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## GABA<sub>A</sub> Receptor Properties in Catastrophic Infantile Epilepsy

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### Abstract

Catastrophic epilepsy due to cortical dysplasia is often intractable to anticonvulsant treatment. Many of the medications used unsuccessfully in treating this disorder are thought to exert at least a portion of their action through enhancement of inhibitory GABA<sub>A</sub> neurotransmission. In the present study, GABA<sub>A</sub> receptor properties in resected brain tissue from four infants with infantile spasms and intractable epilepsy due to cortical dysplasia were measured to determine if this clinical resistance to pharmacologic treatment correlates with alterations in receptor function. Results from epileptic cortex were compared with those from autopsy control samples. To perform these studies, we utilized the technique of injection of brain cellular membrane preparations into the *Xenopus* oocyte, which results in the incorporation of human GABA<sub>A</sub> receptors in their native configuration into the oocyte plasma membrane. Two-electrode voltage clamp electrophysiology analysis was then performed to assess GABA<sub>A</sub> receptor pharmacologic properties. The intrinsic properties of affinity, reversal potential, current decay, and current rundown were unchanged in the epileptic infants. Current enhancement by benzodiazepines was also unaltered, as was the response to barbiturates. However, a significant decrease was found in the degree of GABA<sub>A</sub> current enhancement by neurosteroids in the epileptic infants, along with an increase in current inhibition by zinc. These findings may contribute to the mechanisms of intractability in catastrophic infantile epilepsy due to cortical dysplasia, and suggest alternative therapeutic approaches.

### 1. Introduction

The catastrophic epilepsies of infancy, including infantile spasms, are frequently refractory to pharmacologic treatment and have devastating developmental outcomes (Glauser, 2004, Mackay et al., 2004, Riikonen, 2005). One of the potential underlying etiologies of infantile spasms is cortical dysplasia, particularly if it is widespread (D'Agostino et al., 2004). While surgical resection of dysplastic brain may result in freedom from seizures, this is often at the

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expense of neurologic deficits such as hemiplegia and visual field loss (Wheless, 2004, Gonzalez-Martinez et al., 2005, Lettori et al., 2007).

Abnormalities in inhibitory GABA<sub>A</sub> receptor mediated signaling have been implicated as contributing to the epileptogenicity of cortical dysplasia (Crino et al., 2002, Najm et al., 2004, Calcagnotto et al., 2005). Several of the medications used in the attempted treatment of catastrophic infantile epilepsy, such as barbiturates, benzodiazepines, topiramate, vigabatrin, and ACTH, may exert their actions at least partially through enhancement of GABAergic transmission (Treiman, 2001, Rogawski and Reddy, 2002, Conry, 2004). Investigation of potential alterations in GABA<sub>A</sub> receptor function and causes of anticonvulsant failure in epilepsy due to cortical dysplasia has been limited by a lack of animal models that faithfully replicate the disorder and the inherent difficulties of working with human tissue (Cepeda et al., 2006, Najm et al., 2007). As a result, detailed pharmacologic analysis of GABA<sub>A</sub> receptors from human cortical dysplasia tissue has not previously been reported.

In the present study, we examine the intrinsic physiologic properties and responses to pharmacologic agents of GABA<sub>A</sub> receptors from four infants with infantile spasms and refractory epilepsy who underwent surgical resection of areas of cortical dysplasia. Results from the epileptic infants were compared with those from age matched autopsy control brain samples. To do this, we utilized the technique of injection of brain cellular membrane preparations into the *Xenopus* oocyte, which results in the “microtransplantation” of human GABA<sub>A</sub> receptors in their native configuration into the oocyte plasma membrane (Miledi et al., 2002). The appearance of receptor currents utilizing this method does not depend on mRNA translation, but instead arises from incorporation into the oocyte membrane of the injected membrane vesicles harboring native receptors (Marsal et al., 1995). This allows analysis by standard two-electrode voltage clamp electrophysiology. Receptor properties measured after membrane injection into *Xenopus* oocytes are similar to those measured *in situ* in neurons from brain slices (Palma et al., 2004, Ragazzino et al., 2005) or in stably-expressing cell lines (Palma et al., 2003). Through the use of this innovative technique, this study reveals previously undescribed abnormalities in GABA<sub>A</sub> receptor function in infants with catastrophic epilepsy due to cortical dysplasia, which may contribute to the pathogenesis of this disorder and its resistance to anticonvulsant treatment.

## 2. Methods

### 2.1 Pediatric cortical brain specimens

Infants with intractable epilepsy were evaluated and underwent brain surgery at Children's Hospital and Regional Medical Center in Seattle, WA. Informed consent for the use of a portion of the resected tissue for research purposes was obtained under the guidance of the hospital's Institutional Review Board. After excision, the fresh tissue was frozen in liquid nitrogen and stored at -80°C. Control frozen autaptic brain tissue was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, Maryland.

### 2.2 Membrane isolation

Membrane isolation was performed using the method of (Miledi et al., 2002), with modifications, as follows. Frozen cortical brain tissue from each specimen (50 to 300 mg) was weighed and homogenized in 1 mL of glycine buffer containing (in mM) glycine 200, NaCl 150, EGTA 50, EDTA 50, and sucrose 300 (pH 9), plus 10 µL of protease inhibitor cocktail (Sigma P2714). The samples were centrifuged at 9,500 × g, 4°C, for 15 minutes. The supernatant was then centrifuged at 100,000 × g, 4°C, for 2 hours. The resulting membrane pellet was resuspended in 5 mM glycine, using 100 µL per 100 mg original tissue weight. The membrane samples were frozen and stored at -80°C until use.

## 2.3 Oocyte preparation and injection

Female *Xenopus laevis* were euthanized in a bath of 0.2% tricaine methane sulfonate (MS-222). The ovaries were surgically isolated and dissociated for approximately 1 hour at 18°C by 2 mg/mL collagenase A in a solution containing (in mM) NaCl 96, KCl 2, MgCl<sub>2</sub> 1, and Hepes 5 (pH 7.4). Stage V-VI oocytes were selected and stored at 18°C in the above solution with the addition of 1.8 mM CaCl<sub>2</sub> and 50 µg/mL gentamycin. Oocytes were injected with 50-100 nL of membrane in 5 mM glycine and incubated at 18°C for 1-3 days before use.

