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Experience-dependent modification of a central amygdala fear circuit

Haohong Li[1,4], Mario A Penzo[1,4], Hiroki Taniguchi[1,3], Charles D Kopec[2], Z Josh Huang[1] & Bo Li[1]

The amygdala is essential for fear learning and expression. The central amygdala (CeA), once viewed as a passive relay between the amygdala complex and downstream fear effectors, has emerged as an active participant in fear conditioning. However, the mechanism by which CeA contributes to the learning and expression of fear is unclear. We found that fear conditioning in mice induced robust plasticity of excitatory synapses onto inhibitory neurons in the lateral subdivision of the CeA (CeL). This experience-dependent plasticity was cell specific, bidirectional and expressed presynaptically by inputs from the lateral amygdala. In particular, preventing synaptic potentiation onto somatostatin-positive neurons impaired fear memory formation. Furthermore, activation of these neurons was necessary for fear memory recall and was sufficient to drive fear responses. Our findings support a model in which fear conditioning–induced synaptic modifications in CeL favor the activation of somatostatin-positive neurons, which inhibit CeL output, thereby disinhibiting the medial subdivision of CeA and releasing fear expression.

Extensive evidence indicates that the amygdala is important for the learning and expression of conditioned fear[1–6]. It is well established that synaptic plasticity in the lateral amygdala is critical for the formation and storage of fear memory[7–13]. More recent studies have shown that the CeA is another amygdala component that is actively involved in fear learning[14–19]. Indeed, pharmacological inactivation of CeA[14,16], or specific inactivation of the CeL[17], during conditioning blocks the formation of fear memory. Moreover, fear conditioning induces changes in CeL neuronal activity such that a population of cells (CeL_{on}) becomes excited, whereas another (CeL_{off}) is inhibited in response to the conditioned stimulus[17–19]. These findings have led to the proposal that activity-dependent synaptic plasticity in CeL stores fear memory and underlies the changes in cellular activity during fear conditioning. Nevertheless, fear conditioning–induced synaptic plasticity has not been observed in CeL.

If the presumed CeL synaptic plasticity stores fear memory, an important question is how the memory trace can be read out and translated into fear responses. The CeL, which is composed of several classes of GABA-producing inhibitory neurons[6,18,20,21], gates fear expression by tonically inhibiting the medial subdivision of CeA (CeM)[17], the major output of amygdala[22]. Synaptic plasticity in distinct CeL cell populations, depending on their largely unknown connectivity, may have different roles in shaping CeL output, and therefore in controlling the function of CeM and the expression of fear[6].

Combining electrophysiological, optogenetic and chemical-genetic methods, we found that experience-dependent synaptic plasticity occurred and stored fear memory in the CeL inhibitory circuits following auditory Pavlovian fear conditioning. We further elucidated features of the functional organization of CeA inhibitory circuitry that allow this synaptic plasticity to serve as a link connecting fear learning and fear expression.

RESULTS

Experience-driven CeL synaptic plasticity

The GABA-producing inhibitory neurons in CeL can be classified on the basis of the distinct neurochemical markers that they express[6,18,20,21]. Among these neurons, somatostatin-positive (SOM^+) neurons[21] constituted a major population and displayed heterogeneous electrophysiological properties (**Fig. 1a** and **Supplementary Fig. 1**)[23]. They were intermingled with SOM-negative (SOM^-) neurons, the majority of which expressed protein kinase C-δ (PKC-δ) (**Supplementary Fig. 1c**). SOM^+ and PKC-δ^+ neurons were largely non-overlapping (13 ± 1% of SOM^+ neurons expressed PKC-δ, $n = 3$ mice, mean ± s.e.m.), and may represent functionally distinct populations that have different roles in fear conditioning. Indeed, PKC-δ^+ cells are mainly CeL_{off} neurons, and selective inhibition of these neurons facilitates fear conditioning[18].

To determine whether synaptic plasticity occurs in CeL in response to fear conditioning, we monitored excitatory synaptic transmission onto different classes of CeL neurons. We used a *Som-IRES-cre* knock-in mouse line, in which Cre is driven by the endogenous *Som* (also known as *Sst*) promoter[24]. When crossed with *Ai14* reporter mice[25], SOM^+ neurons in the resulting *Som-IRES-cre; Ai14* mice were readily identified by their red fluorescence (**Fig. 1a**). This strategy allowed us to examine synaptic transmission onto both the SOM^+ and SOM^- neurons in CeL.

We simultaneously recorded pairs of a SOM^+ (red fluorescent) and an adjacent SOM^- (nonfluorescent) neuron in the CeL in acute brain slices. Excitatory postsynaptic currents (EPSCs) were evoked by a stimulating electrode placed in the lateral amygdala (**Fig. 1b**). Inputs from the basolateral amygdala (BLA), which is important for fear learning[26], may also be recruited by the stimulation. The critical advantage of this simultaneous paired-recording technique

[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA. [2]Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA. [3]Present address: Max Planck Florida Institute for Neuroscience, Jupiter, Florida, USA. [4]These authors contributed equally to this work. Correspondence should be addressed to B.L. (bli@cshl.edu).

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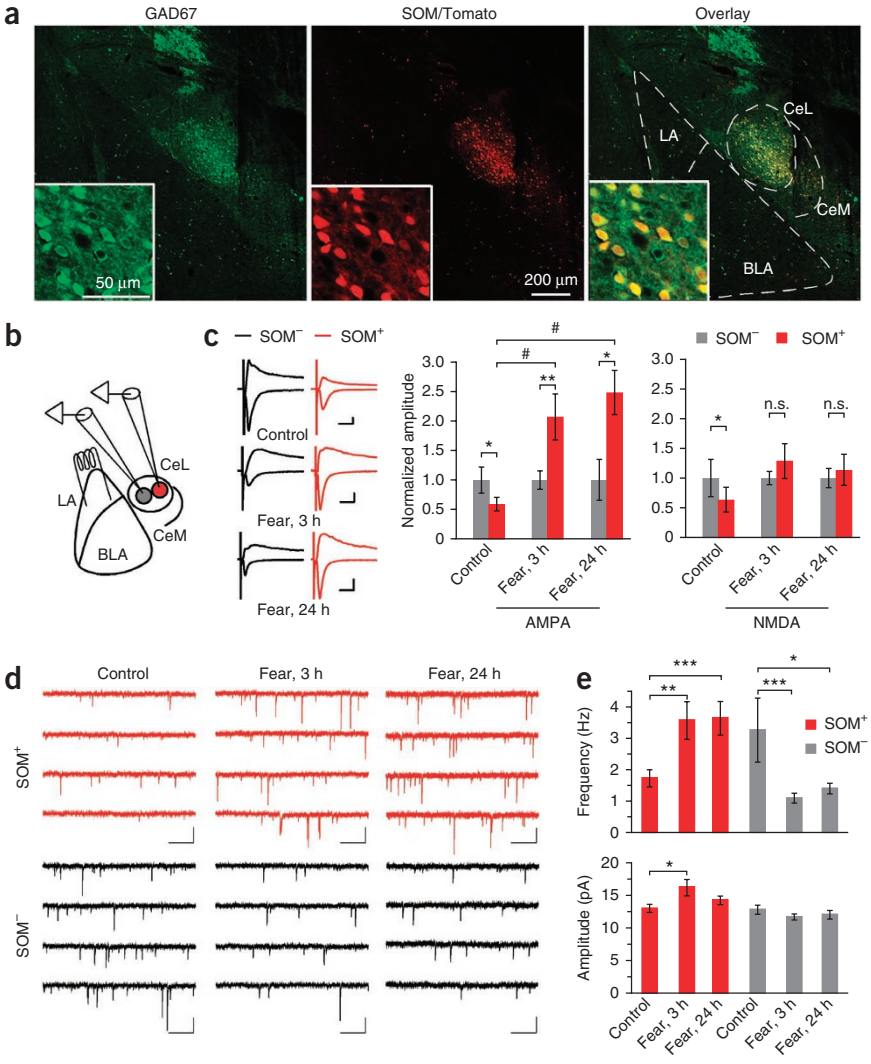

