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Regular Article

T2 relaxation time post febrile status epilepticus predicts cognitive outcome

Jeremy M. Barry [a,b,*,1], ManKin Choy [c,1], Celine Dube [c], Ashlee Robbins [b], Andre Obenaus [e], Pierre Pascal Lenck-Santini [a], Rod C. Scott [a,d], Tallie Z. Baram [c], Gregory L. Holmes [a]

[a] Department of Neurological Sciences, University of Vermont College of Medicine, Burlington, VT, United States
[b] Department of Neurology, Geisel School of Medicine at Dartmouth, Hanover, NH, United States
[c] Department of Neurology, University of California-Irvine, Irvine, CA, United States
[d] Department of Neurology, University College London, Institute of Child Health, United Kingdom
[e] Department of Radiation Medicine, Loma Linda University, Riverside, CA, United States

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ABSTRACT

Evidence from animal models and patient data indicates that febrile status epilepticus (FSE) in early development can result in permanently diminished cognitive abilities. To understand the variability in cognitive outcome following FSE, we used MRI to measure dynamic brain metabolic responses to the induction of FSE in juvenile rats. We then compared these measurements to the ability to learn an active avoidance spatial task weeks later. T2 relaxation times were significantly lower in FSE rats that were task learners in comparison to FSE non-learners. While T2 time in whole brain held the greatest predictive power, T2 in hippocampus and basolateral amygdala were also excellent predictors. These signal differences in response to FSE indicate that rats that fail to meet metabolic and oxygen demand are more likely to develop spatial cognition deficits. Place cells from FSE non-learners had significantly larger firing fields and higher in-field firing rate than FSE learners and control animals and imply increased excitability in the pyramidal cells of FSE non-learners. These findings suggest a mechanistic cause for the spatial memory deficits in active avoidance and are relevant to other acute neurological insults in early development where cognitive outcome is a concern.

Introduction

Febrile seizures (FS) are the most common type of seizures seen in young children occurring in 2–5% of children before the age of 5 years (Huang et al., 1999; Shinnar and Pellock, 2002). Epidemiological clinical studies suggest that most children with FS have normal development and intelligence (Annegers et al., 1987; Chang et al., 2001; Verity et al., 1998) while some children with prolonged FS appear to be at risk for long-term mild cognitive disturbances (Chang et al., 2001; Epstein et al., 2012; Martinos et al., 2012, 2013). Why some children with prolonged FS are predisposed to cognitive deficits is unknown.

Prospective imaging studies in children with prolonged FS have identified early hippocampal edema, within 48 h of the event, as a common finding (Scott et al., 2003; Scott and Neville, 2009; Shinnar et al., 2012; VanLandingham et al., 1998). Although these findings appear to predict hippocampal volume and growth (Lewis et al., 2014; Yoong et al., 2013) it remains uncertain whether these findings predict cognitive disruption. The long follow-up timescale required for human studies has led to the development of animal models to address whether neuronal function within the hippocampal circuit might be permanently affected by a single bout of febrile status epilepticus (FSE), and if these changes can be predicted early in the course of events that follow FSE (Choy et al., 2014; Dube et al., 2004, 2009, 2010; Jansen et al., 2008).

In early animal studies, MR imaging shortly after the seizures failed to demonstrate predictive value for cognitive or epileptogenic outcomes (Dube et al., 2004, 2009, 2010; Jansen et al., 2008). However, these studies were conducted on low-magnetic field MRI scanners. Dube et al. (2009) carried out MRI studies using a higher field magnet (7 T) and showed that increases in T2 relaxation time 1 month following FS can serve as a putative surrogate marker associated with moderate spatial deficits in a sub-population of animals that had experienced FS. In this timescale the T2 time is a marker of long-term brain modification by FS but does not give insight into the dynamic pathophysiological processes that occur around the time of the seizure and whether the degree of change is important for determining outcomes in relation to cognitive abilities. The use of early T2 measurements provides information on brain water content as well as information of oxygen extraction, given the paramagnetic effects of deoxyhemoglobin (Choy et al., 2014).

* Corresponding author at: Department of Neurological Sciences, University of Vermont College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405, United States. Fax: +1 802 656 5678.
E-mail address: jbarry4@uvm.edu (J.M. Barry).
[1] These authors contributed equally to the work.

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Based on clinical observations, we hypothesized that a subset of rats with FSE would have cognitive impairment. Further, we hypothesized that the magnitude of change in the MRI, representative of metabolic demand post FSE, is predictive of cognitive outcome and that such changes are due to permanent alterations in neural networks that underpin spatial performance. Delineating the processes that culminate in normal or impaired cognitive ability provides a powerful tool for directing experimental or proven interventions to an 'at risk' population, thereby minimizing adverse outcomes. To this end, we set out to identify prolonged FS animals that exhibit normal or impaired learning on a complex spatial task as adults and relate these cognitive outcomes to both MRI changes following FSE induction as well as electrophysiological parameters close to the time of behavioral testing.

Methods

Overview

All animals used in the study were born at UC Irvine and shipped to Dartmouth post weaning in groups of 11–12 animals. On postnatal day 10 (P10) a total of 24 male rats experienced febrile status epilepticus (FSE) and 23 rats were used as normothermic controls (littermates of experimental group that were removed from the cage). At P10 FSE rat pups ($n = 24$) underwent induction of febrile status epilepticus while normothermic control animals ($n = 23$) underwent separation from the dam for a matched time period. A total of 25 rats were imaged for quantitative mapping of T2 relaxation time at high-field MRI at P10: 13 FSE rats and 11 normothermic controls were scanned 2 h after the FSE or maternal separation in the controls. At age 2 months, all rats ($n = 47$) were trained to perform an active avoidance task in which they learned to avoid a shock zone on a rotating arena. When the rats were approximately 3 months old they were trained to pellet chase on the stable arena. A subset of control ($n = 8$) and FSE animals ($n = 8$) were then implanted with an array of micro-electrodes in each hippocampus that allowed for the recording of local field potentials and place cells during pellet chasing behavior.

