The cellular mechanisms of memory are modified by experience

Brian J. Wiltgen,1,3 Alynda N. Wood,1 and Brynne Levy2

1Department of Psychology, University of Virginia, Charlottesville, Virginia 22904, USA; 2Department of Biology, University of Virginia, Charlottesville, Virginia 22904, USA

The N-methyl-D-aspartate receptor (NMDAR) is thought to be essential for synaptic plasticity and learning. However, recent work indicates that the role of this receptor depends on the prior history of the research subject. For example, animals trained on a hippocampus-dependent learning task are subsequently able to acquire new information in the absence of NMDAR activation. The current experiments were designed to identify the types of experiences that lead to NMDAR-independent learning. Using contextual fear conditioning in mice, we find that NMDAR-independent learning is only observed when (1) animals are trained on the same behavioral task and (2) initial learning is successfully encoded into long-term memory.

[Supplemental material is available for this article.]
et al. 2010; Tayler et al. 2011). Group Imm B-A was fear-conditioned in context B using an immediate shock procedure prior to training in context A. Shock was presented 5 sec after placement in the conditioning chamber, which does not provide adequate time to learn about the context (Fanselow 1990; Wiltgen et al. 2001; Frankland et al. 2004). As a result, this group should not form a memory of context B and can be used to determine if exposure to footshock is sufficient to produce NMDAR-independent context fear learning in context A.

Figure 2C shows the amount of baseline freezing in context A prior to shock delivery in saline-injected animals. As expected, there was very little baseline freezing in the control, MWM, and Imm B-A groups. Relative to these mice, group B-A showed elevated freezing (main effect of group, $F_{(3,22)} = 5.96, P < 0.05$; Fisher’s protected least significant differences (PLSD) post hoc comparisons, $P$-values < 0.05). This result is consistent with previous work and indicates that prior learning in context B produces a small amount of fear generalization to context A (Hardt et al. 2009; Tayler et al. 2011). The fact that group B-A froze significantly more than animals in group Imm B-A indicates that immediate shock prevented mice from learning about context B.

Twenty-hour hours after training in context A, mice received a test in the same environment (Fig. 2D). A set of planned comparisons (Fisher’s PLSD) revealed that CPP impaired memory for context A in all groups ($P$-values < 0.05) except group B-A ($P > 0.05$). Control mice that received injections of CPP showed significantly less context fear than saline-injected animals, as observed previously (Wiltgen et al. 2010; Tayler et al. 2011). A similar deficit was observed in CPP-injected mice that had undergone water maze training. This result indicates that prior spatial learning in the Morris water maze is not sufficient to produce NMDAR-independent context fear conditioning. Consistent with previous results, mice that were initially fear-conditioned in context B learned normally in context A even in the presence of CPP (Wiltgen et al. 2010; Tayler et al. 2011). Together, these results suggest that NMDAR-independent learning is task specific; only when animals are repeatedly trained on the same behavioral task are they able to learn without NMDARs. Last, we found that immediate shock training in context B (Imm B-A) did not produce NMDAR-independent learning in context A. This result indicates that prior exposure to footshock is not sufficient to produce NMDAR-independent context fear learning. Instead, animals must form a long-term memory of the initial training context.

The current data suggest that NMDAR-independent learning in context A is contingent on prior conditioning in context B. In the next experiment, we used pharmacology to test this idea by blocking learning in context B. Three groups of mice were examined (Fig. 3A). One group received saline injections prior to learning in context B and CPP injections prior to learning in context A (saline–CPP). As observed in previous experiments, CPP should not impair context A learning in these animals. A second group of mice received CPP injections prior to learning in context B and saline prior to training in context A (CPP–saline). In this group, CPP should prevent initial learning about context B but saline should have no effect on subsequent learning in context A. The last group of mice received CPP injections prior to both training in context B and context A (CPP–CPP). As in the previous group, CPP should prevent initial learning about context B. If forming this memory is essential for subsequent NMDAR-independent learning to occur, then CPP should block conditioning in context A. One day after training in context A, all groups received a test in the same environment. A set of planned comparisons (Fisher’s PLSD) revealed that saline–CPP and CPP–saline groups showed similar amounts of freezing during the test in context A ($P > 0.05$). In contrast, context fear was reduced in group CPP–CPP. There was a significant difference between saline–CPP and

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**Figure 1.** Four groups of mice were used to examine the effects of prior experience on NMDAR-independent context fear learning. The control group received no prior experience, the MWM group was trained on the hidden version of the water maze, group B-A was fear-conditioned in context B, and Imm B-A received immediate shocks in context B. One day after these experiences, the mice received injections of saline or CPP and were trained in context A (N’s for saline/CPP groups; control = 5/6, MWM = 7/8, B-A = 8/8, Imm B-A = 6/6).

