stage puller (Narishige PP-83, East Meadow, NY). A 2 mV junction potential was measured between this solution and the bath aCSF. All data were left uncorrected.

Rapid agonist application

Fast application of agonists was performed as described by Jonas (1995). Theta glass was mounted on a piezoelectric transducer (Burleigh, Fishers, NY). Waveform protocols were generated using Clampex 7.0 software (Axon Instruments). Agonists were applied at 10 to 20 s intervals, and traces shown in figures are averaged from at least five applications.

On excision of an outside-out patch, the tip of the patch electrode was positioned in the control solution, approximately 20 μm from the interface separating the control and drug streams, which was visualized by the addition of 25 mM sucrose to the drug solution. The patches yielded GABA (1 mM) currents between 10 and 250 pA. In experiments in which zolpidem and GABA were co-applied, zolpidem was included in control solutions. After rupturing the patch, the 20–80% exchange times of the liquid junction currents between control and a 90% control/10% distilled H_2O solution was typically between 200 and 250 μs (see open tip responses in Fig. 7).

Analysis of mIPSCs

Recorded mIPSCs were reacquired using Dempster software (Strathclyde, Glasgow, UK), which collects events using a manually controlled threshold detector and is capable of detecting events as small as two to three times the baseline noise. To attempt to minimize cases of inadequate space clamp, neurons were used for analysis only when series resistance (R_s) was ≤20 MΩ, and ≥80% series resistance compensation was achieved. R_s was checked frequently throughout experiments, and neurons in which R_s increased >20% were discarded. In addition, event amplitudes were plotted against rise times and examined for a possible correlation, where a significant correlation (r² > 0.5) was assumed to signify inadequate space clamp. Neurons in which this occurred were discarded. This occurred in <0.2% of neurons. The kinetics of mIPSCs i.e., amplitude, rise, and decay times were analyzed using cumulative probability histograms. mIPSC frequency was determined using Mini Analysis software (Synaptosoft, Leonia, NJ).

Peak scaled nonstationary noise analysis (NSNA) of mIPSCs (De Koninck and Mody 1994; Perrais and Ropert 1999; Traynelis et al. 1993) was conducted by averaging 50 events to form a single mean ensemble mIPSC time course for a given cell. Fifty to 75 mIPSCs were then randomly selected from the same cell and used for further analysis. The ensemble average was scaled up or down to the size of each original trace and then subtracted. Subtraction of the average ensemble current left a noise trace which fluctuated around the zero current level (see RESULTS and Fig. 6B). The variance at each time point of every individual trace was calculated, and the mean variance was plotted against the mean current amplitude. Data plotted in this manner were fit by a parabolic curve with the following equation

\[ \text{variance} = \text{amplitude}^2 \times \text{ICC} + \text{background} \]

with

\[ \text{ICC} = \frac{\text{ICC}_{\text{baseline}} \times \text{ICC}_{\text{ensemble}}}{\text{ICC}_{\text{ensemble}} - \text{ICC}_{\text{baseline}}} \]
where \( i \) is the single-channel current and \( N P o \) is the number of open channels activated during the mIPSC. The single-channel conductance \((g)\) was derived by dividing \( i \) by the driving force for \( \text{GABA}_A \)-mediated currents, determined from the Goldman-Hodgkin-Katz equation to be 60 mV \( (E_{\text{GABA}} - E_{\text{GABA}}) \) in our solutions. The basal recording noise, i.e., square basal variance \( (\sigma^2_{\text{basal}}) \) was not subtracted prior to conducting NSNA, but ranged from 2.75 to 3.23 pA\(^2\) for all conditions that were used in this analysis (see Table 2 for details).

**Reagents and statistical tests**

Reagents were purchased from the following vendors: all salts and zolpidem, diazepam, furosemide and bicuculline methiodide from Sigma (St Louis, MO); \( \text{D}-2\text{-amino-5-phosphonopentanoic acid (AP5)} \) and \( \text{6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)} \) from Research Biochemicals International (Natick, MA); tetrodotoxin (TTX) from Calbiochem (La Jolla, CA). All drugs were made as stock solutions and then diluted to their final concentration in the bathing medium. Control cells \((n = 3)\) received sham drug administration, i.e., the flow line was switched for 30 min to the same reservoir used in the drug experiments, but in this case the reservoir contained only normal aCSF (data not shown). This procedure controlled for any switching-induced pressure artifacts that may have affected recording parameters in addition to those caused by the drugs. No switching artifacts were apparent, however.

Statistical significance between cumulative probability distributions in control and drug conditions in individual neurons was assessed at the \( P < 0.05 \) confidence level using the Kolmogorov-Smirnov non-parametric statistical test. Two-tailed unpaired Student’s \( t \)-tests were performed to determine statistical significance in the \( P < 0.05 \) confidence level when comparing different treatment groups. Diazepam concentration-response curves were best fitted employing a nonlinear least-squares method assuming a monophasic sigmoidal diazepam concentration-response relationship with the use of the following equation

\[
\%\text{Potentiation} = \frac{M \cdot C^H (C'' + EC_{50}^H)}{1 + EC_{50}^H}
\]

where \( M \) is the maximal effect, \( C \) is the concentration of diazepam, \( H \) is the Hill coefficient, and \( EC_{50} \) is the diazepam concentration at which half-maximal potentiation is achieved. All data are presented as group means \( \pm \) SD unless otherwise noted.

