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Report

Cortical Representations Are Reinstated by the Hippocampus during Memory Retrieval

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SUMMARY

The hippocampus is assumed to retrieve memory by reinstating patterns of cortical activity that were observed during learning. To test this idea, we monitored the activity of individual cortical neurons while simultaneously inactivating the hippocampus. Neurons that were active during context fear conditioning were tagged with the long-lasting fluorescent protein H2B-GFP and the light-activated proton pump ArchT. These proteins allowed us to identify encoding neurons several days after learning and silence them with laser stimulation. When tagged CA1 cells were silenced, we found that memory retrieval was impaired and representations in the cortex (entorhinal, retrosplenial, perirhinal) and the amygdala could not be reactivated. Importantly, hippocampal inactivation did not alter the total amount of activity in most brain regions. Instead, it selectively prevented neurons that were active during learning from being reactivated during retrieval. These data provide functional evidence that the hippocampus reactivates specific memory representations during retrieval.

INTRODUCTION

Since patient H.M., researchers have known that the hippocampus is essential for memory (Scoville and Milner, 1957). This discovery was confirmed by animal studies showing that dysfunction in this area produces profound amnesia for spatial and contextual information (Kim and Fanselow, 1992; Morris et al., 1986). Despite these facts, it is still unknown why the hippocampus is so fundamental for memory. The dominant idea, based on the work of Marr, is that memory is retrieved when the hippocampus reinstates patterns of cortical activity that were observed during learning (Alvarez and Squire, 1994; Marr, 1971; Treves and Rolls, 1994). This idea is supported by spatial studies in rodents showing that learned sequences are replayed in the hippocampus and cortex after training (Ji and Wilson, 2007). Similar effects have been observed in human patients during free recall of episodic memories (Gelbard-Sagiv et al., 2008). However, it has yet to be determined whether cortical representations formed during learning are reinstated by the hippocampus during retrieval.

Recent studies have used *fos-tTA* reporter mice to tag active neurons in the hippocampus, cortex, and amygdala during context fear learning and show that they are reactivated several days later when memory is retrieved (Liu et al., 2012; Ramirez et al., 2013; Reijmers et al., 2007; Tayler et al., 2013). Similar to recording experiments, these data indicate that specific context representations are reactivated after learning. However, studies to date have not determined whether the hippocampus is responsible for reinstating memory representations in other structures as is widely assumed (Frankland and Bontempi, 2005). To examine this idea, we used *fos-tTA* mice to tag active CA1 neurons with the long-lasting fluorescent protein H2B-GFP and the light-activated proton pump archaerhodopsin (ArchT). These proteins allowed us to identify encoding neurons several days after learning and inactivate them with laser stimulation. When tagged CA1 neurons were silenced, we found that memory retrieval was impaired and representations in the cortex (entorhinal, retrosplenial, and perirhinal) and amygdala (central nucleus) could not be reactivated. These results provide direct evidence that the hippocampus is fundamental for memory because it reinstates patterns of activity that were originally present during learning.

RESULTS

Silencing Encoding Neurons in CA1

To identify and label active neurons, we used *fos-tTA* reporter mice that express the long-lasting fluorescent protein H2B-GFP. We previously found that ≈40% of tagged neurons in the CA1 region of the hippocampus are reactivated when context fear memories are retrieved (Tayler et al., 2013). To selectively silence these cells, we engineered our reporter mice to express Cre recombinase under the control of the *tetO* promoter. In the absence of doxycycline (DOX), c-Fos activity leads to the expression of tTA, H2B-GFP, and Cre in these animals (Figure 1A). Previous studies showed that activity-dependent labeling in *fos-tTA* reporter mice largely recapitulates the expression of endogenous c-Fos in the hippocampus (Liu et al., 2012). To silence CA1 neurons that were active during learning, we infused an adeno-associated virus (AAV) that encodes a double-floxed inverted ArchT gene into the dorsal hippocampus (AAV-FLEX-ArchT) (Figure 1B). Once expressed, ArchT can be activated

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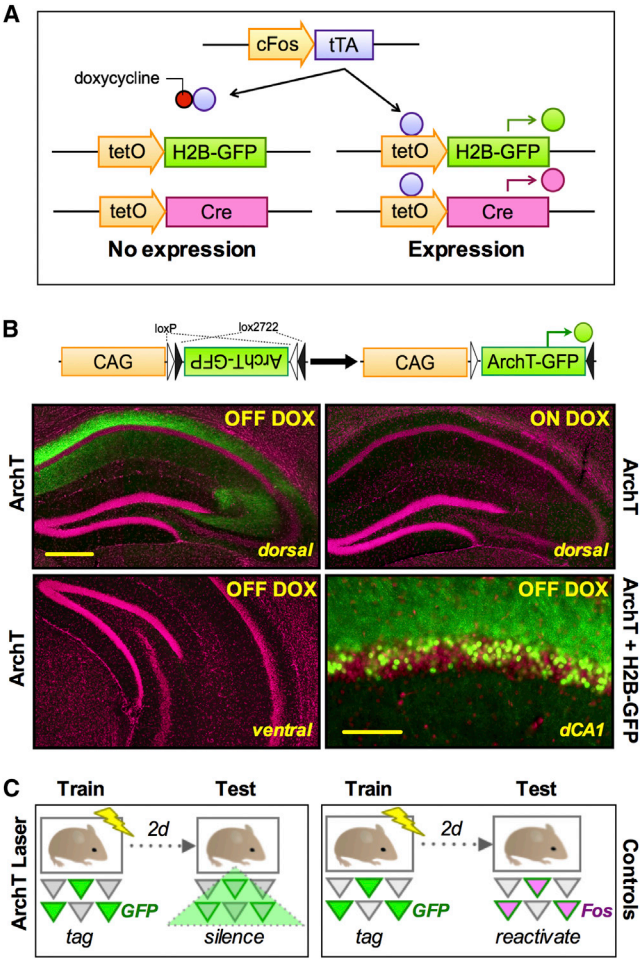

