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Memory retrieval along the proximodistal axis of CA1

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Abstract

The proximal and distal segments of CA1 are thought to perform distinct computations. Neurons in proximal CA1 are reciprocally connected with the medial entorhinal cortex (MEC) and exhibit precise spatial firing. In contrast, cells in distal CA1 communicate with the lateral entorhinal cortex (LEC), exhibit more diffuse spatial firing and are affected by the presence of objects in the environment. To determine if these segments make unique contributions to memory retrieval, we examined cellular activity along the proximodistal axis of CA1 using transgenic reporter mice. Neurons tagged during context learning in proximal CA1 were more likely to be reactivated during testing than those in distal CA1. This was true following context fear conditioning and after exposure to a novel environment. Reactivation was also higher in brain regions connected to proximal CA1 (MEC, distal CA3) than those connected to the distal segment (LEC, proximal CA3). To examine contributions to memory retrieval, we performed neurotoxic lesions of proximal or distal CA1 after training. Lesions of the proximal segment significantly impaired memory retrieval while damage to distal CA1 had no effect. These data suggest that context memories are retrieved by a hippocampal microcircuit that involves the proximal but not distal segment of CA1.

Introduction

The CA1 region of the dorsal hippocampus is important for retrieving spatial and contextual memories (Moser and Moser, 1998; Teixeira et al., 2006; Goshen et al., 2011; Tanaka et al., 2014). This region can be subdivided into several segments that are thought to have distinct functions. For example, the proximal portion of CA1 (adjacent to CA2) is preferentially connected with circuits that determine the animal's position in space like the medial entorhinal cortex and the retrosplenial cortex (Witter et al., 2000; Naber et al., 2001; Knierim et al., 2013). Accordingly, place cells in this region are extremely stable and precise (Henriksen et al., 2010). Distal CA1, in contrast, is connected with brain areas that are important for processing items and objects in the environment like the lateral entorhinal cortex and perirhinal cortex (Witter et al., 2000; Naber et al., 2001; Knierim et al., 2013). Neurons in this segment (adjacent to the subiculum) exhibit less precise spatial firing and are affected by the presence of non-spatial cues (Henriksen et al., 2010; Burke et al., 2011; Ito

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and Schuman, 2012; Igarashi et al., 2014). Based on these findings, it has been suggested that proximal CA1 is part of a circuit that encodes the animal's position in space while distal CA1 contributes to the encoding of object and item location (Knierim et al., 2013). The current experiments examined this idea using activity-dependent reporter mice and selective neurotoxic lesions.

In rodents, damage or inactivation of dorsal CA1 leads to profound retrograde amnesia for context fear (Kim and Fanselow, 1992; Maren et al., 1997; Anagnostaras et al., 1999; Goshen et al., 2011; Tanaka et al., 2014). However, it is currently unknown if this deficit is due to the loss of function in proximal CA1, distal CA1 or both. Given that few non-spatial cues are present in the conditioning environment, it is possible that context fear is driven mainly by a memory of the spatial location where shock occurred. If this is the case, then neurons in proximal CA1 should be essential for memory retrieval. Alternatively, the few non-spatial cues that are present in the context may be sufficient to drive context fear. If this is the case, then memory retrieval should depend critically on the distal segment of CA1. To examine these ideas, we used transgenic reporter mice to examine the reactivation of neural ensembles along the proximodistal axis of CA1 during memory retrieval. We found that neurons in proximal CA1 were more likely to be reactivated during the retrieval of context memories than those in distal CA1. This dissociation was also observed in areas connected with the proximal segment of CA1 (e.g. MEC, distal CA3) compared to those connected with the distal segment (e.g. LEC, proximal CA3). We also examined the functional contribution of these circuits to memory retrieval by performing neurotoxic lesions of proximal or distal CA1 following context fear conditioning. Retrograde amnesia was only observed when proximal CA1 was damaged; distal lesions had no effect on fear memory retrieval. Together, these findings suggest that context memories are retrieved by a hippocampal microcircuit that involves the proximal segment of CA1.

Results

Experiment 1: Reactivation along the proximodistal axis of CA1 following context fear conditioning

In our previous work, we found that 20-40% of CA1 neurons were reactivated when context fear memories were retrieved (Tayler and Wiltgen, 2010; Tayler et al., 2013; Tanaka et al., 2014). However, these analyses were restricted to intermediate CA1 and did not include the proximal or distal segments of this structure. Therefore, in our first experiment, we determined if reactivation differences exist along the proximodistal axis of CA1. To do this, we tagged active neurons during context fear conditioning using H2B-GFP reporter mice as described previously (Tayler and Wiltgen, 2010; Tayler et al., 2013; Tanaka et al., 2014). Immediately after training, these animals were fed high concentration DOX to turn the system off and prevent additional tagging. Two days later, the mice were tested in the same environment and c-Fos expression was used to index cellular activity (Fig. 1*A*). Reactivation was determined by quantifying the percentage of H2B-GFP positive neurons (i.e. tagged) that co-expressed c-Fos. This number was then compared to the percentage of H2B-GFP negative cells (i.e. untagged) that expressed c-Fos during testing. Analyses were conducted in proximal, intermediate and distal portions of CA1 (Fig. 1*B*). A two-way repeated

measures ANOVA revealed a significant difference between c-Fos expression in H2B-GFP tagged and untagged cells ($F(1,5) = 35.24$, $p \leq .05$) that varied along the proximodistal axis of CA1 (Fos in $GFP^{+/-}$ × region interaction, $F(2,10) = 27.86$, $p \leq .05$). Post-hoc tests (Fisher's PLSD) revealed that H2B-GFP positive cells were more likely to express c-Fos than non-tagged cells in proximal and intermediate CA1 (p values ≤ .05) but not in distal CA1 ($p > .05$) (Fig. 1*C*). To control for potential differences in cell activity, we also compared the percentage of reactivated neurons (percent H2B-GFP and c-Fos) to that expected by chance (percent H2B-GFP × percent c-Fos) for each segment. Using this measure, we found greater than chance reactivation in proximal ($t(5) = 7.21$, $p \leq .05$) and intermediate CA1 ($t(5) = 11.34$, $p \leq .05$) but not in distal CA1 ($t(5) = 0.59$, $p > .05$) (Fig. 1*D*). Together, these data indicate that proximal and intermediate CA1 neurons engaged during context fear learning are more likely to be reactivated during memory retrieval than those in distal CA1.