## 2.4 Electrophysiology

Injected oocytes were placed in a recording chamber and bathed in oocyte Ringer's solution containing (in mM) NaCl 82.5, KCl 2.5, CaCl<sub>2</sub> 2.5, MgCl<sub>2</sub> 1, and Hepes 5 (pH 7.4). Two-electrode voltage-clamp recordings were made at room temperature using a GeneClamp 500B amplifier, Digidata 1322A data acquisition system, and pCLAMP9 data analysis software (Axon Instruments, Molecular Devices Corporation, Sunnyvale, CA). Electrodes with a resistance of 0.5 - 2.5 MΩ were fashioned from borosilicate capillary glass and were filled with 3 mM KCl. Oocyte membrane potential was held at -80 mV unless otherwise stated. Solutions were applied using a gravity-driven, valve-controlled perfusion system.

## 2.5 Statistical analysis

The significance of any detected difference between oocytes incorporating control or epileptic receptors was determined through the use of a two-tailed, unpaired t-test, or one way ANOVA with posttests, at a significance level of 0.05 (InStat, GraphPad Software, Inc., San Diego, CA). ANOVA was used whenever multiple comparisons were made. Curve fitting and comparison of best-fit parameters was done using GraphPad Prism software.

# 3. Results

## 3.1 Patient characteristics

Cortical tissue from four epileptic infants was analyzed, with clinical characteristics as summarized in Table 1. All epileptic infants had onset of catastrophic epilepsy within the first weeks of life which progressed to infantile spasms refractory to ACTH and multiple anticonvulsants. All were found to have unilateral abnormalities on EEG and brain MRI, and underwent surgical resection of the epileptogenic regions at less than a year of age (average age at surgery 5.2 months, range 3-7 months). On pathologic analysis, all were found to have extensive areas of focal cortical dysplasia type 2A (dyslaminar, columnar disorganization, immature and dysmorphic neurons, but without balloon cells) (Palmini et al., 2004). Two of the four infants returned to the operating room for completion of functional hemispherectomy due to persistent seizures. All were seizure free at the time of last follow up (14-27 months post surgery).

Frozen postmortem control cortical tissue was analyzed from five infants 2 to 10 months of age (average 6.2 months), with causes of death of sudden infant death syndrome (SIDS) or spinal muscular atrophy type 1, which are not expected to involve significant cortical pathology. Postmortem interval averaged 3.6 hours (range 1-6 hours).

## 3.2 Intrinsic GABA<sub>A</sub> Receptor Properties

After control and epileptic membrane injection into *Xenopus* oocytes, robust GABA-mediated currents were elicited that were not present in uninjected oocytes. These currents were completely blocked by bicuculline and picrotoxin, confirming their mediation by GABA<sub>A</sub> receptors (data not shown). The GABA reversal potential was calculated for each injected oocyte, and was not significantly different between control and epileptic samples at  $-22.2 \pm$

5.3 mV and  $-25.9 \pm 2.5$  mV, respectively. These values are similar to those obtained after expression of GABA<sub>A</sub> receptors in oocytes following injection of brain mRNA (Miledi et al., 1982, Parker et al., 1986) and are equivalent to the chloride reversal potential (Barish, 1983).

Dose-response curves were generated for GABA concentrations between 0.1 and 1000  $\mu$ M in oocytes expressing control and epileptic receptors (Figure 1A, B). The half-maximal effective GABA concentration (EC<sub>50</sub>) was the same in the two groups (Control = 45.6  $\mu$ M, 95% CI = 36.6 - 56.8  $\mu$ M); Epileptic = 38.3  $\mu$ M, 95% CI = 29.8 - 49.2  $\mu$ M;  $p > 0.05$ ), as was the Hill Slope ( $1.5 \pm 0.2$  vs.  $1.6 \pm 0.3$ ;  $p > 0.05$ ). These values are similar to that obtained from dissociated human temporal cortical neurons (25  $\mu$ M) (Gibbs et al., 1996, Gibbs et al., 1998), but substantially lower than that reported from adult temporal cortex using the membrane injection technique (100-120  $\mu$ M) (Palma et al., 2005, Ragozzino et al., 2005). The reasons for these discrepancies are unknown.

At higher GABA concentrations, the current exhibited a significant amount of decay during the 10 second application, the rate of which was again not significantly different between the two groups (Figure 2A, B). Repeated application of a high concentration of GABA results in a decline in current amplitude, described as current rundown, which recovers after prolonged washout (Huang and Dillon, 1998, Ragozzino et al., 2005). The degree of rundown elicited by 1 mM GABA applied every 40 seconds was also not different between the control and epileptic specimens (Figure 2C).

In summary, analysis of several intrinsic physiologic properties of GABA<sub>A</sub> receptors revealed no significant differences between control infants and those with catastrophic epilepsy due to cortical dysplasia.

### 3.3 Pharmacologic GABA<sub>A</sub> receptor properties

Next, the modulation of GABA<sub>A</sub> receptor currents by endogenous and exogenous agents, including antiepileptic medications, was investigated. Three of the four infants had been treated unsuccessfully with the barbiturate phenobarbital. Barbiturates can either potentiate GABA responses, directly activate GABA<sub>A</sub> receptors, or block the GABA chloride channel, depending on concentration and receptor subunit composition (Thompson et al., 1996). In this study, pentobarbital produced a sizeable enhancement of the current elicited by 10  $\mu$ M GABA, which was not different between oocytes incorporating GABA<sub>A</sub> receptors from control or epileptic specimens (Figure 3A, B). There was no significant current elicited by 100  $\mu$ M pentobarbital in the absence of GABA in any specimen tested (Figure 3B), consistent with previous studies demonstrating that its direct agonist effect is primarily seen with receptors expressing the  $\alpha_6$  subunit (Drafts and Fisher, 2006), which are found exclusively in the cerebellum (Fritschy and Brunig, 2003).

Benzodiazepines, which exert anticonvulsant effects through enhancement of GABA current by binding to a site formed by the  $\alpha$  and  $\gamma$  subunits (Barnard et al., 1998, Jones-Davis and Macdonald, 2003, Rudolph and Mohler, 2004), were used chronically in the treatment of two of the four epileptic infants. The effects of zolpidem, which acts preferentially on  $\alpha_1$  containing receptors, and diazepam, which acts equally at receptors containing  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$  subunits, were tested. The degree of enhancement of GABA current by either zolpidem or diazepam was not significantly different between the control and epileptic samples (Figure 3C, D).

