You might find this additional information useful...

This information is current as of July 9, 2007 .

Layer I Ectopias and Increased Excitability in Murine Neocortex

LISA A. GABEL AND JOSEPH J. LoTURCO
Department of Physiology and Neurobiology, University of Connecticut, Storrs, Connecticut 06269

Gabel, Lisa A. and Joseph J. LoTurco. Layer I ectopias and increased excitability in murine neocortex. *J Neurophysiol* 87: 2471–2479, 2002; 10.1152/jn.00828.2001. Cortical dysplasias are associated with both epilepsy and cognitive impairments in humans. Similarly, several animal models of cortical dysplasia show that dysplasia causes increased seizure susceptibility and behavioral deficits in vivo and increased levels of excitability in vitro. As most current animal models involve either global disruptions in cortical architecture or the induction of lesions, it is not yet clear whether small spontaneous neocortical malformations are also associated with increased excitability or seizure susceptibility. Small groups of displaced neurons in layer I of the neocortex, ectopias, have been identified in patients with cognitive impairments, and similar malformations occur sporadically in some inbred lines of mice where they are associated with behavioral and sensory-processing deficits. In a previous study, we characterized the physiology of cells within neocortical ectopias, in one of the inbred lines (NXSM-D/Ei) and showed that the presence of multiple ectopias is associated with the generation of spontaneous epileptiform activity in slices. In this study, we use extracellular recordings from brain slices to show that even single-layer I ectopias are associated with higher excitability. Specifically, slices that contain single ectopias display epileptiform activity at significantly lower concentrations of the GABA$_A$ receptor antagonist bicuculline than do slices without ectopias (either from opposite hemispheres or animals without ectopias). Moreover, because removal of ectopias from slices does not restore normal excitability, enhanced excitability is not generated within the ectopia. Finally, we show that in vivo, mice with ectopias are more sensitive to the convulsant pentylenetetrazole than are mice without ectopias. Together these results suggest that alterations in cortical hemispheres containing focal layer I malformations increase cortical excitability and that even moderately small spontaneous cortical dysplasias are associated with increased excitability in vitro and in vivo.

INTRODUCTION

Cortical malformations have been identified in ≤24% of all cases of epilepsy (Annegers 1994; Meencke and Veith 1992) and ~ 40% of severe or intractable epilepsy cases (Farrell et al. 1992; Hardiman et al. 1988). Cognitive impairments have similarly been associated with disruptions in neocortical development (Barkovich et al. 1989, 1994; Palmini et al. 1991; Ricci et al. 1992). For example, patients with four-layered polymicrogyria show cognitive impairments, including language impairments, mental retardation, and epilepsy (Guerrini et al. 1999; Kuzniecky and Barkovich 1996; Kuzniecky et al. 1989), and patients with layer I ectopias and microgyria show language and learning impairments (Humphreys et al. 1990;

Kaufmann and Galaburda 1989). Although there is a correlation between the presence of cortical malformations and neurological impairments, the particular properties of dysplastic cortex that cause impaired function have not been clearly defined.

To determine the mechanistic relationship between cortical malformations and impaired neurological function, investigators have focused on several animal models that display cortical dysplasias similar to those observed in humans. In one animal model, microgyria are induced by perinatal freeze-lesions, and this causes a pronounced increase in spontaneous hyperexcitability and spontaneous epileptiform activity in slices (Jacobs et al. 1999; Luhmann and Raabe 1996). Hyperexcitability in this model is blocked with *N*-methyl-D-aspartate (NMDA) receptor antagonist, D-2-amino-5-phosphonopentanoic acid (D-AP5) (Jacobs et al. 1999) and correlates with the severity of the malformation (Luhmann and Raabe 1996). However, rats with induced-microgyria have not been shown to spontaneously seize or have an increased sensitivity to proconvulsant drugs. In contrast, a rat genetic model of subcortical band heterotopia (SBH) (*tish* mutant: telencephalic internal structural heterotopia) and a model of micrencephaly (*fh/fh*) show spontaneous electrographic and behavioral seizures in vivo (Chen et al. 2000; Lee et al. 1997; Sarkisian et al. 1999). In vitro, the *tish* mutant exhibits hyperexcitability on exposure to proconvulsant drugs [i.e., 4-aminopyridine (4-AP) and penicillin] in the presence of low magnesium (Chen et al. 2000), but unlike the microgyria model, epileptiform activity in slices from *tish* mutants does not occur without proconvulsant treatment. In addition, in slices from rats with either induced-microgyria or the *tish* mutant, hyperexcitability appears to be greatest within nearby normatopic cortex (Chen et al. 2000; Jacobs et al. 1996, 1999; Luhmann and Raabe 1996), suggesting that the surrounding tissue, not the malformation, is the source of hyperexcitability.

Layer I ectopias are made up of clusters of misplaced neurons and glia and contain small and medium pyramidal cells, some abnormally oriented pyramidal cells, as well as nonpyramidal cells (Caviness et al. 1978; Galaburda et al. 1985; Kaufmann and Galaburda 1989). Ectopias appear to occur as a result of disrupted migration caused by either abnormal interactions between migrating neuroblasts and radial glial fibers (Caviness et al. 1978) and/or disruptions in the pia and layer I (Caviness et al. 1978; McBride and Kemper 1982); however, the mechanisms have not been elucidated. Focal dysplasias in

---

upper cortical layers, including ectopias and microgyria, have been associated with dyslexia (Humphreys et al. 1990; Kaufmann and Galaburda 1989) and ectopias with psychomotor retardation (Caviness et al. 1978). Ectopias, virtually identical to those described in humans with developmental dyslexia and psychomotor retardation, have been identified in three strains of autoimmune mice: NZB/BlNJ, BXSB/MPJ, and NXSM-D/Ei mice (Sherman et al. 1985, 1987, 1990a, 1991). Ectopias in these mice contain ≥50 cells, are located in either the somatosensory (NXSM-D/Ei and NZB/BlNJ) or frontal/motor (BXSB/MPJ) cortices, and occur in 40–85% of mice (Boehm et al. 1996; Denenberg et al. 1991a; Gabel and LoTurco 2001; Sherman et al. 1990a). The sporadic occurrence within a particular line makes them an ideal experimental model to test differences in behavior, anatomy, or physiology associated with spontaneous malformations. For example, ectopias in these mice have been associated with behavioral impairments, such as spatial and nonspatial working-memory deficits (Balogh et al. 1998; Boehm et al. 1996; Denenberg et al. 1991b; Schrott et al. 1993; Spencer et al. 1986) and in processing rapid auditory stimuli (Clark et al. 2000; Frenkel et al. 2000). Similar behavioral disruptions occur in rats with induced microgyria (Fitch et al. 1994, 1997; Herman et al. 1997; Rosen et al. 1995), suggesting a common neurological phenotype for spontaneously occurring ectopias and induced microgyria.

