

GENE EXPRESSION IN TEMPORAL LOBE EPILEPSY

Gene Expression in Temporal Lobe Epilepsy Is Consistent with Increased Release of Glutamate by Astrocytes. Lee TS, Mane S, Eid T, Zhao H, Lin A, Guan Z, Kim JH, Schweitzer J, King-Stevens D, Weber P, Spencer SS, Spencer DD, de Lanerolle NC. *Mol Med.* 2007;13(1–2):1–13. Patients with temporal lobe epilepsy (TLE) often have a shrunken hippocampus that is known to be the location in which seizures originate. The role of the sclerotic hippocampus in the causation and maintenance of seizures in temporal lobe epilepsy (TLE) has remained incompletely understood despite extensive neuropathological investigations of this substrate. To gain new insights and develop new testable hypotheses on the role of sclerosis in the pathophysiology of TLE, the differential gene expression profile was studied. To this end, DNA microarray analysis was used to compare gene expression profiles in sclerotic and non-sclerotic hippocampi surgically removed from TLE patients. Sclerotic hippocampi had transcriptional signatures that were different from non-sclerotic hippocampi. The differentially expressed gene set in sclerotic hippocampi revealed changes in several molecular signaling pathways, which included the increased expression of genes associated with astrocyte structure (glial fibrillary acidic protein, ezrin-moesin-radixin, palladin), calcium regulation (S100 calcium binding protein beta, chemokine (C-X-C motif) receptor 4) and blood-brain barrier function (Aquaaporin 4, Chemokine (C-C- motif) ligand 2, Chemokine (C-C- motif) ligand 3, Plectin 1, intermediate filament binding protein 55kDa) and inflammatory responses. Immunohistochemical localization studies show that there is altered distribution of the gene-associated proteins in astrocytes from sclerotic foci compared with non-sclerotic foci. It is hypothesized that the astrocytes in sclerotic tissue have activated molecular pathways that could lead to enhanced release of glutamate by these cells. Such glutamate release may excite surrounding neurons and elicit seizure activity.

COMMENTARY

Hippocampal sclerosis is a very common feature of temporal lobe epilepsy. It is found in approximately 50 to 75 percent of temporal lobe resections performed to relieve medically intractable limbic epilepsy; the presence of sclerosis is a good indicator for a positive outcome to surgery. Since most of the epilepsy surgery resections consist of temporal lobectomies, electrophysiological and neuropathological investigations of epileptogenesis usually are performed on hippocampus and surrounding temporal cortex. Sclerosis is characterized by gross anatomical and microscopic changes, but the main histological feature is widespread neuronal cell death and gliosis/astrocytosis. As with many pathological findings in neurodegenerative diseases, it is difficult to determine if the changes are a cause or consequence of epileptic seizures. Studies with animal models suggest that hippocampal sclerosis is not necessary for progression to epileptic seizures (1); however, the clinical evidence summarized in the article by Lee et al. suggests a crucial role for the sclerotic hippocampus in determining seizures. If this is the case, then comparing the molecular properties of sclerotic with nonsclerotic hippocampi may provide insight into the cause of seizures in temporal lobe epilepsy.

Modern molecular tools allow genome-wide gene expression analyses to be performed on minute amounts of tissue. One of the first applications of the “gene chip” in human epilepsy was to investigate the correlates of multiple antiepileptic drug resistance (2). Subsequent studies explored epileptogenesis in animal models (3). Gene chip analysis of tissue isolated from animal models of epilepsy has several advantages, including relative low cost of otherwise impractical genome-wide comparisons, availability of impressive data analysis tools to analyze patterns of gene expression, and completion of the human genome project, allowing for cross comparison with patients’ data. In contrast, limitations of animal models include the difficulty of validating thousands of data points as well as reproducibility and correlation with data from different laboratories. As in all studies of epileptic human brain, true control brain tissue is not readily available. Recognizing these limitations, Lee and colleagues set out to use gene chips to analyze gene expression changes in hippocampal tissue obtained from surgical resections. Pitfalls associated with control tissue and interindividual variability were largely prevented by comparing hippocampi removed solely from patients with epilepsy and by focusing on the differences seen between sclerotic and nonsclerotic hippocampi of the same patients. This approach produced a number of results that were then validated with routine immunocytochemical and reverse transcriptase polymerase chain reaction approaches.