The antiepileptic drug topiramate, which has variably been reported to increase GABA<sub>A</sub> currents (Gordey et al., 2000, White et al., 2000, Simeone et al., 2006), was used clinically in two of the four patients. At concentrations of up to 30  $\mu$ M, which includes the expected therapeutic serum drug level (Simeone et al., 2006), topiramate had no significant effect on currents elicited by 10  $\mu$ M GABA (data not shown).

ACTH injections are often effective in the treatment of infantile spasms, which was unfortunately not the case in any of these infants. One mechanism of action proposed for the suppression of spasms by ACTH is through decreasing corticotrophin releasing hormone (CRH) levels (Brunson et al., 2001); however, it also increases levels of endogenous neurosteroids, which are particularly potent enhancers of GABA<sub>A</sub> receptor function (Torres et al., 2001, Belelli et al., 2002, Rogawski and Reddy, 2002). In addition, a synthetic neurosteroid, ganaxolone, has been found to be effective in treatment-resistant infantile spasms (Kerrigan et al., 2000). Enhancement of the current elicited by 10  $\mu$ M GABA by the neurosteroid 5 $\alpha$ -pregnan-3 $\alpha$ -ol-20-one (5 $\alpha$ 3 $\alpha$ ) was found to be present, but reduced, in this group of patients with infantile spasms as compared with controls (E(max) Control =  $546.2 \pm 36.0\%$ ; Epileptic =  $427.8 \pm 17.6\%$ ,  $p < 0.05$ ; Figure 4A). This occurred without a change in the EC50 value (313.8 nM, 95% CI 196.6 - 500.8 nM, vs. 254.7 nM, 95% CI 187.9 - 345.3 nM;  $p > 0.05$ .) or Hill Slope ( $1.2 \pm 0.2$  vs.  $1.4 \pm 0.2$ ,  $p > 0.05$ ). Of note, higher concentrations of the neurosteroid induced a significant amount of GABA current decay (Figure 4B), the degree of which was not different between the control and epileptic samples (5 second decay with 3  $\mu$ M 5 $\alpha$ 3 $\alpha$ :  $28.3 \pm 3.9\%$  vs.  $29.4 \pm 6.1\%$ ,  $p > 0.05$ ). No current was elicited by 3  $\mu$ M 5 $\alpha$ -pregnan-3 $\alpha$ -ol-20-one in the absence of GABA (data not shown).

Zinc is another endogenous modulator of GABA<sub>A</sub> receptors, which interacts directly with the ion channel to inhibit chloride entry (Smart et al., 2004). The antiepileptic medication levetiracetam, used in two of the four infants, has been reported to reverse this zinc-mediated inhibition (Rigo et al., 2002). Increased sensitivity of hippocampal GABA currents to zinc has been reported in several models of temporal lobe epilepsy (Buhl et al., 1996, Gibbs et al., 1997, Brooks-Kayal et al., 1998, Shumate et al., 1998, Cohen et al., 2003). Similarly, in cortical specimens from epileptic infants, the degree of reduction in GABA current by zinc was significantly increased as compared with controls (Figure 5A, B).

In summary, analysis of the functional GABA<sub>A</sub> receptor properties of four infants with infantile spasms and intractable epilepsy due to cortical dysplasia revealed unchanged intrinsic physiologic properties, but altered pharmacology, including a decreased degree of current enhancement by neurosteroids and an increased degree of current inhibition by zinc.

## 4. Discussion

### 4.1 GABA<sub>A</sub> receptor functional studies

In this study we characterized cortical GABA<sub>A</sub> receptor properties in four infants with catastrophic epilepsy including infantile spasms due to widespread areas of cortical dysplasia. These findings demonstrate that the intrinsic physiologic receptor properties of GABA affinity, reversal potential, current decay, and rundown are unchanged between control and epileptic samples. In addition, responses to anticonvulsant barbiturates and benzodiazepines were similar. Conversely, the responses of GABA<sub>A</sub> receptors from the epileptic infants to modulation by neurosteroids and zinc were significantly altered.

ACTH is effective in the short-term treatment of infantile spasms in 42-93% of patients, although with a fairly high relapse rate after completion of treatment and serious side effects (Mackay et al., 2004). One of the possible mechanisms of action of ACTH is via increases in endogenous neurosteroids, which are known to be very potent enhancers of GABA<sub>A</sub> receptor currents (Torres et al., 2001, Belelli et al., 2002, Rogawski and Reddy, 2002). GABA<sub>A</sub> current augmentation by the neurosteroid 5 $\alpha$ -pregnan-3 $\alpha$ -ol-20-one was decreased in these epileptic infants, suggesting that a portion of their resistance to ACTH treatment may be due to this deficit. This also suggests that the synthetic neurosteroid ganaxolone may be less effective in the treatment of infants with infantile spasms due to cortical dysplasia as opposed to other pathologies.

Another significant difference between the infant control and epileptic specimens in this study was an increased sensitivity to GABA<sub>A</sub> current inhibition by zinc. A reversal of this inhibition has been reported as a mechanism of action of the anticonvulsant levetiracetam (Rigo et al., 2002). Based on these results, other agents targeting the interaction between zinc and GABA<sub>A</sub> receptors may be beneficial in the treatment of epilepsy due to cortical dysplasia.

The observed differences in GABA<sub>A</sub> receptor modulation by neurosteroids and zinc in the epileptic infants did occur at fairly high concentrations that are reasonably unlikely to be relevant under normal physiological conditions. However, they may come into play in pathologic states such as during repetitive seizures, exogenous administration of synthetic neurosteroids, or stimulation of endogenous neurosteroid synthesis with ACTH. These situations have been found to increase zinc or neurosteroid levels into the ranges found to differentially affect the epileptic infants in this study (Assaf and Chung, 1984, Kerrigan et al., 2000, Torres et al., 2001).