We recently characterized the electrophysiology of neurons within neocortical ectopias in NZB/BlNJ and NXSM-D/Ei mice and showed that spontaneous epileptiform-like discharges were recorded within ectopias in a slice that contained two adjacent ectopias (Gabel and LoTurco 2001) but not in slices with single ectopias. In the present study, we performed extracellular field potential recordings in partially disinhibited slices containing single ectopias. In addition, we examined the seizure susceptibility of NXSM-D/Ei mice with and without ectopias by injecting them with increasing doses of pentylenetetrazole (PTZ). We show that neocortical slices containing ectopias have a lower bicuculline (BMI) threshold for generation of epileptiform activity as compared with hemispheres either opposite ectopias or taken from mice without ectopias. The epileptiform events induced in slices containing ectopias are reversibly blocked with the NMDA receptor antagonist D-AP5. Furthermore, using knife cuts, we demonstrate that ectopias are not necessary for generating epileptiform activity in slices. Last, NXSM-D/Ei mice with ectopias are more susceptible to PTZ than NXSM-D/Ei mice without ectopias. These results indicate that NXSM-D/Ei mice with layer I neocortical ectopias display an increased level of excitability in vitro and in vivo.

METHODS

Preparation of brain slices

Male and female NXSM-D/Ei mice [37–246 days postnatal (PD); PD 129 ± 11, mean ± SE; Jackson Laboratories, Bar Harbor, ME] were deeply anesthetized with halothane and decapitated. Following decapitation, the brains were removed from the skull and immersed in an ice-cold, oxygenated sucrose-artificial cerebrospinal fluid (sucrose-ACSF) solution containing (in mM): 124.0 sucrose, 5.0 KCl, 2.0 $MgCl_2$, 1.23 NaH_2PO_4 (2 · H_2O), 23.8 $NaHCO_3$, and 2.0 $CaCl_2$; pH = 7.4. The brains were blocked, and 300-µm-thick coronal serial sec-

tions were cut using a Vibroslice (Campden Instruments, London, UK). Slices were transferred to a petri dish containing ice-cold, oxygenated ACSF and examined with oblique illumination under a dissecting microscope to identify slices containing ectopias. ACSF contained (in mM): 124.0 NaCl, 5.0 KCl, 2.0 $MgCl_2$, 1.23 NaH_2PO_4 (2 · H_2O), 23.8 $NaHCO_3$, and 2.0 $CaCl_2$; pH = 7.2, osmolarity = 300 ± 5 mosM/l. Slices were maintained at room temperature (20–22°C) in ACSF for ≥1 h in an oxygenated holding chamber before recording began. Recordings were performed at 35–36°C. The University of Connecticut Institutional Animal Care and Use Committee (IACUC) approved all protocols.

Extracellular recordings

Field potential recordings were made from 55 slices (DC-5 kHz, low-pass filtered at 3 kHz, or AC-5 kHz, low-pass filtered at 0.1 kHz, AM Systems). Recording electrodes, made from glass micropipettes (~5 MΩ), were pulled from capillary tubing (Garner Glass N51A, Garner Glass, Claremont, CA) using a Narishige multi-step electrode puller (Model PP-830) and filled with ACSF. Data were digitally sampled at 10 kHz and acquired and analyzed with Superscope II software (GW Instruments, Sommerville, MA). Extracellular stimuli were delivered through concentric micro-bipolar electrodes (FHC, Bowdoinham, ME). Biphasic 200-µs square current pulses were applied at 0.033 Hz using increasing current intensities in increments of 50 µA until a maximal field potential was reached; baseline recordings were continued at 75% of maximal stimulation. BMI (Sigma Chemicals, St. Louis, MO) was added to the bath in increasing concentrations of 0.1, 0.3, 1.0, and 3.0 µM to determine the threshold of epileptiform responses. Each BMI concentration was applied for 20 min at a flow rate of 1.6 ml/min. In some experiments, 50 µM D-AP5 (Tocris Cookson, St. Louis, MO) was added. In 14 slices, the ectopia was separated from the adjacent cortex and/or cut out of the slice using a finely pulled hand-held glass micropipette. Following extracellular recordings, slices were immediately fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (see *Histological procedures*).

Characterization of epileptiform activity in slices

Epileptiform events were identified based on their large, multiphasic voltage deflections, which lasted for durations that were from 572.8 to 960.3 ms. In this study, epileptiform responses were defined as voltage deflections which were ≥1.5 times the amplitude and >5 times the duration of the baseline field potential responses. Peak amplitudes of baseline field potential responses and epileptiform events were determined at each recording site. Averages from four to seven responses for each recording site were calculated for each slice, and then these values were averaged to obtain mean peak amplitudes for each recording site across slices. Duration of baseline field potentials and epileptiform responses were measured from initial rise (1 SD above or below the mean voltage of the baseline period) to a return either to baseline or steady state following the decay of the responses. The average amplitude of epileptiform response was 3.8 times the amplitude and 37.2 times the duration of baseline responses. Latencies to epileptiform events were calculated from stimulus onset to initial rise of the epileptiform events. Latencies to epileptiform events were determined at each recording site. Averages from four to seven responses for each recording site were calculated for each slice, and then these values were averaged to obtain mean latencies to epileptiform events for each recording site across slices. BMI thresholds were determined by the concentration of BMI needed to induce an epileptiform event.

In vivo PTZ treatment

PTZ (Sigma) was injected intraperitoneally at an initial concentration of 30 mg/kg in 0.9% saline. This initial dose of PTZ did not cause

behavioral seizure activity in any of the mice tested. Additional injections of 10 mg/kg ip were administered every 10 min until the mice exhibited a generalized seizure or a maximum cumulative dose of 130 mg/kg was given. Before testing NXSM-D/Ei mice, we examined the seizure susceptibility of mice with a different genetic background, CD1 mice. In general, myoclonic jerks and generalized seizures were induced in CD1 mice at a lower cumulative dose of PTZ (65 ± 5.0 and 75 ± 15.0 mg/kg, respectively) compared with NXSM-D/Ei mice (75.38 ± 6.76 and 90.83 ± 7.33 mg/kg, respectively), and seizures were always lethal. A generalized seizure was characterized as a wild running episode, major generalized seizure without tonus, and/or tonic/clonic seizure. Postmortem histological analysis of the brains was performed to determine which mice had ectopias (see *Histological procedures*). Four NXSM-D/Ei mice, two with and two without ectopias, did not have generalized seizures. A small percentage of mice had lethal seizures (12.5%, 2/16), one female mouse without and one male mouse with ectopias. The seizure susceptibility of these mice was determined by the dose of PTZ injected and time to first myoclonic jerk (MJ) and to first generalized seizure. Time to first MJ and generalized seizure was calculated from time of dose that induced a MJ or generalized seizure. Statistical significance was determined using single-factor ANOVA with a $P < 0.05$. Values are given as means $\pm$ SE.

Histological procedures

ANALYSIS OF WHOLE BRAINS. Following PTZ treatment, mice were anesthetized using halothane and then perfused with 4% paraformaldehyde. Brains were then postfixed overnight, blocked in 1.9% agar, and sectioned on a vibratome. Sections (40-μm thick) were collected, mounted onto slides, and stained using standard Nissl staining procedures. Following Nissl stain, slices were dehydrated in 70, 95, and 100% ethanol and coverslipped with cytoseal (Stephens Scientific, Kalamazoo, MI). Slices were then scanned for ectopias and images were taken in Adobe Photoshop using a Nikon Eclipse e-400 microscope with a spot camera (Diagnostic Instruments). Ectopias were identified in the somatosensory cortex in 8 of 16 mice tested.

