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The precision of remote context memories does not require the hippocampus

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Although the clarity of many memories fades with time, some memories may maintain their original precision. Here we used a context discrimination procedure to evaluate whether the hippocampus is important in maintaining precision as memories mature. Spared discrimination in hippocampal-lesioned mice indicated that precise, remote context memories may be supported by extra-hippocampal brain regions.

Although the hippocampus may be important in the expression of memories soon after encoding, expression of the same (or at least equivalent) memory may become independent of the hippocampus at later time points[1]. One predominant view is that the transition of the memory from a hippocampus-dependent to hippocampus-independent form reflects a time-dependent process of reorganization that leads to the permanent storage of the memory in the cortex[2]. An alternative view proposes that the transition from a hippocampus-dependent to hippocampus-independent form reflects a transformation of the memory from a precise (or detailed and contextually rich) form to a less precise (or generic and context-free) form in extra-hippocampal regions. According to this latter view, the expression of the original memory in its precise form always requires the hippocampus[3].

In experimental animals, these memory consolidation processes have been most successfully modeled using context fear conditioning, in which mice learn an association between a distinctive place (context) and an aversive event (typically the delivery of a mild foot shock). When returned to the same context, context memory is inferred from an increase in freezing behavior[4]. The precision of this context memory may then be evaluated by comparing freezing levels in the training context to those in an alternate context[5–7] (**Supplementary Note** online). Only hippocampal lesions occurring in the days immediately after training, but not thereafter, disrupt conditioned freezing in the trained context[4], consistent with the view that context memories may be supported by extra-hippocampal brain regions at remote time points. However, levels of generalized freezing in an alternate context also increase over a similar timeframe, suggesting that the precision of context fear memories declines with time[5–7] (**Supplementary Note**). Therefore, as memory age and memory precision tend to covary in these studies, both the reorganization or transformation views can readily account for spared freezing in the training context after hippocampal lesions at remote time points. However, these two views make distinct predictions about the requirement of the hippocampus in the expression of remote context memories that maintain their original precision. The reorganization view predicts that precise memories may eventually become independent of the hippocampus, whereas the transformation view predicts that the hippocampus is always necessary for precision.

To produce durable, precise context memories in mice, we used a context fear discrimination protocol[8] (**Supplementary Methods** and

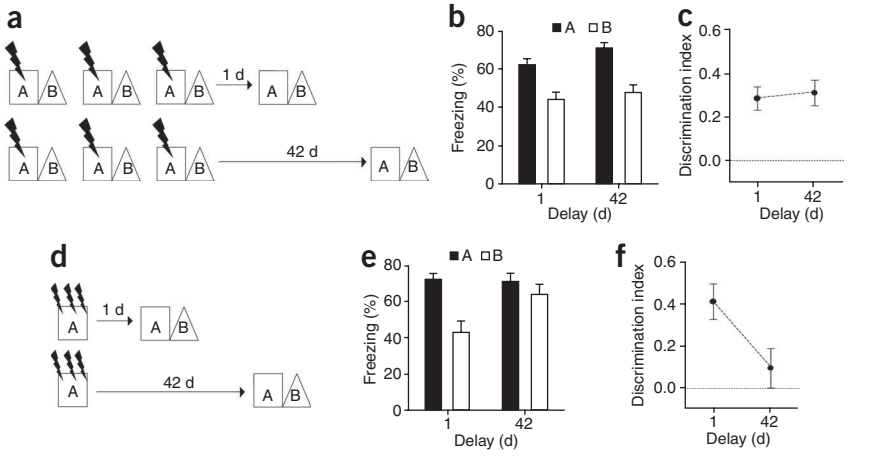

Figure 1 Delay-dependent decline in the precision of context memories depends on training. (**a**) Context discrimination design. (**b**,**c**) Levels of conditioned freezing (**b**) were greater in context A (black bars) than in context B (white bars) when tested either 1 or 42 d following training ($P < 0.001$, by paired t test) and levels of discrimination (**c**) did not differ at these two retention delays following context discrimination training ($P > 0.05$, by t test). (**d**) Contextual fear conditioning design. (**e**) Levels of conditioned freezing were greater in context A (black bars) than in context B (white bars) when tested 1 d ($P < 0.001$, by paired t test), but not 42 d ($P > 0.05$, by paired t test), following training in the standard version of contextual fear conditioning. (**f**) Accordingly, levels of discrimination declined in a delay-dependent manner ($P < 0.05$, by t test). These and subsequent graphs show means ± s.e.m.

