An Inside Look at Hippocampal Silent Cells

John E. Ferguson,[1] Jadin C. Jackson,[2] and A. David Redish[2,*]
[1]Department of Biomedical Engineering
[2]Department of Neuroscience
University of Minnesota, Minneapolis, MN 55455, USA
*Correspondence: redish@umn.edu
DOI 10.1016/j.neuron.2011.03.015

Hippocampal pyramidal cells can be divided into place cells, which fire action potentials when an animal is in specific locations, and silent cells, which are not spatially selective. In this issue of *Neuron*, Epsztein et al. find intracellular differences between place and silent cells by using whole-cell recordings in freely moving rats.

Not long after John O'Keefe and Jonathan Dostrovsky discovered place cells (O'Keefe and Dostrovsky, 1971), hippocampal neurons that preferentially fire action potentials when an animal is located in specific parts of an environment, Gary Lynch complained to John O'Keefe, "I've tested your theory about these place cells and the spatial function of the hippocampus. I put my slice on wheels, moved it around the lab and it made no difference at all" (Seifert, 1983). Although disconnected from natural behaviors, slice preparations have remained the primary method of studying the intracellular dynamics of hippocampal cells until recently because of the daunting challenge of keeping a micropipette stable in a moving animal. In this issue of *Neuron*, a study by Epsztein et al. (2011) is part of an emerging body of literature that uses recently developed methods for intracellular recording of neurons in awake, behaving animals, adding rich details of subthreshold membrane potential dynamics to previous findings from extracellular recording studies.

Obtaining an intracellular recording in an awake, behaving animal is extremely difficult and requires addressing the issue of mechanical stability. In recent years, two different methods have been developed to solve the stability problem. In the first method, which was used by Epsztein et al. (2011), hippocampal neurons are patched while the rat is under anesthesia, and the electrode is rigidly attached to the skull for stability (Lee et al., 2009). Then the anesthesia is rapidly reversed with an injection of an antagonist so the rat can wake up and explore an environment while the intracellular recoding continues for about another 10 min. In the second method, a mouse's skull is attached to a rigid head plate while a neuron is patched (Harvey et al., 2009). While holding the head plate in place, the mouse is allowed to run on a spherical treadmill (essentially, a large floating ball) in front of a video screen displaying a virtual maze. Thus, the head-fixed mouse can run and navigate a virtual environment during the intracellular recording. Both methods have been used to record from hippocampal place cells and have found depolarization peaks surrounding action potentials that fired within place fields.

Methods of intracellular recording in awake, behaving animals can be applied to a range of different investigations but are particularly useful for studying neurons that are difficult to record by using traditional techniques, such as silent cells. Silent cells are hippocampal pyramidal cells that fire few or no spikes in an environment. In any given environment, approximately 40% of hippocampal pyramidal cells are place cells, and the remaining 60% are silent cells (Thompson and Best, 1989). Although silent cells were identified by using extracellular recordings, they are challenging to study extracellularly because of their low (or zero) firing rate during a given task. Although silent cells can be identified by finding cells active in rest states or with barbiturate anesthesia or antidromic stimulation (Thompson and Best, 1989), there is currently no way with extracellular recordings to determine how many silent cells did not fire or if truly silent cells exist and cannot be evoked to fire action potentials. Silent cells also pose a challenge in neural ensemble recordings where a small number of spikes are difficult to assign to a cluster as a putative neuron. Despite the difficulty in identifying and recording from silent cells, they are important to understand in the sparse coding of information in the hippocampus and other brain regions that have significant proportions of silent cells, such as cortex and cerebellum. Some silent cells become place cells in different environments, some could be silent in all environments, and some could be relaying infrequent yet meaningful nonlocal or nonspatial information. However, because of the limitations of extracellular recording studies, there is currently a large gap in our understanding of what makes a place cell or a silent cell and the role that silent cells play in memory, learning, or navigation.

In order to begin addressing some of these questions, Epsztein et al. (2011) studied silent cells and place cells in rats running around a circular maze by using whole-cell recordings (see Figure 1). After classifying silent cells and place cells based on their spiking activity, they calculated subthreshold fields by removing the action potential components and then measured the thresholds that would trigger action potentials. They found that silent cells had higher thresholds than place cells and had flatter subthreshold fields surrounding action potentials. Silent cells also had fewer complex spikes and the complex spikes were not spatially tuned. They also confirmed previous findings that place cells had depolarizations before place field-firing (Harvey et al., 2009). The proportion of hippocampal cells that was silent also agreed with findings from extracellular and immediate-early gene studies (Thompson and Best, 1989; Guzowski et al., 1999). Although

many of these subthreshold differences cleanly separated place and silent cells, it is not yet possible to determine whether intrinsic factors or network factors cause a place cell to be spatially selective.

