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Burst firing of single neurons in the human medial temporal lobe changes before epileptic seizures

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HIGHLIGHTS

- Ipsilateral MTL neurons show an increased degree of burstiness during interictal recordings.
- This effect is found only for putative principal cells, not for interneurons.
- Burstiness may serve as a protective factor and its absence may in turn facilitate seizure emergence.

ABSTRACT

Objective: To better understand the mechanisms that lead to the sudden and unexpected occurrence of seizures, with the neuronal correlate being abnormally synchronous discharges that disrupt neuronal function.
Methods: To address this problem, we recorded single neuron activity in epilepsy patients during the transition to seizures to uncover specific changes of neuronal firing patterns. We focused particularly on neurons repeatedly firing discrete groups of high-frequency action potentials (so called bursters) that have been associated with ictogenesis. We analyzed a total of 459 single neurons and used the mean autocorrelation time as a quantitative measure of burstiness. To unravel the intricate roles of excitation and inhibition, we also examined differential contributions from putative principal cells and interneurons.
Results: During interictal recordings, burstiness was significantly higher in the seizure onset hemisphere, an effect found only for principal cells, but not for interneurons, and which disappeared before seizures.
Conclusion: These findings deviate from conventional views of ictogenesis that propose slowly-increasing aggregates of bursting neurons which give rise to seizures once they reach a critical mass.
Significance: Instead our results are in line with recent hypotheses that bursting may represent a protective mechanism by preventing direct transmission of postsynaptic high-frequency oscillations.

1. Introduction

Epilepsy is not a disease in and of itself, but a chronic condition of the brain characterized by paroxysmal and recurrent epileptic events that occur as a result of chronic structural and/or functional changes. A number of studies have investigated seizure initiation at a neuronal population level using EEG or local field potential (LFP) recordings both in humans and animal models of epilepsy (Szabo et al., 2015). However, it is not possible to extrapolate meaningfully from this population level to the behavior of its cellular elements, even though they underlie this activity. In order to obtain mechanistic insight, it is therefore necessary to record cellular activity, in vivo, during seizure onsets. This technique has only recently become available in humans (Bower et al., 2012; Mormann and Jefferys, 2013 and references therein).

A specific neuronal discharge pattern termed *bursting*, which is characterized by the repeated firing of discrete groups of

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high-frequency action potentials, has been associated with chronic epilepsy both in experimental models of epilepsy and in humans (Wyler and Ward, 1986; Sanabria et al., 2001; Schindler et al., 2006). To date only a few in vivo studies in humans have investigated bursting and its relevance to the pathophysiology of epilepsy. These few studies have yielded conflicting results. Earlier studies compared seizure onset zones to contralateral brain regions during seizure-free (interictal) periods and reported burst discharges to be less frequent ipsilaterally (Colder et al., 1996). In contrast, other studies found higher burst rates in seizure onset zones in MTL epilepsy (MTLE) (Staba et al., 2002). Single cells recorded in close proximity to the seizure onset zone tended to have higher bursting rates if they showed pre-ictal and ictal changes in spiking rates (Keller et al., 2010). Finally, burst interspike interval ratios have been used to predict seizure onset zones from interictal recordings (Valdez et al., 2013).

The relation between interictal epileptiform EEG activity and neuronal bursting has also been investigated. Neurons whose firing rates were modulated by interictal discharges had a significantly higher bursting rate than neurons not showing this modulation (Keller et al., 2010). Alarcón and colleagues reported an increased occurrence of burst firing during spontaneous interictal epileptiform discharges and after single pulse electrical stimulation (Alarcón et al., 2012).

The analysis methods employed by these studies, particularly the definition of bursting, varied considerably, and most were carried out on limited data sets. This might explain why no consistent relationship between burst firing and localization of the seizure onset zone has been demonstrated to date.

We here examined the role of bursting neurons in the emergence of clinical seizures. We analyzed simultaneous recordings from the MTLs ipsi- and contralateral to the seizure onset zone in patients with MTLE both during the seizure-free interval and immediately before seizures. To unravel the intricate and potentially counterintuitive roles of excitation and inhibition, we also studied the differential contributions of putative principal cells and interneurons.

