

Alterations in NMDA Receptors in a Rat Model of Cortical Dysplasia

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Defazio, R. Anthony and John J. Hablitz. Alterations in NMDA receptors in a rat model of cortical dysplasia. *J. Neurophysiol.* 83: 315–321, 2000. Recent studies have demonstrated an important role for the *N*-methyl-D-aspartate receptor (NMDAR) in epilepsy. NMDARs have also been shown to play a critical role in hyperexcitability associated with several animal models of human epilepsy. Using whole-cell voltage clamp recordings in brain slices, we studied evoked paroxysmal discharges in the freeze-lesion model of neocortical microgyria. The voltage dependence of epileptiform discharges indicated that these paroxysmal events were produced by a complex pattern of excitatory and inhibitory inputs. We examined the effect of the NMDAR antagonist D-2-amino-5-phosphopentanoic acid (APV) and the NMDA receptor subunit type 2B (NR2B)-selective antagonist ifenprodil on the threshold, peak amplitude, and area of evoked epileptiform discharges in brain slices from lesioned animals. Both compounds consistently raised the threshold for evoking the discharge but had modest effects on the discharge peak and amplitude. For comparison with nonlesioned cortex, we examined the effects of ifenprodil on the epileptiform discharge evoked in the presence of 2 μ M bicuculline (partial disinhibition). In slices from nonlesioned cortex, 10 μ M ifenprodil had little effect on the threshold whereas 71% of the recordings in bicuculline-treated lesioned cortex showed a >25% increase in threshold. These results suggest that NR2B-containing receptors are functionally enhanced in freeze-lesioned cortex and may contribute to the abnormal hyperexcitability observed in this model of neocortical microgyria.

INTRODUCTION

Neuronal migration disorders resulting in cortical dysplasia, microgyria, and heterotopia are associated with intractable seizure disorders in humans (Palmini et al. 1994). Several animal models have been developed to examine neural mechanisms underlying hyperexcitability in dysplastic cortex (Baraban and Schwartzkroin 1995; Dvorak et al. 1978; Roper 1998). The rat neonatal freeze-lesion model reproduces many of the anatomic findings found in human microgyria (Dvorak et al. 1978). In this model, a freezing probe is briefly placed on the skull of a neonatal rat pup. This procedure destroys the deep layer neurons present near the surface of the cortical plate but preserves progenitor cells and radial glia, which ultimately give rise to superficial neuronal layers in the area of the lesion. The local loss of deep layer cells results in a microsulcus in the otherwise lissencephalic rat brain. Neocortical brain slices from rats >2 wk old containing the microsulcus are hyperexcitable. Epileptiform discharges, which propagate over large distances, are evoked by weak intracortical stimulation (Ha-

blitz and DeFazio 1998; Jacobs et al. 1996, 1999; Luhmann and Raabe 1996; Luhmann et al. 1998; Prince et al. 1997). Electrophysiological studies have indicated changes in intrinsic membrane properties (Luhmann et al. 1998), alterations in GABAergic inhibition (DeFazio and Hablitz 1999; Jacobs et al. 1996; Qu et al. 1998), and modification of glutamate receptors (Qu et al. 1998). It is likely that these factors interact to produce hyperexcitability.

Chronic changes in *N*-methyl-D-aspartate receptors (NMDARs) are involved in human temporal lobe epilepsy (Mathern et al. 1998) and the kindling model of temporal lobe epilepsy (Mody and Heinemann 1987). In addition, acute models of interictal spiking show sensitivity to NMDAR antagonists (e.g., Hablitz and Lee 1992; Lee and Hablitz 1990). Variable results with epileptiform activity in slices from freeze-lesioned cortex have been reported. The NMDAR antagonist D-2-amino-5-phosphopentanoic acid (APV, 50–100 μ M) has been reported to completely block hyperexcitability in slices from freeze-lesioned animals (Jacobs et al. 1996, 1999). In other studies, APV had no effect or only reduced late recurrent epileptiform activity; blockade of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors was necessary to completely abolish hyperexcitability (Luhmann and Raabe 1996; Luhmann et al. 1998). However, in both cases an important role for NMDARs in the initiation and/or propagation of the epileptiform discharge was apparent. It remains to be determined if NMDAR participation reflects pathological function of normal receptors (e.g., caused by diminished inhibition), alterations in the receptors themselves (e.g., expression of receptors with enhanced current flow), and/or abnormal excitatory interconnectivity (e.g., sprouting of horizontal excitatory collaterals).

