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Article

The Hippocampus Plays a Selective Role in the Retrieval of Detailed Contextual Memories

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Summary

Background: It is widely believed that the hippocampus plays a temporary role in the retrieval of episodic and contextual memories. Initial research indicated that damage to this structure produced amnesia for newly acquired memories but did not affect those formed in the distant past. A number of recent studies, however, have found that the hippocampus is required for the retrieval of episodic and contextual memories regardless of their age. These findings are currently the subject of intense debate, and a satisfying resolution has yet to be identified.

Results: The current experiments address this issue by demonstrating that detailed memories require the hippocampus, whereas memories that lose precision become independent of this structure. First, we show that the dorsal hippocampus is preferentially activated by the retrieval of detailed contextual fear memories. We then establish that the hippocampus is necessary for the retrieval of detailed memories by using a context-generalization procedure. Mice that exhibit high levels of generalization to a novel environment show no memory loss when the hippocampus is subsequently inactivated. In contrast, mice that discriminate between contexts are significantly impaired by hippocampus inactivation.

Conclusions: Our data suggest that detailed contextual memories require the hippocampus, whereas memories that lose precision can be retrieved without this structure. These findings can account for discrepancies in the literature—memories of our distant past can be *either* lost or retained after hippocampus damage depending on their quality—and provide a new framework for understanding memory consolidation.

Introduction

The hippocampus is essential for the formation and retrieval of episodic and contextual memories in humans and animals. It is well established that cell loss or dysfunction in this area produces profound amnesia for newly acquired information [1–5]. In contrast, the fate of old memories is less clear. Initial work indicated that the hippocampus was not involved in the retrieval of episodic or contextual memories formed in the distant past. These findings gave rise to the idea, called consolidation theory, that the hippocampal system plays a temporary role in the formation and retrieval of new memories as they are being permanently stored in regions of the neocortex [6–8]. Recent work has presented several challenges to this theory. First, functional imaging studies (fMRI) commonly observe hippocampus activation during the retrieval of both new and old episodic memories [9–11]. Consistent with these findings, damage to the hippocampal system in humans and animals often impairs the retrieval of both recent and remote memories [2, 12–18]. Results such as these have contributed to the development of multiple trace theory (MTT), which argues that episodic and contextual memories are permanently stored in the hippocampus [17]. These findings are currently the subject of intense debate in the field, and a satisfying resolution has yet to be identified.

The current paper seeks to address the discrepancies in the animal literature by examining the relationship between memory quality and hippocampus dependency. In previous experiments, we demonstrated that contextual memories become less specific and more general during the consolidation period [19]. Mice trained to fear a specific context are initially able to discriminate between it and a similar environment. However, as the time between training and testing is increased, memory becomes less accurate, and animals are unable to distinguish between these contexts. On the basis of these results, we suggested that memory quality might be a critical factor that determines whether or not the hippocampus is essential for retrieval. As memories become less detailed and more general across time, they gradually become independent of this structure. In contrast, when the details of memories are retained, the hippocampus continues to be necessary for their retrieval. This idea is consistent with previous theories that argue that the function of consolidation is to gradually integrate memories of specific events with general knowledge that is stored in the neocortex [2, 20, 21].

Results

The Retrieval of Recent but Not Remote Memory Increases Immediate Early Gene Expression in the Hippocampus

To test the idea just described, we used two complementary approaches. Our first approach was to monitor hippocampus activity during memory retrieval by using immediate early gene (IEG) expression as measured by qRT-PCR. This technique has several advantages over immunohistochemistry and in situ hybridization (ISH), which are commonly used for indexing IEG activity. First, very small changes in mRNA expression can be amplified and detected via PCR. Second, the observed changes can be quickly and reliably quantified without the use of stereology or image thresholding. Therefore, we first determined whether qRT-PCR was capable of detecting increased gene expression in the dorsal hippocampus after contextual fear conditioning. To optimize IEG expression, we trained mice with a robust context-conditioning protocol (five 0.75 mA shocks). *Arc*, *c-fos*, and *zif268* expression were analyzed in mice sacrificed immediately, 15 min, 30 min, or 60 min after training (Figure 1A). We found

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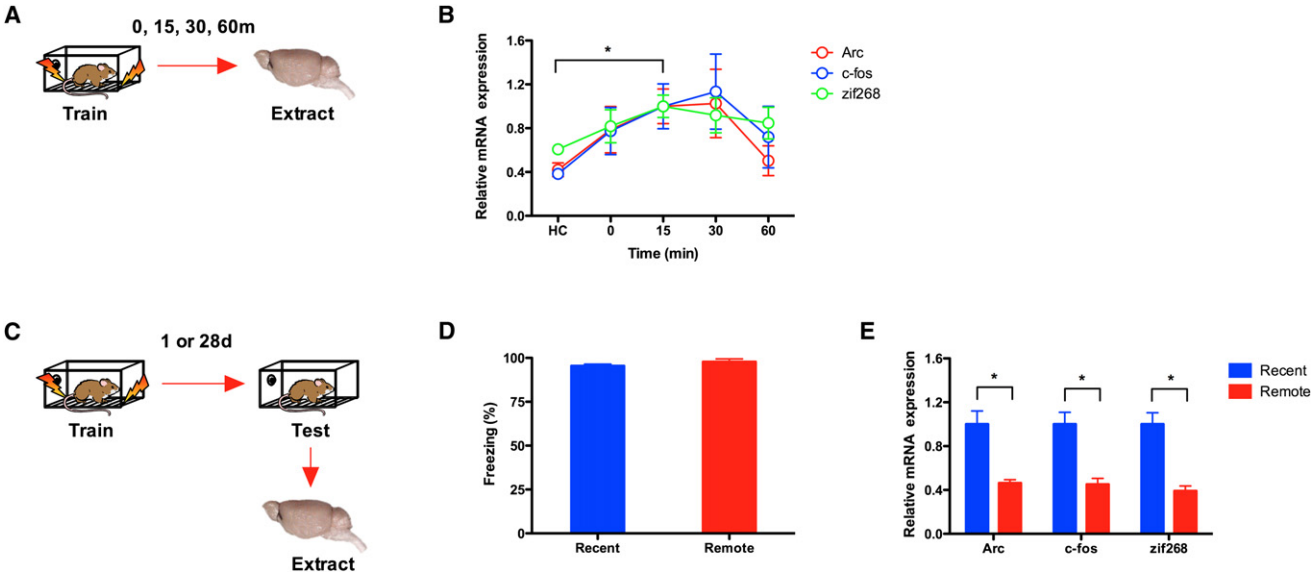

