Prolongation of Hippocampal Miniature Inhibitory Postsynaptic Currents in Mice Lacking the GABA	extsubscript{A} Receptor α1 Subunit

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Goldstein, Peter A., Frank P. Elsen, Shui-Wang Ying, Carolyn Ferguson, Gregg E. Homanics, and Neil L. Harrison. Prolongation of hippocampal miniature inhibitory postsynaptic currents in mice lacking the GABA	extsubscript{A} receptor α1 subunit. J Neurophysiol 88: 3208–3217, 2002. 10.1152/jn.00885.2001. GABA	extsubscript{A} receptors (GABA	extsubscript{A}-Rs) are pentameric structures consisting of two α, two β, and one γ subunit. The α subunit influences agonist efficacy, benzodiazepine pharmacology, and kinetics of activation/deactivation. To investigate the contribution of the α1 subunit to native GABA	extsubscript{A}-Rs, we analyzed miniature inhibitory postsynaptic currents (mIPSCs) in CA1 hippocampal pyramidal cells and interneurons from wild-type (WT) and α1 subunit knock-out (α1 KO) mice. mIPSCs recorded from interneurons and pyramidal cells obtained from α1 KO mice were detected less frequently, were smaller in amplitude, and decayed more slowly than mIPSCs recorded in neurons from WT mice. The effect of zolpidem was examined in view of its reported selectivity for receptors containing the α1 subunit. In interneurons and pyramidal cells from WT mice, zolpidem significantly increased mIPSC frequency, prolonged mIPSC decay, and increased mIPSC amplitude; those effects were diminished or absent in neurons from α1 KO mice. Nonstationary fluctuation analysis of mIPSCs indicated that the zolpidem-induced increase in mIPSC amplitude was associated with an increase in the number of open receptors rather than a change in the unitary conductance of individual channels. These data indicate that the α1 subunit is present at synapses on WT interneurons and pyramidal cells, although differences in mIPSC decay times and zolpidem sensitivity suggest that the degree to which the α1 subunit is functionally expressed at synapses on CA1 interneurons may be greater than that at synapses on CA1 pyramidal cells.

INTRODUCTION

GABA	extsubscript{A} receptors (GABA	extsubscript{A}-Rs) mediate fast synaptic inhibition throughout the mammalian brain (McCormick 1992). Like other members of the ligand-gated ion channel superfamily of receptors, GABA	extsubscript{A}-Rs are presumably pentameric structures consisting of multiple subunits (reviewed by Barnard et al. 1998; Chang et al. 1996; Tretter et al. 1997). Twenty subunits have been identified in mammalian tissues, including six α, four β, three γ, one δ, one ε, one π, three ρ, and one θ subunit (for reviews see Barnard et al. 1998; Sieghart et al. 1999). Allowing for only α, β, and γ subunits in a functional receptor, more than 10,000 configurations are possible, but in reality the final number is much smaller (McKernan and Whiting 1996).

The α subunit contributes to numerous properties of the GABA	extsubscript{A} receptor, including agonist efficacy (Ebert et al. 1994; Krasowski et al. 1997), benzodiazepine pharmacology (Hadingham et al. 1993; Pritchett et al. 1989; Pritchett and Seeburg 1990; Wafford et al. 1993; Wisden et al. 1991), and the kinetics of activation and deactivation (Gingrich et al. 1995; Lavoie et al. 1996; Tia et al. 1996; Vicini et al. 2001). Although the pharmacological profiles of multiple receptor configurations have been extensively studied using heterologous expression systems (reviewed by Sieghart 1995), the contribution of specific GABA	extsubscript{A} receptor subtypes, and of individual subunit species, to synaptic physiology is largely unknown.

The α1 subunit is the most common isoform in the mammalian CNS, contributing to approximately 40 to 65% of the total number of GABA	extsubscript{A}-Rs in the brain (McKernan and Whiting 1996; Sur et al. 2001). The α1 subunit has been investigated using gene targeting techniques, and the study of α1(H101R) knock-in mutant mice suggests that the α1 subunit contributes to the amnestic, sedative, and anticonvulsant properties of benzodiazepines but not to the anxiolysis, myorelaxation, or motor impairment caused by those drugs (McKernan et al. 2000; Rudolph et al. 1999). This evidence, in turn, suggests an important role for the α1 subunit in inhibitory synaptic transmission.