The title of this article does not tell the whole story of what the authors found. In fact, gene expression differences that were

unveiled have more to do with inflammation, vascular changes, and cell cycle/apoptosis than with glutamate uptake or transport by astroglia. These changes cannot be assumed to be exclusively astrocytic, since whole tissue mRNA was used. Furthermore, immunocytochemical detection revealed changes in other cell types, such as vascular endothelial cells. How whole tissue data differ from mRNA isolated from specific cell components has been discussed elsewhere (4). The exact analysis of cytological correlates of the mRNA data also leads to a potential pitfall with the interpretation of data. For tissue comparison, the authors defined gene expression levels of greater or smaller than 50% as a significant change; a definition that possibly leads to an overinterpretation of results, because cell numbers and transcriptional activity were not controlled for. Controlling for transcriptional activity is particularly important for gene expression levels that are not adjusted by any cellular quantification, such as number of astrocytes, ratio of astrocytes/oligodendrocytes/neurons, and contribution of nonbrain or vascular cells. The contributions of vascular cells are particularly important because several of the inflammatory markers and antigens that were reported to be upregulated are expressed by white blood cells, which may extravasate after seizures, leading to hippocampal sclerosis.

One of the unexpected and most experimentally solid findings was that the use of an unsupervised hierarchical cluster analysis was able to organize all samples into two major groups: one constituted specimens isolated from sclerotic hippocampi and the other containing mRNA from temporal lobe epilepsy hippocampi, not characterized by sclerosis. The surprising fact was not so much that sclerotic tissue had a distinct gene expression profile, but rather that sclerotic hippocampi as a group had similar gene expression profiles, regardless of whether the sclerosis was mass-associated (i.e., peritumoral) and nontumoral. The infiltrative nature of some of the tumors (e.g., glioma, oligodendroglioma) and the ischemic or cystic origin of other samples may have predicted a significant difference in mRNA, but this was not the case.

Sclerotic hippocampi demonstrated increased expression of several astrocytic genes, but it was not immediately clear if the finding was a reflection of an increased number of cells or increased transcriptional activity. A few of the upregulated genes that the investigators attributed to astrocytes, in fact, are expressed in many cell types and diseases, other than epilepsy. For instance, the protein S100A6 is upregulated in Alzheimer's and amyotrophic lateral sclerosis, CD44 antigen is expressed in bone marrow-derived cells, and the gene product AHNAK is expressed in blood-brain barrier endothelium. Indeed, a large number of upregulated genes were associated with blood-brain barrier function or systemic inflammation, further supporting a significant role for these two mechanisms in the etiology of seizures (5–7). One of the upregulated genes, *CCL3*, whose macrophage inflammatory protein is involved in the acute in-

flammatory state occurring with the recruitment and activation of polymorphonuclear leukocytes, suggests the presence of an infectious agent in sclerotic tissue. Similarly, interleukin-11 is a mediator of bone marrow-derived cells involved in the immune response. Additional findings are related to the expected loss of mRNA signaling, resulting from widespread neuronal cell death characteristic of sclerotic brain.

The introductory statements by Lee et al. support a role for sclerosis in the pathogenesis of hippocampal epilepsy; yet, the data presented do not clarify which of the observed changes are pathogenic and which are simply the result of seizures. Similarly, it is important to understand whether sclerosis is caused by some of these gene expression changes or is the trigger for altered inflammatory, glial, and cerebrovascular expression profiles. One of the unavoidable pitfalls of static, neuropathological studies is the lack of temporal perspective. For example, inflammation may have preceded sclerosis, and gliosis may be a consequence of neuronal cell loss. Conversely, neuronal death may be a consequence of seizures, which may also constitute the initiating factor for a mopping up intervention by immune cells, including macrophages. Unfortunately, the results presented here do not provide a major leap in understanding the origin of temporal lobe epilepsy, hippocampal seizures, or whether hippocampal sclerosis is a consequence or cause of seizures. This study, however, provides additional support for an immunological component in the pathophysiology of temporal lobe epilepsy and hippocampal sclerosis.

by Damir Janigro, PhD

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EPILEPSY AND FORGETFULNESS: ONE IMPAIRMENT, MULTIPLE MECHANISMS

Impaired Single Cell Firing and Long-term Potentiation Parallels Memory Impairment Following Recurrent Seizures.

