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Research Paper

# Temporal Coordination of Hippocampal Neurons Reflects Cognitive Outcome Post-febrile Status Epilepticus☆,☆☆

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ABSTRACT

The coordination of dynamic neural activity within and between neural networks is believed to underlie normal cognitive processes. Conversely, cognitive deficits that occur following neurological insults may result from network discoordination. We hypothesized that cognitive outcome following febrile status epilepticus (FSE) depends on network efficacy within and between fields CA1 and CA3 to dynamically organize cell activity by theta phase. Control and FSE rats were trained to forage or perform an active avoidance spatial task. FSE rats were sorted by those that were able to reach task criterion (FSE-L) and those that could not (FSE-NL). FSE-NL CA1 place cells did not exhibit phase preference in either context and exhibited poor cross-theta interaction between CA1 and CA3. FSE-L and control CA1 place cells exhibited phase preference at peak theta that shifted during active avoidance to the same static phase preference observed in CA3. Temporal coordination of neuronal activity by theta phase may therefore explain variability in cognitive outcome following neurological insults in early development.



## 1. Introduction

The physiological capacity to coordinate dynamic neural activity within and between neural networks is believed to underlie normal cognitive processes (Fenton, 2015). This theory is largely based upon studies that have observed that the temporal coordination of neuronal firing, with respect to theta oscillations within the hippocampal circuit (Mizuseki et al., 2009), is correlated with learning and memory (Robbe and Buzsaki, 2009; Schomburg et al., 2014; Douchamps et al., 2013; Siegle and Wilson, 2014). Specifically, both modeling and experimental work suggest that the dynamic phase relationships of synaptic current as well as the timing of action potentials during theta rhythm are critical in both encoding and retrieval by organizing the transfer of neural information between the hippocampus and neocortex and within the hippocampal circuit (Hasselmo, 2005; Siegle and Wilson, 2014). Whether neuronal discoordination has a role in cognitive impairment following neurological insults requires demonstrating a link between temporal discoordination within and between components of the hippocampal circuit and cognitive outcome. If neural coordination by theta oscillations is necessary for cognitive processes, we hypothesized that levels of temporal coordination should reflect cognitive outcome in pathologies where learning and memory deficits are known to occur.

We chose to study febrile status epilepticus (FSE) to test this theory as it is the most common cause of seizures lasting 30 min or more in children (Kravljanac et al., 2015), and increases risk for developing cognitive impairment in both pediatric patients (Martinos et al., 2012; Roy et al., 2011; van Esch et al., 1996) and in the animal model (Dube et al., 2009; Barry et al., 2015). While animal models of febrile seizures are not found to be associated with hippocampal cell loss (Toth et al., 1998; Bender et al., 2003; Dube et al., 2004), febrile seizures have been found to persistently modify inhibitory h-channels (Chen et al., 2001) and alter GABAergic inhibition (Chen et al., 1999). Prolonged febrile seizures, in particular, have been shown to lead to long-term increases in network hyperexcitability (Dube et al., 2000). However, it remains to

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be shown that these network changes affect the temporal coordination of action potentials in a manner that could explain cognitive impairment following FSE. To this end we induced prolonged experimental febrile seizures lasting 30 min and investigated seizure-induced changes in temporal coordination through an in vivo study of hippocampal LFPs and CA1 and CA3 place cells. We aimed to evaluate the baseline levels of place cell organization in each region by local theta oscillations for FSE animals that could effectively learn (FSE-L), or were unable to learn (FSE-NL), while simply foraging for food pellets as well as during the active avoidance task.

FSE-NL CA1 place cells did not exhibit phase preference in either foraging or active avoidance contexts and exhibited poor cross-theta interaction between CA1 and CA3. In contrast, FSE-L and control CA1 place cells exhibited a baseline phase preference at peak theta during foraging. However, during performance of the active avoidance task, which necessitated the recall of the shock zone location, the preferred theta phase shifted to the descending phase of theta, matching the static phase preference observed in CA3. Altogether, these results show that dynamic temporal organization of neurons within local theta oscillations, as well as circuit efficacy in local hippocampal networks, reflect cognitive outcome observed post FSE. The results thereby support the notion that neural coordination by local theta oscillations, as well as dynamic theta phase modulation with regard to different aspects of learning and memory, plays an important role in the underpinning of normal cognitive processing as well as cognitive deficits associated with a pediatric seizure model (Hasselmo, 2005; Fenton, 2015).

