OPTICAL METHODS FOR INTERROGATING EPILEPTIC MECHANISMS

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Summary

A set of emerging optical imaging technologies exemplified by mesoscopic two-photon imaging with genetically targeted calcium indicators and a new generation of voltage sensitive dyes, allow us to record the activity in epileptic circuits of awake behaving animals with cell specificity at high spatio-temporal resolution, complementing classical electrophysiological approaches. These methods, in combination with transcriptomic profiling via high-throughput multiplexed error-robust fluorescence in situ hybridization (MERFISH) microscopy and with optogenetic-chemogenetic strategies for testing causality, hold great promise for dissecting the circuit mechanisms of epilepsy.

Key words: Optical methods, Circuit mechanisms of epilepsy, 2-photon imaging, transcriptomic analysis, MERFISH

Optical imaging methods have come of age in neuroscience, allowing chronic *in vivo* monitoring and manipulation of large networks of identified neurons with unprecedented yield and cell specificity. These tools are ideally suited for the study of circuit mechanisms of epilepsy. Understanding how neurons of identified type and circuit location get engaged into abnormal epileptiform patterns of activity requires the 1) study of neuronal networks across time with cell specificity and high spatio-temporal resolution, the 2) causal manipulation of circuit elements of interest, and the 3) cell-specific interrogation of molecular mechanisms. Below we focus on items 1 and 3, briefly summarizing recent work that promises to usher a new era in investigating the mechanisms underlying epileptic disorders.

The new generation of extracellular multi-electrode probes and arrays allow the monitoring of hundreds to thousands of neurons (new silicon probes [1]) or large cortical regions (MEAs) with high temporal resolution, and have successfully been used to gather valuable information about epileptic mechanisms in humans [2] and animals [1]. Silicon probes are however invasive and suffer from a selection bias, inability to accurately identify specific cell types, and poor capacity for monitoring stably the same units over time and for determining their precise localization and connectivity across the cortical circuit. Imaging methods can overcome these problems, albeit at the cost of relatively poor temporal resolution and limited access to deep brain regions. However, these problems have been recently mitigated with the advent of a new generation of *in vivo* voltage sensitive dyes whose temporal resolution is measured in milliseconds [3-5] and specialized miniscope microscopy methods capable of accessing deep nuclei for optical imaging [6, 7].

Wide-field epifluorescence microscopy with spatial resolution in the tens of micrometers and kHz frame rate has been available since the early 90's [8] and continues to yield important information. Rossi et al. recently used this method to argue that acute epileptiform activity induced by chemo-convulsant injection travels along homotypic connections to spread across cortical sensory areas extending several millimeters [9]. However this method lacks the spatial resolution needed to resolve activity arising in individual cells. In the last 2 decades, two-photon (2P) laser scanning microscopy [10] is being increasingly applied to study initiation and propagation of epileptiform activity as it affords single cell resolution at the sub-micrometer scale and can be combined with genetic labeling techniques to image specific cell types. For example, 2P imaging has been used to study genetic syndromes of epilepsy such as the mouse models of Stargazer [11] and Dravet [12], showing that desynchronization of neuronal firing is a feature of certain epileptic syndromes (see also [7]). Direct visualization of acute focal cortical seizure events induced by chemo-convulsant injections with 2P imaging, revealed that seizure events start as local neuronal ensemble hyper-synchronization that spreads in a saltatory fashion to nearby territories [13-15]. Individual excitatory neurons appear to get engaged reliably into acute epileptiform events of variable duration, with supragranular neurons

preceding infragranular ones [16, 17]. Functional connectivity analysis during interictal periods has led to the identification of epileptiform network motifs and putative cell types hypothesized to contribute significantly to the evolution of aberrant activation patterns [18,19]. Multiple other studies and applications, well beyond the scope of our brief review, are currently ongoing.

Recent advances in optical technology allow the simultaneous monitoring of exceptionally large (~5x5mm^2) cortical fields of view (mesoscopic imaging) covering multiple areas lying on the surface of the brain, imaging thousands of neurons in different layers without forgoing the micrometer resolution necessary to identify and localize individual units [20]. Deeper brain regions are also accessible for imaging using miniscope technology [6, 7]. Using this approach, Shuman et al. [7] found that CA1 place cells in mice with temporal lobe epilepsy become unstable, completely remapping their place fields across a period of a week. Importantly, miniscope technology allows imaging in freely moving animals, which is essential in epilepsy models that have low spontaneous seizure frequency. Multiple organisms from rodents to zebrafish to primates can be interrogated with these methods, yielding important mechanistic information at the pre-clinical level. Results can be compared with global EEG and fMRI measurements, which are more appropriate for studying distributed epileptic networks in human patients (see [21]). Combining these techniques with optogenetic or chemo-genetic approaches (not reviewed here) to probe causal relationships by manipulating neuronal activity in specific cell types, is a powerful approach for dissecting the circuit mechanisms of epilepsy.

The combination of new optical imaging and genetic technologies has great promise for unravelling the cellular interactions that generate epileptiform activity, that is, cause neuronal networks to become epileptogenic. Particularly promising is a recently developed high-throughput *ex vivo* mRNA hybridization microscopy method (MERFISH), capable of resolving hundreds to thousands of distinct mRNA transcripts per imaging session while preserving spatial localization, thereby making it possible to attribute in situ mRNA expression profiles to specific cell types [22-24]. Aligning 2P images obtained *in vivo* with *ex vivo* MERFISH images obtained from the same tissue, has the potential to uncover the cell-specific transcriptomic profiles that underlie the aberrant functional activity phenotype exhibited by the same cells *in vivo.* An important question that can be studied with these methods is the mechanism by which specific pharmacologic interventions succeed or fail to contain abnormal patterns of excitability that lead to seizures. In time, information gained will help identify more effective therapeutic targets for pharmaco-resistant epilepsies.

In conclusion, now is a particularly exciting time to work in the field of epilepsy. The optical imaging and spatially resolved transcriptomic microscopy techniques outlined above, as well as other techniques not reviewed here (see [21,25,26]), will undoubtedly be harnessed in the near future to reveal in unprecedented detail the circuit mechanisms of epilepsy.

Acknowledgements

SS and JL have received support from VA Merit Awards I01 RX002981 and I01 BX005221-01.

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