Native NMDARs are thought to contain subunits from two different classes termed NR1 and NR2 (for review see Monaghan et al. 1998). Each class has a number of subtypes that can alter the pharmacological and kinetic properties of the receptor. In heterologous expression systems, NR1 homomers form poorly conducting NMDAR channel complexes compared with NR1-NR2 heteromers. The primary candidates for native NMDAR in rat somatosensory cortex are NR1 (and its splice variants) and NR2A, B, and/or C. During neocortical development, there is a shift from predominately NR2B to both NR2A and NR2B receptor subunit expression (Sheng et al. 1994), with a concomitant decrease in the decay time constant of excitatory postsynaptic currents (Flint et al. 1997; Roberts and Ramoa 1999; Stocca and Vicini 1998). In human cortical dysplasia, evidence exists for an increased expression of NR2 (possibly NR2B) subunits within the region of the dysplasia,

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but not in surrounding or "nonspiking" cortex (Ying et al. 1998). The absence of NR2 immunoreactivity in nonspiking cortex compared with the abundance of NR2 immunoreactivity in actively epileptic cortex has led to the suggestion that increases in NR2 expression alone could explain hyperexcitability in human cortical dysplasia (Ying et al. 1998).

In the present study, we tested the hypothesis that functional alterations in NMDA receptor subunit composition are responsible for the hyperexcitability observed in brain slices from freeze-lesioned rats. NR2B-containing receptors are more sensitive than NR2A-containing receptors to the polyamine-site antagonist ifenprodil (Williams 1993). We therefore compared the effects of APV and ifenprodil in lesioned and nonlesioned animals. A preliminary account of some of these results appeared in DeFazio and Hablitz (1998).

METHODS

Animals were housed and handled according to approved guidelines. Timed pregnant Sprague-Dawley dams arrived on embryonic day 15. Freeze lesions were produced using modifications of the technique of Dvorak and Feit (1977). On postnatal day (PN) 2, rat pups were anesthetized by hypothermia (5 min on ice). After making a midline incision, the cold probe was placed on the surface of the skull near the midline for 3–5 s. The cold probe consisted of a 2 mm diameter copper rod extending from a 60 ml methanol-filled centrifuge tube cooled to about -50°C with dry ice. After the incision was sutured, the animals were placed under a heat lamp and returned to their home cage after 30 min.

Brain slices were prepared from PN 17–27 animals. Rats were

anesthetized with ketamine (100 mg/kg) before decapitation. The brain was rapidly removed and submerged in oxygenated (95% $\text{O}_2/5\% \text{CO}_2$), ice-cold, low-calcium saline (containing, in mM: 125 NaCl, 3.5 KCl, 26 NaHCO_3 , 10 glucose, 4 MgCl_2). Coronal sections (300 μm) containing somatosensory cortex were cut using a Vibratome (TPI). Slices were stored in saline consisting of (in mM) 125 NaCl, 5 KCl, 26 NaHCO_3 , 10 glucose, 2 CaCl_2 , and 2 MgCl_2 bubbled with 95% $\text{O}_2/5\% \text{CO}_2$.

Whole cell voltage clamp recordings were made 1–2 mm lateral to the lesion and at least 1 mm lateral to the stimulation site (Fig. 1A). All records were obtained from visually identified neocortical pyramidal cells in layer II/III. Cells were identified by their location below the pial surface, pyramidal shape, and presence of a prominent apical dendrite (Fig. 1B). Recordings were made at room temperature and at a holding potential of -60 mV . Series resistance was regularly monitored and cells with series resistance $>20 \text{ M}\Omega$ or significant changes in series resistance during the experiment were discarded. The internal solution consisted of 140 K-isethionate or K-methyl sulfate, 10 HEPES, 5 EGTA, 0.1 CaCl_2 , 4 MgATP , 0.4 NaGTP , and 5 QX-314 (all concentrations in mM). Slices were continuously perfused with the storage saline listed above. Whole cell recordings were made with an Axopatch-1B amplifier. No series resistance compensation was employed. Recordings were digitized at 5–10 kHz using a Digidata 1200 data acquisition system and Clampex 7 software (Axon Instruments). Data analysis was performed using custom scripts written for Origin 5 (Microcal Software). Stimulating electrodes were made from a twisted pair of nichrome wires (0.002 in. diameter) and placed in deep cortical layers $\sim 0.5 \text{ mm}$ lateral to the lesion. An example of electrode positioning is shown in Fig. 1B. In slices from control animals, the recording site was $\sim 2\text{--}3 \text{ mm}$ lateral to the medial edge of cortex (corresponding to the longitudinal cerebral fissure) and at least 1 mm lateral to the stimulation site. Threshold stimulus currents ranged