Unfortunately, the findings of this study do not completely explain the refractoriness to treatment of these infants. Three of the four infants were being treated unsuccessfully with the barbiturate phenobarbital at the time of surgery, but did not exhibit reduced GABA<sub>A</sub> current enhancement by barbiturates on direct testing. Somewhat unexpected was the intact receptor responsiveness to benzodiazepines in the epileptic infants, particularly in the two infants with innumerable daily seizures despite scheduled benzodiazepine dosing (one on a continuous midazolam infusion the days prior to surgery). This is in contrast to studies of human and experimental temporal lobe epilepsy, where decreased sensitivity to benzodiazepines, in particular the  $\alpha_1$ -subunit preferring agent zolpidem, has frequently been seen (Buhl et al., 1996, Gibbs et al., 1997, Brooks-Kayal et al., 1998, Shumate et al., 1998, Cohen et al., 2003). The findings of the present study indicate that the failure of barbiturates and benzodiazepines to control seizures in these infants with catastrophic epilepsy due to cortical dysplasia was not due to an inherent inability of their GABA<sub>A</sub> receptors to respond to these agents.

Previous studies using recombinant GABA<sub>A</sub> receptors have demonstrated variable effects of topiramate. Depending on subunit composition, an enhancement or inhibition of current was reported, although generally only at supratherapeutic concentrations (Gordey et al., 2000, White et al., 2000, Simeone et al., 2006). In the control and epileptic infants tested in this study, topiramate at a concentration within the range of the usual therapeutic level had no effect on GABA currents, suggesting that direct enhancement of GABA<sub>A</sub> current is unlikely to be a significant mechanism of action for this anticonvulsant.

#### 4.2 Correlation with previous studies on GABA<sub>A</sub> receptors in human cortical dysplasia

As discussed above, a detailed pharmacologic analysis of GABA<sub>A</sub> receptors from human cortical dysplasia tissue has not previously been reported. Using a single-cell mRNA amplification technique, dysplastic neurons dissected from adult cortical dysplasia specimens were shown to have decreased GABA<sub>A</sub>  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ , and  $\beta_2$  subunit mRNA expression when compared to postmortem control pyramidal neurons, with no change in  $\alpha_3$ ,  $\alpha_4$ ,  $\alpha_5$  or  $\beta_3$  levels (Crino et al., 2001). However, it is difficult to extrapolate these results to predict associated pharmacologic alterations. A recent paper investigated GABA receptor-mediated synaptic function in resected brain slices from pediatric cortical dysplasia patients, identifying a predominance of GABAergic over glutamatergic synaptic activity, spontaneous GABA-mediated depolarizations, and a depolarized GABA reversal potential, all thought to represent features of developmentally immature cortex (Cepeda et al., 2007). Interestingly, increased sensitivity to GABA<sub>A</sub> current inhibition by zinc and decreased sensitivity to current enhancement by neurosteroids, as observed in the present study, are features of immature rat hippocampal dentate granule cells (Kapur and Macdonald, 1999, Brooks-Kayal et al., 2001, Mtchedlishvili et al., 2003). This suggests that these observed pharmacologic properties may

be consistent with a developmental immaturity of the dysplastic tissue. However, immature dentate granule cells are also reported to be less sensitive to diazepam and zolpidem (Kapur and Macdonald, 1999, Brooks-Kayal et al., 2001), which was not the case in the cortical dysplasia samples.

Cellular chloride homeostasis, modulated by the NKCC1 and KCC2 cotransporters, also determines neuronal responses to GABA<sub>A</sub> receptor activation. In immature neurons, high levels of NKCC1 expression and low levels of KCC2 expression result in relatively high intracellular chloride concentrations, and a corresponding depolarizing, excitatory response to GABA during development (Dzhala et al., 2005, Ben-Ari, 2006). Increased NKCC1 and decreased KCC2 levels have been reported in an immunohistochemical study of focal cortical dysplasia (Aronica et al., 2007), as has a depolarizing shift in GABA reversal potential in a subset of neurons in resected dysplastic tissue (Cepeda et al., 2007). In contrast, in the present study, no significant change in GABA reversal potential was found in the epileptic infants. One possible explanation for this disparity is that the *Xenopus* oocyte expresses its own endogenous set of chloride transporters (Mercado et al., 2001), so that a comparably small difference in levels of “transplanted” transporters is insufficient to substantially change intracellular chloride levels and thus affect GABA reversal potential.

### 4.3 Significance of findings

It is important to emphasize that the observed alterations in GABA<sub>A</sub> receptor function in this study may be primary, contributing to epileptogenesis and resistance to pharmacotherapy, or secondary, as a consequence of repeated seizures or antiepileptic medication exposure. Indeed, GABA<sub>A</sub> properties have been shown to change after episodes of status epilepticus or exposure to benzodiazepines, barbiturates, or steroids (Kapur and Macdonald, 1997, Jones-Davis and Macdonald, 2003, Leroy et al., 2004, Zhang et al., 2004, Raol et al., 2005, Shen et al., 2005, Maguire and Mody, 2007).

Although the sample size in the present study is small, these four patients were chosen due to their relative homogeneity in terms of age (less than 1 year), seizure type (infantile spasms), pathology (FCD 2A), and drug exposure (ACTH). The ability of this study to detect significant alterations in receptor properties in the epileptic patients is likely attributable to this homogeneity. Further studies are underway on a larger population of patients in an attempt to correlate clinical factors with observed GABA<sub>A</sub> receptor functional properties.

### 4.4 Discussion of the methods used in this study

The technique of examination of human receptors incorporated in the *Xenopus* oocyte after injection of cellular membranes has several advantages over other methods previously used to study GABA<sub>A</sub> receptor function in epileptic human tissue. Techniques which study acutely dissociated neurons or brain slices require fresh tissue, which degrades rapidly, limiting the number of experiments which can be performed. In contrast, with the oocyte membrane injection technique, fresh or recently frozen tissue can be used, as well as autopsy tissue or tissue maintained frozen for many years. Experiments which have looked at GABA<sub>A</sub> receptor subunit mRNA expression or immunohistochemistry are not able to provide information on the functional status of the detected subunits, which is easily analyzed using this technique. Finally, experiments which isolate mRNA from human tissue and then express it in *Xenopus* oocytes for analysis depend on the translational, post-translational, protein trafficking, and assembly machinery of the oocyte, which likely does not reproduce that of the host tissue. With the technique of membrane injection, the receptors are incorporated into the oocyte plasma membrane in their native configuration, and their functional properties therefore likely better reflect those of the receptors *in situ*. Furthermore, RNA is much more susceptible to degradation than are membrane-embedded proteins.

