Title
Does Acquired Epileptogenesis in the Immature Brain Require Neuronal Death?

Journal
Epilepsy Currents, 11(1)

ISSN
1535-7597

Authors
Baram, Tallie Z
Jensen, Frances E
Brooks-Kayal, Amy

Publication Date
2011

DOI
10.5698/1535-7511-11.1.21

EPILEPSY CURRENTS

Current Review
In Basic Science

Does Acquired Epileptogenesis in the Immature Brain Require Neuronal Death?

Tallie Z. Baram, MD, PhD,[1*] Frances E. Jensen, MD,[2] and Amy Brooks-Kayal, MD[3]
[1]Departments of Anatomy/Neurobiology and Pediatrics, University of California Irvine, Irvine, CA
[2]Children's Hospital Boston and Harvard Medical School, Neurology, Boston, MA
[3]Pediatrics and Neurology, University of Colorado Denver School of Medicine and The Children's Hospital Denver and Aurora, CO
*Address correspondence to Tallie Z. Baram, MD, PhD, Departments of Anatomy/Neurobiology and Pediatrics, University of California Irvine, ZOT 4475, Irvine, CA. E-mail: tallie@uci.edu

Because epilepsy often occurs during development, understanding the mechanisms by which this process takes place (epileptogenesis) is important. In addition, the age-specificity of seizures and epilepsies of the neonatal, infancy, and childhood periods suggests that the processes and mechanisms that culminate in epilepsy might be age specific as well. Here we provide an updated review of recent and existing literature and discuss evidence that neuronal loss may occur during epileptogenesis in the developing brain, but is not required for the epileptogenic process. We speculate about the mechanisms for the resilience of neurons in immature limbic structures to epileptogenic insults, and propose that the type, duration and severity of these insults influence the phenomenology of the resulting spontaneous seizures.

Does Epileptogenesis in the Developing Brain Require Cell Loss?

Loss of specific neuronal populations in hippocampal areas CA1 and CA3/4, and the hilus of the dentate gyrus, is a common feature in hippocampi resected from patients with TLE (1–9). TLE often follows early-life prolonged febrile and other seizures, and it is currently unclear if the neuronal loss found in epileptic tissue precedes or is a result of the TLE itself (7, 8, 16). In adult models of TLE in which status epilepticus (SE) is the inciting insult, cells loss is common and is plausibly required for the epileptogenic process. Because of these associations, cell loss was extensively investigated in several developmental epilepsy models. These include immature rodent models of neonatal seizures (17, 18), prolonged febrile seizures (19, 20) and febrile status epilepticus (21), and pediatric nonfebrile status epilepticus (22–24).

In the model of prolonged febrile seizures or febrile status epilepticus (FS/FSE), initial studies found no increase in acute apoptotic cell death anywhere in the hippocampal formation at 4 to 24 hours after the seizures. However, injury to pyramidal cells in hippocampal CA1 and CA3 was observed in a pattern reminiscent of human mesial temporal sclerosis (MTS). This injury, manifest as augmented Golgi-staining (19), was also apparent in the hilus, yet resolved within weeks without progressing to cell loss. Delayed cell loss was also systematically explored in vulnerable hippocampal regions in four additional animal cohorts (21, 25–27). Methods employed included Cresyl Violet augmented by neurochemical markers for interneurons and pyramidal cells as well as for glia (25). The possibility that the failure to find an appreciable reduction of cell numbers might be a result of neurogenesis was also considered using BrdU cell-birth dating (25). In all cases, blinded analyses using stereologic principles failed to show significant neuronal loss in vulnerable hippocampal regions in rats that sustained the seizures, including those that developed limbic epilepsy (21, 26). Notably, even hippocampi of rats that developed spontaneous motor seizures lasting over 100 seconds were devoid of appreciable neuronal dropout (21). Looking at potentially vulnerable regions elsewhere in the brain (e.g., dorsomedial thalamus [28]), apparent cell loss was absent, whereas injury was found also in limbic cortices (19). These data suggest that significant acute or delayed cell death was not required for the onset of TLE after experimental prolonged febrile seizures. Whether the resulting TLE, that is, the spontaneous seizures in a subgroup of FS/FSE rats, could eventually lead to cell loss in a distribution similar to the loss in MTS and to the injury observed by Toth et al. (19) remains to be determined.