Figure 1 Fear conditioning induces modifications of excitatory synapses onto neurons in CeL. (**a**) A representative coronal brain section from *Som-IRES-cre; Ai14* mice stained with antibody to GAD67 (left). The SOM$^+$ neurons expressed both tdTomato (middle) and GAD67 (see overlay in right). Inset, a portion of CeL shown at higher magnification. The borders of CeL, CeM, lateral amygdala (LA) and BLA are outlined. (**b**) A schematic recording configuration. In red is a SOM$^+$ neuron. (**c**) Left, representative EPSC traces recorded from SOM$^-$ and SOM$^+$ neuronal pairs in CeL of the following groups: control, 3 h following fear conditioning (fear, 3 h) and 24 h following fear conditioning (fear, 24 h). Scale bars represent 50 pA and 20 ms. Middle, quantification of AMPAR-mediated EPSC amplitude, which was normalized to the mean EPSC amplitude of SOM$^-$ neurons. SOM$^+$ neurons had smaller AMPAR-mediated EPSCs than SOM$^-$ neurons in control mice, but this relationship was reversed in fear-conditioned mice (control: SOM$^-$, 1 ± 0.23; SOM$^+$, 0.59 ± 0.12; n = 13 pairs, 4 mice, t = 2.53, degrees of freedom (DF) = 12; fear, 3 h: SOM$^-$, 1 ± 0.16; SOM$^+$, 2.07 ± 0.40; n = 11 pairs, 3 mice, t = –3.42, DF = 10; fear, 24 h: SOM$^-$, 1 ± 0.34; SOM$^+$, 2.49 ± 0.37; n = 8 pairs, 2 mice, t = –2.94, DF = 7). $^*P < 0.05$, paired t test; $^{**}P < 0.01$, paired t test; $^{\#}P < 0.05$, $F_{2,29}$ = 11.7, one-way ANOVA followed by Tukey's test. Right, quantification of NMDAR-mediated EPSCs. SOM$^+$ neurons had smaller NMDAR-mediated EPSCs than SOM$^-$ neurons in control mice, but this difference disappeared in fear-conditioned mice (control: SOM$^-$, 1 ± 0.31; SOM$^+$, 0.63 ± 0.20; n = 13 pairs, 4 mice, t = 2.75, DF = 12; fear, 3 h: SOM$^-$, 1 ± 0.11; SOM$^+$, 1.3 ± 0.29; n = 11 pairs, 3 mice, t = –1.14, DF = 10; fear, 24 h: SOM$^-$, 1 ± 0.16; SOM$^+$, 1.13 ± 0.26; n = 7 pairs, 2 mice, t = –0.40, DF = 6). n.s., non-significant, $P > 0.05$, paired t test. (**d**) Representative mEPSC traces recorded from SOM$^+$ (red) and SOM$^-$ (black) neurons in the CeL of different groups. Scale bars represent 20 pA and 500 ms. (**e**) Top, fear conditioning increased the mEPSC frequency of SOM$^+$ neurons (control: 1.73 ± 0.27 Hz, n = 15 cells, 3 mice; fear, 3 h: 3.58 ± 0.59 Hz, n = 15 cells, 3 mice; fear, 24 h: 3.64 ± 0.53 Hz, n = 12 cells, 3 mice) and decreased that of SOM$^-$ neurons (control: 3.26 ± 1.02 Hz, n = 12 cells, 3 mice; fear, 3 h: 1.11 ± 0.14 Hz; n = 10 cells, 3 mice; fear, 24 h: 1.42 ± 0.16 Hz, n = 13 cells, 3 mice). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, bootstrap with Bonferroni correction. Bottom, fear conditioning increased the mEPSC amplitude of SOM$^+$ neurons at 3 h after fear conditioning (control: 13.03 ± 0.60 pA, n = 15 cells, 3 mice; fear, 3 h: 16.26 ± 1.21 pA, n = 15 cells, 3 mice; fear, 24 h: 14.28 ± 0.63 Hz, n = 12 cells, 3 mice; $^*P < 0.05$, bootstrap with Bonferroni correction), but did not affect that of SOM$^-$ neurons (control: 12.85 ± 0.64 pA, n = 12 cells, 3 mice; fear, 3 h: 11.74 ± 0.44 pA, n = 10 cells, 3 mice; fear, 24 h: 12.06 ± 0.61 Hz, n = 13 cells, 3 mice; $P > 0.05$, bootstrap). Error bars represent s.e.m.

is that it allows the direct comparison of synaptic input strength onto two cells when stimulating the identical group of axons[27]. We measured the amplitude of the evoked synaptic transmission onto both cells and computed its normalized values, which represent the true difference between cells in a pair (**Fig. 1c**).

In control mice, both AMPA receptor (AMPAR)- and NMDA receptor (NMDAR)-mediated EPSCs recorded from SOM$^+$ cells were significantly ($P < 0.05$) smaller than those from SOM$^-$ neurons (**Fig. 1c**), indicating that the strength of excitatory synapses onto SOM$^+$ neurons is weaker than those onto SOM$^-$ neurons under basal condition. Notably, in fear-conditioned mice, the strength of excitatory synapses onto these two populations of neurons, measured at either 3 or 24 h after conditioning, was markedly altered, such that AMPAR-mediated transmission onto SOM$^+$ neurons became much stronger than that onto SOM$^-$ neurons (**Fig. 1c** and **Supplementary Figs. 2** and **3**). NMDAR-mediated transmission onto SOM$^+$ neurons was also enhanced relative to that onto the SOM$^-$ neurons, albeit to a lesser extent.

The reversal of the relative strength of excitatory synaptic transmission onto SOM$^+$ versus SOM$^-$ neurons following fear conditioning could be a result of an increase in transmission onto SOM$^+$ neurons, a reduction of transmission onto SOM$^-$ neurons or a combination of both. To distinguish between these possibilities, we recorded miniature EPSCs (mEPSCs), in the presence of tetrodotoxin to block action potentials and picrotoxin to block $GABA_A$-mediated synaptic currents, from both SOM$^+$ and SOM$^-$ CeL neurons. Fear conditioning increased the frequency of mEPSCs recorded from SOM$^+$ CeL neurons at both 3 and 24 h following conditioning (**Fig. 1d**,**e** and **Supplementary Fig. 2**). It also increased the amplitude of mEPSCs in these neurons (**Fig. 1e**). In contrast, fear conditioning decreased the frequency of mEPSCs recorded from SOM$^-$ CeL neurons, without appreciably affecting their amplitude (mEPSC frequency, $F_{2,71} = 8.62$, $P < 0.001$,

