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Serial MRI after Experimental Febrile Seizures: Altered T2 Signal without Neuronal Death

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Abstract

Whereas most febrile seizures (FSs) carry a benign outcome, a subpopulation of individuals with prolonged FSs are at risk for later temporal lobe epilepsy. Signal changes on magnetic resonance imaging (MRI) may provide early markers for changes in neuronal integrity that may promote epileptogenesis in such individuals. Here, we used serial MRIs, obtained before and at several time points after experimental prolonged FSs, to determine the prevalence and distribution of signal changes on T2-weighted images and to investigate the pathological substrates leading to these changes. Seventy-five percent of immature rats with experimental prolonged FSs had abnormal T2 signal enhancement at 24 hours, and 87.5% at 8 days after the seizures. The altered T2 values involved the dorsal hippocampus (75%), the piriform cortex (87.5%), and the amygdala (25%). However, these changes were not accompanied by evidence of neuronal injury or death in these regions, as assessed using the Fluoro-Jade method. Thus, experimental prolonged FSs lead to relatively frequent abnormal MRI signal in “temporal lobe” structures. Although these changes do not signify cell death, they may denote pathological cellular processes that promote epileptogenesis.

The consequences of prolonged febrile seizures (FSs) in young children have not been fully resolved. Although epidemiological studies suggest that most children have a benign outcome and no subsequent epilepsy¹ (and see Hesdorffer and Hauser² for a recent review), an increased risk of epilepsy has been reported.^{2,3} In addition, retrospective analyses of adults with temporal lobe epilepsy document a high prevalence of a history of prolonged FSs during childhood (eg, Cendes and colleagues⁴ and French and colleagues⁵). These data suggest that a subpopulation of children with prolonged FSs may be at risk for adverse consequences. These are potential candidates for targeted intervention in the future.

Markers for this “at-risk” population are lacking. The ideal marker for potential sequelae of prolonged FSs should be quantifiable and amenable to repeated assessments, so that any progression can be measured. It should also discriminate individuals with evolving disease from those who will have no sequelae. Magnetic resonance imaging (MRI) changes in seizure-prone brain regions may be an attractive tool because imaging can be repeated. Indeed, human studies have demonstrated acute hippocampal MRI changes typical of neuronal injury (increased signal on T2-weighted images), followed by a volume loss and MRI evidence of mesial temporal sclerosis⁶ in children with prolonged FSs.⁷ However, several of these children had other known or suspected predisposing factors or had

experienced prolonged febrile status epilepticus (median, 99 minutes). Thus, the prevalence of MRI changes after prolonged FSs and the nature of the responsible cellular mechanisms have remained unknown.

Therefore, we used longitudinal MRI studies to study signal changes evoked by experimental prolonged FSs in an immature rodent model. We eliminated the possibility of significant pre-existing abnormalities by imaging all subjects before inducing the seizures and obtained MRIs several times after seizures. Finally, we correlated MRI changes with sensitive analyses of neuronal injury and death.

Materials and Methods

Animals and Induction of Experimental Febrile Seizures

Sprague-Dawley-derived rats were born and maintained in quiet facilities under controlled temperatures and light schedule. Experimental procedures were approved by Institutional Animal Care Committees and conformed to NIH guidelines. Prolonged experimental FSs were elicited as described.^{8–10} In brief, on postnatal day (P) 11, pups were placed in a glass container, and their core temperature was raised using a regulated stream of heated air to approximately 41°C (simulating high fever). Core temperatures were measured at baseline, seizure onset, and every 2 minutes during the seizures and maintained in a narrow range. Hyperthermia was induced for 30 minutes resulting in 20-to 22-minute seizures. The behavioral seizures in this paradigm are stereotyped, consisting of arrest of heat-induced hyperkinesis and facial automatisms, often followed by body flexion. They correlate with electrographic hippocampal seizures.^{9–11}

Magnetic Resonance Imaging Procedure

MRIs were performed on a 4T scanner Picker console (Marconi/Philips Medical, Cleveland, OH). Baseline scans were performed on P10, in five control rats and eight in which seizure were evoked on P11. All animals then were imaged on P12 and 7 to 8 days later. Age-matched littermate controls served to account for potential maturational changes in the MRI signal.