Because neonatal seizures may be followed by the onset of epilepsy, several models have been developed to examine these seizures. These include seizure induction by hypoxia as well as by chemoconvulsants including kainate and repeated flurothyl exposure (17, 18, 29, 30). Among these paradigms that employed postnatal day-7 to day-10 rats, the hypoxia-induced seizure model generated later-life spontaneous seizures assessed by behavioral and EEG parameters (31). Recently, Rakhade et al. (31) have reported that over 90% of rat pups experiencing early-life hypoxic seizures develop recurrent spontaneous seizures by P100, as assessed by video-EEG long-term monitoring with intracranial depth electrodes. Moreover,

Epilepsy Currents, Vol. 11, No. 1 (January/February) 2011 pp. 21–26

serial EEG recordings during the juvenile period following neonatal seizures demonstrated that seizures were first detected around 12 to 15 days following the initial hypoxia-induced seizures.

Despite the development of spontaneous seizures, a number of investigations, including the use of Fluoro-Jade B staining in the acute and subacute period up to a week after the initial seizures, have failed to show the presence of increased neuronal death compared with control littermate rats (17, 31). Still, synaptic reorganization in the form of sprouting was observed in several studies (31–34). Taken together, these findings indicate that epileptogenesis, defined as the occurrence of spontaneous seizures, occurs after several types of neonatal seizures in the absence of appreciable acute and subacute cell death.

To probe epileptogenesis during the period of life that is comparable to infancy/early childhood (35), kainic acid (18) or pilocarpine was employed to provoke status epilepticus at postnatal day 20 (P20). Following pilocarpine-induced status epilepticus, approximately two thirds of animals developed later recurrent spontaneous seizures (epilepsy), as determined by long-term (>3 months) video-intracranial EEG monitoring (22). In the report by Raol et al., it was found that in the subset of animals that developed epilepsy, hippocampal cell loss was detectable and quantifiable in two of the nine animals using standard cell staining and counting methods (22). This finding indicates that the methods used were capable of detecting and quantifying cell loss. There was no correlation between the degree of cell loss and the severity of epilepsy (assessed by the frequency of recurrent seizures) (22). In other words, although 2 animals had very severe cell loss, their epilepsy was no more severe than animals that showed no evidence of cell loss. Thus, whereas modest cell loss could have been missed using Cresyl Violet or other standard histopathological staining techniques and traditional neuronal-counting methods, the absence of any difference in epilepsy severity between those animals with a substantial amount of cell loss in the hippocampus and those with no (or possibly minimal) cell loss suggests that hippocampal cell loss alone is unlikely to be a major determinant of epilepsy development in this model.

Collectively, these studies demonstrate that some acquired epilepsy can arise without appreciable cell loss in the developing brain. Consistent with recent opinions (35), the studies do not imply that cell loss is not an important factor in some cases of pediatric epilepsy. Stroke, ischemia, trauma, or severe infection may lead to cell death during development, and to associated epilepsy. In addition, not all seizures cause epileptogenesis, and, in most children, seizures result in neither apparent structural changes nor later epilepsy. Further, clinical observations suggest that more severe brain insults, associated with severe brain injury, might be more likely to provoke epilepsy in children, and probably also in immature animals. Indeed, diverse etiologies can produce epilepsy after early-life insults, and in both humans and animals, there is no reason to assume that the mechanisms leading to epilepsy would be the same in each etiology. Thus, it is quite conceivable that cell loss occurs in some models of developmental epilepsy, such as in hypoxia-ischemia or neonatal stroke, and plays an intrinsic role in epileptogenesis. In other clinical situations and animal models, cell loss may not occur, or, if it is observed, this cell loss may be neither causal nor necessary for epilepsy development. In summary, epileptogenesis early in life *may* be associated with cell loss in vulnerable hippocampal regions and in other brain areas. However, epileptogenesis may arise also *in the absence* of appreciable cell loss: it is not required for acquired epileptogenesis, at least in the developing brain (and potentially in some instances of epileptogenesis taking place in the mature brain).

The Important Questions About Epileptogenesis in the Immature Brain: Why Are Neurons Resilient to Excitotoxicity, and What Are the Key Mechanisms of Epileptogenesis?

Lack of an absolute requirement for cell loss in early-life acquired epileptogenesis is an important conceptual point, because it brings up two important gaps in our current understanding of epileptogenesis in general. These gaps hamper the development of interventional and therapeutic approaches.

The first question relates to the mechanism of the resilience of neurons in the immature hippocampus, limbic cortices, and thalamus to status epilepticus-induced cell death (36–38). Why don't neurons die, when the inciting seizures are prolonged and severe? Can we exploit the underlying mechanisms to protect neurons in adults from similar insults? The mechanisms that contribute to the resilience are not fully understood; two candidates include mitochondrial uncoupling and the relative paucity of inflammation. During adult SE, metabolic demand in neurons results in the overwhelming of mitochondrial function and, hence, accumulation of reactive oxygen species (ROS), with eventual mitochondrial decompensation and runaway cell death (39). In immature brain, a fat-rich diet promotes augmented expression of the mitochondrial uncoupling protein 2 (UCP2). This protein reduces mitochondrial membrane gradient, prevents ROS accumulation and protects from SE-induced neuronal death (40, 41). A second, perhaps complementary protective element is the attenuated inflammatory response to SE during development (42). This is in contrast to the adult hippocampus, where cytokines and related mediators are both released from injured cells and contribute to neuronal death (42). Obviously, numerous other factors, potentially including augmented levels of growth factors such as brain-derived neurotrophic factor (BDNF), might protect neurons in the immature brain from excitotoxic and oxidative injury.