## 2. Methods

### 2.1. Overview

On postnatal day 10 (P10) 7 male Sprague–Dawley rats experienced experimental FSE and 6 littermate rats were removed from the cage and used as controls (Cont). At age 2 months, these animals underwent the training phase for a hippocampal dependent spatial task, the dual reference frame active avoidance task. As described in our previous work, the FSE rats separated into those that met criterion of 5 or fewer consecutive shocks in 2 consecutive sessions (learners [FSE-L]) and those that did not reach criterion by the 15th training session (non-learners [FSE-NL]) (Barry et al., 2015). After acquisition of the active avoidance task, the animals were placed on a food deprivation schedule and exposed to the stable arena for 20 min a day for 5 days while food pellets fell from an overhead feeder every 30 sec. After this period, the animals were trained to alternate from foraging to avoidance contexts in the same arena. At age 3 months, rats were implanted with an array of micro-electrodes in each hippocampus that allowed for the recording of LFPs and hippocampal place cells in the open field and during the active avoidance task. The recording sessions during the foraging task allowed for the assessment of the temporal organization of hippocampal place cells with uniform spatial sampling in the absence of cognitive demand. In contrast, the recording sessions during the active avoidance task allowed for this assessment in a context with cognitive demand that had separated the FSE animals into learners (FSE-L) and non-learners (FSE-NL). Fig. 1 illustrates the experimental protocol and associated analyses used in our study.

### 2.2. Induction of Experimental FSE

All procedures were approved by the University of Vermont and UC—Irvine animal care and use committee and conducted in accordance with guidelines from the National Institutes of Health.

Sample size calculation was based on differences between the Cont and the FSE in phase preference of CA1 neurons during active avoidance. Using estimates of standard deviation from our preliminary data of phase preference, a sample size of 80 cells in the Cont and 30 cells in the FSE-L and FSE-NL has 90% power to detect a difference of 30° between means with a significance level (alpha) of 0.05 (two-tailed). We therefore used 13 male Sprague–Dawley rats for the study. The animals 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.

Experimental FSE was induced for 7 rats as previously described (Dube et al., 2006). On P10, pups were placed in a glass container and their core temperature increased to approximately 40.5 °C using a regulated stream of warm air as a simulation of high fever. Core temperatures were measured at baseline, at seizure onset, and every 2 min during hyperthermia. Hyperthermic seizure onset is heralded by sudden freezing, followed by oral automatisms and forelimb clonus. Seizures progressed to body flexion and one or more tonic stage 5 seizures. In order to more accurately model prolonged FSE in humans that typically last longer than 30 min, hyperthermia (Maximum temperature: 41.5–42.9 °C) was maintained for 34–38 min, resulting in behavioral seizures lasting an average of 36.8 min (±0.24 SEM) (Dube et al., 2010). The Cont group included 6 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. None of the features associated with hyperthermic induction of FSE at P10 (seizure duration, seizure threshold, maximal temperature or hyperthermia duration) were found to influence the categorization of FSE animals as learners or non-learners (Sup

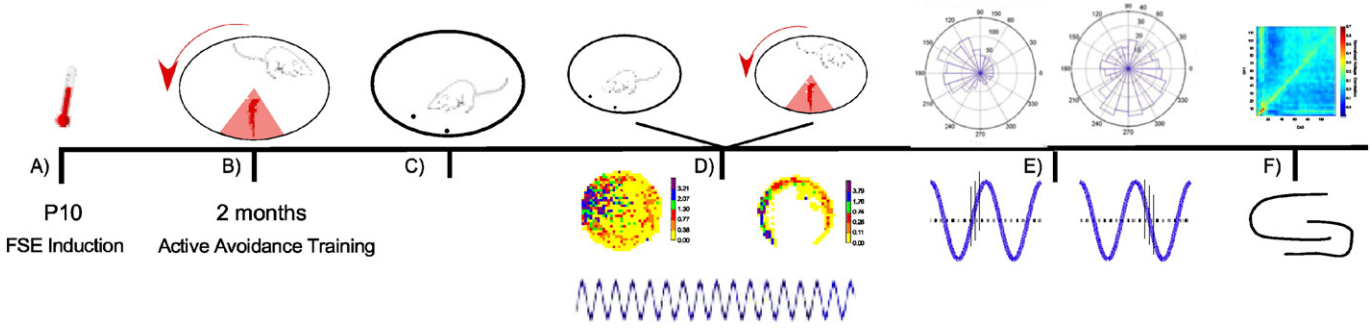


**Fig. 1.** Illustration of experimental design and analyses. A) On P10 7 rats experienced FSE and 6 rats were used as normothermic Cont (underwent separation from the dam for a matched time period). B) At age 2 months, all rats were trained to perform an active avoidance task in which they learned a hippocampal dependent spatial task which involved the avoidance of a shock zone on a rotating arena. Task measurements were made during this task acquisition phase and FSE animals that failed to meet criterion were designated as FSE-NL and those that did were designated as FSE-L. C) When the rats were approximately 3 months old they were trained to forage for food pellets on the stable arena. Cont and FSE animals were then implanted with an array of micro-electrodes in each hippocampus that allowed for the recording of LFPs and place cells during pellet chasing behavior. D) Two recording protocols were used in the study. The first protocol examined the long-term stability of place cell firing fields in two foraging sessions that were separated by two hrs. The second protocol examined place cell firing field and LFP properties when alternating between foraging and avoidance sessions. E) The temporal coordination of CA1 and CA3 place cells by local theta was analyzed for both foraging and avoidance sessions. F) Voltage correlations between theta signals in both CA1 and CA3 against all LFP signals (1–120 Hz) in the opposite region were analyzed as a means of measuring communication efficacy between the two structures.