

# Reactivating Memories for Consolidation

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The consolidation of memory is thought to occur via a hippocampal-neocortical dialog involving reactivation of memory patterns in the hippocampus during sharp-wave ripples. In this issue of *Neuron*, Nakashiba et al. demonstrate that CA3 output is required for consolidation of contextual fear memory. They also show that lack of CA3 output results in a decrease in ripple-related reactivation, providing additional evidence for a role of ripple-related reactivation in the consolidation process.

How animals form event memories and use past experience to guide behavior remains one of the most challenging questions in neuroscience. The formation of these memories is thought to occur in two steps, with initial encoding of novel events occurring in the hippocampus, followed by a consolidation process where some memories are engrained in hippocampal-neocortical circuits for long-term storage (Zola-Morgan and Squire, 1990; Buzsaki, 1996).

The mechanisms by which newly acquired hippocampal memories are transferred gradually into more permanent storage in hippocampal and neocortical circuits remain a subject of intense investigation. Many lines of evidence suggest that reactivation of hippocampal memories in “offline” processing modes during high-frequency sharp-wave ripple activity, which occur in slow-wave sleep and during pauses in awake behavior, may be essential for the consolidation process (Wilson and McNaughton, 1994; Buzsaki, 1996). A causal demonstration is still lacking, however. The role of specific hippocampal circuits in consolidation is also unclear.

The hippocampus consists of two major excitatory circuits: the trisynaptic and the direct pathway. The trisynaptic pathway runs from layer II of entorhinal cortex (EC) to the dentate gyrus (DG), which projects via the mossy fiber pathway to CA3, which in turn sends Schaffer collateral projections to CA1. The direct pathway runs from layer III of EC to CA1. The CA1 region sends output connections from the hippocampus to other structures, including the deep layers of EC. Previous studies using either lesions or transgenic suppression of activity

came to different conclusions about the roles of the two pathways in spatial memory. Lesion studies suggested that the trisynaptic pathway is required for spatial recall (Morris water maze), but not for spatial recognition memory or for development of stable place fields in CA1 during exploration (Brun et al., 2002). In contrast, Tonegawa's group employed specific and inducible control of CA3 output using the CA3-TeTX transgenic mouse to show that the trisynaptic pathway is in fact dispensable for both acquisition of incremental spatial learning and spatial recall. However, they found that CA3 output is necessary for acquisition of one-trial learning in a contextual fear memory task and for development of stable place fields in CA1 during exploration, especially in novel environments (Nakashiba et al., 2008). These contrasting results raise questions about the role of the trisynaptic pathway in the consolidation process. Ripples have been shown to frequently originate in the highly recurrent CA3 network and then propagate out to CA1 (Buzsaki, 1986; Csicsvari et al., 2000), suggesting that the trisynaptic pathway should be important for reactivation, and thus for consolidation. To address whether CA3 output is indeed necessary for consolidation and reactivation, Nakashiba et al. (2009 [this issue of *Neuron*]) used the CA3-TeTX transgenic mouse to block CA3 output specifically during the consolidation process.

In a previous study, Tonegawa's group showed that the CA3-TeTX transgenic mouse shows normal CA3 activity when raised on a doxycycline (Dox) diet, but after 2 weeks of Dox withdrawal, CA3 output is diminished by over 90%, and the Schaffer collaterals are unable to drive CA1 activity (Nakashiba et al., 2008). In the

current study, the authors used this inducible method to block CA3 output during the consolidation phase of a contextual fear memory test, while keeping it intact during the acquisition and recall of memory. Similar to previous results, these animals were impaired in the task if CA3 output was blocked during acquisition. In the first experiment testing consolidation, they began Dox withdrawal 1 week before a single fear conditioning session where animals were trained to associate a tone with a mild foot shock in a novel context. The trisynaptic pathway was therefore turned off during the consolidation phase starting 1 week after training. As expected, transgenics tested 1 day after training with the CA3 output still intact showed no deficit in recent memory in the contextual and cued conditioning tasks. However, transgenics tested 6 weeks after training were significantly impaired in remote contextual memory as compared to controls. Performance on the cued conditioning task remained the same. Since CA3 output was blocked both during the consolidation and recall phase of the task, the observed impairment could be due to a deficit in either or both of these processes. To address this issue, the authors started Dox withdrawal 3 weeks after training in a second experiment. This allowed the transgenic animals to have an intact trisynaptic pathway for 5 weeks of the consolidation phase after training. The remote memory test was again performed at the 6 week time point, at which CA3 output should be diminished by over 90%. These animals showed no deficit in memory, thus establishing that CA3 output is not required for recall of the remote contextual memory but is

essential for consolidation of the memory for a few weeks after training.

Given this behavioral result, the authors went on to examine the physiological consequences of the suppression of CA3 output. Numerous previous papers have demonstrated that, during spatial exploration, activity representing learned spatial associations is reactivated during ripples. It has been posited that strong bursts of hippocampal activity during ripples might be able to drive neocortical areas, allowing memory-related patterns to be incorporated into hippocampal-neocortical networks for long-term storage (Buzsaki, 1996; Wierzynski et al., 2009). This possibility led the authors to examine ripples and the associated reactivation in the transgenics.