To date, specific knowledge of synaptic α1 subunit function is restricted to the cerebellum. In the cerebellum, the level of α1 subunit expression appears to increase between postnatal day 11 (P11) and P35, as evidenced by the fact that miniature inhibitory postsynaptic currents (mIPSCs) recorded in neurons obtained from wild-type mice decayed more rapidly in the P35 age group than in the P11 group and that “switch” was not observed in neurons obtained from α1 subunit knock-out (α1 KO) mice (Vicini et al. 2001).

The degree to which the GABA	extsubscript{A} receptor α1 subunit is incorporated into functional synaptic receptors in the hippocampus is unknown. In the adult rat, mRNA and GABA	extsubscript{A} receptor protein corresponding to the α1, α2, and α5 subunits can be readily detected in the hippocampal formation, the α4 protein is predominantly detected in the molecular layer of the dentate gyrus, and the α3 protein is barely detectable (Fritschy...
and Möhler 1995; Laurie et al. 1992; Pirker et al. 2000; Sperk et al. 1997; Wisden et al. 1992). In the CA1 subfield, Sperk et al. (1997) were unable to detect any of the α subunit proteins on the somata of pyramidal neurons, and only α1 subunit protein could be detected on the somata of CA1 interneurons (see also Gao and Fritschky 1994). More recent data, however, have demonstrated that parvalbumin-containing basket cells form axosomatic synapses with pyramidal cells that are rich in the α1 subunit (Klausberger et al. 2002). In the dendritic field layers of CA1 pyramidal cells (strata radiatum and oriens), the relative subunit protein densities appeared to be α1 > α2 > α2 >> α4 ~ α3, and α6 was undetectable.

Given the predominant role that GABA<sub>A</sub>-Rs play in mediating inhibitory synaptic transmission in the CNS, and their critical relevance to mediating the effects of a host of therapeutically used compounds, including benzodiazepines and general anesthetics (Franks and Lieb 1997; Jones and Harrison 1993; Mihic et al. 1997; Olsen 1998), it is important to further define and understand the molecular nature of functional GABA<sub>A</sub> receptors. Using WT and α1 KO mice, we examined mIPSC frequency, amplitude, decay time, and zolpidem modulation in CA1 interneurons and pyramidal cells in order to evaluate the contribution of the GABA<sub>A</sub>-R α1 subunit to native receptors mediating fast inhibitory synaptic transmission in the CA1 region of the hippocampus.

**METHO D S**

**Mutant mouse production**

Production and initial characterization of GABA<sub>A</sub>-R α1 KO mice have been described (Vicini et al. 2001). Mice that were heterozygous for the floxed, unrecombined allele (F) and the floxed, recombinated allele (f) were interbred to produce control heterozygous floxed unrecombined mice (α1<sup>f/f</sup>), heterozygous mice (α1<sup>f/w</sup>), and homozygous null allele mice (α1<sup>w/w</sup> or α1 KO). For the purposes of this paper, WT, or α1<sup>f/w</sup>; is defined as the floxed, unrecombined targeted locus without neo/Tk (F), as illustrated in Vicini et al. (2001). These mice were of the F<sub>8</sub> generation and the genetic background consisted of a mixture of C57Bl/6J, Strain 129/Sv/SvJ, and FVB/N. It is important to note that the genetic background of all mice is identical, including the α1 gene and flanking DNA. Since the α1 allele was floxed in Strain 129/Sv/SvJ embryonic stem cells (Nagy et al. 1990), the α1 gene and flanking DNA is Strain 129/Sv/SvJ derived in all mice. Mice were genotyped by Southern blot analysis of tail clip DNA as described (Vicini et al. 2001). Breeding and genotype analysis occurred at the University of Pittsburgh and, subsequently, 3- to 4-wk-old animals were shipped to Weill Medical College, Cornell University, for electrophysiological analysis.

**Electrophysiology**

In accordance with institutional and federal guidelines, standard hippocampal sections were prepared from P23–P58 mice. Briefly, mice were anesthetized with isoflurane and decapitated; the brain was rapidly removed and placed in ice-cold (2–4°C) Krebs solution saturated with 95% O<sub>2</sub>-5% CO<sub>2</sub>. The brain was blocked, and three to four 350- to 400-μm coronal sections were obtained using a microslicer (DTK, Kyoto, Japan). Slices were maintained in saturated Krebs solution at 37°C for 30–60 min prior to use and then kept at room temperature (22–24°C). Ascorbic acid (1 mM) was present in the Krebs solution during the dissection and recovery period but was not present during drug application or data acquisition.