Figure 1. TetTag-Cre System

(A) Activation of the c-*fos* promoter drives the expression of H2B-GFP and Cre in a DOX-regulated manner. (B) *Fos-tTA/tetO-Cre* mice received infusions of AAV-FLEX-ArchT into the dorsal hippocampus and were fear conditioned off DOX. In these animals, Cre recombination in active neurons led to the expression of ArchT in the dorsal hippocampus (top left). Expression was not observed in the ventral hippocampus (bottom left) or in mice trained on DOX (top right). In *fos-tTA/tetO-Cre/tetO-H2B-GFP* mice, fear conditioning led to the expression of ArchT and H2B-GFP in dorsal CA1 (bottom right). H2B-GFP (green) was expressed exclusively within the nucleus (magenta, DAPI), while ArchT (green) was expressed only in axons and dendrites. The scale bar represents 400 μm, or 100 μm on the bottom right panel. (C) Experimental procedure. Active CA1 neurons were tagged with H2B-GFP and ArchT during training (left). During testing, laser stimulation was delivered to silence tagged cells. In control animals (right), tagged cells were not inactivated during testing and should therefore be reactivated during testing. See also Figure S1.

with green light to produce robust silencing of neural activity (Han et al., 2011).

In our mice, Cre is expressed in active neurons and causes the ArchT gene to be inverted and expressed under the control of the constitutively active CAG promoter. Figure 1B (top left) shows the expression of ArchT in the dorsal hippocampus of *fos-tTA/tetO-Cre* mice that underwent context fear conditioning off DOX. Expression was restricted to CA1 dendrites and axons and was not observed in cell bodies. We estimated that ArchT was expressed in ≈1.05 mm^3 of tissue in the dorsal hippocampus (Figure S1 available online). Expression was not observed in the ventral hippocampus (bottom left). We also did not observe ArchT expression in mice that were fear conditioned on DOX (top right). Given that ArchT was selectively expressed in CA1 dendrites and axons, we used H2B-GFP to determine which cells were active during learning. Figure 1B (bottom right) shows the expression of H2B-GFP in cell nuclei and ArchT in fibers of *fos-tTA/tetO-Cre/tetO-H2B-GFP* mice that were trained off DOX. Consistent with previous work, expression was observed almost exclusively in excitatory cells (Figure S2) (Liu et al., 2012; Reijmers et al., 2007; Tayler et al., 2013). Given their distinct localization, we could not determine the degree to which H2B-GFP and ArchT overlapped in CA1 neurons. However, the inactivation data presented in the next section provides direct evidence that ArchT is selectively expressed in H2B-GFP-positive cells. We also examined the expression of H2B-GFP in control mice (n = 3) that had DOX removed but were not trained. Consistent with previous work, these animals had significantly fewer H2B-GFP-positive neurons in CA1 (11%) than mice in the ArchT (28%) and No ArchT Laser (23%) groups (main effect of group, $F(2, 11) = 7.2$, $p < 0.05$; planned comparisons, Fisher's PLSD, p values < 0.05) (Tayler et al., 2013).

Reactivation of CA1 Neurons Is Required for Memory Retrieval

To inactivate ArchT-positive neurons during memory retrieval, we infused AAV-FLEX-ArchT into dorsal CA1 (dCA1) and implanted bilateral optic fibers in the same region. During context fear conditioning, DOX was removed to induce the selective expression of ArchT and H2B-GFP in active neurons (Figure 1C, left). After training, we administered high-concentration DOX to suppress additional expression of H2B-GFP and ArchT. A memory test was conducted 2 days later and green laser light (532 nm, 10mW) was delivered to both hippocampi to stimulate ArchT. We compared performance in the ArchT-Laser group to three control conditions. One group received laser stimulation but did not express ArchT (No ArchT-Laser). Another expressed ArchT but did not receive laser stimulation (ArchT-No Laser). The third control group did not express ArchT and did not receive laser stimulation (No ArchT-No Laser). In each of these groups, H2B-GFP-positive neurons should be intact during testing and express c-Fos when memory is retrieved (Figure 1C, right).

