In contextual fear conditioning, experimental subjects learn to associate a neutral context with an aversive stimulus and display fear responses to a context that predicts danger. Although the hippocampal-amygdala pathway has been implicated in the retrieval of contextual fear memory, the mechanism by which fear memory is encoded in this circuit has not been investigated. Here, we show that activity in the ventral CA1 (vCA1) hippocampal projections to the basal amygdala (BA), paired with aversive stimuli, contributes to encoding conditioned fear memory. Contextual fear conditioning induced selective strengthening of a subset of vCA1-BA synapses, which was prevented under anisomycin-induced retrograde amnesia. Moreover, a subpopulation of BA neurons receives stronger monosynaptic inputs from context-responding vCA1 neurons, whose activity was required for contextual fear learning and synaptic potentiation in the vCA1-BA pathway. Our study suggests that synaptic strengthening of vCA1 inputs conveying contextual information to a subset of BA neurons contributes to encoding adaptive fear memory for the threat-predictive context.
n order to survive, animals develop fear responses to dangerous situations. The neural mechanism of learned fear has great survival value for animals, which must predict danger from seemingly neutral contexts. In contextual fear conditioning, an experimental model of fear learning, experimental subjects learn to associate a neutral context with an aversive stimulus and display fear responses to a context that predicts danger. Contextual fear learning requires coordinated activity of the hippocampus and amygdala. Ventral CA1 (vCA1) hippocampal neurons encode and convey contextual representations through monosynaptic projections to the amygdala, which induces defensive behavior. Thus, the vCA1–amygdala pathway can play an essential role in contextual fear learning. Although the vCA1–amygdala pathway has been implicated in the retrieval of contextual fear memory, the mechanism by which contextual fear memory is encoded in this circuit has not been investigated.

Exposure to a context activates a subset of vCA1 hippocampal neurons, which convey contextual representations directly to the amygdala. The contextual information is then integrated with aversive signals in the amygdala for fear memory formation. Strengthening of the hippocampal–amygdala pathway as a consequence of learning can facilitate the activation of the amygdala, resulting in conditioned fear responses to the threat-predictive context during the recall of contextual fear memory. Moreover, selective strengthening of the hippocampal inputs conveying specific contextual information to the amygdala can confer selective fear responses only to the relevant context. However, these hypotheses have not been examined in contextual fear conditioning. Recent studies have identified memory engram cells in the hippocampus and amygdala. Although these studies demonstrate the role of memory engram cells in contextual fear learning, it remains unknown how memory engram cells in the amygdala are connected to hippocampal engram cells encoding specific contextual representations, as well as how the synaptic strength of these connections is modified to encode contextual fear memory. In this study, we determined the mechanism by which contextual fear memory is encoded in the hippocampal–amygdala circuit by testing our hypothesis that fear memory associated with a particular context is encoded by selective strengthening of hippocampal inputs conveying the contextual information to the amygdala.

Results

vCA1–BA activity contributes to contextual fear learning. In the anterograde tracing experiment, eYFP-labeled vCA1 projections were found to be in the basolateral (BLA) and basomedial nuclei of the amygdala (BMA), collectively termed the basal amygdala (BA) (Fig. 1a, b). In the retrograde tracing experiment, hippocampal neurons projecting to the BA were predominantly found in the vCA1 and ventral subiculum (Fig. 1c, d), suggesting monosynaptic connection of vCA1 neurons to BA neurons.

More vCA1 neurons projecting to the BA (vCA1:BA projectors) expressed the immediate early gene c-fos in mice that were exposed to a novel context or recalled contextual fear memory than in mice left in their home cages (Fig. 1c, d, Supplementary Fig. 1a, and Supplementary Table 1), suggesting that a subset of vCA1:BA projectors can encode contextual representations. We next determined the role of the vCA1–BA pathway in the formation of contextual fear memory using a chemogenetic approach (Fig. 1e, f). Application of clozapine N-oxide (CNO) induced hyperpolarization and inhibited action potential (AP) firing in vCA1: BA projectors expressing hM4Di (Supplementary Fig. 2a–c), indicating the validity of our approach to silence vCA1–BA activity. After surgery, mice received a CNO injection and received unconditioned stimuli (US) in Context A 30 min later (Fig. 1g and Supplementary Fig. 1a). After 24 h, mice were tested for freezing behavior in Context A. On the following day, mice were fear conditioned in Context A after a vehicle injection and tested for freezing behavior in Context A 24 h later. In the hM4Di group, mice displayed significantly reduced freezing behavior when they had received a CNO injection on the training day compared with a vehicle injection, whereas in the mCherry group, there was no difference in freezing behavior on the test days between CNO and vehicle injections on the training day (Fig. 1h). The CNO effect in the hM4Di group on conditioned fear response was not due to the order of CNO and vehicle injections before fear conditioning (Supplementary Fig. 2d–e). These results indicate that silencing vCA1–BA activity during contextual fear learning decreased conditioned fear responses to the context 24 h later. Thus, vCA1–BA activity contributes to the acquisition of contextual fear memory.

vCA1–BA activity paired with shocks generated a fear memory. We next examined whether the activation of a random population of vCA1: BA projectors could serve as a conditioned stimulus with which mice could learn to associate an aversive stimulus. We induced Chronos or eYFP expression in vCA1: BA projectors and implanted an optical cannula to the vCA1 for in vivo photostimulation (Fig. 2a, b). Blue light illumination at 20 Hz reliably induced AP firing in Chronos-expressing vCA1 neurons (Supplementary Fig. 3). After surgery, mice were placed in Context C for habituation to photostimulations, which did not induce freezing behavior (Fig. 2c, d and Supplementary Table 2). On the training day, the mice received 20 Hz photostimulation terminating with the US in Context A. On the test days, the mice received the same 20 Hz photostimulation in Context C. In the Chronos group, photostimulation during test sessions significantly increased freezing behavior than photostimulation during habituation, whereas such an effect was not observed in the eYFP group (Fig. 2d, e). However, when presented unpaired with the US on the training day, photostimulation on the test days did not increase freezing behavior in the Chronos group (Fig. 2f, g), suggesting that photostimulation-induced freezing behavior requires temporal association of photostimulation with the US on the training day. Together, our results indicate that the activation of vCA1: BA projectors induced freezing behavior after their activation was temporally paired with the US.

Functional labeling of vCA1 neurons active in a context. We next employed a neural activity-dependent labeling approach with heterozygous Fos-CreERT2 knock-in mice to label vCA1 neurons active in a context. We injected AAV-DIO-eYFP into the vCA1 and retrograde HSV-mCherry into the BA in these mice (Fig. 3a). We exposed the mice to a novel Context A for 12 min 14, 19, and 24 h after tamoxifen administration (Fig. 3b). vCA1 neurons active in Context A expressed CreERT2 under the control of endogenous c-Fos promoter, which then induced the recombination of the DIO in the presence of tamoxifen, resulting in permanent eYFP expression, whereas vCA1 neurons projecting to the BA were retrogradely labeled with mCherry. Our labeling induced eYFP expression in 4.9 ± 0.6% of vCA1 neurons that project to the BA (mCherry+) (mean ± SEM; Fig. 3c), suggesting a small population of vCA1: BA projectors was active in Context A. eYFP-labeled neurons were found predominantly in the vCA1 than in other subregions of the hippocampus and were not detected in adjacent brain areas (Supplementary Fig. 4a–d). Without tamoxifen injection before context exposure, transgene expression was not detected in the vCA1 (Supplementary Fig. 5a). We confirmed the specificity of our labeling with c-Fos immunohistochemistry in Fos-CreERT2 × ROSA-LSL-tdTomato mice.
Fig. 1 Activity in the vCA1–BA pathway contributes to the acquisition of contextual fear memory. a Experimental setup for b. b Images showing eYFP expression in the vCA1 (left, green) and eYFP-labeled vCA1 axons in the amygdala (middle and right). Red, Nissl stain. LA, BLA, BMA, and CeA: lateral, basolateral, basomedial, and central nuclei of the amygdala, respectively. c Experimental setup for d. Top: vCA1 neurons projecting to the BA (vCA1: BA projectors) were retrogradely labeled with HSV-mCherry. Bottom: mice in FC group were fear conditioned in Context A as in Supplementary Fig. 1a. Mice in CTX group were exposed to Context A without a US. After 24 h, they were tested for freezing behavior in Context A. Brain tissues were then fixed 90 min later for c-Fos immunohistochemistry. Mice in HC group were left in their home cages until brain fixation. d Left: image showing vCA1: BA projectors (red) in the dorsal (dCA1), intermediate (iCA1), ventral CA1 hippocampus (vCA1), and ventral subiculum (vSub). vDG, ventral dentate gyrus. LEC, lateral entorhinal cortex. Middle: image showing c-Fos+ cells (green) and vCA1: BA projectors (mCherry+, red). A square indicates a c-Fos+ vCA1: BA projector. Right: quantification of c-Fos+ proportion among vCA1: BA projectors (6 mice per group). ***p = 0.001, **p < 0.01 (one-way ANOVA with post hoc comparisons). e Experimental setup for f–h. vCA1: BA projectors expressed hM4Di-mCherry or mCherry. We bilaterally injected retrograde CAV2-Cre into the BA and AAV-DIO-hM4Di-mCherry (hM4Di group) or AAV-DIO-mCherry (mCherry group) into the vCA1. f Images showing hM4Di-mCherry expression in vCA1: BA projectors (left and middle). Note no hM4Di-mCherry expression in the amygdala (right). g Behavioral training and testing protocols for h. h Quantification of freezing behavior on test days in the hM4Di (left, 10 mice) and mCherry groups (right, 8 mice) on test days. *p < 0.05, **p < 0.01 (two-way ANOVA with post hoc comparisons; group × treatment interaction, p < 0.05). Error bars indicate standard error of the mean (SEM). Source data are provided as a Source Data file. See also Supplementary Figs. 1 and 2.
Fig. 2 The activation of vCA1 neurons projecting to the BA, paired with aversive stimuli, generated a new conditioned fear memory. a An optical cannula was implanted to photostimulate vCA1 neurons projecting to the BA (vCA1: BA projectors) expressing Chronos-GFP (Chronos group) or eYFP (eYFP group). Retrograde CAV2-Cre was injected into the BA, and AAV-DIO-Chronos-GFP or AAV-DIO-eYFP was injected into the vCA1. b Left: image showing eYFP expression (green) in vCA1 neurons and optical cannula tip (arrow). Right: diagrams showing optical cannula implantation sites in d and e.
b Experimental setup for d, e. Mice were habituated to 20 Hz photostimulation in Context C on days 1–3. After a 3-min acclimatization period and baseline recording of freezing behavior for 1 min, 20 Hz photostimulation was applied to the vCA1 through an optical cannula (blue). On day 4, the mice received 20 Hz photostimulation (blue bars) 6 times, each co-terminating with a footshock, in Context A. On days 5–6, the mice were tested for freezing behavior in Context C in the presence and absence of 20 Hz photostimulation.
c Experimental setup for g. Mice in the Chronos: unpaired group underwent surgery with AAV-DIO-Chronos-GFP injected into the vCA1 as in a. On the training day, the mice received 20 Hz photostimulation 6 times in Context A and then received 6 shocks in Context A 30 min later. The mice were tested for photostimulation-induced freezing behavior in Context C. g Left: the time course of freezing behavior during habitation and test sessions in the Chronos: unpaired group (9 mice). Right: summary plot of the difference in the average freezing time in the presence and absence of photostimulation (ON – OFF freezing). p = 0.39, habitation vs. test sessions (two-sided paired t-test). Error bars represent the SEM. Source data are provided as a Source Data file. See also Supplementary Fig. 3.
The c-Fos+ proportion among all tdTomato-labeled vCA1 neurons as well as c-Fos+ and tdTomato+ cell density was significantly higher in mice exposed to the same context (A–A group) than in mice exposed to different contexts (A–B group), whereas there was no significant difference in tdTomato+ or c-Fos+ cell density (Fig. 3e, f and Supplementary Fig. 4e–g). In a separate experiment, more vCA1 neurons were labeled with tdTomato in mice exposed to a novel context than in mice that remained in the home cages after tamoxifen injection (Supplementary Fig. 5b), indicating that a subset of labeled neurons reflected vCA1 neurons active during context exposure. Moreover, a subset of labeled vCA1 neurons stably encoded the context representations over time (Supplementary Fig. 5c–e). Together, these results indicate that Context A exposure after tamoxifen injection induces labeling of a vCA1 neuronal population in FosCreERT2 mice. As a subset of labeled neurons reflects vCA1