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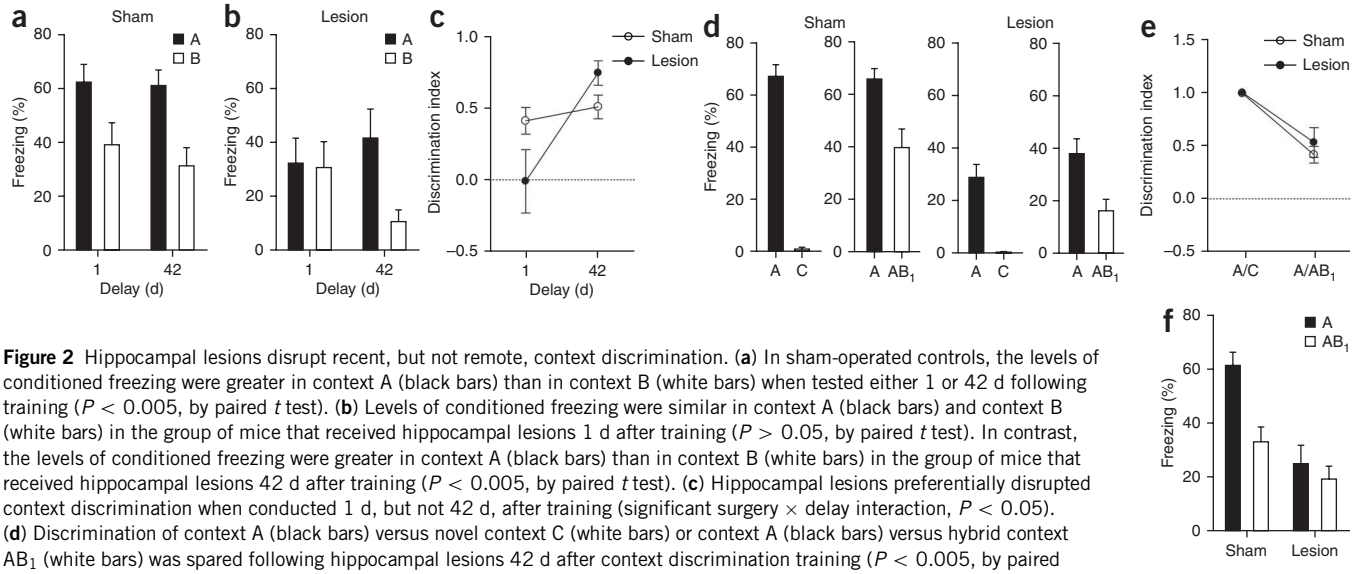

Figure 2 Hippocampal lesions disrupt recent, but not remote, context discrimination. (**a**) In sham-operated controls, the levels of conditioned freezing were greater in context A (black bars) than in context B (white bars) when tested either 1 or 42 d following training ($P < 0.005$, by paired t test). (**b**) Levels of conditioned freezing were similar in context A (black bars) and context B (white bars) in the group of mice that received hippocampal lesions 1 d after training ($P > 0.05$, by paired t test). In contrast, the levels of conditioned freezing were greater in context A (black bars) than in context B (white bars) in the group of mice that received hippocampal lesions 42 d after training ($P < 0.005$, by paired t test). (**c**) Hippocampal lesions preferentially disrupted context discrimination when conducted 1 d, but not 42 d, after training (significant surgery × delay interaction, $P < 0.05$). (**d**) Discrimination of context A (black bars) versus novel context C (white bars) or context A (black bars) versus hybrid context AB_1 (white bars) was spared following hippocampal lesions 42 d after context discrimination training ($P < 0.005$, by paired t test). (**e**) Levels of discrimination were similar in sham and hippocampal-lesioned mice, but differed between experiments ($P < 0.001$, by ANOVA). (**f**) Discrimination of contexts A and AB_1 was abolished in hippocampal-lesioned, but not sham-operated, mice following re-testing ($P < 0.001$, by t test).