Epsztein et al. (2011) also found that before the anesthesia was reversed, the cells that were going to become place cells in the upcoming maze run were much more likely to fire action potentials in bursts than cells that were to become silent cells. However, other differences between place and silent cells, such as differences in thresholds, were not seen at this point. This raises many intriguing questions related to the formation of spatial and contextual maps. What causes a neuron to be a place cell or a silent cell in an upcoming novel environment? Could place cells somehow be primed by the contextual cues present in the room in which both the surgery was performed and the behavior was measured, such as shared odors, acoustics, and visual similarities? After all, it is known that place cells maintain their spatial selectivity during a sleep state, even when the animal is moved (Jarosiewicz and Skaggs, 2004). Also, could the bursting propensity of soon-to-be place cells be preconfigured (Samsonovich and McNaughton, 1997; Dragoi and Tonegawa, 2011)?

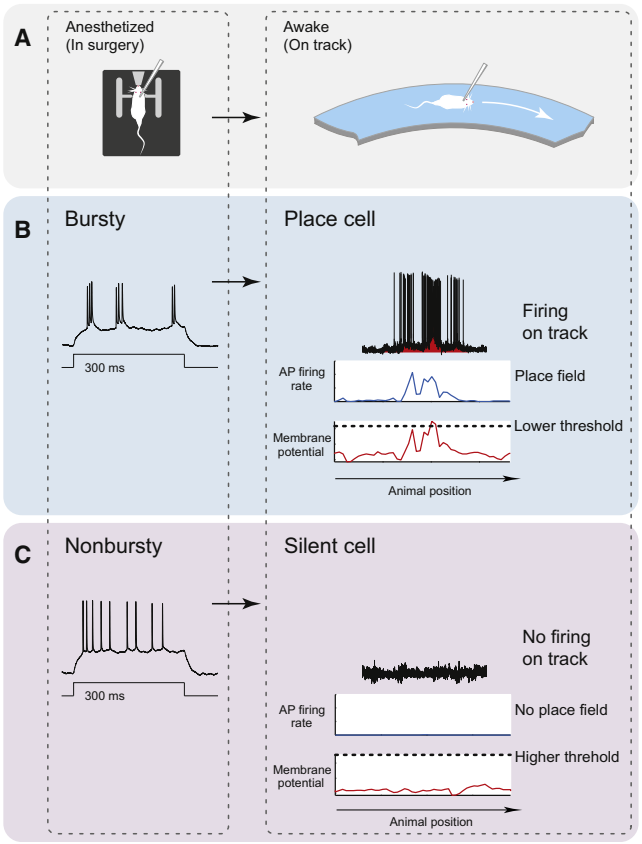

Figure 1. General Results: Intracellular Recordings in Awake, Behaving Animals Reveal Differences in Properties of Place Cells versus Silent Cells

(A) A whole-cell recording of a CA1 pyramidal cell was obtained before transferring the rat to a circular track.

(B) Cells with a propensity to burst when a 300 ms current step was applied (left) tended to become place cells, displaying positional firing (right, blue trace) and subthreshold membrane depolarization (red trace).

(C) Cells that exhibited less bursting when a 300 ms current step was applied (left) tended to be silent when the animal was placed on the track and had higher thresholds for triggering action potentials (right, dashed black line).

Note: data shown were compiled from different neurons from Epsztein et al. (2011).

Another area that this research could impact is in understanding the activity of place cells firing when animals are not located in place fields. These "extra-field" spikes, once considered to be noise, are now understood to be involved in information processing such as replay of recently navigated spaces and sweeps of future potential locations, which are important in learning, memory consolidation, and decision-making (Johnson et al., 2009). In Epsztein et al. (2011), extra-field spikes from place cells appear to occur without the depolarization found with in-field spikes. What network or intrinsic factors are thus responsible for these extra-field spikes? Are extra-field spikes similar at a subthreshold level to the occasional spikes from silent cells? Could subthreshold measurements be a viable way of distinguishing in-field and extra-field spikes from place cells?

Intracellular recording techniques in behaving animals also allow for cell labeling for reconstruction and connectivity studies. Place cells in regions of CA1 that receive input from the medial entorhinal cortex have been shown to be more spatially selective than regions that receive inputs from the lateral entorhinal cortex (Henriksen et al., 2010). Is there a difference between these regions in terms of the selection of place cells and silent cells for novel environments?

Intracellular recording techniques in vivo can also be used to study other nonpyramidal hippocampal cells, such as interneurons or glia, in relation to place and silent cells. Do different types of interneurons (Klausberger and Somogyi, 2008) have different roles in the formation of place cells? Are interneurons involved in selective inhibition of place or silent cells (Thompson and Best, 1989)? Are glia, which have been shown to be involved in information processing in the hippocampus (Perea et al., 2009), involved as well? For example, could the calcium waves seen in networks of astrocytes in the hippocampus (Kuga et al., 2011) contribute to the calcium-related complex spikes of place cells (Harvey et al., 2009; Epsztein et al., 2011)?

Intracellular recording in awake, behaving animals is proving to be a useful new technique in bridging the intracellular and extracellular recording literatures. It is exciting to consider how studies with these recently developed methods will add to our understanding of hippocampal function. Intracellular recordings can serve as a complementary technique to extracellular recordings. While intracellular studies enable detailed analyses of subthreshold phenomena for short durations, extracellular studies allow recordings of multiple neurons simultaneously for long durations. Perhaps it will become possible in the near future to record large neural ensembles extracellularly while simultaneously recording from one or more cells intracellularly. The work by Epsztein et al. (2011) is an important first step to applying these new methods to neurons that are difficult

to study by using traditional methods and will lead to a much more detailed understanding of silent and place cells and the nature of sparse coding in the brain.

