Title: A neural circuit basis for reward-induced suppression of fear generalization

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How positive and negative affective stimuli interact in the brain to influence behavioral outcomes remains poorly understood. Here, we show that recall of a positive reward-associated conditioned stimulus (CS_{Rew}+) can prevent or reverse fear generalization in mice. Modification of generalized fear by recall of a CS_{Rew}+ is dependent on the midbrain dopamine system and the regulation of discriminatory threat encoding by the central amygdala (CeA). Precisely timed, transient elevations in dopamine and activation of dopamine D2 receptors in the CeA are necessary to reverse threat generalization and non-discriminatory threat encoding in the CeA. Recall of a positive association is also effective at enhancing the extinction of a conditioned threat response in a dopamine dependent manner. These data demonstrate that recall of a positive experience can be an effective means to suppress generalized fear and show that dopamine projections to the CeA are an important neural substrate for this phenomenon.

Introduction

The ability to learn stimulus-outcome associations is predicated on effective stimulus discrimination. Stress caused by uncertainty, and vice versa, can impair discriminatory fear learning resulting in maladaptive generalization[1-3]. Negative affective stimuli can potently suppress positive reinforcement[4], but the extent to which positive affective stimuli can impact discriminatory fear learning is less clear.

In generalized fear-related disorders, like post-traumatic stress disorder, the most efficacious therapy is exposure-based treatment where recall of the most salient features of trauma-related memory is repeatedly evoked to neutralize or extinguish those memories[5]. In human subjects, positive emotions have been shown to have an inhibitory effect on fear generalization[6], and positive affect has been shown to enhance fear extinction[7]. In rodents, counterconditioning in which presentation of a fear-associated conditioned stimulus (CS_{Fear}+) is subsequently paired with a reward unconditioned stimulus (US_{Rew}) results in persistent reductions in the fear memory that is associated with immediate early gene induction in reward circuitry[8]. These findings suggest that stimuli associated with positive affect can have a potent influence on fear-related memories, but the exact nature of how a positive stimulus affects the encoding of fear to exact these outcomes is not clear.

The ventral tegmental area (VTA) dopamine system and the CeA have emerged as two key nodes for regulating behavioral responses to both positive and negative affective stimuli[9-12]. There are numerous connections between the CeA and midbrain dopamine systems[13-16]. Among these, the VTA dopamine neurons are the most broadly implicated in associative learning[17] and are potent modulators of discriminatory fear learning[12,13,18], suggesting that the connections between the VTA and CeA may be central to the interactions between positively and negatively

valenced stimuli[19]. Here, we sought to determine whether positive reinforcement learning could be leveraged as a means to suppress or reverse fear generalization, or enhance extinction of a fear memory in a manner that is dependent on dopaminergic modulation of threat encoding in the CeA. We find that precisely timed delivery of a CS_{Rew}+ at the onset of a fear CS can prevent and reverse fear generalization. This effect is associated with a CS_{Rew}+ evoked change in dopamine release in the CeA during fear reconditioning and a shift from non-discriminatory to discriminatory fear encoding by neurons of the CeA. Mechanistically, dopamine release from the VTA and dopamine d2 receptor expression in the CeA are required for CS_{Rew}+ modification of generalized fear memories. Consistent with this observation, the CS_{Rew}+ modifies discriminatory fear encoding by Drd2-expressing neurons in the CeA. Finally, the timed delivery of the CS_{Rew}+ in place of an expected fear US during extinction can enhance the extinction of the fear memory in a dopamine D2 receptor-dependent manner. Collectively, these data provide evidence for a neural substrate through which a reward associated stimulus can potently modify generalized fear states.

A reward conditioned stimulus can prevent and reverse generalized fear.

We first sought to establish whether a CS associated with positive reinforcement can impact threat generalization and whether this is selective for the reward-predictive cue. To achieve this, we utilized a Pavlovian reward reinforcement task (**Extended Data Fig. 1a**) in which a 10 s tone (CS_{Rew}+) co-terminated with the delivery of a food reinforcer (US_{Rew}; 25-CS/US pairings). Interleaved with the CS/US pairings, a second tone was played (10 s) an equal number of times but was not paired with reward delivery (CS_{Rew}-). To assess the efficacy of conditioning, we monitored discriminatory conditioned responses (head entries during CS_{Rew}+

presentation) and recorded dopamine neuron activity during CS_{Rew}+ or CS_{Rew}- presentations. Dopamine neurons were isolated during recording by injecting mice expressing Cre recombinase under the control of the dopamine transporter locus (DAT-Cre)[20] with a virus for conditional expression of the stimulatory light-activated channel rhodopsin[21] (AAV-FLEX-ChR2-mCherry) and implanting an optical fiber and electrodes over the VTA (**Extended Data Fig. 1b**). During the positive reinforcement task, mice learned to discriminate between the CS_{Rew}+ and CS_{Rew}- (**Extended Data Fig. 1c**). Photosensitive dopamine neurons (**Extended Data Fig. 1d-g**) exhibited increased conditioned responses to the CS_{Rew}+ and diminished responses to the CS_{Rew}- (**Fig. 1a,b**; CS response) which coincided with reduced responding to the reward US (**Fig. 1a,b**; Reward response), as predicted[22].