Figure 1. The Retrieval of Recent Contextual Fear Memories Increases Immediate Early Gene Expression in the Dorsal Hippocampus

(A) Experimental design.

(B) Immediate early gene expression (*Arc*, *c-fos*, *zif268*) (n = 4 per group) was significantly increased 15 min after training in trained mice relative to home-cage controls ($p < .05$).

(C) Experimental design.

(D) Freezing levels during 1 day (recent, n = 4) and 28 day (remote, n = 4) contextual fear tests were equivalent ($p > .05$).

(E) Immediate early gene expression (*Arc*, *c-fos*, *zif268*) was reduced in the dorsal hippocampus during the retrieval of remote memory ($p < .05$). Note: mRNA expression is shown relative to the recent memory test.

Data represent mean ± SEM in (B), (D), and (E).

an increase in the expression of all three IEGs [significant effect of time, $F(4, 36) = 3.71$, $p < .05$; no effect of gene, $F(2, 9) = 3.27$, $p > .05$; no time × gene interaction, $F < 1$], and this change in expression was significantly different from that of home-cage controls at 15 min after training (Fisher's PLSD, $p < .05$) (Figure 1B). We next determined whether qRT-PCR was sensitive enough to detect activity changes that occur in the hippocampus during the consolidation period. To do this, mice were trained with five shocks and tested 1 or 28 days later (Figure 1C). Consistent with previous results, we found reduced expression of *Arc*, *c-fos*, and *zif268* in the dorsal hippocampus after the retrieval of remote memories [significant effect of test day, $F(1,6) = 25.35$, $p < .05$); no effect of gene, $F < 1$; no test day × gene interaction, $F < 1$] [22–24] (Figure 1E). Freezing behavior was equivalent during the recent and remote tests, suggesting that memory strength did not change with time [no effect of test day, $F(1,6) = 1.37$, $p > .05$] (Figure 1D).

Temporary Inactivation of the Hippocampus Impairs the Retrieval of Recent but Not Remote Memory

In the next experiment, we determined whether hippocampus inactivation selectively impairs the retrieval of recently formed contextual fear memories. Mice were trained with a single shock and tested 1 or 28 days later (Figure 2). During testing, the hippocampus was transiently inactivated with the AMPA receptor antagonist CNQX. Temporary inactivation was used because, unlike traditional lesion methods, this technique does not damage distal structures [25]. Similar to findings in previous studies, inactivation of the dorsal hippocampus with CNQX impaired the retrieval of recent but not remote contextual fear memories [significant test day × infusion interaction, $F(1, 68) = 5.95$, $p < .05$] (Figure 2B) [3, 8]. After testing, the dorsal hippocampus was extracted from saline-infused animals and analyzed for *Arc* expression via qRT-PCR. Consistent with our previous experiment, *Arc* expression was elevated during the 1 day test relative to home-cage controls ($p < .05$), and this value decreased during the 28 day test ($p < .05$) (Figure 2C).

Because the saline controls froze slightly less during the 28 day memory test, we conducted a regression analysis to determine whether there was a relationship between freezing and *Arc* expression. We found no relationship between these variables at either 1 day (slope not significantly different from zero, $F < 1$, $r^2 = .002$) or 28 days (slope not significantly different from zero, $F < 1$, $r^2 = .00012$). This suggests that decreased *Arc* expression at 28 days is not due to reduced freezing.

To determine whether the reduced IEG expression observed in our experiments reflects less mRNA expression per cell or the activation of fewer neurons during memory retrieval, we performed fluorescent in situ hybridization (FISH) for Homer 1A. Homer is an IEG that reliably indexes cellular activity in the hippocampus and neocortex. The correspondence between *Arc* and Homer expression in activated CA1 neurons is 95% [26, 27]. We examined expression of Homer 1A in the CA1 region of the dorsal hippocampus during memory retrieval 1 or 28 days after training (Figure 2D). There was a significant reduction in the percentage of neurons that expressed Homer at 28 days compared to 1 day [significant effect of test day, $F(1, 6) = 6.41$, $p < .05$] (Figures 2E and 2F). This suggests that reduced IEG expression during remote memory tests reflects the activation of fewer cells in the hippocampus.

