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Granger causality relationships between local field potentials in an animal model of temporal lobe epilepsy

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ABSTRACT

An understanding of the *in vivo* spatial emergence of abnormal brain activity during spontaneous seizure onset is critical to future early seizure detection and closed-loop seizure prevention therapies. In this study, we use Granger causality (GC) to determine the strength and direction of relationships between local field potentials (LFPs) recorded from bilateral microelectrode arrays in an intermittent spontaneous seizure model of chronic temporal lobe epilepsy before, during, and after Racine grade partial onset generalized seizures. Our results indicate distinct patterns of directional GC relationships within the hippocampus, specifically from the CA1 subfield to the dentate gyrus, prior to and during seizure onset. Our results suggest sequential and hierarchical temporal relationships between the CA1 and dentate gyrus within and across hippocampal hemispheres during seizure. Additionally, our analysis suggests a reversal in the direction of GC relationships during seizure, from an abnormal pattern to more anatomically expected pattern. This reversal correlates well with the observed behavioral transition from tonic to clonic seizure in time-locked video. These findings highlight the utility of GC to reveal dynamic directional temporal relationships between multichannel LFP recordings from multiple brain regions during unprovoked spontaneous seizures.

1. Introduction

A better understanding of the spatial–temporal dynamics of brain electrical activity during ictogenesis is an important step toward the development of seizure prevention strategies (Mormann et al., 2007). There are two prevailing theories by which seizures are thought to spatially initiate and propagate; the first being from a discrete seizure focus and the second being from a diffuse hyper-excitable network (Bertram et al., 1998). Furthermore, there are theories on how seizures terminate ranging from increased GABAergic inhibition to "anticonvulsant effects" of specific subcortical brain regions (Lado and Moshe, 2008). However, the mechanisms and subsequent dynamics underlying spontaneous seizures remain largely unknown (Bertram, 2009). A better understanding of the dynamics of how seizures spatially initiate, propagate, and terminate may provide an important step towards the development of successful seizure intervention therapies.

Much of what is known about the circuitry of the hippocampus during ictogenesis is based on *in vitro* experiments (Ang et al., 2006; Avoli et al., 2002; Barbarosie and Avoli, 1997). However, it is difficult to apply *in vitro* electrophysiological recording methods to a freely behaving *in vivo* animal model. Therefore, new methods, models, and tools are necessary to examine spontaneous seizures *in vivo*.

To better examine the spatiotemporal dynamics of spontaneous temporal lobe seizure onset and spread, we use an animal model of chronic temporal lobe epilepsy (TLE) in parallel with continuous multichannel electrophysiological recording. This was accomplished using 32-channel microwire arrays chronically implanted bilaterally into the hippocampus dentate gyrus (DG) and CA1 sub-

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fields. We then use Granger causality (GC) to reveal the magnitude and direction of temporal relationships during 1 s overlapping windows during each seizure. Granger proposed that, "for two simultaneously measured time series, one series can be called causal to the other one if we can better predict the second time series by incorporating past knowledge of the first" (Granger, 1969). The mathematical and statistical framework that underlies GC can be traced back to Wiener (1956) and also has roots in autoregressive (AR) modeling (Chatfield, 1996). Granger has discussed in recent and prior communication (Granger, 2001, 1980; Seth, 2007) how the concept of causality is a controversial philosophical question that is continually debated. Indeed, GC is not a measure of true causality "it is only Granger's causality" (Seth, 2007). However, GC has proven a practical, well-defined statistical tool for quantifying directional temporal relationships between financial time series (Granger, 1969), that has found broad use in the neuroscience community (Bollimunta et al., 2008; Ding et al., 2007; Franaszczuk and Bergey, 1998; Franaszczuk et al., 1998, 1994; Osorio et al., 2008, 1998; Schiff et al., 2005; Sitnikova et al., 2008) to reveal directional relationships between specific brain regions in the context of the underlying anatomy as well as behavior. We will use this practical formulation to reveal directional relationships between multichannel local field potentials (LFP) recorded from the brain during seizure.

This study seeks to measure the directional GC relationships between LFP recorded from the bilateral CA1 and DG subfields of the hippocampus. We test the *hypothesis* that specific directional GC relationships between hippocampal subfields occur during TLE seizure onset (as opposed to global non-directional synchronization) and are detectable using GC. This is based on previous success in elucidating directional relationships in the epileptic brain (Cadotte et al., 2009; Franaszczuk et al., 1994; Sitnikova et al., 2008). We will demonstrate how GC can be used to quantify the magnitude and the direction of these relationships over the time course of the seizure epoch. To our knowledge, this is the first *in vivo* demonstration of the dynamic relationships between time series recorded from multiple bilateral hippocampal subfields before, during, and after seizure onset in an animal model of TLE. We postulate that a better understanding of the dynamics of how LFP propagate *in vivo* during spontaneous seizure onset and termination may provide an important step towards the development of successful seizure intervention therapies.

