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Topic Introduction

Whole-Cell Recording in the Awake Brain

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Intracellular recording is an essential technique for investigating cellular mechanisms underlying complex brain functions. Despite the high sensitivity of the technique to mechanical disturbances, intracellular recording has been applied to awake, behaving, and even freely moving, animals. Here we summarize recent advances in these methods and their application to the measurement and manipulation of membrane potential dynamics for understanding neuronal computations in behaving animals.

INTRODUCTION

Intracellular recording has served as a key technique for investigating the cellular and circuit mechanisms underlying neural activity. Because of its high sensitivity to mechanical disturbances, intracellular recording previously was limited primarily to stable preparations such as brain slices and, more recently, the intact brains of anesthetized animals (Pei et al. 1991; Ferster and Jagadeesh 1992; Metherate et al. 1992; Moore and Nelson 1998; Zhu and Connors 1999; Margrie et al. 2002). Thus, it had not been straightforward to link the accumulated knowledge regarding synaptic and intrinsic mechanisms from experiments conducted mostly in vitro with higher brain functions that generally involve active participation of the animal. The development of intracellular recording methods for awake behaving animals across multiple species (Covey et al. 1996; Fee 2000; Aksay et al. 2001; Margrie et al. 2002; Wilson et al. 2004; Lee et al. 2006, 2014; Harvey et al. 2009; Long et al. 2010; English et al. 2014; Tan et al. 2014) has therefore played and will continue to play a critical role in understanding the neural basis of cognitive processes (for a review, see Long and Lee (2012)).

INTRACELLULAR RECORDINGS IN HEAD-FIXED BEHAVING ANIMALS

Information processing in the brain is affected by vigilance or arousal states. Therefore, some recordings need to be performed during wakefulness to fully understand neuronal computations. Several intracellular recording studies have demonstrated differences in subthreshold membrane potential dynamics during anesthetized or sleep versus awake states (Steriade et al. 2001; Mahon et al. 2006; Constantinople and Bruno 2011; Haider et al. 2013). Furthermore, animals do not simply passively receive sensory input; they actively interact with the environment, which also affects information processing. For instance, whole-cell recordings in the barrel cortex of head-fixed mice revealed

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membrane potential fluctuations synchronized to active whisker movements. Different neurons had different phase offsets, which could contribute to position coding (Crochet and Petersen 2006).

Intracellular recording has also been performed in head-fixed animals engaged in activities involving much larger bodily motions such as walking, running, or wing-beating. It had been shown previously that locomotion affects the firing rate of orientation-tuned visual neurons (Niell and Stryker 2010). Cellular mechanisms for such gain modulation have been investigated using whole-cell recordings in head-fixed mice that alternated between periods of running and sitting still on a spherical treadmill while being presented with visual stimuli (Bennett et al. 2013; Polack et al. 2013). Whole-cell recordings obtained from flies fixed in place at the head but free to move their wings were used to study flight-dependent gain modulation in visual neurons from the lobula plate (Maimon et al. 2010).

Head-fixed preparations that allow intracellular recordings to be performed during locomotion on a treadmill have been combined with visually simulated spatial environments where visual scenes are updated based on the animal's movements. Such virtual reality systems allow the study of the brain's navigation system. Whole-cell recordings from mouse hippocampal CA1 place cells revealed intracellular features such as a ramping subthreshold depolarization of the membrane potential during traversals through place fields and phase precession of the intracellular theta-band oscillation with respect to the extracellular local field potential theta rhythm, providing deeper understanding into place cell mechanisms (Harvey et al. 2009). Similar approaches have been used for measuring intracellular features of entorhinal grid cells, allowing investigation into the mechanisms underlying their periodic firing pattern as a function of the animal's location in space (Domnisoru et al. 2013; Schmidt-Hieber and Häusser 2013), as well as for assessing the role of large calcium-based events in place field plasticity in CA1 (Bittner et al. 2015).

Protocol: **In Vivo Patch-Clamp Recording in Awake Head-Fixed Rodents** (Lee and Lee 2016a) describes a detailed training procedure and an efficient patching method for obtaining whole-cell recordings from awake head-fixed animals (Lee et al. 2014).