(Fig. 3d).
neurons active in Context A, we termed these labeled neurons ‘Context A vCA1 neurons’. We next examined how each BA neuron received inputs from vCA1 neurons active in a context. We labeled vCA1 neurons active in Context A with ChR2-eYFP three times with a 1-week interval for sufficient ChR2 expression (Fig. 3g, h). Three context labeling sessions induced transgene expression in more vCA1 neurons than one labeling session did, whereas the number of labeling sessions did not affect the context specificity (Supplementary Fig. 5c–e). After labeling, ChR2-eYFP expression was detected in 1.3 ± 0.1% of vCA1 neurons (mean ± SEM, 8 mice), and eYFP-labeled axons were sparsely distributed in the BA (Fig. 3h, i). In brain slices, blue light illumination in the BA selectively activated axons of ChR2-expressing Context A vCA1 neurons, which we termed ‘Context A vCA1 inputs’. Thus, photostimulation in the BA induced synaptic responses in the Context A vCA1 inputs to BA pathway, which we termed ‘Context A vCA1–BA pathway’. Using whole-cell patch-clamp technique, we recorded in BA principal neurons excitatory postsynaptic currents (EPSCs), which were mediated by glutamate, an excitatory neurotransmitter (Fig. 3i). EPSC amplitude and the proportion of BA neurons that displayed EPSCs were proportional to the number of context labeling sessions (Supplementary Fig. 6). The peak amplitudes of EPSCs recorded in different BA neurons in the same brain slice were heterogeneous (95% confidence interval: −0.08−0.76 nA, Fig. 3k), and robust EPSCs were detected only in a subset of BA neurons (Fig. 3l). We isolated monosynaptic EPSCs in the Context A vCA1–BA pathway, and their amplitude was also heterogeneous among BA neurons (Fig. 3m, n). However, when vCA1 neurons globally expressed ChR2 and their axons were randomly stimulated, the EPSC amplitudes were much larger and less variable (95% confidence interval: 1.5−8.7 nA, Supplementary Fig. 7a–e). These results suggest that a subset of BA neurons receive more vCA1 inputs conveying specific contextual information than other BA neuronal populations do (Supplementary Fig. 7f−i).

**Contextual fear learning strengthened vCA1 inputs to the BA.** We next examined how associative fear learning for a particular context affected synaptic strength in vCA1 inputs conveying the contextual information to the BA. We labeled vCA1 neurons active in Context A with ChR2 (Fig. 4a, b). We then trained mice to learn to discriminate between contexts and display freezing behavior preferentially in a context that predicts danger. Mice in the fear conditioning (FC) group received a shock in Context A but not in Context B (Fig. 4b and Supplementary Fig. 1b). After multiple trials of fear conditioning, the mice showed fear responses predominantly in Context A (Fig. 4c and Supplementary Fig. 1c−d). Mice in the no shock (NS) control group were exposed to the contexts without the US and did not show fear responses in Context A or B (Fig. 4b, c and Supplementary Fig. 1c). In brain slices, we photostimulated Context A vCA1 inputs and recorded EPSCs in BA principal neurons, which were differentiated from GABAergic interneurons based on their intrinsic membrane properties (Supplementary Fig. 8). Photostimulations induced EPSCs, which reflected postsynaptic responses in the Context A vCA1–BA pathway (Fig. 4a, d). To detect changes in synaptic strength by postsynaptic mechanisms, we recorded both AMPA receptor (AMPAR)- and NMDA receptor (NMDAR)-mediated EPSCs in the same BA neurons and calculated the AMPA/NMDA ratio, which was significantly higher in the FC group than in the NS group (Fig. 4d, e, Supplementary Table 3), suggesting synaptic potentiation in the Context A pathway after fear learning in Context A. However, only 11.5% of 52 BA neurons in the FC group displayed the AMPA/NMDA ratio larger than the average ratio in the NS group by more than two standard deviations (Fig. 4f), suggesting that only a small population of BA neurons underwent synaptic strengthening in the Context A pathway. To detect synaptic changes by presynaptic mechanisms, we compared the rate of progressive block of NMDAR EPSCs by MK-801 and the paired-pulse ratio (PPR), which were not significantly different between groups (Supplementary Fig. 9a−b, 9d; Supplementary Table 4). In the Context A vCA1 inputs to the CeA, we did not
detect significant difference in the AMPA/NMDA ratio between groups (Supplementary Fig. 10), suggesting that synaptic changes associated with contextual fear learning are pathway-specific.

We next examined whether discriminative fear conditioning in Context A altered the strength of the Context B vCA1–BA synapses (Fig. 4g). After labeling vCA1 neurons active in Context B with ChR2, mice in the FC group underwent discriminative fear conditioning in Context A (Fig. 4h, i). Some mice (i.e., discriminators) displayed freezing behavior predominantly in Context A, while others (i.e., generalizers) showed significant freezing behavior in Context B as well (>35% freezing time on Day 5). Because the purpose of this experiment was to examine how ‘discriminative’ fear conditioning in Context A affected synaptic strength in Context B vCA1–BA pathway, only discriminators in the FC groups were included in our analysis. Mice in the NS control group were exposed to the contexts without the US and did not show fear responses in Context A or B (Fig. 4h, i). In brain slices, photostimulations in the BA activated ChR2-expressing Context B vCA1 inputs and induced EPSCs, which reflected postsynaptic responses in the Context B vCA1–BA pathway. There was no difference in the AMPA/NMDA ratio (Fig. 4j, k) or the rate of progressive block of NMDAR EPSCs by MK-801 between the FC and NS groups (Supplementary Fig. 9c), indicating that synaptic efficacy in the Context B pathway did not change after fear learning in Context A. When both discriminators and generalizers were included in our analysis, the average AMPA/NMDA ratio in the Context B pathway was positively correlated with freezing score in Context B on Day 5, whereas the ratio was negatively correlated with the discrimination index (Fig. 4l). These results suggest that the synaptic strength in the Context B pathway is inversely correlated with the ability to discriminate between contexts and show selective fear responses to threat-predictive Context A.
We also induced global expression of Chr2 or Chronos in the vCA1 (Supplementary Fig. 11a–b). The AMPA/NMDA ratio was not significantly different between the FC and NS groups (Supplementary Fig. 11c). There was no significant difference between groups in PPR or the rate of progressive block of NMDAR EPSCs by MK-801 (Supplementary Fig. 11d–e). These results suggest that synaptic efficacy in the vCA1–BA pathway was not globally altered in discriminative contextual fear conditioning. Together, our results suggest that discriminative contextual fear learning selectively strengthens the vCA1–BA pathway that convey relevant contextual information to the BA.