Zhou JL, Shatskikh TN, Liu X, Holmes GL. *Eur J Neurosci* 2007;25(12):3667-3677. Patients with epilepsy are at substantial risk for memory impairment. Animal studies have paralleled these clinical observations, demonstrating impaired hippocampal function as measured by spatial memory in rodents subjected to seizures. However, the mechanism of seizure-induced hippocampal impairment is unclear. Here we investigated the effects of recurrent seizures on water-maze performance, a behavioural measure of learning and memory, long-term potentiation (LTP; considered a test of synaptic plasticity and memory) and place-cell firing patterns, a single-cell indicator of spatial memory. LTP and CA1 place-cell activity were examined in separate groups of freely moving rats, before and after 10 flurothyl-induced seizures. Water maze performance was examined in a third group of rats, five with previously induced seizures and five controls. Recurrent flurothyl seizures were associated with marked impairment in LTP and a reduction in the frequency of the peak theta power. Compared to baseline recordings, place-cell firing patterns following recurrent seizures were significantly less precise, had lower firing rates and were less stable. Impaired place-cell firing was seen as early as after two seizures and persisted at least 72h after the last seizure. Water-maze performance was also significantly impaired in animals that underwent recurrent seizures. No cell loss or synaptic reorganization was observed in the hippocampus or in several other cortical areas that are vulnerable to seizures. These results demonstrate that relatively brief excitatory events, not producing visible cell damage, can nevertheless cause long-lasting changes in hippocampal physiology, observable as impairments in place-cell function, LTP and spatial memory.

COMMENTARY

Impairments of learning and memory in patients with epilepsy represent a significant burden under conditions of an already debilitating disease. While the phenomenon of deficient learning, memory, and cognition in epilepsy has been well established, its place in the pathophysiology of epilepsy continues to be a subject of debates. A number of mechanisms may contribute to the disruption of memory function in epilepsy patients. One commonly cited reason for memory function impairment is hippocampal neuronal cell loss that is due both to the precipitating insult (e.g., status epilepticus or brain trauma) and recurrent seizures. Furthermore, frequent recurrent seizures, even in the absence of neuronal injury, may lead to the decline of learning and memory. It is also possible that chronic, persistent dysfunction of limbic circuits, which is characteristic of epilepsy, may impair memory even in the absence of neuronal injury and seizures. A less frequently contemplated scenario is that memory disorders may develop as a result of other comorbidities of epilepsy (e.g., depression), rather than from major hallmarks of the disease, such as neurodegeneration and seizures. Needless to say, understanding the nature of memory deficits associated with epilepsy is important, because it would define therapeutic approaches to their treatment. Meanwhile, experimental and clinical evidence accumulated to date, although abundant, provides little mechanistic clarification.

Impairments of learning and memory have been documented in animal models of limbic epilepsy triggered by status epilepticus. However, data obtained from poststatus epilepticus models are difficult to analyze, since these models are characterized both by extensive neurodegeneration and by spontaneous seizures, with respective contributions of each of the two factors in memory deficits being hard to interpret. Liu et al. reported that the extent of both memory deterioration and hippocampal place cell activity positively correlated with the severity of hippocampal neuronal injury and mossy fiber sprouting after pilocarpine status epilepticus (1). However, no correlation between hippocampal pathology and memory deficits with frequency of spontaneous seizures was performed; therefore, it was still possible that memory deficits in poststatus epilepticus animals resulted from frequent seizures, which in turn occurred because of severe hippocampal pathology. Stafstrom et al. found that memory deficits were observed after kainic acid status epilepticus induced in rats at postnatal day 20 or later, but not in 5- or 10-day-old pups (2). At these younger ages, animals apparently developed neither hippocampal injury nor spontaneous recurrent seizures; hence, causative relationship between each of the two factors and memory impairment was hard to establish. In clinical observations, the extent of cognitive and memory disorders is more pronounced in patients with a longer history of the disease. Again, however, this finding might reflect a longer history of seizures, progressing neuropathology, or both.

