Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Losing Connections, Losing Memory: AMPA Receptor Endocytosis as a Neurobiological Mechanism of Forgetting

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Review of Migues et al.

Although much is known about how memories are formed (Josselyn et al., 2015), much less research has focused on the neurobiological mechanisms underlying forgetting. This is likely due to the implicit, yet incorrect, belief that forgetting is a failure in neural processing whereby memories are simply broken down as a function of time. In fact, forgetting is a mechanistically controlled and adaptive phenomenon that facilitates future mnemonic processing (Epp et al. 2016). With this recognition, recent years have witnessed a surge in investigations of the neurobiology of forgetting.

Successful memory retrieval is thought to occur by reactivating the spatiotemporal pattern of neuronal activity that was present at encoding (i.e., pattern completion). When the circuitry that underlies a memory is disrupted, the probability of successful pattern completion is decreased, ultimately culminating in forgetting. In other words, at a basic level, making memories involves making connections, and breaking memories involves breaking connections. In line with this idea, recent work by Migues et al. (2016) sought to determine whether the stability of GluA2-containing AMPA receptors

(GluA2/AMPARs), which play an important role in the persistence of memory across time, also regulate the forgetting of consolidated memories, as was predicted by Hardt et al. (2014). Consistent with a general framework in which forgetting is caused by a disruption in the synaptic connectivity upon which memories are dependent, Migues et al. (2016) provide compelling evidence that forgetting ensues as dorsal hippocampal GluA2/AMPAR synapses are lost.

The researchers first used an object–location task in which rats were exposed to two identical objects located in fixed places over a period of many days. After training, one of the objects was moved to a new location. This task takes advantage of rodents' innate tendency to explore novelty. Thus, if the rat remembers the objects' previous location, it will preferentially explore the moved object. Using this task, it was found that rats display memory for a moved object for at least 7 d, but lack this preference by 10 –14 d (Migues et al., 2016, their Fig. 1*B*).

Having established a behavioral protocol that reliably produces forgetting, the authors next asked whether GluA2/AMPAR endocytosis mediates forgetting of object–location memories. To answer this question, the synthetic interference peptide GluA2$_{3Y}$, which obstructs activity-dependent removal of synaptic AMPARs, was used. Following training in the object location task, GluA2$_{3Y}$ (or its inactive control) was infused twice daily into the dorsal hippocampus for the next

13 d, by which point natural forgetting has occurred. Memory retention was tested 24 h following the last infusion. Fascinatingly, whereas control rats once again lacked a preference for the moved object, inhibiting GluA2/AMPAR endocytosis with GluA2$_{3Y}$ completely reversed this forgetting phenotype (Migues et al., 2016, their Fig. 1*E*).

The results above suggest that activity-dependent GluA2/AMPAR endocytosis mediates the forgetting of object–location memory. However, because treatment was begun immediately after training and forgetting did not occur for at least 7 d after training (Migues et al., 2016, their Fig. 1*B*), it is possible that GluA2$_{3Y}$ strengthened consolidation of the memory trace rather than preventing forgetting, per se. Controlling for this possibility, GluA2$_{3Y}$ was only given shortly before the animals began to forget, starting on day 8. As before, memory performance was assessed on day 14. GluA2$_{3Y}$ was once again found to reverse natural forgetting, thereby substantiating the role of GluA2/AMPAR endocytosis in forgetting (Migues et al., 2016, their Fig. 2*E*). However, the possibility that GluA2$_{3Y}$ prevents forgetting by enhancing memory retrieval specifically was not examined. This is possible given that forgetting is often found to be a reversible "failure" in memory retrieval (Li et al. 2014; Roy et al. 2016) and in light of the finding that perirhinal GluA2/AMPA receptor endocytosis is required for the retrieval (but not the encoding or consolidation) of object recognition memory

(Cazakoff and Howland, 2011). By infusing $GluA2_{3Y}$ closer to memory assessment, the potential role of GluA2/AMPAR endocytosis in retrieval of a forgotten memory could have been determined.

Migues et al. (2016) next assessed whether GluA2/AMPAR endocytosis mediates forgetting of associative memories, using a conditioned place preference (CPP) task in which one of two contexts was paired with reward. In this task, animals typically spend more time exploring the food-associated context. Migues et al. (2016) confirmed that the CPP task resulted in a memory that was forgotten over 10 d (Migues et al., 2016, their Fig. 4B). Next, $GluA2_{3Y}$ or its inactive control was infused into the dorsal hippocampus (twice daily) throughout the interval between training and test. As predicted, suppressing synaptic removal of GluA2/ AMPARs increased the amount of time the rats spent in the food-paired context when tested on day 10 (Migues et al., 2016, their Fig. 4E). This suggests that activity-dependent GluA2/AMPAR endocytosis mediates the forgetting of appetitive associative memories.