SLICES USED IN EXTRACELLULAR EXPERIMENTS. To determine if BMI threshold of the epileptiform events was correlated with the size of ectopias in slices, the area of each ectopia was determined. Area was determined by examining the number of pixels in an image of an ectopia that was taken in Adobe Photoshop using a spot camera (Diagnostic Instruments) connected to a Nikon Eclipse e-400 microscope. A correlation analysis was then performed comparing the area of ectopias to the epileptiform threshold, determined by lowest BMI concentration needed to induce an epileptiform event in slices.

RESULTS

Ectopias

Ectopias were identified in 300-μm slices and visualized with the aid of a dissecting microscope and oblique illumination. Ectopias are seen in living slices as cone shaped malformations in layer I with fibers emanating from the base of the malformation (see Fig. 3A). In post hoc histological analysis of brain slices, ectopic clusters of cells were seen predominately in layer I but often extended into and disrupted the laminar pattern in cortical layers II/III (Fig. 1). Fibers emanating from the base of ectopias often disrupted layers II–VI underlying ectopias (see Gabel and LoTurco 2001; Sherman et al. 1990b). Ectopias in slices were identified in 58.8% (30/51) of all mice tested: 67.5% (27/40) of male and 27.3% (3/11) of female NXSM-D/Ei mice. Ectopias were primarily located in somatosensory cortex (SS ctx; 93.3%, 28/30), although a few (6.7%, 2/30) mice had ectopias located in frontal cortex (FR1 ctx). A
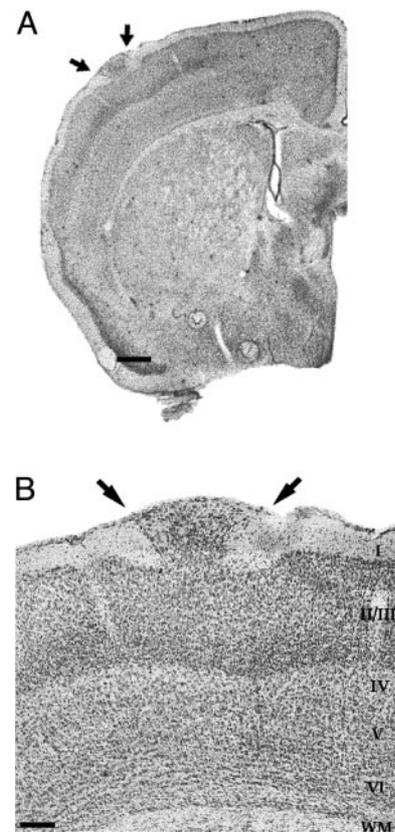

FIG. 1. Representative layer I neocortical ectopias in NXSM-D/Ei mice. *A*: low-magnification image of a layer I neocortical ectopia in the somatosensory cortex of an adult male NXSM-D/Ei mouse. *B*: magnification of the ectopia in *A* shows that ectopias cause minimal disruption of the normal architecture of the cortex. Scale bars (in μm) in $A = 200$ and $B = 50$.

small percentage (13.3%, 4/30) of mice had multiple ectopias located in the same or opposite hemispheres but never with multiple ectopias located in the same hemisphere of a single 300-μm slice. All cases of multiple ectopias involved the SS ctx of male NXSM-D/Ei mice. Field potential recordings were made in a total of 55 slices; 25 slices contained ectopias and 30 slices did not. Ectopias ranged in area from 412.3 to 2234.9 μm^2.

Slices containing ectopias have a lower threshold for epileptiform activity

To compare the threshold for epileptiform activity in slices containing ectopias (E^+), slices from mice without ectopias (E^-), and slices from hemispheres opposite slices containing ectopias (E^{opp}), we recorded field potentials and applied increasing concentrations of BMI (0.1, 0.3, 1.0, and 3.0 μM) until characteristic long-latency (824.01 ± 19.52 ms), large-voltage deflections (1.73 ± 0.14 mV; i.e., epileptiform responses) were observed (Fig. 2). Epileptiform responses were initiated at an average concentration of 2.43 ± 0.37 μM BMI in E^- slices (Fig. 2A), which was not significantly different from E^{opp} slices [2.71 ± 0.29 μM BMI; $F_{(1,13)} = 0.375$; $P = 0.6$; Fig. 2B]. Because there was no significant difference between E^{opp} and E^- slices, collectively they will be referred to as slices without ectopias. Epileptiform responses were observed in slices containing ectopias at significantly lower

A   Without ectopia (E⁻)

(Ctl)

(0.1 μM BMI)

(3.0 μM BMI)

|0.2 mV
100 ms

B   Opposite hemisphere (E^OPP)

(Ctl)

(0.1 μM BMI)

(3.0 μM BMI)

C   Containing ectopia (E⁺)

(Ctl)

(0.1 μM BMI)

D   Application of d-AP5 (50 μM)

pre d-AP5
wash

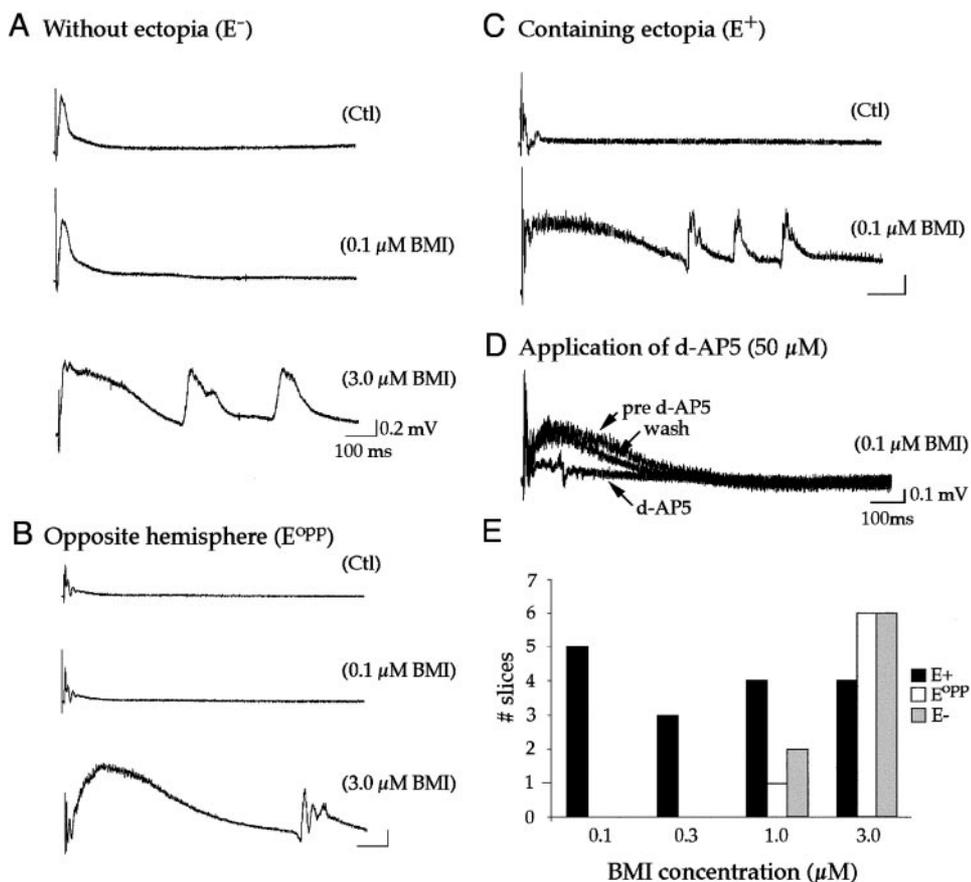(0.1 μM BMI)