The second question is the logical extension of refuting the hypothesis that cell death is the *sine qua non* of epileptogenesis during development. Namely, if cell death is not the principal mechanism for epileptogenesis, then what *is* required for the epileptogenic process during infancy and childhood? If obvious structural defects are not required, then *how does the developing brain become epileptic after insults*? The mechanisms that truly govern the changes in neuronal phenotype and network properties that, in turn, result in epilepsy, must then be sought. Indeed, the study of these mechanisms is at the forefront of our efforts to understand epileptogenesis in the developing brain.

Results that have already been obtained in the models described above as well as by other groups are beginning to answer these questions. For example, neonatal seizures

induced by hypoxia led to dramatic changes in subunit expression of the AMPA subtype of the glutamate receptors, as well as kinase and phosphatase activation and subsequent posttranslational modification of glutamate and GABA receptors (34, 43, 44). Long-lasting changes in the expression patterns of $GABA_A$ receptor subunits have been described after pilocarpine-provoked status epilepticus (45, 46). Similarly, a number of gene-expression changes, including early and enduring reduction of the expression of the hyperpolarization-activation cyclic nucleotide gated (HCN) channel type 1 and augmented endocannabinoid receptors were found after prolonged experimental FS (47–49). These molecular changes have been shown to be associated with changes in ionic currents that promote hyperexcitability and vulnerability to subsequent seizures (43, 44, 49–52). The fundamental basic transcriptional and posttranslational regulatory mechanisms governing the major and persistent changes in gene expression that promote epileptogenesis during development are under intense investigation (53–56).

Does Temporal Lobe–Like Epilepsy Actually Result From Experimental Developmental Seizures?

If cell loss is minimal or absent after some developmental seizures, and if cell loss is considered crucial for epileptogenesis, then it is reasonable to question whether acquired epileptogenesis actually takes place after experimental FSE, chemical SE, or neonatal hypoxia-related seizures. The clinical diagnosis of epilepsy requires the documentation of two or more seizures. Notably, in the clinical situation, prolonged monitoring using video-EEG to capture and document seizures is not required for a diagnosis of epilepsy and is rarely used for this purpose. Using the same criteria (i.e., documentation of two seizures), epilepsy was documented after developmental seizures in the models described here. In addition, prolonged video-EEG monitoring was carried out in all to address additional questions. When does epilepsy arise? How frequent are the seizures? Does the frequency vary? When does abnormal (interictal) brain activity commence? Unlike the confirmation of the presence of epilepsy and the phenotype of seizures, these types of questions may require continuous long-term monitoring in both patients and animal models.

A second point is the definition of a seizure. This is a complex and controversial issue, and conservative approaches consisting of the employment of both EEG and behavioral measures of seizures are preferred in both patients and animals. Galvan et al. (57) and Nissinen et al. (58) defined seizures as EEG abnormalities consisting of rhythmic discharges involving doubling of voltage and lasting over 6 seconds, associated with behavioral phenomena. D'Ambrosio et al. (59) suggested that a minimal time limit might not be needed. Both of these definitions exclude events found on EEG alone. In addition to the video-EEG criteria, seizures can be defined on behavioral criteria alone; for example, when overt motor phenomena consisting of the classic Racine progression (60) are observed. Because behavioral phenomena of limbic seizures in both humans and animal models are often subtle, behavioral approaches alone, as well as the requirement for behavioral change in association with EEG criteria may underestimate the prevalence of seizures.

After experimental long FS (lasting ~20 minutes), spontaneous short seizures consisting of Racine stage 0 to II behaviors and EEG discharges longer than 6 seconds were observed (26). These FS are induced in postnatal day-10 to day-12 rats, where the stage of hippocampal development is roughly equivalent to that of human infants (a comparative table can be found in Avishai-Eliner et al. [36]). Similarly, in the hypoxia-induced seizure model in postnatal day-10 rats, electrographic seizures were always associated with abnormal behavioral activity or motor phenomena. In both cases, these relatively subtle seizures were distinguishable from theta bursts seen in control animals, which were generally shorter and associated with exploratory behavior (26, 31).