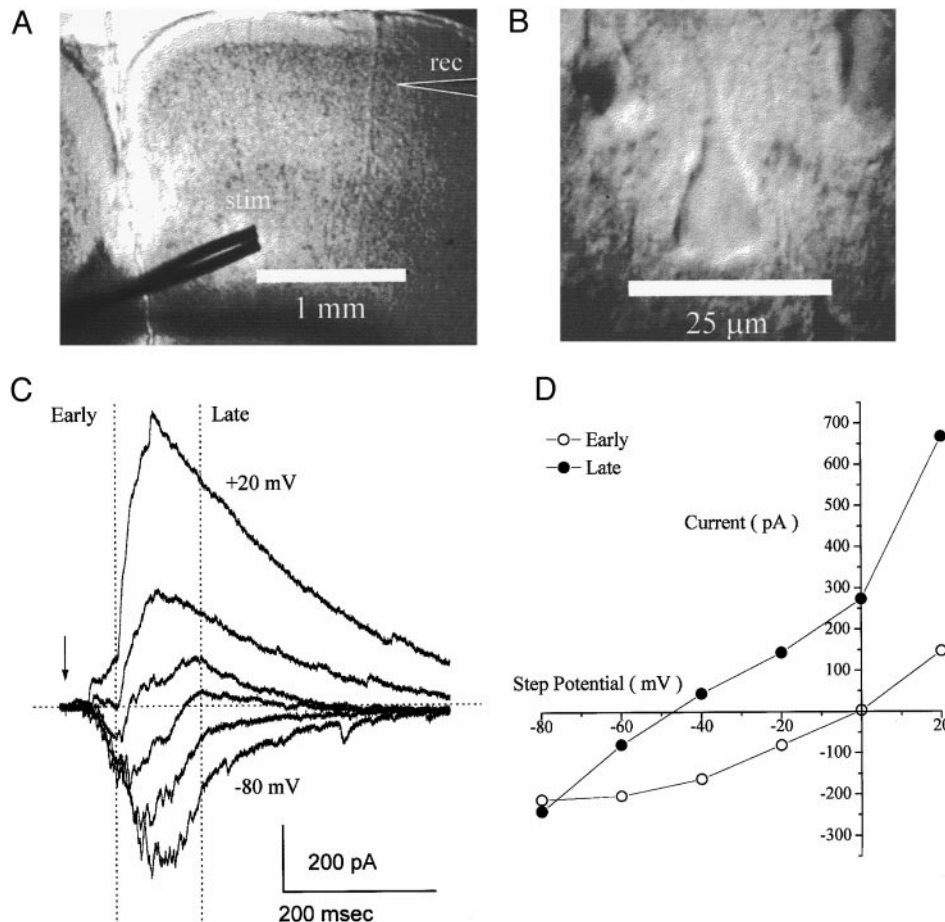


FIG. 1. Evoked paroxysmal discharges have both excitatory and inhibitory components. A: an image of the recording arrangement taken at $4\times$ magnification. The stimulation site was in deep cortical layers $\sim 0.5 \text{ mm}$ lateral to the lesion. The recording site was $\sim 1 \text{ mm}$ lateral to the stimulation site and $1\text{--}2 \text{ mm}$ lateral to the lesion. B: infrared/differential interference contrast image of layer 2/3 pyramidal cells ($160\times$; $40\times$ water immersion, $4\times$ magnification before the camera). Recordings were restricted to pyramidal shaped cells with an apical dendrite projecting to the pial surface. C: voltage clamp recordings of responses evoked at membrane potentials from -80 to 20 mV . Epileptiform discharges at -40 and -20 mV were biphasic, suggesting a mixture of excitatory and inhibitory components. D: plots of the early and late component amplitudes as a function of voltage.

from 30–500 μ A at 80 μ sec and were not statistically significant between the control and lesion groups. A Weco SC-100 constant current source and a WPI pulse generator were employed to deliver current pulses through the stimulating electrode. The threshold for epileptiform discharges was determined by varying the stimulation current (80 μ sec duration). To generate stimulus intensity curves, the stimulus duration was varied, under digital control, from 20 to 300 μ sec in 10–50 μ sec steps. The interstimulus interval (30 s) was kept constant throughout the experiment. To quantify the effects of NMDAR antagonists, epileptiform discharge peak amplitude and area were calculated. Peak amplitude was defined as the greatest absolute value after the stimulus artifact. Area was calculated by zeroing the baseline to the average of 50 ms just before the stimulus artifact, then integrating the trace after the stimulus artifact.

All drugs were bath applied and each cell served as its own control. Concentrated stocks of APV (Tocris) were made in deionized water. Ifenprodil (Sigma) was dissolved in DMSO to make concentrated stocks. These stocks were stored at -20°C .

RESULTS

Voltage dependence of the epileptiform discharge

Previous investigations have shown that there is a hyperexcitable zone adjacent to the microsulcus in freeze-lesioned cortex (Hablitz and DeFazio 1998; Jacobs et al. 1999). Synaptic activation of deep layers adjacent to the lesion evoked a long-lasting epileptiform discharge that was monitored in layer II/III pyramidal cells 1–2 mm lateral to the lesion using whole cell voltage clamp techniques (Fig. 1). The voltage dependence of the epileptiform discharge was examined by stepping the membrane potential between -80 and 20 mV from a holding potential of -60 mV. The voltage step was 1 s in duration, starting 400 ms before the stimulus. In 22 of 26 cells, a biphasic current waveform was apparent at membrane potentials between -40 and -20 mV, whereas the remaining recordings were monophasic with little or no current at 0 mV. Outward currents at these potentials are most likely caused by synaptic activation of chloride-permeable GABA_A receptors that reverse near -70 mV (compared with 0 mV for the expected reversal of excitatory synaptic currents). Figure 1C shows epileptiform discharges evoked at potentials from -80 mV to 20 mV. Both inward and outward currents were present at step potentials between -60 mV and -40 mV, whereas

an outward component predominated at 0 mV. Plots of current amplitude as a function of voltage for the time points indicated by the dashed lines are shown in Fig. 1D. The early component did not change polarity until the voltage step exceeded 0 mV, as would be expected for an excitatory postsynaptic current under the present recording conditions. The late component reversed near -50 mV, closer to the reversal potential for chloride. Exact reversal potentials for the two components could not be measured because of overlapping excitatory and inhibitory components. Pharmacological blockade of individual components was not attempted because this would alter the excitability of the local circuits responsible for generating the epileptiform discharge.