REFERENCES

Dragoi, G., and Tonegawa, S. (2011). Nature *469*, 397–401.

Epsztein, J., Brecht, M., and Lee, A.K. (2011). Neuron *70*, this issue, 109–120.

Guzowski, J.F., McNaughton, B.L., Barnes, C.A., and Worley, P.F. (1999). Nat. Neurosci. *2*, 1120–1124.

Harvey, C.D., Collman, F., Dombeck, D.A., and Tank, D.W. (2009). Nature *461*, 941–946.

Henriksen, E.J., Colgin, L.L., Barnes, C.A., Witter, M.P., Moser, M.B., and Moser, E.I. (2010). Neuron *68*, 127–137.

Jarosiewicz, B., and Skaggs, W.E. (2004). J. Neurosci. *24*, 5070–5077.

Johnson, A., Fenton, A., Kentros, C., and Redish, A.D. (2009). Trends Cogn. Sci. *13*, 55–64.

Klausberger, T., and Somogyi, P. (2008). Science *321*, 53–57.

Kuga, N., Sasaki, T., Takahara, Y., Matsuki, N., and Ikegaya, Y. (2011). J. Neurosci. *31*, 2607–2614.

Lee, A.K., Epsztein, J., and Brecht, M. (2009). Nat. Protoc. *4*, 385–392.

O'Keefe, J., and Dostrovsky, J. (1971). Brain Res. *34*, 171–175.

Perea, G., Navarrete, M., and Araque, A. (2009). Trends Neurosci. *32*, 421–431.

Samsonovich, A., and McNaughton, B.L. (1997). J. Neurosci. *17*, 5900–5920.

Seifert, W. (1983). Neurobiology of the Hippocampus (New York: Academic Press), pp. 601.

Thompson, L.T., and Best, P.J. (1989). J. Neurosci. *9*, 2382–2390.

Allocating Attention in Rank-Ordered Groups

Gregor Rainer[1,*]
[1]Department of Medicine, University of Fribourg, Chemin du Musée 5, CH-1700 Fribourg, Switzerland
*Correspondence: gregor.rainer@unifr.ch
DOI 10.1016/j.neuron.2011.03.016

When confronted with multiple stimuli, it is often necessary to prioritize one's attentional resources. In this issue, Lennert and Martinez-Trujillo (2011) investigate the neural dynamics in dorsolateral prefrontal cortex for stimulus pairs of differing importance and demonstrate that the responses to the lesser stimuli become increasingly suppressed with increasing difference.

Primate groups tend to organize themselves in hierarchical structures where each individual has a specific social rank. It has been well documented that in such groups, high-rank individuals tend to receive more attention than low-rank individuals (Chance, 1967). It is clearly useful to keep an eye on high-rank individuals during social encounters because even small communication signals they send out might have large consequences for one's own well-being. Because direct staring is generally interpreted as a dominant and aggressive gesture (Emery, 2000) much of the attention to high-rank individuals is paid covertly without directing gaze toward them. But how does rank order affect the neural mechanisms that subserve covert attention?

In this issue, Lennert and Martinez-Trujillo set out to answer this question (Lennert and Martinez-Trujillo, 2011), taking as a starting point findings linking activity in the dorsolateral prefrontal cortex, as well as the closely related frontal eye fields (FEF), to control signals that regulate attention allocation in more posterior brain regions (Buschman and Miller, 2007; Moore and Armstrong, 2003). In their task, they did not study social rank, but instead they had monkeys learn a hierarchy among a set of colored moving random dot patterns. Patterns were presented side-by-side, one to each visual hemifield, and monkeys had to detect a small change in the movement direction of the higher rank pattern to obtain a reward while ignoring a change in the lower rank pattern. Monkeys readily learned the rank of the individual patterns by trial and error throughout the course of a training period, which is consistent with a known tendency of monkeys to remember elements in an ordered list by their list rank (Orlov et al., 2000). As a critical control, a new pattern was introduced once the hierarchy had been well learned, and monkeys were indeed able to use transitive inference (A > B and B > C implies that A > C) when faced with this new pattern. This confirms that monkeys had in fact learned a hierarchical structure among the patterns rather than memorizing the appropriate response for all stimulus combinations.

For the recording of neural activity in the dorsolateral prefrontal cortex, they introduced a small but important modification: the presentation of the two stimuli of different rank to each visual hemifield was preceded by presentation of two gray neutral random dot patterns, with no—or therefore indeterminate—rank in the same location of the visual field. These neutral patterns served as placeholders and the actual attention task began only with a color change of these patterns. For the prefrontal cortex, the presentation of these neutral stimuli already evoked robust activity. Their single neuron example quadrupled its activity to these neutral patterns and across the population activation was approximately doubled. If one accepts the notion that these prefrontal activities are related to attentional control in posterior cortices, this enhancement to the