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Neuronal synchrony and the transition to spontaneous seizures

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ABSTRACT

The role of inhibitory neuronal activity in the transition to seizure is unclear. On the one hand, seizures are associated with excessive neuronal activity that can spread across the brain, suggesting run-away excitation. On the other hand, recent in vitro studies suggest substantial activity of inhibitory interneurons prior to the onset of evoked seizure-like activity. Yet little is known about the behavior of interneurons before and during spontaneous seizures in chronic temporal lobe epilepsy. Here, we examined the relationship between the on-going local field potential (LFP) and the activity of populations of hippocampal neurons during the transition to spontaneous seizures in the pilocarpine rat model of epilepsy. Pilocarpine treated rats that exhibited spontaneous seizures were implanted with drivable tetrodes and an LFP electrode and recordings were obtained from the CA3 region. For each recorded seizure, identified single units were classified into putative interneurons or pyramidal cells based on average firing rate, autocorrelation activity and spike morphology. The onset of sustained ictal spiking, a consistent seizure event that occurred within seconds after the clinically defined seizure onset time, was used to align data from each seizure to a common reference point. Ictal spiking, in this paper, refers to spiking activity in the low-pass filtered LFP during seizures and not the neuronal action potentials. Results show that beginning minutes before the onset of sustained ictal spiking in the local field, subpopulations of putative interneurons displayed a sequence of synchronous behaviors. This includes progressive synchrony with local field oscillations at theta, gamma, and finally ictal spiking frequencies, and an increased firing rate seconds before the onset of ictal spiking. Conversely, putative pyramidal cells did not exhibit increased synchrony or firing rate until after ictal spiking had begun. Our data suggest that the transition to spontaneous seizure in this network is not mediated by increasing excitatory activity, but by distinct changes in the dynamical state of putative interneurons. While these states are not unique for seizure onset, they suggest a series of state transitions that continuously increase the likelihood of a seizure. These data help to interpret the link between in vitro studies demonstrating interneuron activation at the transition to seizure, and human studies demonstrating heterogeneous neuronal firing at this time.

Introduction

The heterogeneous interneuron population in the hippocampus plays a crucial role in epilepsy. Selective loss of interneuron subtypes (Dinocourt et al., 2003; Ratte and Lacaille, 2006) and decrease in GABAergic synapses and inhibition (de Lanerolle et al., 1989; Kobayashi and Buckmaster, 2003; Sloviter, 1987) have been observed in animal models and human temporal lobe epilepsy. Despite this loss, physiological data support the survival of inhibition in human and animal epileptic tissues (Esclapez et al., 1997; Wilson et al. 1998). This is attributed to sprouting and synaptogenesis of the existing interneurons in an attempt to compensate for the loss (Cossart et al., 2001; Maglóczky et al., 2000; Wittner et al. 2001; Wyeth et al., 2010). For example, recent reports indicate a selective increase in somatic inhibition combined with the loss of dendritic inhibition in the hippocampus of epileptic rats (Cossart et al., 2001). The functional impact of these changes is not yet fully understood and the physiological impact of this reorganization on the surviving interneurons during the transition to a spontaneous seizure is unknown.

In vitro studies also support an important role for interneurons during the transition to seizure. Although in vitro seizure-like-events are not spontaneous, they can mimic certain acute changes leading up to a spontaneous seizure, including onset of rhythmic ictal spiking. Recently, in vitro studies identified that activation of hippocampal interneuron networks is responsible for initiation of these seizure-like-events (Fujiwara-Tsukamoto et al., 2010; Velazquez and Carlen, 1999; Ziburkus et al., 2006). For example, Ziburkus et al. showed that interneuron activity was maximal just before the onset of rhythmic ictal spikes in the local field of hippocampal slices. Trevelyan et al. showed that cortical pyramidal cells were recruited in seizure-like-events, only after the failure of existing inhibitory restraint mechanisms (Schevon et al., 2012; Trevelyan et al., 2007). Induced seizures have also

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been studied in the intact brain preparation and here too, interneuron activity was shown to be related to the generation of seizure-like-events (Bragin et al., 1997; Gnatkovsky et al., 2008; Timofeev et al., 2002). These results suggest a complex role for interneurons in the generation of seizures, however, such activity during spontaneous seizures has yet to be demonstrated. We hypothesized that hyper-synchronous interneuron activity may also occur during the transition to spontaneous seizures in-vivo. To test this idea, the behavior of hippocampal neurons was examined during the transition to spontaneous seizures in an awake, freely moving animal model of epilepsy.

We recorded extracellular neuronal activity from the CA3 hippocampus of freely moving pilocarpine-treated rats exhibiting chronic recurrent spontaneous seizures. The isolated single units were sorted into fast spiking putative interneurons and regular spiking putative pyramidal cells. We examined changes in neuronal activity from these distinct populations as the network transitioned from interictal to ictal activity as evidenced from LFP. For the sake of simplicity, henceforth in this paper, putative interneurons and pyramidal cells will be referred to as interneurons and pyramidal cells, respectively. Our results show a series of changes in interneuron synchrony that begins minutes before LFP ictal spiking. Ictal spiking, in this paper, refers to spiking activity in the low-pass filtered LFP during seizures and not the neuronal action potentials. First, interneurons undergo changes in synchrony, becoming more correlated with each other and more coherent with theta oscillations in the LFP. In the seconds before onset of ictal spiking, interneurons become coherent with gamma LFP oscillations and display increased firing rates not seen during the prior interictal state. Finally, the onset of sustained ictal spiking is characterized by a further change in synchrony, where interneurons become highly coherent with the frequency of ictal spiking, and more correlated with each other. Only after these synchronous activities occur, and ictal spiking has begun, do pyramidal cells begin to show increases in firing rate and coherence with ictal spikes. These results support the view that synchronous interneuron activity is a hallmark of the transition from interictal to ictal states in CA3.

