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Brief activation of GABAergic interneurons initiates the transition to ictal events through post-inhibitory rebound excitation

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ABSTRACT

Activation of γ-aminobutyric acid ($GABA_A$) receptors have been associated with the onset of epileptiform events. To investigate if a causal relationship exists between $GABA_A$ receptor activation and ictal event onset, we activated inhibitory GABAergic networks in the superficial layer (2/3) of the somatosensory cortex during hyperexcitable conditions using optogenetic techniques in mice expressing channelrhodopsin-2 in all GABAergic interneurons. We found that a brief 30 ms light pulse reliably triggered either an interictal-like event (IIE) or ictal-like ("ictal") event in the *in vitro* cortical 4-Aminopyridine (4-AP) slice model. The link between light pulse and epileptiform event onset was lost following blockade of $GABA_A$ receptors with bicuculline methiodide. Additionally, recording the chronological sequence of events following a light pulse in a variety of configurations (whole-cell, gramicidin-perforated patch, and multi-electrode array) demonstrated an initial hyperpolarization followed by post-inhibitory rebound spiking and a subsequent slow depolarization at the transition to epileptiform activity. Furthermore, the light-triggered ictal events were independent of the duration or intensity of the initiating light pulse, suggesting an underlying regenerative mechanism. Moreover, we demonstrated that brief $GABA_A$ receptor activation can initiate ictal events in the *in vivo* 4-AP mouse model, in another common *in vitro* model of epileptiform activity, and in neocortical tissue resected from epilepsy patients. Our findings reveal that the synchronous activation of GABAergic interneurons is a robust trigger for ictal event onset in hyperexcitable cortical networks.

1. Introduction

Increased activity and synchronization of GABAergic interneurons at ictal event onset has been reported in animal models *in vivo* (Grasse et al., 2013), *in vitro* (Lasztoczi et al., 2009; Uva et al., 2015; Velazquez and Carlen, 1999), in clinical investigations (D'antuono et al., 2004; Mattia et al., 1995), and also comprehensively reviewed (Avoli and de Curtis, 2011; De Curtis and Gnatkovsky, 2009). Converging evidence from animal (Uva et al., 2009; Velazquez and Carlen, 1999) and human (D'antuono et al., 2004; Huberfeld et al., 2011; Mattia et al., 1995) studies suggest that interictal events (IIEs) are also produced by interneuronal activation, specifically relating to $GABA_A$-mediated conductances (Panuccio et al., 2009). These interictal (inhibitory) mechanisms are suspected to recruit neural activity responsible for transition into low-voltage, fast-activity ictal events (LVF ictal events) (De Curtis and Gnatkovsky, 2009; Gnatkovsky et al., 2008; Panuccio et al., 2009; Uva et al., 2015). Optogenetic studies in rodents have indeed demonstrated that sustained (300–1000 ms) activation of GABAergic interneurons can initiate LVF ictal events in the *in vitro* 4-aminopyrmidine (4-AP) model of epileptiform activity (Shiri et al.,

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2015a; Yekhlef et al., 2015).

Interestingly, brief (30 ms) synchronous optogenetic activation of GABAergic interneurons have also been demonstrated to sufficiently trigger LVF ictal events in the *in vivo* 4-AP model (Ritter et al., 2014). Previous *in vitro* brain slice studies have similarly suggested that the synchronous release of GABA is a causal factor for network hyperexcitability and ictal event onset (Klaassen et al., 2006; Medrihan et al., 2014). Recent *in vitro* work revealed that Cl^- gradients are preserved in the 4-AP model of epileptiform activity just prior to the onset of an ictal event (Ellender et al., 2014), suggesting $GABA_A$-mediated inhibitory synapses remain functional. In cortical microcircuits, the synchronous phasic synaptic inhibition provided by GABAergic interneurons (Gibson et al., 2005; Hu et al., 2011) is essential for coordinating postsynaptic pyramidal cells either through inhibitory mechanisms or rebound spiking (Cobb et al., 1995; Gonzalez-Burgos and Lewis, 2008) to form transient neuronal assemblies (Diba et al., 2014) that play a critical role in information processing (Varela et al., 2001). Interneurons are endowed with this ability through their exceptionally powerful and divergent inhibitory connections to multiple postsynaptic cells (Helmstaedter et al., 2008; Hoffmann et al., 2015). For these reasons, the synchronous activation of GABAergic interneurons has been suspected to initiate the highly structured, hypersynchronous ictal events (Engel, 2013; Panuccio et al., 2009).

In this study, we explored the hypothesis that brief activation of GABAergic interneurons in the 4-AP model (an optogenetically well-characterized model of epileptiform activity (Shiri et al., 2015a; Shiri et al., 2015b; Yekhlef et al., 2015) would initiate the transition to ictal events *via* post-inhibitory rebound (PIR) excitation. Our findings demonstrate that ictal events triggered by a brief light pulse are similar to those occurring spontaneously in 4-AP treated cortical brain slices of transgenic mice expressing channelrhodopsin-2 (ChR2) in all subtypes of GABAergic interneurons. By successfully triggering a critical state transition in the neural network with a brief light pulse while recordings in various configurations: local field potential (LFP), intracellular patch-clamp, gramicidin-perforated patch, and multi-electrode array (MEA), we could gather direct evidence to elucidate the chronological order of early events in the transition to epileptiform-like (herein referred to as “epileptiform”) activity. Our findings indicate that synchronous activation of interneurons results in PIR spikes that likely triggers a cascade of regenerative events underlying the generation of IIEs and ictal events in the *in vitro* cortical 4-AP neocortical slice model. These findings contribute further evidence and new mechanistic insight to the growing literature on the complicity of interneurons in the transition to ictal events (Avoli and de Curtis, 2011; De Curtis and Gnatkovsky, 2009).

