The Ins and Outs of Hippocampal Circuits

Susumu Tonegawa[1,*] and Thomas J. McHugh[1]
[1]The Picower Institute for Learning and Memory, Howard Hughes Medical Institute, RIKEN-MIT Neuroscience Research Center, Department of Biology and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
*Correspondence: tonegawa@mit.edu
DOI 10.1016/j.neuron.2008.01.005

The anatomy of the entorhinal-hippocampal circuit suggests how spatial information may flow into and out of the CA1 region. In this issue of *Neuron*, two groups use in vivo physiology to make predictions about the circuit mechanisms involved in the encoding and maintenance of spatial memory. Brun et al. show that lesions of the cells providing direct input from the mEC to CA1 lead to a decrease in spatial tuning, while Cheng and Frank report that the exploration of novel space leads to a transient increase in the temporally correlated firing of pairs of CA1 cells outside of their place fields specifically during ripple-like high-frequency events in the local field potential.

The last 15 years have seen a leap forward in our understanding of how contextual and spatial memory is encoded in the hippocampal-entorhinal network. Behavioral studies have long suggested that the hippocampus is necessary for the encoding of spatial memories, which then, over time, are consolidated to sites downstream of this structure (Squire, 1992). The observation that hippocampal principal neurons fire when an animal visits a specific location in an environment (place cells) has allowed in vivo physiology to become a crucial tool in characterizing the mechanisms of the formation and consolidation of spatial memory (O'Keefe, 1976). Building on an existing knowledge of the anatomy, synaptic plasticity, and place-specific responses of hippocampal and entorhinal neurons, many groups have identified physiological phenomena that correlate with these processes. In parallel, others have combined interventional techniques with in vivo physiology to begin to address the circuits and mechanisms responsible for these mnemonic processes. Classic approaches such as lesions and pharmacology and, more recently, targeted genetic techniques have shed light on the contributions of specific hippocampal subregions or circuits to spatial and contextual memory (Nakazawa et al., 2003, 2004; Rolls and Kesner, 2006). Two papers in this issue of *Neuron* make distinct contributions to the long-term goal of a complete understanding of these circuits.

To understand how memory is encoded we must have physiological correlates of learning. Cheng and Frank (Cheng and Frank, 2008) address this by asking: what physiological activities in the network subserve a rapid acquisition of memory when an animal encounters a novel space? When an animal enters a new space, hippocampal cell assemblies rapidly (within a few minutes) come to express a pattern of place fields unique to the environment. Previous studies suggest that the hippocampal network is designed so that a robust ensemble code will emerge via the temporally coordinated firing of place cells with place fields that overlap in space (Wilson and McNaughton, 1993). This temporal coordination could result in synaptic plasticity in one or more downstream sites, a la Hebb, enabling a rapid formation of a new memory engram. However, in the initial phase of the exploration of a novel space, the place fields in the CA1 region of the hippocampus are highly variable in their firing (Frank et al., 2004; Leutgeb et al., 2004). This led Cheng and Frank to hypothesize that coordination during new learning may not be expressed through spatially organized firing alone, and thus, they examined the coordinated activity of pairs of CA1 cells outside of their shared place fields.

To this end they employed multitetrode recording in the CA1 region as rats moved through both novel and familiar arms of a T maze. Examining the synchrony of pairs of place cells that fired in overlapping locations, they found that pairs in the novel arm demonstrated more near-synchronous firing than familiar arm cells. The "excess correlation," defined as the above-baseline correlation at zero-lag on the cross-correlogram, disappeared when the animal became familiar with the initially novel arm by multiple visits over several days. Counterintuitively, when the analysis was limited to the times that the rat occupied the shared place fields of the cells they examined, the difference of excess correlation disappeared, indicating that the augmented correlation in the novel arm is due to spiking that occurred while the animal was located *outside* of the cells' place fields.

