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Febrile Seizures and Mechanisms of Epileptogenesis: Insights from an Animal Model

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Abstract

Temporal lobe epilepsy (TLE) is the most prevalent type of human epilepsy, yet the causes for its development, and the processes involved, are not known. Most individuals with TLE do not have a family history, suggesting that this limbic epilepsy is a consequence of acquired rather than genetic causes. Among suspected etiologies, febrile seizures have frequently been cited. This is due to the fact that retrospective analyses of adults with TLE have demonstrated a high prevalence (20->60%) of a hisrory of prolonged febrile seizures during early childhood, suggesting an etiological role for these seizures in the development of TLE. Specifically, neuronal damage induced by febrile seizures has been suggested as a mechanism for the development of mesial temporal sclerosis, the pathological hallmark of TLE. However, the statisrical correlation between febrile seizures and TLE does not necessarily indicate a causal relationship. For example, preexisting (genetic or acquired) 'causes' that result independently in febrile seizures and in TLE would also result in tight statistical correlation. For obvious reasons, complex febrile seizures cannot be induced in the human, and studies of their mechanisms and of their consequences on brain molecules and circuits are severely limited. Therefore, an animal model was designed to study these seizures. The model reproduces the fundamental key elements of the human condition: the age specificity, the physiological temperatures seen in fevers of children, the length of the seizures and their lack of immediate morbidity. Neuroanatomical, molecular and functional methods have been used in this model to determine the consequences of prolonged febrile seizures on the survival and integrity of neurons, and on hyperexcitability in the hippocampal-limbic network. Experimental prolonged febrile seizures did not lead to death of any of the seizure-vulnerable populations in hippocampus, and the rate of neurogenesis was also unchanged. Neuronal function was altered sufficiently to promote synaptic reorganization of granule cells, and transient and long-term alterations in the expression of specific genes were observed. The contribution of these consequences of febrile seizures to the epileptogenic process is discussed.

Introduction: The Human Problem

Among the epilepsies, temporal lobe epilepsy is often intractable and is associated with significant morbidity in terms of cognitive and psychosocial dysfunction. The most common pathology identified in resected temporal lobe tissue from patients with intractable TLE is the constellation of mesial temporal lobe sclerosis.[6] This entity is characterized by selective neuronal loss, gliosis and synaptic reorganization in discrete regions of the hippocampal formation and related structures.[6,16,30,56] The neuroanatomical alterations of the mesial temporal lobe, and particularly the hippocampus, can be observed using sophisticated neuroimaging studies, including magnetic resonance imaging[15,17,21,33] permitting their recognition in vivo, in individual patients. The convergence of temporal lobe seizures, MRI changes and the pathological findings of mesial temporal sclerosis have been increasingly recognized as a distinct entity, mesial temporal lobe epilepsy, which is quite likely the most common of all epileptic syndromes in humans.[25]

Whereas the neuroparhological features of mesial TLE, i.e., mesial temporal sclerosis, have been defined and extensively studied for decades, the relationship of the anatomical

abnormalities to the seizures has remained controversial.[27,52,55,57,81] A significant body of evidence, including the presence of early features of mesial temporal sclerosis in young children, suggests that in some patients mesial temporal sclerosis precedes the TLE, and is thus not a consequence of the seizures.[18,31,55] This has been interpreted to suggest that the hippocampal injury is also the cause of the TLE. In contrast, progression of the hippocampal lesion on magnetic resonance imaging in individuals with TLE who were imaged repetitively, and a correlation of the hippocampal atrophy with the number of partial and generalized seizures have also been reported.[13,49,61,78] These observations are in support of the notion of induction of mesial temporal sclerosis by the seizures themselves. These conflicting views illustrate that an understanding of the causal relationship of neuronal loss in the hippocampal formation to temporal lobe seizures remains incomplete, and is further hampered by the difficulty inherent in human studies, i.e., their correlational nature.