An individual slice was transferred to the recording chamber and held in place by nylon threads attached to a platinum frame and continuously superfused with O<sub>2</sub>-CO<sub>2</sub>-saturated Krebs solution. CA1 pyramidal cells and CA1 interneurons in the stratum radiatum and stratum lacunosum-moleculare were visually identified using a Zeiss Axioskop FS microscope fitted with DIC-IR optics. Whole-cell patch clamp recordings were performed under voltage-clamp using either an Axopatch 1D or Axopatch 200A (Axon Instruments, Union City, CA) amplifier. Cells were voltage clamped at −60 mV after correcting for liquid junction potential and compensating for capacitance and series resistance. Access resistance was monitored using a 5-mV test pulse throughout the recording period; cells were included for analysis if the series resistance was less than 25 MΩ and rejected if resistance changed by more than 25% during the experiment. Data were acquired at 10 kHz using pClamp 8 (Axon Instruments) and filtered at 2 kHz. The Krebs solution contained 117 mM NaCl, 3.6 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 11 mM d-glucose, 5 × 10<sup>−4</sup> TTX, and 300–305 mOsm (adjusted with sucrose). Recording electrodes were made of borosilicate glass and had a tip resistance of 3–5 MΩ when filled with intracellular solution containing (in mM) 130 CH<sub>3</sub>SO<sub>3</sub> Cs, 8.3 CH<sub>3</sub>SO<sub>3</sub> Na, 1.7 NaCl, 1 CaCl<sub>2</sub>, 10 EGTA, 2 Mg<sub>2</sub>-ATP, 0.3 Na-GTP, 10 HEPES (pH 7.2 with CsOH and 295 mOsm with sucrose). All compounds were obtained from Sigma (St. Louis, MO) except for TTX, which was obtained from Alomone Labs (Jerusalem, Israel). Zolpidem was prepared as a 5-mM stock solution in 100% ethanol and serially diluted to 30 and 300 nM in Krebs solution; the sequence of drug application was Krebs solution, Krebs containing: TTX, TTX + 30 nM zolpidem, and TTX + 300 nM zolpidem. The sequence of application was always the same, and only one cell per slice was tested. In a number of experiments, bicuculline methiodide (20 μM) was used. Drugs were preapplied for 5 min prior to data acquisition.

**Data analysis**

Off-line analysis was performed using MiniAnalysis 5.5 (Synaptosoft, Decatur, GA), SigmaPlot 6.0 (SPSS, Chicago, IL), and Prism 3 (GraphPad, San Diego, CA). Using the random selection function in MiniAnalysis, ensemble mIPSCs were created by randomly selecting a total of 40–50 individual mIPSCs from each recording condition (in 9 instances, less than 50 events were analyzed due to the low frequency of suitable mIPSCs, and the lowest number of events averaged was 27) and aligning them to the 50% rise time; the 90–10% decay phase was fit to two exponentials with the least-squares simplex method; overlapping events were excluded from the ensemble. The weighted decay time constant (τ<sub>ω</sub>) was calculated as τ<sub>ω</sub> = (A<sub>1</sub> × τ<sub>1</sub> + A<sub>2</sub> × τ<sub>2</sub>)/(A<sub>1</sub> + A<sub>2</sub>), where τ<sub>1</sub> and τ<sub>2</sub> are the time constants of the first and second exponential functions, respectively, and A<sub>1</sub> and A<sub>2</sub> are the current amplitudes measured at time, t, equal to τ<sub>1</sub> and τ<sub>2</sub>, respectively (Banks et al. 1998; Vicini et al. 2001). Data are presented as mean ± SE unless otherwise indicated. Statistical significance was determined using the unpaired t-test.