d-AP5

|0.1 mV
100ms

E

FIG. 2. Slices with ectopias have a lower threshold for epileptiform activity. *A–C*: field potentials in layers II/III elicited in slices without ectopias (*A*), hemispheres opposite slices with ectopias (E^OPP, *B*), and slices containing ectopias by layer VI/white matter stimulation (*C*). The concentration of biculline (BMI) used during each recording is listed in parentheses above each trace. *D*: epileptiform responses induced with 0.1 μM BMI in a slice containing an ectopia were blocked following application of 50 μM D-2-amino-5-phosphonopentanoic acid (D-AP5) and returned after wash. *E*: summary of the number of slices that produced an epileptiform event following application of 0.1, 0.3, 1.0, or 3.0 μM BMI. Slices containing ectopias (E⁺) showed a significantly lower threshold for epileptiform responses induced with BMI compared with slices without ectopias (E⁻ and E^OPP slices).

BMI concentrations (1.2 ± 0.30 μM BMI, Fig. 2*C*) than slices without ectopias [$F_{(1,30)} = 16.57$, $P = 0.0003$; Fig. 2*E*]. Furthermore, 50% (8/16) of slices containing ectopias had epileptiform thresholds of 0.1 and 0.3 μM BMI, whereas none of the slices without ectopias showed epileptiform responses at these low concentrations. In addition, epileptiform events induced with low concentrations of BMI in slices containing ectopias were reversibly blocked with 50 μM D-AP5 (Fig. 2*D*). Together these data suggest that slices containing ectopias are hyperexcitable compared with slices without ectopias. This difference could not be attributed to age differences because the average ages in each group were not significantly different (E⁺ 120.6 ± 12.0, E⁻ 87.3 ± 12.5, E^OPP 126.2 ± 18.8 days postnatal). In addition, there was no significant correlation between the area of ectopias and the BMI threshold ($r = -0.064$, data not shown).

Spread of epileptiform activity in slices containing ectopias

Simultaneous field potential recordings were made in slices containing ectopias to examine the laminar propagation of epileptiform activity (Fig. 3*A*). Epileptiform responses induced by low BMI concentrations of either 0.1 or 0.3 μM were examined in slices containing ectopias (concentrations that never produced epileptiform responses in slices without ectopias). Figure 3 shows an example of epileptiform responses, recorded simultaneously from three sites (superficial, middle and deep layers) medial (Fig. 3*B*), lateral (not shown), or within the column containing an ectopia (Fig. 3*C*). Field potential responses show similar laminar profiles in all three

locations (medial, lateral, or within the column containing an ectopia). All epileptiform responses within layer II/III showed a large, long-latency positivity, and a large, long-latency negativity in layers V/VI. Layers V/VI consistently displayed the shortest latency epileptiform response (Table 1).

Increased excitability remains after ectopia removal

To determine if the epileptiform activity is generated by ectopias, knife cuts were made to remove ectopias from slices. Figure 4*A* shows an example of a recording from a slice containing an ectopia tested both before and after the removal of an ectopia. Epileptiform responses induced by 0.1 μM BMI were still present in the slice after the ectopia was removed. To rule out the possibility that epileptiform responses were initiated within ectopias in slices and then this activity induced plasticity in tissue adjacent to the ectopia, which in turn lowered the threshold to BMI, we performed additional knife cut experiments. Slices containing ectopias were cut before the addition of low concentrations of BMI and the BMI threshold was determined for both halves of the slice. Although separated, both halves of the slice began showing epileptiform activity with application of 0.1 μM BMI (Fig. 4*B*). Together these results show that epileptiform activity in low concentrations of BMI is generated outside of ectopias.

Mice with ectopias have a lower seizure threshold to PTZ

Considering the increased sensitivity to BMI of slices containing ectopias, we hypothesized that mice with ectopias
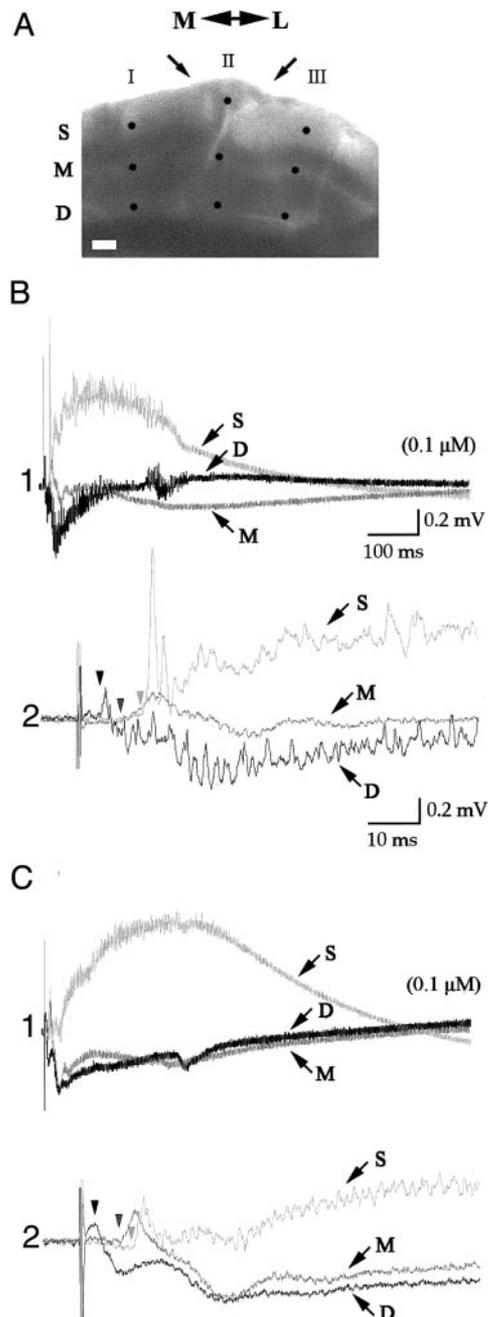
FIG. 3. Laminar pattern of epileptiform activity in slices with ectopias. *A*: image depicting the recording sites (filled circle) in a slice containing an ectopia (arrows). The stimulating electrode (not shown) was placed in the white matter either medial or lateral to the recording electrodes. Scale bar = 200 μm. *B* and *C*: epileptiform responses induced with 0.1 μM BMI in superficial (S), middle (M), and deep (D) cortical layers. Epileptiform events recorded medial (I, *B1*) to and within the area containing an ectopia (II, *C1*). Traces were swept out to show latencies of the epileptiform events in each layer (*B2* and *C2*). Arrowheads point to the onset of the epileptiform activity in each trace. Layer II/III responses (light gray), layer IV responses (dark gray), and layer V/VI responses (black). Epileptiform latencies were consistently shorter in cortical layers V/VI, suggesting that epileptiform activity may be initiated in infragranular layers in slices containing ectopias.

