Large scale *in vivo* recordings to study neuronal biophysics

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Over the last several years, technological advances have enabled researchers to more readily observe single-cell membrane biophysics in awake, behaving animals. Studies utilizing these technologies have provided important insights into the mechanisms generating functional neural codes in both sensory and non-sensory cortical circuits. Crucial for a deeper understanding of how membrane biophysics control circuit dynamics however, is a continued effort to move toward large scale studies of membrane biophysics, in terms of the numbers of neurons and ion channels examined. Future work faces a number of theoretical and technical challenges on this front but recent technological developments hold great promise for a larger scale understanding of how membrane biophysics contribute to circuit coding and computation.

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Introduction

Capable of remarkable feats of computation, our brain regularly translates our complex sensory world into neural codes that allow us to process and interact with our environment. Neural computation begins in single cells, where myriads of ion channels compose the algorithms determining a neuron's output signal. Diverse biophysical profiles thus emerge from unique ion channel expression patterns, affording finely tuned neural computation based on the functional role of the local neural circuit. Remarkably, how this vast ionic and biophysical diversity controls circuit coding and behavior remains unresolved. Answering this question poses a significant challenge, as examining the membrane biophysics of neurons in a behaving animal remains technically difficult. Over the last several years however, technical achievements have improved the ability of researchers to examine membrane biophysics in awake, behaving animals. These studies provided deeper insights into the mechanisms underlying circuit coding by observing membrane dynamics during sensory processing and active exploration. While there are many examples demonstrating how the examination of membrane biophysics can advance our understanding of circuit coding mechanisms, I begin this review by highlighting two recent contributions. Next, I discuss what challenges remain for understanding how biophysics control circuit coding and the boundaries that limit the examination of membrane dynamics in large populations of neurons. Finally, I present some of the more promising technologies for examining biophysics at a larger scale and explore the insights that such approaches could provide.

What can membrane dynamics tell us?

Recent works in several brain systems serve as ideal examples for how the examination of membrane biophysics can lead to significant gains in our understanding of the mechanisms generating circuit codes. First, the non-sensory hippocampal and entorhinal regions contain neurons with spatially selective response properties, offering tractable systems for linking biophysics with functionally defined neural codes. In addition, the hippocampus and entorhinal cortex lay several synapses away from primary sensory inputs, raising the possibility that neural codes in these regions emerge from mechanisms within the circuits themselves. Second, primary visual cortex contains neurons with highly structured coding properties and is one of the longest, most studied regions in the brain. Recent work examining the membrane biophysics of primary visual cortex neurons however, has provided exciting new insights into the mechanisms underlying state-dependent visual coding.

Spatial navigation

Animal survival depends on accurate spatial coding; recalling and navigating to a home or mate intimately depends on an animal's ability to determine their location within a spatial environment. Only in the last few decades however, have researchers begun to discover the building blocks of an internal neural navigation system. This system depends, in part, on spatially selective neurons in the medial entorhinal cortex (MEC) and hippocampus, which translate the external environment into an internal map of space. MEC grid cells provide the neural metric for distance traveled, coding space with a periodic firing pattern that tiles the environment, while hippocampal

place cells fire at a higher rate when the animal is located only in a specific portion of the environment [1,2,3•]. Grid and place codes exist in the parahippocampus of multiple species, from rodents to primates, suggesting they serve as a fundamental and evolutionary conserved coding scheme utilized for memory and navigation [1,4•,5–7].