Next, the authors assessed whether GluA2/AMPAR endocytosis regulates time-dependent memory changes that relate to, but are distinct from, forgetting by examining the generalization of a contextual fear memory. In this paradigm, rats are trained to express fear in a specific context. Over the subsequent ≥2 weeks, rats typically generalize their fear response to contexts in which they were never trained. Although many factors underlie fear generalization, forgetting the details of the original context in which training occurred plays an important role (Riccio and Joynes, 2007; Wiltgen and Silva, 2007; Jasnow et al., 2012). Migues et al. (2016) confirmed that fear generalization occurred over a period of 2 weeks (Migues et al., 2016, their Fig. 5B) and then infused $GluA2_{3Y}$ into the dorsal hippocampus of rats throughout the 13 d between training and test. Consistent with a role of synaptic GluA2/AMPARs in memory generalization, $GluA2_{3Y}$ preserved the specificity (possibly via reducing the forgetting of details) of the context memory across the 2 week period (Migues et al., 2016, their Fig. 5D).

How does GluA2/AMPAR endocytosis mediate forgetting? Previous research has demonstrated that $GluA2_{3Y}$ inhibits the induction of LTD without affecting basal synaptic transmission or induction of LTP (Yu et al., 2008). However, whether GluA2/AMPAR endocytosis plays a role

in synaptic depotentiation (i.e., decay) of LTP is unknown. To address this question, GluA2/AMPAR endocytosis was inhibited during stimulation of the Schaffer collateral (CA3–CA1) pathway in hippocampal slices. Migues et al. (2016) first established that delivering low-frequency stimulation depotentiates LTP (Migues et al., 2016, their Fig. 7A). Next, they demonstrated that intracellular delivery of $GluA2_{3Y}$, but not its inactive control, blocked this depotentiation (Migues et al., 2016, their Fig. 7B). Together, these results suggest that activity-dependent GluA2/AMPAR endocytosis likely mediates forgetting through regulating both the induction of LTD and the depotentiation of LTP (Dong et al. 2015; Migues et al., 2016, their Fig. 7B,C). This explanation is congruent with recent work demonstrating a role for LTD and LTP in failed and successful memory retrieval, respectively (Nabavi et al., 2014). Together, these data suggest that inhibiting the removal of GluA2/AMPARs "locks" that current state of connectivity, preserving synaptic potentiation, thereby preventing the loss of the information stored in those circuits (i.e., forgetting).

Research into the neurobiological mechanisms of forgetting has begun to blossom, with important results being found across a variety of species, including *Caenorhabditis elegans* (Inoue et al., 2013; Hadziselimovic et al. 2014); *Drosophila* (Shuai et al., 2010; Berry et al. 2012; Cervantes-Sandoval et al., 2016); mice, degus, and guinea pigs (Akers et al., 2014; Epp et al., 2016); and rats (Dong et al., 2015; Migues et al., 2016; Sachser et al., 2016). Synthesizing this seemingly diverse collection of results into a unified framework is an important task. The work of Migues et al. (2016) is consistent with a preliminary model, whereby "unlocking" and disrupting the circuit in which a memory is embedded culminates in forgetting. This "destructive circuit remodeling" can occur via the elimination of synaptic connections that are essential to the memory trace or the addition of mnemonically irrelevant synaptic connections into a memory circuit; in either case, the rewiring would render pattern completion more difficult, culminating in forgetting. In the current work, postlearning loss of GluA2/AMPAR connectivity resulted in forgetting. Consistent with this, AMPA-type glutamate receptor-containing synapse size regulates forgetting in *C. elegans*, with synaptic shrinkage (i.e., loss of connectivity) leading to forgetting (Hadziselimovic et al. 2014). These alterations in synaptic architecture were mediated by the Arp2/3 complex, which controls actin

cytoskeletal nucleation and branching (Hadziselimovic et al. 2014), a result that is consistent with the involvement of actin cytoskeleton regulation in AMPAR trafficking more generally (Hanley, 2014). Similarly, the Rho GTPase Rac1 bidirectionally modulates forgetting in *Drosophila*, likely through the regulation of actin cytoskeleton dynamics (Shuai et al., 2010; Cervantes-Sandoval et al., 2016). Following the basic model of forgetting outlined here, one would predict that Rac1-mediated forgetting would result from the shrinkage or elimination of spines relevant to the memory trace, but this has yet to be tested directly. In rodents, we recently found that postlearning neurogenesis causes forgetting of hippocampal-dependent information learned ≥28 d earlier (Akers et al., 2014). We have hypothesized that neurogenesis causes forgetting by rewiring the circuitry in which the memory is embedded through the addition of new synaptic connections as well as the elimination of pre-existing connections (Frankland et al., 2013), a result that likely depends on Rac1-mediated cytoskeletal remodeling (Vadodaria et al. 2013). It is also possible that that the infiltration of new neurons into hippocampal circuitry results in a net increase in circuit excitability. This increased excitability might trigger homeostatic mechanisms that reduce the excitability of surrounding neurons through GluA2/AMPAR endocytosis (Frankland et al., 2013), with the loss of these receptors inducing forgetting, a finding that would complement the results obtained by Migues et al. (2016). Together, these findings converge on the idea that just as making memories involves making connections, breaking memories involves breaking connections.

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