### Fear learning strengthens a subset of vCA1–BA synapses.

Our results demonstrate that contextual fear learning induces vCA1 input-specific synaptic potentiation, which was detected only in a small subset of BA neurons (Fig. 4f). Synaptic plasticity for fear memory formation may be induced preferentially in presynaptic vCA1 inputs and postsynaptic BA neurons that are activated during contextual fear conditioning. To test this possibility, vCA1 neurons active in Context A were labeled with Chr2 during context labeling sessions, whereas BA neurons active during contextual fear conditioning were labeled with tdTomato (tdT) in FosCreERT2 × ROSA-LSL-tdTomato mice (Fig. 5a, b, d). As a subset of BA neurons labeled during contextual fear conditioning was reactivated during memory recall (see below), we termed these labeled neurons ‘BA fear neurons’. After labeling, the mice were trained for discriminative fear in Context A (Fig. 5b, c). We then induced EPSCs with photostimulation of Context A vCA1 inputs and compared EPSCs recorded in tdT-labeled BA neurons with EPSCs recorded in adjacent unlabeled neurons (Fig. 5a). Both the AMPA/NMDA ratio and AMPAR EPSC amplitudes were significantly larger in tdT+ neurons than in tdT− neurons (Fig. 5e–g and Supplementary Fig. 12a–d), whereas NMDAR EPSCs or feed-forward inhibition in the vCA1–BA pathway did not differ between these neurons (Supplementary Fig. 12e–h). However, context exposure without the US did not increase the AMPA/NMDA ratio in tdT+ neurons (Supplementary Fig. 13).

Together, our results suggest that discriminative fear learning in Context A selectively strengthened Context A vCA1 inputs to BA fear neurons. We also examined whether BA fear neurons have intrinsic membrane properties that facilitate synaptic potentiation21,22. There was no significant difference in AP firing, resting membrane potential or input resistance between tdT+ and tdT− BA neurons (Fig. 5h–j), indicating no difference in intrinsic membrane properties between BA fear neurons and other BA neurons 5 days after initial fear conditioning.

We next examined whether discriminative fear learning also induced synaptic strengthening in randomly selected vCA1 inputs to BA fear neurons. We induced global Chr2 expression in the vCA1. For more consistent experimental conditions, the mice received three context labeling sessions as in the previous experiments although these procedures were not necessary for global Chr2 expression in the vCA1. We then labeled BA fear neurons with tdT and trained the mice for discriminative fear in Context A (Fig. 5k, l). There was no significant difference in the AMPA/NMDA ratio between tdT+ and tdT− BA neurons (Fig. 5m, n), suggesting that synapses in randomly selected vCA1 inputs to BA fear neurons were not altered in contextual fear learning. Together, these results suggest that discriminative contextual fear learning selectively strengthened synapses, which connect context-specific vCA1 neurons to BA fear neurons.

### Lack of vCA1–BA potentiation under drug-induced amnesia.

We next examined how inhibition of fear memory consolidation by anisomycin affected synaptic strength in the vCA1–BA pathway23–25. As in the previous experiments, we labeled vCA1 neurons active in Context A with Chr2 and BA fear neurons with tdT (Fig. 6a, b). Mice received systemic injections of anisomycin or saline after fear conditioning in Context A (Fig. 6b and Supplementary Fig. 14a). After 24 h, mice in the anisomycin group displayed less freezing behavior in Context A than mice in the saline group did (Fig. 6c), indicating anisomycin-induced retrograde amnesia. Anisomycin injection after fear conditioning decreased the density of c-Fos+ neurons in the vCA1 and BMA (Supplementary Fig. 14b–d), while it did not prevent tdT
expression in the BA (Fig. 6d), suggesting that the dosage of anisomycin used in our study inhibited protein synthesis but was insufficient to completely block CreERT2 expression in BA fear neurons. To allow for sufficient tdTom expression in BA fear neurons, we performed recording experiments 2 days after the memory recall test. In brain slices, we induced EPSCs by photostimulating Context A vCA1 inputs and compared the AMPA/NMDA ratio. In the saline group, the AMPA/NMDA ratio significantly larger in tdTom+ BA neurons than in tdTom− neurons (Fig. 6e), whereas there was no significant difference in the AMPA/NMDA ratio in the anisomycin group (Fig. 6f). Overall, the average difference in the AMPA/NMDA ratio between tdTom+ and tdTom− neurons in each mouse correlated with freezing behavior during memory recall (Fig. 6g). These results indicate...
Fig. 5 A subset of vCA1–BA synapses was selectively strengthened in discriminative contextual fear conditioning. a Experimental setup for b–j. After surgery, mice received three context labeling sessions with a 1-week interval to induce ChR2 expression in vCA1 neurons active in Context A. After a week, the mice received tamoxifen injection and were fear-conditioned in Context A on Day 1 for tdTomato (tdT) expression in BA fear neurons. On Days 2–5, mice were trained for discriminative fear in Context A as in Supplementary Fig. 1b. c Comparison of freezing responses in Contexts (Ctx) A vs. Context B on Day 5 (p = 0.001, two-sided paired t-test; n = 5 mice). d Images showing ChR2-eYFP-expressing vCA1 neurons (green, circles; left) and tdT-labeled BA neurons (red; right). e Traces of EPSCs recorded in tdT− and tdT+ BA neurons. tdT+ neurons were identified with red fluorescence within the BA (inset; scale bar, 10 μm). EPSCs were induced with photostimulation of Context A vCA1 inputs and recorded as in Fig. 4d. f Left: comparison of the AMPA/NMDA (A/N) ratios between tdT− and tdT+ BA neurons. Two-way ANOVA with post hoc comparisons was used to analyze combined data in f and m. Right: scatter plot of the A/N ratios in 18 pairs of tdT− (x-axis) and tdT+ BA neurons (y-axis) that were adjacent to each other. g Comparison of the amplitude of AMPAR EPSC (EPSCAMPAR) induced by photostimulation of the same intensity (6.4 mW/mm²) and recorded in tdT− vs. tdT+ BA neurons (two-sided paired t-test). h Traces of AP firing induced by depolarizing current injection (500 ms long) in tdT− and tdT+ BA neurons. Baseline membrane potential was adjusted to approximate −85 mV. i Comparison of AP firing in tdT− (18 cells) and tdT+ BA neurons (17 cells) (p = 0.67, two-way ANOVA). j Comparison of resting membrane potential (RMP, p = 0.45) and input resistance (Rin, p = 0.96, two-sided unpaired t-test) in tdT− (18 cells) and tdT+ BA neurons (17 cells). k Experimental setup for l–n. ChR2 was globally expressed in vCA1 neurons. BA fear neurons (tdT+) were labeled with tdT as in b. l Left: mice received three context labeling sessions and were then fear-conditioned in Context A on Day 1 for tdT expression in BA fear neurons as in b. On Days 2–5, the mice were trained for discriminative fear in Context A as in b. Right: quantification of freezing responses on Day 5. n = 5 mice. m Traces of EPSCs induced by global stimulation of vCA1 inputs and recorded in tdT− and tdT+ BA neurons as in e. n Left: comparison of the AMPA/NMDA ratios between tdT− and tdT+ BA neurons (p = 0.35, two-way ANOVA with post hoc comparisons). Right: scatter plot showing the AMPA/NMDA ratio in 10 pairs of tdT− and tdT+ BA neurons. Error bars represent the mean. Source data are provided as a Source Data file. See also Supplementary Figs. 12–13.

that synaptic strengthening in the vCA1–BA pathway was inhibited by post-training anisomycin treatment, which also suppressed the consolidation of contextual fear memory. Moreover, the pharmacological inhibition of NMDAR during contextual fear conditioning prevented the acquisition of contextual fear memory and blocked strengthening of the vCA1–BA synapses (Supplementary Fig. 15). Together, our results suggest that the acquisition and consolidation of contextual fear memory involve synaptic potentiation in the vCA1–BA pathway.

We next examined whether vCA1 and BA neurons active during contextual fear conditioning were reactivated during fear memory recall (Fig. 6h). In the FC group, BA and vCA1 neurons active during fear conditioning were labeled with mCherry. Mice in the HC group remained in their home cages for mCherry expression in BA and vCA1 neurons active in the home cages and were then fear conditioned in Context A on the following day. On the test day, mice in both groups displayed freezing behavior in Context A. The brain tissues were fixed 90 min later for c-Fos immunostaining of neurons active during memory recall. The c-Fos+ proportion among all mCherry+ BA and vCA1 neurons was significantly higher in the FC group than in the HC group (Fig. 6i, j), indicating that BA and vCA1 neurons active during fear conditioning were more readily reactivated during memory recall than neurons active in the home cages. These results suggest that a subset of BA and vCA1 neurons active during contextual fear conditioning were reactivated during fear memory recall, thus they likely included memory engram cells in the BA and vCA1

Synapse-specific encoding of fear memory in vCA1–BA circuit. Our labeling procedures in the previous experiments induced labeling of vCA1 and BA neurons active during contextual fear conditioning as well as those active during context exposure without the US (Figs. 5b, 6b). To avoid such a caveat, we independently labeled context-specific vCA1 neurons and BA fear neurons using Fos-CreERT2 × Fos-tTA double transgenic mice, in which transgene expression in the vCA1 and BA was controlled by different mechanisms. We injected AAV-TRE-ChR2-eYFP into the vCA1 and AAV-DIO-mCherry into the BA (Fig. 7a) in these mice fed with doxycycline (Dox)-containing food. After surgery, the mice were taken off Dox for 48 h and exposed to Context A, which induced the expression of tetracycline transactivator (tTA) under the control of c-Fos promoter and subsequent ChR2-eYFP expression in vCA1 neurons active in Context A (Fig. 7b, c). We confirmed the context specificity of our vCA1 labeling in Fos-tTA mice (Supplementary Fig. 16). After three context labeling sessions with a 1-week interval, the mice received a tamoxifen injection and were fear conditioned in Context A 24 h later, resulting in mCherry expression in BA fear neurons active during fear conditioning (Fig. 7b, d). The mice showed freezing behavior in Context A 24 h later (Fig. 7b). In brain slices, we photostimulated Context A vCA1 inputs and recorded EPSCs in mCherry+ and adjacent mCherry− BA neurons. The AMPA/NMDA EPSC ratio was significantly higher in mCherry+ neurons than in unlabeled BA neurons (Fig. 7e, f), indicating synaptic potentiation in Context A vCA1 inputs to BA fear neurons. Our results suggest that contextual fear learning induces selective strengthening of synapses that connect pre-synaptic vCA1 neurons active in threat-predictive context to postsynaptic BA fear neurons recruited during fear conditioning.

