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Loss of dendritic inhibition in the hippocampus after repeated early-life hyperthermic seizures in rats

Richard Boyce[a], **L. Stan Leung**[a,b,*]

[a] *Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada*
[b] *Program in Neuroscience, University of Western Ontario, London, Ontario, Canada*

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Summary Seizures are relatively common in children and are a risk factor for subsequent temporal lobe epilepsy. To investigate whether early-life seizures themselves are detrimental to the proper function of the adult brain, we studied whether dendritic excitation and inhibition in the hippocampus of adult rats were altered after hyperthermia-induced seizures in immature rats. In particular, we hypothesized that apical dendritic inhibition in hippocampal CA1 pyramidal cells would be disrupted following hyperthermia-induced seizures in early life. Seizure rats were given three hyperthermia-induced seizures per day for three days from postnatal day (PND) 13 to 15; control rats were handled similarly but not heated. At PND 65—75, paired-pulse inhibition in area CA1 was evaluated under urethane anesthesia, using CA3 and medial perforant path (MPP) stimulation to excite the proximal and distal apical-dendrites, respectively, and the evoked field potentials were analyzed by current source density. There was no difference in the CA1 response to single-pulse stimulation of CA3 or MPP. In control rats, a high-intensity CA3 stimulus inhibited a subsequent MPP-evoked CA1 distal dendritic excitatory sink, and the inhibition at 150—200 ms was blocked by a $GABA_B$ receptor antagonist. Seizure as compared to control rats showed a decrease in a CA3-evoked inhibition of the CA1 distal dendritic excitation, 30—400 ms after the CA3 stimulus. In addition, seizure as compared to control rats showed a reduced early (20—80 ms) inhibition of a CA1 mid-apical dendritic sink following paired-pulse CA3 stimulation. In conclusion, long-term alterations in dendritic inhibition in CA1 were found following early-life seizures.

Introduction

Whether early life seizures have long-term functional consequences is controversial (Berg and Shinnar, 1997). Atypical, prolonged and recurrent febrile seizures in children may induce temporal lobe sclerosis (Falconer, 1974) and increase the risk of temporal lobe epilepsy (TLE) later in life

* Corresponding author at: Department of Physiology and Pharmacology, Medical Sciences Bldg, University of Western Ontario, London, Canada N6A 5C1. Tel.: +1 519 850 2400; fax: +1 519 661 3827.
E-mail address: sleung@uwo.ca (L.S. Leung).

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(Annegers et al., 1987; Maher and McLachlan, 1995). However, other epidemiological studies suggest that febrile seizures do not significantly increase the risk for the development of TLE or cognitive deficits without underlying neurological abnormalities (Nelson and Ellenberg, 1978; Verity and Golding, 1991; Verity et al., 1998).

Animal models provide insights into the consequences of seizure activity in the developing brain. As a model of febrile seizures, hyperthermia-induced seizures in immature rats (Baram et al., 1997) were found to increase long-term seizure susceptibility and induce spontaneous seizures (Dube et al., 2010), without significant cell loss or sustained brain damage (Dube et al., 2000). Additionally, long-term behavioral changes have been reported after hyperthermic seizures (Chang et al., 2003; Dube et al., 2000, 2009, 2010). Long-term physiological changes after febrile seizure models include alteration of intrinsic membrane currents and an increased in $GABA_A$ receptor-mediated transmission in hippocampal slices *in vitro* (Chen et al., 1999). *In vivo*, a decreased $GABA_B$ receptor ($GABA_BR$)-mediated inhibition in CA1 was reported 30 days after repeated hyperthermia-induced seizures (Tsai and Leung, 2006) or early-life limbic seizures induced in immature rats (Tsai et al., 2008).