Having confirmed the discriminatory encoding of reinforcement, we next assessed whether the presentation of the CS_{Rew}+ or CS_{Rew}- could influence discriminatory fear learning. To determine whether these effects are dopamine neuron dependent, DAT-Cre mice were injected with a virus for conditional expression of the inhibitory light-activated channel JAWS[23] (AAV-FLEX-JAWS-EGFP) or control virus (AAV-FLEX-EYFP) and an optical fiber was implanted over the VTA (**Extended Data Fig. 1h,i**). Following positive reinforcement learning, mice underwent discriminatory fear conditioning (**Fig. 1c,d** and **Extended Data Fig. 1j,k**) using a 0.5mA foot shock US, which has been shown to induce generalized threat responses[13]. During fear conditioning, CS_{Rew}+ or CS_{Rew}- was played for 1 s at the onset of the CS_{Fear}+ (10 s tone), but not the non-threat predictive stimulus (CS_{Fear}-, 10s tone). In a separate group of mice, VTA dopamine neurons were inhibited during the 1 s of CS_{Rew}+ presentation (JAWS-CS_{Rew}+) (**Fig. 1c**). Mice presented with the CS_{Rew}+ at the onset of the CS_{Fear}+ displayed discriminatory fear learning, whereas mice presented with the CS_{Rew}-, or had dopamine neurons inhibited during the

presentation of the CS_{Rew}+ displayed generalized threat responses following conditioning (**Fig. 1d**).

As threat intensity increases, uncertainty also increases as a result of stress-related signaling that promotes generalization[3,13]. To determine whether a positive stimulus can prevent generalization associated with uncertainty, mice were conditioned using a probabilistic conditioning paradigm (**Extended Data Fig. 2a**) that has been shown to induce generalization when threat intensity is relatively low[8,13]. Brief co-presentation of the CS_{Rew}+, but not the CS_{Rew}-, with the higher probability fear CS ($CS_{0.7}$), promoted discriminatory fear learning that was blocked in mice with inhibition of VTA dopamine neurons during the CS_{Rew}+ presentation (**Extended Data Fig. 2b-e**).

We next asked whether an established generalized fear response could be reversed by reconditioning mice with co-presentation of CS_{Rew}+. Mice were conditioned using the probabilistic conditioning paradigm to induce generalization, followed by two days of reconditioning with co-presentation of the CS_{Rew}+ or CS_{Rew}- with the $CS_{0.7}$ (**Fig. 1e** and **Extended Data Fig. 3a-c**). All groups of mice showed generalized fear after a single conditioning session (**Fig. 1f**; ret1), but mice presented with the CS_{Rew}+ at the onset of the $CS_{0.7}$ during reconditioning displayed improved discrimination (**Fig. 1f**; YFP-CS_{Rew}+; ret2,3). In contrast, mice presented with the CS_{Rew}- or had dopamine neurons inhibited by JAWS during the presentation of the CS_{Rew}+ displayed persistent generalized threat responses (**Fig. 1F**; YFP-CS_{Rew}- and JAWS- CS_{Rew}+; ret2,3).