BA fear cells receive abundant context-specific vCA1 inputs. We next determined the properties of BA fear neurons that facilitate synaptic potentiation in the vCA1–BA pathway in contextual fear learning. For this, we examined the possibility that BA fear neurons may receive more vCA1 inputs that convey threat-predictive contextual information than other BA neurons do, which would facilitate their activation during contextual fear learning, thereby promoting synaptic potentiation and recruitment into a fear memory trace. To test this, we employed a retrograde trans-synaptic tracing approach with rabies virus (RV)26 in Arc-CreERT2 mice, in which BA fear neurons were labeled more efficiently than in Fos-CreERT2 mice (Supplementary Fig. 17a–c). We first injected AAV-DIO-TVA-G-GFP into the BA (Fig. 8a). In the FC group, BA fear neurons were labeled with TVA-G-GFP, whereas BA neurons active in the home cages were labeled in the HC group (Fig. 8a, c). Fear learning-induced synaptic plasticity, which can affect RV-mediated labeling, was blocked with anisomycin in the FC group (Figs. 6f, 8a, b and Supplementary Fig. 18a). More BA neurons were labeled with TVA-G-GFP in the FC group than in the HC group (Supplementary Fig. 18b–d), indicating the recruitment of BA fear neurons by contextual fear conditioning. We then injected EnvA-expressing and G-deficient RV-mCherry into the BA, which infected TVA-G-expressing BA neurons (Fig. 8a, e) and propagated trans-synaptically, resulting in mCherry expression in vCA1 neurons monosynaptically projecting to TVA-G-GFP-labeled BA neurons (Fig. 8d). After 11 days, vCA1 neurons active
in Context A were immunostained for c-Fos (Fig. 8a). The c-Fos+ proportion among all mCherry+ vCA1 neurons was significantly higher in the FC group than in the HC group, whereas there was no difference in the density of mCherry+ or c-Fos+ vCA1 neurons between groups (Fig. 8f, g). Thus, vCA1 neurons projecting to BA fear neurons were more likely to be c-Fos+ than vCA1 neurons projecting to BA neurons active in the home cages. As fear conditioning did not affect the excitability or intrinsic membrane properties of Context A vCA1 neurons (Supplementary Fig. 19), and anisomycin injection after fear learning prevented synaptic changes in the vCA1–BA pathway (Fig. 6f), these results suggest that more vCA1 neurons active in
Context A likely project to BA fear neurons than to BA neurons active in the home cages.

We next used an electrophysiological approach to determine more clearly whether BA fear neurons receive more vCA1 inputs conveying threat-predictive contextual signals than other BA neurons do (Fig. 8h). vCA1 neurons active in Context A were labeled with ChR2, and BA neurons active during fear conditioning in Context A were labeled with tdT (Fig. 8i). In brain slices, monosynaptic AMPAR EPSCs were induced with photostimulation of ChR2-labeled vCA1 inputs and recorded in BA neurons. The peak amplitude of EPSCs recorded in tdT+ BA neurons was significantly larger than in adjacent tdT− BA neurons (Fig. 8k, l). As fear learning-induced synaptic strengthening of the vCA1–BA pathway was blocked by anisomycin injected after contextual fear conditioning (Figs. 6f, 8i, j), EPSC amplitude was proportional to the number of Context A vCA1 inputs to each BA neuron. Thus, these results suggest that BA fear neurons receive more vCA1 inputs that convey threat-predictive contextual information than other BA neurons do (Fig. 8m).

Context-specific vCA1 activity contributes to fear learning. To further determine the role of context-specific vCA1 neurons in contextual fear learning, we next examined how silencing vCA1 neurons active in a context affected the acquisition of fear memory for the context. To silence neural activity in a sufficient number of vCA1 neurons active in a context, we used Arc-CreERT2 mice, in which more vCA1 neurons were labeled than in Fos-CreERT2 mice (Supplementary Fig. 17d–g). We confirmed the context-specificity of vCA1 labeling in Arc-CreERT2 mice (Supplementary Fig. 20). vCA1 neurons active in Context A were labeled with hM4Dι-mCherry or mCherry in Arc-CreERT2 mice (Fig. 9a, b). After 3 weeks, the mice received CNO or vehicle injections, underwent fear conditioning in Context A and were tested for freezing behavior in the same context 24 h later (Fig. 9b). When mice in the hM4Dι group were injected with CNO on the training day, they displayed less freezing behavior on the test day as compared with vehicle injection, whereas in the mCherry group, there was no difference in freezing behavior between CNO and vehicle injections (Fig. 9c). The CNO effect in the hM4Dι group on conditioned fear response was not due to the order of CNO and vehicle injections before fear conditioning (Supplementary Fig. 21). As silencing of Context A vCA1 neuronal activity during fear learning decreased conditioned fear responses to the context, activity in these vCA1 neurons contributes to the acquisition of contextual fear memory. When vCA1 neurons active in different Context B were labeled with hM4Dι-mCherry, however, CNO injection before fear conditioning in Context A did not affect freezing behavior in Context A 24 h later, compared with the vehicle control (Fig. 9d–f), suggesting that silencing vCA1 neurons active in an irrelevant context did not affect contextual fear learning.

We next examined how silencing of Context A vCA1 neurons during fear conditioning affected synaptic strength in the vCA1–BA pathway (Fig. 10). We first performed control experiments with Arc-CreER T2×Fos-tTA mice, in which we independently induced ChR2-eYFP expression in vCA1 neurons active in Context A and mCherry expression in BA fear neurons active during contextual fear conditioning (FC group) or those active in the home cages (HC group). Neurons active during fear memory recall were immunostained for c-Fos (Supplementary Fig. 21). As silencing of AMPA/NMDA ratios between tdT+ and tdT− neurons in the anisomycin group. Middle and Right: comparison of AMPA/NMDA ratios between tdT+ and tdT− neurons in the anisomycin group (p=1.00, two-way ANOVA with post hoc comparisons). The average AMPA/NMDA ratio between tdT+ and tdT− neurons in each mouse positively correlated with freezing behavior during memory recall (Pearson correlation test).

Discussion

In this study, we tested the hypothesis that contextual fear learning involves strengthening of functionally defined synapses, which connect context-encoding hippocampal CA1 neurons to a subset of neurons in the amygdala. The conventional approaches examining randomly selected synapses do not allow the efficient detection of associative learning-induced synaptic changes because associative memory is encoded sparsely in a subset of synapses28−30. We recently developed an innovative approach by combining neural

Fig. 6 Lack of synaptic potentiation of the vCA1-BA pathway under anisomycin-induced retrograde amnesia. a Experimental setup for b–g. b Mice were exposed to Context A to induce ChR2 expression in vCA1 neurons active in Context A. They were then fear conditioned in Context A for tdT expression in BA fear neurons and received anisomycin (ANI, 10 mice) or saline injections (SAL, 7 mice). c Comparison of freezing behavior in Context A 24 h after fear conditioning between groups (two-sided unpaired t-test). d Left: image showing ChR2-eYFP-expressing vCA1 neurons (green, circles). Right: image of a pair of BA neurons, one tdT+ (red) and one tdT−, loaded with bicynotic during recording and labeled with streptavidin-Alexa Fluor 633 (green). e Left: traces of EPSCs recorded in tdT− and tdT+ BA neurons in the saline control group. EPSCs were induced with photostimulation of Context A vCA1 inputs and recorded as in Fig. 4d. Middle: comparison of the AMPA/NMDA (A/N) ratios between tdT− and tdT+ neurons. Two-way ANOVA with post hoc comparisons was used to analyze combined data in e and f. Right: scatter plot of the A/N ratios in 22 pairs of tdT− and adjacent tdT+ cells in the saline control group. f Left: traces of EPSCs recorded in tdT− and tdT+ BA neurons in the anisomycin group. Middle and Right: comparison of AMPA/NMDA ratios between tdT− and tdT+ neurons in the anisomycin group (p=1.00, two-way ANOVA with post hoc comparisons). g The average difference in AMPA/NMDA ratio between tdT+ and tdT− neurons in each mouse positively correlated with freezing behavior during memory recall (Pearson correlation test). h Experimental setup for i and j. mCherry was expressed in BA and vCA1 neurons active during contextual fear conditioning (FC group) or those active in the home cages (HC group). Neurons active during fear memory recall were immunostained for c-Fos. i Images showing BA and vCA1 neurons labeled with mCherry (red) and c-Fos (green). Both mCherry+ and c-Fos+ neurons were marked with circles. j Comparison of c-Fos+ proportion among all mCherry-labeled BA and vCA1 neurons (two-sided unpaired t-test). n = 5–7 mice per group. Error bars represent the SEM. Source data are provided as a Source Data file. See also Supplementary Figs. 14–15.
activity-dependent labeling\textsuperscript{31}, optogenetic, and electrophysiological techniques, enabling efficient detection of synaptic changes in associative learning\textsuperscript{7}. With this approach, we identified a BA neuronal population that receives more inputs from vCA1 neurons active in a context than other BA neurons do. Our study suggests that heterogeneous populations of BA neurons receive vCA1 inputs conveying different contextual information, while the total number of vCA1 inputs to each BA neuron is uniform. Although extensively used in previous studies\textsuperscript{8,9,11,15}, neural activity-dependent labeling approaches have limitations including background labeling. As the labeling window (hours) is substantially longer than the labeling events (e.g., context exposure and contextual fear learning) (minutes), our labeling procedures likely tagged neurons active in the hours before and after the events, including those active in the home cages\textsuperscript{31}. Thus, it is noteworthy that only a fraction of vCA1 and BA neurons labeled
with our approaches represents neurons active during context exposure or contextual fear conditioning.

