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Fiber tract stimulation can reduce epileptiform activity in an *in-vitro* bilateral hippocampal slice preparation

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ABSTRACT

Mesial temporal lobe epilepsy (MTLE) is a common medically refractory neurological disease that has been treated with electrical stimulation of gray matter with limited success. However, stimulation of a white matter tract connecting the hippocampi could maximize treatment efficacy and extent. We tested low-frequency stimulation (LFS) of a novel target that enables simultaneous targeting of bilateral hippocampi: the ventral hippocampal commissure (VHC) with a novel *in-vitro* slice preparation containing bilateral hippocampi connected by the VHC. The goal of this study is to understand the role of hippocampal interplay in seizure propagation and reduction by commissural fiber tract stimulation. LFS is applied to the VHC as extracellular and intracellular recording techniques are combined with signal processing to estimate several metrics of epilepsy including: (1) total time occupied by seizure activity (%); (2) seizure duration (s); (3) seizures per minute (#); and (4) power in the ictal (V^2Hz^{-1}); as well as (5) interictal spectra (V^2Hz^{-1}). Bilateral epileptiform activity in this preparation is highly correlated between hippocampi. Application of LFS to the VHC reduces all metrics of epilepsy during treatment in an amplitude and frequency dependent manner. This study lends several insights into the mechanisms of bilateral seizure reduction by LFS of the VHC, including that depolarization blocking, LTD/LTP and $GABA_A$ are not involved. Importantly, enhanced post-stimulation 1-Hz spiking correlates with long-lasting seizure reduction and both are heightened by targeting bilateral hippocampi *via* the VHC. Therefore, stimulating bilateral hippocampi *via* a single electrode in the VHC may provide an effective MTLE treatment.

Introduction

Epilepsy is a neurological disorder characterized by abnormal electrical activity within the brain, which can result in either generalized or partial seizures. It is a complex disease with varied causes and manifestations that affects more than 50 million people worldwide (Duncan et al., 2006). The most common and medically refractory form of human epilepsy, mesial temporal lobe epilepsy (MTLE), is characterized by seizures of the hippocampus and surrounding structures (Avoli et al., 2002; Barbarosie and Avoli, 1997; Calcagnotto et al., 2000; King et al., 1995; Spencer, 2002; Swanson, 1995). Standard treatments for MTLE include drug therapy and surgery, neither of which is completely effective or risk-free. Only 11–25% of MTLE patients become seizure-free with drug therapy (Jallon, 1997). Although surgery can render 65–75% of selected patients free of seizures, it is only an option for patients with a single, identifiable epileptic focus and traumatic side effects can result (Blume, 2006; Blume and Parrent, 2006). An alternative treatment, deep brain electrical stimulation (DBS), is being tested clinically and shows promise as a safe and effective therapy for medically intractable epilepsy (Durand and Bikson, 2001; Fisher et al., 2010; Morrell, 2006, 2011), including MTLE (Boon et al., 2007; Velasco A. et al., 2000; Velasco M. et al., 2000; Vonck et al., 2002, 2005). DBS offers several advantages to surgical resection in that (1) it is less invasive; (2) it is reversible; and (3) the treatment protocol can be customized to fit the needs of individual patients (Sunderam et al., 2010). However, this treatment is still recent and undergoing optimization. As a result, there are many stimulation paradigms that are currently being investigated targeting a range of locations including the cerebellum, caudate nucleus, thalamus, and substantia nigra. Current trials for MTLE focus

Abbreviations: 4-AP, 4-aminopyridine; ACSF, artificial cerebral spinal fluid; ANT, anterior nucleus of thalamus; AP, action potential; BMI, bicuculline methiodide; CV, cresyl-violet; DBS, deep brain stimulation; DHC, dorsal hippocampal commissure; EC, entorhinal cortex; EP, evoked potential; ff, fimbria–fornix; HC, hippocampal commissures; HFS, high frequency stimulation; IACUC, Institutional Animal Care and Use Committee; LFB, luxol fast blue; LFS, low frequency stimulation; MTLE, mesial temporal lobe epilepsy; PDS, paroxysmal depolarizing shift; SD, Sprague–Dawley; sAHP, slow after-hyperpolarization; UPO, unstable periodic orbit; VHC, ventral hippocampal commissure.