In the model of prolonged FS, when the duration of the inciting FS was increased, generating experimental febrile status epilepticus, long-term video-EEG monitoring using hippocampal and cortical electrodes documented the appearance of longer spontaneous seizures (mean duration 137 seconds; median, 91 seconds) on EEG. Notably, these epileptic events were associated with motor phenomena (Racine stages III–V; 60), including unilateral clonus, bilateral clonus, and rearing and falling (21). These are the classical behavioral sequences observed in limbic seizures that generalize.

In a developmental model of pilocarpine-induced status epilepticus in weanling postnatal day-20 rats, EEG and intracranial video recording demonstrated spontaneous seizures defined as discrete alterations in behavior accompanied by rhythmic electrographic discharges that evolved over time and lasted for 8 or more seconds (22). Sixty-seven percent of the rats (12/18) subjected to lithium-pilocarpine–induced SE at P20 went on to develop spontaneous seizures in adulthood with a latency to spontaneous seizure onset of 45.2 $\pm$ 9.0 days. The behavioral seizure phenotypes included wild running, facial clonus, staring, head bobbing, and tail stiffening lasting from 8 to 20 seconds (stages II–III) as well as generalized tonic-clonic activity with falling (Racine stage V seizures) lasting up to 43 seconds. All behavioral seizures had EEG correlates.

Together, these findings show a spectrum of the behavioral and electrographic seizures incited by early-life insults and enable speculation about the basis of the often more subtle nature of the spontaneous seizures that are provoked by these early-life insults. The duration or severity of the inciting event might be an important factor (21). In addition, unlike the consequences of status epilepticus that provokes TLE in adult models, catastrophic injury to regions that are known to gate seizure propagation, such as the dentate gyrus, is not observed after developmental seizures and status epilepticus. Hence, it is possible that seizures that result from more subtle injury in the context of a relatively preserved hippocampal circuitry may not readily generalize. The limbic phenomenology of freezing, staring, and facial automatisms, as well as focal motor activity, may thus be more typical of TLE that follows developmental inciting events.

In summary, epilepsy, defined as more than two spontaneous seizures (using a conservative definition of seizures), was documented in several models of pediatric epileptogenesis. More work is required to define precisely the temporal evolution of this epileptogenesis.

Are We Using Appropriate Developmental Models to Study Acquired Epileptogenesis in the Immature Brain?

It is an *a priori* assumption that no single animal model can capture the full breadth of the clinical condition (35). Suitable models focus on salient elements of the condition that is being modeled, and on the questions that are being studied. In addition, because acquired epilepsy in infancy and childhood is heterogeneous and can result from numerous etiologies, varied animal models of developmental epilepsies induced by different methods will undoubtedly be needed to fully understand the breadth of mechanisms that may underlie developmental epileptogenesis.

Because prolonged FS and FSE may be a risk factor described in a significant proportion of individuals with TLE (61, 62), it is important to study if and how these developmental seizures might cause epilepsy. However, unlike in young children, fever cannot be induced in immature rats. Therefore, as stated by Reid et al., "the reason that all models employed to date rely on more than just a true fever is that, at least at the ages tested, even in immature common laboratory rat strains, FSs are difficult to evoke...." This has led to a number of models of FS, using hyperthermia (19, 21, 25, 27, 49, 51, 52, 63–68), or lipopolysaccharide combined with kainic acid at ambient temperature of 30°C (20, 69). Whereas the mechanisms for the elevation of brain temperature might differ in these models, hyperthermia per se induces the release of endogenous fever mediators and specifically interleukin 1 beta (IL1-β) in the brain. Indeed, binding of IL1-β to its receptor may be required for "febrile" seizure generation, as found by studying IL1-β-receptor null mice (70, 71). To exclude the possibility that hyperthermia itself may provoke epilepsy, hyperthermic controls have been used (26, 51). In addition, core and brain temperatures during these seizures have been monitored throughout their duration (21, 71, 72), and refinement of the model has lowered these temperatures to ~39 to 40°C. Thus, temperatures employed in these models do not exceed those in children with high fever. In addition, dehydration, a marker of heat stroke or shock does not take place (weight loss is ~2%, whereas a clinically relevant change is >5%).

Neonatal seizures may precede epilepsy in children and are most commonly caused by hypoxic/ischemic encephalopathy (17). Similar to the human, the immature rat responds to hypoxia and hypoxia/ischemia with seizures that can be refractory to conventional anticonvulsants (73, 74) and may thus prove useful for preclinical investigative and therapeutic studies (75).