Neural activity was monitored during exploration on a linear track and during rest periods before and after the exploration period. Surprisingly, the authors found no decrease in the number and length of ripples seen in CA1 of transgenics as compared to controls. This suggests that the CA1 network can show ripple oscillations in the absence of CA3 output. However, the authors did find a decrease in average intrinsic frequency of ripples. Thus, ripples were significantly "slower" when CA3 output was blocked, which might compromise the ability to drive downstream regions in neocortex. Pyramidal cells fired at the same rate and showed similar phase relationship to ripples as in controls. In contrast, the firing rate of interneurons was significantly lower than controls during ripples, indicating a decrease in inhibitory tone. The authors next examined reactivation by comparing coactivation of cells with overlapping place fields before and after the exploration period. Prior work has shown that overlapping pairs of cells show an increase in correlation after experience as compared to before, revealing a memory trace of learned spatial locations (Wilson and McNaughton, 1994). The authors found that this increase in correlation, which was present in control animals, was significantly reduced but not entirely absent in the mutant animals. Thus, although the number of ripples and the firing rate and phase of CA1 place cells are not affected by the blockade of CA3 output, there is impairment in reactivation of firing patterns representing previous experience. Control

analyses show that this impairment was not a result of decrease in spatial specificity in CA1 that is seen in the absence of CA3 output. Further, the transgenic animals still showed greater correlation for overlapping pairs than nonoverlapping pairs, which suggests that some experience-related reactivation is still present in the CA1 network. The authors found that this residual reactivation was effectively limited to the remaining higher-frequency ripples in the transgenics and suggest that this may underlie the residual remote memory observed in the behavior experiments.

The elegant behavior experiments, with specific and inducible genetic blockade of CA3 output to CA1, show for the first time that the trisynaptic pathway is necessary for the consolidation of contextual fear memory. The authors also show that the lack of CA3 output results in a reduction of intrinsic frequency of ripple events and reactivation in CA1. This provides a plausible mechanism for the behavioral impairment and represents the most direct association between ripples and consolidation to date.

At the same time, important questions still remain. The induction experiment used in these experiments did not have the temporal specificity to block specific physiological activity patterns. It is possible that, although the trisynaptic pathway is required for consolidation, the changes in reactivation patterns have no relation to the reduction in remote memory. In order to establish a causal link between ripple-related reactivation and memory, disruption of hippocampal activity specifically during ripples, without affecting activity at other times, is necessary.

It is also worth pointing out that contrasting results in different behavior tasks suggest that memory consolidation is complicated. The Tonegawa group previously found that learning as well as final performance in the Morris water maze was not affected by blocking CA3 output, which showed that the trisynaptic pathway is dispensable for tasks involving incremental spatial learning (Nakashiba et al., 2008). In the current study, they show that the trisynaptic pathway is required for consolidation in the contextual fear task. This raises questions about the role of high-frequency ripples and reactivation in memory formation, since these phenomena are thought to be important for consolidation of spatial

memories (Wilson and McNaughton, 1994; Buzsaki, 1996). Indeed, coactivation of neurons during ripples is also hypothesized to be important during acquisition of memories (Cheng and Frank, 2008). But if ripple-related reactivation is impaired in the absence of CA3 output, why is learning in spatial tasks not affected by lack of CA3 output? One possibility is that the slow ripples and the residual reactivation observed by the authors in the current study could support these processes. How this reactivation is sustained in CA1 in the absence of CA3 output is also a crucial question. It could be that structured reactivation in CA1 may simply reflect structure of direct inputs from the EC, but it is also possible that it is supported by plasticity in inhibitory-excitatory networks (Pelletier and Lacaille, 2008) or in the weak recurrent connections that have been reported in CA1 (Deuchars and Thomson, 1996). Alternative physiological substrates that depend on the direct pathway cannot be ruled out. Thus, in establishing a role for CA3 output and a potential link between ripples and memory, this study has opened up a new set of questions that should lead us closer to understanding the mechanisms of learning and consolidation.

## 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.
- Buzsaki, G. (1986). *Brain Res.* 398, 242–252.
- Buzsaki, G. (1996). *Cereb. Cortex* 6, 81–92.
- Cheng, S., and Frank, L.M. (2008). *Neuron* 57, 303–313.
- Csicsvari, J., Hirase, H., Mamiya, A., and Buzsaki, G. (2000). *Neuron* 28, 585–594.
- Deuchars, J., and Thomson, A.M. (1996). *Neuroscience* 74, 1009–1018.
- Nakashiba, T., Young, J.Z., McHugh, T.J., Buhl, D.L., and Tonegawa, S. (2008). *Science* 319, 1260–1264.
- Nakashiba, T., Buhl, D.L., McHugh, T.J., and Tonegawa, S. (2009). *Neuron* 62, this issue, 781–787.
- Pelletier, J.G., and Lacaille, J. (2008). *Prog. Brain Res.* 169, 241–250.
- Wierzynski, C.M., Lubenov, E.V., Gu, M., and Siapas, A.G. (2009). *Neuron* 61, 587–596.
- Wilson, M.A., and McNaughton, B.L. (1994). *Science* 265, 676–679.
- Zola-Morgan, S.M., and Squire, L.R. (1990). *Science* 250, 288–290.