**RESULTS**

**Spontaneous miniature inhibitory currents are present at all postnatal stages**

To investigate how developmental changes might affect synaptic function in the developing brain, we examined the postsynaptic responses elicited by the spontaneous release of \( \text{GABA} \) from single presynaptic vesicles. These events persist in the presence of TTX and are resistant to blocking \( Ca^{2+} \) entry into the terminal and therefore, by analogy to miniature end-plate potentials first studied by Fatt and Katz (1952) have been termed mIPSCs. The advantage of studying mIPSCs is that they are due to activation of single synapses, in contrast to stimulation studies, which can activate tens or hundreds of synapses simultaneously. Therefore mIPSC studies may provide additional insight into the normal functioning of \( \text{GABA} \)-ergic synapses. In the presence of TTX (400 nM) and the excitatory amino acid antagonists \( \text{D}-\text{AP5} (50 \mu M) \) and CNQX (6 \( \mu M \)), spontaneous inward currents were evident at all ages (Fig. 1). Events occurring with a frequency of \( \geq 0.1 \text{ Hz} \) were only detected in 13 (5/38) and 15% (6/41) of \( P1–7 \) and \( P8–14 \) neurons, respectively (Fig. 1C). mIPSC frequency increased markedly and reached adult values by the third postnatal week (see Fig. 1), which may represent increased innervation by inhibitory interneurons (Dupuy and Houser 1996). The \( \text{GABA} \)-ergic identity of these events was confirmed by their blockade by the \( \text{GABA}_A \)-antagonist bicuculline methiodide (30 \( \mu M \), data not shown). Furthermore the reversal potentials \( (E_{\text{rev}}) \) for adolescent and adult neurons were 3.1 \( \pm \) 2.6 and 1.2 \( \pm \) 4.1 mV, respectively \((n = 3 \) for both populations, not significantly different, \( P > 0.05 \)). Both values were close to the theoretical value of \( E_{\text{GABA}} \) (0.2 mV) as calculated by the Goldman-Hodgkin-Katz equation for a \( \text{GABA} \) conductance (Hodgkin and Katz 1949), assuming a bicarbonate to chloride permeability ratio of 0.025 and an activity coefficient of 0.75 for the chloride solution (Bormann et al. 1987).

**Biophysical properties of miniature IPSCs from neonatal (P1–7), immature (P8–14), adolescent (P15–21), and adult (P > 56) CA1 neurons**

Examples of averaged mIPSCs from neonatal, immature, adolescent and adult neurons are depicted in Fig. 2. Analysis of mIPSCs recorded from \( P1–7 \) and \( P8–14 \) pups revealed significantly larger mIPSC amplitudes, longer decay times, and slower rise times compared with those present in the adult \((P > 0.05, \text{see Fig. 2, B–D, and Table 1})\). Conversely, the decay and amplitude of mIPSCs recorded from adolescent \((P15–21) \) neurons were similar to those present in adult neurons (Fig. 2, B

\[
\sigma^2 = (i - i^2)/NPo
\]
and C; Table 1). However, 10–90% rise times measured in adolescent CA1 neurons were still significantly slower when compared with adult values ($P < 0.05$, Fig. 2D; Table 1). Because the frequency of mIPSCs in neonatal and immature (P1–14, Fig. 1, B and C; Table 1) neurons was so low, conducting pharmacological studies on these age groups was extremely difficult. Therefore we were confined to investigate further potential differences in inhibitory GABA<sub>A</sub> receptor pharmacology in adolescent (P15–21) and adult CA1 neurons.

### Differential effects of zolpidem on mIPSCs from adolescent and adult neurons

GABA<sub>A</sub>Rs are believed to be pentameric (Chang et al. 1996; Tretter et al. 1997) and composed of subunits from several related subunit families. GABA<sub>A</sub>R subunit composition varies developmentally, regionally, and in specific neuronal subtypes. This in turn influences the conductance, channel kinetics, and pharmacological sensitivity of GABA-evoked responses (Macdonald and Olsen 1994; Wisden et al. 1992). For example BDZ sensitivity is conferred by the presence of a γ2 subunit (Pritchett et al. 1989), and given that a γ2 subunit is present, it has been shown that the α subunits in the receptor dictate the specific BDZ ligand that binds to and modulates the GABA<sub>A</sub>R complex (Lüddens and Wisden 1991; Pritchett et al. 1989). For example, GABA<sub>A</sub>Rs in which α1 subunits are expressed together with β<sub>3</sub>γ2 exhibit high affinity for BDZ type 1 (BDZ1) agonists like zolpidem (Arbilla et al. 1986; reviewed in Barnard et al. 1998; Macdonald and Olsen 1994). GABA<sub>A</sub>Rs containing α2, α3, or α5 subunits demonstrate lower affinity for BDZ1-specific ligands, with α4- and α6-containing receptors completely lacking BDZ agonist sensitivity (Wafford et al. 1996). Immunohistochemical (Killisch et al. 1991) and in situ hybridization (Wisden et al. 1992) studies demonstrated that α1 can be detected shortly after birth (P6) in forebrain neurons, and its expression continues to increase significantly until adulthood.