It has been shown that neurons with overlapping place fields tend to fire together during ripple oscillation—brief, large-amplitude high-frequency bursts (150–250 Hz) in the local field potential (LFP)—that occur in subsequent periods of sleep (Nadasdy et al., 1999; Wilson and McNaughton, 1994). The reactivation of place cells during sleep has been studied in relation to its possible role in memory consolidation. However, a recent study has shown that ripples also occur during periods of running (O'Neill et al., 2006). So, Chen and Frank examined whether spiking during ripple events on the T maze could account for the novel arm-associated enhancement of excess correlation. They did confirm that high-amplitude ripples do occur throughout the run sessions, and the spiking during this form of oscillation contributes to the excess correlation, but does not fully account for the difference between cells on

the novel and familiar arms. The authors then focused on spiking during another LFP oscillation, which they term "high-frequency events (HFE)," that have been largely ignored in the past. An HFE has the same frequency composition as ripples but is of lower amplitude. They found that excluding spiking during HFEs abolished the difference in excess correlation, suggesting that HFE activity is responsible for the correlation difference between novel and familiar arm cells. HFEs took up less than 2% of the time spent on the track, so it is quite amazing that the coordination between spikes during HFE can account for most of the difference in the overall correlations.

Cheng and Frank's study represents the first identification of a correlated neural activity pattern that is specific to HFEs. It is also the first demonstration that such a correlated neural activity is augmented in the initial stage of animals' learning in a novel environment. In contrast to the more commonly held view that spatial learning may be driven via the maturation of place fields, they found that the location-associated activities of CA1 neurons is no more coordinated in an early stage of learning than in a familiar environment. Another novel suggestion of this study is that in contrast to previous work that focused on the ripples' role in the systems-level consolidation of hippocampal memories, ripple-like events may be crucial in the initial formation of spatial memories intrinsic in the hippocampal circuit.

As usual, however, a novel discovery such as this generates a number of questions, of which the most fundamental one is the specific function of the HFE-associated, enhanced correlative spiking of CA1 neurons. Cheng and Frank hypothesize that this temporally coordinated firing will allow for effective plasticity in CA1 and its downstream regions, such as the subiculum and the deep layer of the entorhinal cortex, and therefore plays an important role in initial learning. However, as the authors agree, associations of various features of new space are expected to be first formed in the recurrent CA3 network, and it is the activity of the CA3 memory traces that generates ripples or HFEs in CA1 (Buzsaki, 1986). Thus, we seem to have returned to the starting line: how does plasticity at the CA3 recurrent synapses contribute to the formation of spatial memory traces even prior to the plasticity build-up in CA1 or downstream? One possibility is that the recurrent network intrinsically undergoes a similar process observed in CA1, but even more efficiently and rapidly when an animal faces novelty, and the observations of Cheng and Frank are a result of this process. Another hypothesis, not incompatible with the first one, is that the increased temporal correlation in CA1 may serve as a "novelty signal" that could activate a feedback loop involving subcortical areas that could acutely and transiently provide to the hippocampus neuromodulators, such as dopamine, that could enhance plasticity throughout the structure (Lisman and Grace, 2005). These and other ideas triggered by this elegant work will undoubtedly be pursued in coming months. In addition, while this study uncovered a very interesting physiological correlate of the initial stage of novel space learning, their causal relationship will have to be tested by highly specific and rigorous intervention methods.

In addition to the indirect input from the entorhinal cortex (EC) that is conveyed by CA3 via the Schaffer collaterals to CA1, each principal neuron in CA1 receives excitatory inputs directly from EC layer III. While Cheng and Frank focused on the input from CA3 as the source of the highly coordinated, nonspatial firing in CA1 during an early stage of spatial learning, a second paper in this issue from Brun, Moser, and their coworkers addresses the question of the relative contributions of these direct and indirect inputs to the maintenance of the spatial firing in CA1 in a familiar environment (Brun et al., 2008). Earlier, Moser's group examined the effect of an ibotenic acid-mediated lesion of CA3 or a resurrection of the CA3-CA1 connections on the spatial firing of CA1 pyramidal cells in a familiar environment (Brun et al., 2002). They found that although there were small changes in the tuning of the CA1 cells, the sizes of the CA1 place fields were normal, indicating that the direct projections are mostly sufficient for spatial firing in CA1.