A second striking correlation found in patienrs with TLE is the frequent history of childhood febrile seizures, and particularly prolonged ones.[72] Thus, whereas the overall frequency of febrile seizures of the general population in western countries is 2-5%,[42,74] retrospective analyses of populations with intractable TLE indicate a frequency of a febrile seizure history of 20->60%.[1,17,35,40,65] This remarkable statistical relationship has raised the hypothesis that febrile seizures—particularly complex ones (i.e., focal, prolonged or repetitive)—may produce hippocampal injury that evolves into mesial temporal sclerosis.[41,48,76,79] However, the high concordance of childhood febrile seizures in patients with mesial TLE is also consistent with a functional or structural ‘predisposing factor’ that leads independently to both conditions. Put differently, a genetically determined malformation or molecular dysfunction, or an early ‘acquired’ lesion or insult (e.g., pre- or perinatal injury or infection) may predate and actually cause both the hippocampal injury/mesial temporal sclerosis, as well as the complex febrile seizures.[8,17,18,31,52,73]

Given the high frequency of both febrile seizures and TLE, understanding the true impact of the former, and specifically their causal relationship to TLE, is of enormous clinical significance. However, studying the key questions relating to the acute and chronic effects of febrile seizures on neuronal integrity and function cannot be achieved in the human, for obvious reasons: Ethical considerations prevent the induction of febrile seizures in humans, and naturally occurring febrile seizures are typically sudden and unexpected, and rarely occur in circumstances where electrographic monitoring is possible. Funhermore, the evolution of molecular and fine structural alterations cannot be studied in the live human with currently available technology. Thus, studying febrile seizures and their consequences on the immature brain requires controlled and reproducible experiments which can only be achieved in an appropriate animal model. Here we describe such an immature rat model for prolonged febrile seizures, and discuss the contribution of data obtained using this model to the understanding of the consequences of complex febrile seizures on the developing hippocampal circuit.

The ‘Optimal’ Animal Model

Animal models used for the study of human conditions are by their nature only an approximation of the ‘real’, actual disorder. Therefore, care should be taken to define the key characteristics which are essential for any meaningful modeling of the human condition. In addition, the features and nature of a given model dictate the scope of questions that can be addressed using it. For example, substitution of hyperthermia for fever (it should be noted that hyperthermia, typically drug-induced, is a not uncommon cause of seizures in children[22,47,54]) does not permit determination of the mechanisms involved in fever generation. Thus, it is conceivable that separate models might be needed ro address different questions related to the same human problem. In addition to reproducing certain key

elements of the human condition, it is advantageous for a model to be relatively simple and inexpensive, and much should be known about the relevant brain structures. These considerations have led to the choice of rodents over primates. In the following paragraphs we discuss several other characteristics of a meaningful animal model for prolonged febrile seizures.

Age Specificity

Febrile seizures are seen almost exclusively in infants and young children, specifically between 6 months and ~ 5 years of age, with peak incidence at ~ 18 months.[42] An appropriate rat model for febrile seizures should therefore employ rats which are in a developmental age equivalent to the seizure-sensitive period in humans. However, data comparing rodent and human brain development are rare. In addition, different brain regions develop at variable rates and chronological ages, in terms of neurogenesis, migration, connectivity and function, and these processes are not necessarily parallel in human and rat. These facts are important considerations in defining the 'appropriate age' of an animal model of febrile seizures.

Early, detailed studies correlated rat and human brain development based on neuronal birth dates, myelination and saltatory growth stages, and suggested that the 5-7 day old rat may be "equivalent" to the human full-term newborn.[24,39] More selective comparative neuroanatomical scudies have focused on maturational milestones in discrete limbic regions, specifically the hippocampus and the prefrontal cortex.[7,43] Overall, these converging and complementary studies suggest that in the rat the first postnatal week may be comparable to the third trimester gestational period of the human fetus, and the second postnatal week to the first year of human life. For the hippocampal formation, specifically, we have recently summarized the information on comparative developmental milestones in human and rodent (see Table 1).[7] For example, comparison of the maturation of synaptic communication indicates that the maturational state of the hippocampus of a 8 day old rat[4,64] is roughly equivalent to the maturational state of the human hippocampus at 7 months of age (see refs. 7, 71 for analysis of hippocampal neurogenesis, connectivity and maturation of select synapses). Thus, based on anatomical data, rat hippocampal development during the second postnatal week seems to correspond best to me developmental stage at which human infants and young children are most susceptible to febrile seizures.