would be more sensitive to proconvulsant treatment in vivo. To test sensitivity to PTZ, 16 NXSM-D/Ei mice (9 males and 7 females) were given an initial dose of 30 mg/kg ip, then 10 mg/kg of PTZ was injected every 10 min until a generalized

seizure was observed or a maximum cumulative dose of 130 mg/kg was given. Because mice with ectopias can only be identified with postmortem histological analysis, the experimenter was blind to which mice have an ectopia. Postmortem histological analysis of the brains showed that 50% (8/16) of the mice tested had ectopias in the somatosensory cortex: 75% (6/8) of mice with ectopias were male and 25% (2/8) were female. Behaviorally, seizure activity consisted of MJ, which was often followed by a generalized seizure. Generalized seizures occurred between 6 s and 60 min after the first MJ. Generalized seizure activity ranged from forelimb clonus and wild running episodes to major generalized seizures (with or without tonus) in both mice with and without ectopias. One-third of mice with ectopias exhibited generalized seizure activity at cumulative doses of PTZ (40–50 mg/kg), a dose that never induced a generalized seizure in mice without ectopias. In addition, 3/8 mice with ectopias had more severe generalized seizures (major generalized seizures), whereas all mice without ectopias exhibited only minor generalized seizure activity (clonus of the forelimbs and/or wild running episodes). Despite behavioral differences between these groups, there were no significant differences in the dose of PTZ to induce generalized seizures between mice with (78.33 ± 11.95 μM) and without ectopias [103.33 ± 5.58 μM; $F_{(1,11)}$ = 3.59, P = 0.09; Fig. 5*B*]. The time between the PTZ injection and the resultant seizure response (either myoclonic jerk or generalized seizure) was also not significantly different between mice with and mice without ectopias [$F_{(1,12)}$ = 3.02, P = 0.11, MJ; $F_{(1,11)}$ = 0.14, P = 0.72, generalized seizure]. However, it took significantly lower doses of PTZ to induce myoclonic jerks in mice with ectopias (58.3 ± 6.01 μM) compared with mice without ectopias [90 ± 8.16 μM; $F_{(1,12)}$ = 9.18, P = 0.01; Fig. 5*A*]. This difference could not be attributed to sex differences between groups because there was no significant difference in the dose of PTZ to induce myoclonic jerks in female compared with male mice [90.0 ± 11.79, male; 80.0 ± 9.76, female; $F_{(1,12)}$ = 0.52, P = 0.48]. These results show that mice with ectopias have an increased sensitivity to PTZ when compared with mice without ectopias.

DISCUSSION

Possible mechanisms of increased excitability

Previously we showed that the presence of two ectopias in the same slice is associated with the generation of spontaneous epileptiform activity, evoked in the absence of BMI application, in vitro (Gabel and LoTurco 2001). Here we show that slices containing single ectopias display epileptiform activity at significantly lower concentrations of BMI than slices without ectopias. These results are consistent with findings from other

TABLE 1. *Average latencies of epileptiform responses*

Lamina	Column		
	Medial	Containing ectopia	Lateral
II/III or Ectopia	19.2 ± 2.3	26.5 ± 6.6	27.0 ± 8.7
IV	17.0 ± 1.4	24.3 ± 8.1	22.7 ± 8.9
V/VI	15.6 ± 2.7	13.0 ± 1.8	17.2 ± 6.9

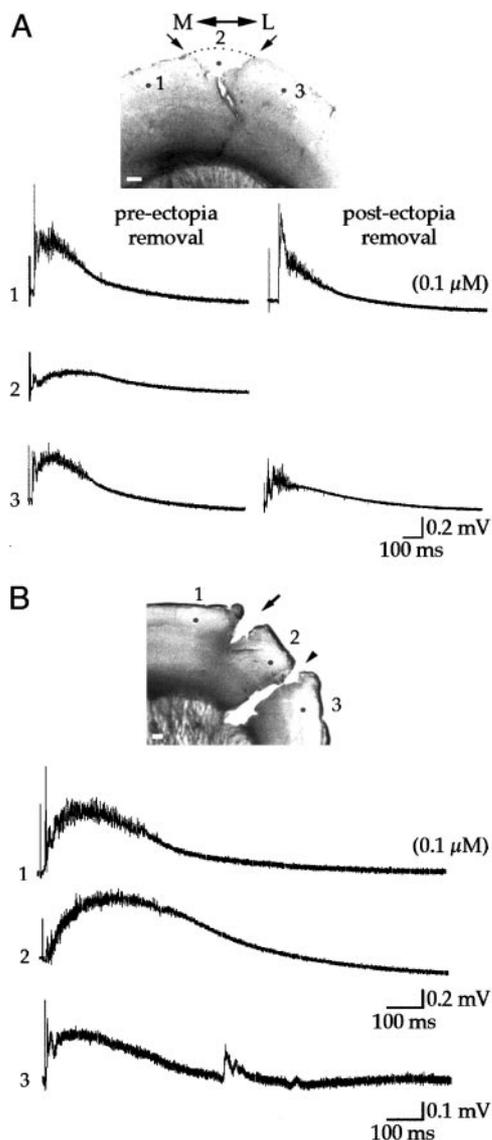Latencies (ms) are listed as means ± SE.

FIG. 4. Epileptiform responses persist in low BMI concentrations within the cortex following removal of ectopias. *A* and *B*: image depicts the location of the recording electrodes (●) in a slice containing an ectopia (↓). Scale bar = 200 μm. *A*: epileptiform responses, induced with 0.1 μM BMI, recorded simultaneously from sites medial (*1*), within (*2*), and lateral (*3*) to an ectopia is shown before and following removal of an ectopia. *B*: prior to recording a vertical cut was made through layers I–VI (including white matter) 1,000 μm from the lateral edge of the ectopia (▲). Application of 0.1 μM BMI induced epileptiform activity medial (*1*) and lateral (*2*) to an ectopia and also on the lateral side (*3*) of the distal cut. Stimulating electrodes were placed in the white matter on both sides of the cortical transection.

animal models of cortical dysplasias that exhibit increased excitability in slices containing malformations (Baraban et al. 2000; Chen et al. 2000; Jacobs et al. 1996, 1999; Luhmann and Raabe 1996; Roper et al. 1997).

Animal models of both SBH and microgyria have provided evidence that epileptiform activity is initiated in the normatopic rather than the dysplastic cortex (Chen et al. 2000; Jacobs et al. 1996, 1999; Luhmann and Raabe 1996). Similarly, we have shown that removal of ectopias in partially disinhibited slices does not restore normal excitability. Therefore epileptiform activity in slices containing ectopias also appears to be generated in the normatopic cortex, suggesting that this area

contains anomalous structures or connections that initiate epileptiform activity. Mechanisms underlying the increased excitability in the normatopic cortex surrounding dysplasias remains unclear, but disruptions in cortical circuitry (Rosen et al. 1989, 2000) and an imbalance of excitation and inhibition (Babb et al. 2000; Defazio and Hablitz 2000; Hablitz and Defazio 1998; Luhmann et al. 1998; Prince and Jacobs 1998; Redecker et al. 2000; Roper 1998; Rosen et al. 1998; Zhu and Roper 2000) have been suggested as possible causes of hyperexcitability in animal models of cortical dysplasias.