Rats were anesthetized (pentobarbital, 40mg/kg, IP) 30 minutes before acquisitions. For each scan, a pair of rats was positioned prone inside the radiofrequency coil on a handmade support, along with several phantoms (distilled water and mixed water-gadolinium). Sagittal and coronal localizing acquisitions were obtained using a fast spin-echo sequence (repetition time [TR]: 3,000 milliseconds; echo time [TE], 104 milliseconds; eight echoes; field of view [FOV], 120mm; acquisition matrix size, 128 × 256). Brains then were scanned coronally: posterior cuts corresponded to A −0.4 to −0.1mm (referring to bregma¹²) for entorhinal cortex visualization; most anterior cuts corresponded to A −5.5 to −5.6 mm, at the septal level, and five to six contiguous 1.1mm slices were imaged. T2-weighted anatomical images were acquired using fast spin-echo: TR, 5,500 milliseconds; TE, 105 milliseconds; eight echoes; FOV, 65mm; matrix size, 128 × 256; eight excitations.

Magnetic Resonance Imaging Analysis

Images were analyzed without knowledge of group (“blindly”): regions of interest, delineated manually from anatomical images,¹² included dorsal and ventral hippocampus, amygdala, piriform and entorhinal cortices, and medial ventroposterior thalamic nucleus. T2-weighted signal *intensity* was determined using standards calibrated using “phantoms” of water and water-gadolinium mixtures falling within, as well as outside of, the range of observed values. The ratio of intensity of the structure of interest and a reference structure

(corpus callosum) then was calculated, permitting normalization among sections and animals.

Determination of Neuronal Injury

A total of 39 animals (not those undergoing MRI) were analyzed for the presence of neuronal death. Because the time course of injury visualizable by Fluoro-Jade¹³ in the immature rat typically commences at 24 hours, peaks at 3 to 4 days, and dissipates after a week,^{14,15} eight animals were perfused at 24 hours, five at 48 hours, six at 4 days, and eight at 7 days and compared with three controls per time point. Animals were perfused transcatheterially with saline followed by buffered 4% paraformaldehyde under pentobarbital anesthesia.¹⁰ Brains were cryoprotected and sectioned coronally (30µm), and sections were processed for Fluoro-Jade visualization of neuronal death.^{13,15}

Statistical Considerations

Seizure-experiencing animals were compared with age-matched controls. Effects of the seizures and of age (maturation) were evaluated using analysis of variance (ANOVA), with post hoc tests as indicated. Significance was set at *p* value of 0.05, and values are reported as means ± SEM.

Results

Prolonged Experimental Febrile Seizures Induce Acute T2 Signal Enhancement in Limbic Regions of Immature Rat Brain

Prolonged experimental FSs led to increased T2 signal in 75% of the rats 24 hours later, compared with age-matched controls. In general, signal changes were more pronounced at 8 days, distinguishing 87.5% of the seizure group (seven of eight animals) from their age-matched controls (Figs 1 and 2). These signal changes involved dorsal hippocampus, amygdala, and piriform cortex (see Fig 1) and were quantitatively analyzed (see Fig 2). In dorsal hippocampus, seizures increased T2 signal in a single animal at 24 hours and caused significant increase in T2 signal of the aggregate seizure group at 8 days (*p* < 0.01, Bonferroni's post hoc test; see Fig 2A). At that time point, the ratio of T2-weighted signal intensity in hippocampus over that of corpus callosum exceeded "1" only in rats experiencing seizures (see Fig 2F).