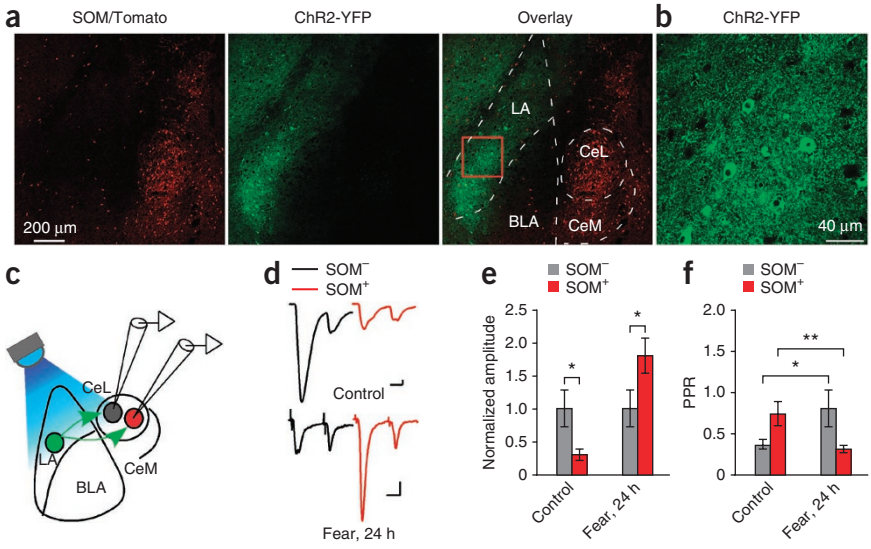

Figure 2 The fear conditioning–induced synaptic modifications in CeL are expressed presynaptically at the lateral amygdala–CeL pathway. (**a**) Confocal images of a coronal brain section, which was recovered after electrophysiological recording, from a *Som-IRES-cre; Ai14* mouse injected with AAV-CAG-ChR2(H134R)-YFP into the lateral amygdala (middle). The SOM$^+$ neurons expressed tdTomato (left, and overlay in right). The borders of CeL, CeM, lateral amygdala and BLA are outlined. (**b**) ChR2-YFP was expressed in many lateral amygdala neurons. A higher magnification image of the boxed region in **a** (see overlay) is shown. (**c**) A schematic recording configuration. A ChR2-YFP$^+$ neuron in lateral amygdala is shown in green and a SOM$^+$ neuron in CeL is shown in red. A blue LED (λ = 470 nm) was used to activate ChR2 and evoke the lateral amygdala neuron–driven synaptic transmission, which was simultaneously recorded from SOM$^-$ and SOM$^+$ neuronal pairs in CeL. (**d**) Representative EPSCs, which were evoked by light stimulation of the lateral amygdala–CeL pathway, were recorded from SOM$^-$ and SOM$^+$ neuronal pairs in the CeL, in control (top) and 24 h following fear conditioning (bottom) groups. A paired-pulse stimulation protocol (50-ms inter-stimulus interval) was used. Scale bars represent 20 pA and 20 ms. (**e**) Quantification of AMPAR-mediated EPSC amplitude (peak of the EPSC in response to the first pulse in the paired pulse), which was normalized to the mean EPSC amplitude of SOM$^-$ neurons. SOM$^+$ neurons had smaller EPSCs than SOM$^-$ neurons in control mice (control: SOM$^-$, 1 ± 0.28; SOM$^+$, 0.3 ± 0.08; n = 14 pairs, 2 mice, t = 2.98, DF = 13), but this relationship was reversed in fear-conditioned mice (fear, 24 h: SOM$^-$, 1 ± 0.19; SOM$^+$, 1.8 ± 0.27; n = 28 pairs, 3 mice, t = –2.42, DF = 27). $^*P < 0.05$, paired t test. (**f**) Quantification of PPR. SOM$^-$ neurons increased, whereas SOM$^+$ neurons decreased, PPR after fear conditioning (SOM$^-$, control: 0.37 ± 0.06, n = 14 cells, 2 mice, fear, 24 h: 0.99 ± 0.36, n = 22 cells, 3 mice; SOM$^+$, control: 0.74 ± 0.15, n = 11 cells, 2 mice, fear, 24 h: 0.31 ± 0.04, n = 26 cells, 3 mice). $^*P < 0.05$, $^{**}P < 0.01$, bootstrap. Error bars represent s.e.m.

two-way analysis of variance (ANOVA); mEPSC amplitude, $F_{2,71}$ = 3.72, $P < 0.05$, two-way ANOVA; **Fig. 1d,e**). These results demonstrate that fear conditioning strengthened the excitatory synapses onto SOM$^+$ neurons while weakening those onto SOM$^-$ neurons in the CeL.

To determine whether the synaptic modifications in CeL neurons occurred in synapses driven by inputs originating from, or axons passing through, lateral amygdala, we injected lateral amygdala with an adeno-associated virus, AAV-CAG-ChR2(H134R)-YFP, that

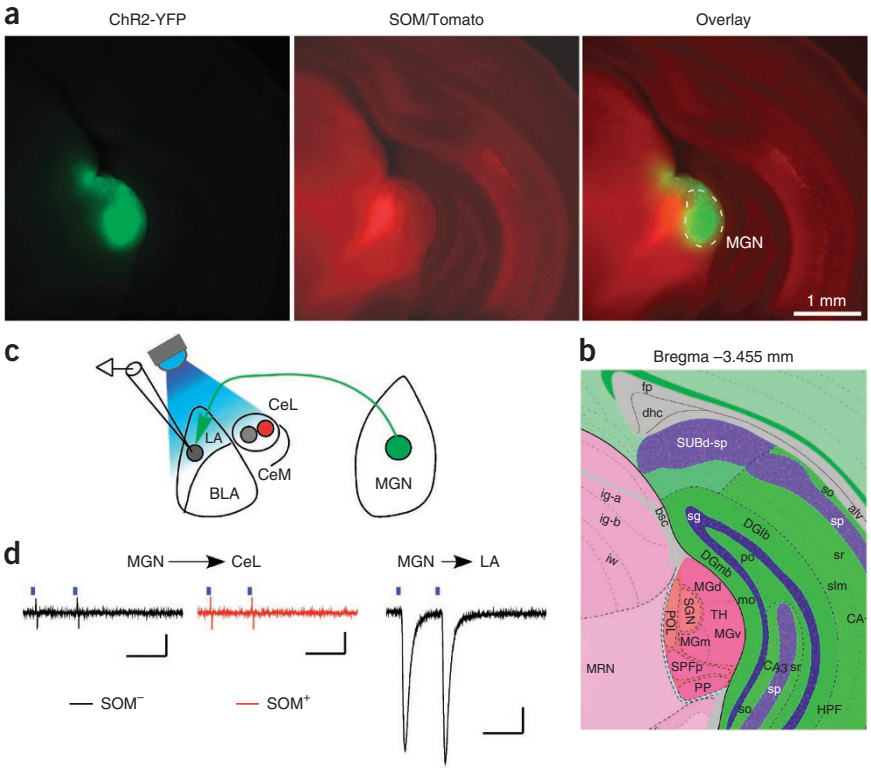

Figure 3 Auditory thalamus does not drive excitatory synaptic transmission onto CeL neurons. (**a**) Images of a coronal brain section that was recovered from a brain used for making acute slices for recording, from a *Som-IRES-cre; Ai14* mouse injected with AAV-CAG-ChR2(H134R)-YFP into the medial geniculate nucleus (MGN) (left, and overlay in the right). Images were taken using an Olympus BX41 histology microscope with a 5× lens. Note the area infected with the virus was large and covered the entire MGN. (**b**) A section of a brain atlas that contains the auditory thalamus, which is at Bregma –3.455 mm, a level approximately the same as that of the brain section shown in **a**. The atlas is adapted from the Allen Mouse Brain Atlas (http://mouse.brain-map.org/). (**c**) A schematic recording configuration. A ChR2-positive neuron in MGN is shown in green and a SOM$^+$ neuron in CeL is shown in red. (**d**) Left, representative EPSC traces recorded from a SOM$^-$ (black) and a SOM$^+$ (red) neuron in the CeL. Out of 11 SOM$^-$ (2 mice) and 11 SOM$^+$ (2 mice) neurons recorded in CeL, none had any measurable EPSCs. Right, a representative EPSC trace recorded from a lateral amygdala neuron in the same slice as that shown on the left. EPSCs could be readily evoked for all 17 neurons (2 mice) recorded in lateral amygdala (EPSC amplitude: 154.2 ± 36.6 pA, mean ± s.e.m.). Blue bars indicate light pulses (2 ms). Scale bars represent 20 pA and 50 ms.