A reward-associated cue can modify fear-associated dopamine release in the CeA

Our results demonstrate that VTA dopamine neurons play an important role in the ability of a stimulus associated with positive reinforcement to modify generalized fear behavior. The CeA has emerged as an important site for regulating discriminatory fear learning[13,24-26], and dopamine signaling in the CeA is critical for discriminatory fear[27]. However, the dynamics of dopamine release in the CeA during either positive or negative reinforcement remain unknown. To begin to address this, we first assessed whether activation of VTA dopamine neurons evokes dopamine release in the CeA. DAT-Cre mice were injected with a virus to conditionally express the red-shifted stimulatory opsin Chrimson[28] (AAV-FLEX-Chrimson-tdTomato) in dopamine neurons in the VTA and a virus to express the dopamine sensor dLight1.3b[29] in the CeA (AAV-dLight1.3b, **Fig. 2a**). Stimulation of dopamine neurons evoked transient dopamine release in a frequency- and stimulus duration-dependent manner (**Fig. 2b,c** and **Extended Data Fig. 3d,e**). Next, we tested whether a CS_{Rew}+ modifies dopamine release in the CeA to the $CS_{0.7}$ or $CS_{0.3}$ following the induction of generalization and reconditioning (**Fig. 2d**). Similar to our findings with single-unit recording of VTA dopamine neurons during positive reinforcement learning, dopamine was initially released in the CeA to the US (**Fig. 2e-h**). As learning progressed (**Extended Data Fig. 3f**), dopamine signals emerged to the CS_{Rew}+ but not the CS_{Rew}- and responses to the reward US diminished (**Fig. 2e-h**). Interestingly, in response to the reward US activation to the reward retrieval (head entry to the food dispenser; HE_{Rew}) became smaller and more transient but was followed by the emergence of a robust decrease in dopamine (**Fig. 2g,h**). During probabilistic fear conditioning (**Extended Data Fig. 3g,h**), dopamine release was initially detected in response to the US_{Fear} (**Fig. 2i**; FC1). Co-presentation of the CS_{Rew}+ during

reconditioning did not alter dopamine release to the fear $CS_{0.7}$ but significantly reduced dopamine release to the $CS_{0.3}$ (**Fig. 2i,j**; FC2 and FC3).

VTA dopamine modifies fear encoding in the CeA

How does dopamine regulate fear encoding in the CeA? Subpopulations of genetically distinct CeA neurons are activated (Fear-On) or inhibited (Fear-Off) by conditioned threat stimuli and contribute to associative fear learning[25,26,30-33]. However, how these neurons respond during generalized fear or the impact of dopamine on this encoding is not known. To address this question, we sought to simplify our approach. First, we established whether VTA dopamine projections to the CeA are sufficient to facilitate the reversal of threat generalization. To test this, we expressed ChR2 (AAV-FLEX-ChR2) or a control virus mCherry (AAV-FLEX-mCherry) in VTA dopamine neurons (DAT-Cre mice) and implanted optical fibers over the CeA (**Extended Data Fig. 4a**) to allow for precise stimulation of dopamine terminals for 1 s (20 Hz) during reconditioning (**Extended Data Fig. 4b**). Fear conditioning with a high-intensity US (0.5 mA) induced generalized responses as observed previously. Stimulation of VTA dopamine terminals in the CeA effectively reduced generalized fear responses with just a single reconditioning session (**Extended Data Fig. 4c-e**). Next, we asked whether this stimulation was sufficient to reverse generalization under increased uncertainty, and if so, whether the effect was dopamine-dependent. To achieve this, we expressed ChR2 in VTA dopamine neurons and conditionally mutated the gene encoding tyrosine hydroxylase (*Th*), the rate limiting enzyme in dopamine production, in these same cells using CRISPR/SaCas9 mutagenesis (**Fig. 3a-c**)[34]. Optical fibers were implanted over the CeA (**Fig. 3a** and **Extended Data Fig. 4f**) to deliver 1 s of dopamine terminal stimulations (20 Hz) at the onset of the high probability $CS_{0.7}$ during reconditioning

(**Fig. 3d** and **Extended Data Fig. 4g**). Again, stimulation of dopamine terminals effectively induced discrimination between $CS_{0.7}$ and $CS_{0.3}$ in a dopamine-dependent manner (**Fig. 3e,f**).

To assess effects of dopamine signaling on fear encoding in the CeA, DAT-Cre mice were injected with ChR2 and *Th*-CRISPR virus or *Rosa26* control virus in the VTA and implanted an optical fiber and recording electrodes in the CeA (**Fig. 3g** and **Extended Data Fig. 4h**). Stimulation of dopamine-releasing terminals (10 pulses at 20 Hz) in the CeA resulted in both neuronal excitation and inhibition (**Fig. 3h**). The proportion of neurons responsive to dopamine terminal stimulation was significantly reduced in mice with mutated *Th* (**Fig. 3i**). Within our control group (sg*Rosa26*), stimulation of dopamine terminals (1s, 20 HZ) enhanced discriminatory fear following reconditioning (**Fig. 3j** and **Extended Data Fig. 4i,j**), as above. Within the CeA, a subset of neurons was phasically excited by both CSs during the pre-test phase (**Fig. 3k**), but no neurons were detected that were inhibited by either CS (**Fig. 3k** and **Extended Data Fig. 4k**). Following the first conditioning session when mice displayed generalized freezing responses, we observed equivalent transient excitations and prolonged inhibitions (**Fig. 3k,l**; ret1). After reconditioning with dopamine terminal stimulation, we observed discriminatory freezing behavior, which was associated with discriminatory encoding of the $CS_{0.7}$ and $CS_{0.3}$ by CeA neurons in sg*Rosa26* control mice (**Fig. 3k,l**; ret2). In contrast, reconditioning mice with *Th* mutagenesis (sg*Th* group) in VTA dopamine neurons with dopamine terminal stimulation in the CeA did not promote discriminatory behavior (**Fig. 3m**). During the pre-test and first retention test, CeA neurons from sg*Th* mice showed a similar response to those from the sg*Rosa26* group (**Fig. 3n,o** and **Extended Data Fig. 4k**). However, sg*Th* mice had a significantly smaller proportion of inhibited neurons during the retention tests (**Extended Data Fig. 4k**), suggesting that inhibited responses may be mediated in part by