The ontogenetic profile of physiological responses of the developing rat brain to hyperthermia supports the end of the second postnatal week as the 'rat equivalent' of the human susceptibility period for febrile seizures: The threshold temperatures required to generate experimental febrile seizures are lowest during postnatal days 10-13, and rise rapidly thereafter[44] (Eghbal-Ahmadi & Baram, unpublished observations). Thus, because febrile seizures are a developmental phenomenon confined to infants and young children, and because limbic neuronal circuits (and particularly the hippocampal formation) are those suspected of involvement in and vulnerability to febrile seizures, models of febrile seizures should employ developing animals in which the stage of development of these limbic structures corresponds to the state of maturation of the human infant and young child, i.e., the middle of the second postnatal week in rat, and the end of the second postnatal week in mouse.

Temperature

Febrile seizures are defined as those occurring in children with fever (rectal temperarure of at least 38.4°C) but without evidence of intracranial infection.[60] In addition, it has been suggested that in most normal children, threshold temperatures for febrile seizures exceed ~ 41°C.[50] Hence, animal models for febrile seizures should involve temperatures which are

relevant to the human condition, and are observed in children with fever and should not rely on extreme temperatures (e.g., ref 59). The ability to tightly regulate the temperature is an advancage of a hyperthermia-based model compared to fever-inducing agents. In addition, the majority of established pyrogens do not induce substantive fever in the immature rat[32,51] (Hatalski & Baram, unpublished observations).

The relationship of brain and core temperatures should be carefully considered. In animal models, core temperature is routinely monitored, rather than direct measurements of brain temperatures. It should be noted that the relationship between the two may not be consistent throughout the range of temperatures induced to provoke a hyperthermic seizure.[75] This may result in core temperatures that do not reflect actual brain temperatures. Therefore, calibration and standardization of parameters that influence the relationship of core and brain temperatures: the rate of heating, and volume, direction and diffusion of the heat, should carefully be standardized. Ideally, chronic measurement of brain temperature should be employed, but this is not feasible in the immature rat.

Ascertainment and Localization the Seizures

The behaviors induced by hyperthermia may resemble those seen during seizures, but automatisms, stiffening and other motor phenomena may result from nonconvulsive discharges in brainstem, basal ganglia or other subcortical regions. Therefore, electrophysiological correlation of such observed behaviors should be obtained from behaving animals. In infant rats, hyperthermia induces stereotyped seizure behaviors[27,44] consisting of tonic body flexion accompanied by biting and chewing (‘facial myoclonus’). These are typical for seizures of limbic origin.[9,46] Electrographic correlates should therefore be sought also in limbic regions, particularly in amygdala and hippocampus. In addition to pinpointing the likely source of the seizures, electrophysiological recording also from limbic rather than only from cortical regions is justified by the normal sequence of maturation in these regions: cortical maturation is incomplete during the second postnatal week in the rat, resulting typically in poorly organized and low-voltage cortical EEG activity.[9,69] It should be noted that because EEG correlations of genuine febrile seizures in humans are exceedingly rare,[59] they are not helpful in guiding placement of electrodes in experimental animals.

Absence of Immediate Morbidity and Mortality

Febrile seizures, whether single, short and nonfocal (simple) or recurrent, more prolonged or focal (complex) are typically not associated with immediate morbidity or mortality. Therefore, an appropriate animal model of febrile seizures should demonstrate similar low morbidity and mortality. In addition, a fundamental question related to hyperthermic seizures in the young human is whether they result in long-term effects, i.e., loss of hippocampal neurons and/or alteration of hippocampal circuitry leading to epilepsy. Therefore, an optimal model should be suitable for long-term survival without the confounding effects of major stress, burns, infection or general moribund states. An optimal model should use a defined, benign mechanism for increasing brain and core temperatures, which is suitable for repeated exposures. In summary, benign outcome not only reproduces the human situation, but permits meaningful prospective long-term studies of the long-term consequences of these seizures on epileprogenesis and neuronal function in general.