Experiment 2: Changes in memory stability along the proximodistal axis of CA1

In experiment 1, neurons were tagged with H2B-GFP during training and then labeled with c-Fos during testing. It is possible that behavioral/procedural differences between these sessions (e.g. mice explored and received shock during training but were motionless and did not receive shock during testing) contributed to the reduced reactivation observed in distal CA1. To examine this possibility, we tagged active neurons during two identical testing periods during which behavior was equated. This design also allowed us to determine how stable context representations are across multiple tests. Mice were first conditioned across 3 days to produce robust context fear that would not extinguish across testing sessions. Two days after the last training session, animals were tested off DOX to label active cells. After this session, animals were placed on high concentration DOX to prevent additional tagging. Three days later, the mice were tested again and c-Fos was used to index cellular activity (Fig. 2*A*). The amount of freezing observed during the two testing sessions was equivalent ($t(4) = 1.25$, $p > .05$) (mean test 1 = 88%, mean test 2 = 78%). Under these conditions, increased c-Fos expression in H2B-GFP tagged vs untagged cells was once again greater in proximal than distal CA1 (Fos in $GFP^{+/-}$ × region interaction, $F(1,4) = 17.67$, $p \leq .05$ (Fig. 2*B*). Reactivation in proximal CA1 also exceeded that expected by chance ($t(4) = 5.26$, $p \leq .05$) while reactivation in distal CA1 did not ($t(4) = 2.07$, $p > .05$) (Fig. 2*C*). These data suggest that contextual representations are more stable in proximal than distal CA1.

The total number of reactivated H2B-GFP neurons in the current experiment was less than that observed in experiment 1. This was unexpected as the procedures and behavior during the testing sessions were identical. However, subsequent analyses revealed that the use of extended training sessions led to a reduction in c-Fos expression ($t(9) = 9.78$, $p \leq .05$) that in turn decreased the total number of reactivated neurons. This finding is not without precedent as several studies have found that extended training or testing procedures reduce the expression of IEGs in the hippocampus (Milanovic et al., 1998; Guzowski et al., 2006). Given this fact, we cannot directly compare the percentage of c-Fos labeled H2B-GFP neurons in experiments 1 and 2 to determine if similar testing sessions increases the amount of reactivation in CA1.

Experiment 3: Reactivation in CA1 following exposure to a novel environment

To determine if proximodistal differences in reactivation are also observed following spatial exploration, we exposed mice to the conditioning chambers in the absence of shock. During the first session, DOX was removed and mice were allowed to explore the environment for 5 minutes. Animals were then placed on high concentration DOX to prevent additional tagging. Two days later, the mice were allowed to explore the same environment again and c-Fos expression was used to index cellular activity (Fig. 2*D*). As in our fear conditioning experiments, we found that tagged neurons were more likely to express c-Fos than non-tagged cells in proximal CA1 compared to distal CA1 (Fos in $GFP^{+/-}$ × region interaction, $F(1,3) = 21.78$, $p \leq 0.05$) (Fig. 2*E*). We also found greater than chance reactivation in the proximal ($t(3) = 2.32$, $p \leq .05$) but not distal segment of CA1 ($t(3) = 0.32$, $p > .05$) (Fig. 1*F*). These data indicate that differences in reactivation can be observed in proximal and distal CA1 when mice are re-exposed to a previously experienced neutral context.

The percentage of reactivated neurons in this experiment was less than that observed in experiment 1 even though the exposure sessions were identical. An analysis of c-Fos expression in proximal and distal CA1 revealed that it was reduced compared to that observed in experiment 1 ($t(8) = 7.07$, $p \leq .05$). This reduction cannot be attributed to differences in context exposure as the number of testing sessions were the same in experiments 1 and 3. Instead, the lack of fear in the current experiment likely reduced the total amount of c-Fos expression during testing as has been reported previously (Strekalova et al., 2003; Frankland et al., 2004).

Experiment 4: Reactivation in two distinct hippocampal microcircuits

A major difference between proximal and distal CA1 is that the former is reciprocally connected with MEC while the latter communicates with LEC (Witter et al., 2000). Interestingly, these CA1 segments also have unique connections within the hippocampus. Proximal CA1 receives direct input from the distal portion of CA3, which in turn receives projections exclusively from the enclosed blade of DG (e.g. suprapyramidal). In contrast, distal CA1 receives input from the proximal portion of CA3, which is preferentially innervated by the exposed blade of DG (e.g. infrapyramidal) and the adjacent portion of the enclosed blade (Claiborne et al., 1986; Witter, 2007; Knierim et al., 2013). This anatomy suggests that information reaching the hippocampus is routed into one of two streams that terminate in either MEC or LEC (Fig. 3*A*). It is currently thought that output from the ‘MEC pathway’ is used to determine the animal's location in space while output from the ‘LEC pathway’ helps calculate the position of items and objects in space (Knierim et al., 2013). The current experiment determined if there are reactivation differences between these microcircuits during the retrieval of context fear memories. To do this, mice were trained and tested as described in experiment 2. Neurons activated during an initial fear conditioning test were tagged with H2B-GFP while c-Fos expression was used to index cellular activity during a subsequent memory test. Freezing levels were, once again, equivalent in each of these test sessions ($t(4) = 1.4$, $p > .05$) (Fig. 3*B*). To examine reactivation, we calculated the percentage of reactivated neurons in each brain region and compared it to that expected by chance. In DG, we found that reactivation was greater than chance in the suprapyramidal blade (sDG) ($t(4) = 2.91$, $p \leq .05$) and significantly lower than chance in the infrapyramidal

