

Reduction of Zolpidem Sensitivity in a Freeze Lesion Model of Neocortical Dysgenesis

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DeFazio, R. Anthony and John J. Hablitz. Reduction of zolpidem sensitivity in a freeze lesion model of neocortical dysgenesis. *J. Neurophysiol.* 81: 404–407, 1999. Early postnatal freeze lesions in rat neocortex produce anatomic abnormalities resembling those observed in human patients with seizure disorders. Although in vitro brain slices containing the lesion are hyperexcitable, the mechanisms of this alteration have yet to be elucidated. To test the hypothesis that changes in postsynaptic inhibitory receptors may underlie this hyperexcitability, we examined properties of γ -aminobutyric acid type A receptor (GABA_AR)-mediated miniature inhibitory postsynaptic currents (mIPSCs). Recordings were obtained in layer II/III pyramidal cells located 1–2 mm lateral to the lesion. mIPSC peak amplitude and rate of rise were increased relative to nonlesioned animals, whereas decay time constant and interevent interval were unaltered. Bath application of zolpidem at a concentration (20 nM) specific for activation of the type 1 benzodiazepine receptor had no significant effect on decay time constant in six of nine cells. Exposure to higher concentrations (100 nM) enhanced the decay time constant of all cells tested ($n = 7$). Because mIPSCs from unlesioned animals were sensitive to both concentrations of zolpidem, these results suggest that freeze lesions may decrease the affinity of pyramidal cell GABA_ARs for zolpidem. This could be mediated via a change in α -subunit composition of the GABA_AR, which eliminates the type 1 benzodiazepine receptor.

INTRODUCTION

Cortical malformations such as polymicrogyria have been observed in patients with epilepsy and other disorders. The etiology of microgyria is thought to be injury during the “inside-out” development of cortex, which leads to a local loss of cells that were in the process of migration (reviewed in Humphreys et al. 1991). In the rat, transcranial freeze-induced lesions early in postnatal development produce a microsulcus consisting of a region of four-layered cortex similar to that observed in human polymicrogyria (Dvorak and Feit 1977; Humphreys et al. 1991; Jacobs et al. 1996). Recent studies have demonstrated that acutely prepared, lesion-containing, neocortical brain slices are hyperexcitable (Hablitz and DeFazio 1998; Jacobs et al. 1996; Prince et al. 1997). The mechanisms underlying this increase in excitability are still in debate.

To identify possible changes in GABA_AR pharmacology in this model, we examined the effects of the type 1 benzodi-

azepine receptor (BZ1) agonist zolpidem on miniature inhibitory postsynaptic currents (mIPSCs). Preliminary results from this study have appeared in abstract form (DeFazio and Hablitz 1997).

METHODS

Animals were housed and handled according to approved guidelines. Timed pregnant Sprague Dawley rats arrived on *embryonic day 15*. Freeze lesions were generated using modifications of the technique of Dvorak and Feit (1977). On *postnatal day 2* (P2) rat pups were anesthetized by hypothermia (5 min on ice). The cold probe consisted of a 2-mm-diam copper bar extending from a 50-ml centrifuge tube filled with methanol cooled to about -50°C with dry ice. After the scalp was opened along the midline, the cold probe was placed on the surface of the skull near the midline for 3–5 s. After the incision was sutured, the animals were placed under a heat lamp and, after 30 min, returned to their home cage.

All procedures for recording and slice preparation are described in detail in DeFazio and Hablitz (1998). Briefly, slices were prepared from eight lesioned animals 17–27 days postnatal. Rats were anesthetized by intraperitoneal ketamine injection (100 mg/kg) before decapitation. The brain was rapidly removed and submerged in ice-cold, low-calcium saline (containing, in mM, 125 NaCl, 3.5 KCl, 26 NaHCO₃, 10 glucose, and 3.8 MgCl₂) bubbled with 95% O₂-5% CO₂. Coronal sections (300 μm) containing somatosensory cortex were cut using a Vibratome. Slices were stored in saline consisting of (in mM) 125 NaCl, 3.5 KCl, 26 NaHCO₃, 10 glucose, 2.5 CaCl₂, and 1.3 MgCl₂, bubbled with 95% O₂-5% CO₂.