When tagged CA1 neurons were silenced, we found that memory retrieval was significantly impaired in the ArchT-Laser group relative to controls (Figure 2A; main effect of group $F(3,19) = 5.15$, $p < 0.05$; planned comparisons, Fisher's PLSD, p values < 0.05). Freezing levels did not differ between any of the control groups (planned comparisons, Fisher's PLSD, p values > 0.05). These results provide functional evidence that memory retrieval requires the reactivation of previously engaged CA1 neurons. To determine the degree to which reactivation was disrupted, we quantified the expression of H2B-GFP and c-Fos 90 min after the memory test. As in our previous work, we compared the percentage of double-labeled neurons observed in dCA1 to that expected by chance alone (percent H2B-GFP × percent c-Fos) (Tayler et al., 2013). We found a significant

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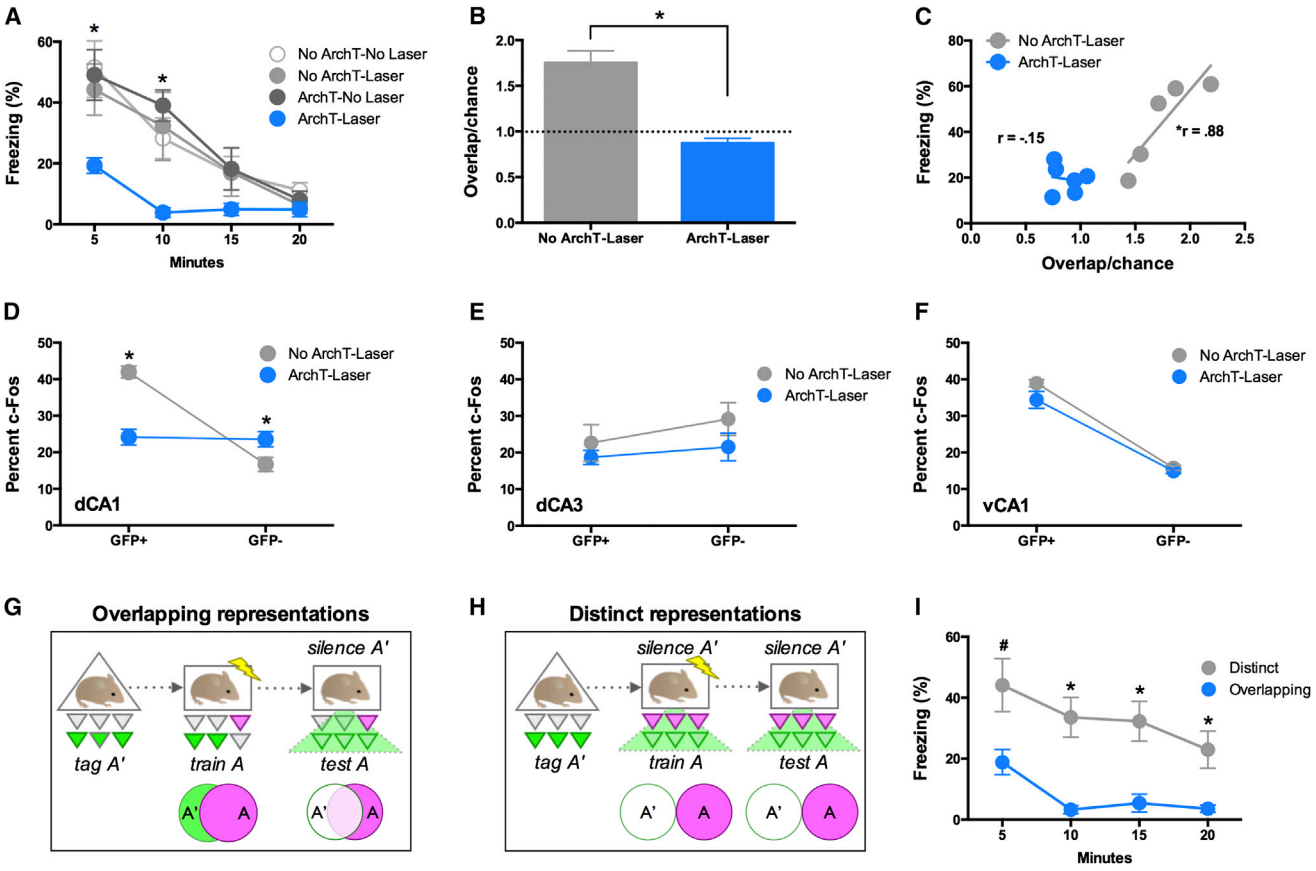