If an apparent developmental up-regulation of the α1 subunit was occurring in CA1 neurons, we hypothesized that mIPSCs recorded in adult CA1 neurons would demonstrate higher BDZ1 sensitivity relative to adolescent neurons. Therefore, we examined the effects of zolpidem (ZOL), a specific BDZ1 receptor agonist (Biggio et al. 1989; Wafford et al. 1993). Bath application of ZOL (200 nM) affected both the amplitude and decay of mIPSCs recorded from P15–21 CA1 pyramidal neurons (Fig. 3A, 1 and 2, and inset). The median amplitude was significantly enhanced from −34.7 ± 6.8 (n = 15) to −44.8 ± 7.1 pA (a 30.6% enhancement, n = 6, P < 0.05). The median T50 decay time was also significantly increased from 12.2 ± 2.5 to 24.3 ± 5.0 ms (Fig. 3A and inset). Furthermore, the slow component of the decay, $\tau_{\text{slow}}$, was significantly augmented as well from 19.5 ± 2.8 to 32.1 ± 3.2 ms. The contribution of $\tau_{\text{slow}}$ to the entire decay was significantly enhanced (from 51 ± 11 to 90 ± 4%, P < 0.05). The fast component of the decay was unaffected by ZOL application ($\tau_{\text{fast}}$ = 4.8 ± 1.9; 6.0 ± 3.5 ms for control and ZOL, respectively). These results contrast with ZOL effects on mIPSCs recorded from adult pyramidal CA1 neurons, where bath application of ZOL significantly slowed only the decay of the mIPSCs (Fig. 3B, 1 and inset). T50 increased from 10.8 ± 1.5 to 16.9 ± 3.9 ms, n = 6, 4 respectively, and $\tau_{\text{slow}}$ increased from 19.7 ± 3.4 to 28.4 ± 3.1 ms, P < 0.05. Interestingly, mIPSC amplitude and contribution of $\tau_{\text{slow}}$ to the

### Table 1. Biophysical properties for mIPSCs recorded from neonatal, immature, adolescent, and adult neurons

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Amplitude (pA)</th>
<th>10–90% Rise Time (μs)</th>
<th>T50 Decay Time (ms)</th>
<th>Charge Transfer, pC</th>
<th>Capacitance, pF</th>
<th>Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal</td>
<td>714 (5)</td>
<td>−61.5 ± 14.8†‡</td>
<td>2460 ± 63†‡</td>
<td>24.5 ± 6.1†‡</td>
<td>2239.3 ± 659.7†‡</td>
<td>31.94 ± 12.8</td>
</tr>
<tr>
<td>Immature</td>
<td>831 (6)</td>
<td>−47.3 ± 8.3†‡</td>
<td>1480 ± 50†‡</td>
<td>19.71 ± 3.2†‡</td>
<td>1587.9 ± 573.9†‡</td>
<td>43.35 ± 7.0</td>
</tr>
<tr>
<td>Adolescent</td>
<td>3541 (16)</td>
<td>−34.3 ± 6.4</td>
<td>670 ± 80†</td>
<td>12.1 ± 2.5</td>
<td>675.5 ± 134.7</td>
<td>28.8 ± 7.2</td>
</tr>
<tr>
<td>Adult</td>
<td>2167 (15)</td>
<td>−39.0 ± 4.7</td>
<td>530 ± 40†‡</td>
<td>10.7 ± 1.7</td>
<td>654.7 ± 289.0</td>
<td>33.9 ± 17.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. Neonatals are postnatal (P) days 1–7, immatures on days P8–14, adolescents are days P15–21, adults are days P56+. * Number of events analyzed; numbers of cells are in parentheses. MIPSC, miniature postsynaptic current. † Denotes significance at the $P < 0.05$ confidence level compared to adult values. ‡ Denotes significance at the $P < 0.05$ confidence level compared to adolescent values.
entire decay were not altered (Fig. 3B1 and inset). As has been previously shown, mIPSC amplitudes recorded in adult neurons in the presence of BDZs were unchanged from that recorded in control solution (Otis and Mody 1992; Poncer et al. 1996). The total mIPSC charge transfer was significantly enhanced by ZOL application from \(-654.7 \pm 289.0\) pC to \(-1013.5 \pm 187.2\) pC (a 54.8% augmentation, \(P < 0.05\)).

The mean increase in mIPSC amplitude observed during ZOL exposure to P15–21 CA1 pyramidal neurons profoundly affected total mIPSC charge transfer in these cells, relative to ZOL effects on mIPSCs in adult neurons. The mean charge transfer was augmented 110.1% (from \(-675.5 \pm 134.7\) to \(-1419.1 \pm 237.1\) pC, Fig. 3A2 and B2, insets). When the amplitude effects were normalized by scaling the control sweep to the ZOL sweep, the ZOL-induced enhancement was markedly smaller (56.3%; range 41.2–70%, normalized ZOL-Cont, Fig. 3A2, inset), and comparable to the augmentation induced by ZOL on mIPSCs in adult neurons (50.5%; range 32.1–71.8%, Fig. 3B2, inset). Thus the large increase in charge transfer during ZOL application demonstrates that ZOL is more efficacious in enhancing inhibitory neurotransmission in P15–21 CA1 neurons primarily due to the specific mIPSC amplitude effects in these neurons. Furthermore the mean mIPSC frequency was not significantly altered in the presence of ZOL, suggesting that the ZOL-induced mIPSC enhancement is due to a postsynaptic mechanism in both adolescent and adult CA1 neurons. The mIPSC frequency in the adolescent group was 3.1 \pm 1.2 and 3.6 \pm 2.0 Hz for control and ZOL, respectively, while the frequency in the adult group was 3.0 \pm 1.8 and 3.7 \pm 1.2 Hz in control and ZOL (unpaired t-test \(P > 0.05\)).

