Memory Consolidation and NMDA Receptors: Discrepancy Between Genetic and Pharmacological Approaches

Using third-generation molecular engineering techniques, Shimizu et al. (1) claimed that inducible targeted deletion of the N-methyl-D-aspartate (NMDA) NR1 receptor gene in area CA1 of the hippocampus interferes with memory consolidation for two hippocampus-dependent learning tasks. Although we acknowledge the elegance of these techniques, we are skeptical of some aspects of the claim.

The effects of interference with NMDA receptors upon acquisition have previously been established using pharmacological techniques (2, 3) and both first-generation (4) and second-generation (5) genetic knockout techniques. Inducible gene deletion using doxycycline (doxy) makes it possible, as with timed administration of a drug, to study memory encoding, consolidation, and retrieval processes in isolation (6). The new claim of Shimizu et al. (1) is that reversible deletion of the NR1 subunit in area CA1 of the hippocampus interfered with spatial memory when the deletion was induced during days 1 to 7 of the posttraining memory consolidation period (doxy 1- to 7-day mice) but not when deletion was induced during days 9 to 14 (doxy 9- to 14-day mice). Shimizu et al. also reported an analogous finding, with a more extended time course, for contextual fear without reference to NMDA receptor involvement in memory consolidation. [No statistical analysis of these data was presented in (1).]

3) Admittedly, such a relearning or reminding effect would likely have been greater in the doxy 9- to 14-day group than the doxy 1- to 7-day group. It is unclear, however, whether the doxy 9- to 14-day mice or their controls were above chance in their transfer test [figure 3D in (1)], which raises the possibility of nonspecific effects of doxycycline administration on performance in mice trained without doxycycline.

4) It is not clear why different durations of doxycycline treatment were used for the two time-of-deletion conditions.

We have recently completed a similar study, using rats rather than mice and using pharmacological rather than genetic interference, according to an experimental design identical to that of Riedel et al. [experiment 2 in (9)]. After 4 days of spatial training using an Atlantis Platform, chronic posttraining intracerebroventricular (icv) infusion of D-AP5 (30 nmol, 0.5 μl/hour for 7 days) had no effect on memory retention in a single transfer test given 16 days later. This lack of an effect occurred irrespective of whether the 7-day infusion occurred 1 to 7 days after training or 5 to 12 days after training (mean time spent in target quadrant = Immediate-AP5, 52.5 ± 12.9%; Delayed-AP5, 50.5 ± 10.6%; ACSF, 55.5 ± 6.6%; chance = 25%; ± 1 SEM). This dose was sufficient to block CA1 long-term potentiation (LTP) in vivo measured in the same animals as given behavioral training (AP5, 104.9 ± 2.9%; ACSF, 126.5 ± 5.3%). This lack of effect of NMDA receptor blockade on memory consolidation contrasts with the striking effect observed with intrahippocampal AMPA/kainate receptor blockade (9), which in turn suggests to us that posttraining neural activity in the hippocampus, but not synaptic plasticity, is essential for guiding memory consolidation.

The findings of Shimizu et al. (1) are a challenge to current theoretical perspectives. Posttraining cortical NMDA receptor-dependent plasticity might play a role in memory consolidation if it triggered gradual strengthening of intracortical connections guided by a previously established hippocampal trace that served an “indexes” or “pointing” function (10, 11). The concept of “synaptic reentry reinforcement” (SRR) that Shimizu et al. propose is puzzling in relation to the hippocampus, unless memory consolidation is conceived as a process through which cortical traces are reactivated and then retrieved back to the hippocampus for further association with the initial hippocampal trace.

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References and Notes
7. There is a discrepancy between the labeling of figure 3 in (1) and the accompanying legend. Hereafter, we refer to the figure labeling.

Response: Day and Morris claim that, in a test using the Atlantis water maze (a different water maze task than used in our study), a posttraining icv infusion of AP5 had no effect on memory retention in rats. To draw the correct conclusion from these experiments, it is prudent to consider several technical issues that may affect the interpretation of their data.

First, the Atlantis water maze paradigm involves extensive pret raining and training trials, which makes it less than ideal for temporal dissection of memory processes (1). Commonly, the task consists of 3 days of pret raining (6 trials per day for 3 days, during which animals learn to approach the location of the hidden platform indicated by a visible hanging sign above the platform), followed by 4 days of spatial training (10 trials each day, 40 trials total). Such a training design could be problematic for the study of memory consolidation in three ways: (i) Pret raining involves tremendous amounts of learning and memory about the task and thus signifi-

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cantly alters the cognitive processes and neural substrates of the task. (ii) Pretraining overcomes the requirement of the NMDA receptors in subsequent spatial learning (2–6). (iii) The excessive trial sessions (a total of 58 trials) likely resulted in sufficient intertrial consolidation for memory to be retained for at least 16 days and, thus, likely diminished the effect of posttraining AP5 infusion on memory consolidation.