Hyperthermic Controls

The goals of setting up models of febrile seizures are to study the mechanisms or the outcomes of these seizures. However, by definition, each model involves subjecting me brain to hyperthermia, to simulate fever. Therefore, the effects of the hyperthermia per se must be distinguished from those of the associated seizures. The potential effects of

hyperthermia may not be inconsequential Hyperthermia induces a significant number of genes (e.g., heat shock proteins), and both deleterious and protective effects on neuronal function and integrity. For example, hyperthermia enhances seizure severity and the neuronal loss induced by kainic acid,[53] and when extreme, results in neuronal injury by itself.[38] Therefore, experiments using febrile seizure models should compare three sets of animals: normothermic controls, hyperthermic controls, in which hyperthermia was induced, but seizures were prevented[14,19,27,76] and the experimental group which has experienced both hyperthermia and seizures.

The Immature Rat Model

Based on the criteria outlined above, a rat model for prolonged febrile seizures was developed:[27,28,78] In this model, 10-11 day-old Sprague-Dawley rats are subjected to hyperthermia which raises body and brain temperatures gradually via a regulated stream of moderately heated air (using a hair-dryer, low-intermediate settings, air temperature ~43°C). The air stream is directed ~ 30 cm above the rats, which are placed (1-2 at a time) on a towel in a 3 liter glass jar. Core temperatures are measured prior to initiating the hyperthermia, then every two minutes as well as at the onset of hyperthermia-induced seizures. These core temperatures have been extensively correlated with brain temperatures (Eghbal-Ahmadi and Baram, unpublished observations). In over 400 animals, we have found that raising core and brain temperatures to an average 40.88°C resulted in behavioral seizures in over 98%.

As mentioned above, the behavioral seizures are stereoryped, consisting of arrest of the heat-induced hyperkinesis, body flexion and biting of an extremity, occasionally followed by clonus. The epileptic nature of these seizures was confirmed by electrophysiological recording from the hippocampi of behaving pups, using bipolar electrodes. As shown in (Fig. 1), hyperthermia induces a change in hippocampal activity from a nonrhythmic pattern in the theta range (Fig. 1A) to the onset of rhythmic epileptiform spikes (Fig. 1B), which correlate wim the onset of behavioral seizures. In hyperthermic controls, given the rapid- and short-acting barbiturate pentobarbital prior to the procedure, the behavioral as well as the electrophysiological seizures are blocked.

Animals are maintained hyperthermic (39-41.5°C) for 30 minutes, which is designed to generate seizures lasting about 20 minutes. This reproduces the human condition of prolonged, or complex febrile seizures (defined as longer than 15 minutes, and comprising only ~ 10% of all febrile seizures.[12] It is these longer seizures which have been statistically implicated in the development of TLE.[5,12,76] The 20-minute duration also avoids the onset of status epilepticus (defined as continuous seizures for 30 minutes), which may carry distinct implications for outcome (see refs. 2, 23). Following the hyperthermia, animals are moved to a cool surface to regain normal body and brain temperatures, then returned to their mothers for rehydration. It should be noted that weighing the animals before and after the procedure indicates little evidence of dehydration < 3% change in body weight). Furthermore, animals regain normal activity rapidly after the procedure, and mortality has been <1%.

In summary, the immature rat model described above reproduces key features of prolonged febrile seizures, those that have been statistically correlated with the development of epilepsy and/or the presence of mesial temporal sclerosis. Using this model, we have started addressing the question of whether the relationship of these prolonged febrile seizures to neuronal loss and hyperexcitability is causal. In other words, the experiments described below query whether prolonged experimental febrile seizures cause epilepsy. Furthermore, if these seizures do induce hippocampal hyperexcitability, what are the underlying mechanisms?

Do Prolonged Experimental Febrile Seizures Increase Seizure Susceptibility?

To determine whether the susceptibility to seizures is altered after prolonged febrile seizures, rats were allowed to mature (three months), and then underwent extensive hippocampal-electrophysiology and behavioral seizure monitoring.[27] Both the hippocampal tracings and the behavioral measures to date have failed to demonstrate the occurrence of spontaneous seizures. However, when challenged with a sub-convulsant dose of the AMPA/kainate-type glutamate receptor agonist kainic acid, adult animals which had sustained developmental febrile seizures were far more sensitive than age-matched controls to the development of further seizures. In essence, a dose that failed to provoke seizures in normothermic and hyperthermic littermate controls led to severe seizures in all adult animals which had sustained prolonged experimental febrile seizures early in life (Fig. 2), demonstrating a ~four-fold increased sensitivity to kainic acid. This increased susceptibility to limbic convulsants was confirmed in vitro:[27] Spontaneous epileptiform discharges were not observed in hippocampal-entorhinal cortex slices derived from either control or experimental groups. However, Schaffer collateral stimulation induced prolonged, self-sustaining, status-epilepticus-like discharges exclusively in slices from experimental rats. These data indicate that experimental prolonged febrile seizures do not cause spontaneous limbic seizures during adulthood. However, they induce persistent enhancement of hippocampal excitability that may facilitate the emergence of subsequent seizures in response to even a mild (and perhaps not clearly demonstrable in the human situation) trigger later in life.