dopamine signaling. Following reconditioning, sg*Th* mice exhibited equivalent responses to the $CS_{0.7}$ and $CS_{0.3}$, consistent with their persistent generalized fear responses (**Fig. 3m-o**).

Differential actions of dopamine receptors in the CeA in the reversal of fear generalization.

We now have clear evidence to support a mechanism whereby dopamine can influence discriminatory fear learning, but we do not know which dopamine receptors are responsible for these observed effects. Within the CeA, dopamine D1 and D2 receptors are broadly expressed on subpopulations of inhibitory GABAergic neurons[27,35-37] where they potently modulate neuronal activity. Related to threat processing, dopamine D2 receptor agonist infused into the CeA can prevent fear generalization, and infusion of dopamine D2 receptor antagonists can promote fear generalization[27], strongly implicating the importance of this receptor. However, in addition to their ability to signal at the somatodendritic level to modulate neuronal activity, dopamine D2 receptors are also localized to dopamine neuron terminals[38] and cortical projection neuron terminals[39]; thus, making it difficult to assess the source of D2 receptor that is important for facilitating fear discrimination. To determine whether dopamine receptor expression in the GABA-releasing neurons of the CeA are important for the ability of dopamine terminal stimulation to reverse threat generalization and enhance fear extinction, we generated CRISPR guides to selectively mutate the genes encoding D1 (sg*Drd1*) and D2 (sg*Drd2*) receptors in these neurons. DAT-Cre::VGAT-Flp double transgenic mice were injected with AAV-FLEX-ChR2-mCherry into the VTA and either AAV-FLEXfrt-SaCas9-U6-sg*Drd1*, AAV-FLEXfrt-SaCas9-U6-sg*Drd2* or AAV-FLEXfrt-SaCas9-U6-sg*Rosa26* (control) into the CeA with optical fibers were placed above the bilateral CeA (**Extended Data Fig. 5a,b**). Both sg*Drd1* and sg*Drd2* CRISPRs resulted in significant reductions in mRNA levels in the CeA relative to the sg*Rosa26*

control CRISPR (**Fig. 4a,b** and **Extended Data Fig. 5c**). Dopamine receptor mutagenized mice and controls were fear conditioned using the probabilistic conditioning paradigm followed by reconditioning with optical stimulation of dopamine terminals in the CeA (1s, 20Hz) (**Fig. 4c**). Reconditioning facilitated discrimination in the sg*Rosa26* and sg*Drd1* mice, but not in the sg*Drd2* mice, indicating that dopamine signaling mediating the reversal of threat generalization is D2 receptor-dependent (**Fig. 4d** and **Extended Data Fig. 5d,e**). In separate cohorts of mice, we induced fear generalization and reconditioned with co-presentation of the CS_{Rew}+ or CS_{Rew}- (**Fig. 4e**). The sg*Rosa26* control mice displayed discriminatory fear after the second reconditioning day (**Fig. 4f**). In contrast, the sg*Drd1* group showed discriminatory fear after a single reconditioning, but this effect was not persistent (**Fig. 4f**) and the sg*Drd*2 group did not display discriminatory fear after either reconditioning day.