Contextual fear learning requires coordinated activity in the hippocampus and the amygdala. Contextual representations encoded in the dorsal CA1 (dCA1) hippocampus are conveyed through the entorhinal and perirhinal cortices to the BA, where they are integrated with aversive signals for fear memory formation. Contextual information is also relayed to the BA through monosynaptic inputs from the vCA1, which encodes a context with larger place fields and lower spatial resolution.

**Figure Legend**

- **a** Flowchart showing the experimental timeline and procedures.
- **b** Graph showing freezing behavior over time.
- **c** Diagram illustrating the experimental setup and labeling strategies.
- **d** Graph showing c-Fos expression in vCA1 neurons.
- **e** Image showing the expression of mCherry and TVA-G-GFP in amygdala regions.
- **f** Image showing immunohistochemistry of c-Fos localization.
- **g** Graph showing synaptic responses under different conditions.
- **h** Diagram showing the experimental setup for contextual fear conditioning.
- **i** Flowchart showing the experimental timeline and procedures.
- **j** Graph showing freezing behavior over time.
- **k** Image showing synaptic currents in BA neurons.
- **l** Graph showing synaptic responses under different conditions.
- **m** Diagram showing the experimental setup and labeling strategies.

**References**

1. Contextual fear learning requires coordinated activity in the hippocampus and the amygdala. Contextual representations encoded in the dorsal CA1 (dCA1) hippocampus are conveyed through the entorhinal and perirhinal cortices to the BA, where they are integrated with aversive signals for fear memory formation. Contextual information is also relayed to the BA through monosynaptic inputs from the vCA1, which encodes a context with larger place fields and lower spatial resolution.

**Discussion**

- **Flowchart (a)**: The experimental timeline and procedures are depicted, showing the 1st surgery, AAV: TVA-G, 1 wk, FC group, 24 h, A, Recall, A, 2 wks, 2nd surgery, RV-mCherry, 11 d, BA label, Fix, Fos IHC, 4-OHT + Anisomycin.
- **Graph (b)**: Freezing behavior over time is shown, with significant differences indicated.
- **Diagram (c)**: The experimental setup and labeling strategies are illustrated, showing Arc-CreERT2 mice, Arc promoter, CreERT2, AAV-DIO-TVA-G, pSyn, Neural activity, 4-OHT, EnvA-ΔG-RV-mCherry, ΔG-mCherry, EnvA.
- **Graph (d)**: c-Fos expression in vCA1 neurons is shown, with significant differences indicated.
- **Image (e)**: The expression of mCherry and TVA-G-GFP in amygdala regions is shown.
- **Image (f)**: Immunohistochemistry of c-Fos localization in ventral CA1 hippocampus is shown.
- **Graph (g)**: Synaptic responses under different conditions are shown, with significant differences indicated.
- **Diagram (h)**: The experimental setup for contextual fear conditioning is illustrated, showing Fos-CreERT2 x ROSA-LSL-tdTomato, AAV-DIO-ChR2-eYFP, Context A, vCA1, BA: tdT+, BA: tdT–, Label 3x, Label, Recall, E-phys, 1 d, Anisomycin.
- **Graph (i)**: Freezing behavior over time is shown, with significant differences indicated.
- **Image (j)**: Synaptic currents in BA neurons are shown, with significant differences indicated.
- **Graph (k)**: Synaptic responses under different conditions are shown, with significant differences indicated.
- **Diagram (l)**: The experimental setup and labeling strategies are illustrated, showing Context A, vCA1 inputs, BA: tdT–, BA: tdT+, Label, Label, BA: tdT–, BA: tdT+, Light power density (mW/mm²), EPSC (nA).

**Conclusion**

Contextual fear learning involves coordinated activity in the hippocampus and the amygdala, with contextual representations encoded in the dorsal CA1 (dCA1) hippocampus being conveyed through the entorhinal and perirhinal cortices to the BA, where they are integrated with aversive signals for fear memory formation. Contextual information is also relayed to the BA through monosynaptic inputs from the vCA1, which encodes a context with larger place fields and lower spatial resolution.
selectivity than the dCA1. Previous studies have implicated the vCA1 in contextual fear learning. We found that silencing vCA1–BA activity during fear conditioning decreased conditioned fear responses 24 h later, indicating that vCA1–BA activity is involved in the acquisition of contextual fear memory, consistent with a previous report. Moreover, optogenetic activation of vCA1 neurons projecting to the BA induced conditioned fear responses after it was paired with the US in a different context. Together, our results support the role of the vCA1–BA pathway in contextual fear learning.

As the vCA1 conveys contextual information directly to the amygdala, which generates conditioned fear responses, contextual fear memory can be encoded by long-term potentiation (LTP) of vCA1–BA synapses, which subsequently facilitates the activation of the amygdala during fear memory recall in a threat-predictive context. Our study demonstrates that synaptic potentiation was induced selectively in the vCA1 inputs conveying threat-predictive contextual information to the BA in discriminative contextual fear conditioning. Notably, LTP was not detected in vCA1 inputs conveying irrelevant contextual information to the BA or randomly selected vCA1–BA synapses in discriminative fear learning. Thus, the input specificity of LTP could confer the selectivity of contextual fear memory, enabling adaptive fear responses only to the relevant context, together with a well-documented role of the medial prefrontal cortex in memory consolidation. Therefore, the input specificity of synaptic potentiation may serve as the universal mechanism for selective fear responses to a context or sensory cue that predicts danger.

We also found that input-specific LTP in contextual fear learning was confined to a subset of BA neurons active during contextual fear conditioning, which we termed BA fear neurons. vCA1 neurons active in a context are monosynaptically connected to BA fear neurons. Oure results suggest that LTP was selectively induced in context-specific vCA1 inputs to BA fear neurons in contextual fear conditioning, which is analogous to a previous report that contextual fear learning strengthens a subset of dorsal hippocampal CA3–CA1 synapses. Thus, the locus of LTP for contextual fear memory is determined by both presynaptic vCA1 inputs and postsynaptic BA neurons, supporting the Hebbian model of associative learning and memory. Moreover, strengthening of these vCA1–BA synapses was prevented by either post-training anisomycin or pretraining MK-801 injection. Notably, both anisomycin and MK-801 treatment also prevented conditioned fear responses to the context 24 h later, consistent with previous reports. Therefore, impaired acquisition and consolidation of fear memory by MK-801 and anisomycin correlate with the lack of LTP in the vCA1–BA pathway, further supporting the role of LTP in the vCA1–BA pathway in contextual fear learning.

Which BA neuronal population is recruited into a contextual fear memory trace, and which neuronal or synaptic properties determine the allocation of contextual fear memory into a subset of BA neurons? A subset of BA neurons may receive more hippocampal inputs conveying threat-predictive contextual information and be recruited into a fear memory trace. Our trans-synaptic tracing and electrophysiological studies suggest that BA fear neurons active during fear conditioning receives more vCA1 inputs conveying threat-predictive contextual information than other BA neurons do. Although these results are correlational, our results raise the possibility that a BA neuronal population that receives more inputs from vCA1 neurons encoding relevant contextual representations could be more readily activated during contextual fear learning and, thus, would be preferentially recruited into a fear memory trace. Therefore, the number of vCA1 inputs to each BA neuron could contribute to determining which BA neurons are recruited into the contextual fear memory trace, together with a well-documented role of the transient changes in neuronal excitability in the selection of memory engram cells. We did not detect increase in the membrane excitability in BA fear neurons 5 days after initial fear conditioning. This suggests that the neuronal excitability of BA fear neurons may be transiently increased during fear conditioning and then return to baseline once these cells have been recruited into a fear memory trace. This possibility is supported by previous reports that the excitability state of engram cells is dynamic, and a transient increase in neuronal excitability contributes to memory allocation and a more precise and effective retrieval of contextual fear memory.

Previous studies suggest that functional heterogeneity exists in the vCA1. Depending upon their projection targets, vCA1 neuronal populations play distinct roles in various behavioral tasks. vCA1 neurons projecting to the mPFC or CeA contribute to...
context-dependent expression of extinguished fear memory for an auditory cue. Moreover, vCA1 neurons projecting to the nucleus accumbens play a role in social memory, goal-directed behaviors, and conditioned place preference for cocaine. Previous studies also implicate granule cells in the ventral dentate gyrus and vCA1 neurons projecting to the lateral hypothalamus in innate anxiety and avoidance behavior. In our study, silencing of context-specific vCA1 neurons during fear conditioning inhibited both the acquisition of contextual fear memory and synaptic strengthening of the vCA1–BA pathway, suggesting the role of the vCA1 in contextual fear learning. Our findings also implicate strengthening of the vCA1–BA pathway in the formation of contextual fear memory. Thus, our study provides important insights into the mechanism by which an associative fear memory for a particular context is encoded in the hippocampal–amygdala circuit.