Supplementary Fig. 1 online). Mice were trained for three consecutive days in context A (always paired with shock) and context B (never paired with shock), according to protocols approved by the Animal Care Committee at The Hospital for Sick Children. Conditioned freezing, defined as the absence of all movement except for breathing, was then examined in both contexts either 1 or 42 d later (**Fig. 1a**). When trained in this manner, mice froze more in context A (the shock context) than in context B (the no shock context) at both delays (**Fig. 1b**). Notably, the degree of discrimination did not differ at these two time points (**Fig. 1c**), suggesting that the mice maintained precise representations of the two contexts over this period. The persistent discrimination contrasted with a second group of mice that were trained using a more standard context fear–conditioning protocol[6]. During training, these mice received three shocks in context A and were then tested in both context A and context B either 1 or 42 d later (**Fig. 1d**). Conditioned freezing levels were greater in context A than in context B when tested 1 d after training. At the longer delay, however, conditioned freezing levels were similar in both contexts (**Fig. 1e**). The delay-dependent decline in discrimination (**Fig. 1f**) suggests that the precision of context memories declined over time following training in this more standard context fear–conditioning protocol[5–7] (significant training protocol × delay interaction, $F_{1,88} = 5.68$, $P < 0.05$). Because the discriminative (rather than non-discriminative[5,9]; **Supplementary Results** and **Supplementary Fig. 2** online) fear conditioning protocol prevented the time-dependent decline in the precision of context memories, we used these procedures to evaluate the hippocampal dependence of precise context fear memories at different times following training.

Mice received complete NMDA lesions of the hippocampus either 1 or 42 d following training in the context discrimination procedure (for training data, see **Supplementary Results** and **Supplementary Fig. 3**

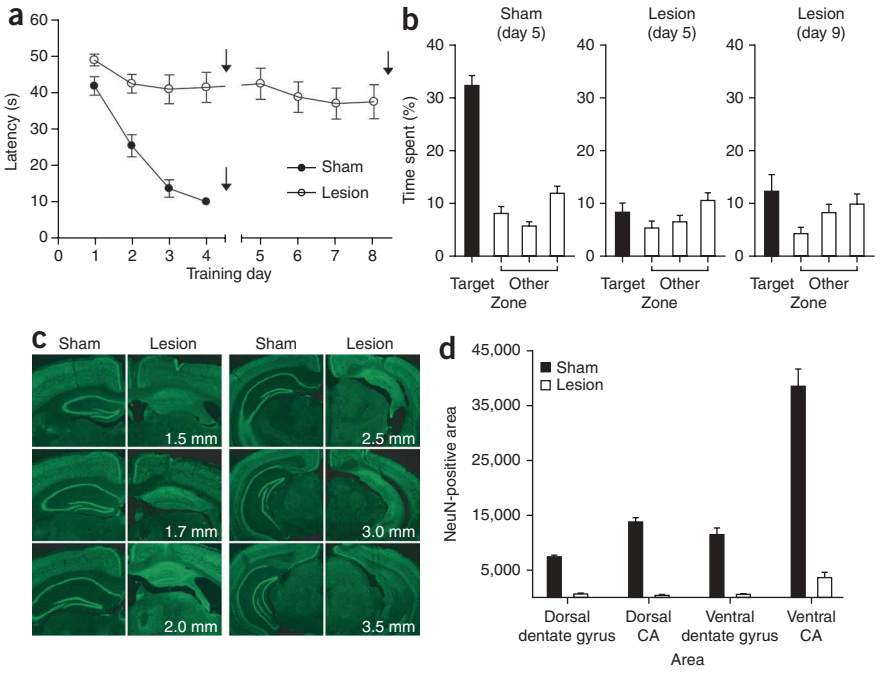

Figure 3 Behavioral and histological quantification of hippocampal lesions. (**a**) In the water maze, the time required to reach the escape platform declined in sham-operated mice (closed circles), but not in hippocampal-lesioned mice (open circles), across days ($P < 0.001$, by ANOVA, significant day × surgery interaction). Probe tests (arrows) were conducted on days 5 and 9. (**b**) Only sham-operated mice spent more time searching the target zone (black) compared with other (white) zones in the probe test on day 5 ($P < 0.001$, by paired t test). (**c**) Representative images from sham- and lesioned-mice at different levels of the hippocampus stained for the neuronal specific marker NeuN (mm posterior to bregma). (**d**) NMDA lesions led to almost complete neuronal depletion in the dorsal and ventral CA fields and dentate gyrus.