Whereas neonatal and febrile seizure models are intrinsically age specific, chemical convulsant-induced status epilepticus has been extensively used in the study of adult epileptogenesis. The most common chemoconvulsants include pilocarpine (with or without lithium) (76–79), and kainic acid. Developmental models have relied on these chemoconvulsants as well (22, 28, 80, 81), and it is reasonable to propose that, in this case, the validity of these models does not vary with age.

Conclusions

In summary, epilepsy affects primarily children and young adults, so that studying how it arises is of paramount clinical importance. Models have been established and validated for several of the many and varied insults to the developing brain that can lead to epilepsy, including neonatal hypoxia, hypoxia-ischemia, fever/hyperthermia, and status epilepticus. The resulting data indicate that epileptogenesis in the developing brain has features that are distinct from those in the adult. Therefore, extrapolating principles established from the study of adult epileptogenesis (including cell loss) to developmental epileptogenesis may not be justified. In addition, careful consideration of the state of maturation of specific brain regions across ages and across species might be warranted (a comparative table can be found in Avishai-Eliner [36]). The emerging consensus further suggests that epileptogenic insults early in life might act by changing the gene expression repertoire and functional phenotype of neurons, rather than by killing them. These data are exciting because if we understand these mechanisms and identify the "master switches" that govern them, we will have made a major advance in preventing developmental epileptogenesis.

Acknowledgments

The authors appreciate the excellent editorial help of Mrs. Barbara Cartwright. Authors' research was supported by NIH awards R37 NS35439 and NS35439-S1 ARRA (TZB), RO1 NS 38595 (ABK), and RO1 NS31718 and DP10OD003347 (FEJ), as well as awards from the American Epilepsy Society and Epilepsy Foundation of America.

References

1. de Lanerolle NC, Kim JH, Williamson A, Spencer SS, Zaveri HP, Eid T, Spencer DD. A retrospective analysis of hippocampal pathology in human temporal lobe epilepsy: Evidence for distinctive patient subcategories. *Epilepsia* 2003;44:677–687.
2. Sloviter RS. The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. *Ann Neurol* 1994;35:640–654.
3. Engel J Jr. Introduction to temporal lobe epilepsy. *Epilepsy Res* 1996;26:141–150.
4. Liu Z, Mikati M, Holmes GL. Mesial temporal sclerosis: Pathogenesis and significance. *Pediatr Neurol* 1995;12:5–16.
5. Blümcke I, Beck H, Lie AA, Wiestler OD. Molecular neuropathology of human mesial temporal lobe epilepsy. *Epilepsy Res* 1999;36:205–223.
6. Cendes F. Febrile seizures and mesial temporal sclerosis. *Curr Opin Neurol* 2004;17:161–164.
7. Theodore WH. Recent advances and trends in epilepsy imaging: Pathogenesis and pathophysiology. *Rev Neurol Dis* 2004;1:53–59.
8. Bender RA, Dubé C, Baram TZ. Febrile seizures and mechanisms of epileptogenesis: Insights from an animal model. *Adv Exp Med Biol* 2004;548:213–225.
9. Thom M, Eriksson S, Martinian L, Caboclo LO, McEvoy AW, Duncan JS, Sisodiya SM. Temporal lobe sclerosis associated with hippocampal sclerosis in temporal lobe epilepsy: Neuropathological features. *J Neuropathol Exp Neurol* 2009;68:928–938.
10. Pitkänen A, Nissinen J, Nairismägi J, Lukasiuk K, Grohn OH, Miettinen R, Kauppinen R. Progression of neuronal damage after status epilepticus and during spontaneous seizures in a rat model of temporal lobe epilepsy. *Prog Brain Res* 2002;135:67–83.
11. Nadler JV. Kainic acid as a tool for the study of temporal lobe epilepsy. *Life Sci* 1981;29:2031–2042.