As with all human studies, in the present study ideal control brain tissue could not be obtained, as healthy children do not generally undergo brain surgery. In previous electrophysiology studies of human cortical dysplasia, histologically “normal” brain tissue from the “least epileptogenic area” was used as a control (Mathern et al., 2000, Cepeda et al., 2003). However, there are problems with this approach, as the tissue is still from a brain that experienced repetitive seizures and which was exposed to antiepileptic medications, and therefore does not serve well as a “control”. Because of the techniques used in the present study, autopsy control cortical tissue was able to be used, allowing examination of age-matched tissues from infants without known central nervous system disease. Of course, this does introduce possible complicating factors of perimortem and postmortem events which could themselves alter receptor function. We have tried to limit this by using tissue with the shortest available postmortem periods before freezing (< 6 hrs).

One drawback to this technique is its inability to adequately determine GABA efficacy; i.e. the maximal GABA<sub>A</sub> current evoked by GABA application. This is due to the large variability which has been observed to occur in the amplitude of the currents measured (> 10 fold), even when the same membrane preparation is used to inject oocytes from the same frog on the same day. The amplitude of the GABA current does not directly depend on the volume or protein concentration of the membrane preparation injected (Miledi et al., 2002). As a result, these currents do not accurately reflect amplitudes within the source tissue. In contrast, relative responses to experimental perturbations can be reliably determined by normalizing current amplitudes to the current obtained under specified conditions for each oocyte.

A final inherent limitation of this technique is that it does not allow discrimination of GABA<sub>A</sub> receptor properties based on cell type, e.g. neuronal versus glial, pyramidal cell versus interneuron, or immature versus dysmorphic neuron. However, the actions of the antiepileptic drugs given to patients with epilepsy also do not discriminate in these ways. Therefore, these experiments function to provide global information about cortical GABA<sub>A</sub> receptor function in pediatric epilepsy, rather than cell-specific or synapse-specific information. As such, alterations observed in GABA<sub>A</sub> receptor pharmacology in children with intractable epilepsy using this technique can provide guidance for further studies or targeted development of new therapies.

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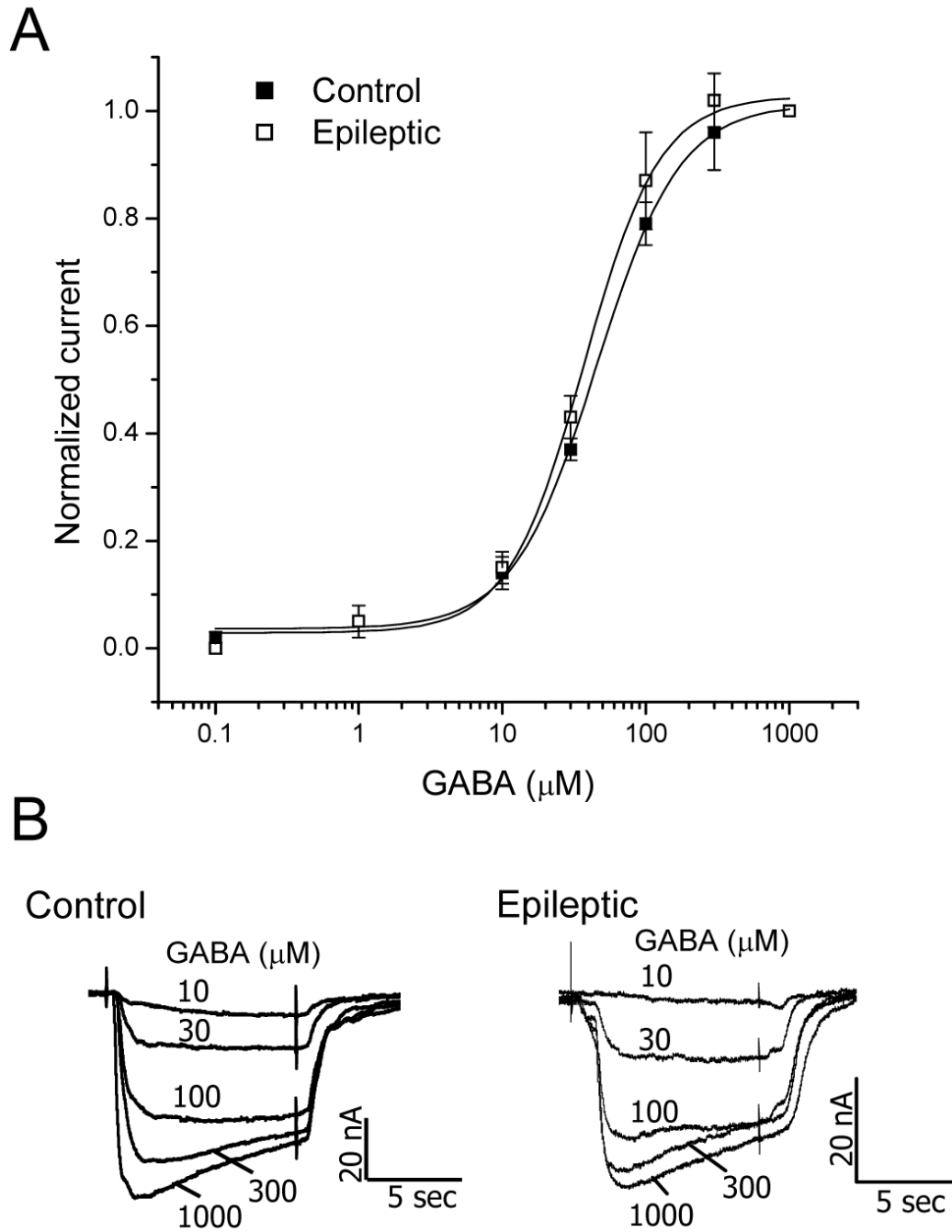