Diazepam, like ZOL, differentially modulates mIPSCs from adolescent and adult CA1 neurons

What factor(s) could possibly be mediating differential mIPSC amplitude effects of BDZ agonists present in adolescent inhibitory synapses? Could it simply be due to enhanced ZOL sensitivity in the adolescent mIPSCs due to dissimilar expression levels of BDZ-sensitive subunits in the adolescent receptors? To examine this possibility, we pharmacologically probed adolescent and adult GABA\(_A\)Rs with a second drug, the broad-spectrum BDZ diazepam (DZ). Concentration-response curves were constructed for effects of DZ on CA1 mIPSCs in both populations. It was technically difficult to record in control aCSF and then bath apply each DZ concentration to each cell due to the necessary long duration of the recordings. Therefore we preincubated slices in varying diazepam concentrations (1–300 nM) and plotted the mean values of the median amplitudes and T50s of mIPSCs in neurons for each individual concentration. When plotted as log [DZ] versus percentage increase in T50, the decay time was found to increase in sigmoidal manner as greater concentrations of diazepam were applied to both adolescent and adult slices (Fig. 4A).

The concentration-response relationships representing DZ-induced effects on mIPSC decay time and amplitude contrasted markedly with each other. In the decay plot (Fig. 4A), both curves are sigmoidal in shape and are not significantly different from each other. The efficacy (or maximal effect) of DZ on mIPSC decay time was not significantly different in adolescent and adult neurons (\(P > 0.05\), unpaired t-test, 100 and 300 nM concentrations). Prolongation of mIPSC decay in both populations is not surprising since both ZOL and midazolam have been reported to slow mIPSC decay time in adult DG (Otis and Mody 1992) and CA3 (Poncer et al. 1996) neurons. The Hill coefficients for these curves (Fig. 4A) were 1.8 and 1.5 for adolescent and adult cells, respectively.

In the amplitude plot (Fig. 4B), the two curves are notably dissimilar. In the adult population, there is no significant amplitude potentiation as the concentration of DZ was increased up to 300 nM (Fig. 4B), consistent with previous studies (Otis and Mody 1992; Poncer et al. 1996). On the other hand, the amplitude plot in the adolescent population is sigmoidal, i.e., mIPSC amplitude increases with increasing DZ concentrations. In addition, the efficacy of DZ-induced mIPSC amplitude augmentation in the P15–21 group is significantly higher than...
that exhibited in the adult population ($P < 0.05$, unpaired $t$-test, 100 and 300 nM concentrations). Adolescent mIPSC amplitude augmentation induced by DZ was similar to that previously demonstrated with ZOL (Fig. 3), both of which suggest that postsynaptic GABAA Rs in adolescent synapses are not fully occupied by the release of a single quantum.

Possible developmental increases in BDZ-insensitive receptors do not appear to contribute to altered BDZ sensitivity

BDZ-induced mIPSC amplitude effects, which we are hypothesizing may be due to partial occupancy of postsynaptic GABA$\alpha$Rs in response to quantal GABA release, may also be due to different receptor subunit composition present in inhibitory synapses in adolescent neurons. Therefore we next considered whether overall BDZ sensitivity might be diminished due to an up-regulation of $\alpha 4$ in the adult population. If $\alpha 4$ was being substituted for either $\alpha 1$ or $\alpha 5$, then this should result in an overall decrease in BDZ sensitivity of mIPSCs recorded from adult neurons because inclusion of $\alpha 4$ in the pentameric receptor leads to BDZ insensitivity (Wieland et al. 1992). To test this hypothesis, we examined mIPSC sensitivity to an $\alpha 4$ and $\alpha 6$ subunit-specific modulator. The diuretic furosemide has been shown to specifically block GABA$\alpha$Rs composed of $\alpha 4$ or $\alpha 6$ subunits in addition to $\beta$ and $\gamma$ (Tia et al. 1996; Wafford et al. 1996). Bath application of furosemide (600 $\mu$M) for 40 min significantly altered the amplitude and decay of mIPSCs from both adolescent and adult neurons ($P < 0.05$; Fig. 5). Application of furosemide reduced the median mIPSC amplitude by 33 $\pm$ 3 and 28 $\pm$ 4%.

![Diagram](image1.png)

**FIG. 4.** Concentration-response curve of diazepam (DZ) effects on mIPSCs in adolescent (P15–21) and adult (>P56) CA1 neurons. A: log/linear plot of the concentration-dependent augmentation in mIPSC T50 decay time by increasing concentrations of DZ. B: similar log/linear plot of the DZ concentration-dependent augmentation in mIPSC amplitude. Values were plotted as a percentage increase calculated by dividing the population median value in a given [diazepam] by the population median T50 or amplitude obtained in control artificial cerebrospinal fluid (aCSF). Note the amplitude scale difference in B. All slices were preincubated in their specific DZ concentration for 20–30 min before transfer to the recording chamber for experiments. The number of cells recorded ($n$) for each DZ concentration is given in parentheses above each curve in B. Values are expressed as a percentage of the median values obtained in control aCSF (different slices). Curves are unweighted nonlinear least-squares fits of the data. The EC$_{50}$ and Hill coefficients for the mIPSC decay curves were 12.0 $\pm$ 7.8 and 25.5 $\pm$ 6.8 nM and 1.8 $\pm$ 1.5; 1.5 $\pm$ 0.4 for the adolescent and adult groups, respectively. The EC$_{50}$ and Hill coefficient for the mIPSC amplitude curve obtained in adolescent CA1 neurons were 12.0 $\pm$ 1.6 nM and 1.4 $\pm$ 0.4. Higher concentrations of DZ were not assayed because it has been reported that the agonist will begin to decrease the frequency of channel openings (Rogers et al. 1994).