Remote Memories Are Less Precise Than Recently Formed Memories

Next, we determined whether hippocampus activation is correlated with memory quality (Figure 3A). To do this, we

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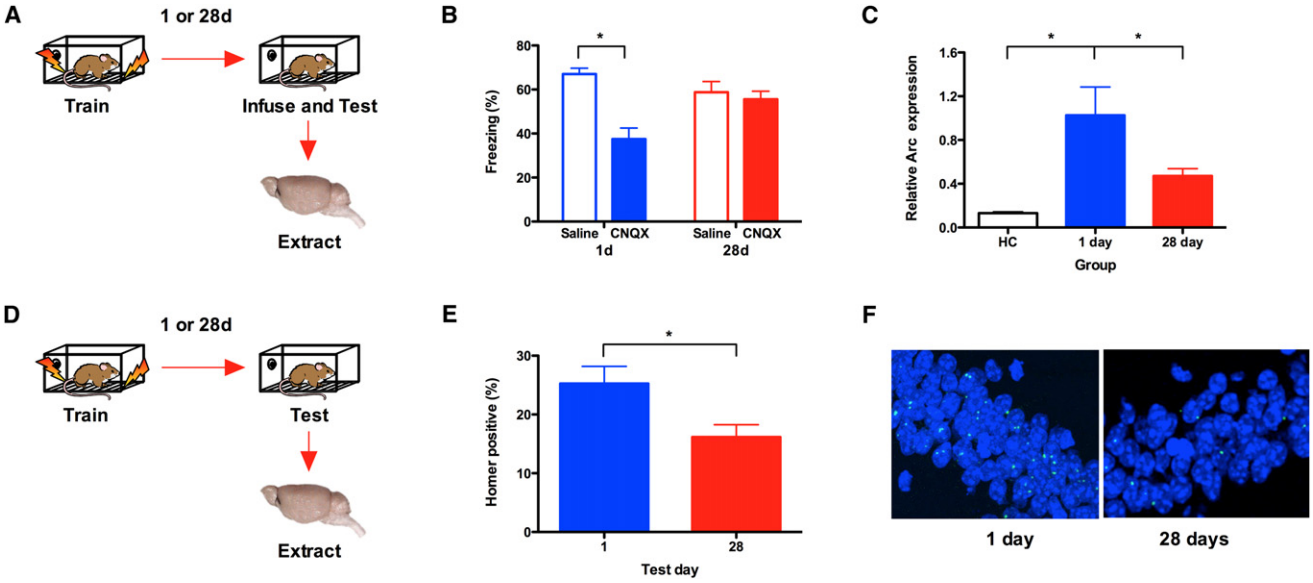

Figure 2. The Retrieval of Remote Contextual Fear Memories Does Not Require the Dorsal Hippocampus

(A) Experimental design.

(B) Inactivation of the dorsal hippocampus with CNQX impaired memory retrieval at day 1 (saline, n = 16, CNQX, n = 16) but not day 28 (saline, n = 20, CNQX, n = 20) ($p < .05$).

(C) *Arc* expression in the saline animals increased in comparison to that in home-cage controls (HC) (n = 3) during the day 1 test ($p < .05$) and decreased during the day 28 test ($p < .05$). Note: mRNA expression is shown relative to the 1 day test.

(D) Experimental design.

(E) Fewer cells expressed Homer 1A in the CA1 region of the hippocampus during the day 28 test (n = 4) than during the day 1 test (n = 4) ($p < .05$).

(F) Representative samples of Homer1A expression after retrieval at days 1 and 28.

Data represent mean ± SEM in (B), (C), and (E).

conditioned mice in the training context (context A) and then tested them in a similar, yet novel environment (context B) 1, 7, 14, or 28 days later. We tested separate groups of animals at each interval. We previously showed that generalization to context B increases over time [19]. As a result, animals are able to discriminate between context A and B shortly after training but are unable to do so at longer test intervals. This suggests that memory for the training environment becomes less precise with the passage of time [28–30]. As a control, we tested an additional four groups of mice in a context that was completely distinct from the training environment (context C). Mice showed significantly more generalization to the similar context than to the distinct environment [significant effect of context, $F(1, 49) = 169.2$, $p < .05$]. In addition, generalization to the similar context increased over time [1 versus 28 day, significant effect of test day, $F(1, 12) = 4.81$, $p < .05$]. whereas no change was observed in generalization to the distinct environment ($F < 1$) (Figure 3B). The fact that generalization only increased in context B suggests that nonspecific mechanisms (e.g., sensitization, incubation) do not contribute to this change. In our previous experiment (Figure 2B), fear of context A also did not increase over time. This implies that changes in memory strength are not responsible for the observed increases in fear generalization [19]. Together, these data demonstrate that increases in fear generalization reflect animals' inability to discriminate between the training context and a similar environment.

After the context test, we examined IEG expression and found that generalization was correlated with reduced activation of the dorsal hippocampus. As generalization to context B increased, the hippocampus expressed less *Arc* and *c-fos* and remained activated by context C at all test intervals [significant context × test day interaction ($F(3, 49) = 2.86$, $p < .05$] (Figures 3C and 3D). Post hoc tests (Fisher's PLSD) revealed that IEG expression was significantly reduced in context B compared to context C at 14 and 28 days ($p < .05$). These data suggest that the hippocampus is less activated by the retrieval of generalized contextual fear memories.

Previous work has shown that the excitability of hippocampal neurons is increased for several days after new learning [31, 32]. Consequently, an alternative interpretation of the current data is that decreased IEG expression reflects a return of hippocampal excitability to baseline after fear conditioning. This does not appear to be the case, however, because exposure to context C continued to produce robust IEG expression at all test intervals (probably as a result of new learning). Therefore, the current experiment demonstrates that reduced hippocampus activation after fear conditioning is contingent on memory retrieval.

A regression analysis conducted on the freezing scores in context B and IEG expression revealed that there was a significant relationship between these factors for both *Arc* [slope significantly different from zero, $F(1, 24) = 4.32$, $p < .05$, $r^2 = .15$] and *c-fos* (slope significantly different from zero, $F(1, 24) = 9.95$, $p < .05$, $r^2 = .29$) (Figures 3E and 3F). The more mice generalized to context B, the less IEG expression was observed in the dorsal hippocampus.

