EPILEPSY CURRENTS

Current Literature
In Basic Science

Dyeing to Be Fired: Firing Order Distinguishes Two Types of Bursting Activity

Deterministic and Stochastic Neuronal Contributions to Distinct Synchronous CA3 Network Bursts.

Takano H, McCartney M, Ortinski PI, Yue C, Putt ME, Coulter DA. *J Neurosci* 2012;32:4743– 4754.

Computational studies have suggested that stochastic, deterministic, and mixed processes all could be possible determinants of spontaneous, synchronous network bursts. In the present study, using multicellular calcium imaging coupled with fast confocal microscopy, we describe neuronal behavior underlying spontaneous network bursts in developing rat and mouse hippocampal area CA3 networks. Two primary burst types were studied: giant depolarizing potentials (GDPs) and spontaneous interictal bursts recorded in bicuculline, a $GABA_A$ receptor antagonist. Analysis of the simultaneous behavior of multiple CA3 neurons during synchronous GDPs revealed a repeatable activation order from burst to burst. This was validated using several statistical methods, including high Kendall's coefficient of concordance values for firing order during GDPs, high Pearson's correlations of cellular activation times between burst pairs, and latent class analysis, which revealed a population of 5–6% of CA3 neurons reliably firing very early during GDPs. In contrast, neuronal firing order during interictal bursts appeared homogeneous, with no particular cells repeatedly leading or lagging during these synchronous events. We conclude that GDPs activate via a deterministic mechanism, with distinct, repeatable roles for subsets of neurons during burst generation, while interictal bursts appear to be stochastic events with cells assuming interchangeable roles in the generation of these events.

Commentary

Decades of research have helped to characterize many electrophysiological patterns in normal and in epileptic brain tissue. However, this characterization has primarily been on the scale of either a very large or very small number of neurons. Neurophysiological recordings—from EEG down to local fields—record electrical phenomena occurring in a large population of neurons: spikes, slow waves, seizures, etc. While these phenomena form the basis of clinical decision making, little is known about their complex dynamics at the cellular level. On the other side of the spectrum, techniques such as patch clamping and spike sorting monitor individual action potentials, but typically follow fewer than 10 cells at a time. Multi-electrode arrays can monitor up to 100 cells but become technically challenging beyond that number. One method to bridge this gap and monitor action potentials from larger numbers of cells simultaneously is to use voltage- or calcium-sensitive dyes. The key to this technology is that the number of recorded cells depends upon optics and the camera resolution rather than electrode placement. Different forms of this technology have been under development for several years, but it has been challenging to apply it to the fast waveforms seen in epilepsy. In this article, Takano and colleagues use a combination of fast confocal microscopy and multicellular calcium imaging to monitor a large number of neurons during network bursts, then use sophisticated statistics to process the data. They are able to track the firing order of action potentials of individual cells during two different types of bursting activity, and find intriguing differences between these bursts.

Over the past 2 decades, several technologies have been developed that strive to monitor the activity of neuronal networks in the brain. Most of these exploit advanced imaging technology: fMRI, SPECT, PET, MEG, and microscopic imaging of voltage-sensitive dyes. These technologies have been revolutionary but often have limited spatial and temporal resolution. Relatively brief events such as epileptic spikes are fast and small enough that it has been very difficult to investigate network behavior at the cellular level. A more recent development is functional multicellular (or multineuronal) calcium imaging (fMCI) (1). In this technique, which has been under development for just over a decade (2), a calcium-sensitive fluorescence indicator is bulk-loaded across a large area of brain cortex, and it can be performed in vivo (3). The fluorescent molecule is designed so that intracellular concentration rises to much higher levels than in the extracellular space, which produces good signal fidelity. The intracellular indicator changes fluorescence very quickly and strongly in response to calcium influx, allowing imaging of action potentials in individual cells.

The current work uses this technology to analyze population firing during epileptiform bursts in the hippocampal

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CA3 region from postnatal day (P) 4-9 rat and mouse brain slices. Two types of bursting activity were seen: spontaneous synchronous activity consistent with giant depolarizing potentials (GDPs) (4) and epileptiform bursts that arose when the $GABA_A$ antagonist bicuculline was added. Although these two bursts are not necessarily analogous to human physiology, they present a fundamental question in epilepsy whose answer has been waiting for the correct technology—how are they different? The GDPs are spontaneous events that likely represent normal activity, while the epileptiform bursts appear when the slice loses all $GABA_A$ activity; there ought to be fundamental differences in their neurophysiology. To analyze these two phenomena, the authors had to image the slice very rapidly (down to 3 ms per frame) with a confocal microscope, and then devise a statistical method to process the data. This latter method is the most innovative portion of the work and illustrates the two-edged sword of studying network dynamics: the amount of information can be overwhelming.

Most experiments tracked action potentials from 60 to 70 cells. The first challenge was to determine the firing time of each of these cells, but it was much more difficult to translate those data into a statement about network dynamics. Were there groups of cells firing together? Did some groups initiate the burst? Was firing order consistent during subsequent bursts? Were there any spatial patterns to inform about network structure? Such questions, which are at the heart of network dynamics in epilepsy, require detailed statistical analysis. The authors utilized three statistical tools: Kendall's W, Pearson's correlation, and a latent class analysis. The first two tested whether firing order was consistent (deterministic) within a small cluster and while different bursts propagated across the slice. The latent class analysis tested whether certain cells tended to be "early responders." The authors conclude that GDP bursts are deterministic: cells tend to fire at similar times whenever a burst occurs. There was strong evidence that a population of cells were early responders, suggesting an ordered pattern underlying the GDP activity. This finding is corroborated by another recent publication concluding that certain cells are responsible for driving and synchronizing gamma oscillations (5). In contrast, the current article finds that epileptiform bursts did not have structured firing order; they were stochastic, suggesting they are generated through different network pathways.

There are some important limitations to the data. These P4-9 rodents have different physiology than humans or even mature rodents: the wiring is still developing, and both GDPs and the bicuculline-induced bursts are somewhat unique to this preparation. GABAergic channels have unusual properties in this age group, which is right at the transition of depolarizing to hyperpolarizing chloride reversal potentials. This effect, which appears to be present primarily in neonatal brain slices (6), creates a mixture of excitatory and inhibitory GABA channels in a developing glutamatergic network. This leads to the paradoxical finding that bicuculline normally blocks the GDPs, except in a small subset of mice in which they become higher amplitude and less frequent, and are considered epileptiform (7). Both of these waveforms have somewhat limited applicability to human neurophysiology, and similar research is necessary in other models of normal and epileptic behavior in the future.

There are two main contributions from this work. First, the analysis demonstrated a fundamental difference in the network dynamics of a "normal" versus "epileptic" burst. Despite the similarities in the two types of bursts at the field potential level, these two phenomena appear to be very different neurophysiologically. This finding leads to many additional questions about epileptic activity and networks. The second contribution is that this method opens the door for many future studies in epileptic networks to explore these questions. As technology evolves to allow recording of more and more cells simultaneously, automated algorithms and statistical methods such as these are critical for both interpretation and analysis. Can this method discern normal from epileptic brain activity in other phenomena such as high frequency oscillations (8)? Can it characterize firing dynamics of other models of epilepsy, and distinguish them from other normal brain activities? Do seizures originate from multiple potential foci, functioning as a network phenomenon rather than a focal onset? These questions have been asked for many years; perhaps there are tools to start answering them.

by William Stacey, MD, PhD

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