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Corneal kindled C57BL/6 mice exhibit saturated dentate gyrus long-term potentiation and associated memory deficits in the absence of overt neuron loss

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ABSTRACT

Memory deficits have a significant impact on the quality of life of patients with epilepsy and currently no effective treatments exist to mitigate this comorbidity. While these cognitive comorbidities can be associated with varying degrees of hippocampal cell death and hippocampal sclerosis, more subtle changes in hippocampal physiology independent of cell loss may underlie memory dysfunction in many epilepsy patients. Accordingly, animal models of epilepsy or epileptic processes exhibiting memory deficits in the absence of cell loss could facilitate novel therapy discovery. Mouse corneal kindling is a cost-effective and non-invasive model of focal to bilateral tonic-clonic seizures that may exhibit memory deficits in the absence of cell loss. Therefore, we tested the hypothesis that corneal kindled C57BL/6 mice exhibit spatial pattern processing and memory deficits in a task reliant on DG function and that these impairments would be concurrent with physiological remodeling of the DG as opposed to overt neuron loss. Following corneal kindling, C57BL/6 mice exhibited deficits in a DG-associated spatial memory test – the metric task. Compatible with this finding, we also discovered saturated, and subsequently impaired, LTP of excitatory synaptic transmission at the perforant path to DGC synapse. This saturation of LTP was consistent with evidence suggesting that perforant path to DGC synapses in kindled mice had previously experienced LTP-like changes to their synaptic weights: increased postsynaptic depolarizations in response to equivalent presynaptic input and significantly larger amplitude AMPA receptor mediated spontaneous EPSCs. Additionally, there was evidence for kindling-induced changes in the intrinsic excitability of DGCs: reduced threshold to population spikes under extracellular recording conditions and significantly increased membrane resistances observed in DGCs. Importantly, quantitative immunohistochemical analysis revealed hippocampal astrogliosis in the absence of overt neuron loss. These changes in spatial pattern processing and memory deficits in corneal kindled mice represent a novel model of seizure-induced cognitive dysfunction associated with pathophysiological remodeling of excitatory synaptic transmission and granule cell excitability in the absence of overt cell loss.

1. Introduction

Epilepsy is a neurological disorder that affects one in 26 individuals worldwide (Hesdorffer et al., 2011). Besides spontaneous recurrent seizures, patients with epilepsy frequently experience behavioral, emotional, and cognitive comorbidities (Jensen, 2011). For example, approximately one in four individuals with temporal lobe epilepsy exhibit cognitive impairment during neuropsychological evaluation, most notably on memory tests (Hermann et al., 2006). These cognitive comorbidities are increasingly recognized as an equal (or even more disabling) part of epilepsy; cognitive dysfunction does not universally

Abbreviations: ACSF, artificial cerebral spinal fluid; DG, dentate gyrus; DGC, dentate granule cell; fEPSP, field excitatory post-synaptic potential; GFAP, glial fibrillary acidic protein; HS, hippocampal sclerosis; IEI, interevent interval; LTP, long-term potentiation; NeuN, neuronal nuclear antigen; PBS, phosphate-buffer saline; PFA, paraformaldehyde; pp, perforant path; PPR, paired-pulse ratio; PTP, post-tetanic potentiation; PTX, picrotoxin; RID, recognition index; sEPSC, spontaneous excitatory post-synaptic current; sIPSC, spontaneous inhibitory post-synaptic currents; TBS, theta-burst stimulation.

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disappear once seizures are well controlled which leads to a poor quality of life for patients and their families, even in the absence of seizures (Jacobs et al., 2009). Furthermore, there are currently no treatments for memory deficits experienced by patients with epilepsy (Giovagnoli and Avanzini, 2000; Hrabok et al., 2013). Accordingly, the NINDS Epilepsy Research Benchmarks recognizes this as a significant challenge and calls for increased efforts aimed at establishing animal models that will in turn aid in the development of optimal treatments for cognitive comorbidities in epilepsy (Kelley et al., 2009; Long et al., 2016). The discovery of treatments for this constellation of debilitating cognitive symptoms would be greatly facilitated by such animal models and an improved understanding of the underlying pathophysiology contributing to these symptoms.

When considering the pathophysiology of memory dysfunction in epilepsy, neuron death and atrophy of the hippocampus may be a reasonable underlying cause in many cases. Indeed, patients with temporal lobe epilepsy (TLE) exhibit varying degrees of cell death and hippocampal sclerosis (HS) (see review (de Lanerolle et al., 2012)), and similar neuronal damage can be recapitulated in a number of animal models (Chauvière et al., 2009; Covolan and Mello, 2000; Friedman et al., 1994; Gröticke et al., 2008; Rao et al., 2006). However, not all epilepsy patients with memory impairment have severe hippocampal damage (Aikiä et al., 1995; Giovagnoli and Avanzini, 1999; Lah et al., 2014; Loiseau et al., 1983; Martins et al., 2015), and hippocampal cell loss with or without HS is not always associated with memory impairment (Castro et al., 2013; Schmidt et al., 2015). Moreover, associations between hippocampal volume loss and performance on memory tests are highly variable (Äikiä et al., 2001). These observations suggest, at least in some epilepsy patients (e.g. cryptogenic TLE), more subtle changes in hippocampal physiology may underlie memory dysfunction, and this patient population in particular might be expected to benefit most from pharmacological therapies aimed at restoring normal memory. Thus, a better understanding of these physiological changes is needed, and animal models of epileptic processes that exhibit memory dysfunction without overt cell loss or HS would be particularly useful.