Figure 2. CA1 Silencing during Memory Retrieval

(A) During the memory test, ArchT-stimulated mice (n = 6) froze significantly less than the control groups (ArchT-No Laser n = 6; No ArchT-Laser n = 5; No ArchT-No Laser n = 6). The control groups did not differ from one another. (B) The reactivation index (overlap/chance) was significantly reduced in the CA1 region of ArchT-Laser mice compared to the No ArchT-Laser group. (C) The amount of freezing was strongly correlated with the reactivation index (r = 0.88) in CA1 in the No ArchT-Laser group. This correlation was eliminated in ArchT-Laser mice (r = −0.15). (D) c-Fos activity was reduced in H2B-GFP-tagged neurons in the Arch-Laser group and increased in untagged neurons. (E and F) There was no effect on c-Fos expression in tagged or untagged neurons in (E) dCA3 or (F) vCA1. (G) Experimental procedure to silence overlapping context representations. Active neurons in dCA1 were tagged with ArchT during exposure to context A' (off DOX) (left). Two days later, animals were fear conditioned (on DOX) in context A, an environment whose representation overlaps with context A' (middle). Two days after training, mice were tested in context A, while tagged neurons were silenced (right). (H) Experimental procedure to silence distinct context representations. Active neurons in dCA1 were tagged with ArchT during exposure to context A' (off DOX) (left). Two days later, animals were fear conditioned (on DOX) in context A, while tagged neurons were silenced (middle). This manipulation should result in a distinct representation for context A that does not overlap with context A'. Two days after training, mice were tested in context A while tagged neurons were once again silenced (right). (I) When ArchT-labeled neurons were silenced, memory retrieval was selectively impaired in mice that had overlapping memory representations (n = 5) and had no effect in animals with distinct representations of context A and A' (n = 9). Values are represented as means ± SEM, *$p \leq 0.05$, #$p = 0.06$. See also Figure S2.

reduction in double labeling (normalized to chance) in the ArchT-Laser group compared to that observed in No ArchT-Laser mice (Figure 2B; main effect of group $F(1,9) = 43.83$, $p < 0.05$). Reactivation of dCA1 neurons exceeded chance levels in No ArchT-Laser mice (paired t test, $p < 0.05$) but did not in the ArchT-Laser group (paired t test, $p > 0.05$). These results indicate that ArchT stimulation prevented the reactivation of dCA1 neurons during memory retrieval. Laser stimulation in the No-ArchT group did not appear to disrupt reactivation as the degree of overlap was nearly identical to that seen in nonstimulated control animals (Figure S2).

The amount of freezing observed during testing was strongly correlated with the degree of reactivation in the dCA1 region of No ArchT-Laser mice (Figure 2C; $r = 0.88$, $p < 0.05$). This correlation was completely eliminated in the ArchT-Laser group ($r = -0.15$, $p > 05$.). Expression levels of H2B-GFP (no effect of group, $F(1,9) = 2.18$, $p > 0.05$) and c-Fos (no effect of group, $F(1,9) = 1.21$, $p > 0.05$) in dCA1 were similar in both groups. However, as expected, c-Fos expression was selectively reduced in tagged neurons relative to untagged cells in ArchT-Laser animals (Figure 2D; group × cell type interaction, $F(1,9) = 127.4$, $p < 0.05$). This result demonstrates that ArchT was selectively expressed in H2B-GFP-tagged neurons. Interestingly, c-Fos expression in the ArchT-Laser group was decreased in tagged cells (Fisher's PLSD, $p < 0.05$) and increased in untagged neurons (Fisher's PLSD, $p < 0.05$) compared to No ArchT-Laser

controls. This finding is consistent with the idea that active CA1 neurons can suppress the activity of neighboring cells (Hirase et al., 2001).

To determine the anatomical specificity of our manipulation, we also examined reactivation in dorsal CA3 (dCA3) and ventral CA1 (vCA1). We found that ArchT stimulation in dCA1 had no effect on c-Fos expression in H2B-GFP-positive or -negative neurons in dCA3 (Figure 2E; no effect of group $F(1, 9) = 1.51$, $p > 0.05$; no group × cell type interaction $F < 1$) or vCA1 (Figure 2F; no effect of group $F(1, 9) = 4.07$, $p > 0.05$; no group × cell type interaction $F(1, 9) = 1.5$, $p > 0.05$). These results indicate that ArchT-mediated silencing was restricted to neurons in the dorsal segment of CA1.

Our data suggest that a specific subset of dCA1 neurons mediate memory retrieval. However, an alternative possibility is that retrieval is impaired anytime hippocampal activity is disrupted (independent of which neurons are affected). To test this idea, we silenced dCA1 neurons that were either part of the trained context representation (overlapping) or orthogonal to it (distinct). If memory retrieval requires the reactivation of specific CA1 cells, then performance should only be impaired when an overlapping representation is silenced. Inactivation of a distinct context representation should have no effect. To test this idea, we trained mice in similar environments (A and A') that activate many of the same cells in dCA1 (Figure S3). Mice were first exposed to A' (off DOX), where active neurons were tagged with ArchT (Figure 2G). Two days later, animals were fear conditioned (on DOX) in context A. Neurons were not tagged during this session. Memory was then tested in context A while neurons from A' were silenced via ArchT stimulation. Given the significant overlap between these representations, we predicted that memory retrieval would be impaired. In a second procedure, we prevented overlap between context representations by inactivating A' neurons while mice were fear conditioned in A. Once again, active neurons were first tagged with ArchT in context A'. To prevent overlap, these cells were silenced during subsequent training in A (Figure 2H). Silencing A' cells during testing in this group should not alter the representation of context A and, as a result, memory should be intact.