![Diagram](image2.png)

**FIG. 5.** Furosemide affects mIPSCs in adolescent and adult CA1 neurons similarly. A: representative mIPSCs for a P18 neuron in control (A1) and in furosemide (A2, 600 $\mu$M, 20 min). B: representative mIPSCs for an adult (P58) neuron in control (B1) and in furosemide (B2). A similar decrease in the median mIPSC amplitude in both populations during furosemide exposure can be readily seen in the cumulative probability histograms (A3 and B3). Data were reacquired at 10 kHz.
and increased the median T50 31 ± 5 and 16 ± 4%, respectively, for adolescent and adult neurons. These effects are very similar to what has been reported previously in developing CA1 pyramidal neurons (P14–42) (Banks et al. 1998). Since α6 is only expressed in the cerebellum, the similar alterations in mIPSC kinetics induced by furosemide suggests that BDZ-induced mIPSC amplitude effects are not due to differential alterations in α4 expression, which is making a similar contribution to GABA_ARs involved in synaptic transmission at both developmental stages.

**Amplitude augmentation of mIPSCs in adolescent neurons by allosteric modulators is due to an increased number of activated synaptic channels**

The significant BDZ augmentation of mIPSC amplitude recorded in adolescent neurons could be a result of either an increase in single GABA_A channel conductance (g), as reported in cultured hippocampal neurons (Eghbali et al. 1997), or due to an increase in the number of activated postsynaptic channels (N) or the open probability of an activated channel (P_o). We conducted peak scaled nonstationary noise analysis to elucidate the mechanism(s) underlying the ZOL-induced increase in adolescent (P15–21) mIPSC amplitude (see Fig. 6). According to nonlinear least-squares fitting of the resulting noise curves under control and ZOL-exposed conditions (Fig. 6, C and D, respectively), the mean single-channel conductance g for neurons under control conditions was 26.7 ± 1.8 pS, not significantly different from g for the cells in the presence of ZOL (25.0 ± 0.9 pS) or g of control adult neurons (25.6 ± 1.0 pS, n = 4, Table 2). This is similar to the predominant conductance state of 30 pS measured in single-channel studies (Schonrock and Bormann 1993). Hence ZOL-induced augmentation of mIPSC amplitude is probably not due to an increase in subsynaptic receptor single-channel current. Since two main variables determine peak amplitude, g and N_Po, and g has not changed, then N_Po must have. Accordingly, N_Po (the number of activated channels) during ZOL application was 32 ± 0.5 (n = 3), significantly larger than N_Po in control conditions (21 ± 0.5, n = 3). Because peak scaling was used to minimize some of the inherent variability in mIPSC amplitudes, it is not possible to determine whether N or P_o was enhanced by ZOL in this analysis; however, see following text.

**Patch currents**

From the data described in the preceding text, we hypothesized that during synaptic activity, GABA cleft concentration may be lower or P_o might be decreased in adolescent synapses compared with that present in adult synapses. An alternate explanation for the preceding data is that there could be some occult difference between the properties of receptors in the two developmental stages that are not manifest in whole-cell synaptic recordings, and these subtle distinctions account for the specific amplitude effects of GABA modulators in adolescent neuron mIPSCs. To further explore this issue, we conducted additional experiments, examining ZOL-induced effects on GABA-evoked currents in perisomatic outside-out patches pulled from adolescent and adult CA1 neurons. In these experiments, saturating concentrations of GABA or GABA + ZOL were applied using ultra-rapid agonist application tech-

### Table 2. Parameters of single GABA_A channel conductance (g) and number of open channels (N_Po) and baseline recording noise (σ^2 recording noise) derived from non-stationary noise analysis of mIPSCs conducted in adult and adolescent neurons under control conditions and during ZOL exposure

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>g (pS)</th>
<th>N_Po</th>
<th>σ^2 recording noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>4</td>
<td>25.64 ± 0.8</td>
<td>27.00 ± 4.0</td>
<td>2.80 ± 0.6</td>
</tr>
<tr>
<td>Adult + ZOL</td>
<td>4</td>
<td>26.61 ± 1.8</td>
<td>28.80 ± 2.9</td>
<td>3.20 ± 0.3</td>
</tr>
<tr>
<td>Adolescent</td>
<td>3</td>
<td>26.70 ± 1.8</td>
<td>21.10 ± 0.5*</td>
<td>2.75 ± 0.6</td>
</tr>
<tr>
<td>Adolescent + ZOL</td>
<td>3</td>
<td>25.23 ± 0.9</td>
<td>32.30 ± 0.5</td>
<td>3.23 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SD; n is the number of cells. * Significance at the P < 0.05 confidence level compared to adolescent zolpidem (ZOL) values.
niques, to better mimic GABA application kinetics during synaptic responses.