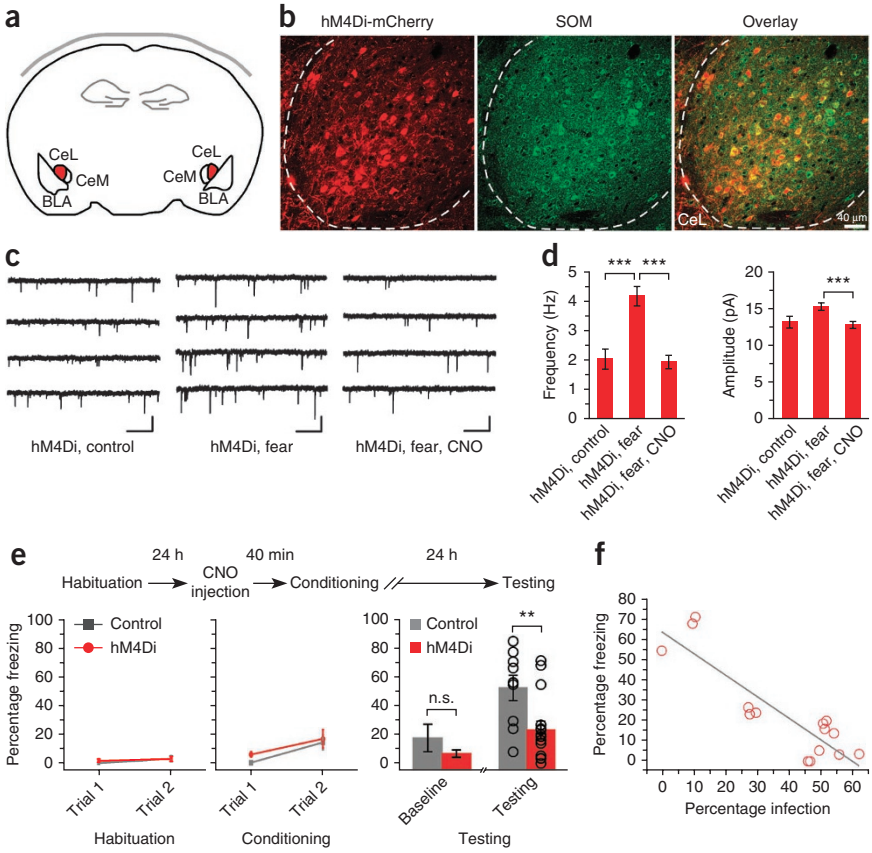

Figure 4 Synaptic potentiation onto SOM$^+$ neurons in CeL is required for the formation of fear memory. The *Som-IRES-cre* mice were used in these experiments. (**a**) A schematic experimental design. hM4Di-mCherry was expressed in SOM$^+$ neurons in CeL (shown in red) by viral infection. (**b**) An example image of the SOM$^+$ neurons in CeL infected with the AAV-DIO-hM4Di-mCherry virus. Left, expression of hM4Di (red) was detected on the basis of the intrinsic fluorescence of mCherry. Middle, SOM$^+$ neurons (green) were recognized by an antibody to SOM. Right, only SOM$^+$ neurons expressed hM4Di, as indicated by the overlapping of red and green signals. Dashed lines mark the border of CeL. (**c**) Representative mEPSC traces recorded from SOM$^+$ CeL neurons expressing hM4Di-mCherry. For the fear-conditioning groups, acute brain slices were prepared 3 h following fear conditioning. Scale bars represent 20 pA and 500 ms. (**d**) Left, CNO pre-treatment suppressed the fear conditioning–induced increase in mEPSC frequency in SOM$^+$ neurons that expressed hM4Di (control: 2.02 ± 0.35 Hz, $n = 11$ cells, 3 mice; fear: 4.18 ± 0.33 Hz, $n = 15$ cells, 3 mice; fear, CNO: 1.93 ± 0.22 Hz, $n = 12$ cells, 3 mice). $^{***}P < 0.001$, bootstrap with Bonferroni correction. Right, CNO pre-treatment also suppressed the fear conditioning–induced increase in mEPSC amplitude in SOM$^+$ neurons that expressed hM4Di (control: 13.1 ± 0.81 Hz, $n = 11$ cells, 3 mice; fear: 15.24 ± 0.51 Hz, $n = 15$ cells, 3 mice; fear, CNO: 12.75 ± 0.47 Hz, $n = 12$ cells, 3 mice). $^{***}P < 0.001$, bootstrap with Bonferroni correction. (**e**) Top, a schematic experimental procedure. Bottom left and middle, freezing behavior during habituation and conditioning. Control: *Som-IRES-cre* mice that received AAV-DIO-GFP injection bilaterally into CeL. hM4Di: *Som-IRES-cre* mice that received AAV-DIO-hM4Di injection bilaterally into CeL. Freezing responses were similar for the two groups at the end of conditioning (control, 14.2 ± 4.9%, $n = 9$ mice; hM4Di, 16.6 ± 6.1%, $n = 15$ mice; $P > 0.05$, t test). Bottom right, the hM4Di mice showed impaired fear memory recall compared with control (control, 52.36 ± 8.76%, $n = 9$ mice; hM4Di, 23.24 ± 6.05%, $n = 15$ mice; $t = 2.82$, DF = 22). $^{**}P < 0.01$, t test. The two groups did not differ significantly in their baseline freezing levels (control, 17.56 ± 9.62%, $n = 9$ mice; hM4Di, 6.67 ± 2.37%, $n = 15$ mice; $P > 0.05$, t test). (**f**) The freezing level of individual animals correlated with the extent of infection, measured as the fraction of SOM$^+$ CeL neurons that expressed hMD4i-mCherry ($R^2 = 0.76$, $F_{1,13} = 45.62$, gray line; $P < 0.001$ by a linear regression; $n = 15$ mice). The extent of infection for the left and right CeL was averaged. Error bars represent s.e.m.

expresses channelrhodopsin-2 (ChR2), which can activate neurons in response to light[28]. ChR2 was mainly expressed in lateral amygdala neurons as a result of targeted viral injection (**Fig. 2**). Excitatory synaptic transmission onto CeL neurons was reliably evoked by light (**Fig. 2**), consistent with the existence of anatomical connection from lateral amygdala to CeL[29]. In control mice, the light-evoked EPSCs in SOM$^+$ CeL neurons were much smaller than those in the simultaneously recorded SOM$^-$ CeL neurons; however, 24 h after fear conditioning, this relationship was reversed, and the EPSCs in SOM$^+$ CeL neurons became larger (**Fig. 2** and **Supplementary Figs. 2** and **4**). These results demonstrate that the fear conditioning–induced synaptic plasticity in CeL (**Fig. 1**) is located at synapses driven by the inputs from lateral amygdala. On the other hand, ChR2 stimulation of axons originating from the auditory thalamus, another potential source of input to CeL[30,31], failed to evoke any detectable excitatory synaptic transmission onto CeL neurons, although it did evoke transmission onto lateral amygdala neurons (**Fig. 3**). These results suggest that the fear conditioning–induced synaptic plasticity in CeL is in series with that in the lateral amygdala[16].

To probe the nature of the fear conditioning–induced synaptic plasticity in the lateral amygdala–CeL pathway, we employed a paired-pulse stimulation protocol using light to evoke transmission (**Fig. 2**). The paired pulse ratio (PPR), measured as the amplitude of the second EPSC relative to that of the first in response to the paired-pulse stimulation, reflects presynaptic release probability; a lower PPR correlates with higher release probability[32]. In naive mice, SOM$^+$ CeL neurons had higher PPRs than SOM$^-$ neurons (**Fig. 2**). Notably, after fear conditioning, SOM$^+$ CeL neurons showed a marked decrease in PPR, whereas SOM$^-$ neurons showed an increase in PPR (**Fig. 2**). These results corroborate the changes in mEPSC frequency (**Figs. 1d,e** and **4**), and indicate that changes in presynaptic release probability can, at least in part, account for the fear conditioning–induced synaptic plasticity in CeL. Because fear conditioning also increased the amplitude of mEPSC in SOM$^+$ CeL neurons (**Figs. 1e** and **4**), an additional postsynaptic process likely contributes to the enhancement of excitatory synaptic transmission onto these neurons.

CeL synaptic potentiation stores memory

We wished to test whether the fear conditioning–induced synaptic plasticity in CeL is essential for the storage of fear memory. Synaptic plasticity, including both long-term potentiation and long-term depression of synaptic transmission, can be induced *in vitro* in CeL neurons[33,34] and is, in general, dependent on postsynaptic neuronal activation[35]. To specifically test whether the fear conditioning–induced synaptic strengthening onto SOM$^+$ CeL neurons is dependent on postsynaptic

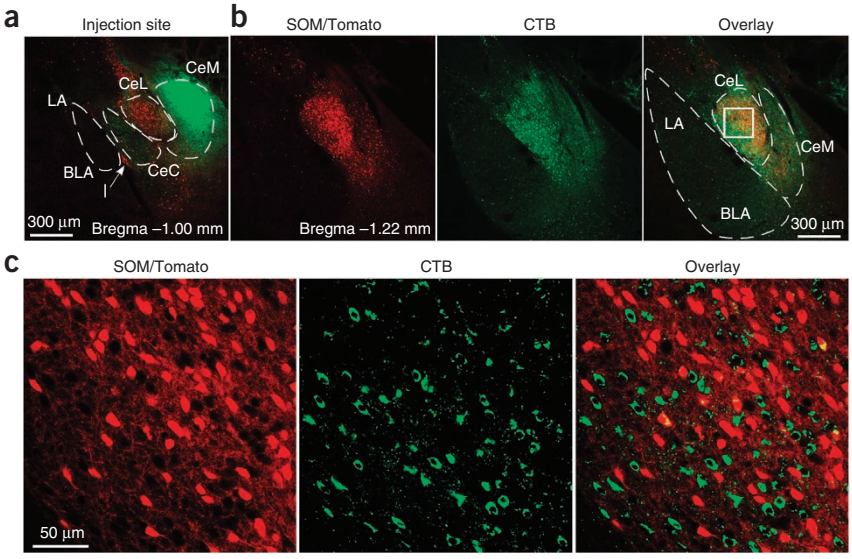

Figure 5 SOM+ CeL neurons do not project to CeM. (**a**) CTB (green) was injected into the CeM of a *Som-IRES-cre; Ai14* mouse (similar results were obtained from 2 mice). Shown is a coronal brain section (at ~Bregma –1.00 mm). (**b**) Images of a brain section from the same mouse (at ~Bregma –1.22 mm). Left, the SOM+ neurons expressed tdTomato (red). Middle, extensive labeling by CTB (green) was seen in CeL. Right, overlay. (**c**) Higher magnification images of the boxed region in CeL in **b**. The vast majority of the CTB-labeled neurons (green) in CeL were not SOM+ (red; see overlay at right).

activity and whether it is required for fear memory formation, we selectively suppressed SOM+ neurons in CeL during learning via a chemical-genetic method[36]. We bilaterally injected the CeL of *Som-IRES-cre* mice with an adeno-associated virus, AAV-DIO-hM4Di-mCherry, that expresses hM4Di, an engineered inhibitory G protein–coupled receptor that can suppress neuronal activity[36,37], in a Cre-dependent manner (**Fig. 4a**,**b** and **Supplementary Fig. 5a**,**b**). Two to three weeks after surgery, mice received treatment with CNO, the agonist of hM4Di, followed by fear conditioning (**Fig. 4** and **Supplementary Fig. 5**).