NMDAR antagonists raise epileptiform discharge threshold

NMDAR antagonists have been shown to either block a late recurrent component of the evoked epileptiform discharge (Luhmann et al. 1998) or abolish the discharge entirely (Jacobs et al. 1999). In the present experiments, bath application of APV at concentrations of 2–10 μ M reversibly raised the threshold for evoking epileptiform discharges, in addition to reducing peak amplitude and response area in lesioned animals. Figure 2A illustrates the multiple effects of antagonist on evoked epileptiform discharges. In the presence of 10 μ M APV, the epileptiform discharge was reduced in amplitude and decayed more rapidly. These effects were reversible. Epileptiform discharges can show trial-to-trial variation in peak amplitude and area. A $>25\%$ change in the measured properties was considered significant because this represented a change in threshold greater than that produced by the minimum stimulus duration increment employed in determining input-output curves. APV caused a reduction of $>25\%$ in the peak amplitude in 6 of 14 recordings and in the area in 9 of 14 recordings. A reduction in area without a $>25\%$ change in amplitude was observed in 3 of 9 cells.

APV also raised the threshold for evoking an epileptiform discharge. A plot of response area as a function of stimulus duration is shown in Fig. 2B. In both control and wash, the epileptiform discharge could be reliably evoked at a 100 μ sec stimulus duration. In APV, a stimulus of 180 μ sec was re-

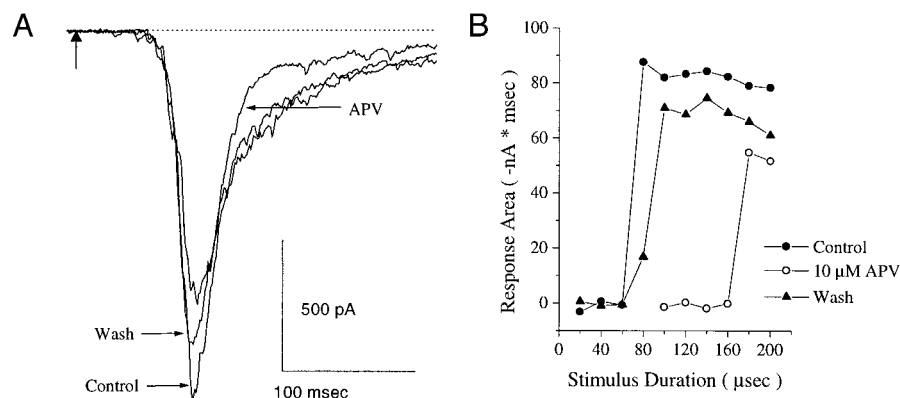


FIG. 2. The *N*-methyl-D-aspartate receptor (NMDAR) antagonist D-2-amino-5-phosphopentanoic acid (APV) increases the threshold and reduces the amplitude and area of evoked epileptiform discharges. *A*: specimen records showing that 10 μ M APV reversibly reduced the peak amplitude and area of the evoked epileptiform discharge. Traces are aligned on the rising phase to demonstrate changes in area. *B*: effects of APV on input-output relations. Stimulus intensity was varied by changing the duration of the current pulse. Brief pulses (≤ 60 μ sec) did not evoke a detectable current. Under control conditions, stimulus durations exceeding 60 μ sec evoked all-or-nothing events (\bullet). 10 μ M APV increased the threshold threefold in this recording. After 30 min wash, the threshold was still slightly elevated, with a small event evoked at a stimulus duration of 80 μ sec. The holding potential was -60 mV for this and all the following figures.