Rodent kindling models that employ direct electrical stimulation of limbic structures may represent an ideal model of human complex partial seizures (focal aware or impaired awareness seizures (Fisher et al., 2017)), with comorbid memory dysfunction. However, the underlying pathological changes leading to memory dysfunction in these models can vary considerably depending on the age of the animal, the location of stimulation, and the extent of kindling (Hannesson and Corcoran, 2000; Morimoto et al., 2004). While some kindling studies employing direct electrical stimulation methods report minimal neuronal loss (Brandt et al., 2004; Haas et al., 2001; Singh et al., 2013; Tooyama et al., 2002), others report striking neuronal loss, sometimes resembling hippocampal sclerosis, after more extensive kindling protocols (Cavazos et al., 1994; Cavazos and Sutula, 1990; Chen et al., 2010; Sutula, 1990). Furthermore, all of these models suffer from the time- and labor-intensive nature of invasive electrode implantation surgeries and post-operative care. However, the corneal kindling model of seizures in mice, which has been validated as a rapid screening model of partial limbic seizures that secondarily generalize in humans (focal to bilateral tonic-clonic seizures, (Fisher et al., 2017)), has become a useful tool in the discovery of novel anti-seizure drugs, in part, because of its non-invasive, cost-effective and relatively easy methodology (Matagne and Klitgaard, 1998; Potschka and Löscher, 1999; Rowley and White, 2010).

While the pharmacological responsiveness of corneal kindled seizures in CF1 mice has been thoroughly examined and shown to both correlate well with that of the hippocampal kindled rat and be consistent with human focal limbic seizures (Rowley and White, 2010), their cognitive comorbidities and underlying pathophysiology are only now beginning to be examined. Recently, corneal kindled CF1 mice were shown to be cognitively indistinguishable from control mice in a behavioral paradigm that relies on both hippocampal and extra-hippocampal areas – the novel object/place recognition task (Barker-Haliski et al., 2016). Additionally, Loewen et al. (2016) recently reported that corneal kindled CF1 mice do not exhibit neuronal loss within the hippocampus but do exhibit significant astrogliosis in both area CA1 and the dentate gyrus (DG). These reactive astrocytes indicate that corneal kindled seizures may induce pathological changes in important hippocampal areas such as the DG; the full nature of these changes, and whether they are beneficial or detrimental, remains to be determined. Taken together, these data suggest that corneal kindled mice lack overt hippocampal neuron loss and, despite showing no cognitive dysfunction in a task that relies on extra-hippocampal function, may yet still exhibit cognitive dysfunction in other tests of learning and memory that rely heavily on hippocampal function. Furthermore, it is unknown whether corneal kindling in other strains, particularly C57BL/6 mice, similarly spare hippocampal neurons.

In the present study, we tested the hypothesis that corneal kindled C57BL/6 mice exhibit spatial pattern processing deficits in a task reliant on DG function and that these impairments are concurrent with physiological remodeling of the DG as opposed to overt neuron loss. To accomplish this, we evaluated neurodegeneration in the hippocampus of corneal kindled C57BL/6 mice as well as their performance in a DG-mediated spatial pattern processing test that relies on the rodents' natural tendencies to explore changes in the distance between two objects – the "metric task" (Ennaceur, 2010; Goodrich-Hunsaker et al., 2008; Lee et al., 2005). Next, we examined physiological changes in the DG of corneal kindled mice that may contribute to memory impairment. We used in-vitro extracellular and whole-cell patch-clamp electrophysiology to evaluate changes in synaptic transmission, granule cell excitability, short- and long-term synaptic plasticity (paired pulse depression, post-tetanic potentiation, and long-term potentiation), and saturation of LTP in the DG. Our results demonstrate that corneal kindled C57BL/6 mice, in the absence of overt cell loss, exhibit DG-associated cognitive deficits associated with pathophysiological remodeling of excitatory synaptic transmission and granule cell excitability that is consistent with saturation of LTP at the perforant path – dentate granule cell synapse.

2. Methods

2.1. Animals

Fifty, five- to six-week-old male C57BL/6 mice (15–20 g, Charles River, Raleigh, NC, U.S.A.) were used in this study. The first cohort ($n = 20$) of mice was used for the metric task, immunofluorescence, and synaptic plasticity experiments. The second cohort ($n = 20$) was used for basal synaptic transmission and patch-clamp electrophysiology experiments. The third cohort ($n = 10$) was used for LTP saturation experiments. All experiments were conducted between three days and 2 weeks after kindling. All mice were group housed in a light- and temperature-controlled (12 h on/12 h off) environment and permitted access to food and water ad libitum throughout the study. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Utah Institutional Animal Care and Use Committee. All efforts were made to minimize the number and suffering of animals used.

2.2. Corneal kindling

C57Bl/6 mice were corneal kindled using a protocol adapted from CF1 mice (Rowley and White, 2010). Briefly, a 0.9% saline solution containing 0.5% tetracaine hydrochloride was applied to each eye to provide local anesthesia and electrical conductivity. Mice were stimulated twice daily (4 h apart) with corneal stimulation of 1.5 mA (60 Hz) for 3 s. Mice were considered fully kindled when five consecutive stage five seizures were achieved according to a modified Racine scale