Methods

Subjects. We obtained the heterozygous Fos-CreERT2 mice used in this study by crossing wild-type C57BL6/J (Jackson Laboratory Stock # 000664) and Fos-CreERT2 (+/−) mice (Jackson Laboratory Stock # 201882). We obtained heterozygous Arc-CreERT2 mice by crossing wild-type C57BL6/J and Arc-CreERT2 (+/−) mice (Jackson Laboratory Stock # 022357). We obtained the heterozygous Fos-CreERT2 mice used in this study by crossing wild-type C57BL6/J and Fos-tTA/Fos-shGFP (+/−) mice (Jackson Laboratory Stock # 018306). Fos-CreERT2 (+/−) and Ai9 ROSA-LSL-tTmato (+/−) mice (Jackson Laboratory Stock # 007909) were crossed to generate Arc-CreERT2 (+/−) x Fos-tTA/Fos-shGFP (+−) mice. Fos-tTA/Fos-shGFP (+−) mice were crossed to generate Arc-CreERT2 (+/−) x Fos-tTA/Fos-shGFP (+−) mice. GAD2-ires-Cre (+/−) mice were obtained from the Jackson Laboratory (Stock # 010802). Mice were singly housed in home cages on a 12-h light/dark cycle with food and water continuously available. The light cycle was from 8 AM to 8 PM. Six- to eight-week-old mice of both sexes underwent stereotaxic brain surgery. All of the animal procedures were approved by the Institutional Animal Care and Use Committee of the University of California, Riverside.

Fig. 9 Context-specific vCA1 neuronal activity is involved in the acquisition of contextual fear memory. a Left: experimental setup for b and c. vCA1 neurons active in Context A expressed hM4Di-mCherry (hM4Di group) or mCherry (mCherry group) in Arc-CreERT2 mice. Right: images showing mCherry-labeled vCA1 neurons (yellow). b Behavioral training and testing protocols for c. After labeling Context A vCA1 neurons, mice received a CNO or vehicle injection 30 min before fear conditioning in Context A and were tested for fear memory in Context A 24 h later. c Comparison of freezing behavior in Context A on the test days in the hM4Di (12 mice; **p = 0.004, CNO vs. vehicle; *p = 0.016) and mCherry control groups (8 mice; p = 1.00, CNO vs. vehicle) (two-way ANOVA with post hoc comparisons; group × treatment interaction, p = 0.023). d Left: experimental setup for e and f. vCA1 neurons active in Context B were labeled with hM4Di-mCherry. Right: images showing mCherry-labeled vCA1 neurons (yellow). e After labeling Context B vCA1 neurons, mice received a CNO or vehicle injection 30 min before fear conditioning in Context A and were tested for fear memory in Context A 24 h later. f Comparison of freezing behavior in Context A on the test days (7 mice; p = 0.18, CNO vs. vehicle; two-sided paired t-test). Error bars represent the SEM. Source data are provided as a Source Data file. See also Supplementary Figs. 20–21.
**Virus constructs.** The recombinant adeno-associated viruses (AAVs) were packaged by the Vector Core at the University of North Carolina. The AAV titers were 4.3 × 10^{12} genome copies (GC)/mL for AAV5-pCaMKIIα-eYFP, 6.5 × 10^{12} GC/mL for AAV5-pEF1α-DIO-eYFP, 5.3 × 10^{12} GC/mL for AAV5-pEF1α-DIO-mCherry, 4.0 × 10^{12} GC/mL for AAV5-pEF1α-DIO-hmCherry, 5.5 × 10^{12} GC/mL for AAV5-pSyn-DIO-hM4Di-mCherry, 4.6 × 10^{12} GC/mL for AAV5-pCaMKIIα-hChR2(H134R)-eYFP, 4.2–7.0 × 10^{12} GC/mL for AAV5-pEF1α-DIO-hChR2(H134R)-eYFP, 5.7 × 10^{12} GC/mL for AAV5-pSyn-Chronos-GFP, 3.6 × 10^{12} GC/mL for AAV5-pEF1α-DIO-hChR2(H134R)-eYFP, and 5.7 × 10^{12} GC/mL for AAV5-pSyn-Chronos-GFP, 3.6 × 10^{12} GC/mL for AAV5-pEF1α-DIO-Chronos-GFP, and 3.9 × 10^{12} GC/mL for AAV1-pSyn-DIO-TVA-G-GFP. AAV9-TRE-hChR2(H134R)-eYFP and AAV9-TRE-mCherry constructs were obtained from Dr. Susumu Tonegawa at MIT and was packaged by Dr. Joung-Hun Kim’s laboratory at POSTECH (serotype 9, titer: 8 × 10^{13} and 8 × 10^{12} GC/mL for AAV-TRE-hChR2(H134R)-eYFP and AAV-TRE-mCherry, respectively). Herpes simplex virus (HSV-pEF1α-mCherry) for the retrograde.
tracing experiments was packaged by Dr. Rachael Neve at the Gene Delivery Technology Core of Massachusetts General Hospital, and the titer was 3.3 × 10^{12} viral particles/mL. Rabies virus (EmVA-ΔG-RV-mCherry) was obtained from Dr. John Naughton at the Gene Transfer, Targeting and Therapeutics Core of the Salk Institute for Biological Studies, and the titer was 1.4–2.3 × 10^{14} transducing units/mL.

**Surgery.** Six- to eight-week-old mice underwent stereotaxic surgery. Prior to surgery, general anesthesia was induced by placing the mice in a transparent anesthetic chamber filled with 5% isoflurane with intramuscular injection of ketamine and xylazine (30 mg/kg and 2 mg/kg body weight, respectively). The anesthesia was maintained during surgery with 1% isoflurane applied to the nostrils of the mice using a precision vaporizer. Mice were checked for the absence of the tail-pinch reflex as a sign of sufficient anesthesia. The mice were then immobilized in a stereotaxic frame with non-rupture ear bars (David Kopf Instruments), and ophthalmic ointment was applied to prevent eye drying. After an incision was made along the midline of the scalp, small unilateral or bilateral craniotomies were performed using a microdrill with 0.5-mm-diameter drills. The tips of glass capillaries loaded with AAV were placed into the vCA1 (3.4 mm caudal to bregma, 3.7 mm lateral to the midline, and 3.2 mm ventral to the pial surface) or BA (1.5 mm caudal to bregma, 3.2 mm lateral to the midline, and 3.5 mm ventral to the pial surface). AAV-containing solution was injected at a rate of 0.1 μL/minute using a 10 μL Hamilton microsyringe and a syringe pump. The total volume of injected virus-containing solution was 0.15 μL for AAV5-pCmKfla-eYFP, 0.5 μL for AAV5-pEFla-Dio-eYFP, 1.0 μL for AAV5-pEFla-Dio-mCherry, 1.0 μL for AAV5-pSyn-Dio-mCherry, 0.15 μL for AAV5-pCmKfla-ChR2(H134R)-eYFP, 1.0 μL for AAV5-pEFla-DIO-Chronos-GFP, 0.5 μL for AAV5-pEFla-DIO-Chronos-F, 0.5 μL for AAV5-pSyn-DIO-TVA-G-GFP, 0.5 μL for AAV9-TRE-hChR2(H134R)-eYFP, 0.5 μL for AAV9-TRE-mCherry 1.0 μL for HSV-pEFla-mCherry, 1.0 μL for AAV5-pCmKfla-chR2(134H)-YFP, and 0.5 μL for EnVAΔG-RV-mCherry. In Fig. 10b–n, a mixture of 0.5 μL AAV5-pEFla-Dio-ChR2(134H)-YFP and 0.5 μL AAV5-pSyn-DIO-mCherry was used. mCherry was mixed with AAV5 or AAV9 virus and was injected into the vCA1. After injection, the capillary was left in place for an additional 5 min to allow diffusion of the virus solution and then withdrawn. The scalp incision was closed with surgical sutures, and the mice were given buprenorphine-containing saline (1 mL, 0.13 mg buprenorphine/kg body weight) for postoperative analgesia and hydration.

For the experiments described in Fig. 2, an optical cannula (200 μm in diameter, numerical aperture of 0.53, Duric Lenses) was implanted above the left vCA1 (3.4 mm caudal to bregma, 3.8 mm lateral to the midline, and 2.15 mm ventral to the pial surface) and secured with dental cement. To minimize light leakage during photostimulation, which can act as a visual cue, we painted all optical pathways, including the dental cement securing the cannula, with black nail polish. We verified the cannula implantation site in each animal (Fig. 2b).

