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Regular Article

Seizure reduction through interneuron-mediated entrainment using low frequency optical stimulation

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ABSTRACT

Low frequency electrical stimulation (LFS) can reduce neural excitability and suppress seizures in animals and patients with epilepsy. However the therapeutic outcome could benefit from the determination of the cell types involved in seizure suppression. We used optogenetic techniques to investigate the role of interneurons in LFS (1 Hz) in the epileptogenic hippocampus. Optical low frequency stimulation (oLFS) was first used to activate the cation channel channelrhodopsin-2 (ChR2) in the Thy1-ChR2 transgenic mouse that expresses ChR2 in both excitatory and inhibitory neurons. We found that oLFS could effectively reduce epileptiform activity in the hippocampus through the activation of GAD-expressing hippocampal interneurons. This was confirmed using the VGAT-ChR2 transgenic mouse, allowing for selective optical activation of only GABA interneurons. Activating hippocampal interneurons through oLFS was found to cause entrainment of neural activity similar to electrical stimulation, but through a $GABA_A$-mediated mechanism. These results confirm the robustness of the LFS paradigm and indicate that GABA interneurons play an unexpected role of shaping inter-ictal activity to decrease neural excitability in the hippocampus.

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Introduction

Epilepsy is a chronic disorder of the central nervous system characterized by recurrent, unprovoked seizures. Mesial temporal lobe epilepsy (MTLE) involving the hippocampus is a common type of epilepsy, often medically refractory and necessitating surgical resection if an epileptic focus can be identified (Wiebe et al., 2001). A less invasive alternative to surgery shown to reduce disease burden is deep brain electrical stimulation (DBS) (Fisher et al., 2010; Jobst et al., 2010; Morrell, 2011), although the optimal location and stimulus paradigm is highly debated and the mechanisms of seizure suppression remain poorly understood (Sunderam et al., 2010). Nonetheless, recent studies have shown low frequency electrical stimulation to be effective at suppressing epileptiform activity in animal models (Rashid et al., 2012) and reducing seizure frequency in patients (Koubeissi et al., 2013).

Despite the advantages offered by a low frequency electrical treatment strategy, the inherent lack of cell type-specificity in using an electrical stimulus to modulate neuronal activity may limit overall efficacy and create undesired side effects. The confounding effects from non-specific cell activation also make it difficult to determine the mechanisms responsible for the therapeutic effect. There is much debate over whether stimulation of the neurons at the seizure focus (Bragin et al., 2002), afferent connections (Yang et al., 2006), or even local glial cells (Tawfik et al., 2010) are important for reducing seizure activity. The emerging field of optogenetics can help address these questions by providing tools that allow for cell-specific activation (Boyden et al., 2005) or inhibition (Zhang et al., 2007; Chow et al., 2010) in a reversible manner with millisecond time resolution. These optogenetic constructs have recently been shown to suppress neuronal hyperactivity and seizure in various models epilepsy (Paz et al., 2013; Wykes et al., 2012; Krook-Magnuson et al., 2013) but can also be applied to elucidate the mechanisms seizure suppression.

In the present study, we used optogenetic techniques to investigate the role of interneurons in low-frequency stimulation paradigms that have been shown to suppress epileptiform activity in the hippocampus (Rashid et al., 2012; Koubeissi et al., 2013). Two different optogenetic transgenic mouse models expressing ChR2 were used to study the effects of selectively activating hippocampal neurons or only GABA interneurons to assess the role these cell types play in suppressing seizure activity. The pro-epileptogenic compound 4-aminopyridine (4-AP) was used as a model of epilepsy (Perreault and Avoli, 1989) in both in vitro (Zhang et al., 2014) and acute in vivo (Gonzalez-Reyes et al., 2013) preparations. This compound has been shown to trigger epileptiform activity in vivo after local (intra-hippocampal) (Gonzalez-Reyes et al., 2013) and systemic (Lévesque et al., 2013) administration. While this work was primarily aimed to gain insight into the mechanisms of seizure reduction using LFS, the efficacy of this approach

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in vivo adds to the growing body of evidence that translation of optogenetic techniques can provide more targeted therapy for future clinical applications.