blade (iDG) (t (4) = 4.61, p ≤ .05) (Fig. 3*C*). The latter result may be an indication of extreme pattern separation, which has been reported in this region (Schmidt et al., 2012). We also found that reactivation exceeded chance in the distal (t (4) = 4.1, p ≤ .05) but not proximal portion of CA3 (t (4) = 2.64, p > .05) (Fig. 3*D*). This result is consistent with recent reports that suggest proximal CA3 is involved in pattern separation while distal CA3 is important for pattern completion (Lee et al., 2015; Lu et al., 2015). Similar to our initial findings, reactivation in proximal CA1 was significantly greater than that expected by chance (t (4) = 4.05, p ≤ .05). We observed less reactivation in distal CA1, although it did slightly exceed chance levels in the current experiment (t (4) = 2.93, p = .042) (Fig. 3*E*). This result indicates that context memory retrieval does induce some reactivation in the distal portion of CA1. Finally, we analyzed reactivation in the deep layers of the entorhinal cortex, which receive direct projections from CA1. In these regions, we found that reactivation exceeded chance in MEC (t (4) = 4.29, p ≤ .05) but not in LEC (t (4) = 2.54, p > .05) (Fig. 3*F*). Together, these data indicate that reactivation is more robust in the 'MEC pathway' than the 'LEC pathway' during the retrieval of context fear memories.

Experiment 5: Functional contributions of proximal and distal CA1 to memory retrieval

Our reactivation data suggests that memory retrieval for context fear engages a specific microcircuit in the hippocampus that sends projections to MEC. To determine if this pathway is required for memory retrieval, we tried to silence H2B-GFP tagged cells using optogenetic techniques, as we had done in a previous study (Tanaka et al., 2014). However, we were unable to confine our AAV infusions to the proximal or distal segment of CA1 (data not shown). Next, we tried micro-infusions of NMDA and found that damage could be restricted to each CA1 segment (Fig. 4*A*; Supplemental Fig. 1). In our behavioral experiment, mice were trained on context fear conditioning and then bilateral, fiber-sparing NMDA lesions were made 1 day later. Following a 7 day recovery period, the animals were tested in the conditioning context (Fig. 4*B*). An analysis of c-Fos expression after testing revealed that distal lesions did not affect c-Fos activity in proximal CA1 (t (3) = 1.62, p > .05) and proximal lesions did not affect c-Fos activity in distal CA1 (t (4) = 0.25, p > .05) (Fig. 4*C*-*F*). During the memory test, mice with damage to distal CA1 showed intact retrieval (t (8) = 0.48, p > .05) (Fig. 4*G*) while animals with proximal lesions exhibited a significant impairment (t (9) = 2.26, p ≤ .05) (Fig. 4*H*). This finding suggests that context fear memories are retrieved by a specialized hippocampal microcircuit that involves the proximal segment of CA1.

Discussion

The proximal and distal segments of CA1 have distinct physiological properties and connectivity. Place cells in proximal CA1 have higher spatial selectively (Henriksen et al., 2010) while neurons in distal CA1 are more sensitive to the presence of non-spatial cues in the environment (Burke et al., 2011; Ito and Schuman, 2012). These differences likely exist because the former receives direct input from MEC while the latter is directly connected with LEC. Based on these findings, it has been suggested that proximal CA1 is part of a circuit that encodes the animal's position in space while distal CA1 contributes to the encoding of object and item location (Knierim et al., 2013). In the current study, we

examined the contribution of these distinct CA1 segments to memory retrieval for context fear.

Using transgenic reporter mice, we showed that H2B-GFP tagged neurons in the proximal segment of CA1 are more likely to be reactivated during context memory retrieval than those in distal CA1. This result is consistent with the fact that place cells in proximal CA1 have higher spatial selectivity and are more stable than those in distal CA1 (Henriksen et al., 2010). We found a similar pattern of reactivation in brain regions that are connected to each these segments. Proximal CA1 receives direct input from the distal segment of CA3, which in turn receives projections from the suprapyramidal blade of the DG. Reactivation exceeded that expected by chance in each of these regions as well as in the deep layers of MEC, which receive return projections from proximal CA1. In contrast, reactivation did not exceed chance in regions connected to distal CA1, including infrapyramdial DG, proximal CA3 and the deep layers of LEC. These results suggest that the hippocampal formation consists of two discrete microcircuits that are differentially engaged during memory retrieval.

To determine if these microcircuits make distinct functional contributions to memory retrieval we made fiber-sparing, neurotoxic lesions that were confined to the proximal or distal segment of CA1. Damage to neurons in proximal CA1 significantly impaired the expression of context fear while lesions of distal CA1 had no effect. This fining provides the first direct evidence that memory retrieval for context fear depends on a specific segment of CA1. The fact that distal CA1 lesions did not affect memory retrieval could be interpreted in several ways. One possibility is that this region is not involved in context fear conditioning because distinct objects are not present in the environment. However, our conditioning chambers did contain some non-spatial cues (e.g. odors) that are known to influence processing in LEC and distal CA1 (Xu and Wilson, 2012; Igarashi et al., 2014). Therefore, it may be the case that non-spatial cues normally contribute to context fear but are not necessary or sufficient for freezing to occur. Consistent with this idea, the training odor that we used to clean the context (ethanol) does not produce robust fear when it is presented in a different environment (Wiltgen and Silva, 2007; Wiltgen et al., 2010). Based on these results, we suggest that context fear is largely controlled by an animal's knowledge of its spatial location, which is primarily processed in proximal CA1, MEC and associated circuitry.