Context Generalization Predicts Memory Precision

The previous experiments demonstrate that the hippocampus is less activated by remote contextual fear memories that have lost details. Consequently, reduced activity could be due to changes in memory quality or the passage of time. To discriminate between these possibilities, we first examined

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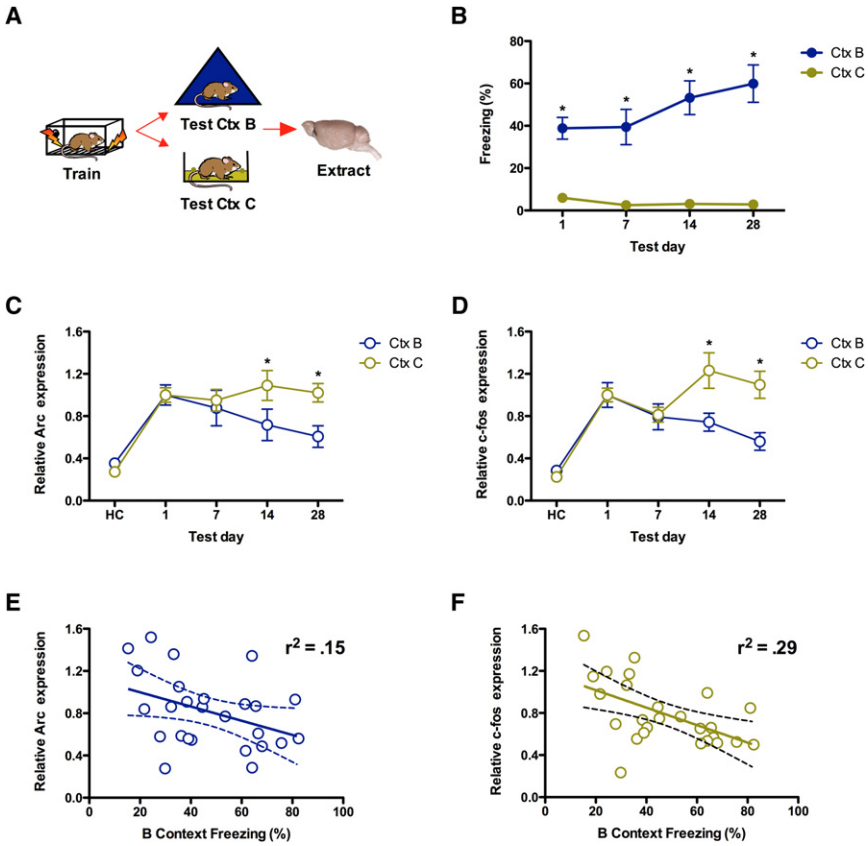

Figure 3. The Dorsal Hippocampus Is Less Activated by the Retrieval of Generalized Contextual Fear Memories

(A) Experimental design.

(B) Mice showed more generalized fear to context B, which was similar to the training environment, than to context C, which was distinct ($p < .05$). Fear generalization to the similar environment increased over time (days 1, 7, 14, and 28, n = 8, 6, 6, and 6) ($p < .05$), whereas fear of the distinct context did not (n = 8, 8, 8, and 7) ($p > .05$).

(C) *Arc* activation in the dorsal hippocampus decreased as generalization to context B increased ($p < .05$). Activation in context C did not change over time ($p > .05$). Note: mRNA expression is shown relative to the day 1 test.

(D) C-fos activation in the dorsal hippocampus decreased as generalization to context B increased ($p < .05$). Activation in context C did not change ($p > .05$). Note: mRNA expression is shown relative to the day 1 test.

(E) Linear regression analysis found a significant relationship between freezing in context B and *Arc* expression in the dorsal hippocampus ($p < .05$).

(F) Linear regression analysis found a significant relationship between freezing in context B and c-Fos expression in the dorsal hippocampus ($p < .05$).

Data represent mean ± SEM in (B), (C), and (D).

contextual-fear generalization in more depth (Figure 4). We trained a large group of mice (n = 185) and tested them in context A and context B 1 or 14 days after training. Consistent with our previous results, context discrimination was significantly better at 1 day than 14 days (significant test day × context interaction, $F(1, 183) = 28.878$, $p < .05$] (Figure 4A) [19]. We then calculated a discrimination index [A/(A+B)] for each animal and constructed a frequency distribution (Figure 4B). As expected, the mean discrimination ratio was significantly higher at day 1 ($\bar{x} = 0.67$) than day 14 ($\bar{x} = 0.58$) [$F(1, 183) = 20.964$, $p < .05$]. However, even though the majority of mice could discriminate at day 1, a few animals were unable to do so (discrimination ratio < .5). In addition, a large number of mice tested at day 14 froze more in context A than context B (discrimination ratio > .5). If the hippocampus is necessary for the retrieval of detailed memories, then inactivating it should only impair freezing in animals that can discriminate, regardless of the test interval. In the next section we describe a method that allows us to test this prediction.

A regression analysis conducted on the freezing scores in context A and the discrimination index revealed that there was no relationship between these factors [slope not significantly different from zero, $F(1, 183) = 3.47$, $p < .05$, $r^2 = .018$] (Figure 4C). In contrast, there was a robust relationship between freezing in context B and the ability to discriminate [slope significantly different from zero, $F(1, 183) = 318$, $p < .05$, $r^2 = .634$] (Figure 4C). The less that mice generalized to context B, the better they were able to discriminate. This was not simply a performance artifact (e.g., more/less freezing makes it harder/easier to discriminate) because the same relationship was not observed in context A. This suggests that discrimination is largely driven by the amount of freezing in context B. As a result, one can use freezing scores in context B to identify mice that are able to discriminate between contexts. If our main hypothesis is correct, hippocampus inactivation should selectively impair memory retrieval in these animals.

To categorize animals prior to hippocampus inactivation, we analyzed the frequency distributions of freezing scores in context A and context B. A single large peak was observed in context A (Figure 4E), whereas multiple peaks were observed in context B (Figure 4F). The mean of the first peak in context B was 16.7, which correlates with a discrimination index of 0.74. The mean of the second peak was 66.1, which correlates with a discrimination index of 0.53. Therefore, mice from the first distribution should be able to discriminate between contexts, whereas those in the second distribution should not. Accordingly, we chose the intersection of these curves (42% freezing) as our threshold to distinguish between mice belonging to each of these two groups. Mice that froze less than this value in context B were categorized as discriminators, and those that froze more were categorized as generalizers.