Selective suppression of SOM+ CeL neurons by hM4Di during conditioning completely abolished the fear conditioning–induced synaptic strengthening (**Figs. 1d**,**e** and **4c**,**d** and **Supplementary Fig. 2**), and markedly impaired fear memory, which was measured as a reduction in the freezing behavior that is characteristic of fear[22], in response to the conditioned stimulus 24 h after conditioning (**Fig. 4e** and **Supplementary Fig. 5c**). Furthermore, the impairment of fear memory was significantly ($P < 0.001$) correlated with the extent of infection of SOM+ CeL neurons with AAV-DIO-hM4Di-mCherry (**Fig. 4f**). Notably, hM4Di-mCherry was selectively expressed in the SOM+ neurons in CeL (**Fig. 4b** and **Supplementary Fig. 5a**), and activation of hM4Di by CNO reversibly induced membrane hyperpolarization and suppressed neuronal firing (see Online Methods). These results indicate that the fear conditioning–induced synaptic strengthening onto SOM+ CeL neurons is dependent on postsynaptic activity and that SOM+ CeL neurons are required for fear learning. The most parsimonious explanation for these results is that the experience- and activity-dependent strengthening of excitatory synapses onto SOM+ CeL neurons (**Figs. 1**, **2** and **4**) is necessary for the formation and storage of fear memory.

The organization of CeA circuits

CeL tonically inhibits CeM[6,17–20,38], the main output nucleus of the amygdala[22], thereby gating the expression of fear[17,18]. To understand

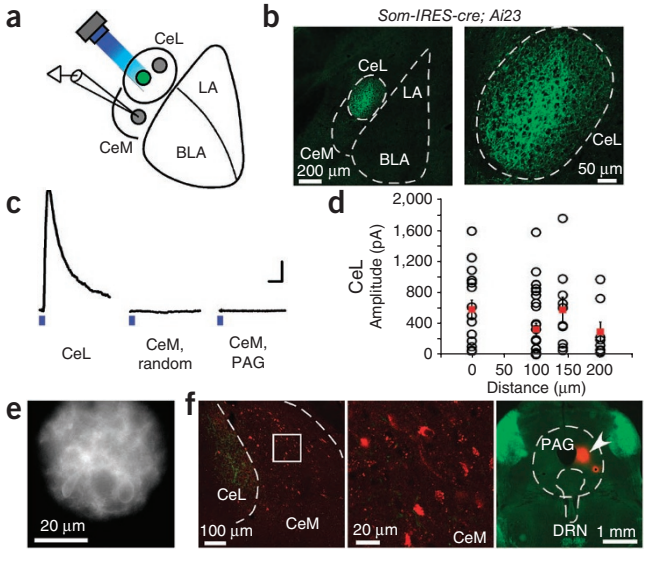

Figure 6 SOM+ CeL neurons do not inhibit CeM neurons that project to PAG. (**a**) A schematic recording configuration. A SOM+ neuron that expresses ChR2-YFP is shown in green. Recordings were made onto CeL or CeM neurons that did (SOM+ cells) or did not (SOM− cells) express ChR2-YFP. Holding potential was set at 0 mV, which is the reversal potential of ChR2. A blue LED ($\lambda = 470$ nm), the beam size of which was restricted to ~50 µm in diameter by a shutter (see **e**), was used to activate ChR2-expressing cells in multiple locations that together covered the entire CeL. (**b**) A coronal brain section from a *Som-IRES-cre; Ai32* mouse in which the ChR2-YFP was specifically and uniformly expressed in SOM+ cells. Prominent labeling with ChR2-YFP was seen in cell bodies and fibers in CeL. Weak labeling of fibers was occasionally observed in CeM. Right, higher magnification image of the CeL area. (**c**) Representative IPSC traces recorded from a CeL neuron, a randomly recorded CeM neuron (CeM, random) and a PAG-projecting CeM neuron (CeM, PAG). IPSCs were evoked by 2-ms light pulses (denoted by the blue bars) from an LED set at a constant power for all recordings. Scale bars represent 100 pA and 50 ms. (**d**) Quantification of the amplitude of IPSCs in CeL neurons in response to the focal stimulation of SOM+ cells in different locations of CeL. *x* axis is the distances (in µm) between the soma of the recorded cells and the center of stimulation. Each circle represents an IPSC of one cell in response to the stimulation of one location (red squares, mean ± s.e.m., $n = 17$ cells, 3 mice). All neurons recorded in CeL showed robust IPSCs for all stimulation locations. In contrast, only 4 of 40 randomly recorded CeM cells (from 3 mice) had measurable IPSCs (data not shown), whereas none (0 of 16 cells, 1 mouse) of the PAG-projecting CeM cells showed any measurable IPSCs. (**e**) An image showing the field of illumination by the LED light beam used for the light stimulation in CeL. The ChR2-YFP–expressing cells are clearly visible. (**f**) Left, image of a coronal brain section containing CeM. The brain section was prepared from a *Som-IRES-cre; Ai32* mouse, in which the CTB conjugated to the dye Alexa Fluor 594 was injected into the PAG. Middle, the boxed region in CeM is shown in higher magnification. The CTB-labeled PAG-projecting neurons are red fluorescent. Right, an image of the brain section containing PAG. Arrowhead indicates the site of CTB injection. DRN, dorsal raphe nucleus. Error bars represent s.e.m.