Examination of cortical and subcortical circuitry in microgyric rats indicated that callosal (Rosen et al. 1989, 2000) and thalamic (Rosen et al. 2000) afferents normally made to the missing laminae in the microgyric cortex were re-routed to the paramicrogyrial cortex. This disruption may have increased the strength of excitatory connections to the paramicrogyrial zone, which would be consistent with increased excitability in areas surrounding the dysplasia (Jacobs et al. 1999). Similarly, aberrant cortical and subcortical circuitry has also been identified in animal models of ectopias. Studies using retrograde and anterograde tracers have shown ectopias make aberrant connections with thalamus, receive input from subcortical structures (Jenner et al. 2000), and extend axons along the axon fascicles underlying ectopias (Gabel and LoTurco 2001; Jenner et al. 2000), which terminate in cortical areas ipsilateral to ectopias, as well as in thalamus (Jenner et al. 2000). Further-
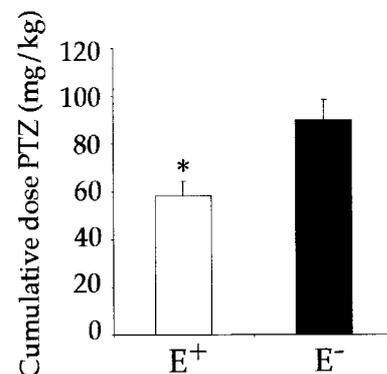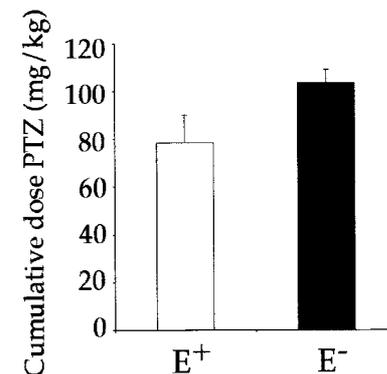

FIG. 5. Mice with ectopias have an increased susceptibility to seizure activity induced with pentylenetetrazole (PTZ). Bar graph showing the cumulative dose of PTZ given that induced a myoclonic jerk (*A*) and generalized seizure (*B*) in mice with and without ectopias. *, significant difference between groups ($P = 0.01$).

more, recent histological data have suggested that horizontal axonal projections across the dysplastic cortex may be disrupted by ectopias (Gabel and LoTurco 2001). These data suggest that ectopias are anomalously connected to cortical as well as subcortical structures and cause disruptions in circuitry. Similar to animals with microgyria, it is possible that increased excitability in slices with neocortical ectopias is also due to disruptions in cortical circuitry.

An imbalance between excitation and inhibition has also been suggested to underlie hyperexcitability in slices containing focal cortical malformations. A reduction in the number of interneurons (Roper et al. 1999; Sarkisian et al. 2001), a downregulation of GABA receptors (Luhmann et al. 1998; Redecker et al. 2000), and an increase in NMDA receptor subunits (Babb et al. 2000; Defazio and Hablitz 2000) and AMPA receptors (Luhmann et al. 1998) have all been shown to occur in models of cortical dysplasias. Our recent electrophysiological data did not suggest that there is a functional increase in NMDA, AMPA, or $GABA_A$ receptor-mediated events within ectopias compared with normatopic cortex (Gabel and LoTurco 2001), but a comparison between the normatopic cortex surrounding ectopias and the normatopic cortex in mice without ectopias has not been performed. An increase in the number of vasoactive intestinal peptide (VIP) immuno-positive neurons was reported within the dysplastic cortex of NZB/BlNJ mice, another mouse strain with spontaneously occurring ectopias in layer I of neocortex (Sherman et al. 1990b). More specifically, an increase in the number of VIP immuno-positive neurons was identified medial to and within the column containing an ectopia compared with a homologous area in the opposite hemisphere. It is unclear how an increase in VIP immuno-positive interneurons in slices containing ectopias could create an increased sensitivity to BMI; however, potentiation in GABA-mediated synaptic transmission is suggested to promote interneuronal synchrony and circuitry excitability (Kohling et al. 1998; Lopantsev and Avoli 1998; Velazquez and Carlen 1999). These results suggest that both disruptions in circuitry and alterations in synaptic transmission may underlie increased excitability in slices containing ectopias.

Comparison to other cortical dysplasias

Current models of cortical dysplasias range from global malformations (either genetic or induced) to induced focal cortical dysplasias. Despite anatomical differences between animal models of cortical dysplasias, most exhibit increased excitability in slices containing the malformation (Baraban et al. 2000; Chen et al. 2000; Jacobs et al. 1996, 1999; Luhmann and Raabe 1996; Roper et al. 1997). Most models also have either spontaneous seizure activity or an increased susceptibility to seizure-inducing drugs (Baraban and Schwartzkroin 1996; Baraban et al. 2000; Chen et al. 2000; Germano and Sperber 1997, 1998; Germano et al. 1996; Lee et al. 1997; Roper et al. 1995; Sarkisian et al. 1999). Similarly, we have shown that slices containing ectopias display an increased level of excitability, and mice with ectopias have an increased sensitivity to PTZ. Unlike other models of cortical dysplasias, ectopias are moderately small malformations, cause a minimal amount of disruption of the

normal cortical architecture, and occur spontaneously within the frontal and somatosensory corticies. Ectopias may provide insight into the minimal amount of disruption to the normal cortical architecture that leads to cortical dysfunction. Despite the small size of ectopias, they may cause global disruptions in cortical function that predisposes mice with ectopias to sensitivity to pro-convulsants, and behavioral and sensory deficits. In addition, the increased excitability in the ectopia model represents another similarity with the microgyria model, which shows concordant behavioral and anatomical abnormalities. Similarities between ectopia and microgyria models include behavioral impairments in discrimination learning (Rosen et al. 1995; Schrott et al. 1992) and processing rapid auditory stimuli (Clark et al. 2000; Fitch et al. 1994, 1997; Frenkel et al. 2000; Herman et al. 1997), changes in size of thalamic neurons (Herman et al. 1997; Jenner et al. 1996), as well as the aforementioned disruptions in cortical circuitry (Jenner et al. 2000; Rosen et al. 1989, 2000). This could suggest that epilepsy and cognitive deficits caused by cortical dysplasias are linked by common mechanisms such as connectivity or receptor changes that alter the excitability of cortical networks.

REFERENCES

ANNEGERS JF. The natural course of epilepsy: an epidemiologic perspective. In: *The Surgical Management of Epilepsy,* edited by Wyler AR and Hermann BP. Boston: Butterworth-Heinemann, 1994, p. 3–7.

BABB TL, YING Z, MIKUNI N, NISHIYAMA K, DRAZBA J, BINGAMAN W, WYLLIE E, WYLIE CJ, AND YACUBOVA K. Brain plasticity and cellular mechanisms of epileptogenesis in human and experimental cortical dysplasia. *Epilepsia* 41, *Suppl*: 76–81, 2000.

BALOGH SA, SHERMAN GF, HYDE LA, AND DENENBERG VH. Effects of neocortical ectopias upon the aquisition and retention of a non-spatial reference memory task in BXSB mice. *Dev Brain Res* 111: 291–293, 1998.

BARABAN SC AND SCHWARTZKROIN PA. Flurothyl seizure susceptibility in rats following prenatal methylazoxymethanol treatment. *Epilep Res* 23: 189–194, 1996.