One possible way to address the role of spontaneous seizures and hippocampal neuronal injury in memory deterioration is to examine how either neuroprotection or elimination of spontaneous seizures by anticonvulsant treatment would affect memory performance. Such studies are complicated

because neuroprotective and anticonvulsant interventions are often hard to dissociate from one another. Furthermore, post-status epilepticus spontaneous seizures exhibit remarkable resistance to antiepileptic medications, and complete eradication has never been achieved.

In their study Zhou and colleagues employed a model of repetitive seizures in the absence of gross hippocampal pathology. Such an approach allowed the authors to show that brief, recurrent seizures themselves may induce impairment of memory and cognition. Furthermore, observed behavioral alterations paralleled several key correlates of learning and memory, such as long-term potentiation and the activity of hippocampal place cells.

Kindling is a model of epilepsy that affords examining the chronic epileptic state, particularly the sustained increase of excitability and seizure susceptibility in the absence of both extensive neurodegeneration and spontaneous seizures. Interestingly, there appears to be a consensus that the kindling state per se, does not affect memory and learning. Such deficits, when observed in kindled animals, most likely result from kindled seizures rather than from tonic changes in the excitability of limbic circuits (3).

The possibility that cognitive disorders are secondary to other comorbidities of epilepsy has received little attention. For example, depression, a very common comorbidity in epilepsy patients, also is known to have significant impact on cognitive and memory performance. Thus, under conditions of an experimental model of depression, animals developed deficits in spatial memory tasks; these deficits were successfully corrected by the antidepressant fluoxetine (4). In contrast, a recent clinical study failed to reveal any correlation between depression and cognitive deficits in epilepsy patients (5). More studies are necessary to definitively address this issue. If memory disorders in epilepsy patients are indeed related to depression, it is possible that the correction of cognitive and memory deficits paradoxically might be achieved through antidepressant medication.

In this regard, the kindling state, which as discussed, does not lead to memory impairments, is characterized by persistent anxiety, fear, altered emotional tone, and depression (6,7). Such dissociation between memory and mood impairments in kindled animals by itself is interesting, as it outlines two different

patterns of epilepsy comorbidities: those that are associated with discrete epileptic events (such as seizures) and those that depend on tonic dysfunction of limbic neuronal network. Furthermore, even if one comorbid state, for example depression, does not directly disrupt memory function, it may still exacerbate it.

Given the complexity of memory and cognition mechanisms, as well as the diversity of underlying neuronal processes, it is unlikely that impaired memory and cognition in epilepsy can be explained by a single mechanism. Indeed, a variety of factors (e.g., neuronal cell loss, recurrent seizures, interictal perturbations, and sustained tonic dysfunction of limbic circuits) likely contribute to the impairments of learning and memory. However, in spite of accumulated data, the question still remains to be answered: do learning and memory impairments require special dedicated treatment or would merely getting rid of the epileptic foci or seizures be sufficient?

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EARLY-LIFE SEIZURES AND COGNITIVE IMPAIRMENT: A SPINY PROBLEM?

Recurrent Seizures and the Molecular Maturation of Hippocampal and Neocortical Glutamatergic Synapses. Swann JW, Le JT, Lee CL. *Dev Neurosci* 2007;29(1–2):168–178. Recurrent seizures in animal models of early-onset epilepsy have been shown to produce deficits in spatial learning and memory. While neuronal loss does not appear to underlie these effects, dendritic spine loss has been shown to occur. In experiments reported here, seizures induced either by tetanus toxin or flurothyl during the second postnatal week were found to reduce the expression of NMDA receptor subunits in both the hippocampus and neocortex. Most experiments focused on alterations in the expression of the NR2A subunit and its associated scaffolding protein, PSD95, since their expression is developmentally regulated. Results suggest that the depression in expression can be delayed by at least 5 days but persists for at least 3–4 weeks. These effects were dependent on the number of seizures experienced, and were not observed when seizures were induced in adult mice. Taken together, the results suggest that recurrent seizures in infancy may interrupt synapse maturation and produce persistent decreases in molecular markers for glutamatergic synapses - particularly components of the NMDA receptor complex implicated in learning and memory.

COMMENTARY

Abundant data from experimental seizure models and clinical studies demonstrate that recurrent seizures early in life are associated with long-term cognitive and behavioral problems (1,2,3). Whether these chronic cognitive deficits are due to the etiology of the seizure or to the seizures themselves has been a controversial topic. If the mechanism of seizure-induced cognitive impairment could be identified, perhaps treatment strategies could be devised to circumvent those deficits.