online). We assessed conditioned freezing 7 d after surgery in both context A and context B. In control mice, conditioned freezing levels were greater in context A versus context B at both delays (**Fig. 2a**), confirming that the precision of context memories can be maintained for several weeks following this type of training. In contrast, hippocampal lesions that occurred 1 d after training reduced freezing and abolished discrimination, with mice showing similar levels of freezing in context A and context B. However, discrimination was preserved when these lesions were performed 42 d after training, with hippocampal-lesioned mice freezing significantly more in context A than in context B ($P < 0.005$; **Fig. 2b**). The spared discrimination in these lesioned mice suggests that the expression of precise context memories does not require the hippocampus at remote time points (significant surgery × delay interaction, $F_{1,30} = 7.31$, $P < 0.05$; **Fig. 2c**).

This temporally graded pattern of retrograde amnesia resembles that observed in memory-impaired patients with hippocampal damage, where recently acquired memories are typically more affected than remotely acquired memories. However, one critical distinction between the reorganization and transformation views of memory consolidation is whether spared remote memories are equivalently detailed in memory-impaired patients and control subjects, and it is possible that more thorough interrogation might reveal differences[10,11]. Accordingly, we trained additional groups of mice in the context discrimination procedure (for training data, see **Supplementary Results** and **Supplementary Fig. 4** online), lesioned the hippocampus 42 d later and examined conditioned freezing in either context A versus a novel context (C) or in context A versus a hybrid context (AB_1) that contained components of both context A and context B (**Supplementary Fig. 1**). Both sham-operated and hippocampal-lesioned mice froze significantly more in context A than in the novel or hybrid contexts ($P < 0.005$; **Fig. 2d**). Notably, the degree of discrimination did not differ between sham-operated and hippocampal-lesioned mice (**Fig. 2e**), indicating that discrimination was preserved in hippocampal mice even under these more rigorous testing conditions. However, additional testing led to pronounced reductions in conditioned freezing levels and discrimination in the hippocampal-lesioned mice (**Fig. 2f**; **Supplementary Fig. 3**). Therefore, although precise context memories may be supported by extra-hippocampal regions at remote time points, these additional analyses revealed that these memories are nonetheless not identical to those in control mice; without the hippocampus, the memory is more fragile, suggesting that the integrity of the hippocampus is important for robust memory expression[12].

To verify the effectiveness of the lesion, we next trained mice in the water maze. As expected, hippocampal-lesioned mice were severely impaired relative to controls, taking longer to locate the platform (days 1–4; **Fig. 3a**). In a probe test on day 5, control mice searched selectively in the region of the pool that formerly contained the platform, whereas hippocampal-lesioned mice searched nonselectively (**Fig. 3b**). Even with an additional 4 d of training, there was no further improvement in escape latency (**Fig. 3a**) or in probe test performance in hippocampal-lesioned mice (**Fig. 3b**). Subsequent histological analyses revealed that lesions resulted in more than 95% depletion of neurons in the dorsal and ventral CA fields and dentate gyrus, with negligible extra-hippocampal damage (**Supplementary Methods** and **Fig. 3c,d**). The completeness of these lesions therefore excludes the possibility that spared discrimination might be supported by residual hippocampal tissue[3].

In conclusion, spared discrimination following hippocampal lesions is inconsistent with the view that the hippocampus is always required for the expression of precise (contextually rich) memories[3]. However, the observed fragility of this remote context memory is also counter to the view that such a memory can be supported independently by extra-hippocampal regions[2]. Indeed, our data point to a prolonged requirement for the hippocampus in the expression of these types of memory[12], with the hippocampus perhaps being involved in the integration of new information into existing extra-hippocampal networks[13,14]. The fragility of the spared memory in our study contrasts with two recent reports that involved much more extensive pre-operative experience in the training environment[14,15]. This suggests that the amount of pre-operative experience determines whether memories become partially or completely independent of the hippocampus at remote time points.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

S.-H.W. and P.W.F. conceived and designed the experiments. S.-H.W., C.M.T. and A.L.W. performed the behavioral studies and carried out the statistical analyses. S.-H.W. conducted the lesions and histological analyses. P.W.F. supervised the project and wrote the paper with S.-H.W.

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