In the current study, Brun et al. questioned whether the direct projections are not only sufficient but also necessary for normal spatial firing in CA1. They prepared rats with unilateral lesion of the medial entorhinal cortex (MEC) mediated by local application of neurotoxin γ-acetylenic GABA (GAG). Earlier, Wu and Schwarcz had shown that application of GAG to the MEC leads to a series of epileptic seizures over several days that results in the relatively selective death of the principal cells in layer III (Wu and Schwarcz, 1998). Taking advantage of their anatomical knowledge, Brun et al. recorded place cells in the portion of the CA1 cell layer that is the postsynaptic target of the lesioned MEC. Because the specificity and completeness of any chemical or physical lesion method is suspect, they made a substantial effort to evaluate the extent of the damage incurred in the GAG-treated rats. Histological analysis following recording indicated some variation in the size of the lesions, with patches of spared tissue in most animals. However, in most rats the lesion typically included the majority of the intermediate MEC, and in MEC the lesions were confined to layer III, with minor or no damage to layers II and V. The authors also used a specific marker for neuronal degeneration, Fluoro-Jade B, as well as a marker of gliosis, vimentin, to confirm that there was no substantial neuronal degeneration in the hippocampal fields.

Brun et al. then went on to determine the quality of the spatial activity of CA1 pyramidal cells of the lesioned rats in a familiar environment. Place fields recorded from the lesioned rats were generally wider and more dispersed than those of control rats. The authors presented the distributions of the information density (an estimate of the amount of spatial information that a single spike conveys) of 49 and 44 individual cells from lesioned and control rats, respectively, and showed that the distribution of the lesioned group was significantly shifted downward compared to that of the controls. However, there were many individual CA1 cells from the lesioned group that maintained relatively high information density similar to controls. In contrast to the parameters that reflect spatial tuning, the parameters reflecting the basic physiological properties such as the average firing rate, peak firing rate, or percentage burst activity were not significantly affected by the lesion.

The indirect projections reach CA1 through CA3, but originate from the layer II of EC. Since this layer is directly adjacent to the layer EC III to which the GAG

was applied, there was a genuine concern that the occasional loss of layer II cells observed by the NeuN staining and/or hidden physiological impairments in these cells may have contributed to the reduced spatial firing of some CA1 cells. In order to evaluate this possibility, Brun et al. recorded from the part of CA3 that is substantially connected to both the CA1 recording area and the MEC layer II adjacent to the area of the main site of GAG-induced lesion. In contrast to the CA1 cells, the distributions of the spatial information density of CA3 cells were not significantly different between the two experimental groups, suggesting that if there were any adverse effect of indirect projections on the CA1 spatial firing in the lesioned rats it would not be substantial.

On the basis of these data, the authors justifiably conclude that the direct projection from the EC is necessary for precise spatial firing in the CA1 place cell population in a familiar environment. When one combines these data and conclusion with those of the earlier report from the same group—CA3 lesion resulted in only a minor impairment in the spatial tuning of CA1 cells in a familiar environment—one might be led to a conclusion that the direct projections are necessary and sufficient and the indirect projections are dispensable for the spatial tuning of CA1 cells. While the authors seem to share our view that such a conclusion is premature, the data presented are valuable in suggesting future experiments that would address a number of important issues. First, it would be interesting to assess the behavioral consequences of the loss of direct EC input to CA1. Does the decrease in spatial tuning of CA1 pyramidal cells caused by the lesion of the direct input have observable effects on spatial or contextual learning? Unfortunately, the small, unilateral lesions cleverly used by the authors to evaluate the role of EC input in CA1 activity would not allow such an experiment. An alternative method that accomplishes specific and complete suppression of the direct input would be necessary.