Among seizure-related neuronal alterations, certain changes are known *not* to explain cognitive deficits, including cell death, axonal reorganization, and neurogenesis. While each of those cellular alterations can occur after early-life seizures, cognitive deficits are seen even when none of those cellular structural alterations are documented. Since learning and memory are hippocampal based and are dependent upon glutamatergic synaptic plasticity, it has been hypothesized that glutamatergic synaptic function (especially NMDA receptors) is altered by seizures and causes subsequent cognitive deficits. Thus, investigations focusing on these synapses and the spine-like structures that house them are extremely relevant.

The experiments described here address the hypothesis that early-life seizures cause cognitive impairment by altering the expression of NMDA receptors, particularly those located at synapses on dendritic spines. Swann and colleagues induced seizures in rodents in the second postnatal week using two methods with very different mechanisms of action that both impair GABAergic (inhibitory) synapses: intrahippocampal injection of tetanus toxin (TNTX) or inhalation of the volatile convulsant flurothyl. TNTX causes several brief limbic seizures (by block-

ing presynaptic GABA release) over a discrete time period, lasting about a week after injection. Therefore, this method assesses the effect of repeated but time-limited seizures; here, TNTX was delivered to postnatal (P) day 10 rats and spontaneous seizure frequency was monitored for the next week. Flurothyl induces seizures only during exposure to the inhalant compound (by blocking postsynaptic GABA action). This method allows the experimenter to determine the exact number and duration of seizures; in these experiments, 15 flurothyl seizures were induced in mice between P9–P13. The authors subsequently used Western blotting to examine the expression of NMDA receptor subunit subtypes NR1, NR2A, and NR2B as well as the dendritic spine-associated scaffold protein, PSD95, which has a developmental expression similar to NR2A.

The authors found that both TNTX and flurothyl seizures were associated with down-regulation of NR1, NR2A, and NR2B as well as of PSD95. Decreases in NR2A were most prominent and persistent, which is of significance given the central importance of this receptor subtype in learning and memory and its unique developmental profile. These receptor changes were sustained in both hippocampus and neocortex, but only when seizures were induced during the neonatal period. Seizures induced in adult rodents by the same methods did not alter the expression of any of those proteins. Furthermore, in the flurothyl model, the changes were only found when at least three daily seizures (15 total), but not when a single daily seizure (5 total) occurred across the five treatment days.

It was concluded that seizures early in life impair subsequent learning and cognition by disrupting the developmental trajectory of glutamate synapses. One possible mechanism for these effects on synaptic function is that network hyperexcitability engendered by the seizures interferes with normal glutamatergic synapse development (4). The finding of altered scaffold protein PSD95 points toward dendritic spines as a potential localization for the defect. Since PSD95 is the most abundant excitatory synaptic protein, down-regulation implies

either synaptic loss (5,6), altered synaptic function, or both. PSD95 directly interacts with NR2A and indirectly with other elements of the subsynaptic machinery involved in plasticity.

These results provide additional data that seizures early in life are associated with long-term cognitive impairment. Cognition was not tested directly in these experiments, though the authors' previous work established cognitive deficits in neonatal TNTX-exposed rats (7). Interestingly, down-regulation of NR2A and alteration of PSD95 expression also was documented in recent experiments in which a single neonatal seizure in rats was induced by subcutaneous kainate on P7, but in the opposite direction—PSD95 expression was increased in those experiments with no loss of dendritic spines (8). While these apparently discrepant findings confirm the inherent complexity of the experimental problems of seizure models, they now bring to the forefront the role of subsynaptic scaffolds (9), which provides a novel and unexpected target for potential pharmacologic intervention in seizure-induced cognitive changes.