If Po was enhanced by ZOL application, then even under saturating GABA concentrations, an increase in the current amplitude and/or kinetics should have been evident. Therefore outside-out patches were exposed to 1-ms pulses of a high concentration (1 mM) of GABA that are thought to be saturating (Lavoie and Twyman 1996; Mellor and Randall 1997; Oh et al. 1995) in the absence and presence of 200 nM ZOL to examine ZOL’s effects on maximally activated GABA 2 receptors (Fig. 7B). In six patches excised from adolescent CA1 neurons, no significant alterations were induced by co-application of ZOL and 1 mM GABA (on peak amplitude or decay time) when compared with the current transient evoked by 1 mM GABA alone (P > 0.05). In similar fashion to previous investigations (Hill et al. 1998), decay kinetics of the GABA-evoked currents were best fit using two exponentials. The fast, and slow decay constants and percentage fast decay component of GABA (1 mM)-evoked responses measured in patches pulled from adolescent neurons were not significantly different (P > 0.05 t-test, see Table 3) from the values obtained when GABA was co-applied with ZOL. In similar fashion the fast and slow decay constants, as well as the percentage fast decay component exhibited in patches excised from adult CA1 neurons in response to 1 mM GABA responses, were not significantly different (see Table 3). Interestingly, τfast in the adult patches (in GABA alone and GABA + ZOL) was significantly slower than that measured in adolescent patches (P < 0.05, see Table 3). The data derived from excised patches exposed to saturating concentrations of GABA co-applied with ZOL clearly showed no effect on either the amplitude or kinetics of the response (Fig. 7B, Table 3). Therefore, it is unlikely that Po is enhanced by ZOL application, but rather a single GABA quantum activates a larger number of channels in the presence of ZOL.

When low (50 μM), nonsaturating concentration of GABA was applied to patches pulled from adolescent neurons, this resulted in peak current amplitudes evoked that were only 36.2 ± 4.6% (n = 4) of the peak amplitudes evoked during maximal GABA 2R activation (to 1 mM GABA), confirming 50 μM GABA is nonsaturating (Fig. 7A). Figure 7C depicts the currents evoked by 1-ms pulses of low (nonsaturating) concentrations (50 μM) of GABA on the same patch. When co-applied with 50 μM GABA, 200 nM ZOL induced a 17.6 ± 5.9% augmentation of peak response amplitude (n = 3) with no significant effects on decay kinetics (GABA: τfast = 6.4 ± 0.8 ms, τslow = 149.8 ± 23.1; GABA + ZOL: τfast = 6.0 ± 0.7 τslow = 130.5 ± 5.8 ms, P > 0.05). The potentiation observed with co-application of ZOL and saturating concentrations of GABA (Fig. 7C) is similar to the ZOL-induced mIPSC augmentation observed in whole cell experiments, further supporting the concept that GABA cleft concentrations during the peak of the mIPSC may be subsaturating in adolescent (P15–21) CA1 neurons.

**DISCUSSION**

The primary findings of this CA1 pyramidal neuron study are GABAergic inhibitory synapses are functional immediately after birth; mIPSCs are larger and slower in neonatal and immature (P1–14) neurons compared with those present in the adult; mIPSC frequency is extremely low early in development but reaches adult values by the beginning of the third postnatal week; mIPSC amplitudes and decay times in adolescent (P15–21) neurons are not significantly different from adult values; however, 10–90% rise times are still significantly slower; and sub-synaptic GABA 2 receptors in adolescent CA1 neurons are differentially sensitive to modulation by BDZs, reflecting a protracted postnatal immaturity of inhibitory synapses in these neurons.

**TABLE 3.** Kinetic parameters of GABA-mediated currents (1 mM) in outside-out patches excised from CA1 pyramidal neurons

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>τfast, ms</th>
<th>τslow, ms</th>
<th>Percent τfast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>5</td>
<td>31.56 ± 5.9*</td>
<td>207.72 ± 31.9</td>
<td>47.03 ± 3.1</td>
</tr>
<tr>
<td>Adult + ZOL</td>
<td>5</td>
<td>32.68 ± 4.1*</td>
<td>290.82 ± 12.4</td>
<td>53.84 ± 6.4</td>
</tr>
<tr>
<td>Adolescent</td>
<td>6</td>
<td>13.10 ± 4.4</td>
<td>131.70 ± 18.9</td>
<td>44.12 ± 11.2</td>
</tr>
<tr>
<td>Adolescent + ZOL</td>
<td>6</td>
<td>10.85 ± 3.8</td>
<td>149.32 ± 19.7</td>
<td>33.13 ± 4.2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = number of patches. * Significance at the P < 0.05 confidence level compared to adolescent ZOL values.
In early postnatal development, GABA functions as an excitatory neurotransmitter