BARABAN SC, WENZEL HJ, HOCHMAN DW, AND SCHWARTZKROIN PA. Characterization of heterotopic cell clusters in the hippocampus of rats exposed to methylazoxymethanol. *Epilep Res* 39: 87–102, 2000.

BARKOVICH AJ, GUERRINI R, BATTAGLIA G, KALIFA G, N'GUYEN T, PARMEGGIANI A, SANTUCCI M, GIOVANARDI-ROSSI P, GRANATA T, AND D'INCERTI L. Band heterotopia: correlation of outcome with magnetic resonance imaging parameters. *Ann Neurol* 36: 609–617, 1994.

BARKOVICH AJ, JACKSON DE, AND BOYER RS. Band heterotopias: a newly recognized neuronal migration anomaly. *Radiology* 171: 455–458, 1989.

BOEHM GW, SHERMAN GF, HOPLIGHT BJ, HYDE LA, WATERS NS, BRADWAY DM, GALABURDA AM, AND DENENBERG VH. Learning and memory in the autoimmune BXSB mouse: effects of neocortical ectopias and environmental enrichment. *Brain Res* 726: 11–22, 1996.

CAVINESS VS JR, EVRARD P, AND LYON G. Radial neuronal assemblies, ectopia and necrosis of developing cortex: a case analysis. *Acta Neuropathol (Berl)* 41: 67–72, 1978.

CHEN ZF, SCHOTTLER F, BERTRAM E, GALL CM, ANZIVINO MJ, AND LEE KS. Distribution and initiation of seizure activity in a rat brain with subcortical band heterotopia. *Epilepsia* 41: 493, 2000.

CLARK MG, SHERMAN GF, BIMONTE HA, AND FITCH RH. Perceptual auditory gap detection deficits in male BXSB mice with cerebrocortical ectopias. *Neuroreport* 11: 693–696, 2000.

DEFAZIO RA AND HABLITZ JJ. Alteration in NMDA receptors in a rat model of cortical dysplasia. *J Neurophysiol* 83: 315–321, 2000.

DENENBERG VH, MOBRAATEN LE, SHERMAN GF, MORRISON L, SCHROTT LM, WATERS NS, ROSEN GD, BEHAN PO, AND GALABURDA AM. Effects of the autoimmune uterine/maternal environment upon cortical ectopias, behavior, and autoimmunity. *Brain Res* 563: 114–122, 1991a.

DENENBERG VH, SHERMAN GF, SCHROTT LM, ROSEN GD, AND GALABURDA AM. Spatial learning, discrimination learning, paw preference, and neocortical ectopias in two autoimmune strains of mice. *Brain Res* 562: 98–104, 1991b.

FARRELL MA, DeROSA MJ, CURRAN JG, LENARD SD, CORNFORD ME, COMAIR YG, PEACOCK WJ, SHILEDS WD, AND VINTERS HV. Neuropathologic findings in cortical resections (including hemispherectomies) performed for the treatment of intractable childhood epilepsy. *Acta Neuropathol (Berl)* 83: 246–259, 1992.

FITCH RH, BROWN C, TALLAL P, AND ROSEN GD. Effects of sex and MK-801 on auditory-processing deficits associated with developmental microgyric lesions in rats. *Behav Neurosci* 111: 404–412, 1997.

FITCH RH, TALLAL P, BROWN C, GALABURDA AM, AND ROSEN GD. Induced microgyria and auditory temporal processing in rats: a model for language impairment? *Cereb Cortex* 4: 260–270, 1994.

FRENKEL M, SHERMAN GF, BASHAN KA, GALABURDA AM, AND LoTURCO JJ. Neocortical ectopias are associated with attenuated neurophysiological responses to rapidly changing auditory stimuli. *Neuroreport* 11: 575–579, 2000.

GABEL LA AND LoTURCO JJ. Electrophysiological and morphological characterization of neurons within neocortical ectopias. *J Neurophysiol* 85: 495–505, 2001.

GALABURDA AM, SHERMAN GF, ROSEN GD, ABOITIZ F, AND GESCHWIND N. Developmental dyslexia: four consecutive cases with cortical anomalies. *Ann Neurol* 18: 222–233, 1985.

GERMANO IM AND SPERBER EF. Increased seizure susceptibility in adult rats with neuronal migrational disorders. *Brain Res* 777: 219–222, 1997.

GERMANO IM AND SPERBER EF. Transplacentally induced neuronal migration disorders: an animal model for the study of the epilepsies. *J Neurosci Res* 51: 473–488, 1998.

GERMANO IM, ZHANG YF, SPERBER EF, AND MOSHE SL. Neuronal migration disorders increase susceptibility to hyperthermia-induced seizures in developing rats. *Epilepsia* 37: 902–910, 1996.

GUERRINI R, ANDERMANN E, AVOLI M, AND DOBYNS WB. Cortical dysplasias, genetics, and epileptogenesis. *Adv Neurol* 79: 95–121, 1999.

HABLITZ JJ AND DEFAZIO T. Excitability changes in freeze-induced neocortical microgyria. *Epilep Res* 32: 75–82, 1998.

HARDIMAN O, BURKE T, PHILLIPS J, MURPHY S, O'MOORE B, STAUNTON H, AND FARRELL MA. Microdysgenesis in resected temporal neocortex: incidence and clinical significance in focal epilepsy. *Neurology* 38: 1041–1047, 1988.

HERMAN AE, GALABURDA AM, FITCH RH, CARTER AR, AND ROSEN GD. Cerebral microgyria, thalamic size, and auditory temporal processing in male and female rats. *Cereb Cortex* 7: 453–464, 1997.

HUMPHREYS P, KAUFMANN WE, AND GALABURDA AM. Developmental dyslexia in women: neuropathological findings in three patients. *Ann Neurol* 28: 727–738, 1990.

JACOBS KM, GUTNICK MJ, AND PRINCE DA. Hyperexcitability in a model of cortical maldevelopment. *Cereb Cortex* 6: 514–523, 1996.

JACOBS KM, HWANG BJ, AND PRINCE DA. Focal epileptogenesis in a rat model of polymicrogyria. *J Neurophysiol* 81: 159–173, 1999.

JENNER A, GALABURDA A, AND SHERMAN G. Neuronal size is altered in the ventrobasal complex of newborn mice with cortical ectopias. *Soc Neurosci Abstr* 22: 485, 1996.

JENNER AR, GALABURDA AM, AND SHERMAN GF. Connectivity of ectopic neurons in the molecular layer of the somatosensory cortex in autoimmune mice. *Cereb Cortex* 10: 1005–1013, 2000.

KAUFMANN WE AND GALABURDA AM. Cerebrocortical microdysgenesis in neurologically normal subjects: a histopathologic study. *Neurology* 39: 238–244, 1989.

KOHLING R, LUCKE A, STRAUB H, SPECKMANN E, TUXHORN I, WOLF P, PANNEK H, AND OPPEL F. Spontaneous sharp waves in human neocortical slices excised from epileptic patients. *Brain* 121: 1073–1087, 1998.

KUZNIECKY R, ANDERMANN F, TAMPIERI D, MELANSON D, OLIVIER A, AND LEPPIK I. Bilateral central microgyria: epilepsy, pseudobulbar palsy, and mental retardation—a recognizable neuronal migration disorder. *Ann Neurol* 25: 547–554, 1989.