In contrast to context fear conditioning, behavioral tasks that require an animal to learn about non-spatial features of the environment are likely to depend on distal CA1 and related structures. Recent work suggests that this is the case. In one experiment, rats were trained to use odor cues to find food in specified spatial locations (Igarashi et al., 2014). As animals learned this odor-place association task, firing patterns in LEC and the distal segment of CA1 showed increased coherence (in the 20-40 Hz frequency band). Coupling was not observed between LEC and proximal CA1 or between MEC and distal CA1. In a different study, rats were trained on a non-spatial delayed non-match to sample task where previously encountered odors needed to be recognized in order to receive a food reward (Nakamura et al., 2013). The authors found that memory retrieval was associated with significant increases in the expression of the IEG Arc in distal CA1 and proximal CA3 but not in proximal CA1 or distal CA3. Subsequent studies (using lesion or inactivation techniques) will be needed to

determine if non-spatial odor tasks such as these require direct communication between the distal segment of CA1 and LEC.

Materials and Methods

Subjects

All animals used in this study were the F1 hybrids generated by crossing TetTag mice (C57BL/6J) with wild-type 129S6 mice (Taconic) as described previously (Tayler et al., 2013; Tanaka et al., 2014). TetTag mice express a histone 2B-GFP fusion protein (H2B-GFP) that is under the control of the tetO promoter and a tetracycline-transactivator (tTA) protein under the control of the c-Fos promoter. Mice were born and raised on doxycycline (DOX) chow (Harlan; 40mg/kg) to prevent H2B-GFP expression. All experiments were approved by the UC Davis, Institutional Animal Care and Use Committee (IACUC).

Conditioning chambers

The contextual fear conditioning equipment used in these experiments was described previously (Tayler et al., 2013; Tanaka et al., 2014). Briefly, mice were trained in conditioning chambers that were housed in sound-attenuated boxes. The chambers contained a stainless steel grid floor, overhead LED lighting (providing broad spectrum and infrared light), and a scanning change-coupled device video camera (Med Associates). The chamber and drop pan were cleaned with 95% ethanol before each behavioral session. Context fear memory was assessed by placing the mice in the context and measuring the freezing response. Freezing measurements were automated using the VideoFreeze system (Med Associates).

Immunohistochemistry

Ninety minutes after testing, mice were transcardially perfused with 4% PFA. Following 24-hours of post-fixation in the same solution, brains were sectioned and stained. Forty μm sections were incubated for 15 min in a solution containing 0.1% BSA, 0.2% Triton X, 2 % normal goat serum. This was followed by incubation with anti-c-Fos rabbit primary antibody (P38; Calbiochem) diluted in the same blocking solution at 1:1250 or goat primary antibody (sc52-G; Santa Cruz) at 1:400, and anti-NeuN mouse primary antibody (MAB377; Millipore) at 1:200 for 1hr at room temperature. After a series of 0.1M phosphate buffer washes, slices were stained with the biotinylated goat anti-rabbit secondary antibody (Jackson Immuno Research) diluted in the blocking solution at 1:500 and Alexa-fluor 647 anti-mouse secondary antibody (Invitrogen) at 1:500 for 1hr at room temperature followed by Streptavidin-Cy3 incubation (1:500 dilution for 45 min at room temperature; Jackson Immuno Research). Finally, sections were stained with DAPI (Invitrogen) diluted in the blocking buffer at 1:10,000 for 10 min and mounted on slides.

Cell quantification

A confocal microscope (LSM 510 META, Zeiss) was used for imaging in Figures 2*B-C*. A fluorescence virtual slide microscopic scanner (BX61VS, Olympus) was used in all other experiments. Thirty-five μm z-stacks were used for quantification. An estimate of the total number of neurons in a z-stack was calculated by dividing the total volume of the cell layer

by that of single nucleus. The total volume was quantified in the DAPI channel using a macro (Fiji, Objects Counter plugin, http://fiji.sc/3D_Objects_Counter). To determine the volume of a single nucleus, a smaller z-stack was cropped from each stack. The total number of nuclei in the smaller z-stack was hand-counted and used for calculation (single volume = total volume (by macro) / total number (by hand-count)). Neurons were determined to be c-Fos positive, H2B-GFP positive, H2B-GFP+c-Fos double positive, or signal negative. More than 1500 neurons were examined per region for each animal. Coronal sections from 1.82 mm to 2.18 mm posterior to bregma were used for CA1 in Figures 1, 2, 4. In the coronal sections, proximal CA1 was quantified ≈ ML ±2.4 mm and DV −1.6 mm from bregma. The border between CA1 and CA2 was identified by the pronounced thinning of the pyramidal cell layer as described previously (Andersen et al., 2007). Distal CA1 was quantified ≈ ML ±0.75 mm and DV −1.5 mm from bregma. The Mouse Brain Atlas (Allen Brain Atlas Resources) was used to identify each structure for coronal sections. In Figure 3, horizontal sections were used for quantification. Sections from 1.5 mm to 1.7 mm ventral to bregma were used for CA1. Proximal CA1 was quantified ≈ AP −2.4 mm and ML ±3.15 mm and distal CA1 was quantified ≈ AP −2.25 mm and ML ±0.6 mm. MEC (layer 5/6) was quantified from 2.16 mm to 2.56 mm ventral to bregma ≈ AP −4.75 mm and ML ±3.25 mm. LEC (layer 5/6) was quantified from 2.80 mm to 3.16 mm ventral to bregma ≈ AP −4.75 mm and ML ±4.25 mm. The coordinates for the entorhinal cortex were chosen to include areas that received direct projections from dorsal CA1. Specifically, we used the Allen Mouse Connectivity atlas (http://connectivity.brain-map.org/) to trace axons from fluorescently labeled dorsal CA1 neurons to the entorhinal cortex. We then selected LEC and MEC coordinates based on the presence of fluorescently labeled terminals from dorsal CA1. CA3 was quantified from 1.88 mm to 2.08 mm ventral to bregma; ≈ AP −2.0 mm and ML ±3.0 mm for the distal segment and ≈ AP −2.0 mm and ML ±1.75 mm for the proximal segment. DG was quantified from 1.88 mm to 2.08 mm ventral to bregma; the suprapyramidal blade was quantified ≈ AP −2.0 mm, ML ±1.25 mm and at AP −3.0 mm, ML ±2.25 mm. The infrapyramidal blade was quantified ≈ AP −2.25 mm, ML ±1.25 mm and AP −3.0 mm, ML ±2.25 mm. 0.