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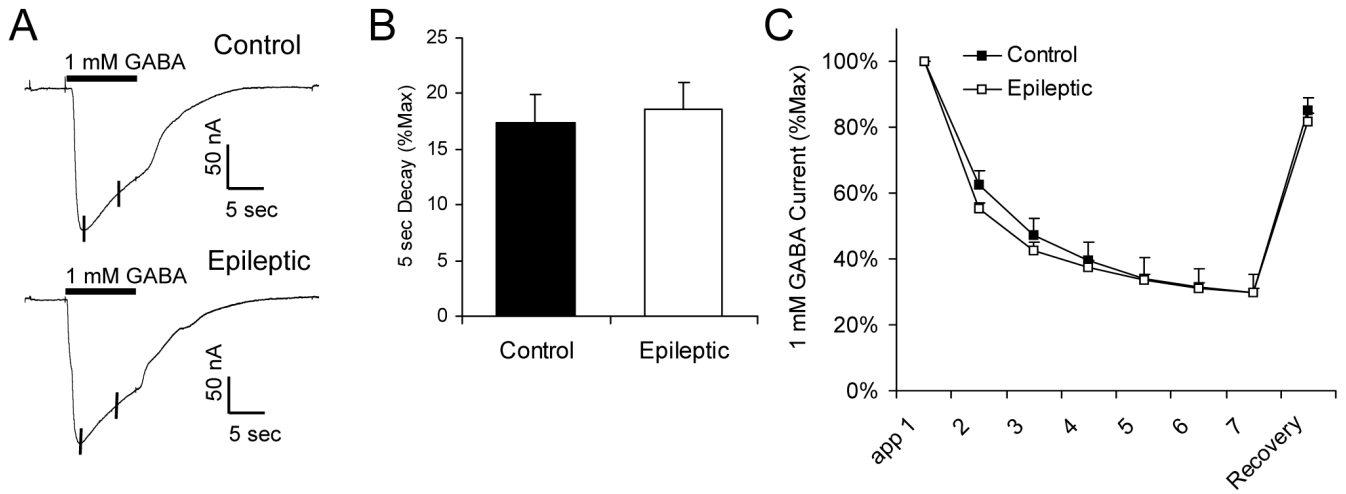
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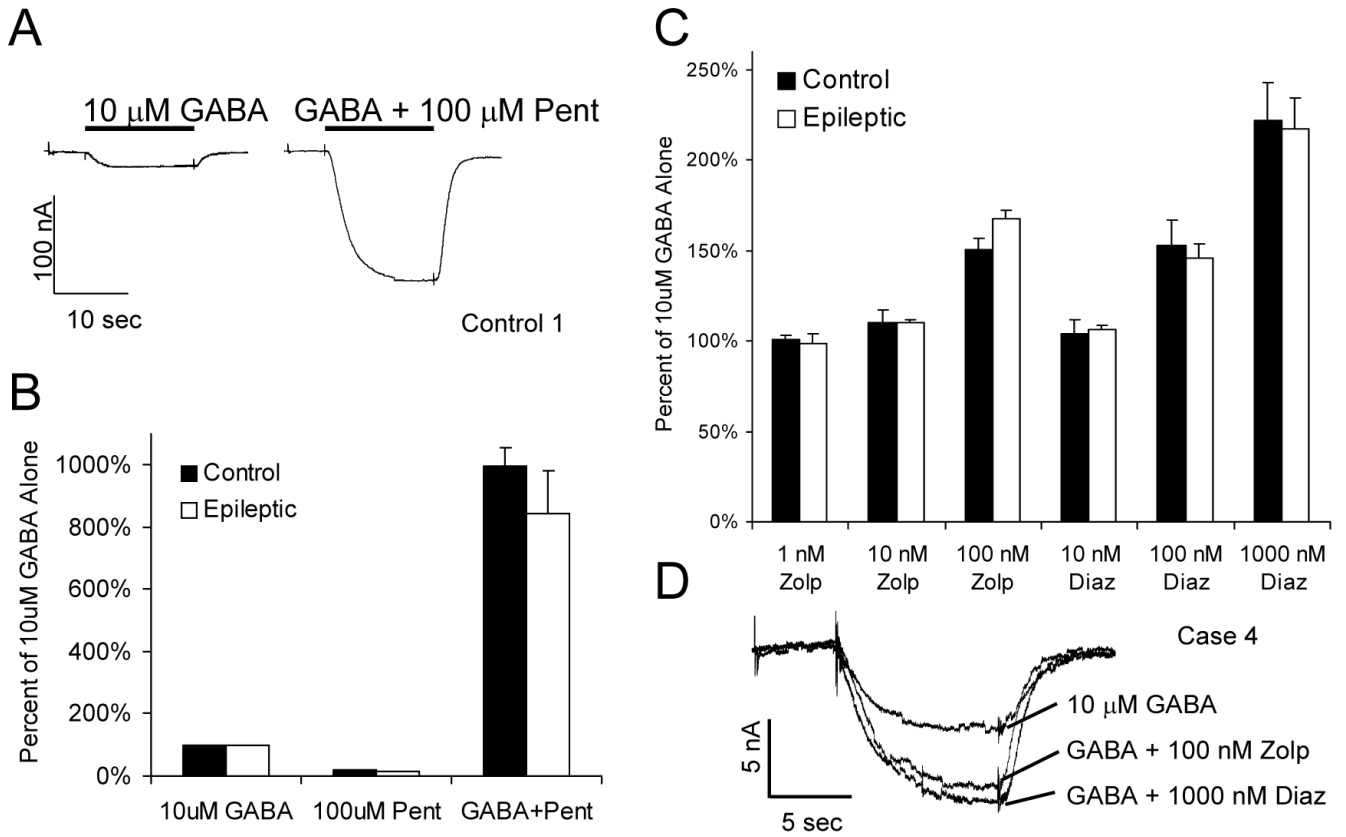
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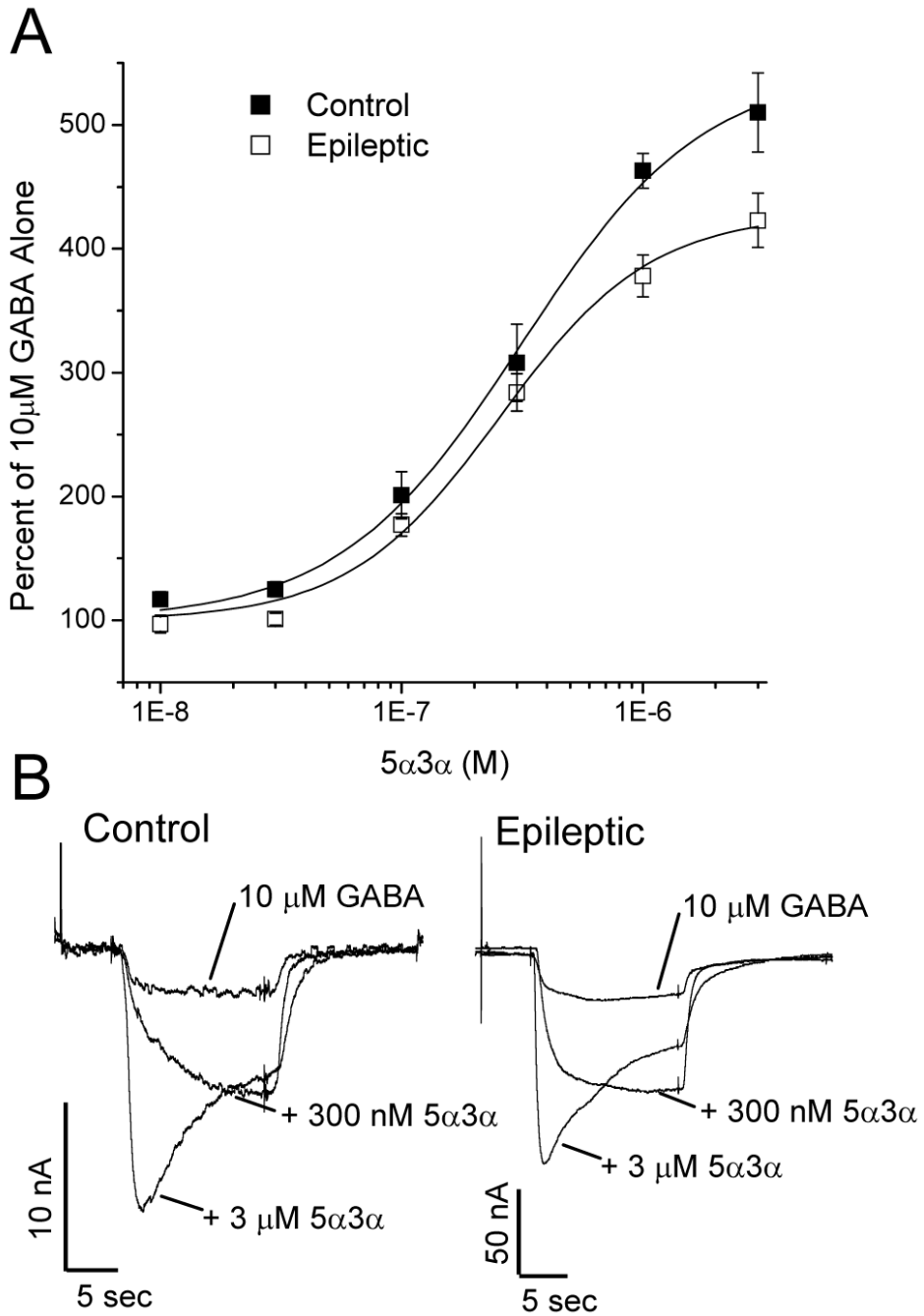
**Figure 1.** GABA affinity is unchanged in oocytes incorporating GABA<sub>A</sub> receptors from infants with catastrophic epilepsy due to cortical dysplasia. *A*, GABA<sub>A</sub> receptor current produced by GABA concentrations from 0.1 to 1000  $\mu\text{M}$ . Each point represents the average current  $\pm$  SEM normalized to that produced by 1000  $\mu\text{M}$  GABA, obtained from five control and four epileptic patients (at least 4 injected oocytes analyzed per patient). *B*, Representative traces of current elicited by increasing concentrations of GABA from oocytes incorporating receptors from a Control (Control 5) and an Epileptic (Case 1) specimen.