Materials and methods

Animals

Thy1-ChR2-YFP (B6.Cg-Tg(Thy1-COP4/EYFP)9Gfng/J Stock Number:007615) and VGAT-ChR2-YFP (B6.Cg-Tg(Slc32a1-COP4*H134R/EYFP)8Gfng/J Stock Number: 014548) transgenic mice (both of C57BL/6 background) were bred from founder animals obtained from the Jackson Laboratory. We used these two transgenic animals since the promoters Thy-1 (Arenkiel et al., 2007) and VGAT (Zhao et al., 2011) control the expression of the ChR2 opsin only in neurons and in GABAergic cells, respectively. Animals were housed no more than 5 adult animals per cage and maintained in a SPF room under light (12-h light/12-h dark cycle), temperature and humidity controlled conditions. Animals used for in vitro brain slice experiments were studied at approximately postnatal day 14 (P14, range P11 to P16) and P90. For acute in vivo experiments, adult animals were used (range P90 to P110). Both male and female animals were used for experiments as no differences due to animal gender were observed. All experimental procedures performed in this study followed the NIH animal use guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) at Case Western Reserve University.

Histology

Transgenic and wildtype mice from both mouse lines were transcardially perfused with ice-cold 4% paraformaldehyde (PFA) under isoflurane anesthesia at age P14 and P100. Brains were then removed, post-fixed in 4% PFA at 4 °C overnight, and cryoprotected in 30% sucrose in phosphate-buffered saline (PBS) for 2 days before being flash-frozen in 2-methylbutane on dry ice and stored at −80 °C. Brains were then sectioned at a thickness of 40 μm using a cryostat (Leica CM3050S), and free-floating sections were blocked in 5% horse serum for 1 hr then stained for GAD-67 (rabbit, Santa Cruz (sc-5602), 1:100 dilution) or GAD-65/67 (rabbit, ABcam (ab49832), 1:1000 dilution) at 4 °C overnight. Sections were then incubated with secondary antibodies conjugated to Cy3 (donkey α-rabbit, 1:700, Jackson ImmunoResearch, West Grove, PA). After rinsing in PBS, sections were mounted on Fisherbrand Superfrost/Plus microscope slides in VECTASHIELD mounting media (Vector Laboratories, Burlingame, CA). For 40 μm sections, TOTO-3 (1:2000 dilution, Invitrogen) was included to visualize nuclei and confocal microscopy was used to capture Z-stacked images on a Zeiss LSM 510 META laser-scanning microscope. All YFP- and GAD-positive neurons in the hippocampus that could be identified were counted from $n = 6$ transgenic (P14, $n = 3$; P100, $n = 3$) and $n = 2$ wildtype mice from each mouse line. Immunohistochemical images presented represent a typical IHC staining result.

Acute in vivo recording and optical stimulation

Adult transgenic and wildtype mice were anesthetized with isoflurane and mounted to a stereotaxic frame (Stoelting, Wood Dale, IL). Anesthesia was maintained throughout the stereotaxic surgery by delivering 3% isoflurane in a carbogen gas mixture (95% O_2/5% CO_2) via a constant pressure mask, and depth of anesthesia was assessed by monitoring animal respiratory rate and response to toe pinch. Screw electrodes were placed in the skull for use as the reference (positioned caudally in the occipital bone) and system ground (positioned rostrally in the frontal/coronal bones). Depth electrodes (tungsten, A-M Systems) were positioned in the septal CA3 region of the hippocampus bilaterally (bregma −1.7 mm, lateral ±2.0 mm, depth 2.1 mm). Local field potential neural activity was bandpass filtered (1 Hz to 5 kHz) and amplified (gain of 1000, A-M Systems Model 1700 Differential AC Amplifier), then digitized at 20 kHz (PowerLabs/16SP) using LabChart7 software (ADInstruments, Dunedin, New Zealand) and stored for offline analysis. Epileptiform activity was induced by 1 μL bolus injections of 40 mM 4-AP (in nACSF) in the left septal CA3 region (bregma −1.8 mm, lateral 2.0 mm, depth 2.1 mm). Additional injections were administered every 10 min until the development of sustained electrographic seizure activity for the duration of the experiment, with