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ANTIEPILEPTOGENESIS THERAPY WITH LEVETIRACETAM: DATA FROM KINDLING VERSUS STATUS EPILEPTICUS MODELS

Prophylactic Treatment with Levetiracetam after Status Epilepticus: Lack of Effect on Epileptogenesis, Neuronal Damage, and Behavioral Alterations in Rats. Brandt C, Glien M, Gastens AM, Fedrowitz M, Bethmann K, Volk HA, Potschka H, Löscher W. *Neuropharmacology* 2007;53(2):207–221. Levetiracetam (LEV) is a structurally novel antiepileptic drug (AED) which has demonstrated a broad spectrum of anticonvulsant activities both in experimental and clinical studies. Previous experiments in the kindling model suggested that LEV, in addition to its seizure-suppressing activity, may possess antiepileptogenic or disease-modifying activity. In the present study, we evaluated this possibility by using a rat model in which epilepsy with spontaneous recurrent seizures (SRS), behavioral alterations, and hippocampal damages develop after a status epilepticus (SE) induced by sustained electrical stimulation of the basal amygdala. Two experimental protocols were used. In the first protocol, LEV treatment was started 24h after onset of electrical amygdala stimulation without prior termination of the SE. In the second protocol, the SE was interrupted after 4h by diazepam, immediately followed by onset of treatment with LEV. Treatment with LEV was continued for 8weeks (experiment #1) or 5weeks (experiment #2) after SE, using continuous drug administration via osmotic minipumps. The occurrence of SRS was recorded during and after treatment. In addition, the rats were tested in a battery of behavioral tests, including the elevated-plus maze and the Morris water maze. Finally, the brains of the animals were analyzed for histological lesions in the hippocampal formation. With the experimental protocols chosen for these experiments, LEV did not exert antiepileptogenic or neuroprotective activity. Furthermore, the behavioral alterations, e.g., behavioral hyperexcitability and learning deficits, in epileptic rats were not affected by treatment with LEV after SE. These data do not support the idea that administration of LEV after SE prevents or reduces the long-term alterations developing after such brain insult in rats.

COMMENTARY

The mechanisms responsible for the development of spontaneous recurrent seizures after brain injury remain unknown, but considerable research has been directed at attempts to identify antiepileptogenic drugs. Clinical studies have found that treatment with traditional antiepileptic drugs (AEDs) after brain injury does not affect the development of epilepsy, leading to the conclusion that these AEDs do not alter the course of epileptogenesis (1). In animals, levetiracetam reduces the duration of kindled seizures and the rate of kindling, suggesting that levetiracetam is not only an AED but also might be antiepileptogenic (2). It is not known if these animal studies are predictive of human antiepileptogenic activity, although a clinical trial currently in progress is attempting to find out if levetiracetam can prevent post-traumatic epilepsy. This study by Brandt and colleagues sought to determine whether chronic administration of levetiracetam, after status epilepticus induced by sustained electrical stimulation of the basolateral amygdala, would block or reduce the subsequent epilepsy, mitigate behavioral deficits, and be neuroprotective in the hippocampus. The key result was that chronic treatment with levetiracetam did not reduce the subsequent epilepsy, as measured by the occurrence of spontaneous seizures. In two separate experiments, which used slightly different protocols, seizures were still observed several weeks after status epilepticus. Also, neither the behavior nor neuronal damage was altered with levetiracetam. These results are at odds, as the authors note, with earlier work by Löscher et al. using the kindling model (2).

An important issue in this study is that levetiracetam, an effective AED, did not appear to reduce the frequency of spontaneous recurrent seizures during the treatment protocol, which could explain the failure to observe an antiepileptogenic effect. In this study, levetiracetam was first administered after the stimulation-induced status epilepticus. This protocol is in contrast to the experiments with the kindling model in which the drug was administered during the kindling stimulation. Therefore, it is conceivable that an effect of the drug on the seizures during treatment is necessary for an antiepileptogenic effect. A second important issue is how the drug was administered. Both experiments had an initial brief treatment period with intraperitoneal injections, followed by chronic treatment via osmotic minipumps. Thus, the initial treatment with intraperitoneal injections of levetiracetam was given immediately after the status epilepticus (i.e., a single injection in the first experiment, and three-times-daily injections for 5 days in the second experiment); however, animals that have experienced status epilepticus and then receive repeated drug injections over prolonged periods are difficult to handle and often develop peritonitis (3), which is presumably one reason why Brandt and colleagues shifted from injections to osmotic minipumps in both experi-

ments (4). Because the frequency of spontaneous seizures was not reduced, it is possible that the dose of levetiracetam was too low, even though the authors measured the level of levetiracetam in the blood. Furthermore, the minipumps had to be replaced three times, which involved additional surgeries and death of some animals; thus, the approach of using osmotic minipumps for long-term chronic treatment is not without problems. The study essentially tested a single chronic dose of levetiracetam during either the 5- or 8-week infusion of levetiracetam (first and second experiments, respectively), which is indicative of the significant difficulty in designing animal model studies that guarantee that the required drug levels are maintained during the chronic drug treatment protocol. This unresolved problem might be better addressed with a dose-response protocol that maintains higher dose levels capable of blocking or at least suppressing the spontaneous seizures during treatment.