**TABLE 1. mIPSC characteristics in interneurons and pyramidal cells**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Number of Cells</th>
<th>Frequency, Hz</th>
<th>Amplitude, pA</th>
<th>τ&lt;sub&gt;ω&lt;/sub&gt;, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interneurons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>19</td>
<td>2.5 ± 0.5</td>
<td>23.7 ± 1.9</td>
<td>17.9 ± 0.9</td>
</tr>
<tr>
<td>α1 KO</td>
<td>10</td>
<td>1 ± 0.1*</td>
<td>19 ± 2.2</td>
<td>28.5 ± 2.9†</td>
</tr>
<tr>
<td>Pyramidal cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>14</td>
<td>2.4 ± 0.5</td>
<td>19.6 ± 1.2</td>
<td>19.1 ± 1.1</td>
</tr>
<tr>
<td>α1 KO</td>
<td>15</td>
<td>1.2 ± 0.2*</td>
<td>16.1 ± 1.4</td>
<td>27.4 ± 1.4†</td>
</tr>
<tr>
<td>Percent change from WT</td>
<td>−50.0</td>
<td>−17.9</td>
<td>+43.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. mIPSC, miniature inhibitory post synaptic current. *P ≤ 0.03 compared to WT (unpaired t-test). †P < 0.001 compared to WT (unpaired t-test).
using paired and unpaired two-way $t$-tests within cell groups as appropriate, Kolmogorov-Smirnov test for comparing cumulative probabilities, and one-way ANOVA with Tukey’s post hoc test for comparisons between interneurons and pyramidal cells; significance was assumed for $P < 0.05$. To minimize experimental bias, recordings were performed without knowledge of the genotype, and the genotype was not revealed until after the data were analyzed.

Peak-scaled nonstationary noise analysis (Brickley et al. 1999; De Koninck and Mody 1994; Perrais and Ropert 1999; Silver et al. 1996; Traynelis et al. 1993; Yoshimura et al. 1999) was performed using MiniAnalysis 5.5 (Synaptosoft). Validity of this approach requires that the current decay time is stable over the course of the recording and that there is no correlation between mIPSC amplitude and decay time; plots of decay time and peak amplitude were created for each group of cells tested and no correlation was observed.

For each data subset obtained as described above, mIPSCs were reexamined and only those within 25% of the mean amplitude were included in the noise analysis. The unitary current ($i$) and total number of channels ($N$) were estimated by fitting the following equation

$$\sigma^2(i) = \sigma^2(t) - \sigma^2(N) + \sigma^2_{\text{hold}}$$

where $\sigma^2$ is the variance at given time, $t$, of the mIPSC, $i$ is the unitary current, $\sigma^2$ is the current at given time, $t$, $N$ is the total number of channels, $p$ is the probability of channel opening at the peak of the mIPSC, and $\sigma^2_{\text{hold}}$ is the variance in the baseline noise measured before the peak. It should be noted that the algorithm forces $p$ to approach unity, and consequently, $p$ is not reported. Fitting was performed without including the offset and after subtracting the baseline variance.

An estimate of single channel conductance ($\gamma$) was calculated as

$$\gamma = \frac{i}{(V_{\text{hold}} - E_{\text{rev}})}$$

where $i$ is the calculated unitary current, $V_{\text{hold}}$ is the amplifier holding potential, and $E_{\text{rev}}$ is the measured mIPSC reversal potential.

**RESULTS**

**mIPSC characteristics**

CA1 interneurons and CA1 pyramidal cells in the stratum radiatum and s. lacunosum-moleculare were visually identified by their relatively bright somata and proximal dendrites. We selected as candidate interneurons those cells that were at least 10 $\mu$m away from the border between the s. pyramidal and s. radiatum. The majority of a sampling of neurons recorded within s. pyramidale displayed action potential accommodation during depolarizing current injection that is characteristic of CA1 pyramidal neurons (Madison and Nicoll 1984; Thompson et al. 1985).