12. Ben-Ari Y. Limbic seizures and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* 1985;14:375–403.
13. Sloviter RS. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: The "dormant basket cell hypothesis" and its possible relevance to temporal lobe epilepsy. *Hippocampus* 1991;1:41–66.
14. Houser CR, Esclapez M. Vulnerability and plasticity of the GABA system in the pilocarpine model of spontaneous recurrent seizures. *Epilepsy Res* 1996;26:207–218.
15. Buckmaster PS, Dudek FE. Neuron loss, granule cell axon reorganization and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol* 1997;385:385–404.
16. Lewis DV. Febrile convulsions and mesial temporal sclerosis. *Curr Opin Neurol* 1999;12:197–201.
17. Jensen FE, Applegate CD, Holtzman D, Belin TR, Burchfiel JL. Epileptogenic effect of hypoxia in the immature rodent brain. *Ann Neurol* 1991;29:629–637.
18. Stafstrom CE, Thompson JL, Holmes GL. Kainic acid seizures in the developing brain: Status epilepticus and spontaneous recurrent seizures. *Brain Res Dev Brain Res* 1992;65:227–236.
19. Toth Z, Yan XX, Heftoglu S, Ribak CE, Baram TZ. Seizure-induced neuronal injury: Vulnerability to febrile seizures in an immature rat model. *J Neurosci* 1998;18:4285–4294.
20. Heida JG, Teskey GC, Pittman QJ. Febrile convulsions induced by the combination of lipopolysaccharide and low-dose kainic acid enhance seizure susceptibility, not epileptogenesis, in rats. *Epilepsia* 2005;46:1898–1905.
21. Dubé CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, Andres AL, Nalcioglu O, Obenaus A, Vezzani A, Baram TZ. Epileptogenesis provoked by prolonged experimental febrile seizures: Mechanisms and biomarkers. *J Neurosci* 2010;30:7484–7494.
22. Raol YS, Budreck EC, Brooks-Kayal AR. Epilepsy after early-life seizures can be independent of hippocampal injury. *Ann Neurol* 2003;53:503–511.
23. Zhang G, Raol YS, Hsu FC, Brooks-Kayal AR. Long-term alterations in glutamate receptor and transporter expression following early-life seizures are associated with increased seizure susceptibility. *J Neurochem* 2004;88:91–101.
24. Porter BE, Maronski M, Brooks-Kayal AR. Fate of newborn dentate granule cells after early life status epilepticus. *Epilepsia* 2004;45:13–19.
25. Bender RA, Dubé C, Gonzalez-Vega R, Mina EW, Baram TZ. Mossy fiber plasticity and enhanced hippocampal excitability, without hippocampal cell loss or altered neurogenesis, in an animal model of prolonged febrile seizures. *Hippocampus* 2003;13:399–412.
26. Dubé C, Richichi C, Bender RA, Chung G, Litt B, Baram TZ. Temporal lobe epilepsy after experimental prolonged febrile seizures: Prospective analysis. *Brain* 2006;129:911–922.
27. Dubé CM, Zhou JL, Hamamura M, Zhao Q, Ring A, Abrahams J, McIntyre K, Nalcioglu O, Shatskih T, Baram TZ, Holmes GL. Cognitive dysfunction after experimental febrile seizures. Exp Neurol 2009;215:167–177.
28. Kubová H, Druga R, Lukasiuk K, Suchomelová L, Haugvicová R, Jirmanová I, Pitkänen A. Status epilepticus causes necrotic damage in the mediodorsal nucleus of the thalamus in immature rats. *J Neurosci* 2001;21:3593–3599.
29. Koh S, Storey TW, Santos TC, Mian AY, Cole AJ. Early-life seizures in rats increase susceptibility to seizure-induced brain injury in adulthood. *Neurology* 1999;53:915–921.