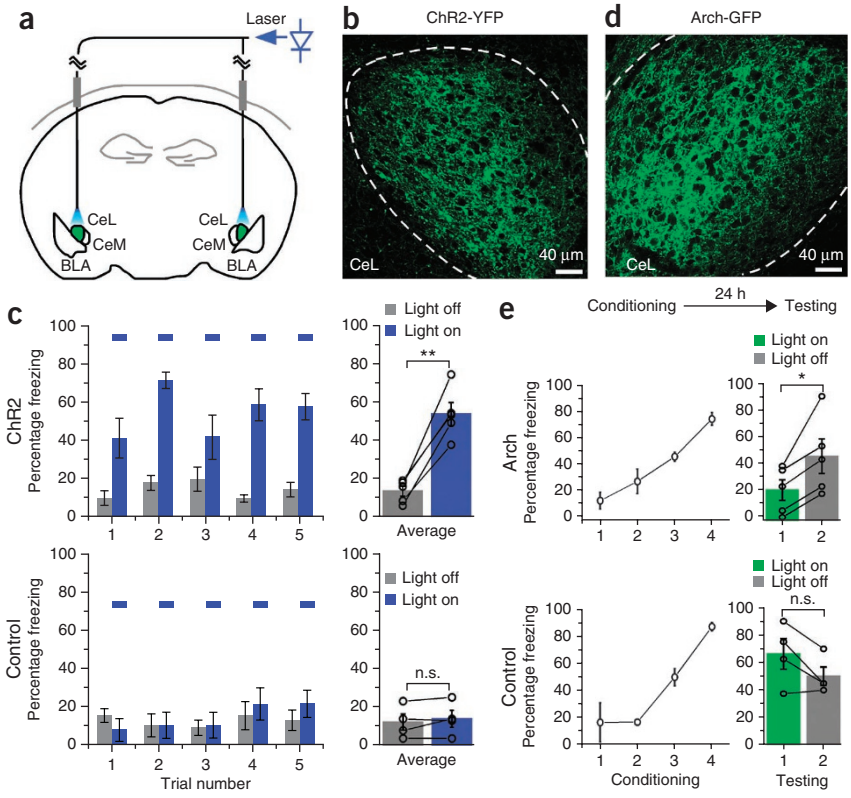

Figure 7 SOM$^+$ neurons in CeL control the expression of fear. The *Som-IRES-cre* mice were used in these experiments. (**a**) A schematic diagram showing the experimental design. ChR2-YFP (or Arch-GFP) was expressed in SOM$^+$ neurons in CeL (shown in green) by viral infection. Optic fibers were chronically implanted bilaterally in CeL and were connected to a blue (for ChR2 activation) or a green (for Arch activation, not shown) laser source. (**b**) An example image of the SOM$^+$ neurons in CeL infected with AAV-DIO-ChR2(H134R)-YFP. Dashed lines mark the border of CeL. (**c**) Activation of SOM$^+$ CeL neurons induced fear-like responses in naive mice. Left, freezing behavior was measured, in repeated trials, before and during the delivery of blue light pulses (blue bars) into the CeL. Delivery of light into CeL induced freezing behavior in mice injected with AAV-DIO-ChR2(H134R)-YFP (ChR2 group, top), but not in animals injected with AAV-GFP (control group, bottom). Right, level of freezing was averaged for ChR2 mice (top; light off, 13.50 ± 2.63%; light on, 53.50 ± 5.90%; $n = 5$ animals, $t = -7.95$, DF = 4, $^{**}P < 0.01$, paired t test) and control mice (bottom; light off, 12.25 ± 4.13%; light on, 14.00 ± 4.30%; $n = 4$ mice, $t = -1.13$, DF = 3, $P > 0.05$, paired t test). (**d**) An example of the SOM$^+$ neurons in CeL infected with AAV-DIO-Arch-GFP. Dashed lines mark the border of CeL. (**e**) Inhibition of SOM$^+$ CeL neurons suppressed conditioned fear expression. Top, a schematic experimental procedure, in which mice were first trained with the fear-conditioning procedure and tested 24 h later for fear memory recall. Middle and bottom, mice injected with AAV-DIO-Arch-GFP (Arch group, middle) or AAV-GFP (control group, bottom) were fear conditioned (left) and then tested (right). During testing, conditioned freezing was measured in two trials, the first of which was conducted during the delivery of a green light into CeL (light on). Delivery of light into CeL suppressed conditioned freezing in the Arch group (light on, 20.20 ± 7.69%; light off, 45.3 ± 12.91%; $n = 5$ mice, $t = -3.72$, DF = 4, $^*P < 0.05$, paired t test), but not in the control group (light on, 66.25 ± 11.11%; light off, 50.00 ± 6.77%; $n = 4$ mice, $t = 2.39$, DF = 3, $P > 0.05$, paired t test). n.s., not significant ($P > 0.05$). Error bars represent s.e.m.

how the fear conditioning–induced modifications of CeL circuits can be read out and used to control fear expression, we examined the organization of CeA inhibitory circuitry. We injected the retrograde tracer cholera-toxin B (CTB) into CeM of *Som-IRES-cre; Ai14* mice (**Fig. 5** and **Supplementary Fig. 6**). CTB extensively labeled CeL neurons, revealing their direct projection to CeM (**Fig. 5b**,**c**). Notably, only 15.7 ± 3.8% of the CTB-labeled neurons in CeL expressed SOM (**Fig. 5c**; similar results were obtained from two mice), indicating that the majority (~85%) of CeM-projecting neurons in CeL are SOM$^-$ cells. This result is likely an overestimation of the contribution of SOM$^+$ neurons to the CeM-projecting cell population, as CTB can leak into CeL from the adjacent CeM. Consistent with the above observations, axonal fibers, which can be readily followed from neurons expressing ChR2-YFP, originating from SOM$^+$ CeL neurons, occupied and filled the entire CeL, but not CeM (**Fig. 6** and **Supplementary Fig. 7b**). These results suggest that the vast majority of SOM$^+$ CeL neurons do not directly inhibit CeM.

To directly assess the spatial range of SOM$^+$ CeL neuron-mediated inhibition, we crossed the *Som-IRES-cre* mice with *Ai32* mice, which express ChR2-YFP in a Cre-dependent manner[39]. In the resulting *Som-IRES-cre; Ai32* mice, ChR2-YFP was selectively and uniformly expressed in SOM$^+$ neurons (**Fig. 6**). We focally stimulated SOM$^+$ neurons by shining light onto small areas, ~50 μm in diameter, in CeL in acute slices prepared from these mice (**Fig. 6a–e**). For each slice, we systematically stimulated multiple areas that together covered the entire CeL (**Fig. 4a**,**e**). Inhibitory postsynaptic currents (IPSCs) in response to the light stimulation were recorded from neurons in either CeL or CeM in the same slice. Robust IPSCs were detected in all of the recorded CeL neurons, including both SOM$^+$ neurons (identified by the expression of ChR2-YFP) and SOM$^-$ neurons (identified by the lack of ChR2-YFP). Moreover, all CeL neurons responded to the stimulation of every CeL location (335 ± 93 pA, $n = 17$ cells, 3 mice, mean ± s.e.m.; **Fig. 6c**,**d**). Consistent results were also obtained in a complementary experiment (**Supplementary Fig. 7**). These IPSCs were not driven by neurons from the lateral amygdala or BLA, as SOM$^+$ neurons in these areas did not synapse onto CeL neurons (**Supplementary Fig. 8**). These results, together with the finding that PKC-δ$^+$ neurons, which are the major SOM$^-$ neurons in CeL (**Supplementary Fig. 1c**), inhibit PKC-δ$^-$ neurons[18], indicate that SOM$^+$ and SOM$^-$ neurons in CeL mutually inhibit.

In contrast with the neurons in CeL, only 10% (4 of 40) of randomly recorded neurons in the CeM showed detectable IPSCs, which were rather small (24 ± 9 pA, $n = 4$ cells, 3 mice, mean ± s.e.m.; **Fig. 6c**), and these neurons did not respond to the stimulation of all CeL locations. Notably, none (0 of 16) of the retrogradely labeled periaqueductal gray (PAG)-projecting CeM neurons responded to the same stimulation with any measurable IPSC (**Fig. 6c**,**f**). Thus, these results demonstrate that SOM$^+$ CeL neurons provide potent inhibition in CeL, but do not appreciably inhibit CeM neurons. In particular, they do not inhibit the PAG-projecting CeM neurons. These results also indicate that

SOM$^+$ and SOM$^-$ CeL neurons have different connectivity, as the PKC-δ$^+$ (and thus SOM$^-$) CeL neurons inhibit all of the identified PAG-projecting CeM neurons[18].

SOM$^+$ CeL neurons control fear expression

It has been shown that pharmacological inhibition of CeL elicits freezing behavior through the disinhibition of CeM[17]. Our results indicate that SOM$^+$ CeL neurons can inhibit CeL output via local inhibition and that they do not inhibit CeM neurons that project to PAG (**Figs. 5** and **6** and **Supplementary Fig. 7**), the effector that triggers freezing behavior[22]. On the basis of these findings, we reasoned that activation of SOM$^+$ neurons might be sufficient to induce freezing behavior in naive mice. To test this hypothesis, we selectively expressed ChR2 in SOM$^+$ neurons by injecting AAV-DIO-ChR2(H134R)-YFP bilaterally into the CeL of *Som-IRES-cre* mice (**Fig. 7a**,**b** and **Supplementary Fig. 7b**). Optic fibers were implanted bilaterally into CeL to allow the activation of ChR2 in behaving mice with a blue laser (**Fig. 7a**)[40,41]. Light activation of SOM$^+$ neurons in CeL of naive freely moving mice induced robust freezing that disappeared on the cessation of light (**Fig. 7c** and **Supplementary Movie 1**), indicating that activation of SOM$^+$ neurons in CeL is sufficient to induce a fear-like response.

We reasoned that activation of SOM$^+$ CeL neurons might also mediate conditioned fear responses. This is because, following fear conditioning, the strengthening of the excitatory synapses onto SOM$^+$ CeL neurons and the weakening of those onto SOM$^-$ CeL neurons altered the balance of excitation onto these two populations, favoring the activation of SOM$^+$ neurons in response to excitatory synaptic inputs (**Figs. 1** and **2** and **Supplementary Figs. 3** and **4**). Indeed, SOM$^+$ CeL neurons were preferentially activated in fear-conditioned mice in response to conditioned stimulus, as measured by the expression of c-Fos (**Supplementary Fig. 9**), a marker of neuronal activation[42].