**FIG. 1.** Characteristics of miniature inhibitory postsynaptic currents (mIPSCs) in interneurons. A: representative sweeps showing mIPSCs recorded from a CA1 hippocampal interneuron obtained from a wild-type (WT) mouse. B: continuous sweeps showing mIPSCs recorded from a CA1 hippocampal interneuron from an $\alpha_1$ subunit knock-out ($\alpha_1$ KO) mouse. C: superimposed averaged mIPSC traces from the neurons in A and B. Note the small reduction in the $\alpha_1$ KO mIPSC averaged amplitude. D: scaled superimposed averaged mIPSC traces from the neurons in A and B. Note the increase in the decay time of the averaged $\alpha_1$ KO mIPSC trace. E: bar graph showing the averaged mIPSC amplitude for both WT ($n = 19$) and $\alpha_1$ KO ($n = 10$) interneurons. F: bar graph showing the averaged $\tau_m$ for WT and $\alpha_1$ KO interneurons. * $P < 0.05$ (unpaired $t$-test) compared to WT. G: cumulative probability curves for mIPSC amplitude in WT interneurons and $\alpha_1$ KO interneurons; the median amplitudes were 23.4 and 19.8 pA, respectively, and the populations were significantly different ($P < 0.001$). Total number of events analyzed was 927 in WT interneurons and 462 in $\alpha_1$ KO interneurons. H: cumulative probability curves for mIPSC decay times in WT interneurons and $\alpha_1$ KO interneurons; the median $\tau_m$ were 20.2 and 34.5 ms, respectively, and the populations were significantly different ($P < 0.001$).
mIPSCs recorded in neurons from WT mice were compared with those recorded in neurons from α1 KO mice. mIPSC frequency in WT interneurons and pyramidal cells was essentially identical (2.5 ± 0.5 and 2.4 ± 0.5 Hz, respectively). In neurons from α1 KO mice there was a 50–60% decrease in mIPSC frequency, and this decrease was significant for both cell groups (Table 1). Mean mIPSC amplitude was decreased by about 20% in neurons from α1 KO mice compared to WT littermates, while the mean mIPSC decay time increased by ~59% in α1 KO interneurons (Fig. 1, A–H) and by ~44% in α1 KO pyramidal cells (Fig. 2, A–H, Table 1).

The observed mIPSCs were always outward and were blocked by 20 μM bicuculline, indicating that they were GABA A-R mediated (Fig. 3). The measured reversal potential of the average mIPSC was ~95 mV. The I-V curve shows outward rectification that results from the large chloride gradient that was present between the intra- and extracellular solutions as predicted by the Goldman–Hodgkin–Katz current equation (Hille 2001).

**Effect of zolpidem on interneurons**

We next examined the effect of zolpidem on mIPSCs. Zolpidem, an imidazopyridine that is structurally unrelated to benzodiazepines but binds to the benzodiazepine site on the GABA A-R, has a high affinity for GABA A-Rs containing the α1 subunit (Pritchett and Seeburg 1990). In functional studies of recombinant receptors, zolpidem potentiates GABA-evoked whole-cell currents generated by α1-containing GABA A-Rs at nanomolar concentrations (Table 2).

Zolpidem (30 nM) had no appreciable effect on mIPSC amplitude. At a concentration of 300 nM, zolpidem significantly increased mIPSC amplitude in WT interneurons compared to KO interneurons (Fig. 4A, Table 3). Zolpidem (300 nM) also significantly increased mIPSC frequency in interneurons from WT but not KO mice.

At a concentration of 30 nM, zolpidem increased the current decay time in WT interneurons by 30%. In the presence of 300 nM zolpidem, the current decay time in WT interneurons increased by ~93%, and this is significantly greater than the ~41% increase seen in interneurons from KO mice (Fig. 4B, Table 3).

**Effect of zolpidem on pyramidal cells**

As was the case for interneurons, 30 nM zolpidem had no appreciable effect on pyramidal cell mIPSC amplitude. Zolpidem (300 nM) increased mIPSC amplitude in WT, but not α1 KO pyramidal cells; median amplitudes were 21.0 and 17.2 pA, respectively, and populations were again significantly different (P < 0.001). Total number of events analyzed was 665 in WT and 647 in α1 KO pyramidal cells; median decay times were 23.2 and 32.2 ms, respectively, and populations were again significantly different (P < 0.001).
mIPSC decay time measured in H9251 observed in interneurons, zolpidem had less of an effect on both WT and H9251 KO pyramidal cells (Fig. 4). The single channel conductance (γ) estimate was the same in all cell groups (Table 4), and the pooled unitary conductance for all WT control mIPSCs was 23.7 ± 0.9 pS (1,437 mIPSCs from 54 cells). Zolpidem had no effect on unitary conductance.

Estimates of the number of channels open at the peak of the mIPSC were also obtained using noise analysis. In α1 KO interneurons, there was a significant decrease in the number of open channels at the peak of the mIPSC (Table 4). In α1 KO pyramidal cells, there was nearly a 20% decrease in the number of open receptors compared to WT; this was in excellent agreement with the ~18% reduction seen in the mean mIPSC amplitude in α1 KO compared to WT pyramidal cells.

Consistent with its effects on mIPSC amplitude, 300 nM zolpidem significantly increased the number of open channels in neurons from WT mice (Fig. 5B, Table 4). In WT interneurons, 300 nM zolpidem increased the number of open channels by ~36%; this is in excellent agreement with the ~34% zolpidem-induced increase in the mean mIPSC amplitude seen in these cells (Table 3). In WT pyramidal cells, zolpidem again significantly increased the number of open channels.