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mainly on hippocampal and cortical seizure foci (Morrell, 2006; Parrent and Almeida, 2006; Theodore and Fisher, 2004) utilizing a variety of stimulation patterns and frequencies (Blume and Parrent, 2006; Duncan et al., 2006; Durand and Bikson, 2001; Engel, 2001; Jallon, 1997; Morrell, 2006; Sadler, 2006; Vonck et al., 2005; Yamamoto et al., 2006) with variable success that does not approach surgical results (Rolston et al.). Therefore, there is need for development of specific stimulation parameters as well as identification of an effective target for stimulation.

In this study, we tested a low-frequency electrical stimulation paradigm based on the observation that LFS has been shown to decrease neural excitability. Low-frequency electrical stimulation (LFS) ranging from 0.1 to 10 Hz (D'Arcangelo et al., 2005; Jerger and Schiff, 1995) has shown promising reduction of epileptic activity *in vitro* (Durand and Bikson, 2001; Jerger and Schiff, 1995; Khosravani et al., 2003; Schiller and Bankirer, 2006; Toprani et al., 2008, 2010), in animal (Ghorbani et al., 2007; Goodman et al., 2005; Kile et al., 2010; Rashid et al., 2011; Sun et al., 2010; Tang and Durand, 2012; Velisek et al., 2002; Zhang et al., 2009), and in human models (Fisher et al., 2010; Kinoshita et al., 2005; Schrader et al., 2006; Yamamoto et al., 2006). Moreover, interictal activity, characterized by low-frequency periodic spiking events (Bonaventura et al., 2006; Holmes et al., 2000; Lees et al., 2006; Schiller and Bankirer, 2006), has been suggested as a potential defense mechanism evolved in epileptic brains that protects against ictal onset (Avoli, 2001; Barbarosie and Avoli, 1997; Cohen et al., 2002, 2003; D'Arcangelo et al., 2005; Swartzwelder et al., 1987). An explanation for this interictal–ictal interplay may be that the interictal events force the hippocampal network to spike at a particular interval or unstable periodic orbit (UPO), thereby making the network more resistant to seizures. This notion is supported by the success of chaos control strategies that use electrical stimulation to entrain epileptic spiking events (Horgan, 1994; Schiff et al., 1994; Slutzky et al., 2003). Another advantage of LFS is that it inherently requires less power than its high-frequency counterpart, which may result in less tissue and electrode damage. Therefore, LFS, applied at a frequency mimicking interictal events, was chosen for the seizure reduction paradigm.

The LFS paradigm was applied to a clinically neglected target, the hippocampal commissures (HC), which include a large, accessible fiber tract whose stimulation can evoke bilateral hippocampal responses in humans (Gloor et al., 1993; Koubeissi et al., 2009). We chose this fiber tract as a target for seizure reduction by electrical stimulation due to its potential to bilaterally affect large portions of the hippocampi as well as its accessibility. While the hippocampus has the lowest seizure threshold of any brain region and is a well known seizure focus of MTLE (Bikson et al., 2001; Durand and Bikson, 2001; Morrell, 2006; Parrent and Almeida, 2006; Theodore and Fisher, 2004), specific subfields of the hippocampus can be difficult to target stereotactically. Furthermore, direct hippocampal targeting has shown limited efficacy (Jobst et al., 2010), perhaps because hippocampal seizure foci, like cortical foci, are often multiple, evolving, and difficult to characterize (Derchansky et al., 2006). Even if foci can be identified, horizontal spread of synchronized activity often occurs too quickly to curtail with stimulation at the onset zone (Chagnac-Amitai and Connors, 1989). Targeting a decussating axonal tract may curtail seizure spread and reduce the risk of developing generalized seizures (Engel, 2001; Gastaut, 1970; Sadler, 2006). It has been shown that when MTLE events cross over into the contralateral hemisphere, they can usually be traced from the seizure focus to the contralateral hippocampus before evolving further (Gloor et al., 1993; Lima et al., 1990). A case study of rapid epileptic intrahippocampal-propagation with marked seizure amplification in the hippocampus contralateral to the seizure focus highlights the functional role of this fiber tract in humans (Rosenzweig et al.). Bilateral hippocampal communication is important in MTLE and changes in hippocampal coherence can predict seizure onset (Meier et al., 2007). Furthermore, the healthy hippocampus in MTLE can evolve into an independent seizure focus through communication with the diseased hippocampus *via* the hippocampal commissures (HC) (Khalilov et al., 2003), bundles of axons that run in the fimbria–fornix (ff) before decussating to connect bilateral hippocampi (Demeter et al., 1985, 1990; Gloor et al., 1993; O'Keefe and Nadel, 1978; Vann et al., 2000; Wilson et al., 1990, 1991). Given this evidence that the hippocampi directly communicate and influence one another's activity in animals (Feng and Durand, 2005) and in patients (Lacruz et al., 2007), there may be benefits to broadly targeting both simultaneously as opposed to just the one that is perceived to be the epileptic focus.