Whole cell voltage-clamp recordings were obtained from visually identified neocortical pyramidal cells in layer II/III, 1–2 mm lateral to the lesion. Recordings were made at room temperature and at a holding potential of -60 mV. Records were digitized in 40- to 120-s epochs. Series resistance was regularly monitored between epochs. The internal solution consisted of (in mM) 140 CsCl, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 1 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 0.1 CaCl₂, 4 MgATP, and 0.4 NaGTP. Slices were continuously perfused with an external solution consisting of the storage saline listed above with the addition of 0.5 μM tetrodotoxin (TTX), 20 μM 2-amino-5-phosphonopentanoic acid (APV), and 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Zolpidem was dissolved in 100% ethanol to make concentrated stock solutions each week. These stocks were stored at -20°C . All compounds were obtained from Sigma Chemical with the exception of zolpidem, which was purchased from Research Biochemicals. Drugs were bath applied, and each cell served as its own control.

Cells with series resistance >20 M Ω or significant changes in series resistance during the duration of the experiment were not used for this analysis. Events were detected off-line, and decay time constants, peak amplitudes, rate of rise, and interevent inter-

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TABLE 1. Basic properties of mIPSCs

	Nonlesioned Cortex	Lesioned Cortex
Decay time constant, ms	17.71 \pm 0.91	17.7 \pm 1.2
Peak amplitude, pA	-40.0 \pm 2.1	-56.1 \pm 5.7*
Rate of rise, pA/ms	70.8 \pm 3.7	110 \pm 13*
Interevent interval, ms	585 \pm 160	973 \pm 320

Values are means \pm SE; number of cells in Nonlesioned Cortex was 13 and in Lesioned Cortex was 9. Basic properties were measured under control conditions before the application of zolpidem. Values from nonlesioned cortex were obtained in a concurrent study (DeFazio and Hablitz 1998). Both peak amplitude and rate of rise were significantly increased in cells from lesioned cortex. The large apparent difference in interevent interval was due to two large outliers in the lesioned cortex group. mIPSCs, miniature inhibitory postsynaptic currents. * $P < 0.02$.

vals were measured as previously described (DeFazio and Hablitz 1998). The Kolmogorov-Smirnov test was used to determine whether two distributions were significantly different. Each distribution contained a minimum of 70 events (mean 123, range 70–277). In a previous study in unlesioned cortex, a level of $P < 0.01$ was used for testing the significance changes in decay time constant distributions (DeFazio and Hablitz 1998); however, for this study a significance level of $P < 0.05$ was to reduce the possibility of false negatives. t -Tests were used to compare averaged results between nonlesioned and lesioned cortex with a significance level of $P < 0.05$.

RESULTS

Properties of mIPSCs in lesioned cortex

Whole cell voltage-clamp recordings were made from visually identified layer II/III pyramidal cells located 1–2 mm lateral from the lesion. The results from nine cells from eight freeze-lesioned animals (2 cells came from 2 slices from 1 animal) are reported below. Four properties of mIPSCs, peak amplitude, rate of rise, decay time constant, and interevent interval were measured using identical analysis parameters employed previously in nonlesioned animals (DeFazio and Hablitz 1998). The results obtained during the control period before exposure to zolpidem are summarized in Table 1, where they are compared with values obtained in nonlesioned cortex (DeFazio and Hablitz 1998). Consistent with previous studies (Prince et al. 1997), peak amplitude and rate of rise were enhanced in cells from lesioned cortex.

Effects of zolpidem in cells from lesioned animals

In six of nine neurons from lesioned animals, 20 nM zolpidem had no significant effect on the basic mIPSC properties measured in this study, whereas 100 nM zolpidem significantly enhanced the decay time constant in all cells tested under this condition ($n = 7$ of 7). The six cells lacking a significant increase in decay time constant by 20 nM zolpidem will be referred to as “less zolpidem-sensitive” cells. Results from a representative less zolpidem-sensitive cell are shown in Fig. 1. No effect of 20 nM zolpidem on decay time constant is apparent in the normalized traces shown in Fig. 1B. Batch application of 100 nM zolpidem significantly enhanced both amplitude and decay time constant (Fig. 1, A and B, respectively). Cumulative probability plots (Fig.