Do Prolonged Experimental Febrile Seizures Cause Neuronal Death and/or Synaptic Reorganization?

Neuronal loss and resulting changes in hippocampal circuitry (e.g., mossy fiber sprouting) in specific hippocampal subfields are characteristic of mesial temporal sclerosis in patients with TLE (reviewed in refs. 6, 45). The loss of seizure-sensitive neuronal populations can critically alter the balance of excitation and inhibition in the hippocampus, which may lead co long-term hyperexcitability and a reduced seizure threshold later in life, as indeed found in this model of prolonged febrile seizures. Therefore, we studied the short- and long-term effects of experimental febrile seizures on neuronal survival and synaptic connectivicy.

Acute neuronal death was studied using the in situ end labeling (ISEL) technique for visualizing apoptotic cell death, as well as using the Gallyas silver stain method (“dark” neuron[36]) for visualizing neuronal injury. ISEL demonstrated no evidence for acute neuronal death in the hippocampus when studied 1, 4, 8.5, 24 or 48 hours after the seizures. However, the seizures did impact neuronal structure: the Gallyas method demonstrated dark, argyrophilic neurons starting within 24 hours and lasting as long as two weeks after the seizures. Whereas the precise mechanisms which render neurons argyrophilic are not known, selective uptake of the silver stain is considered to arise from alterations in proteins constituting the cytoskeleton. These changes have often been suggested to signify cell death. However, such ‘dark’ neurons can also be generated by subjecting the brain to postmortem trauma, indicating that this process is independent from the process of cell death.[37] Indeed, neuronal counts carried out in the central nucleus of the amygdala, where ~30% of neurons became silver-stained after experimenral febrile seizures, demonstrated no loss of cells.[78] These findings suggest that the onset of the avidity to silver may not necessarily mean neuronal death: the changes or injury which render a cell argyrophilic may be reversible and not lead to cell loss.

That experimental prolonged febrile seizures do not cause neuronal cell death was further confirmed in a long-term study,[11] in which neuronal densities in the hippocampal formations of seizure-experiencing animals and age-matched controls were analyzed three months after the seizures. No difference was evident in the neuronal numbers of specific, seizure-sensitive hippocampal cell populations of these experimental groups. However, the density of the mossy fibers, the axons of granule cells, in granule cell and molecular layers was significantly increased in seizure-experiencing compared to control rats 3 months after the seizures. These findings indicate that despite the absence of seizure-induced neuronal loss, reorganization of the hippocampal circuit, evident by mossy fiber sprouting, did occur.

Do Prolonged Experimental Febrile Seizures Alter the Rate of Granule Cell Neurogenesis?

Altered neurogenesis of dentate gyrus granule cells, promoting aberrant, excitatory connectivity in the hippocampus, has recently been proposed as an additional mechanism by which seizures can modulate the hippocampal network.[63] Seizure-induced neurogenesis may be particularly disruptive during hippocampal development, since neurogenesis in the dentate gyrus peaks during the first and second postnatal weeks.[3,70] Therefore, we examined the influence of experimental prolonged febrile seizures on granule cell proliferation: Rats experiencing experimental febrile seizures and age-matched controls were injected with BrdU 3, 7 or 28 days after the seizures, and numbers of BrdU-labeled cells were determined 48 hrs later. No differences were found between seizure-experiencing and control animals at any of the time-points studied.[11] Thus, although granule cell neurogenesis in the immature hippocampus may be influenced by seizures during development.[11,58,66] prolonged febrile seizures had no significant effect on this process. This might be due to the relatively short duration of these seizures, or to other, as yet unresolved model-specific factors.