Figure 2I shows the freezing levels during the context A memory test when ArchT-labeled neurons were silenced. As predicted, inactivating these cells impaired retrieval in mice that had overlapping context representations and had no effect on animals with distinct representations of A and A' (Figure 2I; main effect of group $F(1,12) = 9.42$, $p < 0.05$). The amount of freezing in the Distinct memory group was comparable to that observed in the control groups shown in Figure 2A. An analysis of overlap in H2B-GFP-positive mice from each group suggests that ArchT stimulation silenced a similar number of tagged neurons in both conditions (Figure S3). Together, these data demonstrate that hippocampal inactivation impairs retrieval only when the same neurons that were active during encoding are silenced during retrieval.

CA1 Silencing Disrupts Reactivation of Cortical Representations

We next examined the assumption that the hippocampus reactivates cortical representations during memory retrieval. To do this, we quantified the expression of H2B-GFP and c-Fos in regions that receive monosynaptic projections from dCA1: subiculum (SUB), lateral entorhinal cortex (LEC), perirhinal cortex (PER), and retrosplenial cortex (RSC) (Cenquizca and Swanson, 2007). We observed a large number of ArchT-labeled fibers in each of these regions (Figures 3A, 3D, and 3G). We used these fibers to trace the axons of tagged CA1 cells and examine activity in directly connected cortical regions. When dCA1 neurons were silenced, we found that the total amount of c-Fos expression in the SUB and RSC were unaltered (Figures 3B and 3E; no effect of group, SUB $F < 1$; RSC $F < 1$). However, there was a significant reduction in c-Fos expression in tagged neurons (Fisher's PLSD, $p < 0.05$) compared to untagged cells (Fisher's PLSD, $p > 0.05$) in the ArchT-Laser group (Figures 3C and 3F; significant group × cell type interaction, SUB $F(1, 9) = 44.73$, $p < 0.05$; significant group × cell type interaction RSC $F(1, 9) = 4.63$, $p = 0.05$). In LEC/PER, there was a slight reduction in the total amount of c-Fos expression (Figure 3H; main effect of group, $F(1,9) = 6.54$, $p < 0.05$) that was observed in both tagged (Fisher's PLSD, $p < 0.05$) and untagged (Fisher's PLSD, $p < 0.05$) cells (Figure 3I). However, similar to the results in SUB and RSC, the size of this reduction was significantly larger in tagged neurons compared to untagged cells (significant group × cell type interaction, $F(1, 9) = 16.83$, $p < 0.05$). Together, these data demonstrate that the hippocampus is fundamental for memory because it can reinstate patterns of cortical activity that were originally observed during learning.

CA1 Silencing Disrupts Reactivation in the Central but Not Basolateral Amygdala

In our final analyses, we examined activity in two regions of the amygdala that are known to be essential for context fear; the central nucleus (CeA) and the basolateral nucleus (BLA) (Figure 4A) (Maren, 2001). The CeA receives dense inputs from SUB, LEC, and PER and light inputs from vCA1 (Pitkänen et al., 2000). We found that ArchT stimulation in dCA1 did not affect the total amount of c-Fos expression in this region (Figure 4B; no effect of group, $F(1,9) = 3.05$, $p > 0.05$). However, c-Fos expression in tagged neurons was significantly reduced (Fisher's PLSD, $p < 0.05$) compared to that observed in untagged cells (Fisher's PLSD, $p > 0.05$) (significant group × cell type interaction, $F(1, 9) = 17.86$, $p < 0.05$) (Figure 4C). These data suggest that dCA1 modulates activity in the CeA via its projections to the SUB, LEC, and/or PER.

In contrast to the CeA, the BLA exhibited no change in overall levels of c-Fos (Figure 3E, top; no effect of group, $F(1,8) = 0.34$, $p > 0.05$) or c-Fos expression in tagged (Figure 3E, bottom; Fisher's PLSD, $p > 0.05$) and untagged neurons (Fisher's PLSD, $p > 0.05$) (no group × cell type interaction, $F(1, 9) = 1.4$, $p > 0.05$; no effect of group, $F < 1$) when dCA1 was silenced. This result is consistent with the fact that the BLA receives dense projections from vCA1, which exhibited normal reactivation in our experiments (Pitkänen et al., 2000). Together, these data suggest that dorsal and ventral CA1 can independently modulate the retrieval of context fear memories in the amygdala, a finding that is not predicted by traditional models (Wiltgen and Fanselow, 2003).