1, C and D) further illustrate the lack of sensitivity to 20 nM zolpidem. At a concentration of 100 nM, zolpidem shifted both the decay time constant and peak amplitude distributions to the right; 20 nM zolpidem had no significant effect on either distribution.

One of the three cells sensitive to 20 nM zolpidem is shown in Fig. 2. Both 20 and 100 nM zolpidem enhanced the decay time constant as shown in the normalized traces (Fig. 2B). This enhancement is reflected in the decay time constant probability distributions in Fig. 2C. The results shown in Fig. 2 are very similar to the effects of zolpidem in control animals (DeFazio and Hablitz 1998).

The effects of 20 and 100 nM zolpidem on decay time constant are summarized in Fig. 3. The percent increase in decay time constant, relative to control mIPSCs recorded before exposure to zolpidem, was averaged across cells to allow a direct comparison of the results from lesioned and nonlesioned cortex. A significantly smaller enhancement of the decay time constant by 20 nM zolpidem was observed in neurons from lesioned animals. The cells from lesioned animals were then grouped by the results of the Kolmogorov-Smirnov test of the effect on the decay time constant of 20 nM zolpidem (less zolpidem-sensitive cells with no significant effect, $n = 6$ of 9; zolpidem-sensitive cells with degree of significance $P < 0.05$, $n = 3$ of 9). All cells in both nonlesioned and lesioned cortex demonstrated similar responsiveness to 100 nM zolpidem, as shown in the second set of bar graphs in Fig. 3. In addition to the effect of zolpidem on decay time constant, 20 and 100 nM zolpidem significantly enhanced the distributions of peak amplitudes of a subset of cells ($n = 1$ of 9 and $n = 3$ of 7, respectively).

DISCUSSION

mIPSCs were recorded from neocortical pyramidal cells in an area adjacent to the freeze-induced microsulcus. Both the peak amplitude and rate of rise were significantly enhanced in cells from lesioned cortex. It is clear that inhibitory innervation of layer II/III pyramidal cells is present in lesioned cortex and may be functionally enhanced. This is consistent with previous work demonstrating a prominent inhibitory component in the evoked epileptiform discharge (Hablitz and DeFazio 1998) and enhancement of both evoked and spontaneous IPSCs (Prince et al. 1997) in this model of cortical maldevelopment.

The majority of cells in this study (6 of 9 cells) were insensitive to low concentrations of zolpidem, a benzodiazepine type 1 selective agonist. Our previous work in nonlesioned cortex demonstrated a significant enhancement of decay time constants in the presence of 20 nM zolpidem in all cells studied (9 of 9) (DeFazio and Hablitz 1998). A decrease in the sensitivity to 20 nM zolpidem can be explained by a change in affinity of the benzodiazepine site for zolpidem. The $\alpha 1\beta\gamma 2$ -subunit combination is thought to give rise to the type 1 benzodiazepine receptor with high affinity for zolpidem. In recombinant systems, this subunit combination has pharmacological properties similar to those we observed in unlesioned cortex (DeFazio and Hablitz 1998). The reduction in sensitivity to zolpidem observed in lesioned cortex may be due to a change in the pattern of expression of α -