In α1 KO interneurons, 300 nM zolpidem did not alter the number of open receptors, and this agrees with the lack of a zolpidem effect on mIPSC amplitude in these cells (Fig. 4, Table 3). In α1 KO pyramidal cells, however, 300 nM zolpidem produced a significant increase in the number of open channels (Table 4).

**DISCUSSION**

**Summary of observations**

We examined mIPSCs in CA1 hippocampal interneurons and pyramidal cells in WT and α1 KO mice. mIPSCs recorded from interneurons and pyramidal cells obtained from α1 KO mice were detected less frequently, were smaller in amplitude, and were significantly slower in the decay phase compared to mIPSCs recorded in neurons from WT mice. The α1-selective ligand zolpidem increased mIPSC amplitude and prolonged the current decay time in both WT interneurons and WT pyramidal cells but had a markedly reduced effect on those parameters in α1 KO cells.

**TABLE 2.** Zolpidem EC50 (nM) in heterologously expressed GABA_A receptors

<table>
<thead>
<tr>
<th>α1β3γ2</th>
<th>α2β3γ2</th>
<th>α3β3γ2</th>
<th>α5β3γ2</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 ± 11*</td>
<td>127 ± 10*</td>
<td>467 ± 72*</td>
<td>No effect up to 10 μM*</td>
</tr>
<tr>
<td>α1β1γ2</td>
<td>α3β1γ2</td>
<td>57 ± 7.5†</td>
<td>410 ± 55.4†</td>
</tr>
</tbody>
</table>

Values are means ± SD. * Mouse Ltk cells stably transfected with human GABA_A receptor combinations (Dr. Keith Wafford, personal communication). † Wafford et al. (1993).
number of open channels in neurons from WT animals without altering estimates of single channel conductance.

Kinetics of α1 containing GABA<sub>A</sub>-Rs

α1 subunit expression influences the decay time of synaptic currents mediated by GABA<sub>A</sub> receptors. Synaptic GABA<sub>A</sub> receptor-mediated currents recorded in neurons from WT mice decayed more rapidly than those recorded in neurons from α1 KO mice. This suggests that the loss of the α1 subunit in synaptically localized receptors leads to a population of synaptic receptors with slower deactivation kinetics, such as those primarily containing the α2 subunit (Okada et al. 2000). This is consistent with results obtained from transfected HEK cells, in which GABA receptors consisting of α1β1γ2 subunits had deactivation kinetics that were ~6.5 times faster than receptors consisting of α2β1γ2 (Lavoie et al. 1997).

The current decay time constants reported here in hippocampal neurons in WT mice are slower than the weighted decay time constant reported in cerebellar granule cells (τ<sub>w</sub> ~ 10 ms; Vicini et al. 2001) but are similar to the unweighted decay time constants obtained from cerebellar granule cells in the study by Vicini et al. (2001). The differences in the decay time constants reported here and those reported by Vicini et al. (2001) could be due to the different methodologies used to estimate the decay time constants. In our study, we used the unweighted decay time constant, which is calculated by simply averaging the time points at which the current amplitude is equal to 63% of its maximum amplitude. This method is less accurate than the weighted decay time constant, which takes into account the probability of channel opening and closure at each time point. However, the weighted decay time constant is more difficult to calculate and requires more data points than the unweighted decay time constant.

### Table 3: Zolpidem modulation of mIPSC amplitude and weighted decay time

<table>
<thead>
<tr>
<th></th>
<th>mIPSC Frequency, Hz</th>
<th>%Change in Amplitude</th>
<th>%Change in τ&lt;sub&gt;w&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zolp 300 nM</td>
<td>Zolp 30 nM</td>
<td>Zolp 300 nM</td>
</tr>
<tr>
<td>Interneurons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>3.6 ± 0.7</td>
<td>6.0 ± 5.1 (18)</td>
<td>34.4 ± 9.3&lt;sup&gt;a&lt;/sup&gt; (17)</td>
</tr>
<tr>
<td>α1 KO</td>
<td>1.4 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>−3.5 ± 6.7 (10)</td>
<td>−1.8 ± 8.8&lt;sup&gt;e&lt;/sup&gt; (10)</td>
</tr>
<tr>
<td>Pyramidal cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>3.7 ± 0.8</td>
<td>−1.7 ± 5.8 (14)</td>
<td>17.3 ± 7.8&lt;sup&gt;h&lt;/sup&gt; (13)</td>
</tr>
<tr>
<td>α1 KO</td>
<td>1.5 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.3 ± 3.4 (15)</td>
<td>11.1 ± 5.9 (14)</td>
</tr>
</tbody>
</table>