More important, the technique employed by Day and Morris is prone to misinterpretation. The hippocampus is a banana-shaped structure in the brain (Fig. 1A); the vast majority of brain areas surrounding the ventricular zones, where AP5 was infused in the experiments of Day and Morris, are frontal cortical tissues, striatum, and thalamus (Fig. 1, B to D). Only a tiny fraction of the ventricular zones has a limited exposure to a small part of the dorsal hippocampus. The rapid circulation of drugs in the ventricular system, as well as uneven diffusion of drugs into nearby tissues, would create highly undesirable scenarios in which the toxicity of AP5 in some regions (near the infusion cannula) intermingles with varying degrees of partial inhibition of NMDA receptors in other areas. Because the posterior portion of the hippocampus, which constitutes more than 70% of total hippocampal structure, is not physically located anywhere near ventricular zones (Fig. 1, E and F), we are skeptical of their claim that AP5 (only one dose used) delivered by icv infusion produced no side effects, yet completely inhibited CA1 LTP (no animal numbers in their experiments were given). Other laboratories (2–11) have also raised concerns about AP5’s toxicity and behavioral interpretation of the water maze data and about the validity of the original claims by Morris (12, 13) that blocking LTP by AP5 icv infusion is the reason for impairing spatial learning in the water maze. And we, along with others, think that it is misleading to use artificial LTP as a substitute for all aspects of NMDA receptor-mediated function in vivo (14–16).

These important technical issues lead us to question the accuracy of the interpretation of Day and Morris. Our iCA1-KO mouse experiments (17) used a typical hidden-platform water maze, which involves fewer than half the training trials (with no pretraining and with a total of 28 training trials) of the Day and Morris experiments. Our statistical analysis (student t test) clearly found a significant difference between the control and doxy-treated iCA1-KO mice. This difference is further confirmed by the transfer test (figure 3B in (17)). Although we did consider the potential “reminding effect” in the transfer test, it is unlikely to be responsible for the poor memory recall in iCA1-KO mice, as Day and Morris themselves acknowledge, because the doxy 9- to 14-day group, which also had same “reminding” experience, did not show a difference between doxy-treated iCA1-KO mice and controls. Moreover, the statistical analysis [figure 3D in (17)] clearly revealed that the doxy 9- to 14-day mice showed significant spatial learning (18). We have also ruled out toxicity of doxy [reference 35 in (17)], because more extensive treatment of doxy (1 month) did not produce any observable side effects on learning behaviors (17).

Although the water maze task that we used contains fewer trials, some intertrial consolidation nonetheless may still occur. To completely avoid this problem, we conducted a second behavioral test, contextual fear conditioning, which is also a hippocampus-dependent task and is ideal for temporal dissection of memory consolidation, because learning occurs in a single trial (in seconds) and fear memory is robust and long-lasting. Furthermore, by measuring both contextual and cued memory responses, we can examine the hippocampal specificity of our inducible genetic manipulation in the same animals. As with the water maze results, our contextual fear conditioning experiments demonstrated the requirement for the reactivation of the NMDA receptor during the consolidation period, and suggested SRR as a general mechanism for the formation of hippocampus-dependent long-term memory (17).

We believe that Day and Morris may have misunderstood some aspects of our SRR model. The reentry reinforcement that we have proposed is at the synaptic level and describes the key cellular feature by which repeated reinforcement of synaptic connectivity must occur (or recur/reenter) at the same set of synapses or neurons that were involved in creating the major memory traces in the network. Thus, the NMDA receptor-mediated SRR process, which permits postlearning association of memory traces in neurons, can occur both within the hippocampus

**Technical Comment**

Fig. 1. Hippocampus and surrounding brain areas in rat. (A) Overview of the hippocampus. Cross sections are marked by labeled lines. (B) The ventricular zone (VZ, black shaded area) in ventricular brain tissue are surrounded by nonhippocampal tissues, such as the caudate putamen (CPu), cingulum, stria medullaries of thalamus, triangular septal nucleus (TS), and corpus callosum (CC). (C and D) Anterior part of the hippocampus (HP). Only small area of the ventricular zone (black shaded area) has direct access to the edge of some of the dorsal hippocampal tissue. (E and F) Posterior portion of the hippocampus. No ventricular zones are present in this part, which suggests that the vast portion of the hippocampus is unlikely to be the primary target for icv infusion. [Adapted from (22) with permission of Academic Press, San Diego, CA].

755a
and in the cortex. In fact, emerging evidence of coordinated reactivation of neuron pairs in those areas during sleep (19–21)—which can produce ideal physiological situations in which the NMDA receptor is reactivated, thus allowing the occurrence of synaptic reinforcement—is consistent with hallmark features of SRR. SRR may thus serve as a novel conceptual framework to redefine the molecular and cellular distinction between long-term memory and short-term memory in the mammalian brain.

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References and Notes
18. The dotted line in figure 3D of (17), intended to be at 25% level, was inadvertently shifted during publication process.

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