2. Materials and methods

2.1. Patients

Human tissue was collected from temporal lobectomy candidates who had undergone standard diagnostic assessment and surgical treatment of their medically refractory epilepsy (Mansouri et al., 2012) having provided written informed consent. The epileptogenic onset zone located within the mesial temporal structures was far removed from the temporal neocortex that was used in our study (Valiante, 2009; Mansouri et al., 2012; Florez et al., 2015). Human tissue (temporal neocortex) was sectioned into 500 μM slices using procedures described previously (Florez et al., 2015).

2.2. Animals

Procedures involving animals were in accordance with guidelines by the Canadian Council on Animal Care and approved by the Institutional Animal Care Committee. VGAT-ChR2 mice (JAX Stock#:014548) obtained from The Jackson Laboratory (JAX; Maine, USA) expressed channelrhodopsin tagged with enhanced yellow fluorescent protein (EYFP) in all GABAergic interneuronal subtypes (Zhao et al., 2011). Wild-type (WT) C57BL/6 mice (JAX Stock#:000664) were used as experimental controls. *In vitro* brain slices were prepared from juvenile mice of either sex, postnatal day 13–21 (p13–p21) as 500 μM coronal slices from the somatosensory cortex using procedures described previously (Jin et al., 2011). Briefly, the brain was rapidly removed, and sliced in ice-cold oxygenated (95% O_2/5% CO_2) sucrose solution (in mM): sucrose (248), KCl (2), NaH_2PO_4 (1.25), $MgSO_4{\cdot}7H_2O$ (3), glucose (10), $CaCl_2{\cdot}2H_2O$ (1), and $NaHCO_3$ (26). Slices were recovered in standard artificial cerebrospinal fluid (ACSF) (in mM): NaCl (123), KCl (4), $NaH_2PO_4{\cdot}H_2O$ (1.2), $CaCl_2{\cdot}2H_2O$ (1.0), $MgSO_4{\cdot}7H_2O$ (3.0), $NaHCO_3$ (26), and glucose (10), initially at 35 °C for 30 min, then at room temperature for 60 min prior to recording. Acute *in vivo* experiments were performed on adult mice of either sex (p35–p60) anesthetized with Ketamine (95 mg/kg) and Xylazine (5 mg/kg) and mounted in a stereotactic frame. The scalp was removed to expose the frontoparietal cortex after application of local anesthesia (Lidocaine). A 4 mm diameter craniotomy was subsequently performed at coordinates: 2.0 mm lateromedial and − 2.0 mm rostrocaudal to expose somatosensory cortex, which was covered with warmed isotonic saline solution throughout the experiment.

2.3. Immunohistochemistry

Co-immunostaining was performed according to procedures previously described (Zhao et al., 2011). Briefly, coronal slices 50 μM thick were prepared from cryoprotected brains of three VGAT-ChR2 mice (p21–30) using a cryotome. Slices were co-immunostained for anti-GFP (Invitrogen #A11122, 1:2000) and anti-GAD67 (MAB 5406, 1:2000) and then incubated with conjugated secondary antibodies, Alexa 488 (A11008) or Alexa 594 (A11005), respectively. Areas (300 × 300 μm) of the cortical region from five slices were imaged with a Nikon A1R laser scanning confocal microscope using a 40 × objective (*N*.A. = 0.6). The GFP and TRITC filter sets were used to visualize the Alexa 488 (green fluorescence) and Alexa 594 (red fluorescence), respectively. Images were processed with ImageJ (NIH, USA) and analyzed by three experimenters who each independently identified neurons fluorescently labeled with Alexa 488 and Alexa 594.

2.4. Local field potential (LFP), Intracellular, and Gramicidin perforated-patch Recordings

In vitro extracellular recordings were performed at 35 °C in either an interface type chamber (Haas et al., 1979) or a submerged multi-electrode array (MEA) system USB-MEA60 (Multi Channel Systems, Germany) using a TiN array with 200 μm inter-electrode spacing. LFP (*in vitro* and *in vivo*) was recorded from the superficial layer (2/3) of the somatosensory cortical area using an ACSF-filled glass electrode under the control of a micromanipulator. Whole-cell patch recordings of neurons from the somatosensory cortex were visualized using a combination of YFP fluorescence and infrared differential interference contrast microscopy (Olympus BX51/BX61 microscope; OLY-150IR camera-video monitor unit; QImaging 4000R). Patch electrodes (3–5 MΩ) were filled with solution containing (in mM): potassium gluconate (135), NaCl (10), NaHEPES (10), $MgCl_2$ (1), NaGTP (0.3), and Na_2ATP (2); pH 7.2, 280–290 mOsm. Based upon the Nernst equation, the chloride ($Cl-$) reversal potential was calculated to be − 68 mV. Cells were discarded if the series resistance changed by > 20%. Gramicidin perforated-patch recordings were performed as previously described (Ellender et al., 2014) using an intracellular solution containing (pH 7.3, 280–290 mOsm): 135 potassium gluconate, 10 NaCl, 0.0001 $CaCl_2$, 10 NaHEPES, 1 $MgCl_2$, 0.3 NaGTP, 2 NaATP, and 80 μg/ml gramicidin (Calbiochem). Gramicidin was initially dissolved in DMSO and the final concentration of DMSO in the intracellular solution was < 0.1%. Recorded signals were digitized at