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**Figure 2.** (A) Both groups of mice trained in the Morris water maze learned to find the hidden platform across five training days. (B) Spatial memory was assessed on day 6 by removing the platform and conducting a probe test. Both groups of mice searched selectively in the target quadrant where the platform had been located during training. (C) Baseline freezing prior to shock presentation in context A. Group B-A showed elevated freezing relative to all other groups, indicating a small amount of generalization between context B and context A. (D) Freezing during the memory test in context A. CPP injections during training in context A impaired memory for this context in all groups except B-A. In all panels, error bars represent SEM. (*) $P < 0.05$. 

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Cortical mechanisms that are used to encode new information. However, only the difference between saline–CPP and CPP–CPP groups reached statistical significance. A was tested on day 3. Group CPP–CPP showed reduced freezing relative to the other groups.

The existing data suggest that the NMDAR plays a selective role in learning the basic rules or framework of a new task (Bannerman et al. 1995; Sanders and Fanselow 2003). Once this framework has been acquired, animals can learn the same task in a new environment without activating NMDARs. In the Morris water maze the rules are quite clear; find a hidden platform that is located in a fixed spatial position. Once learned, it is easy to see how this information could transfer to a new environment. Context fear conditioning, in contrast, is Pavlovian in nature and does not require the learning of any explicit rules (Bolles and Riley 1973). Nonetheless, it is possible that forming an expectation of shock could generalize to a new environment and lead to NMDAR-independent learning. This does not appear to be the case, however, as simply exploring context B (in the absence of shock) is enough to produce subsequent NMDAR-independent learning in context A (Tayler et al. 2011).

Based on these data, we argue that forming a representation of the initial training environment is the critical factor that allows subsequent learning to occur in the absence of NMDAR activation. Once a representation of the environment is formed, it can be transferred to a similar context and modified in an NMDAR-independent manner. If a new environment is sufficiently dissimilar from previously experienced ones (e.g., water maze vs. fear conditioning chamber) then NMDARs are once again required for learning.

In order for existing memory representations to influence later learning they must be reactivated in new contexts. Several pieces of data suggest that this is the case. In a previous study we used Tet/Tag mice to permanently label hippocampal neurons activated by context fear conditioning with a long-lasting green fluorescent protein (GFP). When mice were subsequently trained in a new context, a number of these same neurons were reactivated (Tayler et al. 2011). A recent electrophysiological study showed that previously learned spatial sequences are spontaneously replayed in the hippocampus when animals explore a new environment (Carlsson and Frank 2009). It is therefore possible that stored representations are used as templates in new environments that can be modified via NMDAR-independent plasticity mechanisms.

The cellular mechanisms that mediate NMDAR-independent learning are currently unknown. Likely candidates include recept or proteins that are upregulated by experience and contribute to synaptic plasticity. Calcium-permeable (CP) AMPARs, for example, are expressed in hippocampal neurons following fear learning and can mediate NMDAR-independent long-term potentiation (LTP) (Jia et al. 1996; Wiltgen et al. 2010; Mitsushima et al. 2011). In addition, conditional knockout mice that express high levels of CP–AMPARs in the CA1 region of the hippocampus are able to acquire context fear in an NMDAR-independent fashion (Wiltgen et al. 2010). These data raise the possibility that CP–

**Figure 3.** (A) Mice received injections of saline or CPP prior to initial conditioning in context B and subsequent learning in context A. Three groups of mice were examined (context B injection–context A injection): Saline–CPP (n = 10), CPP–saline (n = 8), and CPP–CPP (n = 10). Memory for context A was tested on day 3. Group CPP–CPP showed reduced freezing relative to the other groups. However, only the difference between saline–CPP and CPP–CPP groups reached statistical significance. (B) Mice were trained in context B followed 15 d or 30 d later by conditioning in context A. Injections of CPP prior to training in context A had no effect in either group (n = 10 for all groups). In all panels, error bars represent SEM. (*) P < 0.05.

NMDAR-independent learning

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AMPARs mediate learning in the absence of NMDAR activation. However, a recent study found that expression of CP–AMPARs after fear conditioning was transient and, as we show in the current paper, NMDAR-independent learning is still observed several weeks after initial learning (Mitsushima et al. 2011). Therefore, future studies will need to identify changes in synaptic plasticity mechanisms that are induced by prior learning and stable over long periods of time.

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References


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