The Retrieval of Precise Contextual Memories Requires the Hippocampus

In the next experiment, mice were implanted with guide cannulae that targeted the dorsal hippocampus. They were then trained in context A and tested in context B 14 days later (Figure 5A). Using the threshold from our previous analysis, we divided the mice into two groups: discriminators and generalizers (Figure 5B). The next day all of the animals were tested in the original training environment. Some of the animals in each group received an infusion of saline into the dorsal hippocampus before this test, whereas the others received CNQX.

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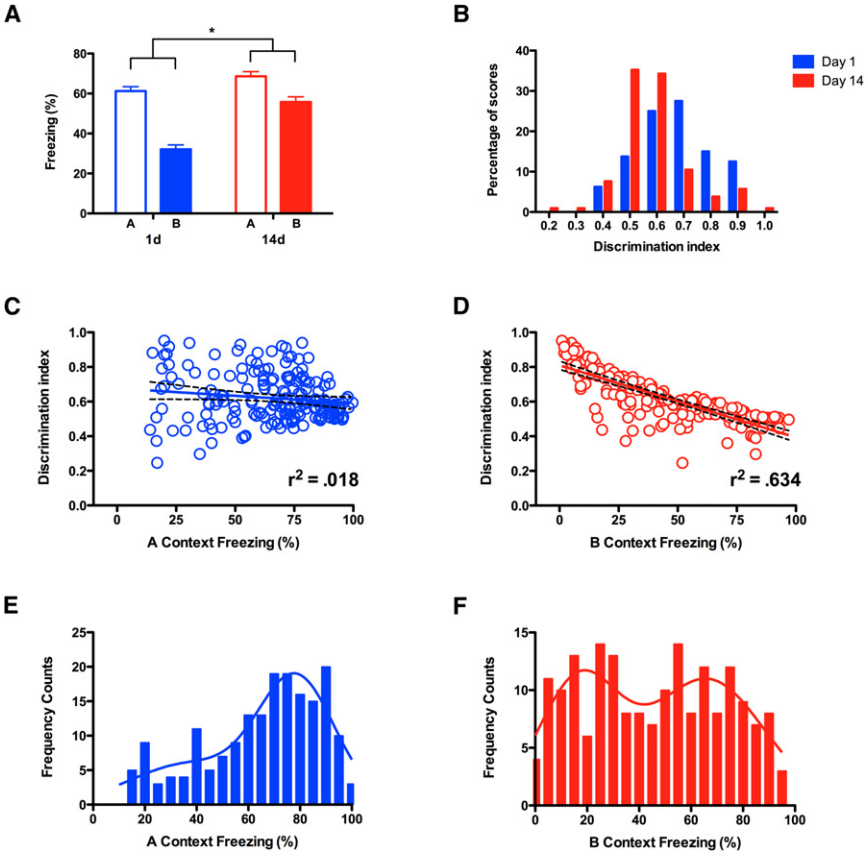

Figure 4. Context Generalization Can Be Used to Index Memory Precision

(A) A large group of mice were tested in contexts A and B, 1 day (n = 80) or 14 days (n = 105) after training. Discrimination was significantly better at day 1 than at day 14 ($p < .05$). Data represent mean ± SEM.

(B) Frequency distributions of the discrimination index [A/(A + B)] for mice tested at day 1 or day 14. The mean discrimination index was significantly higher at day 1 than at day 14 ($p < .05$).

(C) Linear regression analysis found no relationship between freezing in context A and the discrimination index ($p > .05$).

(D) Linear regression analysis found a significant relationship between freezing in context B and the discrimination index ($p < .05$).

(E) Nonlinear regression analysis of freezing scores in context A found a single distribution with a mean of 77.72.

(F) Nonlinear regression analysis of freezing scores in context B found a bimodal distribution with means of 16.7 and 66.1. The intersection of these two distributions occurred at a freezing value of 42.

We found that hippocampus inactivation with CNQX selectively impaired memory retrieval in mice that could discriminate between contexts, whereas those that generalized were not affected [significant group × drug interaction ($F(1, 33) = 4.768$, $p < .05$] (Figure 5C). These results demonstrate that the hippocampus plays a selective role in the retrieval of detailed contextual memories. This effect was independent of memory age: all animals were tested at 14 days.

To confirm that our threshold for categorization was valid, we compared freezing scores in context A and context B for mice that received saline infusions (Figure 5D). As predicted, the discriminators froze substantially more in the training context than in the novel environment, whereas the generalizers froze the same amount in both contexts [significant group × context interaction ($F(1, 12) = 7.762$, $p < .05$]. This difference in discrimination ability cannot be explained by different amounts of learning or

