

SINGLE-NEURON ACTIVITY AND IMAGING DURING EPILEPTOGENESIS: WHAT CAN BE SEEN AND HOW BEST TO SEE IT?

Evolving into Epilepsy: Multiscale Electrophysiological Analysis and Imaging in an Animal Model

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Epilepsy research for the design of seizure detection/prediction neuroprosthetics has been faced with the search for electrophysiologic control parameters that can be used to infer the epileptic state of the animal and be leveraged at a later time to deliver neurotherapeutic feedback. The analysis presented here uses multi-microelectrode array technology to provide an electrophysiologic quantification of a hippocampal neural ensemble during the latent period of epileptogenesis. Through the use of signal processing system identification methodologies, we were able to assess the spatial and temporal interrelations of ensembles of hippocampal neu-

rons and relate them to the evolution of the epileptic condition. High-field magnetic resonance (MR) imaging was used to determine the location of electrode placement and to evaluate hippocampal pyramidal cell structural damage. Long-term single unit activity analysis suggests that hippocampal neurons in both CA1–2 and dentate regions increase the number of occurrences and duration of their bursting activity after injury to the contra-lateral hippocampus. The trends inferred from both single neuron and ensemble analysis suggests that the evolution into epilepsy is not abrupt but modulates gradually from the time of injury.

COMMENTARY

Long-term electrophysiological analyses and high-field MRI of the network mechanisms and brain damage associated with epileptogenesis have the potential to reveal the progressive changes in single neurons within neural circuitry. The many difficulties in this general approach include, but are not limited to: (i) recording from single neurons for prolonged periods; (ii) defining the identity and precise location of the recorded neurons; and (iii) relating the recorded electrical activity to the neuronal damage, gliosis, and the epileptogenic focus or zone. The present paper by Sanchez and coworkers combines multielectrode arrays with high-bandpass recording to isolate, identify, and analyze single-neuron activity in the hippocampus. Subsequent high-field (17.6 Tesla) MRI of fixed brains identified the electrode tracks, recording sites, and areas of histopathological damage. The combination of simultaneous single-cell recordings from many hippocampal neurons and post hoc MRI represents a new technical approach to studying epileptogenesis; thus, it is important to consider the strengths and potential limitations of these techniques.

The electrophysiological experiments employed electrically induced status epilepticus to study epileptogenesis, and the single-neuron electrical recordings with 16-electrode arrays

were analyzed at five different times after status epilepticus. The identification of single neurons was based on the waveform of the action potentials; and using this criterion, the authors found that action potential firing increased in frequency across the recording sessions and bursting was more prominent as the time after status epilepticus progressed. This finding is consistent with long-standing views that epileptogenesis involves hyperactivity and enhanced burst generation, and these experiments suggest that epileptogenesis is a gradual, progressive process. However, the study by Sanchez et al. may best be viewed as the beginning of a single-neuron analysis of the time-dependent changes that occur during epileptogenesis.

At least two key issues regarding electrophysiological research deserve further consideration. First, how can there be confirmation that the same set of single neurons is being recorded throughout the analysis? And, how important is this issue? Second, how often are recordings needed to analyze accurately the temporal progression of seizure activity and/or a related physiological mechanism during epileptogenesis? After electrically inducing status epilepticus, Sanchez and colleagues studied three neurons in CA1–CA2 as well as three other neurons in the dentate gyrus for about 35 days. Although it appears that prolonged or nearly continuously recordings (16 channels at 24 kHz) were obtained during epileptogenesis, the data analyses were based on specific recording sessions. The interspike intervals clearly decreased between the two early recording sessions (days 1 and 3) and the two late recording sessions

(days 32 and 36), and the coefficient of variation was quite high, which the authors attributed to and used as an indicator of burst firing. Plots of the coefficient of variation increased overall across the 35-day duration of the experiments. However, the regression analysis of the coefficient of variation for individual neurons appeared to show a time-dependent decrease in two neurons. Furthermore, the values of coefficient of variation for all of the neurons seemed nearly as dependent on the particular recording session as on time after status epilepticus. One concern is that this latter observation could imply that the same neurons were not recorded in the different recording sessions. To address this possibility, the authors analyzed the waveform of the individual action potentials over time for each hypothetical neuron. The raw data attest to the similarity of the waveforms for each “single neuron.” However, the similarity of waveforms across some of the different neurons suggests that the recording from one neuron could have been lost as another one with the same or similar waveform began to be recorded.

Would lost recordings (i.e., a shift from recording of one neuron to recording of another neuron) affect the outcome of the analysis? In identifying individual neurons by their waveforms, it is possible that slight movements of the brain and/or recording electrodes could lead to changes in the waveform of the action potentials from a single neuron. In other words, the same neuron could still be recorded, but its action potential waveform could have changed substantially. This type of gradual change is often seen in hours-long recordings from neurons in brain slice experiments and could reasonably occur in an experiment over several weeks on an awake and intact animal. Is taking a sample from several neurons of the same type during each recording session another equally valid approach? Or, is it necessary to definitively record from precisely the same neuron over the 5-week period? The paper by Sanchez and coworkers does not answer this question and points out the difficult issues confronting researchers studying time-dependent changes during epileptogenesis with electrophysiological recordings from

single neurons in intact, freely behaving animals. For studies involving interspike interval and coefficient of variation as a function of time after status epilepticus, it would seem that mean values from a population of single-neuron recordings might yield the same results as continuous recording from the same single neurons in the dentate or CA1.

One of the strengths of this study is the simultaneous use of long-term single-neuron recording with MRI. Although it would be optimal to combine recording and imaging at many different time points during the process of epileptogenesis, such a procedure is not feasible because electrophysiological recordings require metal electrodes, which are incompatible with MRI. Sanchez et al. imaged the fixed brain at the end of the electrophysiological experiments to assess recording site and brain damage and were able to determine the precise location of their stereotaxically guided electrodes and relate this information to the sites of brain damage. However, both of these pieces of information can be obtained with traditional anatomical techniques. The question, then, is whether this new approach is actually better, and if so, for what? Although the exact recording site is valuable data, it is not clear that one subregion of the CA1 area or dentate gyrus is consistently different from another—although further studies are needed to address this issue. The use of electrophysiological criteria to determine whether a recorded neuron is a principal neuron versus an interneuron might be more useful than the precise location of the recorded neuron in the hippocampus. In regard to brain damage, future research is needed to ascertain quantitatively how neuronal injury and gliosis are related (or not related) to seizure activity and altered electrophysiological mechanisms. The MRI approaches employed by Sanchez and coworkers are interesting and novel, particularly when linked to electrophysiological data during epileptogenesis, but the key methodological question for future research is how best to implement these approaches in a manner that allows quantitative assessment of the mechanisms of epileptogenesis.

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