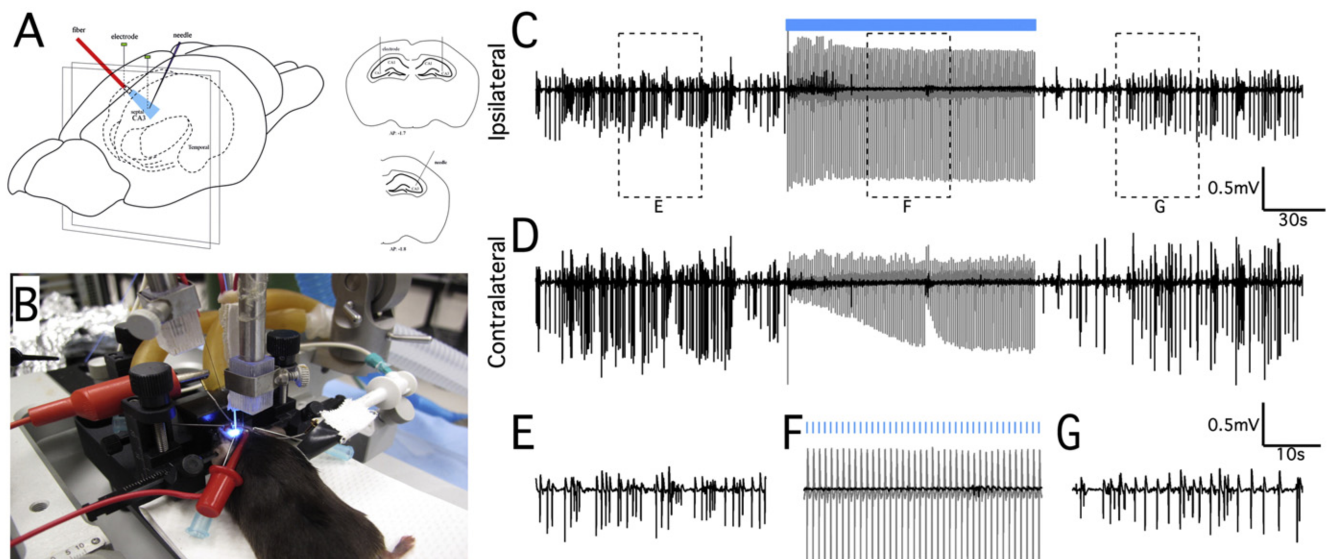

Fig. 1. Optical stimulation at 1 Hz can suppress in vivo epileptiform activity. (A) Schematic representation of in vivo optical stimulation in the mouse hippocampus. Actual experimental preparation is shown in (B). Recording electrodes were positioned bilaterally in the septal CA3 regions. Epileptiform activity was induced by unilateral bolus injection of 40 mM 4-AP, and an optical fiber was positioned to maximally illuminate the dorsal hippocampus near the injection site. Neural recordings from electrodes positioned in the ipsilateral (C) and contralateral (D) septal CA3 regions showing hippocampal seizure activity before, during, and after a 2 min period of optical stimulation at 1 Hz (indicated by blue bar). Stimulation was only applied to the site of 4-AP injection (ipsilateral site), but reduction of epileptiform activity occurred bilaterally. (E–G) Magnification of the regions identified in (C) to show the seizure and suppressed activity in greater detail. Note that the optical evoked potential during the stimulation period (gray traces) was removed from these recordings by template subtraction (black trace, see Methods). Stimulation trials in wild-type control animals did not elicit an optical evoked potential and had no effect on epileptiform activity (data not shown).

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