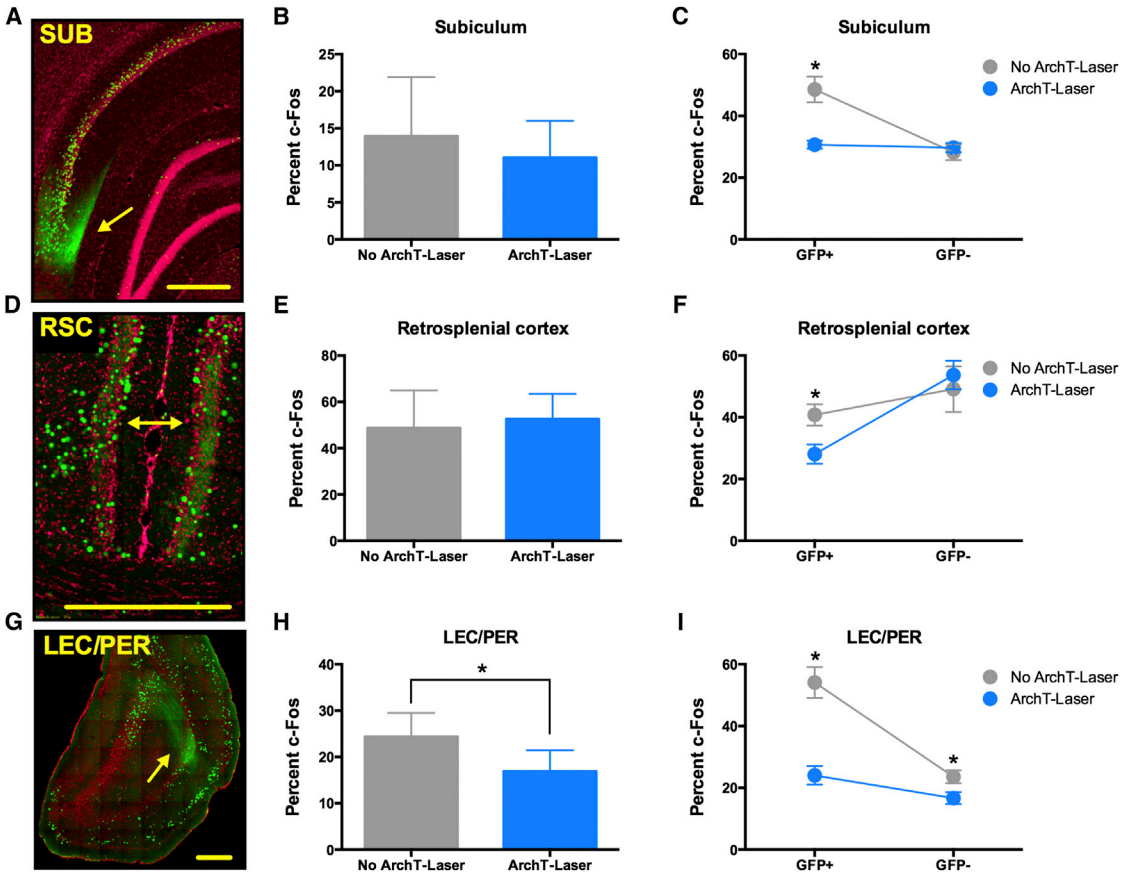

Figure 3. Effects of CA1 Silencing on Cortical Activity

Mice from the ArchT-Laser (n = 6) and No ArchT-Laser (n = 5) groups were used for these analyses. (A) ArchT fibers (green) from tagged dCA1 neurons terminating in SUB. H2B-GFP-expressing nucleus in green; DAPI-stained nucleus in magenta. (B) When dCA1 was silenced, there was no effect on total c-Fos expression in SUB. (C) c-Fos activity was selectively reduced in tagged neurons in SUB. (D) ArchT fibers (green) from tagged dCA1 neurons terminating in RSC. (E) When dCA1 was silenced, there was no effect on total c-Fos expression in RSC. (F) c-Fos activity was selectively reduced in tagged neurons in RSC. (G) ArchT fibers (green) from tagged dCA1 neurons terminating at the border of LEC/PER. (H) When dCA1 was silenced, there was a slight reduction in total c-Fos expression in LEC/PER. (I) There was a reduction in c-Fos activity in both tagged and untagged neurons LEC/PER. However, the magnitude of this reduction was significantly larger in H2B-GFP-tagged neurons. Values are represented as means ± SEM, *$p \leq 0.05$. The scale bar represents 400 μm. See also Figure S3.

DISCUSSION

The hippocampus is essential for retrieving spatial and contextual memories (Kim and Fanselow, 1992; Moser and Moser, 1998). It is thought to mediate this process by activating unique representations of the environment that were formed during learning. To test this idea, we combined *fos-tTA/tetO-Cre* mice with hippocampal infusions of AAV to induce ArchT expression in an activity-dependent manner. This allowed us to selectively silence CA1 neurons that were sufficiently active to drive the c-*fos* promoter. When these cells were inactivated with laser stimulation, mice were unable to retrieve a previously formed context fear memory. Silencing CA1 neurons that were not active during learning had no effect on retrieval. These results are consistent with the idea that specific ensembles of CA1 neurons are used to encode context memories (Guzowski et al., 1999).