To test whether the activation of SOM$^+$ CeL neurons in fear-conditioned mice is required for the expression of learned fear in response to conditioned stimulus presentations, we inhibited these neurons during fear memory recall. To achieve this goal, we selectively expressed Archaerhodopsin (Arch), the light-sensitive inhibitory proton pump[43] (**Supplementary Fig. 10**), in SOM$^+$ neurons by injecting AAV-DIO-Arch-GFP bilaterally into CeL of *Som-IRES-cre* mice (**Fig. 7a**,**d**). Optic fibers were implanted bilaterally into CeL to allow the activation of Arch with a green laser (**Fig. 7a**). Mice were fear conditioned and then tested 24 h later for fear memory recall (**Fig. 7e**). Light-induced inhibition of SOM$^+$ neurons in CeL suppressed the conditioned freezing behavior, which was subsequently revealed following the cessation of light (**Fig. 7e** and **Supplementary Movie 2**). Together, these results suggest that activation of SOM$^+$ neurons in CeL is not only sufficient to drive freezing behavior, but is also necessary for the expression of conditioned fear.

DISCUSSION

We examined the manner in which the inhibitory circuits of CeL respond to fear conditioning and contribute to both the learning and expression of fear. Fear conditioning potentiated the excitatory synaptic transmission onto SOM$^+$ CeL neurons while weakening that onto SOM$^-$ CeL neurons. These modifications occurred, largely through a presynaptic mechanism, in synapses driven by the inputs from lateral amygdala. The opposing, cell-specific changes rendered the SOM$^+$ neurons more sensitive to excitatory synaptic inputs than SOM$^-$ neurons, reversing the relationship found in naive mice. Given that CeL neurons exhibited mutual inhibition, the fear conditioning–induced synaptic modifications biased the competition between mutually inhibitory CeL populations for excitatory inputs, and SOM$^+$ neurons were preferentially activated. Once activated, SOM$^+$ neurons were sufficient to release fear responses, an outcome that is explained by the capacity of these neurons to inhibit CeL output without inhibiting the PAG-projecting CeM neurons. These results are consistent with, and complement, the finding that pharmacological inactivation of CeL disinhibits CeM and elicits freezing behavior[17].

Although fear conditioning modifies multiple synapses, the fear conditioning–induced potentiation of excitatory synaptic transmission onto SOM$^+$ CeL neurons appeared to be crucial, as suppression of this potentiation severely impaired fear memory. The synaptic potentiation was detected at 3 h and persisted for at least 24 h following fear conditioning, suggesting that it is involved in both fear memory acquisition and consolidation[8]. Thus, our results support the notion that the experience-dependent strengthening of excitatory synapses onto SOM$^+$ CeL inhibitory neurons stores fear memory and enables the expression of conditioned fear.

Our results are consistent with a model in which CeA stores fear memory in series with lateral amygdala[15–17]. Such serial organization of fear memory allows the regulation of fear conditioning at multiple levels. Moreover, as transmission was potentiated following fear conditioning, both at the auditory thalamus–lateral amygdala synapses[44] and at the lateral amygdala–CeL synapses, the signal carrying conditioned stimulus information can be reliably transmitted from the auditory thalamus to CeA via lateral amygdala while maintaining specificity. Parallel pathways may also participate in fear conditioning. For example, inputs from the brainstem parabrachial nucleus or the insula cortex to CeL may be recruited and be involved in fear conditioning.

Our findings delineate cellular and circuit mechanisms that may explain previously reported observations. First, pharmacological inactivation of CeL during conditioning impairs fear learning[14,16,17]. Second, fear conditioning is followed by the appearance of two functionally distinct cell populations in CeL, the CeL_{on} and CeL_{off} neurons, which show opposite responses to conditioned stimulus[17–19]. Third, the appearance of CeL_{on} neurons is associated with CeM activation, rather than inhibition[17]. Further studies will be required to elucidate the detailed cellular and molecular changes in distinct CeL inhibitory circuits during fear conditioning and to determine how they are related to fear memory acquisition, consolidation and expression.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

H.L. and M.A.P. performed the experiments. H.L., M.A.P. and B.L. analyzed the data. H.T. and Z.J.H. provided critical reagents and advice. C.D.K. developed the

MatLab programs for statistical (bootstrap) and behavioral analysis. H.L., M.A.P. and B.L. designed the study. B.L. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. Mice were group-housed under a 12-h light-dark cycle (9 a.m. to 9 p.m. light), with food and water freely available. Only animals with optic fiber implants were housed singly. The *Som-IRES-cre* mice and the *Rosa26-loxP-STOP-loxP-H2B-GFP* reporter line were generated as described[24,45]. The *Ai14* reporter mice[25] and *Ai32* mice[39] were purchased from the Jackson Laboratory. All mice were bred onto C57BL/6J genetic background. Male and female mice of 40–60 d of age were used for all the experiments. All procedures involving animals were approved by the Institute Animal Care and Use Committees of Cold Spring Harbor Laboratory and carried out in accordance with US National Institutes of Health standards.

Immunohistochemistry. Immunohistochemistry experiments were performed following standard procedures[46]. Images were taken using a LSM 710 laser-scanning confocal microscope (Carl Zeiss). For primary antibodies, we used antibodies to PKC-δ (mouse, BD Biosciences, cat. no. 610397, 1:500), somatostatin (rabbit, Bachem cat. no. T4103, 1:2,000), GAD67 (mouse, Millipore cat. no. MAB5406, 1:800) and c-Fos (rabbit, Santa Cruz cat. no. sc-52, 1:2,500).

Fear conditioning. Fear-conditioning procedures were performed as previously described[47]. Briefly, mice were first handled and habituated to the conditioning cage and testing cage. Habituation and conditioning were performed, in separate days (**Supplementary Fig. 2**), in a mouse conditioning cage (Test-A, 18 cm × 18 cm × 30 cm) with an electrifiable floor connected to a H13-15 shock generator (Coulbourn Instruments). The Test-A cage was situated in a larger sound-attenuated cabinet (H10-24A, Coulbourn Instruments). On day 1, mice were individually habituated in a Test-A cage with five pure tones (4 kHz, 75 dB, 30 s each) delivered at variable intervals (60–120 s). The entire duration of this session was 600 s. On day 2, mice were conditioned individually using a similar protocol, except that each of the five tones co-terminated with a 2-s, 1-mA foot shock (or 0.3 mA for experiments described in **Fig. 4e**,**f**). The FreezeFrame software (Coulbourn Instruments) was used to control the delivery of tones and foot shocks. The floor and walls of the cage were cleaned with 70% ethanol for each mouse. During habituation and conditioning, the cabinet was illuminated and the behavior was captured with a monochrome CCD camera (Panasonic WV-BP334) at 4 Hz and stored on a personal computer. The test for fear memory was performed in a testing cage, Test-B, in darkness 24 h after conditioning. Test-B (the testing cage) had a different shape (22 cm × 22 cm × 21 cm) and floor texture compared with Test-A (the conditioning cage). The floor and walls of Test-B were wiped with 0.5% (vol/vol) acetic acid for each mouse before testing to make the scent distinct from that of Test-A. Behavioral response to two 4-kHz, 75-dB tone (the conditioned stimulus) delivered with a 120-s interval was recorded. The entire duration of the test session was 340 s. Freezing behavior in response to the two conditioned stimulus presentations during the test session were scored and averaged. Freezing behavior was analyzed by using the FreezeFrame software (Coulbourn Instruments) or a MATLAB (MathWorks)-based software[47], with the evaluator being blind to the treatment of the animals.

Stereotaxic surgery. All AAV viruses, such as AAV-DIO-ChR2(H134R)-YFP, AAV-DIO-Arch-GFP and AAV-DIO-hM4Di-mCherry, were produced by the University of North Carolina Vector Core Facilities. The retrograde tracer Alexa-Fluor 488–conjugated CTB was purchased from Invitrogen. Standard surgical procedures were followed for stereotaxic injection[46]. Briefly, mice were anesthetized with ketamine (100 mg per kg of body weight) supplemented with dexmedetomidine hydrochloride (0.4 mg per kg) and positioned in a stereotaxic injection frame (myNeuroLab.com). A digital mouse brain atlas was linked to the injection frame to guide the identification and targeting of CeL (Angle Two Stereotaxic System, myNeuroLab.com). CTB or viruses were delivered with a glass micropipette through a skull window (1–2 mm^2) by pressure application (5–12 psi, controlled by a Picrospritzer III, General Valve). The injections were performed using the following stereotaxic coordinates for CeL: −1.22 mm from Bregma, 2.5 mm (4-week-old mice) or 2.9 mm (6–7-week-old mice) lateral from the midline, and 4.6 mm vertical from the cortical surface; for CeM: −1.00 mm from Bregma, 2.36 mm lateral from the midline and 5.10 mm vertical from the cortical surface; for lateral amygdala: −1.80 mm from Bregma, 3.4 mm lateral from the midline and 4.9 mm vertical from the cortical surface; for auditory thalamus: −3.16 mm from Bregma, 1.90 mm lateral from the midline and 3.20 mm vertical from the cortical surface; for PAG: −4.48 mm from Bregma, 0.36 mm lateral from the midline and 2.60 mm vertical from the cortical surface. During all surgical procedures, mice were kept on a heating pad and were brought back to their home cages after regaining movement. For postoperative care, mice were hydrated by intraperitoneal injection with 0.3–0.5 ml of lactated ringers. We used metacam (meloxicam, 1–2 mg per kg) as an analgesic and to reduce inflammation. For injection of CTB, we injected 0.1–0.3 μl (2% in phosphate-buffered saline) into CeM and waited 3–5 d to allow the retrograde labeling of CeL neurons. For the injection of viruses, we injected 0.3–0.8 μl of viral solution (~10^{12} virus molecules per ml) bilaterally into CeL and waited approximately 2–3 weeks to allow maximal viral expression.