The primary purpose of the present study was to evaluate the long-term effects of hyperthermic seizures on dendritic excitation and inhibition of CA1 pyramidal cells in rats *in vivo*. Specifically, we hypothesized that dendritic excitation and inhibition at the mid-apical and distal apical dendrites in CA1 are disrupted in rats that experienced early life seizures as compared to control rats. Repeated hyperthermic seizures were used as a model of early-life seizures, in particular for children with recurrent febrile seizures (Berg et al., 1997). Excitability following entorhinal cortex excitation of the CA1 distal apical dendrites was shown to be altered following status epilepticus in adult rats (Wu and Leung, 2003; Ang et al., 2006), but to our knowledge, synaptic transmission at the entorhinal-CA1 synapse *via* the medial perforant path (MPP) has not been studied following early-life seizures.

Methods

Subjects

All procedures were approved by the Animal Use Committee at the University of Western Ontario (London, Ontario, Canada) and were conducted according to the guidelines set by the Canadian Council for Animal Care. Litters of immature Long-Evans rats (9 days old) were acquired from Charles River, Quebec, Canada, and were kept with the mother in a 51 cm × 41 cm × 22 cm (width × length × height) Plexiglas cage until weaning on postnatal day 21. For the entire duration of housing rats were given *ad libitum* access to food and water, and were kept on a 12 h:12 h light:dark cycle with lights on at 7:00 AM.

Repeated heated-air (hyperthermia)-induced seizures

On PND 13, male rats with similar weight were paired together, and each assigned pair was then arbitrarily divided with one rat being placed in the hyperthermic seizure group and the other in the control group. Rats belonging to the seizure group were individually placed into a 3-l glass cylinder which was partly immersed in a water bath kept at room temperature to help prevent overheating of the walking surface (Chang et al., 2003). A hair dryer at a moderate heat setting (500 W) was used to blow hot air down from the top of the container, ∼50 cm above the head of the rat. The ambient air temperature within the container (measured at a height level with the rat's head) was kept between 46 and 49 °C (Chang et al., 2003). Temperature was measured by gentle insertion of a thermometer into the external ear canal before and after each hyperthermia/control session for every rat. In preliminary experiments, we monitored the relation between ambient, external ear and rectal temperature in rat pups of PND15. These data indicated that rectal temperatures of 38.4, 39.7, 41.4 and 42.7 °C were attained in 5, 10, 20 and 30 min of heating, respectively, with ambient temperature reaching a plateau of 47 °C after 10 min of heating. External ear temperature was ∼1 °C lower than rectal temperature. No recordings of brain temperature were performed in the rats that underwent hyperthermia treatment. In two pentobarbital anesthetized rat pups of similar age subjected to similar hyperthermia, the brain temperature measured by a thermistor of 1 mm diameter inserted 2 mm into the sensorimotor cortex averaged 1.3 ± 0.1 °C higher than the rectal temperature during hyperthermia (38.4—42.2 °C rectal temperature). Thus, brain temperature was estimated to be ∼2.3 °C higher than external ear temperature. A hyperthermia-treated rat was kept in the container for 10 min following the first clearly observed behavioral seizure (typically hindlimb extension), or for a total duration of 30 min if no obvious seizure behavior was observed within the first 20 min of heating. Paroxysmal electrical activity in the hippocampus and amygdala was previously recorded with the hyperthermia treatment in both our laboratory (Tsai and Leung, 2006) and the laboratory of others (Baram et al., 1997). The control rat was given similar treatment for the same duration as the seizure rat, except no heated air was applied. Upon completion of a given session, the hyperthermic and control rats were returned to the home cage with the mother. In total, each hyperthermia and control rat underwent 3 hyperthermic/control sessions per day (4 h between each session) for 3 consecutive days from PND 13 to 15. Following early life seizure treatment, small notches were cut in the ears of both control and seizure rats to allow for individual identification later at the time of electrophysiological recording. A hyperthermia without seizure group was not used in the present study.

Surgical procedures

Approximately 50—60 days following early life treatment (PND 65—75), rats were put under urethane anesthesia (1.5 g/kg i.p.) and atropine methyl nitrate (0.15 mg/kg, i.p.) was injected to prevent fluid accumulation in the airway. Urethane anesthesia enhanced $GABA_A$ receptor inhibition and decreased glutamatergic excitation (Hara and Harris, 2002) but the entorhinal cortex to hippocampus circuit was preserved (Wu and Leung, 2003). After shaving the top of