**Figure 2.** GABA<sub>A</sub> current decay and rundown are unchanged in oocytes incorporating receptors from epileptic infants. *A*, Sample traces from oocytes incorporating receptors from a Control (Control 4) and an Epileptic (Case 2) specimen showing current decay during a 10 second application of 1 mM GABA. The amount of decay was measured over the 5 seconds following attainment of the peak current, as shown by the vertical lines. *B*, Average current decay over 5 seconds, shown as the percent of the peak current (%Max) ± SEM. Data for each point was obtained from five control and four epileptic patients (at least 3 injected oocytes analyzed per patient). *C*, Rundown of GABA<sub>A</sub> current during repeated 10 second applications of 1 mM GABA every 40 seconds, followed by recovery of current after 5 minute wash-out. Each point represents the average current ± SEM normalized to that produced by the first GABA application, obtained from five control and four epileptic patients (at least 5 injected oocytes analyzed per patient).

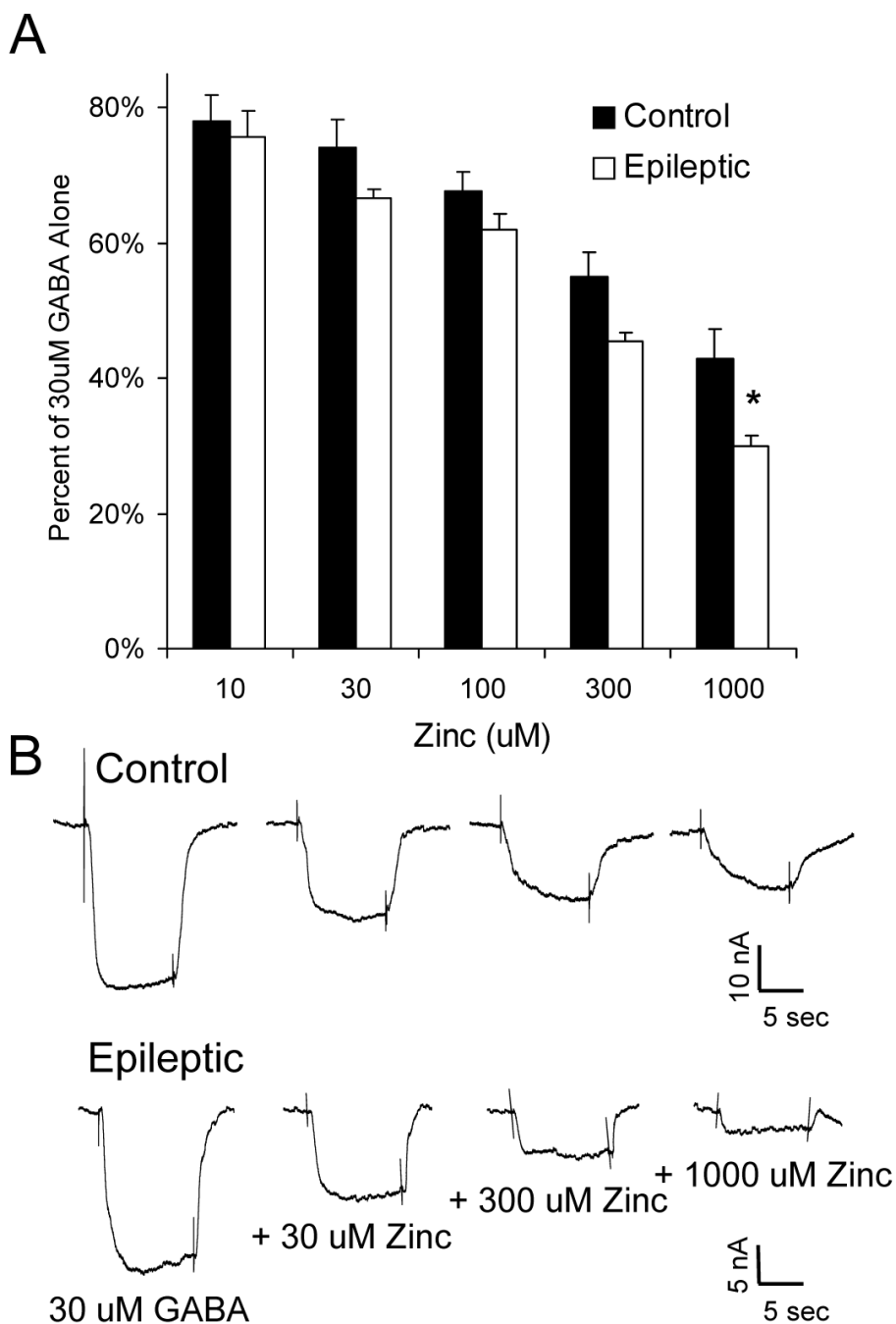


**Figure 3.** GABA<sub>A</sub> current enhancement by barbiturates and benzodiazepines. *A*, Representative traces from an oocyte incorporating receptors from Control 1 showing current generated by 10  $\mu$ M GABA in the absence and presence of 100  $\mu$ M pentobarbital (Pent). *B*, Enhancement of current by pentobarbital. Average current  $\pm$  SEM produced by 10  $\mu$ M GABA, 100  $\mu$ M pentobarbital alone, and 10  $\mu$ M GABA + 100  $\mu$ M pentobarbital, shown as the percent of the current produced by 10  $\mu$ M GABA alone. Data was obtained from five control and four epileptic patients (at least 5 injected oocytes analyzed per patient). *C*, Enhancement of current by the benzodiazepines zolpidem (Zolp) and diazepam (Diaz). Average current  $\pm$  SEM produced by 10  $\mu$ M GABA plus the indicated benzodiazepine concentrations, shown as the percent of the current produced by 10  $\mu$ M GABA alone. Data was obtained from five control and four epileptic patients (at least 5 injected oocytes analyzed per patient). *D*, Sample traces from an oocyte incorporating receptors from Case 4, demonstrating the increase in GABA current by zolpidem and diazepam.



**Figure 4.** GABA<sub>A</sub> current enhancement by neurosteroids is reduced in oocytes incorporating receptors from infants with catastrophic epilepsy. *A*, Current produced by 10 μM GABA in the presence of 5α-pregnan-3α-ol-20-one (5α3α) at concentrations from 10 nM to 3 μM. Each point represents the average current ± SEM normalized to that produced by 10 μM GABA alone, obtained from five control and four epileptic patients (at least 4 injected oocytes analyzed per patient). *B*, Sample traces from oocytes incorporating receptors from a Control (Control 3) and an Epileptic (Case 2) specimen, demonstrating the increase in GABA current by 5α3α.





**Figure 5.** GABA<sub>A</sub> current inhibition by zinc is increased in oocytes expressing receptors from infants with catastrophic epilepsy. **A**, Current produced by 30  $\mu$ M GABA in the presence of ZnCl<sub>2</sub> (Zinc) at concentrations from 10 to 1000  $\mu$ M. Each point represents the average current  $\pm$  SEM normalized to that produced by 30  $\mu$ M GABA alone, obtained from five control and four epileptic patients (at least 5 injected oocytes analyzed per patient). \*  $p < 0.05$  as compared with corresponding control. **B**, Sample traces from oocytes incorporating receptors from a Control (Control 2) and an Epileptic (Case 3), demonstrating the inhibition of GABA current by ZnCl<sub>2</sub>.

Table 1

## Clinical data

Case	Sex	Seizure Onset	Seizure Types	Seizure Frequency	Age at surgery	AED at Surgery	Previous AED	Tissue Studied
1	F	2 mo	CP, IS	25-50/day	7 mo	PB, ZON	ACTH, CBZ, TPM	L. Lateral Temporal
2	M	3 days	CP, IS, M, T	75-100/day	3 mo	PB, ZON, LEV, MDZ	ACTH, PYR, CLZ	R. Posterior Temporal
3	F	4 mo	IS	3/day	8 mo	LTG, ZON, LEV	ACTH	L. Parietal
4	M	3 wk	CP, IS	1-4/day	3 mo	ACTH, ZON, CLZ, PB	TPM, PYR	L. Lateral Temporal

Control	Sex	Age	Cause of Death	Postmortem Interval	Tissue Studied
1	F	6 mo	SIDS	1 hr	Occipital cortex
2	M	5 mo	SMA I	3hr	Parietal cortex
3	F	6 mo	SIDS	6hr	Parietal cortex
4	M	10 mo	SMA I	3hr	Occipital cortex
5	F	2 mo	SIDS	5hr	Temporal cortex

Abbreviations: AED, Antiepileptic drugs; PB, Phenobarbital; ZON, Zonisamide; LEV, Levetiracetam; MDZ, Midazolam; LTG, Lamotrigine; ACTH, Adrenocorticotropic hormone; CBZ, Carbamazepine; TPM, Topiramate; CLZ, Clonazepam; PYR, Pyridoxine; CP, Complex partial; IS, Infantile spasms; M, Myoclonic; T, Tonic; SIDS, Sudden Infant Death Syndrome; SMA, Spinal Muscular Atrophy