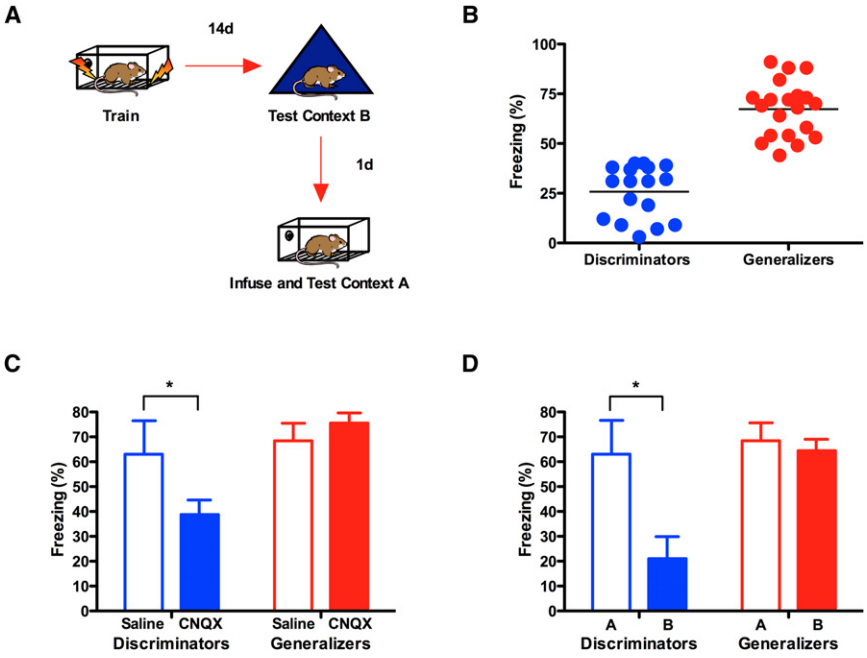

Figure 5. The Hippocampus Is Required for the Retrieval of Precise but Not Generalized Contextual Memories

(A) Experimental design.

(B) Fourteen days after training, mice were tested in context B. After this test, animals were divided into two groups: discriminators and generalizers.

(C) Inactivation of the dorsal hippocampus with CNQX impaired memory retrieval in the discriminators (saline n = 4, CNQX n = 13) ($p < .05$) but had no effect in the generalizers (saline n = 10, CNQX n = 10) ($p > .05$).

(D) An analysis of freezing scores in mice that received saline infusions confirmed that discriminators were able to distinguish between context A and context B ($p < .05$), whereas generalizers could not ($p > .05$).

Data represent mean ± SEM in (C) and (D).

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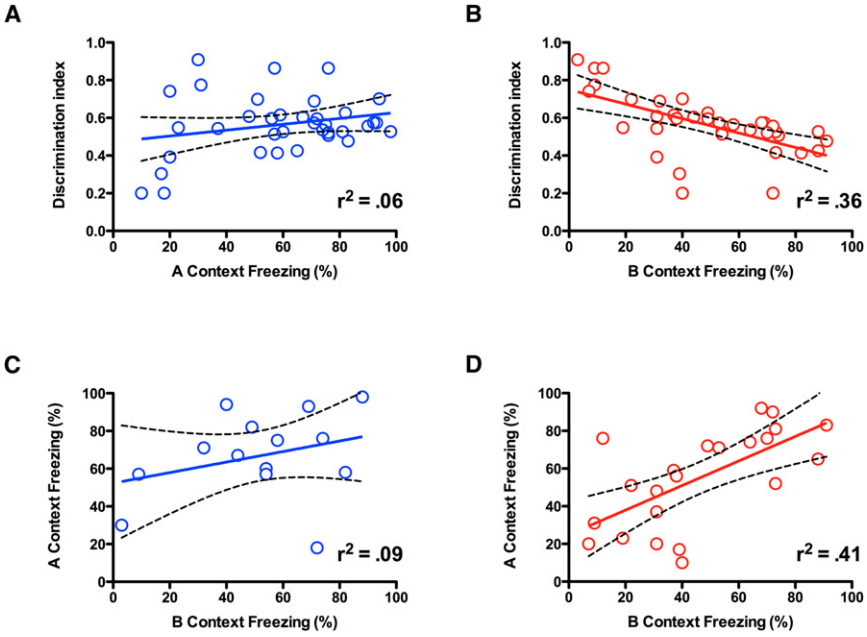

Figure 6. Generalization Predicts which Mice Will Be Affected by Hippocampus Inactivation

(A) Linear regression analysis found no relationship between freezing in context A and the discrimination index ($p > .05$).

(B) Linear regression analysis found a significant relationship between freezing in context B and the discrimination index ($p < .05$).

(C) Linear regression analysis found no relationship between freezing in context B and freezing in context A for mice that received saline infusions ($p > .05$).

(D) Linear regression analysis found a significant relationship between freezing in context B and freezing in context A for mice that received CNQX infusions ($p > .05$).

performance; freezing in context A was identical across groups ($F < 1$).

To further validate these results, we analyzed the same data set by using regression analysis. Similar to our previous experiment, we found no relationship between freezing in context A and the discrimination index [slope not significantly different from zero, $F(1, 35) = 2.238$, $p > .05$, $r^2 = .06$] (Figure 6A) but a strong relationship between freezing in context B and discrimination [slope significantly different from zero, $F(1, 35) = 20.54$, $p < .05$, $r^2 = .36$] (Figure 6B). Next, we examined the relationship between freezing in context A and context B in mice that received infusions of saline or CNQX. In the saline mice, no relationship was observed between these variables [slope not significantly different from zero, $F(1, 12) = 1.251$, $p > .05$, $r^2 = .09$] (Figure 6C). In contrast, there was a significant relationship between freezing in context A and context B in mice that received infusions of CNQX [slope significantly different from zero, $F(1, 21) = 15.12$, $p < .05$, $r^2 = .41$] (Figure 6D). The less animals froze in context B, the more their freezing scores were reduced by CNQX when tested in context A. This relationship is not simply a performance artifact (more/less freezing in context B leads to more/less freezing in context A) because it was not observed in mice that received infusions of saline. This analysis strengthens our conclusion that the hippocampus plays a selective role in the retrieval of detailed contextual fear memories.