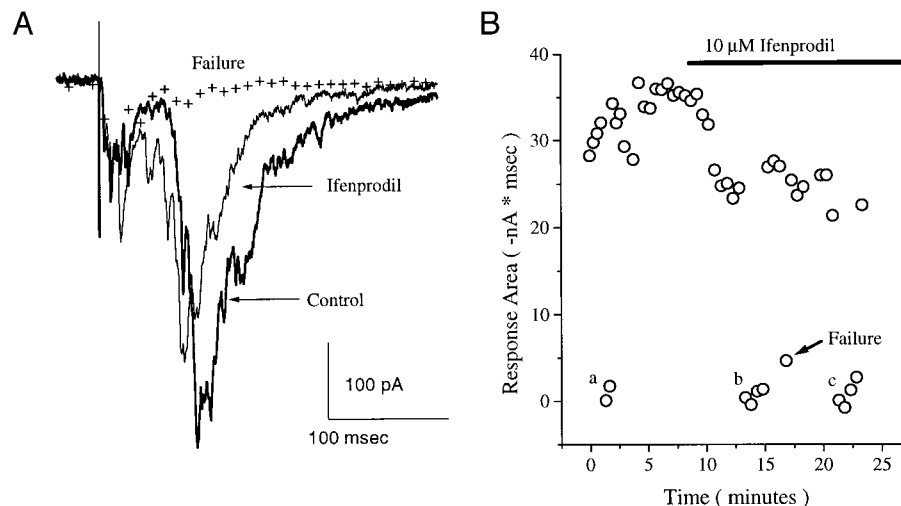


FIG. 3. The NR2B subunit-selective antagonist ifenprodil reduces epileptiform discharges in slices from lesioned animals. **A:** in these recordings, evoked responses consisted of a rapid, possibly monosynaptic, response followed by an epileptiform discharge. These short latency responses were observed in 20% of the recordings in slices from lesioned animals with distal stimulation (>1 mm medial to recording site, adjacent to lesion). Similar stimulation in slices from nonlesioned animals in normal saline did not evoke appreciable currents at the recording site. **B:** the time course of the experiment summarizes the effects of ifenprodil in slices from lesioned animals. The first 5 min of the experiment reflects the stimulus duration dependence of the epileptiform discharge. Data points (a) represent subthreshold pulses of 20 and 40 μ sec. Stimulus durations ≥ 60 μ sec evoked a maximal response. Ifenprodil reduced the area of the epileptiform discharge as well as the threshold. At (b) and (c), stimulus duration dependence was tested again. Stimulus durations <100 μ sec did not evoke a late discharge. \leftarrow indicates a failure at 100 μ sec (also shown in A).

quired to evoke a discharge. APV increased the threshold for evoking an epileptiform discharge by $>25\%$ in 8 of 9 slices tested. As shown in Fig. 2B, elevated stimulus intensity decreased the area of the discharge, but not to the same extent as APV.

To examine the possibility of enhanced expression of NR2B-containing NMDARs, the polyamine-site antagonist ifenprodil was tested. The effects of ifenprodil (10 μ M), which has a 400-fold greater affinity at NR2B-containing receptors (Williams 1993), on epileptiform discharges are shown in Fig. 3. Epileptiform discharges in this cell exhibited an early fast component followed by a multiphasic late component. The fast component was unaffected by ifenprodil whereas peak amplitude and area of the late component of the epileptiform discharge were decreased. The time course of this experiment is shown in Fig. 3B. After two stimuli at 100 μ sec, a series of stimuli at 20, 40, 60, 80, 100, 150, and 200 μ sec was delivered to determine threshold (a).

Threshold for this cell was 60 μ sec. A stimulus duration of 100 μ sec was employed before and during the application of 10 μ M ifenprodil. At (b) and (c), a second threshold determination was made. Epileptiform discharges could not be evoked at stimulus durations <100 μ sec. One stimulation at 100 μ sec resulted in a failure (arrow). Peak amplitude ($n = 4$ of 8 slices) and area ($n = 8$ of 8 slices) of epileptiform discharges were reduced by ifenprodil. Ifenprodil raised the threshold $>25\%$ in all recordings examined for threshold effects ($n = 6$ slices). Thus the NR2B-selective antagonist had effects similar to those observed in APV.

Partial disinhibition reveals differences in sensitivity to ifenprodil in lesioned and nonlesioned cortex

Partial disinhibition resulting from bath application of low doses of bicuculline produces a hyperexcitable state characterized by propagating epileptiform discharges (Chagnac-Amitai

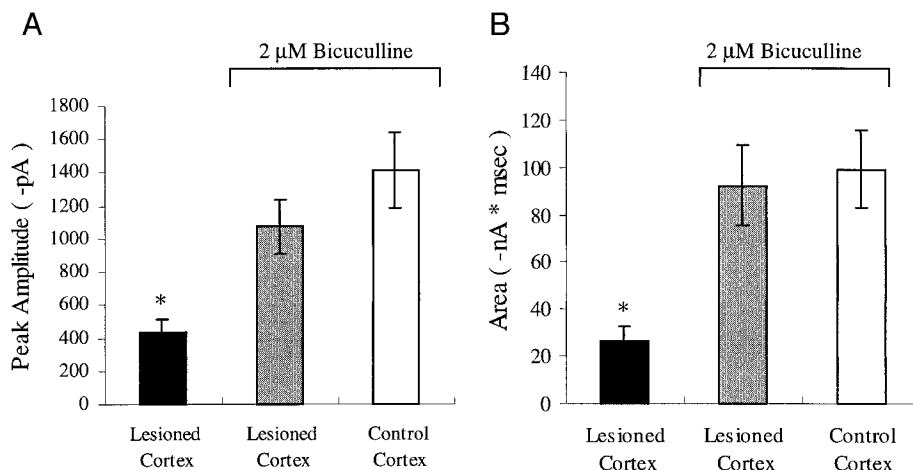


FIG. 4. Partial disinhibition revealed a larger discharge that was similar in waveform in lesioned and nonlesioned cortex. **A:** peak amplitude of the epileptiform discharge under control conditions in lesioned cortex was significantly smaller ($P < 0.001$, $n = 10$) than the response recorded in the presence of 2 μ M bicuculline in both lesioned and nonlesioned cortex (both $n = 7$). **B:** the area was also significantly smaller ($P < 0.001$). These data support the observation that the mechanisms underlying hyperexcitability in lesioned cortex are inconsistent with a loss of inhibition.