Materials and methods

Overview of experimental procedure

Extracellular single units and LFPs were recorded from spontaneously seizing rats. Recordings were made during a continuous 48 hour period in order to catch spontaneous seizures and ensure sufficient interictal data for a complete analysis. To minimize potential baseline contamination from post-seizure effects, seizures were excluded from analysis if another seizure occurred less than 2 h prior. For this study, a total of 25 seizures (1.7 ± 2.9 seizures per 48 hour recording period) were analyzed from 5 rats (4–8 seizures per rat). All procedures were approved by the Institutional Animal Care and Use Committee and followed the National Institute of Health guidelines. All recorded seizures were generalized, as indicated by behavior ranging from 3 to 5 on Racine's scale (Racine, 1972). For each seizure that occurred, the data during the period beginning 10 min before and ending 15 s after ictal spiking onset was used to assess changes in neuronal behavior near the onset of rhythmic ictal spiking compared to activity during a background period defined from 1 h before to ending 10 min before rhythmic ictal spiking onset. For each seizure, single units were putatively classified into pyramidal cells ($n = 169$) or interneurons ($n = 54$) based on waveform shape and firing characteristics (Csicsvari et al., 1998) for the entire period of analysis (1 h before ictal spiking onset to 15 s after). The shapes of single-unit waveforms were tested to ensure stationarity. Offline analysis of the LFP and single unit activity was performed to identify changes in the dynamical state of neurons during the transition to ictal spiking (from 10 min before to 15 s after).

Epilepsy model

Male Long Evans rats (225–275 g) were initially given scopolamine methyl nitrate (1 mg/kg, i.m.) to reduce cholinergic effects, and supplementary doses were given every 2 h. Status epilepticus was induced by a systemic injection of pilocarpine (400 mg/kg, i.p.). Rats that exhibited status (~40%) were clearly distinguished by nearly continuous myoclonic seizures which ranked 3–5 on the Racine scale. Status was allowed to persist for 2 h before administration of diazepam (10 mg/kg, i.p.) and pentobarbital (20 mg/kg, i.p.). Rats were given Lactated Ringer's solution and kept on a heating pad until they recovered. Beginning 3 weeks after pilocarpine injection, rats that experienced status were monitored for spontaneous seizures by recording video for 8 h per day. Only rats that exhibited at least one spontaneous behavioral seizure (Racine 3–5) were implanted with arrays of tetrodes and further studied.

Microdrive implantation surgery

Approximately 1 month after pilocarpine injection, rats that displayed spontaneous seizures were chronically implanted with drivable tetrodes. Tetrodes were made from 4 HFV-coated tungsten wires (12.7 μm), twisted and fused together. Each tetrode was tested to ensure no electrical shorts were present between the individual wires. 7 tetrodes were used for recording single units and a separate electrode was used for recording LFP. The LFP electrode was identical to the other tetrodes, except all 4 wires were connected to the same channel. The separate LFP electrode allowed for a nearby recording of the local field without contamination from unit activity, which is important for high frequency coherence measurements between action potentials and LFP. Tetrodes were loaded into a microdrive (Neuralynx 9-drive) and arranged so that they formed a circle (1 mm diameter) around the LFP electrode. Electroplating was considered unnecessary since the final impedance of wires was 200–500 kΩ. Rats were given scopolamine (1 mg/kg, i.m.), anesthetized with isoflurane, and intubated for sustained isoflurane anesthesia (1.5–2.5%) in a stereotaxic apparatus. The microdrive was implanted above the dorsal hippocampus ($AP = -3.25$, $ML = -3.0$), and tetrodes extended to a depth of 2 mm ventral to bregma. The ground wire for all tetrodes was connected to a skull screw placed at $AP = 5.0$, $ML = 1.0$. After implantation, the microdrive was sealed in place with dental acrylic.

Data collection

Rats were given at least 3 days to recover from surgery before driving the tetrodes. Tetrodes, along with the LFP electrode, were advanced slowly (500 μm per day) until they reached the pyramidal cell layer of CA3 at approximately 3.8 mm ventral to bregma. This position was confirmed by the noticeable appearance of large amplitude unit action potentials indicating close proximity to pyramidal cells. Once the tetrodes were positioned in the pyramidal layer, rats were given at least 1 day for waveforms to stabilize before recordings commenced. Repeated recordings were taken, each being 24–48 h in duration, with 48 h between each recording. This was facilitated by the use of a wireless headstage amplifier (Triangle Biosystems 31-channel, ×600 gain) powered by an external battery attached to a rat jacket. Wideband (0.8 Hz–6.5 kHz) neural signals were broadcast to a receiver, acquired at 40 kHz sampling rate, and written to a hard drive. After each recording, tetrodes were adjusted to obtain new units. However, no recordings were taken less than 24 h after moving the tetrodes.

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