All procedures were approved by local institutional animal care and use committee and conducted in accordance with guidelines from the National Institutes of Health.

Induction of experimental FSE

Forty-seven male Sprague–Dawley rats were born and maintained in quiet facilities under controlled temperatures and light–dark cycle. Their birth was timed within 12 h and the date of birth was considered postnatal day (P) 0. On P2, mixed litters were culled to 10 pups, if needed. When weaned (on P21), male rats were housed 2–3 per cage and used in the current study. Experimental procedures were approved by UC-Irvine or Loma Linda University Institutional Animal Care Committees and conformed to NIH guidelines.

FSE was induced as previously described for 24 rats (Chen et al., 1999; Dube et al., 2006, 2010). Briefly, on P10, pups were placed in a glass container and their core temperature, which is highly correlated with brain temperature (Dube et al., 2005), increased to approximately 40.5 °C (simulating high fever) using a regulated stream of warm air. Core temperatures were measured at baseline, at seizure onset, and every 2 min during the hyperthermia. Hyperthermic seizures manifest as characteristic behaviors: seizure onset is heralded by a sudden loss of motion (freezing), followed by oral automatisms and forelimb clonus. Seizures progress to body flexion with chewing of an extremity and one or more tonic stage 5 seizures. Hyperthermia (maximum temperature: 41.5–42.9 °C) was maintained for less than 34–38 min (Dube et al., 2010) resulting in behavioral seizures lasting an average of 36.8 min (±0.24 SEM). This was done in order to enhance clinical applicability. We modeled our experiment on clinical studies (Bassan et al., 2013; Scott et al., 2006; Scott and Neville, 2009; Shinnar et al., 2008) which defined febrile status epilepticus as a seizure associated with fever lasting longer than 30 min.

The control group included 23 littermates of the experimental group that were removed from the cage for the same duration to control for potential stress and their core temperatures kept within normal range for age (normothermic controls).

MRI protocol

MRIs were performed on a Bruker Avance 11.7 T MR scanner (Bruker Biospin, Billerica) as recently described (Choy et al., 2014). Thirteen FSE-sustaining rats and 11 littermate controls were scanned at 2 h following FSE or, in the case of normothermic controls, removal from the home cage for a duration equivalent to hyperthermia and normal body temperatures were maintained. Rats were anesthetized for the duration of the imaging using 1.5% isoflurane in 100% O_2. For each scan the animal was positioned prone on a cradle and body temperature was maintained using a thermostat-controlled warm water cushion. Scout images were obtained to accurately position the brain. A field-of-view of 2.3 cm and a slice thickness of 0.75 mm were used for all scans. T2-weighted images were acquired using 2D-Multi-Echo-Spin-Echo Sequence (2D-MESE) with the following parameters: TR = 4697 ms, TE = 10.21, 20.42, 30.63, 40.84, 51.05, 61.26, 71.47, 81.68, 91.89, and 102.1 ms, matrix size = 192 × 192, number of averages = 2, and 20 slices. Absolute T2 relaxation time values (ms) were calculated by log transform followed by a least squares fit on a pixel-by-pixel basis, and T2 maps were generated using in-house software (Matlab, Mathworks). All images were coded and analyzed without knowledge of treatment group. Regions were delineated manually and measured using ImageJ software (version 1.25l, NIH, Bethesda), and T2 times for bilateral regions were averaged for analysis. Regions delineated include brain, dorsal hippocampus, basolateral and medial amygdala, medial thalamus, cerebellum, prelimbic cortex, and auditory cortex.

Active place avoidance behavioral task

The active place avoidance task (Biosignal; Brooklyn, New York) was developed to measure spatial memory and cognition (Pastalkova et al., 2006; Popp et al., 2011). In this task, animals learn to associate an unmarked region of space with a mild shock on a constantly rotating arena. The rats must attend to their ever-changing position in the room frame lest they be rotated into a pre-determined shock zone where they receive a mildly painful electrical shock.

At approximately 2 months old, 23 control and 24 FSE rats were lightly anesthetized and implanted with a stainless steel swivel (Eagle Claw, USA) in the skin between the shoulders. This allowed attachment of a cable with an LED at the end that allows for automated tracking and also the delivery of shock. Experimenters were blind as to which animals were control and FSE.

The arena consists of a steel disc 82 cm in diameter and is lighted from both above and below. A white cue card with black diagonal lines was placed on the West wall (1.2 m long and 0.75 m high) and a white cue was placed on the East wall (1.2 m long and 0.75 m high). Two proximal cue cards (45 cm long × 62 cm wide), one black (Color-Aid 9.5 gray) and one white (Color-Aid 2.5 gray) were placed approximately 20 cm from the periphery of the arena and were 135° apart from center to center.

On day one of training, the animal was connected to the cable and allowed to explore the arena for 10 min. On the second day, a clear Plexiglas barrier was placed around the perimeter of the arena that rotated at a rate of one revolution per minute. The rat was again allowed to explore the arena for 10 min. On all subsequent days, rats received a 0.2 ma shock in an unmarked 876 cm^2 wedge-shaped sector of the arena with a 60° arc on the Southern edge of the arena (Fig. 1A). This shock zone was stable in the room frame while the arena rotated. The entrance latency of the shock was 1 ms, the shock duration was 0.5 s and the inter-shock latency was 2 s.