Next, due to the location and size of the lesions, the recordings in this study were limited to the intermediate to ventral portion of CA1, thus it will be important that future work confirms that the dorsal CA1, where the vast majority of CA1 place cells have been observed, is similarly dependent on direct EC input for precise spatial activity. In addition, it will be interesting to see whether the cells that remained highly tuned in the GAG-lesioned rats do so due to spared MEC input or rather because they are more dependent on CA3 input. Indeed, the 2002 Brun et al. study suggests that the indirect projections also play some role in shaping CA1 spatial activity. While there may be a quantitative aspect in the contributions of the two inputs, it is likely that each input exerts a qualitatively different influence on CA1 cells' spatial activities in light of the very different circuits from which they arise. In an interesting additional experiment, the authors shifted the rats from a familiar to a novel environment. As they expected, they found that in both lesioned and control animals spatial tuning was weaker in the novel environment than in the familiar environment, but found no clear evidence for an additional role of the direct input in the spatial tuning of CA1 cells in the novel arena. An examination of the parameters they measured in the control rats during novelty suggests that the fidelity of spatial coding in the lesioned animals in a familiar space is quite similar to that of the controls in a new space. This suggests that the indirect (CA3) projections may play a crucial role in the rapid tuning of CA1 (and CA3) place fields when an animal faces a novel environment, which is consistent with Cheng and Frank's idea that activation of the spatial representation formed in CA3 is crucial for the learning of novel space. On the other hand, both direct and indirect projections may be needed to maintain CA1 spatial tuning in a familiar environment. It is hoped that future studies with highly specific and complete intervention methods will allow rigorous testing of these ideas.

REFERENCES

Brun, V.H., Otnass, M.K., Molden, S., Steffenach, H.A., Witter, M.P., Moser, M.B., and Moser, E.I. (2002). Science *296*, 2243–2246.

Brun, V.H., Leutgeb, S., Wu, H.-Q., Schwarcz, R., Witter, M.P., Moser, E.I., and Moser, M.-B. (2008). Neuron *57*, this issue, 290–302.

Buzsaki, G. (1986). Brain Res. *398*, 242–252.

Cheng, S., and Frank, L.M. (2008). Neuron *57*, this issue, 303–313.

Frank, L.M., Stanley, G.B., and Brown, E.N. (2004). J. Neurosci. *24*, 7681–7689.

Leutgeb, S., Leutgeb, J.K., Treves, A., Moser, M.B., and Moser, E.I. (2004). Science *305*, 1295–1298.

Lisman, J.E., and Grace, A.A. (2005). Neuron *46*, 703–713.

Nadasdy, Z., Hirase, H., Czurko, A., Csicsvari, J., and Buzsaki, G. (1999). J. Neurosci. *19*, 9497–9507.

Nakazawa, K., Sun, L.D., Quirk, M.C., Rondi-Reig, L., Wilson, M.A., and Tonegawa, S. (2003). Neuron *38*, 305–315.

Nakazawa, K., McHugh, T.J., Wilson, M.A., and Tonegawa, S. (2004). Nat. Rev. Neurosci. *5*, 361–372.

O'Keefe, J. (1976). Exp. Neurol. *51*, 78–109.

O'Neill, J., Senior, T., and Csicsvari, J. (2006). Neuron *49*, 143–155.

Rolls, E.T., and Kesner, R.P. (2006). Prog. Neurobiol. *79*, 1–48.

Squire, L.R. (1992). Psychol. Rev. *99*, 195–231.

Wilson, M.A., and McNaughton, B.L. (1993). Science *261*, 1055–1058.

Wilson, M.A., and McNaughton, B.L. (1994). Science *265*, 676–679.

Wu, H.Q., and Schwarcz, R. (1998). Exp. Neurol. *153*, 203–213.