Experiment 1

Behavior—All mice (N = 5) were habituated to handling (5 days, 5 min per day) prior to training. Mice were taken off DOX for 3 days prior to training to label active neurons with H2B-GFP. During context fear conditioning, mice were allowed explore context for 3 min prior to the onset of 2 footshocks (0.3 mA, 2 s) separated by a 20 s intertrial interval. One minute after the last shock, the mice were removed from the conditioning chamber and given high concentration DOX chow (1 g/kg) for 24 h to suppress further H2B-GFP expression and then remained on 40 mg/kg DOX chow. Memory was assessed 2 days later by returning the mice to the training context for 5 min and measuring the freezing response.

Experiment 2

Behavior—Mice (N = 5) were trained for 3 consecutive days to produce robust context fear that would not extinguish across multiple testing sessions. During conditioning, mice were allowed to explore context for 5 min prior to the onset of 3 footshocks (0.6 mA, 2s) each separated by a 120 s intertrial interval. Mice were removed from the conditioning chamber

60s after the last shock. After training on day 3, DOX was immediately removed. Memory was assessed 2 days later (Test 1) in the same way as Experiment 1. After Test 1, mice were given high concentration DOX chow for 24 h and then put back on regular DOX chow. Memory was assessed again 3 days later (Test 2).

Experiment 3

Behavior—Mice (N = 4) were habituated and taken off DOX in the same way as Experiment 1, and then exposed to the context without footshock for 5 min (Exposure 1). After this exposure, mice were given high concentration DOX chow for 24 h and then put back on regular DOX chow. Two days later, mice were returned to the same context for 5 min (Exposure 2).

Experiment 4

Behavior—Mice (N = 5) were trained and tested in the same way as Experiment 2. Ninety minutes after the second test, horizontal sections were taken for quantification of H2B-GFP and c-Fos in DG, CA3, CA1, MEC and LEC.

Experiment 5

Behavior—Mice (N = 21) were trained on context fear conditioning in the same way as Experiment 1 and then underwent surgery 1 day later. Memory was assessed 7 days after surgery by placing the animals back into the training context for 5 minutes. *Lesions*. All mice were anesthetized with isoflurane, and mounted in a stereotaxic apparatus (Kopf Instruments). A small craniotomy was performed and NMDA (10 mg/ml; Sigma) in 0.1 M PB was infused using a 10 µl Hamilton syringe (4.5 µl/minute) mounted in an infusion pump (Harvard Apparatus) and connected to an injection cannula (28G; Plastic One) with polyethylene tubing (Tygon Tubing). Infusions were made at 2 sites for distal CA1 lesions (0.2 µl per site) (AP-2.0 mm from bregma, ML ±0.4 mm, DV - 1.4 mm) and 4 sites for proximal CA1 lesions (0.1 µl per site) (AP −2.0 mm from bregma, ML ±2.25 mm, DV −1.3 mm and AP −2.2 mm, ML ±2.6 mm, DV −1.3 mm). The drug was allowed to diffuse for 3 min after each infusion. Mice were randomly assigned to receive a lesion or sham surgery.

Histology—Sham and lesioned mice were transcardially perfused with 4% PFA 90 min after testing followed by a 24 h post fixation period in the same solution. Coronal sections (40 µm thick) were cut and mounted on glass slides. After drying, the sections were stained with cresyl violet to verify the lesion locations. Three animals were excluded from data analysis in the distal CA1 lesion group: 2 mice had unilateral damage and 1 animal exhibited reduced hippocampal volume in all subregions. In the proximal CA1 lesion group, 3 animals were excluded: 1 mouse had little to no visible damage, 1 animal had a unilateral lesion and 1 had extensive damage that included CA3 and distal CA1.

Statistics

Group differences were analyzed with repeated-measure factorial ANOVAs followed by post-hoc comparisons (Fisher's PLSD) when necessary. Planned comparisons between