An effect of the seizures, with significant increases of signal intensity, was found also for other limbic regions including piriform cortex (see Fig 2B) and the medial ventroposterior thalamic nucleus (see Fig 2C). In amygdala, variability among seizure-experiencing animals was notable. Striking enhancement of T2 signals occurred in two seizure animals at both 24 hours and 8 days (see Fig 1). In these animals at 8 days, normalized signal was 1.34 ± 0.03 versus 1.16 ± 0.05 in the controls (*p* = 0.08; see Fig 2D). Limbic regions without seizure-evoked T2 signal changes included entorhinal cortex (see Fig 2E) and ventral hippocampus (not shown).

T2 Signal Intensity Changes in Limbic Regions Do Not Result from Neuronal Death

Enhancement of T2 signal on MRI within hours or days of a neurological insult is generally considered to indicate increased tissue water content (edema), associated with neuronal injury that may progress to death. Such progression from enhanced T2 signal to subsequent tissue atrophy and cell loss has been documented in animal models of status epilepticus.^{16–18} Therefore, we studied whether cell injury and death occurred in immature rats after experimental prolonged FSs, specifically in regions demonstrating altered T2 MRI signal. We used the Fluoro-Jade method, shown by several groups to sensitively detect irreversible injury of individual neurons^{13–15} and validated its use by concurrently analyzing

sections from adult rats where excitotoxic death was provoked by kainic acid–induced status epilepticus.

Prolonged, kainic acid–provoked limbic seizures in adult rats resulted in the typical pattern of limbic cell death,^{19,20} which was well visualized using Fluoro-Jade in hippocampal cornu ammonis 3 (CA3) region pyramidal cells, piriform cortex, and basolateral amygdala (Fig 3A–C). In contrast, prolonged experimental FSs in immature rats did not lead to observable neuronal injury either at 24 hours (see Fig 3D–F) or at other time points (not shown). These findings suggest that profound neuronal injury, such as detected by Fluoro-Jade and similar methods^{8,14} is not a prerequisite for the occurrence of T2 signal intensity changes. They also affirm the relative resistance of limbic structures of infant rats to seizure–provoked cell injury.^{8,15,21}

Discussion

The major findings of these studies are (1) prolonged experimental FSs induce increased signal intensity on T2-weighted MRI 24 hours and 8 days later; (2) the mechanisms for these MRI changes do not involve cell death but are indicative of transient injury. Importantly, these changes may constitute a marker that might be helpful for teasing out divergent outcomes of prolonged FSs, or for an epileptogenic process.

Prolonged experimental FS-induced T2-weighted signal changes in limbic regions, including dorsal hippocampus, amygdala, and piriform cortex. Behavioral manifestations and electrographic activity of these seizures involve these same areas. Interestingly, although the neuroanatomical location of circuits involved in human FSs are not known, increased T2 signal in the hippocampus has been reported in some children after prolonged complex FSs and have been interpreted as acute edema,⁷ a notion supported by transient increased volume (swelling) of the hippocampal formation in these children. Whereas such quantitative volume measurements of hippocampus and amygdala are feasible in humans,^{4,6,22,23} the resolution of the T2-weighted images obtained from the 4T magnet did not permit us to perform them in infant rats. Our attempts to demonstrate increased water content in hippocampus and amygdala of immature rats experiencing seizures using trypan blue were not successful.

However, the experimental model permitted us to assess the mechanisms for the T2 signal changes, which is not possible for acute changes in the human. Although Briellman and colleagues⁶ correlated *chronic* T2 signal changes on MRI with neuronal loss and the neuropathological features of mesial temporal sclerosis, the degree of neuronal injury underlying *acute* T2 signal changes has remained unclear. Here, we found that significant T2 signal increases 24 hours and 8 days after experimental FSs were not associated with irreversible neuronal injury or death, in contradistinction to the effect of limbic seizures on mature rats. Fluoro-Jade detected irreversible cell injury in our adult tissue and has been shown to visualize it in P12 rats.¹⁴ In our hands, this method is less sensitive to transient structural changes in vulnerable cell populations, which can be detected in immature animals using modified silver stains.⁸ The absence of Fluoro-Jade–labeled “dying” neurons also supports our previous studies demonstrating that experimental prolonged FSs do not lead to “dropout” of neuronal populations shown to be vulnerable to seizure–induced death in several adult limbic seizure models.^{8,10} Thus, the T2 signal increases found in hippocampus after experimental prolonged FSs likely represent reversible changes. In this context, both evolution of the acute MRI changes into atrophy^{24–26} and their resolution after prolonged seizures have been reported in human studies.^{21,27,28}

In summary, these studies demonstrate frequent T2 signal changes in limbic regions after experimental prolonged FSs in immature rats. These commence already at 24 hours, are clearer a week later, and do not signify neuronal death. Future studies will determine whether they are associated with an epileptogenic process.