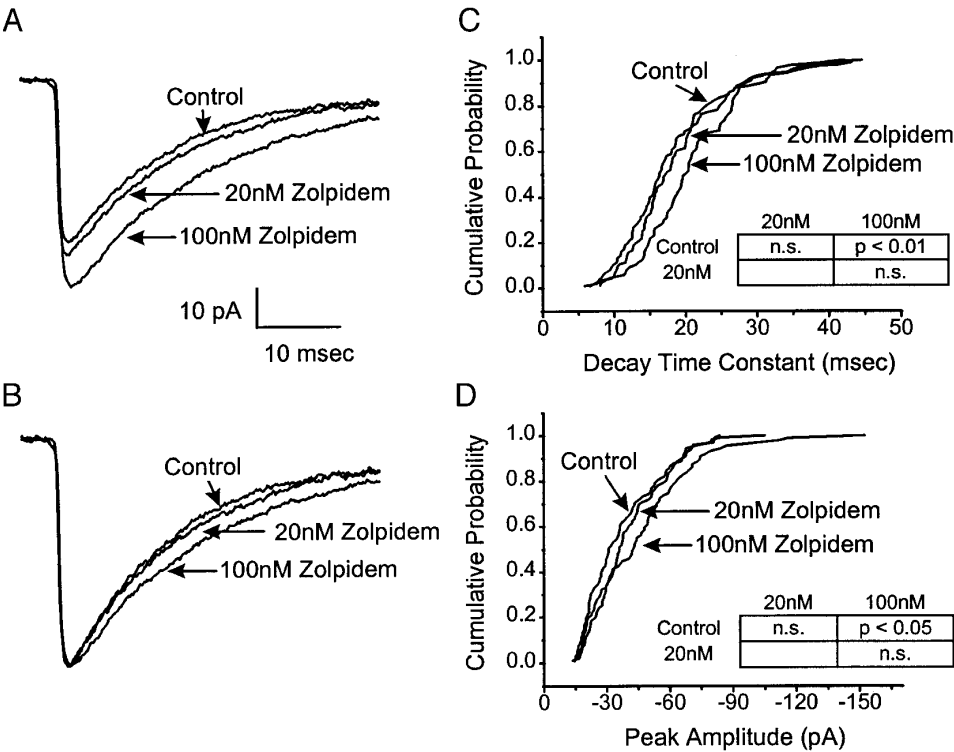


FIG. 1. Most cells from lesioned cortex were insensitive to 20 nM zolpidem. *A*: averaged miniature inhibitory postsynaptic current (mIPSC) records before and after zolpidem application. An increase in peak amplitude during 100 nM zolpidem exposure is apparent in these traces. *B*: averaged traces were normalized to their peak values. Enhancement of the decay time constant in 100 nM zolpidem is apparent. *C* and *D*: probability distributions and the results of the Kolmogorov-Smirnov tests for decay time constant and peak amplitude, respectively. The number of events in each distribution was 106, 98, and 108 for control, 20 and 100 nM zolpidem, respectively.

subunits. A switch in expression of primarily $\alpha 1$ -subunits to $\alpha 2$ - or $\alpha 3$ -subunits would result in a lower affinity for zolpidem, because the pharmacology of recombinant $\alpha 2$ - and $\alpha 3$ -subunits resembles the type 2 benzodiazepine (for review, see Luddens et al. 1995). Expression of $\alpha 2$ -5 subunits, which give rise to GABA_ARs less sensitive or insensitive to

zolpidem, predominates in early postnatal rat development (*P0*–*P6*) with the $\alpha 3$ -subunit eclipsing even $\alpha 1$ -subunit expression intensity during this period (Laurie et al. 1992). It is possible that the freeze lesion somehow delays or arrests maturation of the GABA_AR.