Preparation of acute brain slices and electrophysiology. Experiments were always performed on interleaved naive and fear-conditioned mice. Mice were anesthetized with isoflurane and decapitated, and their brains were quickly removed and chilled in ice-cold dissection buffer (110.0 mM choline chloride, 25.0 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2.5 mM KCl, 0.5 mM $CaCl_2$, 7.0 mM $MgCl_2$, 25.0 mM glucose, 11.6 mM ascorbic acid and 3.1mM pyruvic acid, gassed with 95% O_2 and 5% CO_2). Coronal slices (300 μm) containing the amygdaloid complex were cut in dissection buffer by using a HM650 Vibrating Microtome (MICROM International GmbH), and subsequently transferred to a storage chamber containing artificial cerebrospinal fluid (ACSF; 118 mM NaCl, 2.5 mM KCl, 26.2 mM $NaHCO_3$, 1 mM NaH_2PO_4, 20 mM glucose, 2 mM $MgCl_2$ and 2 mM $CaCl_2$, at 34 °C, pH 7.4, gassed with 95% O_2 and 5% CO_2). After at least 40 min, recovery time, slices were transferred to room temperature (20–24 °C) and were constantly perfused with ACSF.

In acute slices, the major subdivisions of the amygdala can be easily identified under trans-illumination[23,48]. In addition, we took advantage of the *Som-cre; Ai14* line, in which the CeL had very high density of SOM^+ neurons that were red fluorescent (**Fig. 1a** and **Supplementary Fig. 1a–c**), to facilitate the identification of CeL under epifluorescence illumination.

Simultaneous whole-cell, patch-clamp recordings from SOM^+ and SOM^- neuronal pairs in CeL were obtained with Multiclamp 700B amplifiers (Molecular Devices). Recordings were under visual guidance using an Olympus BX51 microscope equipped with both transmitted light illumination and epifluorescence illumination. The SOM^+ cells were identified on the basis of tdTomato fluorescence. For evoked EPSCs, synaptic responses were evoked with a bipolar stimulating electrode placed in the lateral amygdala approximately 0.2 mm away from the recorded cell bodies in CeL. Electrical stimulation was delivered every 30 s and synaptic responses were low-pass filtered at 1 kHz and recorded at holding potentials of −70 mV (for AMPAR-mediated responses), +40 mV (for NMDAR-mediated responses) or 0 mV (for $GABA_A$ receptor–mediated responses). NMDAR-mediated responses were quantified as the mean current between 110 ms and 160 ms after stimulation. Recordings were performed in the ACSF. The internal solution for voltage-clamp experiments contained 115 mM cesium methanesulphonate, 20 mM CsCl, 10 mM HEPES, 2.5 mM $MgCl_2$, 4 mM Na_2-ATP, 0.4 mM Na_3GTP, 10 mM sodium phosphocreatine and 0.6 mM EGTA (pH 7.2). For current-clamp experiments, the internal solution consisted of 130 mM potassium gluconate, 5 mM KCl, 10 mM HEPES, 2.5 mM $MgCl_2$, 4 mM Na_2ATP, 0.4 mM Na_3GTP, 10 mM sodium phophocreatine and 0.6 mM EGTA (pH 7.2). Evoked EPSCs were recorded with picrotoxin (100 μM) added to the ACSF. mEPSCs were recorded in the presence of tetrodotoxin (1 μM) and picrotoxin (100 μM). Electrophysiological data were acquired and analyzed using pCLAMP 10 software (Molecular Devices). mEPSCs were analyzed using Mini Analysis Program (Synaptosoft).

To evoke synaptic transmission using ChR2, we used a single-wavelength LED system (λ = 470 nm, CoolLED.com) connected to the epifluorescence port of the Olympus BX51 microscope. To restrict the size of the light beam for focal stimulation, a built-in shutter along the light path in the BX51 microscope was used. The smallest light beam achieved using this method is ~50 μm in diameter. Light pulses of 2 ms, triggered by a TTL (transistor-transistor logic) signal from the Clampex software (Molecular Devices), were used to evoke synaptic transmission. To measure Arch-mediated neuronal inhibition, a CBT-40 Green LED (λ = 530 nm, Luminus Devices) was used.

***In vivo* optogenetic and chemical-genetic manipulations.** For *in vivo* optogenetic manipulations in awake behaving animals, *Som-IRES-cre* mice were

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bilaterally implanted with optical fiber cannulae (Doric Lenses) during the same surgery procedure for viral injection. Optical fibers (100 μm in diameter) were placed 0.5 mm dorsal to the virus injection site and were secured to the skull with C&B-Metabond Quick adhesive luting cement (Parkell Prod) followed by dental cement (Lang Dental Manufacturing). Viruses were allowed to express for 2–3 weeks. The optic fibers were connected to a laser source using an optic fiber sleeve (Doric Lenses), and the mice were subjected to behavioral tests after habituation. Naive mice that were injected with the ChR2 virus, or a control virus that expresses GFP, into CeL were tested for freezing behavior following the delivery of blue light pulses (λ = 473 nm, OEM Laser Systems) through the optic fibers to activate ChR2. The light stimuli consisted of 5-ms light pulses delivered at 50 Hz for 20 s, and were repeated five times with a 2-min inter-train interval. Freezing behavior was measured during a 20-s period immediately before the delivery of light pulses (light off), and the 20-s period of light presentation (light on; **Fig. 7c**). Mice injected with the Arch virus, or a control virus that expresses GFP, were trained with the fear-conditioning procedure and were then tested for conditioned fear expression 24 h later as described above. We measured the conditioned freezing behavior in response to two 20-s tones, the first of which was presented during the delivery of a constant green light (λ = 532 nm, OEM Laser Systems) through the optic fibers to activate Arch (**Fig. 7e**). The power of both the blue and green lasers was 5–10 mW measured at the tip of the optic fiber.

For the chemical-genetic manipulation, *Som-IRES-cre* mice that received bilateral injections of either the AAV-DIO-hM4Di-mCherry (**Supplementary Fig. 10**) or the AAV-DIO-GFP (a control virus) into CeL were intraperitoneally injected with CNO (10 mg per kg), followed by fear conditioning 40 min later. In addition to a standard conditioning procedure (**Fig. 4c**,**d** and **Supplementary Figs. 2** and **5c**), we also used a mild procedure (**Fig. 4e**,**f**) in which the 4-kHz tones, each co-terminating with a 2-s, 0.3-mA foot shock, were delivered twice at an interval of 120 s. This was to increase our ability to detect an effect of the manipulation on fear memory by avoiding potential compensation resulting from overtraining[9].

Statistics and data presentation. We used a bootstrap procedure[46], which makes no assumptions on the data's distribution, to compare the means of data sets with non-normal distribution that was determined by the Shapiro-Wilk test. Two data sets (*N* of size *n* with mean *Nm* and *M* of size *m* with mean *Mm*) were randomly sampled *n* and *m* times, respectively, allowing resampling, and means *Ni* and *Mi* were generated, respectively. This procedure was repeated 10,000 times. If *Nm* was greater than *Mm,* it was considered to be significant if *Mi* was greater than *Ni* less than 5% of the time, for $P < 0.05$, or 1% of the time, for $P < 0.01$. All other statistical tests are indicated when used. The sample sizes used in this study, such as the numbers of cells or animals, are about the same as those estimated by power analysis (power = 0.9, $\alpha = 0.05$). No mice or data points were excluded from analysis. All data are presented as mean ± s.e.m.

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