In the hippocampus, GABAergic interneurons mature and cease to divide before their principal neuron counterparts (i.e., pyramidal and granule cells) (Amaral and Kurz 1985). Moreover, hippocampal inhibitory networks are well developed at birth (Rozenberg et al. 1989) contrasting markedly with excitatory inputs, which develop during the first postnatal week of life (Richter and Wolf 1990). Previous studies have demonstrated that GABA application to CA3 (Ben-Ari et al. 1994; Cherubini et al. 1991) and CA1 (Janigro and Schwartzkroin 1988) neurons elicits depolarizing responses at resting membrane potentials during the first postnatal week, which subsequently reverse to hyperpolarizing during the second week of development. The depolarizing responses induced by GABA are thought to be important in causing sufficient depolarization to activate voltage-gated Ca\(^{2+}\) channels promoting a rise in intracellular Ca\(^{2+}\) that is essential for neuronal growth and differentiation (Miller 1988). Hippocampal neurons grown in the presence of a GABAA antagonist were stunted with respect to dendritic length, number of primary neurites, and branch points (Ben-Ari et al. 1994). Although whole cell recording-induced dialysis in the present study precludes examination of altered intracellular chloride levels, changes in the kinetics of inhibitory synaptic function were evident during early postnatal development. mIPSCs recorded from neonatal and immature (P1–14) CA1 neurons were significantly slower and larger compared with adult values. Since GABA\(_{A}\)R subunit composition undergoes significant changes during the first two weeks of postnatal development (Killisch et al. 1991; Laurie et al. 1992), the kinetic changes most notably observed in the present study between P1 and P14 are probably due to receptor subunit rearrangement. This hypothesis could not be further tested because a detailed pharmacological analysis probing neonatal and immature mIPSCs was unfeasible due to the low frequency of recordable events in these populations (see Fig. 1C).

However, in the periphery, at the neuromuscular junction, nicotinic acetylcholine receptors are diffusely present along the entire myotube, but on innervation, these receptors migrate and concentrate only in the area directly underneath the presynaptic terminal (Frank and Fischbach 1979). If CNS synapses develop in similar fashion, then developmental transitions in postsynaptic receptor clustering under the presynaptic terminal from diffuse to highly concentrated would not be surprising. In addition, having loosely packed sub synaptic GABA\(_{A}\)Rs encompassing more area might be crucial in the CNS by generating a wider spread depolarization facilitating robust Ca\(^{2+}\) entry necessary for neuronal growth and differentiation. Furthermore, it is reasonable to hypothesize that the larger and slower neonatal and immature mIPSCs may also offer a longer effective temporal window for the requisite summation of depolarizing GABA\(_{A}\)-mediated signals facilitating intracellular calcium accumulation essential for the aforementioned neuronal growth and differentiation.

Age-dependent disparate mIPSC modulation by BDZ agonists

Previous studies have demonstrated age-dependent changes in BDZ binding sites, GABA\(_{A}\)R immunohistochemistry, and expression of mRNAs encoding GABA\(_{A}\)R subunits during postnatal development (Brooks-Kayal et al. 1998a; Killisch et al. 1991; Laurie et al. 1992). Little is known about how these changes in subunit composition and density of GABA\(_{A}\)Rs may impact inhibitory synaptic function during development. In the present study, when CA1 inhibitory neurotransmission was probed with allosteric agonists, we found a marked discrepancy between the modulatory responses of adolescent and adult inhibitory synaptic events. Bath application of broad spectrum and BDZ1-specific BDZ agonists significantly increased the mIPSC decay time in both adult and adolescent populations to an equivalent extent. However, in addition to the expected observation of slowing mIPSC decay time (De Koninck and Mody 1994; Otis and Mody 1992; Poncer et al. 1996), both agonists enhanced mIPSC amplitude at adolescent synapses. Thus the kinetics and sensitivity of adolescent and adult mIPSCs to modulation by BDZ agonists undergoes significant transformation during postnatal development.

Possible mechanisms mediating BDZ-induced mIPSC amplitude effects

What factors could potentially be causing the differential BDZ sensitivities evident in adolescent and adult neurons? Specifically what properties of adolescent (P15–21) synapses could lead to BDZ-induced mIPSC peak effects? First, the subsynaptic receptors in the two populations could be different i.e., the specific subunit composition of GABA\(_{A}\)Rs could be dissimilar; thereby, conferring disparate pharmacological sensitivities in adolescent and mature CA1 pyramidal neurons (reviewed in Barnard et al. 1998; Macdonald and Olsen 1994; Mody et al. 1994). Second, BDZ application could be causing modulator-induced alterations in single-channel conductance (Eghbali et al. 1997). Finally, the anatomical structure of the synapses could differ, altering receptor clustering dynamics and/or transmitter concentration in the cleft.