groups were done with paired or unpaired t-tests depending on the experimental conditions. Statistical significance for all tests was set at $p \leq 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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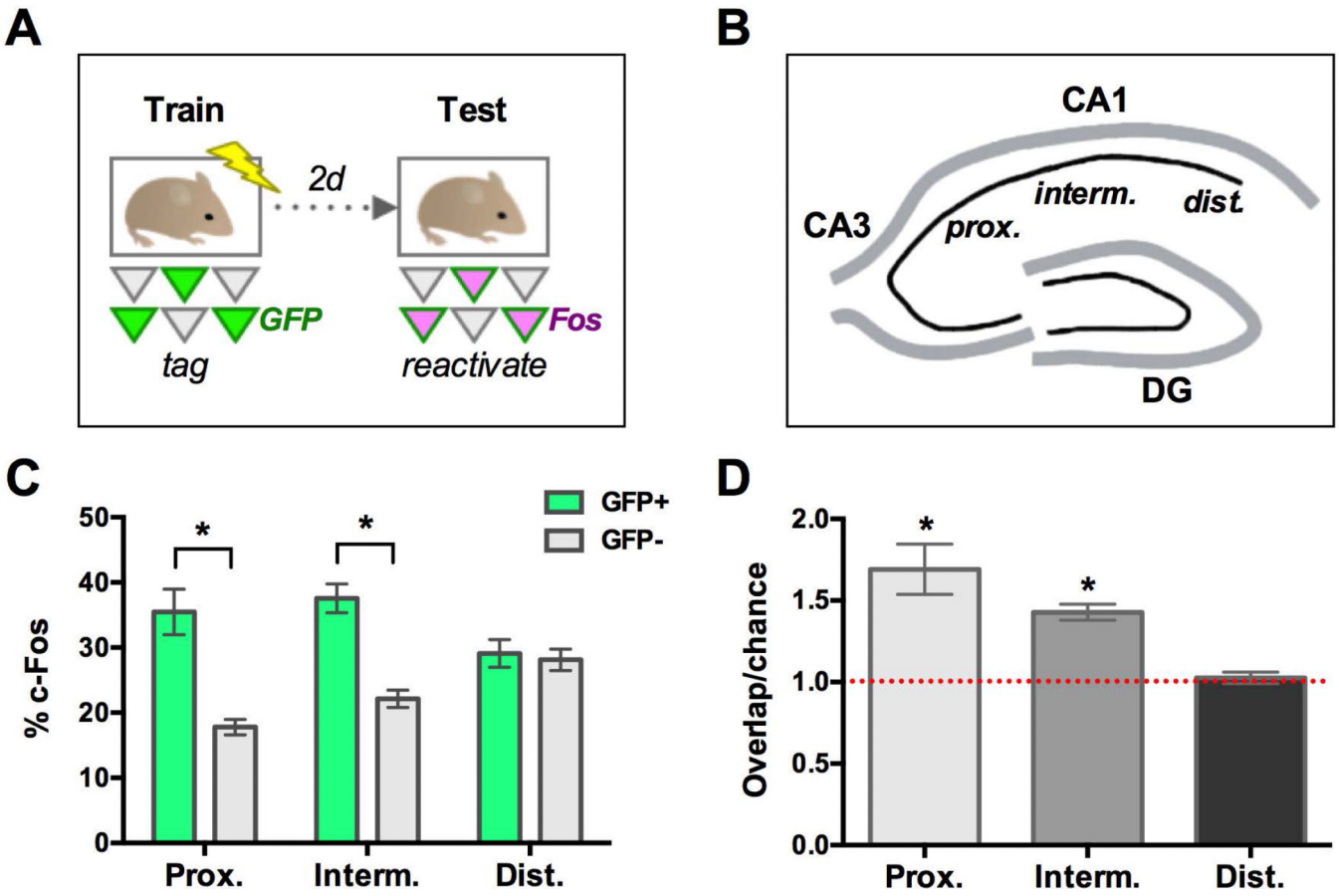

Figure 1.

Reactivation differs along the proximodistal axis of CA1 during memory retrieval. ***A***, Experimental procedure. Active neurons were tagged with H2B-GFP during training and c-Fos expression was used to index activity during testing (n=5). ***B***, Schematic diagram of the mouse hippocampus. Reactivation was quantified in three distinct segments of CA1 (distal, intermediate, and proximal). ***C,*** The percentage of c-Fos positive neurons in GFP-tagged and untagged neurons in proximal, intermediate, and distal CA1. Expression of c-Fos was significantly greater in GFP-tagged neurons than untagged neurons in proximal and intermediate CA1, but not in distal CA1. ***D***, The percentage of GFP and c-Fos double positive (overlapping) neurons relative to chance (percent H2B-GFP × percent c-fos). Significant reactivation was observed in proximal and intermediate CA1, but not in distal CA1. Error bars represent ± SEM. *$p \leq 0.05$.