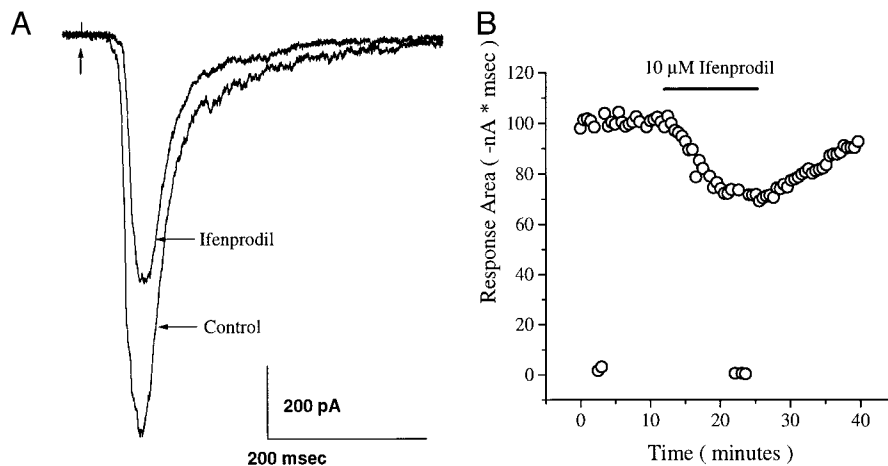


FIG. 5. Ifenprodil modifies the epileptiform discharge in slices from nonlesioned animals in the presence of $2 \mu\text{M}$ bicuculline. The discharge amplitude was reduced with little effect on threshold. **A:** $10 \mu\text{M}$ ifenprodil reduced the amplitude and area of the epileptiform discharge. **B:** time course of ifenprodil antagonism and a small increase in threshold are shown. Two $60 \mu\text{sec}$ stimuli did not evoke the all-or-nothing current response under control conditions; however, stimulus durations $\geq 80 \mu\text{sec}$ reliably evoked the discharge. In the presence of ifenprodil, 3 stimulations at $80 \mu\text{sec}$ were subthreshold whereas $100 \mu\text{sec}$ continued to reliably evoke the response. Thus a 25% increase in threshold was observed in this recording. This was the average increase in threshold by ifenprodil in nonlesioned animals.

and Connors 1989). In brain slices bathed in $2 \mu\text{M}$ bicuculline, we examined the ifenprodil sensitivity of the evoked epileptiform discharge threshold in slices from lesioned and nonlesioned animals. Bicuculline significantly enhanced the peak amplitude and area of the epileptiform discharge in lesioned animals (Fig. 4). No significant differences in bicuculline-induced epileptiform discharges in slices from nonlesioned and lesioned animals were observed. In slices from nonlesioned animals, bath application of $10 \mu\text{M}$ ifenprodil reduced the area of the epileptiform discharge by $>25\%$ in 2 of 7 cells. Figure 5A illustrates the effect of ifenprodil on bicuculline-induced epileptiform discharges in a slice from a nonlesioned animal. In this cell, both the area and the peak amplitude were reduced $>25\%$. The time course of the experiment is shown in Fig. 5B. Symbols near zero represent responses to subthreshold stimulation. The threshold was unaffected by ifenprodil in this cell. Overall, ifenprodil had little effect on the threshold ($<25\%$ increase in threshold) for evoking the epileptiform discharge ($n = 5$ of 6 slices tested).

In contrast, ifenprodil raised the threshold of the bicuculline-induced epileptiform discharge in slices from lesioned animals, as shown in Fig. 6. For this recording, stimuli of $60 \mu\text{sec}$ duration reliably elicited epileptiform discharges (Fig. 6A), but in the presence of ifenprodil, stimuli of $100 \mu\text{sec}$ or greater were required to evoke the epileptiform discharge (Fig. 6B). After washout, the $60 \mu\text{sec}$ stimulus again reliably evoked the epileptiform discharge. In lesioned animals, ifenprodil raised

the threshold by $>25\%$ in 5 of 7 slices examined, but had little effect ($<25\%$) on peak or area of the epileptiform discharge ($n = 7$ of 7). These results are summarized in Table 1 and Fig. 7. The mean of the percent changes observed for each experimental group is shown in Fig. 7. On average, ifenprodil had significantly less effect on threshold in control animals ($P < 0.05$) in bicuculline. The amplitude and area of the discharges evoked in slices from control and lesioned animals in bicuculline were also less sensitive to ifenprodil. The number of slices is the same as that given in Table 1.