Values are means ± SD with number of cells given in parentheses. mIPSC, miniature inhibitory postsynaptic current. <sup>a</sup>P < 0.01 compared to control (one sample t-test). <sup>b</sup>P < 0.05 compared to control (one sample t-test). <sup>c</sup>P < 0.001 compared to control (one sample t-test). <sup>d</sup>P < 0.05 compared to WT (unpaired t-test). <sup>e</sup>P < 0.01 compared to WT (unpaired t-test).
constant in laterodorsal thalamic neurons (τ \sim 16 \text{ ms}; Okada et al. 2000) and CA1 pyramidal cells (τ \sim 18 \text{ ms}; Banks and Pearce 2000). Synaptic receptors in all three cell groups have now been shown to contain the α1 subunit. Differences in the mIPSC decay time constant recorded in neurons from separate anatomic regions may reflect underlying differences in the degree to which the α1 subunit is incorporated into synaptic GABA A-Rs; variations in the temperature at which the recordings were made are unlikely to be the cause of those differences (see, for example, Thompson et al. 1985) as all recordings were made at room temperature.

**Zolpidem modulation of α-containing GABA A-Rs**

Zolpidem increased mIPSC amplitude and current decay time in WT interneurons and pyramidal cells. Receptors containing the α1 subunit have a binding affinity for [3 H]zolpidem that is one to three orders of magnitude greater than that for other α subunit–containing receptors (Pritchett and Seeburg 1990). The differences in affinity are reflected in differences in the functional EC50 for zolpidem at different receptor configurations (Table 2), (KA Wafford, unpublished observations). The pronounced effect of zolpidem on mIPSC amplitude and current decay time recorded in neurons from WT mice is most likely mediated by synthetically localized receptors containing the α1 subunit, although α2 subunit–containing receptors may also have contributed to the observed effects, especially at 300 nM. The persistent effects of zolpidem seen in neurons from α1 KO mice were likely mediated by receptors containing the α2 subunit, rather than the α3 subunit given the differences in the EC50 of zolpidem for heteroligomers containing those subunits (Wafford et al. 1993; KA Wafford, unpublished observations).

Baseline mIPSC frequency in neurons from WT mice was \sim 2.5 \text{ Hz}, and zolpidem increased both frequency and amplitude in those cells. In contrast, zolpidem had no effect on either mIPSC frequency or amplitude in neurons from α1 KO mice. The observed increase in mIPSC frequency recorded in WT neurons is likely to reflect an increase in event detection (rather than an increase in quantal release) as a consequence of increased mean mIPSC amplitude.

In neurons from α1 KO mice, mIPSC frequency was significantly decreased compared to that recorded in WT mice. Cumulative probability plots demonstrated a significant decrease in mIPSC amplitude in α1 KO interneurons and pyramidal cells (compared to WT). In addition, noise analysis demonstrated a significant decrease in the number of receptors open at the peak of the mIPSC in cells from α1 KO compared to WT mice. We suggest that a loss in the number of receptors at the synapse in α1 KO mice accounts for smaller mIPSCs, leading to decreased event detection and an apparent decrease in mIPSC frequency.

**Noise analysis and synaptic GABA A-Rs**

Nonstationary fluctuation analysis revealed a number of differences and similarities between mIPSCs recorded in WT interneurons and pyramidal cells. Synapses on interneurons appeared to contain slightly more GABA A-Rs contributing to the peak of the mIPSC than did synapses on WT pyramidal cells (\sim 57 vs. \sim 28, respectively), and this parallels the larger mIPSC amplitudes in interneurons.

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FIG. 5. Peak-scaled nonstationary fluctuation analysis of mIPSCs. A: the average waveform of 22 mIPSCs (solid line) is shown scaled to the peak of an individual mIPSC (dotted line) recorded in an interneuron from a WT mouse. B: plots of variance against mean current for the neuron in A. Closed circles are the average pooled variance for control mIPSCs while open circles are the measured average reversal potential; for this cell, driving force of 35 mV based on the membrane holding potential and the and pS; while in the presence of zolpidem, the values were i = 0.884 pA, f = 19.8 pA, Np = 21.4, and γ = 18.7 pS. Note the relatively small increase in i and Np in this cell compared to the changes observed in the WT interneuron.
There was a marked decrease in open channel number in neurons from α1 KO mice. The loss of open channels is of the same magnitude as the decrease in mIPSC amplitude (Table 1). Interestingly, the decrease in the number of synaptic receptors is less than the total loss of hippocampal GABA_A receptors in mice lacking the α1 subunit (reportedly on the order of 53%; Sur et al. 2001).