The expression of immediate-early genes (IEGs) requires high-frequency activity that is sufficient to activate NMDARs (Steward and Worley, 2001; Tayler et al., 2011). This type of activity is observed in the hippocampus when animals enter a place field (O'Keefe, 1978). Consistent with this idea, the number of neurons expressing IEGs after spatial exploration is similar to the number of place cells that are found in recording studies (Guzowski et al., 1999; Leutgeb et al., 2004). In addition, manipulations that alter the activity of place cells produce similar effects on IEG-labeled (e.g., *Arc*) neurons (Vazdarjanova and Guzowski, 2004). Given that gene expression in *fos-tTA* mice largely recapitulates endogenous c-Fos expression (Liu et al., 2012), it is likely that place cell activity played a major role in the induction of H2B-GFP, Cre, and ArchT in our experiments.

The current study also examined the idea that the hippocampus retrieves memory by reinstating patterns of cortical activity that were observed during learning. This assumption has been central to theories of hippocampal function for decades (Frankland and Bontempi, 2005). Previous work showed that hippocampal and cortical neurons are reactivated after learning during

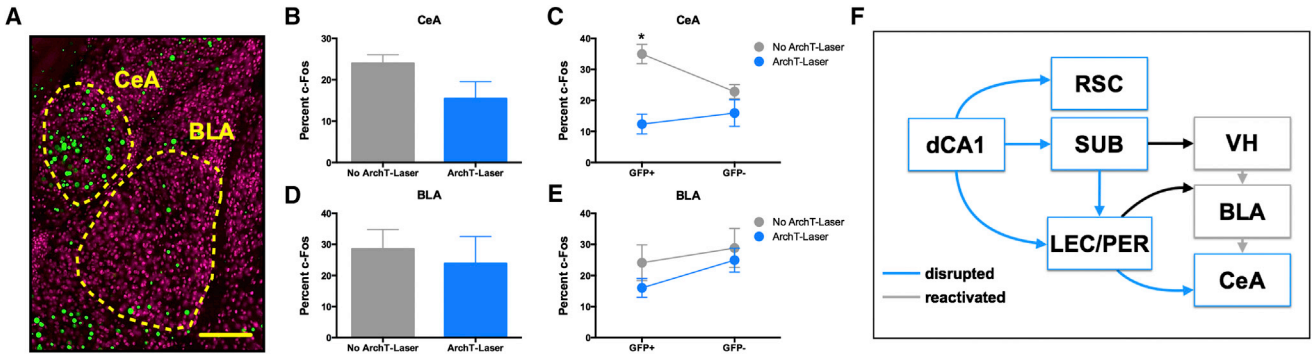

Figure 4. Effects of CA1 Silencing on Amygdala Activity

Mice from the ArchT-Laser (n = 6) and No ArchT-Laser (n = 5) groups were used for these analyses. (A) H2B-GFP expression in CeA and BLA. H2B-GFP-expressing nucleus in green; DAPI-stained nucleus in magenta. The scale bar represents 200 μm. (B) When dCA1 was silenced there was no effect on total c-Fos expression in CeA. (C) c-Fos activity was selectively reduced in tagged neurons in CeA. (D) When dCA1 was silenced there was no effect on total c-Fos expression in BLA. (E) There was no effect on c-Fos expression in tagged or untagged neurons in BLA. (F) Selective silencing of tagged dCA1 neurons disrupted reactivation in RSC, SUB, and LEC/PER. This manipulation also resulted in reduced reactivation in CeA, while activity in the BLA and vCA1 was unaltered. Values are represented as means ± SEM, *$p \leq 0.05$. See also Figure S4.

memory retrieval and sleep (Ji and Wilson, 2007; Tayler et al., 2013). However, these studies did not determine whether the hippocampus is required to induce reactivation in the cortex as is assumed by current models. To test this idea, we traced ArchT-labeled fibers from tagged dCA1 neurons to their targets in SUB, LEC, PER, and RSC. When CA1 neurons were silenced during retrieval, reactivation was disrupted in these regions (Figure 4F). Importantly, the disruption was selective; activity was normal in cortical neurons that were not engaged during learning (i.e., H2B-GFP-negative cells). Therefore, when dCA1 is prevented from retrieving a specific context memory, representations in connected cortical regions also cannot be reactivated. This finding implies that extrahippocampal inputs to these cortical areas cannot fully activate representations that were established during learning.

The fact that reactivation was reduced in the cortex while overall activity was maintained suggests that new cortical neurons came online during the retrieval test. We observed a similar effect in CA1 (Figures 2 and S3). These data suggest that memory retrieval depends on the reactivation of specific cells in the hippocampus and cortex. Our previous work (showing reactivation in the training context but not a distinct environment) is consistent with this finding (Tayler et al., 2013). Increased activity in new cortical cells may have been difficult to detect in our experiments because the number of H2B-GFP-negative neurons is quite large compared to that observed in CA1 (90%–95% in cortex versus 75%–80% in CA1) (Tayler et al., 2013). As a result, a large number of H2B-GFP-negative cells in the cortex would need to express c-Fos to significantly increase activity in this population.