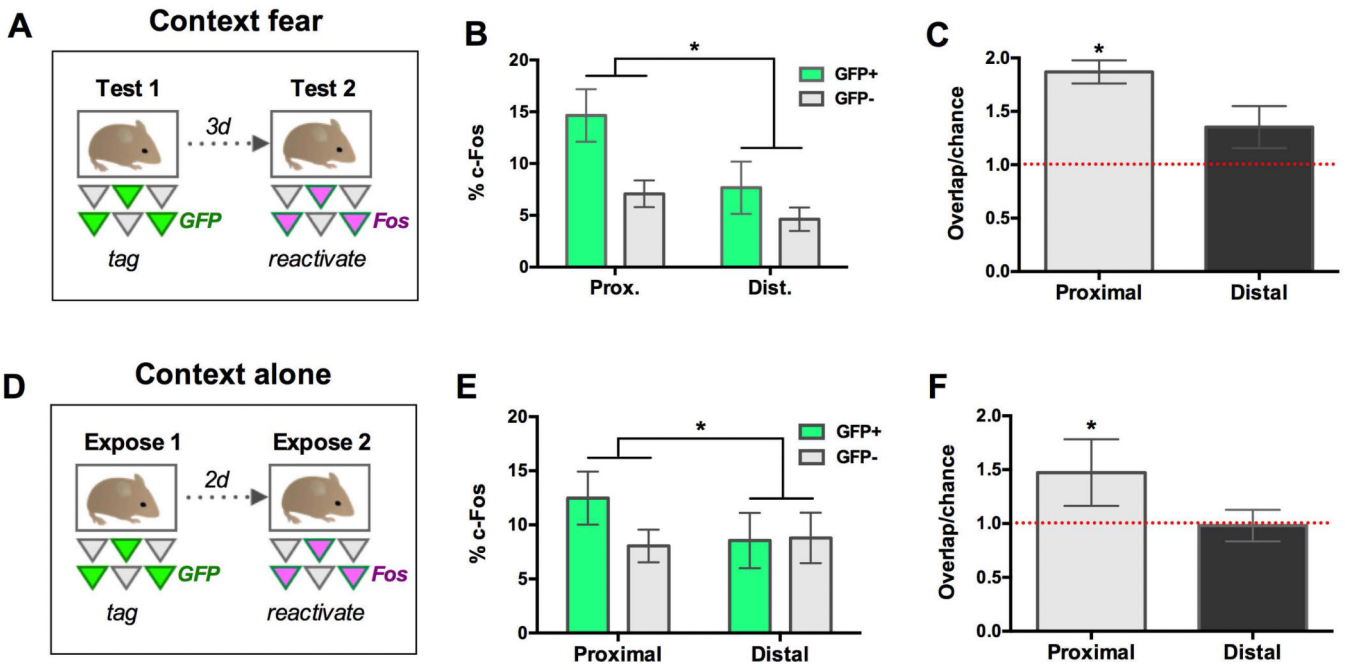

Figure 2.

Reactivation differs along the proximodistal axis of CA1 during identical experiences. ***A***, Experimental procedure. After context fear conditioning (n = 5) mice were tested in the same context twice. Active neurons were tagged with H2B-GFP during the first test and c-Fos was used to index activity during the second test. ***B***, c-Fos expression in GFP-tagged and untagged neurons in proximal and distal CA1. The expression of c-Fos was significantly greater in GFP-tagged neurons than untagged neurons in proximal CA1, but not in distal CA1. ***C***, The percentage of double positive neurons relative to chance level. Significant reactivation was observed in proximal but not distal CA1. **D**, Experimental procedure. Mice (n = 4) were allowed to explore the same novel environment twice. Active neurons were tagged with H2B-GFP during the first session and c-Fos was used to index activity during the second session. ***E***, c-Fos expression in GFP-tagged and untagged neurons in proximal and distal CA1. The expression of c-Fos was significantly greater in GFP-tagged neurons than untagged neurons in proximal CA1, but not in distal CA1. ***F***, The percentage of double positive neurons relative to chance level. Significant reactivation was observed in proximal but not distal CA1. Error bars represent ± SEM. *p ≤ 0.05.

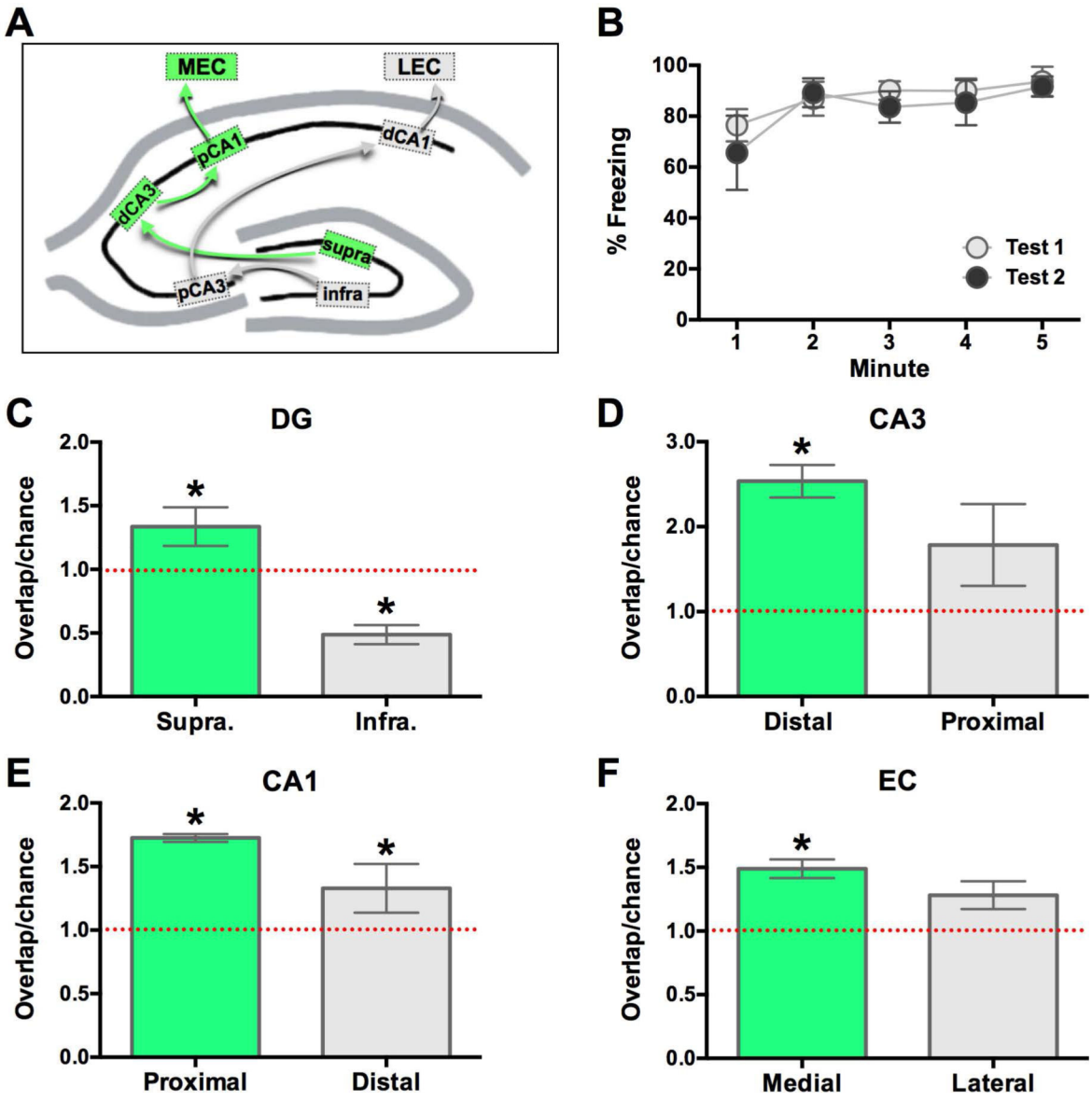

Figure 3.