Decrements in the benzodiazepine receptor-mediated

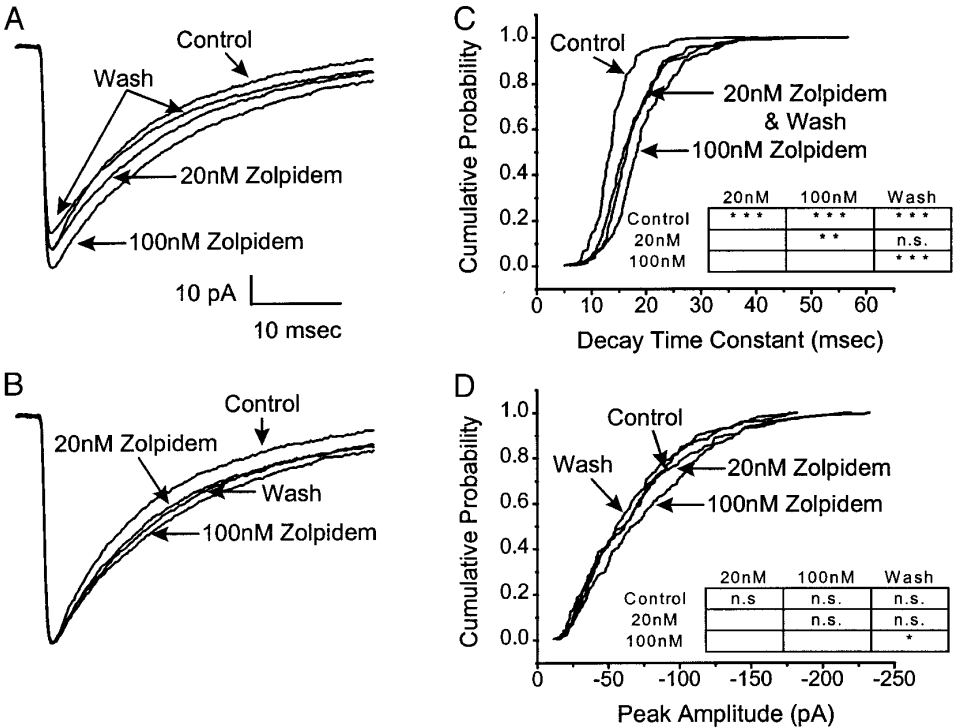


FIG. 2. Example of a cell from a lesioned animal sensitive to 20 and 100 nM zolpidem. *A*: averaged traces illustrate a modest increase in peak amplitude. *B*: normalized traces showing the large increases in decay time constant in both 20 and 100 nM zolpidem. *C* and *D*: decay time constant and amplitude probability distributions. Asterisks indicate degree of significance: ****P* < 0.001, ***P* < 0.005, and **P* < 0.05. Each distribution contained 166, 164, 160, and 157 events for control, 20 nM, 100 nM, and wash.

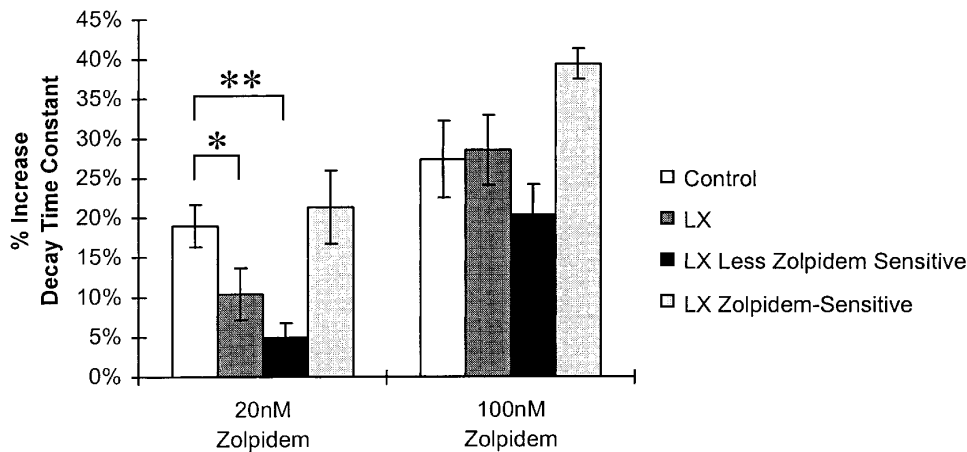


FIG. 3. Comparison of the effects of zolpidem on decay time constants from nonlesioned and lesioned animals. The percent change relative to the prezolpidem control period for each cell was averaged and compared with results obtained in our previous study in nonlesioned cortex. The control (from nonlesioned animals) study consisted of 9 cells exposed to 20 nM zolpidem and 5 cells exposed to 100 nM zolpidem. A total of 9 cells from lesioned cortex were exposed to 20 nM zolpidem, and 7 of those cells were also exposed to 100 nM zolpidem. Six of 9 cells from lesioned cortex were insensitive to 20 nM zolpidem. Asterisks indicate level of significance: * $P < 0.03$ and ** $P < 0.001$. Error bars indicate SE.

augmentation of GABA-evoked currents in hippocampal neurons have been observed in models of temporal lobe epilepsy (Gibbs et al. 1997) and acute status epilepticus (Kapur and Macdonald 1997). Chronic alterations have also been observed in *N*-methyl-D-aspartate and metabotropic glutamate receptors following kindling induced epilepsy (Holmes et al. 1996; Mody and Heinemann 1987). The present results suggest that subtle differences in modulatory influences on neuronal receptors may be a general feature in the chronically hyperexcitable brain.

The authors thank A. Margolies for excellent technical assistance.

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-22373.

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Received 30 July 1998; accepted in final form 22 September 1998.

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