Estimates of single channel conductance in all WT and α1 KO neurons were the same, indicating that channel conductance was independent of α1 subunit expression (see also Verdoorn 1990). The pooled conductance for all cells was ∼24 pS and is similar to that reported in dentate granule cells (De Koninck and Mody 1994), internal cerebellar granule cells (Brickley et al. 1999), cortical neurons (Perrais and Ropert 1999; this study), hippocampal neurons may express synaptic receptors containing α3 (Brüning et al. 2002) and α4 (Choi et al. 1998; but see Thomson et al. 2000) subunits despite limited expression in this region (Pirker et al. 2000; Sperk et al. 1997), protein detection (Fritschy and Mohler 1995; Pirker et al. 2000; Sperk et al. 1997), and localization to a subset of synapses on pyramidal cell somata and dendrites and most synapses on pyramidal cell axon initial segments (Nusser et al. 1996; see also Brüning et al. 2002).

Hippocampal neurons may express synaptic receptors containing α3 (Brüning et al. 2002) and α4 (Banks et al. 1998; but see Thomson et al. 2000) subunits despite limited expression in this region (Pirker et al. 2000; Sperk et al. 1997). Hippocampal neurons also express GABA_A receptors containing the α5 subunit; some of this protein may be incorporated into synaptic receptors (Pawelzik et al. 1999; Collinson et al. 2002), but most α5 subunit-containing GABA_A-Rs appear to be extrasynaptic (Brüning et al. 2002).

α Subunit contribution to synaptically localized GABA_A receptors

Our data indicate that CA1 hippocampal interneurons and pyramidal cells express populations of synaptically localized GABA_A receptors that contain the α1 subunit. This conclusion is consistent with results obtained using immunogold labeling (Nusser et al. 1996). Interneurons had nearly twofold greater increases in mIPSC amplitude and current decay time in response to 300 nM zolpidem than did WT pyramidal cells, suggesting that WT interneurons contain a greater percentage of receptors expressing the α1 subunit than WT pyramidal cells. This view is supported by immunohistochemical data demonstrating that interneurons had a greater density of synaptic α1 protein than did pyramidal cells (Klausberger et al. 2002).

The α2 subunit is likely to be included in hippocampal GABA_A-Rs based on high levels of mRNA expression (Laurie et al. 1992; Wisden et al. 1992), protein detection (Fritschy and Mohler 1995; Pirker et al. 2000; Sperk et al. 1997), and localization to a subset of synapses on pyramidal cell somata and dendrites and most synapses on pyramidal cell axon initial segments (Nusser et al. 1996; see also Brüning et al. 2002).

In α1 KO mice, α2, α3, and/or α5 subunits are again possible candidates for inclusion in the postsynaptic receptor. Given that 300 nM zolpidem prolonged the current decay time even in KO mice (Figs. 3 and 4), it is unlikely that the postsynaptic receptor in α1-null mice contains the α5 subunit in significant amounts since it has a Ki for zolpidem in excess of 15 μM (Pritchett and Seeburg 1990) and is insensitive to zolpidem at the concentrations used in this study. Excluding the α5 subunit, that leaves the α2 and α3 subunits as the most likely α subunits to be expressed in synaptically localized GABA_A-Rs in the hippocampus in KO mice.
Conclusions

CA1 interneurons and pyramidal cells from WT mice express GABA_A-Rs containing the α1 subunit; these receptors are not saturated following quantal release of transmitter at room temperature. In α1 KO mice, the synaptic receptor population likely contains a significant proportion of α2β2/3y2 and/or α3β2/3y2 heteroligoromers. Anatomic data have indicated that the α1 subunit is incorporated into synaptic GABA_A-Rs expressed by CA1 pyramidal cells (Klausberger et al. 2002; Nusser et al. 1996); the physiological data now confirm a contribution of the α1 subunit to synaptically localized GABA_A-Rs expressed by both hippocampal pyramidal cells and interneurons.

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