Reactivation during memory retrieval differs between MEC and LEC pathways. ***A***, Schematic diagram showing the brain regions quantified in this experiment. Regions of the MEC pathway are colored green and those in the LEC pathway are colored gray. ***B***, Freezing data during the two test sessions (n = 5). ***C,*** The percentage of double positive neurons relative to chance in suprapyramidal and infrapyramidal DG ***D,*** proximal and distal CA3 ***E,*** proximal and distal CA1 ***F,*** MEC and LEC. Greater than chance reactivation was consistently observed in regions of the MEC pathway (green), but not in the LEC pathway (gray). The one exception was distal CA1, which showed slightly greater than chance reactivation. Error bars represent ± SEM. *$p \leq 0.05$.

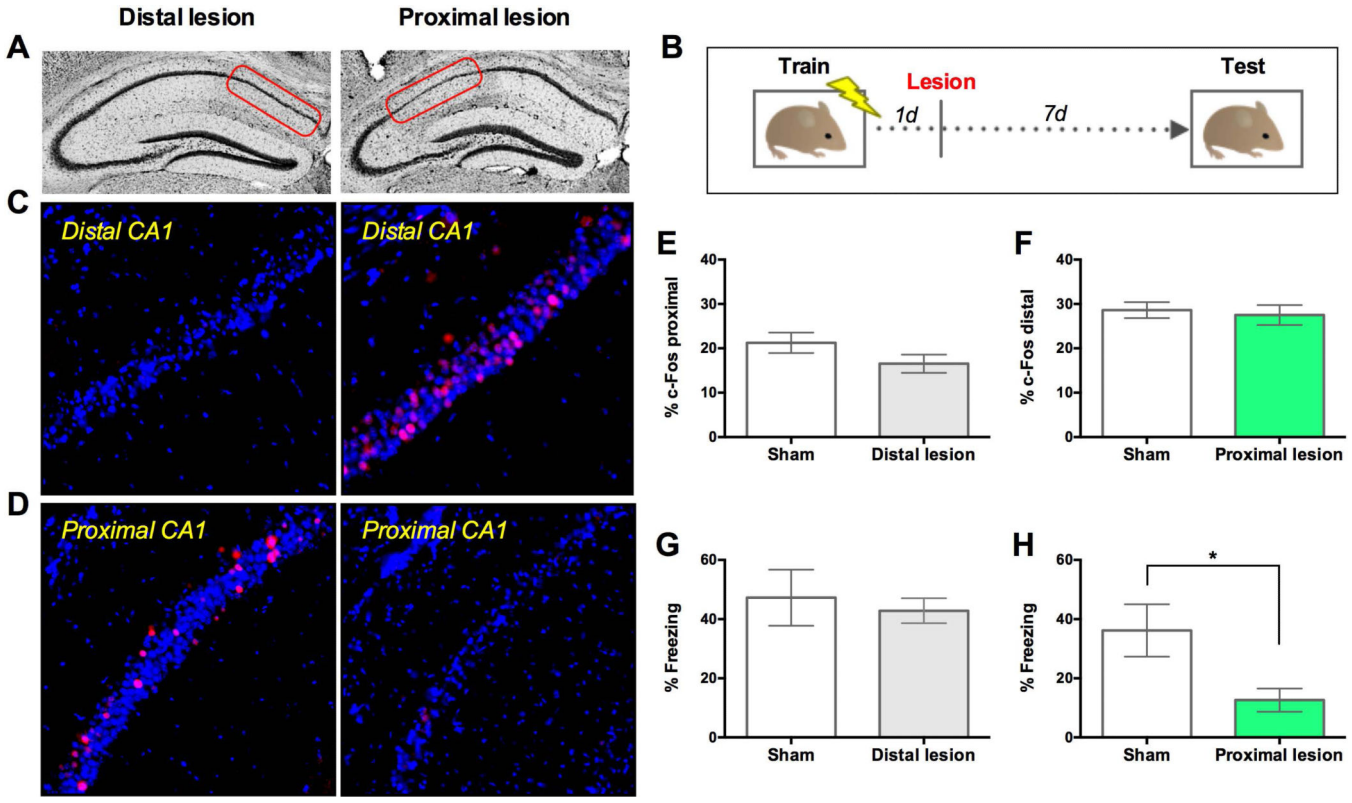

Figure 4.

Damage to proximal CA1 impairs memory retrieval for context fear. ***A***, Representative images showing coronal sections of the dorsal hippocampus stained with crystal violet after a distal CA1 lesion (left) or a proximal CA1 lesion (right). ***B***, Experimental procedure. Mice received lesions or sham surgery one day after training and were then tested 7 days later. ***C, D,*** Representative fluorescence images showing c-Fos expression in distal (C) and proximal CA1 (D) after a distal lesion (left) or a proximal lesion (right). c-Fos immuno-stained nuclei in red; DAPI stained nuclei in blue. ***E***, c-Fos expression in proximal CA1 was similar between shams (n = 4) and mice with a lesion of distal CA1 (n = 6). ***F***, c-Fos expression in distal CA1 was similar between shams (n = 6) and mice with a lesion of proximal CA1 (n = 5). ***G***, Lesions of distal CA1 had no effect on context memory retrieval. ***H***, Lesions of proximal CA1 significantly impaired memory retrieval. Error bars represent ± SEM. *p ≤ 0.05.