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Fig 1.

Signal intensities on T2-weighted magnetic resonance images (MRIs) are increased in rats subjected to experimental prolonged febrile seizures (FSs). MRIs were obtained 24 hours and 8 days after the seizures and compared with those of age-matched controls. T2-weighted images were acquired using a 4T magnet, using TR, 5,500 milliseconds, TE, 105 milliseconds (see Materials and Methods for further details). Both the control and the seizure group were imaged before the seizures on postnatal day [P] 10, to exclude preexisting injury and provide baseline intensities, and on P12 (24 hours after seizures in the seizure group) as well as on P18 to P19. Regions used for analysis are delineated in red in the “baseline” image. Increased signal intensity is evident in the dorsal hippocampus (*red arrows*), piriform cortex (*red arrowheads*), and amygdala (*asterisk*).

Fig 2.

Quantitative analysis of the effects of experimental prolonged febrile seizures (FSs) on T2 signal intensity. (A) In dorsal hippocampus, a significant increase of T2 signal occurred by 8 days after the seizures (ANOVA with Bonferroni's post hoc test). (B) In piriform cortex, a significant overall effect of the seizures was found in six of eight animals, (two-way ANOVA; $F = 5.2$; $p = 0.03$), and signal intensity increased significantly at 24 hours and 8 days. (C) A significant effect of the seizures ($F = 5.3$; $p = 0.028$; two-way ANOVA) was found also in the medial ventroposterior thalamic nucleus (eight of nine animals), with increased signal at the 8 days time point ($p = 0.02$) and a strong trend for increased signal at 24 hours (*diamond*, $p = 0.09$). (D, E) No significant effect of the seizures on T2 signal intensities occurred in the amygdala (D) or the entorhinal cortex of the whole group (E). (F) An example of the T2 intensity values and of the method used to calculate and normalize T2 signal intensities in the developing rat. As shown graphically for the dorsal hippocampus, for each animal at every age, signal in the region of interest and of the corpus callosum (reference region) was measured. Intensity ratios then were derived: signal in region of interest/signal in the corpus callosum. Note that this ratio exceeded "1" only in P18 to P19 rats that had sustained experimental febrile seizures a week earlier. Bars represent group means $\pm$ SEM (except panel F); (*asterisk*) $p < 0.05$.

Fig 3.

Neuronal injury/death is not detectable in hippocampus, amygdala, or piriform cortex of immature rats that had experienced experimental prolonged febrile seizures (FSs), whereas such injury can be visualized using Fluoro-Jade after prolonged seizures in adult rats. (A–C) Coronal sections from adult rats that had sustained prolonged seizures induced by kainic acid (because experimental prolonged FSs do not occur in adults). Animals were perfused 24 hours after the seizures, and brains were cut and subjected to the Fluoro-Jade method for visualizing irreversible neuronal injury¹³ as described in Sullivan and colleagues.¹⁵ Abundant cell injury/death (arrow) is apparent in hippocampal cornu ammonis 3 region (CA3) (A), piriform cortex (B), and basolateral amygdala (C). (D–F) In contrast, coronal sections from immature rats experiencing prolonged FSs and perfused 24 hours (n = 8; see Materials and Methods for other time points) or 2, 4, or 7 days later (not shown) did not contain Fluoro-Jade–labeled neurons in regions where increased T2 signal was consistently found. sp = stratum pyramidale; sr = stratum radiation; so = stratum oriens; BL = basolateral.