2. Materials and methods

2.1. Animal model of TLE and data acquisition

2.1.1. Animal model

Several animal models of epilepsy have been developed in recent years in order to study chronic TLE. Progress in developing a functional connectivity theory to explain human epilepsy has been impeded by the difficulties of obtaining sufficient samples of LFP recordings of patients and the many uncontrollable intervening variables that occur in the clinical setting. A valid animal model that exhibits the essential dynamical features of the human condition is clearly necessary. The self-sustaining electrical status epilepticus (SSESE) rat model (Lothman et al., 1990) was developed to study the pathophysiological and molecular effects of a defined injury (i.e. status epilepticus) on the susceptibility to develop TLE (Bertram, 1997). This animal model has many of the features associated with human TLE including similar electrophysiological correlates, etiology, pathological changes in the limbic system, and seizure induced behavioral manifestations (Bertram and Cornett, 1994; Quigg et al., 1997, 1998; Sanchez et al., 2006). The seizures in this model are recurrent, spontaneous, and chronic in nature. This model is characterized by a progressive strengthening of recurrent spontaneous temporal lobe seizures beginning 4–6 weeks after induced status epilepticus.

2.1.2. Electrode implantation surgery

Fifty-day-old male Sprague–Dawley rats weighing 210–265 g were used using protocols and procedures approved by the University of Florida Institutional Animal Care and Use Committee (IACUC protocol D710). The methods used to create this animal model were similar to those reported by our laboratory elsewhere (Cadotte et al., 2009; Sanchez et al., 2006; Talathi et al., 2009, 2008). Xylazine (10 mg/kg, SQ) and isoflurane (1–3%) in oxygen was used for anesthesia. Inhalation anesthesia was continued via a nose mask during surgery where the animals were secured in a Kopf stereotactic frame. The top of the rat's head is shaved and chemically sterilized with iodine and alcohol. The bregma and lambdoidal suture is exposed by a midsagittal incision that begins between the eyes and extends caudally to the ears. Extraneous soft tissue is removed from the skull using a peroxide wash. Four stainless steel screws (0.8 mm, Small Parts, Miami Lakes, FL) are placed in the skull to anchor an acrylic headset. Two screws were anterior to bregma by 2 mm and bilaterally 2 mm and one of which served as a reference electrode. The remaining two screws were posterior by 2 mm to lambda and bilateral 2 mm and one of which served as a ground electrode. A roughly 3 mm by 1.5 mm craniotomy is created using a rotary tool and the dura was removed for microelectrode array placement such that the long axis extended from 1.7 mm lateral (left and right) to 3.5 mm lateral from bregma. Two independent 16-channel microelectrode arrays (2 by 8 electrode arrays of 50 μm polyimide insulated tungsten microwire, 5 mm long, with 250 μm in row spacing with 500 μm separation between the two rows, Tucker Davis Technologies, Alachua, FL) were positioned to record LFP from the bilateral CA1 and DG subfields. The center of each 2×8 array was placed 4.4 mm back from bregma, 3.2 mm right or left from the midline, and at a depth of 3.1 mm using a stereotaxic instrument based on coordinates from a rat brain atlas (Paxinos, 1997). An independent custom fabricated bipolar twist electrode (Teflon-sheathed stainless steel 330 μm diameter wire) is placed in the right posterior ventral hippocampus (−5.3 mm posterior, 4.9 mm lateral (right) of bregma, 5 mm ventral) for the sole purpose of electrical stimulation into status epilepticus (Lothman et al., 1990). All electrodes are then chronically secured with Cranioplast (Plastics One, Inc., Roanoke, VA) and anchored to the previously mentioned 4 ground and anchor screws. The animals were allowed to recover for 1-week post-surgery, and then induced into status epilepticus using the bipolar twist electrode.

2.1.3. Induction of seizures

Induction into status epilepticus to create the animal model of TLE was achieved by electrical stimulation of the bipolar twist electrode (Lothman et al., 1990). Stimulation consisted of waveform trains composed of biphasic square wave pulses at a frequency of 50 Hz with a pulse duration of 1 ms, intensity of 300–400 μA and was delivered for 50–70 min with a duty cycle of 10 s on and 2 s off. During the stimulus the animal increased exploratory activity and displays 'wet dog shakes'. After approximately 20–30 min of stimulation, convulsive seizures of up to 1 min in duration are observed on average about every 10 min. Post-stimulation, intracranial LFP are assessed for evidence of slow wave activity in all recorded electrodes. In the absence of slow wave activity, the stimulus is re-applied for 10-min intervals (up to 3 times) until continuous slow waves appeared following termination of the stimulus.

Rats were observed for behavioral seizure activity and adequate food and water intake for 12–24 h after stimulation. Post-electrical stimulation, the LFP recordings were characterized by activity below 5 Hz for 12–24 h and occasional spontaneous 30–60 s elec-