**Activity-dependent neuronal labeling.** For functional labeling of vCA1 neurons in a context for labeling of vCA1 neurons active in a context in Fos-CreERT2 mice in Figs. 3, 4, 5, 6, 7a–b, 9, 10, 11, 12, 13, 15, 17d, 19, mice received an intraperitoneal injection of tamoxifen (150 mg/kg of body weight) 1 week after surgery. The mice were exposed to novel Context A (dimension: 30 cm × 24 cm × 21 cm; stainless steel grid floor, white light illumination, and benzaldehyde odor) or Context B (dimension: 30 cm × 24 cm × 21 cm; acrylic plate floor, dim red light illumination, and acetic acid odor) within a standard fear conditioning chamber (Med Associates) three times for 12 min each at 14, 19, and 24 h after tamoxifen injection. In some experiments, the mice received 1 or 2 additional labeling procedures with a 1-week interval. To minimize neuronal labeling by background noise, mice were kept in the dark in their home cage in a quiet place with minimal traffic within a satellite animal care facility for 36 h after tamoxifen injection. Our context labeling procedure induced the most abundant transient expression in the vCA1, the virus injection site, compared with other subregions of the hippocampus (ChR2-eYFP-labeled CA1 neurons/mm^2): 0.2 ± 0.01, 0.34 ± 0.11, 0.97 ± 0.17, and 0.66 ± 0.18 for the dorsal CA1, intermediate CA1, ventral CA1, and ventral subiculum, respectively; average ± SEM (10 mice).

**To label vCA1 neurons active in a context in Arc-CreERT2 mice in Figs. 9, 10 and Supplementary Figs. 17e, 20, 21, mice were exposed to Context A or Context B once for 12 min and returned to their home cages. After 10–20 min, the mice received an intraperitoneal injection of 4-hydroxytamoxifen (4-OHT; 15–30 mg/kg of body weight). To minimize neuronal labeling by background noise, mice were kept in the dark in their home cages in a quiet place for 12 h before and after context exposure. To avoid the confounding effect of the 4-OHT injection on fear memory formation, we injected 4-OHT 10–20 min after context exposure or fear conditioning, which induces neuronal labeling with a sensitivity and specificity comparable to that observed when 4-OHT is administered before the labeling event.**
were then put back on Dox. To minimize neuronal labeling by background noise, mice were kept in the dark in their home cage in a quiet place from 2 h before the first context exposure 24 h after the last Dox exposure. In Fig. 7, the mice received two additional labeling procedures with a 1-week interval.

Labeling BA fear neurons for electrophysiological recording: To label BA fear neurons, which refer to BA neurons active during fear conditioning, in Fos-CreERT2 mice in Figs. 5, 7 and Supplementary Figs. 12, 15, we injected the mice intraperitoneally (150 mg/kg of body weight) to inhibit the consolidation of contextual fear memory in the experimental group (ANI group) or were injected with the same volume of saline (SAL group). Mice received 3 more injections of anisomycin (150 mg/kg of body weight) to inhibit protein synthesis-dependent synaptic strengthening in context-specific vCA1–BA pathway contributors to the selectivity of conditioned fear responses to a context that predicts danger (i.e., Context A).

For discriminative contextual fear conditioning in Fig. 5 and Supplementary Fig. 12, mice were injected with tamoxifen and given 5 US in Context A 24 h later (Day 1). After 14 h, the mice were fear conditioned in Context A. Mice were put back in the home cage (HC) group were kept in their home cages and were given an anisomycin injection as in the FC group. Ten minutes later, the mice also received an intraperitoneal injection of vehicle (the same volume of saline containing 10% DMSO as in the FC group). A day after contextual fear conditioning, the mice also received an intraperitoneal injection of anisomycin (150 mg/kg of body weight) at 2-hour intervals as in the FC group. Ten minutes later, the mice also received a 4-OHT injection and 3 more injections of anisomycin (50 mg/kg of body weight) at 2-hour intervals as in the FC group. To minimize neuronal labeling by background noise, mice were kept in the dark in their home cages in a quiet place for 12 h before and after 4-OHT injection.

Single-trial contextual fear conditioning. In the experiments described in Figs. 1, 6a–i, 9, 10 and Supplementary Figs. 12–19, mice were singly housed in their home cages on a 12-h light/dark cycle starting a week before behavioral training, with food and water continuously available. Mice were randomly assigned to behavioral groups. On the training day, mice were placed in Context A between 9 AM and 10 AM. After 3 min, the mice received the first US (electric footshock, 0.5 mA, 2 s duration) and were given 2 more US with a 1-min interval as in Supplementary Fig. 1a. The temperature in the fear conditioning chamber was 22–24 °C. On the test day, freezing behavior was quantified as the percentage of time immobile in the first 5 min in Context A. The movement of the mice in the fear conditioning chamber was recorded using a near-infrared camera and analyzed in EthoVision XT 11 software (Noldus). Freezing score was calculated as the percentage of time for which the mice remained immobile. Immobility for more than 2 s was counted as freezing behavior.

In Figs. 6a–g, 8a–g and Supplementary Figs. 14, 18b–d, mice were placed in Context A on the training day. After 3 min, the mice received the first US (electric footshock, 0.5 mA, 2 s duration) and were given 2 more US with a 1-min interval. After fear conditioning, the mice received intraperitoneal injections of saline. In Fig. 8a–g and Supplementary Fig. 18b–d, mice in the HC group remained in their home cages on the training day. In Supplementary Fig. 15, mice in the MK-801 group received an intraperitoneal injection of MK-801 (0.3 mg/kg body weight) or saline 30 min before fear conditioning with three US in Context A.

Discriminative contextual fear conditioning. In the experiments described in Fig. 4 and Supplementary Figs. 9, 10, 11, mice were singly housed in their home cages on a 12-h light/dark cycle starting a week before behavioral training, with food and water continuously available. Mice were randomly assigned to either the experimental group (ANI group) or were injected with the same volume of saline (SAL group). Mice received 3 more injections of anisomycin (150 mg/kg of body weight) to inhibit the consolidation of contextual fear memory in the experimental group (ANI group) or were injected with the same volume of saline (SAL group). Mice received 3 more injections of anisomycin (150 mg/kg of body weight) to inhibit protein synthesis-dependent synaptic strengthening in context-specific vCA1–BA pathway contributors to the selectivity of conditioned fear responses to a context that predicts danger (i.e., Context A).

For discriminative contextual fear conditioning in Fig. 5 and Supplementary Fig. 12, mice were injected with tamoxifen and given 5 US in Context A 24 h later (Day 1). After 14 h, the mice were fear conditioned in Context A. Mice were put back in the home cage (HC) group were kept in their home cages and were given an anisomycin injection as in the FC group. Ten minutes later, the mice also received an intraperitoneal injection of anisomycin (150 mg/kg of body weight) at 2-hour intervals as in the FC group. Ten minutes later, the mice also received a 4-OHT injection and 3 more injections of anisomycin (50 mg/kg of body weight) at 2-hour intervals as in the FC group. To minimize neuronal labeling by background noise, mice were kept in the dark in their home cages in a quiet place for 12 h before and after 4-OHT injection.

In vivo chemogenetic silencing of vCA1 activity. For experiments in Fig. 1e–h, retrograde CAV2-pCMV-Cre was bilaterally injected into the basolateral amygdala (BA), and AAV-pEFla-DIO-hmDi-mCherry (hmDi group) or AAV-pEFla-DIO-mCherry (mCherry group) was bilaterally injected into the vCA1. Four weeks after surgery, mice received an intraperitoneal injection of clozapine N-oxide (CNO, 10 mg/kg body weight) on Day 1 and were given 5 shocks in Context A 30 min later as in Supplementary Fig. 1a. CNO was dissolved in DMSO (10 mg/mL) and then further dissolved in saline at 1 mg/mL. After 24 h, the mice were tested for freezing behavior in Context A on Day 2. On Day 3, the mice received an intraperitoneal injection of vehicle (the same volume of saline containing 10% DMSO as in the CNO group on Day 1) and were given 5 shocks in Context A 30 min later as in Supplementary Fig. 1a. After 24 h, the mice were tested for freezing behavior in Context A on Day 2. In Supplementary Fig. 2d–e, mice received a vehicle injection 30 min before fear conditioning in Context A on both Day 1 and Day 3.

For the experiments presented in Figs. 9 and 10, AAV-pEFla-DIO-hmDi-mCherry (hmDi group) or AAV-pEFla-DIO-mCherry (mCherry group) was bilaterally injected into the vCA1 in Arc-CreERT2 mice. A week after surgery, the mice were exposed to Context A or Context B for 12 min and received an intraperitoneal injection of 4-OHT (15–30 mg/kg of body weight) to label vCA1 neurons active in Context A or Context B. Three weeks after behavioral labeling, mice received an intraperitoneal injection of CNO (Day 1) or vehicle (Day 3) and were placed in Context A 30 min later as in Supplementary Fig. 1a. A day after contextual fear conditioning, the mice were tested for freezing behavior in Context A on Days 2 and 4. In Supplementary Fig. 21, after surgery and labeling of vCA1 neurons in Context A, mice received a vehicle injection 30 min before fear conditioning in Context A on both Day 1 and Day 3.