This study raises the question of which animal model is better for antiepileptogenesis studies: the electrically evoked seizures of kindling or the spontaneous seizures that occur after status epilepticus? Although several studies have suggested that kindling might be a useful model for testing antiepileptogenic and neuroprotective agents, the model has not been validated. Furthermore, this study raises the question of whether results from kindling can be generalized to other models. As discussed above, because the drug under investigation is present during the electrically stimulated seizures associated with the kindling process, it is possible that the drug blunts the electrically stimulated seizures. Thus, a mild anticonvulsant effect in the kindling model could lead to an *apparent* antiepileptogenic effect.

A problem with animal models of chronic epilepsy that depend on status epilepticus is that the experimental design must include enough monitoring of spontaneous seizures to have sufficient statistical power to demonstrate that the intervention has no effect (i.e., avoiding a false negative). In this, seizure monitoring entailed: (i) "recording of all observed seizures," (ii) regular but intermittent video monitoring, and (iii) continuous video-EEG for 1 week, which occurred 10 weeks after status epilepticus (the latter, performed only in the second study). The "recording of all observed seizures" represented only the detection of seizures during handling and animal care procedures; therefore, this monitoring protocol and the video monitoring (i.e., without EEG) would have missed many seizures. Thus, the 1 week of video-EEG was the strongest seizure-monitoring data in the study; the limited amount of seizure monitoring raises the question of how much monitoring is enough, which ultimately is a statistical problem that depends on the actual magnitude of the drug effect to be detected and on the mean and variance of the baseline interseizure intervals. Unfortunately, long interseizure intervals and large interval variances are the rule for animal models of chronic epilepsy, and both of these features substantially increase the time intervals over which ani-

imals must be monitored in order to avoid false-negative conclusions. A possible false-negative conclusion, in this circumstance, is the finding that there was no effect from the intervention, when in fact the intervention may have had an effect but was not detected with the monitoring protocol. Therefore, the answer to the question of how much monitoring is enough is probably that a great deal more would have been much better. Thus, techniques and protocols are needed that allow extensive, if not continuous, monitoring, because it cannot be determined with certainty that an antiepileptogenic therapy is effective or ineffective unless the monitoring has been long enough to reliably show an effect or no effect of the intervention on epileptogenesis.

The Brandt et al. study raises yet another question: why would AED treatment alter epileptogenesis? Most proposed mechanisms (e.g., neuronal death, axon sprouting) for epileptogenesis do not involve mechanisms believed to be involved in protection against seizures. The hypotheses that invoke an important role for ion channels in epileptogenesis (including ion channels that the AEDs may target) do not necessarily predict that a transient period of AED treatment will have a subsequent effect on spontaneous seizures. Furthermore, part of the rationale for studying levetiracetam is that it may block seizures via a new and different mechanism (i.e., neurotransmitter release), but it is unclear why this potential mechanism for levetiracetam might also be considered antiepileptogenic. One possible antiepileptogenesis hypothesis based on an antiseizure mechanism of an AED is that reducing spontaneous seizures with an AED early in the process of epileptogenesis alters the subsequent progressive evolution of the epilepsy. That is, do early seizures beget later seizures, and does blocking the early seizures reduce the occurrence of later seizures? Because levetiracetam was not shown to depress seizures during treatment, this study does not address this hypothesis.

The study by Brandt et al. does not provide a definitive answer as to whether levetiracetam has an antiepileptogenic effect. The issues of appropriate dose, timing of dose, and outcome measures are sufficiently complex that it would take years to work out the answer with any true certainty. This initial study (as substantive as the efforts by Brandt and colleagues were) suggests, within its important limitations, that it does not have an antiepileptogenic effect. Although by no means a final word on the matter, the article does raise the question of whether classes of compounds, other than AEDs, should be considered in the search for epilepsy prophylaxis, as AEDs to date have shown no real promise in this area.

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