An alternative account of the data presented in Figures 5 and 6 is that inactivation of the hippocampus reduces freezing by changing the internal state of the animal and causing a context shift. Mice that are sensitive to a shift in the external context (i.e., discriminators) may also be more sensitive to an internal shift caused by drug infusion. Although plausible, this account is unable to explain the results of our other experiments or the findings of previous studies and is thus unlikely. For example, in Figure 4 we show that IEG expression is reduced in the hippocampus as generalization to context B increases. This finding is consistent with our hypothesis that generalized contextual fear memories are not retrieved by the hippocampus. In contrast, a context-shift account makes no predictions about hippocampus activity during memory retrieval, nor does it explain the decrease in IEG expression that accompanies generalization. In addition, previous work from our lab has shown that context discrimination depends on the hippocampus [33]. In the absence of this structure, mice show significantly increased generalization to a nonshocked environment. Consistent with this finding, fluorescent in situ hybridization (FISH) studies have shown that exposure to two distinct contexts activates nonoverlapping groups of neurons in the hippocampus [34]. Together, these results demonstrate that (1) cells in the hippocampus are able to detect external context-shifts and (2) these cells are essential for animals to utilize contextual information and discriminate between environments. The implication is that the hippocampus plays an important role in detecting and encoding unique environments, which is consistent with our hypothesis. The data are inconsistent with the idea that hippocampus inactivation impairs freezing because it produces a large state change.

Discussion

In summary, our data help to resolve the current debate about the role of the hippocampus in memory retrieval. First, we demonstrate that the dorsal hippocampus plays a prolonged role in the retrieval of detailed contextual fear memories. Second, we show that contextual memories tend to lose details with the passage of time; when they do so, they can be retrieved without this structure. Memories that retain details continue to be retrieved by the hippocampus. Therefore, study designs that promote the retention of detailed memories should find that the hippocampus plays a permanent role in their retrieval. In contrast, conditions that result in memory generalization over time will lead to a temporal gradient of hippocampal involvement; this structure will be important shortly after training but not later. Both of these findings are described in the animal literature, although the quality of memory was not examined [3, 8, 13].

The current results can also explain why memory does not become independent of the hippocampus in tasks such as the Morris water maze [35]. Unlike contextual fear conditioning, successful performance in the water maze requires the retrieval of a precise memory about the spatial location of the platform. An inability to remember this specific

information will impair searching during the probe test [36]. Consequently, if hippocampus lesions impair the retrieval of detailed spatial information, then both recent and remote memory will be disrupted in this task. In contrast, the current experiments demonstrate that retrieval of an imprecise contextual memory is sufficient to support freezing. As a result, contextual fear conditioning can be expressed without the hippocampus [5, 33].

The results of our study are consistent with a transformation account of memory consolidation. This account argues that episodic memories lose details and become more schematic as they are permanently stored in regions of the neocortex [29, 30, 37]. In contrast, the standard model of consolidation states that detailed features of episodic memories are retained over time [37, 38]. This distinction has proven difficult to resolve even though the content of remote episodic memory has been studied in some detail in humans. Several studies have observed a decrease in the number of details recalled by subjects with hippocampus damage, whereas others have found no change [17, 38, 39]. However, the number of details recalled may not reflect memory accuracy. As autobiographical memories get older, subjects are more likely to remember and believe false details about these events [40–44]. Given this fact, future studies that examine memory accuracy rather than the number of generated details will be informative. The use of more sensitive measuring devices might reveal that the accurate recall of detailed information activates the hippocampus more than detailed, but inaccurate, information.

A potential interpretation of the current results is that generalized contextual fear memories are similar to semantic memories. Semantic memories, however, are thought to represent the extraction of general features from multiple learning events, and our animals were only trained with a single conditioning trial [20]. Although semantic memories are often formed across many learning events, the studies described above demonstrate that specific episodes can become more schematic and "semantic-like" over time [37, 45]. This is thought to occur as regions of the neocortex extract the general features of the original episodic memory as it is repeatedly retrieved and replayed over time [37, 45]. A similar replay process occurs for hippocampus-dependent memories in rodents and could produce a related effect: the extraction of general contextual features that occur during multiple replay events [21, 46, 47].

Although the current results demonstrate that hippocampus independence is related to memory quality, other important factors have also been identified. For example, studies with rats have shown that the incorporation of new information into existing frameworks or schemas allows it to become independent of the hippocampus within 24 hours [48]. This suggests that the rate of memory consolidation depends on the prior experience of the subjects. Interestingly, we found that mice undergo a shift from precise to general memories at different rates. In a small number of animals, memory for the training context appeared to be generalized as early as 24 hr after training, whereas in others it remained precise even after a month. Distinguishing between discriminators and generalizers allowed us to test hippocampal dependency in each of these groups. On the basis of these results, future studies should be able to identify individual factors that influence the rate of memory consolidation prior to fear conditioning.

A recent paper found that with extended training, mice with hippocampus damage are able to discriminate between contexts at remote time points, although freezing performance is significantly reduced [16]. Similarly, when animals are given extensive experience (e.g., 3 months) in a complex environment, they are able to form and retain spatial memories after hippocampus lesions [49, 50]. These data suggest that structures outside the hippocampus can be recruited to support the retrieval of precise memories under some conditions [5, 33].

Experimental Procedures

Subjects

F1 hybrids for all experiments were generated from breedings between C57BL/6 (Taconic) males and 129SvE (Jackson) females. Mice ranged from 3–6 months of age, were group housed with free access to food and tap water, and were maintained on a 12:12 hr light:dark cycle in the Herbert L. Washington Vivarium in the Department of Psychology at UCLA. All experiments were performed during the light phase of the cycle.

Fear Conditioning

The general apparatus and procedures used in these experiments have been described previously [19]. In all experiments, mice were allowed to explore the training environment (context A) for 2 min before a shock was delivered. Mice received five footshocks (2 s, 0.75 mA) in the experiment depicted in Figure 1 and a single footshock (2 s, 0.5 mA) in the experiments depicted in Figures 2–5. Thirty seconds after the last shock, mice were removed from the training context and returned to their home cages. After training, we conducted a 5 min context test during which we scored freezing behavior as previously described [19]. Testing was conducted in context A, context B (both described previously [19]), or context C. Context C consisted of a small plastic cage (27.5 cm × 17 cm × 12.5 cm) with fresh bedding and was located down the hall from the training environment in a dimly lit room.