The HC consist of dorsal and ventral hippocampal commissures. The dorsal hippocampal commissure (DHC) is the prominent tract in primates, whereas the ventral hippocampal commissure (VHC) is prominent in rodents (Gloor et al., 1993; Wilson et al., 1990, 1991). The efficacy of LFS of the VHC has been demonstrated in rats *in vivo* with chemical (Tang and Durand, 2012), electrical (Rashid et al., 2011), and genetic models of epilepsy (Kile et al., 2010). However, an *in-vitro* study is crucial to determine the role of the commissure in mediating seizure reduction by eliminating other possible pathways, such as the corpus callosum or fiber tracts through the cortices. Furthermore, the ability to separate the hippocampi *in vitro* is crucial to determine the contribution of bilateral propagation of epileptic activity to MTLE severity and LFS efficacy. Therefore, the goal of this study is to test *in-vitro* a novel electrical stimulation paradigm for reduction of MTLE seizures, modeled using 4-aminopyridine (4-AP) (Perreault and Avoli, 1991; Tapia and Sitges, 1982; Traub et al., 1996). A chronic open-loop LFS paradigm implemented at a fixed frequency of 1 Hz is applied to the hippocampal commissures for a broad bilateral effect on the hippocampi. The stimulation paradigm is tested in a new *in vitro* slice preparation that maintains anatomical and functional connectivity of the hippocampi solely through an intact VHC in order to test the bilateral efficacy of the LFS paradigm.

Materials and methods

Ethical approval and animal handling

All procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Case Western Reserve University. 71 Sprague–Dawley (SD) rats from Charles River (12–21 days) were used for this study. Animals were housed according to IACUC guidelines. All rats were anesthetized using ethyl ether or isofluorane before decapitation for brain harvesting.

Functionally connected bilateral hippocampal slice preparation

The brain was removed and placed in cold (3–4 °C) oxygenated (O_2 95%, CO_2 5%) sucrose-rich artificial cerebrospinal fluid (ACSF), consisting of (in mM): 220 sucrose, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 g/L D-glucose (pH 7.45). The cerebellum was detached and the ventral surface of the brain was secured in a vibrating-blade microtome (VT1000S, Leica) containing sucrose-based cold, oxygenated ACSF. A novel bilateral hippocampal slice preparation was developed in which the hippocampi, ECs (for contribution to seizure generation (Barbarosie and Avoli, 1997)), and connecting VHC were preserved after other tissues were carefully dissected away. 750 or 350 μm axial slices were cut and immediately preserved in oxygenated ACSF consisting of (in mM): 124 NaCl, 3.75 KCl, 1.25 KH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_2$, 2 $CaCl_2$, and 10 g/L D-glucose for at least 60 min before being transferred to an interface-recording chamber (Harvard Apparatus). Slice viability was confirmed by the presence of distinct, healthy cell layers marked by cresyl violet (CV) staining in select slices and by extracellular field recordings of evoked potentials from CA3 and CA1 larger than 1 mV for all preparations in ACSF (Fig. 1) that did not diminish over the course of the experiment (Fig. 5). The axonal anatomy of the VHC was examined histologically for select slices using luxol fast blue (LFB), while the functional connection was established in