2. Methods

2.1. Patients

22 consecutive patients with pharmacologically intractable epilepsy were included in this study. In these patients, non-invasive methods had been unable to unequivocally localize a region of seizure onset, and they chose to undergo intracranial long-term EEG monitoring to be evaluated for epilepsy surgery (Rosenow and Lüders, 2001). All patients had given written informed consent to participate in this study, which was approved by the Medical Institutional Review Board in Bonn. In 11 of the 22 patients, seizure onset was bilateral, extratemporal, or could not be unequivocally determined. Data from the remaining 11 patients were analyzed in this study (Table 1).

2.2. Electrodes and recordings

LFPs were recorded with hybrid depth electrodes (AdTech, Racine, WI) containing a bundle of eight microwires protruding approximately 4 mm from their tips. The differential LFP signals were amplified using a 256-channel Neuralynx ATLAS system (Bozeman, MT), filtered between 0.1 and 9000 Hz, and sampled at 32 kHz. Action potential (spike) detection and sorting was performed using the WaveClus package (Quiroga et al., 2004). Sorted units were manually confirmed and classified as single units (SU), multi-units, or artifacts based on spike shape and variance, peak-amplitude-to-noise level, the inter-spike interval (ISI) distribution of each cluster, and presence of a refractory period. Multi-units and artifacts were excluded from further analysis. A total of 249 SU were recorded interictally and 210 pre-ictally (mean number per patient 23 interictally and 30 pre-ictally). Fig. 1 shows an example of an intracranial LFP recorded with a microwire capturing two different SU.

Electrode locations were determined by clinical criteria and verified by MRI and CT. All patients had bilaterally implanted depth electrodes, each equipped with 8 cylindrical contacts, in multiple sites of the MTL, including hippocampus, amygdala, entorhinal cortex and parahippocampal cortex. Total recording time analyzed from these patient (interictal and pre-ictal) was 300 min (see Table 1).

Recordings were performed continuously for the entire duration of the epilepsy monitoring (typically 7–14 days). Eleven interictal sessions were recorded one or two days after electrode implantation. In 8 of the 11 patients, a total of 21 seizures were recorded and analyzed. All seizures occurred at least one day after the interictal recordings. Pre-ictal recordings covered the period of 10 min before seizure onset as determined from the intracranial macro electrode recordings via joint visual inspection by two board-certified electroencephalographers (H.G. and F.M.). Electroclinical evaluation of interictal and ictal recordings confirmed that seizures typically started near the most medial contacts of the clinical depth electrodes (Supplementary Table S1).

Based on electrographical seizure onset, or postoperative seizure control in those cases where no seizure could be recorded, electrodes were labeled ipsilateral or contralateral, depending on their location with respect to the seizure onset and/or resection zone. Note that not all of the 11 patients included in this study went on to have surgery, and of those who did, not all became seizure-free (Table 1). Nevertheless, at the time of electro-clinical evaluation all 11 patients were diagnosed as unilateral MTLE, and therefore included in this study.

2.3. Analysis of electrophysiological data

To assess the neurons' spontaneous firing patterns, we computed autocorrelation histograms with 1 ms bins and time lags ranging from −100 to 100 ms (Fig. 1C). To quantify burstiness, i.e. the firing of dense clusters of action potentials, we calculated the mean autocorrelation time (MAT) for every SU. MAT denotes the mean of each neuron's autocorrelogram (Csicsvari et al., 1998). Low MAT values thus characterize high burstiness, regardless of individual firing rates (Supplementary Fig. S1).

Following Ison and coworkers (Ison et al., 2011), we classified all SU based on their action potential duration (spike width, measured from maximum to minimum, Fig. 1B), as either putative interneurons (width $\leqslant$ 0.65 ms) or putative principal cells (width > 0.65 ms).

Two-way ANOVA was used as an omnibus test to check for any statistical difference in burstiness (as characterized by MAT) with respect to location (ipsi- or contralateral) and time of recording (interictal or pre-ictal). Post hoc analysis between groups was performed using Wilcoxon ranksum tests with Bonferroni correction for multiple (four) comparisons.

3. Results

Classification of SU as either principal cells or interneurons based on spike width yielded a total of 415 principal cells and 44 interneurons. The plausibility of this classification was verified by comparing mean baseline firing rate and burstiness using a Wilcoxon ranksum test. Since it is a priori unclear how these