RT-PCR

Brains were extracted after behavioral training or testing and flash frozen on dry ice. They were then placed in a brain block (kept at −20°C) and sliced in 2 mm sections, from which the dorsal hippocampus was microdissected. Total RNA was extracted by RNeasy Mini Kit and treated with DNase (QIAGEN, Valencia, CA, USA). Total RNA was reverse transcribed into cDNA via oligo (dT) 20 primers and the Superscipt III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Expression of *Arc* and *c-fos* were determined by real-time PCR. *Arc* and *c-fos* primers were designed by Primer Express 2.0 (Applied Biosystems), and the following primer sequences were used: *Arc*, 5′-TATTCAGGCTGGGTCCTGTC-3′ (forward) and 5′-TGGAGCAGCTTATCCAGAGG-3′ (reverse); *c-fos*, 5′-TCACCC TGCCCCTTCTCA-3′ (forward) and 5′-CACGTTGCTGATGCTCTTGAC-3′ (reverse); and *zif268* 5′-TTGCCGATGGCTTGACATG-3′ (forward) and 5′-TAA GGCTAAGGTGAGCGTGTCC-3′ (reverse). Real-time PCR was performed in an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Cycling parameters were as follows: initial denaturation at 95°C for 3 min followed by 40 cycles (95°C for 30 s and 60°C for 1 min). The data were quantified by the 2^CT method [51], and mRNA expression was analyzed relative to that observed in home-cage controls. The expression of 36B4 and HPRT genes were measured and used as housekeeping controls for all samples.

FISH

Fluorescent-in situ hybridization was performed as previously described [27]. Mice were trained, tested, and then immediately sacrificed with isoflurane. Brains were extracted, flash frozen on dry ice, and stored at −80°C until sectioning. Sections of 20 μM were taken on a cryostat (−20°C) and mounted on slides. Fluorescein-labeled riboprobes were made with commercial transcription kits (MaxiScript; Ambion, Austin, TX) and RNA labeling mixes (Roche Products, Hertforshire, UK). The H1a antisense riboprobe was generated with an H1a cDNA clone (gift from Paul Worley) and was directed to the 4.4 kb 3′-untranslated region (UTR) of the H1a mRNA [27]. Single-label FISH was performed as previously described [27, 34, 52]. Fluorescein-labeled H1a probe was detected with anti-fluorescein HRP (Roche Products) and a cyanine-5 substrate kit (CY5 DirectFISH; PerkinElmer Life Sciences). Nuclei were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA).

Slides were imaged with an Olympus FluoView 1000 Laser Scanning Confocal Microscope (LSCM). Laser line (633 nm) from HeNe was used for

imaging Cy5, and 700 nm femtosecond pulses from Ti:Saphire (Mai Tai, Spectra Physics) were used for two-photon excitation of DAPI. Olympus 60×/1.2 oil-immersion objective lens was used for imaging the CA1 region of the dorsal hippocampus. Laser intensity, pinhole size, PMT dynode voltage, amplifier gain, and offset were kept constant for imaging of all slides. Slices from the CA1 region of the hippocampus were Z sectioned in 0.48 μM optical sections from top to bottom. Twenty sections from the middle of the slice (approximately 9.6 μm) were used for analysis. The software ImageJ (ImageJ 1.42 g, NIH, USA) was used for obtaining the distribution of Homer puncta and setting a threshold that separated Homer signal from background noise. Only cells completely localized in the middle 20 optical sections and with Homer particles above threshold in maximum projection were counted as Homer-positive cells. Total cells were counted with DAPI staining. Only whole cells were counted. An average of 831 cells in the dorsal CA1 were counted for each mouse. The results are shown as the percentage of Homer-positive cells per total counted cells. All image analysis was done blind.

Intra-Hippocampal Infusions

Mice were anesthetized with sodium pentobarbital (90 mg/kg) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp of each animal was incised and retracted, and the skull was adjusted so that bregma and lambda were in the same horizontal plane. Small burr holes were drilled at the appropriate injection sites. Plastic guide cannulae (22 gauge; Plastics One, Roanoke, VA) were inserted bilaterally at the following positions relative to bregma (mm): AP, −2; ML, ± 1.5; DV, −1. These were then affixed with dental cement (Harry J. Bosworth Company, Skokie, IL). Dummy cannulae (28 gauge) were inserted into the guide cannulae after surgery. Mice were allowed to recover for one week before undergoing behavioral testing. Twenty minutes prior to testing, the dummy cannulae were removed and replaced with injection cannulae (28 gauge) that projected an additional 1 mm from the tip of the guide cannulae. CNQX (Sigma-Aldrich, St. Louis, MO) (.83 mg/ml) or saline (0.9%) was infused into the hippocampus (.5 ul/side; 0.1 ul/minute). The injectors were left in place for 2 min after the end of the infusion to allow for diffusion. The mice were then returned to their home cage until testing.

Histology

Histological verification of the cannula locations was performed at the end of behavioral testing. Mice were perfused transcardially with 0.9% saline, followed by 4% PFA. After extraction from the skull, the brains were post-fixed in 4% PFA and then transferred to a 30% sucrose solution until sectioning. Coronal sections (40 μm thick, taken every 120 μm) were cut on a cryostat (–16°C) and mounted on glass microscope slides. After drying, the sections were stained with cresyl violet so that neuronal cell bodies could be identified. Cannula tips were verified by visual inspection of the stained sections reconstructed on the mouse Allen Reference Atlas [53]. We were unable to analyze the brains of four mice from each CNQX group because of damaged sustained during extraction and/or slicing.

Supplemental Information

Supplemental Information include one figure and can be found with this article online at doi:10.1016/j.cub.2010.06.068.

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