30. McCabe BK, Silveira DC, Cilio MR, Cha BH, Liu X, Sogawa Y, Holmes GL. Reduced neurogenesis after neonatal seizures. *J Neurosci* 2001;21:2094–2103.
31. Rakhade S, Huynh T, Marya N, Chang M, Jensen F. Development of later life spontaneous seizures in a rodent model of hypoxia induced neonatal seizures. *Epilepsia*. In press.
32. Holmes GL, Sarkisian M, Ben-Ari Y, Chevassus-Au-Louis N. Mossy fiber sprouting after recurrent seizures during early development in rats. *J Comp Neurol* 1999;404:537–553.
33. Anderson AE, Hrachovy RA, Antalffy BA, Armstrong DL, Swann JW. A chronic focal epilepsy with mossy fiber sprouting follows recurrent seizures induced by intrahippocampal tetanus toxin injection in infant rats. *Neuroscience* 1999;92:73–82.
34. Rakhade SN, Jensen FE. Epileptogenesis in the immature brain: Emerging mechanisms. *Nat Rev Neurol* 2009;5:380–391.
35. Dudek FE, Ekstrand JJ, Staley KJ. Is neuronal death necessary for acquired epileptogenesis in the immature brain? *Epilepsy Curr* 2010;10:95–99.
36. Avishai-Eliner S, Brunson KL, Sandman CA, Baram TZ. 'Stressed out?' Or in (utero). *Trends Neurosci* 2002;25:518–524.
37. Sperber EF, Stanton PK, Haas K, Ackermann RF, Moshé SL. Developmental differences in the neurobiology of epileptic brain damage. *Epilepsy Res* Suppl 1992;9:67–80; discussion 80–81.
38. Holmes GL, Khazipov R, Ben-Ari Y. Seizure-induced damage in the developing human: Relevance of experimental models. *Prog Brain Res* 2002;135:321–334.
39. Waldbaum S, Patel M. Mitochondria, oxidative stress, and temporal lobe epilepsy. *Epilepsy Res* 2010;88:23–45.
40. Sullivan PG, Dubé C, Dorenbos K, Steward O, Baram TZ. Mitochondrial uncoupling protein-2 protects the immature brain from excitotoxic neuronal death. *Ann Neurol* 2003;53:711–717.
41. Sullivan PG, Rippy NA, Dorenbos K, Concepcion RC, Agarwal AK, Rho JM. The ketogenic diet increases mitochondrial uncoupling protein levels and activity. *Ann Neurol* 2004;55:576–580.
42. Vezzani A, French J, Bartfai T, Baram TZ. Inflammation and immunity in epilepsy. *Nature Rev Neurol*. 2010, Advance online publication.
43. Sanchez RM, Dai W, Levada RE, Lippman JJ, Jensen FE. AMPA/kainite receptor-mediated downregulation of GABAergic synaptic transmission by calcineurin after seizures in the developing rat brain. *J Neurosci* 2005;25:3442–3451.
44. Rakhade SN, Zhou C, Aujla PK, Fishman R, Sucher NJ, Jensen FE. Early alterations of AMPA receptors mediate synaptic potentiation induced by neonatal seizures. *J Neurosci* 2008;28:7979–7990.
45. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat Med* 1998;4:1166–1172.
46. Raol YH, Zhang G, Lund IV, Porter BE, Maronski MA, Brooks-Kayal AR. Increased GABA(A)-receptor alpha1-subunit expression in hippocampal dentate gyrus after early-life status epilepticus. *Epilepsia* 2006;47:1665–1673.
47. Brewster A, Bender RA, Chen Y, Dube C, Eghbal-Ahmadi M, Baram TZ. Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform- and cell-specific manner. *J Neurosci* 2002;22:4591–4599.
48. Brewster AL, Bernard JA, Gall CM, Baram TZ. Formation of heteromeric hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in the hippocampus is regulated by developmental seizures. *Neurobiol Dis* 2005;19:200–207.
49. Chen K, Ratzliff A, Hilgenberg L, Gulyás A, Freund TF, Smith M, Dinh TP, Piomelli D, Mackie K, Soltesz I. Long-term plasticity of endocannabi-