To assess whether subunit composition in the two populations differed, we assayed several subunit specific modulators. Application of both broad-spectrum BDZ and BDZ1-specific modulators elicited mIPSC peak enhancement. Therefore, differential expression of subunits regulating GABA\(_{A}\)R BDZ sensitivities (γ2, α1, and α5) (see Barnard et al. 1998) are probably not responsible for the altered BDZ responses in the two receptor populations. Furthermore, using furosemide to probe the receptors for potential increased contributions of BDZ-insensitive subunits (α4) (Banks et al. 1998; Tia et al. 1996; Wafford et al. 1996) demonstrated no significant differential sensitivities between adult and adolescent GABA\(_{A}\)Rs (see RESULTS). Finally, no significant differences in biophysical parameters (besides 10–90% rise times) or disparate agonist-induced effects on mIPSC kinetics (specifically decay times) were evident in the two populations, and the noise-analysis-derived single-channel conductance in adolescent and adult synaptic GABA\(_{A}\)Rs was not significantly different (see RESULTS). These data suggest that GABA\(_{A}\)R subunit composition in adolescent and adult CA1 neurons are not markedly different. This suggests two things: first, most of the large scale developmental transitions in GABA\(_{A}\)R subunit composition may be occurring prior to adolescence, and second, other factors may be responsible for the differential BDZ sensitivity in adolescent versus adult synapses.
Several recent reports have reported similar agonist-induced effects on mIPSC amplitude. High concentrations of zolpidem (10 μM) enhanced both mIPSC amplitude and duration in layer V cortical neurons (Perrais and Ropert 1999), rat and mouse CA1 pyramidal cells and interneurons, as well as mouse dentate granule cells (Hajos et al. 2000). Diazepam increased autaptic mIPSC amplitude by 125% in cultured amacrine cells (Frerking et al. 1995). Furthermore, flunitrazepam increased mIPSC amplitude in cultured cerebellar granule cells (Mellor and Randall 1997), and the γ-butyrolactone, diethyl-lactam, enhanced mIPSCs in cultured hippocampal neurons (Hill et al. 1998). Finally, the imidazole etomidate increased mIPSC amplitude by 143% in cultured hippocampal neurons (Hill et al. 1998). Interestingly, all the aforementioned studies were undertaken in developing animals (P13–26) (Hajos et al. 2000; Nusser et al. 1997; Perrais and Ropert 1999) or in neuronal cultures where developmental status is difficult to assess (Frerking et al. 1995; Hill et al. 1998; Mellor and Randall 1997; Yang and Uchida 1996). Interestingly, all the aforementioned studies were undertaken in developing animals (P13–26) (Hajos et al. 2000; Nusser et al. 1997; Perrais and Ropert 1999) or in neuronal cultures where developmental status is difficult to assess (Frerking et al. 1995; Hill et al. 1998; Mellor and Randall 1997; Yang and Uchida 1996). These data all suggest that subsynaptic GABA_{A,R} in developing neurons are not fully occupied by the release of a single quantum.

If the postsynaptic receptor-containing area is larger in adolescent neurons, distributing subsynaptic receptors over a wider postsynaptic area, then transmitter released from a punctate source, e.g., a single quantum, may not reach saturating levels for those receptors distant from the vesicular release site. Examining synaptic ultrastructure in developing (P14) and mature brains (P90), Blue and Parnavelas (1983) reported a 20% decrease in mean postsynaptic density length in putative inhibitory Gray’s type II synapses accompanying development. Furthermore, Markus and Petit (1989) showed a shift in synaptic curvature from a balance between concave and convex synapses during development (P15–30) to a predominance of convex synapses in adult (P60–90). This is intriguing because concave or “smile” synapses have larger postsynaptic areas compared with convex or “frown” synapses (Markus and Petit 1989). The latter study did not discriminate between type I and type II synapses. However, both of these findings suggest a consolidation in subsynaptic area may occur during postnatal development, consistent with a putative transition from partial to full synaptic receptor occupancy during activity.

Developmental decreases in synaptic area may be accompanied by increases in subsynaptic receptor density. Other anatomical and functional alterations besides subsynaptic area and receptor number could also impact subsynaptic GABA concentrations. For example, transmitter clearance mechanisms could be different in adolescent neurons. This is unlikely, however, since GABA transporters appear to have matured by the second week of ontogenesis (Draguhn and Heinemann 1996), and mIPSCs have been shown to be unaffected by GABA uptake inhibitors (Otis and Mody 1992; Thompson and Gähwiler 1992). An additional anatomical mechanism could involve alterations in the synaptic cleft, where the cleft area could diminish in size on maturity, thereby decreasing cleft volume and increasing the GABA cleft concentration following quantal release. Interestingly, even though the mean mIPSC amplitude was not significantly different in the adult and adolescent populations, there was a tendency demonstrating that mIPSCs recorded in adult neurons were larger than their adolescent counterparts. Moreover GABAergic synapses are difficult to identify morphologically in early postnatal dentate gyrus (Dupuy and Houser 1996), supporting a possible increased synaptic cleft distance during development. Furthermore, in the present study, 10–90% rise times in adolescent mIPSCs were significantly slower than those measured in adults, suggesting that activation of adolescent postsynaptic GABA_{A,R} is retarded because the effective transmitter cleft concentration is less in these synapses, delaying channel activation.

In summary, these results demonstrate that spontaneous miniature inhibitory currents become faster and smaller as they mature and reach adult values by the third postnatal week. This maturation may be due to alterations in GABA_{A,R} subunit composition as well as fine tuning of synaptic ultrastructure/cytarchitecture and the resultant condensing of receptor clusters apposing presynaptic active zones. Although adolescent synapses appear to function similarly to adults, significant differences in their modulatory responses to BDZs are evident. The pharmacological disparity existing between inhibitory synapses in adolescent and adult CA1 pyramidal neurons may be conferred by anatomical alterations in synaptic structure in the absence of significant receptor changes and may represent a novel mechanism for regulating transmitter efficacy by receptor modulators in adolescent inhibitory CNS synapses. These data demonstrate that inhibitory synaptic transmission undergoes a markedly protracted postnatal development in rat CA1 pyramidal neurons, possibly reflecting continuing immaturity in synaptic structure persisting until adolescence. Alterations in inhibitory function can contribute to the generation of seizure disorders (Brooks–Kayal et al. 1998b, 1999; Buhl et al. 1996; Gibbs et al. 1997). Moreover, since GABA_{A,R}s are a primary site of action for many anti-epileptic drugs, e.g., BDZs and barbiturates, developmental changes in GABA_{A} receptor function may have critical implications for the development of treatment regimens in the pediatric population. The data presented here may offer insight facilitating the development of novel therapeutic strategies for age-specific seizure disorders.

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