Molecular Plasticity after Experimental Prolonged Febrile Seizures

Electrophysiological analyses in acute hippocampal slices from seizure-experiencing rats revealed a surprising result: Despite the increased network hyperexcitability in the hippocampus, the inhibitory perisomatic drive onto CA1 pyramidal cells was increased, rather than decreased.[19] Conversion of enhanced, GABA-mediated hyperpolarization into neuronal depolarization may be mediated by activation of the hyperpolarization-activated, cyclic nucleotide-gated (I_h) current.[26,62] This led to the hypothesis that the I_h-current was altered after prolonged febrile seizures.[20,77] Indeed, whole-cell patch clamp recordings from CA1 pyramidal cells demonstrated that the biophysical properties of the I_h-current were altered by the experimental febrile seizures.[20] In slices from seizure-experiencing rats, the I_h-current was activated and deactivated much more slowly, and its half-maximal activation (V_{50}) was shifted towards a more depolarized membrane potential. Both changes opposed the increased presynaptic hyperpolarizing input, and could convert it to a depolarizing overshoot and action potential burst firing.[20,77] These changes persisted for at least 3 months.

In teasing out the mechanisms which might mediate these changes of the I_h-current, it was found that, unlike typical short-term modulation of the properties of the current, they did not depend on alteration of cellular cyclic nucleotides. This led to the notion that this long-lasting alteration of the I_h-current might derive from transcriptional regulation of the molecules which constitute the h-channels: The I_h-current is generated by a specific type of channels, the hyperpolarization-activated cyclic nucleotide-gated cation channels (HCNs). Recently, four different genes encoding HCN isoforms (HCN1-4) have been discovered, each isoform forming channels with significantly differing physiological properties (reviewed in ref. 67). Three of these isoforms (HCN1, HCN2, HCN4) are expressed in CA1

pyramidal cells of the immature rat during the age when febrile seizures can be provoked.[10] The relative abundance of each of these isoforms in a given cell has been shown to be critical for the physiological properties of the channels, which, in turn, govern the overall HCN properties of the cell[34,68] In CA1 pyramidal cells, the HCN1 isoform, forming fast activating and deactivating channels with limited conductance, seems to be dominant under normal conditions.[34,68] However, recent results indicate that prolonged experimental febrile seizures—occurring during a period of rapid evolution of the HCN isoform expression pattern[10]—influence the mRNA and protein expression of these channel molecules. The expression of HCN1 mRNA was significantly decreased and the expression of HCN2 mRNA significantly increased in seizure-sustaining animals by one week later.[14] Both of these changes increase the relative abundance of HCN2 compared to HCN1 in a given neuron, favoring the formation of slower kinetics (and potentially larger-conductance) HCN2-homomeric channels, with altered biophysical properties. This alteration in the molecular make-up of the HCNs would promote neuronal activity-dependent depolarization and enhance the excitability of the hippocampal circuit.

Summary

What Has the Immature Rat Model of Prolonged Febrile Seizures Taught Us so Far?

The original working hypotheses driving these studies of experimental febrile seizures suggested that these seizures would either kill vulnerable neurons, or lead only to transient injury, without long-term effects on the hippocampal circuit. As evident from the data above, both hypotheses were refuted. The scenario emerging from the experimental data indicates that the process of epileptogenesis in the immature rat—the transformarion of a ‘normal’ limbic network to a pro-epileptic one—is far more intricate and subtle than a simple composite of direct or compensatory changes in response to cell death.

Indeed, the data cearly indicate that experimental prolonged febrile seizures do not result in death of neurons in amygdala and hippocampus. Vulnerable populations, such as the mossy cells or specific interneuronal subtypes, were specifically labeled and counted, and no loss or reduction in their numbers were found.[11] The preservation of neuronal numbers was not due to the birth of new neurons, since BrdU analyses demonstrated that the rate of neurogenesis was not altered.

However, prolonged experimental febrile seizures were not “benign”. In the aftermath of the seizures, the hippocampus was far more susceptible to minor excitatory input (electrical current in the slice, kainic acid in vivo) compared with a hippocampus not previously involved in febrile seizures. These striking and long-lasting changes rendered the animal more likely to generate seizures. Thus, pro-epileptogenic changes may occur without the requirement for neuronal death.