DISCUSSION

Three main findings were obtained in the present experiments. The voltage dependence of epileptiform discharges indicated that these paroxysmal events were produced by a complex pattern of excitatory and inhibitory inputs. NMDAR antagonists were found to be effective in raising the threshold for evoking epileptiform discharges. Finally, the differential ifenprodil sensitivity of threshold for evoked bicuculline-induced discharges in control and lesioned animals indicated functional differences in NMDARs expressing NR2B subunits.

The presence of a GABA_A receptor-mediated component in epileptiform discharges in the freeze lesion model is consistent with previous observations. These earlier studies, using local stimulation $<0.5 \text{ mm}$ distal to the recording site, demonstrated the presence of inhibitory potentials in layer II/III pyramidal

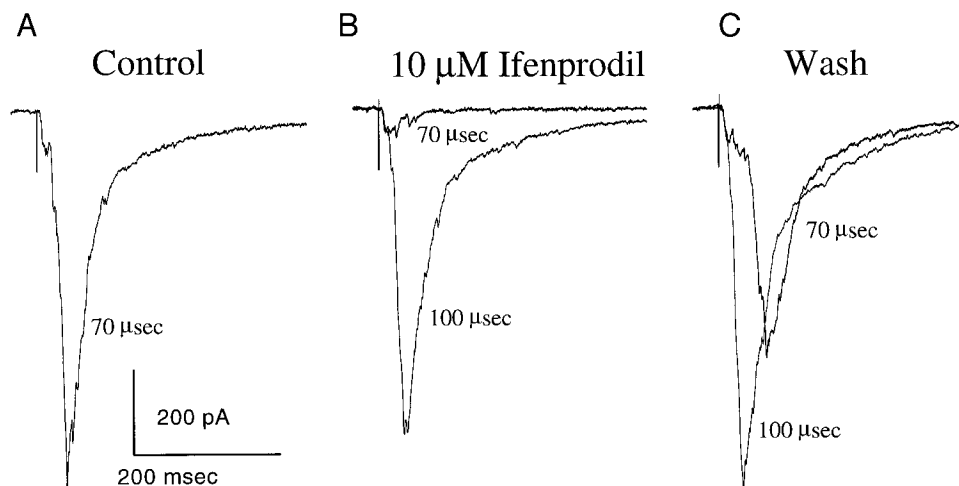


FIG. 6. Ifenprodil increased the threshold by an average of 49% in slices from lesioned animals exposed to bicuculline. **A:** in $2 \mu\text{M}$ bicuculline, lesioned slices displayed an epileptiform discharge similar in waveform and amplitude to that observed in control animals (compare with Fig. 5). **B:** ifenprodil raised the threshold for evoking the discharge from $70 \mu\text{sec}$ in **A** to $100 \mu\text{sec}$ in **B**, a 43% increase. **C:** on washout, the $70 \mu\text{sec}$ stimulation evoked a response similar to the control response in **A**. The amplitude to the response to $100 \mu\text{sec}$ was increased relative to that in the presence of ifenprodil.

TABLE 1. Percent of recordings showing a >25% change in threshold, peak amplitude, or area

	Antagonist	Threshold	Peak	Area
<i>Intrinsic hyperexcitability</i>				
Lesion	APV	89% (9)	43%	64% (14)
Lesion	Ifenprodil	100% (6)	50%	100% (8)
<i>Partial disinhibition</i>				
Nonlesion	Ifenprodil	17% (6)	14%	29% (7)
Lesion	Ifenprodil	71% (7)	0%	0% (7)

Summary of results obtained under control conditions and after partial disinhibition with 2 μ M bicuculline. One recording was obtained from each slice. Number in parenthesis indicates number of slices tested. Concentrations of 2–10 μ M APV and 10 μ M ifenprodil were employed. Differences in the number of slices in threshold and peak/area measurements were caused by the lack of stimulus-intensity curves in some recordings.

cells (Luhmann et al. 1998) and early and late inhibitory currents in layer 5 pyramidal cells (Prince et al. 1997). Our data demonstrate that even during the propagation of epileptiform discharges (1–2 mm from the stimulation site), a prominent inhibitory component is detectable in layer II/III pyramidal cells. In addition, studies of inhibitory postsynaptic currents (IPSCs) in brain slices from lesioned animals have indicated an enhancement of unitary inhibitory events (DeFazio and Hablitz 1999) and enhanced excitatory drive on inhibitory cells (Prince et al. 1997). Although disinhibition has often been suggested as a basis for epileptogenesis (Ribak and Reiffenstein 1982; Schwartzkroin and Prince 1980), epileptiform activity can be observed when inhibitory synaptic activity is normal (Malouf et al. 1990) or enhanced (Rutecki et al. 1987). The occurrence of complex long-lasting epileptiform discharges in dysplastic neocortex suggests alterations in local excitatory circuits. The presence of some degree of inhibitory control may explain the failure to observe spontaneous epileptiform activity in this model.