In vivo optogenetic stimulation for fear memory formation. To activate vCA1 neurons projecting to the BA (vCA1–BA projectors) in Fig. 2a–e, we injected retrograde CAV2-pCMV-Cre into the BA and AAV-DIO-Chronos-GFP (Chronos group) or AAV-DIO-eYFP (eYFP group) into the vCA1. An optical cannula was implanted dorsal to the vCA1 to illuminate vCA1: BA projectors expressing Chronos-GFP or eYFP. Three weeks after surgery, mice were habituated to confinement and the optical cannula was positioned before behavioral training. Each mouse underwent the freezing test once per day for 2 days. Freezing scores in the presence or absence of blue light illumination during habituation and test sessions were calculated separately on each test day and averaged. For each mouse, we then calculated the difference in the average freezing scores in the presence and absence of photostimulation (ON – OFF freezing) during habituation (Days 1–3) and testing (Days 5–6). In the Chronos+ eYFP group in Fig. 2f, g, we injected AAV-CreERT2 mice with an AAV-Chronos-GFP into the vCA1 and implanted an optical cannula dorsal to the vCA1 as in the Chronos: paired group. After surgery, the mice were habituated in Context C as in the Chronos: paired and eYFP group on Days 1–3. On Day 4, the mice received 6 pairings of the same 20 Hz photostimulation (20 s duration) and an electric footshock (0.5 mA, 2 s duration, co-terminating with photostimulation) in Context A. On Days 5–6, freezing behavior was monitored in Context C in the presence and absence of the 20 Hz photostimulations (1-minute duration). Each mouse underwent the freezing test once per day for 2 days. Freezing scores in the presence or absence of blue light illumination during habituation and test sessions were calculated separately on each test day and averaged. For each mouse, we then calculated the difference in the average freezing scores in the presence and absence of photostimulation (ON – OFF freezing) during habituation (Days 1–3) and testing (Days 5–6). In the Chronos+ eYFP group in Fig. 2f, g, we injected AAV-Chronos-GFP into the vCA1 and AAV-DIO-Chronos-GFP into the vCA1 and implanted an optical cannula dorsal to the vCA1 as in the Chronos: paired group. After surgery, the mice were habituated in Context C as in the Chronos: paired and eYFP group on Days 1–3. On Day 4, the mice received 6 pairings of the same 20 Hz photostimulation (20 s duration) and an electric footshock (0.5 mA, 2 s duration, co-terminating with photostimulation) in Context A. On Days 5–6, freezing behavior was monitored in Context C in the presence and absence of the 20 Hz photostimulations.
The pipette was then withdrawn slowly, and the brain slices were permeabilized in PBS-T at room temperature for 4 days. Brain sections were then blocked with PBS containing 5% goat serum at 4 °C for an hour. The slices were then incubated with a polyclonal rabbit anti-c-Fos antibody (1:200 dilution of 0.1 mg/mL stock antibody in PBS-T, 226033/Synaptic Systems) at 4 °C overnight. The slices were then washed three times with PBS-T for 10 min each and mounted on glass slides for confocal microscopic imaging.

For each mouse, we captured Z-series confocal microscopic images of 4 representative fields (0.56 mm² each) of the vCA1 or the BA and averaged the proportions for each mouse. In Supplementary Fig. 14b–d, we manually counted c-Fos+ cells in Z-stacked confocal microscopic images of 4 representative fields (0.56 mm² each) of the vCA1, BMA or BA and calculated the density of c-Fos+ cells by dividing total number of c-Fos+ cells with the volume of interest.

Verification of the specificity of neuronal labeling. To examine the specificity of behavioral labeling of the vCA1 in ArcCreER²×Fos-ERT²×ROSA-Lsl-tdT mice in Fig. 3d–f, we labeled vCA1 neurons at two different time points such that tdTomato+ cells reflected neurons labeled during the first Context A exposure, whereas c-Fos+ cells reflected neurons labeled during the second Context A exposure to either Context A or B one week later. We captured confocal microscopic images of 3-4 representative fields (0.56 mm² each) in the vCA1, where tdTomato+ neurons were most abundant within the vCA1. tdTomato+ or c-Fos+ vCA1 neurons were counted manually. The proportion of c-Fos+ neurons among all tdTomato+ neurons was calculated and used as a reference.

To examine the context-specificity of labeling of the vCA1 in ArcCreER²×Fos-ERT²×ROSA-Lsl-tdT mice in Supplementary Fig. 18a, we labeled vCA1 neurons at two different time points such that ChR2-eYFP+ cells reflected vCA1 neurons active during the first Context A exposure, whereas c-Fos+ cells reflected vCA1 neurons active during the second Context A exposure in the A-HC group 9 days after the first context exposure. We captured confocal microscopic images of 3-4 representative fields (0.56 mm² each) in the vCA1, where ChR2-eYFP+ neurons were most abundant within the vCA1. The proportion of c-Fos+ cells among all ChR2-eYFP+ vCA1 neurons within the field of view was calculated and averaged for each mouse.

Whole-cell patch-clamp recording in brain slices. To electrophysiological recording in brain slices, mice were deeply anesthetized with 5% isoflurane and decapitated. Brains were dissected quickly and chilled in ice-cold artificial cerebrospinal fluid (ACSF) containing 130 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl₂, 1 mM MgSO₄, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, and 10 mM glucose with 95% O₂ and 5% CO₂. Coronal brain slices containing the amygdala (300 μm thick) were prepared with a vibratome and continuously perfused with ACSF at a rate of 1 mL per minute. The unbound streptavidin was then washed out with PBS three times for 20 min each, and the slices were mounted onto slides. Images of the labeled neurons were taken using a Leica TCS SP5 confocal microscope, and neuronal morphology and locations were analyzed.

To examine what proportion of BA and vCA1 neurons active during contextual fear conditioning were reactivated during recall of the fear memory in Fig. 6–h, we identified BA and vCA1 neurons active during contextual fear conditioning or in the home cages with mCherry expression. BA and vCA1 neurons active during fear memory recall were immunostained for c-Fos (see Fig. 3). We captured confocal microscopic images of 3-4 representative fields (0.56 mm² each) of the BA and vCA1, where mCherry-labeled neurons were most abundant. The proportion of c-Fos+ neurons among all mCherry+ BA or vCA1 neurons within the field of view was calculated and averaged for each mouse.
The Clamp 10 (Molecular Devices). Results from electrophysiological experiments are summarized in Supplementary Tables 3 and 4.

Progressive block of NMDAR EPSCs by MK-801: To compare presynaptic release probability in the vCA1 inputs to the BA between behavioral groups, we recorded AMPAR EPSCs (3–5 traces) at −80 mV and then recorded NMDAR EPSCs (3–5 traces) at −40 mV. Then, the holding potential was reduced to −80 mV to record second set of AMPAR EPSCs (3–5 traces). We also recorded EPSCs at 0 mV. This recording protocol minimized the effect of time-dependent EPSC changes on the AMPA/NMDA ratio. To quantify AMPAR EPSCs, we averaged the first and second sets of AMPAR EPSCs recorded before and after the recording of NMDAR EPSCs and calculated the peak amplitude of averaged AMPAR EPSCs. To quantify NMDAR EPSCs (within 80 mV to record the peak amplitude of averaged NMDAR EPSCs), we averaged NMDAR EPSC traces and measured the mean amplitudes of the averaged NMDAR EPSCs between 47.5 ms and 52.5 ms after the onset of photostimulation. As AMPAR EPSCs completely disappeared 50 ms after photostimulation, EPSCs recorded at −40 mV 30 ms after stimulation onset were not contaminated with AMPAR EPSCs and reflected NMDAR EPSCs. Then, we calculated the amplitude ratio of AMPAR EPSCs to NMDAR EPSCs.

Paired-pulse ratio: In order to calculate the paired-pulse ratio (PPR), AMPAR EPSCs were evoked by paired photostimulation (50 ms interval, 0.5 ms duration) of Chronos-expressing presynaptic axons and recorded in BA neurons at −80 mV in voltage-clamp mode. PPR was calculated as the peak amplitude ratio of the averaged AMPAR EPSCs to NMDAR EPSCs. To quantify the rate of NMDAR EPSC decay by MK-801, we calculated decay constant (τ) in stimulus number for each BA neuron by fitting the curve of NMDAR EPSC decrease to a single-exponential equation, \( n(t) = I_e \cdot e^{-t/\tau} \), where \( n \) is stimulus number, \( n(t) \) is the peak amplitude of the nth NMDAR EPSC, and \( I_e \) is the peak amplitude of the first NMDAR EPSC recorded in the presence of MK-801.

Reproducibility. Micrographic images presented in figures are representative ones from at least 3 independent experiments. The injection currents were independently confirmed every 18 times, if (9 times), 2b (13 times), 3b (5 times), 3e (6 times per group), 3h (8 times), 3i (5 times), 5d (5 times), 5e (18 times per group), 6d (3 times), 6i (11 times/BA; 13 times/vCA1), 7c–d (5 times), 7e (12 times per group), 8e–f (10 times/FC; 9 times/HC), 9a (12 times), 9d (7 times), 10d (6 times), 10f (11 times per group), 10k (5 times per group). The ramp injection currents were independently confirmed every 10 times. 1d (18 times), 3b (6 times), 4c–d (5 times), 5a (3 times), 7c (3 times), 8a (5 times), 8b (22 times), 8c (5 times), 10d (12 times), 14c (5 times per group), 16a (6 times), 17b (6 times/Fos-CreERT2; 3 times/Arc-CreERT2), 17c (3 times), 17f (4 times per group), 18c (10 times/FC; 9 times/HC), 19b (3 times), 19c (17 times per group), and 20b (5 times).

Statistical analysis. Data are presented as the means ± the standard error of the mean (SEM) unless indicated otherwise. For statistical comparisons, we used Welch’s t-test or ordinary or repeated measures ANOVA. For post hoc analysis, we used Bonferroni’s simultaneous comparisons. In Fig. 4c, k and Supplementary Figs. 6f, 10e, 11c, nonparametric statistics were used as data did not follow a normal distribution (p < 0.05, Anderson-Darling test). All statistical tests were two-sided. Statistical analysis was performed with Minutab 18 software (Minitab), and p < 0.05 was considered statistically significant. Details of the statistical analyses are summarized in Supplementary Tables 1 and 2.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All data reported in this study are available from the corresponding authors upon request. The source data underlying all Figures and Supplementary Figures are provided as a Source Data file.

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Author contributions
W.B.K. performed experiments, analyzed data, and co-wrote the manuscript. J.-H.C. conceived the study, designed experiments, performed experiments, analyzed data, and co-wrote the manuscript.

Competing interests
The authors declare no competing interests.

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