Insight into the mechanisms contributing to the hyperexcitability resulting from prolonged experimental febrile seizures was derived from electrophysiological and molecular analyses, which, to date, have provided specific clues. Early (within hours of the seizures) regulation of calcium entry is modified, due to transient reduction of GluR2 expression and creation of calcium-permeable AMPA channels.[29] By several days after the seizures, striking changes in ion channels, specifically in the molecular make-up—and hence the kinetics and voltage-dependence—of the HCNs, emerge and persist long-term. These contribute significantly to conversion of augmented GABA-induced hyperpolarization to activity-dependent hyperexcitation. Many questions remain: How do the seizures lead to down-regulation of GluR2? What other critical alterations result from altered calcium entry? What are the mechanisms governing HCN expression in a spatially and temporally constrained pattern?

These and related questions are the focus of ongoing studies. These studies are carried out in the hope that they will lead to further clues and to the discovery of the specific molecular targets, the key determinants, of this seizure-induced long-term hyperexcitability. It is the discovery of such specific molecular targets which could lead to the design of compounds which will specifically prevent the consequences of the seizures, and thus perhaps prevent these pro-epileptogenic effects of prolonged febrile seizures.

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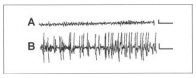

Figure 1.

Electrophysiological characteristics of hyperthermia-induced seizures in immature rats. Records were performed via bipolar hippocampal electrodes in freely-moving 11-day-old rats. A) Baseline tracing of hippocampal activity during normothermia, showing a nonrhythmic pattern in the theta range. B) The hyperthermia procedure provoked hippocampal electrographic seizures, manifest as trains of spike-waves. Calibration: vertical, 50 mV; horizontal 1 sec.

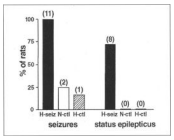

Figure 2.

Differential induction of seizures and of status epilepticus in adult rats by low-dose kainic acid as a function of prolonged hyperthermic seizures early in life. Kainic acid led to seizures in all adult rats that had experienced prolonged hyperthermic seizures on postnatal days 10-11 (H-seiz; n=11). The majority of these (n=8) developed status epilepticus. In contrast, only 2 out of 8 normothermic (N-ctl) and 1 out of 6 hyperthermic control rats developed brief seizures, none of them status epilepticus (reproduced from ref. 27, with permission).

Table 1

Selected milestones in hippocampal development: Human and rat

Category	Event	Human	Rat
General	Maximal growth velocity	2-3 postnatal months	8-12 postnatal days
	Hippocampal volume approximates that of adult	10 postnatal months	11-30 postnatal days
	Hippocampal-dependent learning/ memory function	4-5 postnatal years	15-16 postnatal days
Neuronal	Birth of pyramidal cells	1st half of gestation	2nd half of gestation
Formation	Onset:	before 15th week	~15th day
	End:	24th week	~19th day
	Birth of dentate gyrus (DG) granule cells	~70% prenatal (majority by 34th week)	~85% postnatal (majority by 1st postnatal month)
	Onset:	13-14th weeks	~18th prenatal day
	End:	throughout life	throughout life
Differentiation, Synaptogenesis	'Thorny excrescences' on proximal CA3 pyramidal cell dendrites.	postnatal years	1st postnatal month
	Onset:	3-7 months	~9th day
	Maturation:	3-5 years	21st day
	Pedunculate spines: distal CA3 pyramidal cell dendrites	postnatal years	1st postnatal month
	Onset:	birth	7th day
	Maturation:	3-5 years	21st day
	Peak synapse overshoot (DG)	1-2 postnatal years	9-14 postnatal days
	Synapse density reaches adult levels: DG molecular layer	7-10 postnatal months	21st postnatal day
	Period of maximal mossy cell differentiation (DG)	7-30 postnatal months (adult-like by 5 years)	7-14 postnatal days (adult-like by 14th day)
Afferent input	Entorhinal cortex toDG	Prenatal: 19-20th weeks	17th prenatal day
	Supramammillary afferents reach juxtagranular and CA2 pyramidal cell layers	Prenatal: ~20th week	Presumed 1st postnatal week

Note: Gestation lasts 270-280 and 21 days in human and rat, respectively.

Modified from ref. 7, with permission.