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INTRACELLULAR RECORDINGS IN FREELY MOVING ANIMALS

Although many questions regarding input–output transformations within individual neurons in different behavioral paradigms can be addressed using head-fixed animals, other topics require animals to be non-head-fixed—for example, processing that depends on vestibular input or requires rapid head movements (Monaco et al. 2014). Lee et al. (2006) were the first to demonstrate that intracellular recording (in this case using the whole-cell method) was possible in freely moving animals. These recordings were obtained in anesthetized animals using a head-mounted linear microdrive to advance the pipette electrode; the pipette was subsequently fixed (i.e., "anchored") in place with dental acrylic and the animal awakened for unrestrained behavior. The recordings could survive severe mechanical disturbances and remain stable for ~20 min while rats explored their environment freely. Other groups have shown that sharp intracellular recordings could be obtained and maintained in freely moving birds (Long et al. 2010; Hamaguchi et al. 2014; Kosche et al. 2015; Vallentin and Long 2015) and mice (English et al. 2014; Schneider et al. 2014) using a head-mountable linear microdrive. The first such recordings were made from bird premotor HVC neurons to measure the subthreshold membrane potential dynamics during singing, and the resulting recordings supported a synaptic chain-like mechanism for birdsong generation (Long et al. 2010). Using this method in freely moving mice, Schneider et al. (2014) demonstrated that the membrane potential of excitatory neurons in auditory cortex depolarizes and decreases its variability immediately before and during bodily movements including locomotion, head movements, and grooming. Additional intracellular recordings in head-fixed mice running in place in concert with optogenetic manipulations revealed that these membrane potential dynamics reflect motor-related corollary discharge originating from secondary motor cortex.

An alternate version of the freely moving whole-cell recording technique uses pipette anchoring combined with a conventional micromanipulator located to the side of the animal (Lee et al. 2009)

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instead of a head-mounted microdrive. Conventional micromanipulators not only allow the recording pipette to be held in place more strongly during the anchoring process but also allow the pipette to move horizontally, thus providing a higher probability of obtaining high-quality whole-cell recordings (see Lee and Lee [2016a] for a description of how horizontal movement is used for in vivo patching). This technique has been applied to the study of hippocampal place cells in freely moving rats exploring spatial environments. One such study focused on spikelets, which are all-or-none events with similarities to the action potential but much smaller in amplitude and thus not easily detectable extracellularly. Recordings of hippocampal CA1 pyramidal neurons showed that spikelets occur in the behaving animal and can contribute directly to place cell firing during spatial exploration (Epsztein et al. 2010). In another study, the intracellular features of place cells and silent cells were compared in rats exploring a novel environment. Contrary to expectations from the basic model of neuronal integration, silent cells—unlike place cells—did not display large, spatially tuned inputs at the soma. Furthermore, the excitability of these cells measured even before the rats explored the environment (i.e., for the first time) could partially predict which cells would become place cells, suggesting that intrinsic excitability plays a significant role in place field origin and memory allocation (Epsztein et al. 2011). This method using conventional micromanipulators initially used dental acrylic to fix pipettes in place. However, a limitation of this pipette stabilization technique was that the anchoring process took a relatively long time (~10 min) and thus required that the whole-cell recording be obtained in an initially anesthetized animal that was subsequently awakened for free behavior (Lee et al. 2009). In addition, recording losses during the anchoring process limited the overall success rate of the method (Lee et al. 2009).

To improve this method, the use of dental acrylic was replaced with an ultraviolet (UV)-cured adhesive and UV-transparent collar to anchor the whole-cell pipette in place. This provides highly efficient pipette stabilization with no recording losses. Furthermore, it anchors a pipette in 15 sec, thus allowing the whole-cell recording to be obtained in both anesthetized and awake animals (Lee et al. 2014). This technique has also been applied to the study of cellular mechanisms underlying place cell activity. In particular, injecting a spatially uniform holding current into a silent cell reversibly converted it into a place cell during spatial exploration. Injecting different amounts of current into the silent cell revealed the sudden emergence of large, spatially tuned inputs and place cell spiking above a cell-specific threshold, supporting the idea that nonlinear dendritic mechanisms underlie place cell firing by gating the propagation of spatial inputs to the soma (Lee et al. 2012). These observations were reproduced in drug-free, awake-patched recordings in freely moving animals (Lee et al. 2014).

Protocol: **Efficient Method for Whole-Cell Recording in Freely Moving Rodents Using UV-Cured Collar-Based Pipette Stabilization** (Lee and Lee 2016b) describes a detailed step-by-step procedure for awake-patched whole-cell recording in freely moving, drug-free rats and mice.

We end this introduction with a comment on whole-cell and sharp intracellular recording methods for freely moving animals. High-quality and long-duration recordings of both types have been obtained successfully. There is, at present, no indication of differences in overall success rates when considering the latest versions of the methods applied to the same brain region (e.g., hippocampal subregion CA1) of the same species (e.g., mice) (English et al. 2014; Lee et al. 2014), although differences might emerge with increased use. However, there are some technical differences inherent to each approach. The ability to achieve low series resistance recordings with the whole-cell configuration makes voltage-clamp experiments possible. On the other hand, multiple recordings can be obtained with the same sharp microelectrode, whereas whole-cell pipettes must be replaced after each recording, as well as each attempt in which negative pressure has been applied.

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