In the current experiments, reactivation was only analyzed in cortical regions that contained ArchT-labeled fibers. These areas were quantified because they received direct projections from the tagged CA1 neurons that were silenced during retrieval. However, models of hippocampal function predict widespread disruption in the cortex when the hippocampus is compromised (Frankland and Bontempi, 2005). This assumption was difficult to assess because indirectly connected cortical regions did not contain ArchT-labeled fibers. Given that only a portion of CA1 was silenced (Figure S1), nonlabeled regions probably contain areas that lost input from the hippocampus as well as areas that retained it. Therefore, in the absence of ArchT-labeled fibers, it is difficult to know where reactivation should be examined. Despite this fact, we did observe activity changes in amygdala nuclei that are indirectly connected to dCA1. We believe that this was the case because the CeA and BLA are relatively small and their role in context fear conditioning has been thoroughly described (Goosens and Maren, 2001).

Current models of fear conditioning assume that contextual information is relayed from the ventral hippocampus to the BLA, where it can be associated with aversive events (Maren and Fanselow, 1995). The CeA is typically viewed as an output structure that receives input from the BLA and induces fear responding via its projections to the midbrain (LeDoux, 2000) (but see Balleine and Killcross, 2006). However, the CeA also receives dense projections from the SUB, LEC, and PER that could influence fear responding (Pitkänen et al., 2000). Consistent with this idea, we found that silencing dCA1 neurons disrupted reactivation in each these cortical regions and in the CeA (Figure 4F). Activity in vCA1 and the BLA were not affected. In contrast to traditional models, these data suggest that the dorsal hippocampus can modulate fear responding independent of the ventral hippocampus.

To summarize, three main discoveries were made in the current study. First, we found that a subset of dCA1 neurons (those that were engaged during learning) must be reactivated for context fear memories to be retrieved. This result provides functional evidence that specific CA1 ensembles are used to encode context memories. Second, we showed that silencing previously active CA1 neurons prevents reactivation in cortical regions that are known to be important for context memory. This result provides direct evidence that the hippocampus is fundamental for memory because it can reinstate patterns of cortical activity that were observed during learning. Third, we found that silencing dCA1 neurons did not affect activity in the ventral hippocampus or BLA but, instead, disrupted reactivation in CeA.

This result suggests that the dorsal hippocampus can modulate fear responding independent of the ventral hippocampus. Together, these data illustrate the utility of new genetic tools that can be used to answer fundamental questions about the hippocampus and memory, some of which have remained intractable for decades.

EXPERIMENTAL PROCEDURES

Subjects

Triple transgenic *fos-tTA/tetO-H2B-GFP/tetO-Cre* (TetTag-Cre) mice were generated by crossing heterozygous double transgenic mice expressing H2B-GFP under control of the tetO promoter (*tetO-H2B-GFP*) and a tetracycline-transactivator (tTA) protein under control of the c-*fos* promoter (*fos-tTA*) (Tayler et al., 2013) with heterozygous transgenic mice expressing Cre recombinase under control of the *tetO* promoter (*tetO-Cre*, JAX 006234). TetTag-Cre animals were maintained in a C57BL/6J background. B6/129 F1 hybrids were generated by breeding TetTag-Cre animals with 129S6 mice (Taconic). All of the mice used in the current experiments were F1 hybrids. All experiments were approved by the UC Davis, Institutional Animal Care and Use Committee (IACUC).

Surgery

At 8–12 weeks of age, mice received stereotaxic infusions of AAV and optic fiber implantation. Briefly, AAV2/5-CAG-FLEX-ArchT-GFP was microinjected bilaterally into the CA1 region of the dorsal hippocampus (anterioposterior [AP] −2.0 mm from bregma, mediolateral [ML] ± 1.5 mm, dorsoventral [DV] 1.5 mm). Optic fibers were constructed as previously described (Sparta et al., 2012) and implanted into the same stereotaxic coordinates.

Behavioral Experiments

TetTag-Cre mice were born and raised on low-concentration DOX chow (40 mg/kg, Harlan Laboratories). To label active neurons with H2B-GFP and ArchT, we removed DOX 3 days prior to fear conditioning in context A or exposure to context A'. After the conditioning/exposure session, mice were immediately given high-concentration DOX chow (1 g/kg, Harlan Laboratories) to suppress further H2B-GFP and Cre expression. Memory was assessed 2 days after conditioning by returning the mice to the training context for 30 min and measuring the freezing response. The automated Video Freeze System (Med Associates) was used to quantify freezing as previously described (Anagnostaras et al., 2010). Animals in the Laser groups received stimulation (532 nm, 10 mW) that was directed at dorsal CA1.

See Supplemental Information for detailed procedures.

Statistics

Group differences were analyzed with one-way ANOVAs or repeated-measure factorial ANOVAs followed by planned comparisons (Fisher's PLSD). The percentage of double-labeled neurons ([H2B-GFP and c-Fos]/[DAPI]) was compared to that expected by chance ([H2B-GFP/DAPI] × [c-Fos/DAPI]) using paired t tests. Effects with p values ≤ 0.05 are reported as significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2014.09.037.

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