KUZNIECKY RI AND BARKOVICH AJ. Pathogenesis and pathology of focal malformations of cortical development and epilepsy. *J Clin Neurophysiol* 13: 468–480, 1996.

LEE KS, SCHOTTLER F, COLLINS JL, LANZINO G, COUTURE D, RAO A, HIRAMATSU K, GOTO Y, HONG S, CANER H, YAMAMOTO H, CHEN Z, BERTRAM E, BERR S, OMARY R, SCRABLE H, JACKSON T, GOBLE J, AND EISENMAN L. A genetic animal model of human neocortical heterotopia associated with seizures. *J Neurosci* 17: 6236–6242, 1997.

LOPANTSEV V AND AVOLI M. Participation of GABA$_A$-mediated inhibition in ictallike discharges in the rat entorhinal cortex. *J Neurophysiol* 79: 352–360, 1998.

LUHMANN HJ, KARPUK N, QU M, AND ZILLES K. Characterization of neuronal migration disorders in neocortical structures. II. Intracellular in vitro recordings. *J Neurophysiol* 80: 92–102, 1998.

LUHMANN HJ AND RAABE K. Characterization of neuronal migration disorders in neocortical structures. I. Expression of epileptiform activity in an animal model. *Epilep Res* 26: 67–74, 1996.

McBRIDE MC AND KEMPER TL. Pathogenesis of four-layered microgyric cortex in man. *Acta Neuropathology (Berl)* 57: 93–98, 1982.

MEENCKE H AND VEITH G. Migration disturbances in epilepsy. *Epilep Res (Suppl)* 9: 31–40, 1992.

PALMINI A, ANDERMANN F, AICARDI J, DULAC O, CHAVES F, PONSOT G, PINARD JM, GOUTIERES F, LIVINGSTON J, TAMPIERI D, ANDERMANN E, AND ROBITAILLE Y. Diffuse cortical dysplasia, or the "double cortex" syndrome: the clinical and epileptic spectrum in 10 patients. *Neurology* 41: 1656–1662, 1991.

PRINCE DA AND JACOBS K. Inhibitory function in two models of chronic epileptogenesis. *Epilep Res* 32: 83–92, 1998.

REDECKER C, LUHMANN HJ, HAGEMANN G, FRITSCHY J-M, AND WITTE OW. Differential down-regulation of GABAA receptor subunits in widespread brain regions in the freeze-lesion model of focal cortical malformations. *J Neurosci* 20: 5045–5053, 2000.

RICCI S, CUSMAI R, FARIELLO G, FUSCO L, AND VIGEVANO F. Double cortex: a neuronal migration anomaly as a possible cause of Lennox-Gastaut syndrome. *Arch Neurol* 49: 61–64, 1992.

ROPER SN. In utero irradiation of rats as a model of human cerebrocortical dysgenesis: a review. *Epilep Res* 32: 63–74, 1998.

ROPER SN, EISENSCHENK S, AND KING MA. Reduced density of parvalbumin- and calbindin-D28-immunoreactivity neurons in experimental cortical dysplasia. *Epilep Res* 37: 63–71, 1999.

ROPER SN, GILMORE RL, AND HOUSER CR. Experimentally induced disorders of neuronal migration produced an increased propensity for electrographic seizures in rats. *Epilep Res* 21: 205–219, 1995.

ROPER SN, KING MA, ABRAHAM LA, AND BOILLOT MA. Disinhibited in vitro neocortical slices containing experimentally induced cortical dysplasia demonstrate hyperexcitability. *Epilep Res* 26: 443–449, 1997.

ROSEN GD, BURNSTEIN D, AND GALABURDA AM. Changes in efferent and afferent connectivity in rats with induced cerebrocortical microgyria. *J Comp Neurol* 418: 423–440, 2000.

ROSEN GD, GALABURDA AM, AND SHERMAN GF. Cerebrocortical microdysgenesis with anamalous callosal connections: a case study in the rat. *Int J Neurosci* 47: 237–247, 1989.

ROSEN GD, JACOBS KM, AND PRINCE DA. Effects of neonatal freeze lesions on expression of parvalbumin in rat neocortex. *Cereb Cortex* 8: 753–761, 1998.

ROSEN GD, WATERS NS, GALABURDA AM, AND DENENBERG VH. Behavioral consequences of neonatal injury of the neocortex. *Brain Res* 681: 177–189, 1995.

SARKISIAN MR, FRENKEL M, LI W, OBORSKI JA, AND LoTURCO JJ. Altered interneuron development in the cerebral cortex of the *flathead* mutant. *Cereb Cortex* 11: 734–743, 2001.

SARKISIAN MR, RATTAN S, D'MELLO SR, AND LoTURCO JJ. Characterization of seizures in the flathead (FH) rat: a new genetic model of epilepsy in early postnatal development. *Epilepsia* 40: 394–400, 1999.

SCHROTT LM, DENENBERG VH, SHERMAN GF, WATERS NS, ROSEN GD, AND GALABURDA AM. Environmental enrichment, neocortical ectopias, and behavior in the autoimmune NZB mouse. *Dev Brain Res* 67: 85–93, 1992.

SCHROTT LM, WATERS NS, BOEHM GW, SHERMAN GF, MORRISON L, ROSEN GD, BEHAN PO, GALABURDA AM, AND DENENBERG VH. Behavior, cortical ectopias, and autoimmunity in BXSB-Yaa and BXSB-Yaa+ mice. *Brain Behav Immun* 7: 205–223, 1993.

SHERMAN GF, GALABURDA AM, BEHAN PO, AND ROSEN GD. Neuroanatomical anomalies in autoimmune mice. *Acta Neuropathol (Berl)* 74: 239–242, 1987.

SHERMAN GF, GALABURDA AM, AND GESCHWIND N. Cortical anomalies in brains of New Zealand mice: a neuropathologic model of dyslexia. *Proc Natl Acad Sci USA* 82: 8072–8074, 1985.

SHERMAN GF, MORRISON L, ROSEN GD, BEHAN PO, AND GALABURDA AM. Brain abnormalities in immune defective mice. *Brain Res* 532: 25–33, 1990a.

SHERMAN GF, STONE JS, ROSEN GD, AND GALABURDA AM. Neocortical VIP neurons are increase in the hemisphere containing focal cerebrocortical microdysgenesis in New Zealand Black mice. *Brain Res* 532: 232–236, 1990b.

SHERMAN GF, STONE LV, ROSEN GD, AND GALABURDA AM. The development of neuronal ectopias in fetal New Zealand Black mice. *Soc Neurosci Abstr* 17: 765, 1991.

SPENCER DG, HUMPHRIES K, MATHIS D, AND LAL H. Behavioral impairments related to cognitive dysfunction in the autoimmune New Zealand black mice. *Behav Neurosci* 100: 353–358, 1986.

VELAZQUEZ JLP AND CARLEN PL. Synchronization of GABAergic interneuronal networks during seizure-like activity int he rat horizontal hippocampal slice. *Eur J Neurosci* 11: 4110–4118, 1999.

ZHU WJ AND ROPER SN. Reduced inhibition in an animal model of cortical dysplasia. *J Neurosci* 20: 8925–8931, 2000.