Several classes of computational models propose mechanistic explanations for the ontogenesis of grid and place cell firing patterns. The coding principles, parameters and assumptions of these models however, differ for grid versus place cells. Computational models of grid cells historically fall into one of two classes, although some convergence between these two classes has occurred in more recent models [8–10]. In one class of models, multiple velocity-driven oscillations constructively and destructively interfere, giving rise to spatial firing patterns reflective of oscillatory interference [11]. In a second class of attractor-based network models, recurrent connectivity supports the emergence of periodic activity bumps across a neuronal lattice, with inputs that reflect the animal's velocity translating the activity bumps across the lattice [12]. Critically, each model generates specific predictions regarding the subthreshold membrane dynamics of neurons as an animal approaches and passes through a grid firing field. Oscillatory models predict that amplitude modulated membrane oscillations will drive the formation of a grid firing field. Attractor-based network models, on the other hand, predict that up and down ramps of activity will determine the spatial location of grid firing fields [13••]. Recently, a series of whole-cell patch clamp recordings in awake, behaving animals tested these computational predictions. Recordings of grid cells in head-fixed mice running on a virtual linear track demonstrated that large depolarizing ramps predict spatially specific firing and oscillatory membrane dynamics do not [9••,13••]. While not predictive of spatial coding, membrane potential oscillations were observed to correlate with grid cell spike timing [13••]. The temporal spiking dynamics of grid cells however, may be driven by rhythmic synaptic input rather than oscillatory membrane biophysics, which depend on ion channel kinetics [14•]. Combined, these studies revealed membrane dynamics more consistent with network models of grid cell formation, providing critical insight into the mechanisms generating spatial signals in a high-order brain region.

Computational models of place cell formation fall into a more diverse set of classifications. Many however, propose that place cell firing reflects summed input from external sensory features at the location the cell is active (for a recent review, see [15]). Consistent with this hypothesis, hills of depolarization that correlate with the shape of the place field have been observed in whole-cell patch clamp recordings of place cells in behaving animals [16,17]. Recently, whole-cell recordings in freely moving animals additionally demonstrated that silent neurons in the hippocampus convert to spatially selective place cells after the application of a depolarizing current injection, suggesting that these neurons receive spatially modulated inputs that normally cannot drive spikes [18••]. This result is highly suggestive of a role for single-cell biophysics, such as active dendritic conductances, in determining the response of individual place cells during navigation.

Visual processing

Behavioral state, such as sleeping versus attending, significantly impacts sensory processing. One of the more recent examples of this was the discovery that locomotion, an active behavioral state, modulates the spiking response of primary visual cortical neurons to visual stimuli [19–21]. The development of virtual reality for head-fixed mice allows the examination of neural responses in an awake, behaving animal in combination with strictly controlled visual stimuli [22]. Under these conditions, researchers found that neurons in V1 of mice show orientation and spatial frequency selectivity, with firing rates increasing twofold between resting and running behavioral states [19,23]. The cellular mechanisms underlying enhanced visual responsiveness with locomotion however, remained unresolved. Recent whole-cell patch clamp recordings from visual cortex in head-fixed mice running on a spherical treadmill have provided insight into the biophysics underlying state-dependent changes in visual processing [24••]. During locomotion, the subthreshold membrane response increases in amplitude and reliability, improving the signal to noise ratio and enhancing performance on visual detection tasks [24••,25]. This decrease in membrane potential variability results from a shift toward excitation in the balance between inhibition and excitation [24••], possibly due to state-dependent changes in neuromodulation. Consistent with this hypothesis, recent work reported that V1 pyramidal cells enhance their response after nicotinic activation of interneurons [26] and that noradrenergic input is a necessary component of the tonic depolarization of V1 neurons observed during locomotion [25]. Taken together, the application of whole-cell patch clamp recordings to V1 neurons in an awake, behaving animal provided a deeper mechanistic understanding of state-dependent sensory processing.

The critical nature of examining biophysics in behaving animals

Despite the powerful insights gained by observing membrane dynamics in awake, behaving animals, theoretical and technical challenges remain. Even so, the importance of moving toward the examination of biophysics in behaving animals cannot be overstated. One example for why this movement is critical comes from a series of recent studies on the temporal dynamics of MEC neurons. In MEC, several *in vitro* whole-cell patch clamp studies have