The effects of NMDAR antagonists on the threshold of the epileptiform discharge indicate that NMDARs play an important role in the initiation and/or propagation of epileptiform discharges. In lesioned cortex, NMDAR antagonists potently raised the threshold for evoking discharges in both the intrinsic hyperexcitable state and after partial disinhibition. These results may help to resolve the apparent differences reported in previous studies of APV effects in this animal model. A previous study (using relatively local stimulation, ~ 0.5 mm lateral to the lesion) demonstrated that APV at concentrations >20 μ M blocked only a late component of the discharge (Luhmann et al. 1998). Our study suggests that APV-induced changes in the threshold for evoking the propagating discharge could lead to an apparent abolition of the late component with little effect on the local (early) response if only a single stimulation intensity was tested. Another group reported that 100 μ M APV completely blocked late field potential discharges recorded at 2, 4, and 8 times threshold stimulus intensities (Jacobs et al. 1999). An early component representing the locally evoked postsynaptic potential and population action potential was not obviously affected by 100 μ M APV (see Fig. 10 in Jacobs et al. 1999). The combination of these results with the present study suggests that NMDARs play an important role in the initiation and propagation of the epileptiform discharge.

The inhibitory effect of ifenprodil, an antagonist with higher affinity for receptors containing the NR2B subunit, was unexpected because NR2A receptors are thought to predominate at the stage of development examined in this study (Flint et al. 1997; Sheng et al. 1994). Although ifenprodil sensitivity has been demonstrated in a subpopulation of isolated neocortical cells from animals of this age group (Kew et al. 1998), differences between control and lesioned animals under conditions of partial disinhibition support the hypothesis of alterations in NMDAR subunit composition. These results suggest that NR2B subunit-containing NMDARs predominate in at least a subset of the cells responsible for the initiation of the discharge, possibly conferring hyperexcitability through the prolongation of excitatory postsynaptic currents (EPSCs). The lack of effect of ifenprodil on the discharge observed in the presence of bicuculline in slices from control and lesioned animals suggests that such changes in NMDAR subunit composition are not widespread.

Studies of resected human dysplastic cortex have demonstrated the presence of NR2 subunit immunoreactivity in cortical resections characterized as “spiking” with subdural electrodes. The immunoreactivity was localized to “dysplastic neurons,” or cells that appear disordered compared with the normal laminar structure of cortex (Ying et al. 1998). It should be noted that the morphological abnormalities observed in human polymicrogyria and this freeze-lesion model represent only one type of cortical dysplasia. Consequently, our results cannot be directly compared with the dysplasia observed in human epilepsy. However, our data support the hypothesis that a subset of cells near the lesion and responsible for the initiation of the discharge are sensitive to ifenprodil. These cells could represent the “dysplastic” neurons in human tissue that are observed to express NR2 immunoreactivity.

NMDARs are known to participate in local recurrent excitation in neocortex (Hess et al. 1994; Sutor and Hablitz 1989). Abnormalities in these horizontal connections could also con-

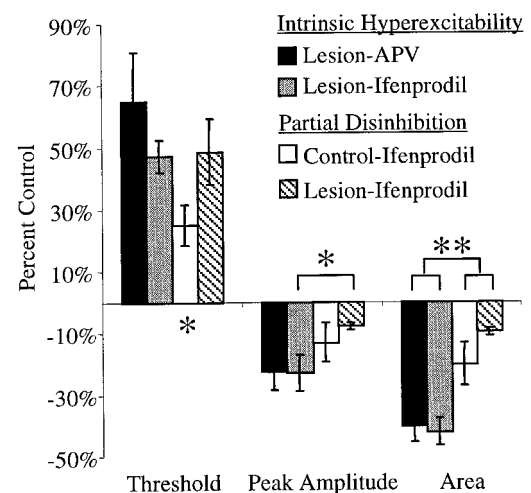


FIG. 7. Summary graph showing the results from the 4 experimental conditions. The ifenprodil-induced increase in threshold was significantly less in control animals in the presence of 2 μ M bicuculline ($P < 0.05$). The effects of NMDAR antagonists on peak amplitude and area were more variable; however, ifenprodil had significantly greater effect on peak amplitude in normal saline than in bicuculline ($P < 0.05$). Both APV and ifenprodil had greater effects on area in the absence of bicuculline ($P < 0.01$). See Table 1 for the numbers of slices in each group.

tribute to hyperexcitability. Alterations in gene expression and changes in receptor subunits have been reported in several models of epilepsy. Metabotropic glutamate receptors are differentially modulated in the kindling model of temporal lobe epilepsy (Akbar et al. 1996; Neugebauer et al. 1997). Our previous studies on miniature IPSCs in dysplastic cortex demonstrated a predominance of $\alpha 2$ over $\alpha 1$ subunits (DeFazio and Hablitz 1999), a pattern found in immature neocortex. Those observations, coupled with the present findings with NMDARs, suggest that a delay or arrest of the developmental pattern of neurotransmitter receptor expression on neocortical cells may be a hallmark of freeze-induced cortical dysplasia.

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