noid signaling induced by developmental febrile seizures. *Neuron* 2003;39:599–611.
50. Sanchez RM, Koh S, Rio C, Wang C, Lamperti ED, Sharma D, Corfas G, Jensen FE. Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. *J Neurosci* 2001;21:8154–8163.
51. Dube C, Chen K, Eghbal-Ahmadi M, Brunson K, Soltesz I, Baram TZ. Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. *Ann Neurol* 2000;47:336–344.
52. Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med* 2001;7:331–337.
53. Roberts DS, Raol YH, Bandyopadhyay S, Lund IV, Budreck EC, Passini MA, Wolfe JH, Brooks-Kayal AR, Russek SJ. Egr3 stimulation of GABRA4 promoter activity as a mechanism for seizure-induced up-regulation of GABA(A) receptor alpha4 subunit expression. *Proc Natl Acad Sci U S A* 2005;102:11894–11899.
54. Richichi C, Brewster AL, Bender RA, Simeone TA, Zha Q, Yin HZ, Weiss JH, Baram TZ. Mechanisms of seizure-induced 'transcriptional channelopathy' of hyperpolarization-activated cyclic nucleotide gated (HCN) channels. *Neurobiol Dis* 2008;29:297–305.
55. Lund IV, Hu Y, Raol YH, Benham RS, Faris R, Russek SJ, Brooks-Kayal AR. BDNF selectively regulates GABAA receptor transcription by activation of the JAK/STAT pathway. *Sci Signal* 2008;1:ra9. Abstract.
56. McCelland S, Dubé CM, Yang J, Baram TZ. Epileptogenesis after prolonged febrile seizures: Mechanisms, biomarkers and therapeutic opportunities. *Neurosci Lett*. In press.
57. Galvan CD, Hrachovy RA, Smith KL, Swann JW. Blockade of neuronal activity during hippocampal development produces a chronic focal epilepsy in the rat. *J Neurosci* 2000;20:2904–2916.
58. Nissinen J, Lukasiuk K, Pitkänen A. Is mossy fiber sprouting present at the time of the first spontaneous seizures in rat experimental temporal lobe epilepsy? *Hippocampus* 2001;11:299–310.
59. D'Ambrosio R, Hakimian S, Stewart T, Verley DR, Fender JS, Eastman CL, Sheerin AH, Gupta P, Diaz-Arrastia R, Ojemann J, Miller JW. Functional definition of seizure provides new insight into post-traumatic epileptogenesis. *Brain* 2009;132:2805–2821.
60. Racine RJ, Burnham WM, Gartner JG, Levitan D. Rates of motor seizure development in rats subjected to electrical brain stimulation: Strain and inter-stimulation interval effects. *Electroencephalogr Clin Neurophysiol* 1973;35:553–556.
61. French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD. Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. *Ann Neurol* 1993;34:774–780.
62. Cendes F, Andermann F, Dubeau F, Gloor P, Evans A, Jones-Gotman M, Olivier A, Andermann E, Robitaille Y, Lopes-Cendes I, et al. Early childhood prolonged febrile convulsions, atrophy and sclerosis of mesial structures, and temporal lobe epilepsy: An MRI volumetric study. *Neurology* 1993;43:1083–1087.
63. Holtzman D, Obana K, Olson J. Hyperthermia-induced seizures in the rat pup: A model for febrile convulsions in children. *Science* 1981;213:1034–1036.
64. Morimoto T, Nagao H, Sano N, Takahashi M, Matsuda H. Electroencephalographic study of rat hyperthermic seizures. *Epilepsia* 1991;32:289–293.
65. Lemmens EM, Lubbers T, Schijns OE, Bulls EA, Hoogland G. Gender differences in febrile seizure-induced proliferation and survival in the rat dentate gyrus. *Epilepsia* 2005;46:1603–1612.
66. Kamal A, Notenboom RG, de Graan PN, Ramakers GM. Persistent changes in action potential broadening and the slow afterhyperpolarization in rat CA1 pyramidal cells after febrile seizures. Eur *J Neurosci* 2006;23:2230–2234.
67. Schuchmann S, Schmitz D, Rivera C, Vanhatalo S, Salmen B, Mackie K, Sipilä ST, Voipio J, Kaila K. Experimental febrile seizures are precipitated by a hyperthermia-induced respiratory alkalosis. *Nat Med* 2006;12:817–823.
68. Oakley JC, Kalume F, Yu FH, Scheuer T, Catterall WA. Temperature- and age-dependent seizures in a mouse model of severe myoclonic epilepsy in infancy. *Proc Natl Acad Sci U S A* 2009;106:3994–3999.
69. Reid AY, Galic MA, Teskey GC, Pittman QJ. Febrile seizures: Current views and investigations. *Can J Neurol Sci* 2009;36:679–686.
70. Dubé C, Vezzani A, Behrens M, Bartfai T, Baram TZ. Interleukin-1beta contributes to the generation of experimental febrile seizures. *Ann Neurol* 2005;57:152–155.
71. Dubé CM, Brewster AL, Richichi C, Zha Q, Baram TZ. Fever, febrile seizures and epilepsy. *Trends Neurosci* 2007;30:490–496.
72. Dubé C, Brunson KL, Eghbal-Ahmadi M, Gonzalez-Vega R, Baram TZ. Endogenous neuropeptide Y prevents recurrence of experimental febrile seizures by increasing seizure threshold. *J Mol Neurosci* 2005;25:275–284.
73. Koh S, Tibayan FD, Simpson JN, Jensen FE. NBQX or topiramate treatment after perinatal hypoxia-induced seizures prevents later increases in seizure-induced neuronal injury. *Epilepsia* 2004;45:569–575.
74. Kadam SD, White AM, Staley KJ, Dudek FE. Continuous electroencephalographic monitoring with radio-telemetry in a rat model of perinatal hypoxia-ischemia reveals progressive post-stroke epilepsy. *J Neurosci* 2010;30:404–415.
75. Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ. NKCC1 transporter facilitates seizures in the developing brain. *Nat Med* 2005;11:1205–1213.
76. Scharfman HE, Goodman JH, Sollas AL. Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: Functional implications of seizure-induced neurogenesis. *J Neurosci* 2000;20:6144–6158.
77. Pathak HR, Weissinger F, Terunuma M, Carlson GC, Hsu FC, Moss SJ, Coulter DA. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. *J Neurosci* 2007;27:14012–14022.
78. Becker AJ, Pitsch J, Sochivko D, Opitz T, Staniek M, Chen CC, Campbell KP, Schoch S, Yaari Y, Beck H. Transcriptional upregulation of Cav3.2 mediates epileptogenesis in the pilocarpine model of epilepsy. *J Neurosci* 2008;28:13341–13353.
79. Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. *Science* 2004;305:532–535.
80. Cilio MR, Sogawa Y, Cha BH, Liu X, Huang LT, Holmes GL. Long-term effects of status epilepticus in the immature brain are specific for age and model. *Epilepsia* 2003;44:518–528.
81. Sankar R, Shin DH, Liu H, Mazarati A, Pereira de Vasconcelos A, Wasterlain CG. Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *J Neurosci* 1998;18:8382–8393.