Based on the effects of CRISPR mutagenesis of *Drd1* and *Drd2*, we investigated how neurons in the CeA that express these receptors respond to appetitive and aversive stimuli, and how they encode generalized or discriminatory fear. To achieve this, we performed fiber photometry recordings of GCaMP6m *Drd1*-expressing CeA neurons or pro-enkephalin (*Penk*)-expressing neurons (**Fig. 5a** and **Extended Data Fig. 6a**) during appetitive conditioning, fear generalization, and the reversal of generalization (**Fig. 5b**). We chose to utilize *Penk*-Cre mice, as a large proportion of *Drd2*-expressing neurons in the CeA co-express *Penk*[35] (**Extended Data Fig. 6b,c**), and inactivation of *Penk* impacts fear learning and anxiety[40]. During Pavlovian appetitive conditioning, we observed the emergence of a decrease in calcium in *Drd1* CeA neurons, followed by the emergence of a transient increase at CS_{Rew}+ onset (**Fig. 5c,d** and **Extended Data Fig. 6d**). This effect was not observed in *Penk* CeA neurons (**Fig. 5c,d** and **Extended Data Fig. 6d**). In response to head entry and reward retrieval, both populations

showed the emergence of an increase in calcium that was slightly larger in the *Penk* CeA population (**Fig. 5e,f**). During fear conditioning, *Drd1* neurons responded to the US but not the $CS_{0.7}$ or $CS_{0.3}$ and reconditioning with the CS_{Rew}+ or CS_{Rew}- did not affect these responses (**Fig. 5g-i** and **Extended Data Fig. 6e-g**). In contrast, *Penk* CeA neurons responded to the fear US and weakly to the fear CSs during FC1; however, with reconditioning discriminatory responses between the $CS_{0.7}$ or $CS_{0.3}$ emerged in the CS_{Rew}+ group but not CS_{Rew}- group (**Fig. 5j-l** and **Extended Data Fig. 6e-g**).

A reward conditioned stimulus can facilitate fear extinction.

During extinction training, dopamine neurons that are inhibited by the CS+ are activated by the omission of the predicted US, generating a type of negative prediction error (NPE) that has been proposed as a type of positive reinforcement[13,41-43]. We found that photosensitive dopamine neurons that are either inhibited or excited by the CS_{Fear}+ are activated by omission of the US (foot shock) (**Fig. 6a,b** and **Extended Data Fig. 7a-d**), indicating that these neurons encode both an NPE and the salience of the omitted threat US. To test whether a CS_{Rew}+ could also facilitate extinction, mice were conditioned in a simple Pavlovian fear conditioning paradigm and presented with the CS_{Rew}+ or CS_{Rew}- at the offset of the CS_{Fear}+ and CS_{Fear}-during extinction training (**Fig. 6c**). Mice presented with the CS_{Rew}+ but not the CS_{Rew}-, or those with dopamine neurons inhibited during the CS_{Rew}+ presentation, displayed enhanced fear extinction and extinction memory recall (**Fig. 6d,e** and **Extended Data Fig. 7e**). The timing of the CS_{Rew}+ presentation during the CS_{Fear}+ is critical for facilitating extinction, as a random presentation during the intertrial interval did not affect extinction training or extinction memory recall (**Extended Data Fig. 7f-h**).

Similar to how stimulation of dopamine terminals prevents or reverses fear generalization, brief (1 s) stimulation of dopamine terminals in the CeA at the offset of the CS_{Fear}+ and CS_{Fear}- (**Fig. 7a**) facilitated extinction and enhanced extinction memory recall in a dopamine-dependent manner (**Fig. 7b,c**). With regard to fear encoding in the CeA, *Th* mutagenized and control mice that had previously undergone fear conditioning and reconditioning (**Fig. 3**) underwent extinction training (**Fig. 7d** and **Extended Data Fig. 7i**). Half of the control mice received dopamine terminal stimulation at the offset of the $CS_{0.7}$ and $CS_{0.3}$, while the other half received no stimulation. All *Th* mutagenized mice received optical stimulation. CeA neurons responsive to the CS were significantly reduced following extinction in mice with dopamine terminal stimulation, compared to non-stimulated controls and *Th* mutagenized mice (**Fig. 7e-g**), indicating that the enhanced extinction observed in the sg*Rosa26* mice that received dopamine terminal stimulation is associated with a greater loss of responsive CeA neurons.

To assess the roles of *Drd1* and *Drd2* expression, mice underwent extinction training with optical stimulation of dopamine terminals in the CeA (1 s, 20Hz) at the offset of the $CS_{0.7}$ and $CS_{0.3}$ following fear conditioning (**Fig. 7h**). The sg*Rosa26* control mice showed reduced freezing across the extinction session to both CSs (**Fig. 7i**). In contrast, the sg*Drd1* mice displayed reduced freezing from the onset of the extinction training (**Fig. 7i**) and sg*Drd2* mice showed persistent freezing across the extinction session (**Fig. 7i**), indicating that D1 and D2 receptor signaling have opposing actions during extinction training. During extinction recall, all mice showed similar freezing levels (**Fig. 7j**), suggesting that loss of D1 or D2 receptor signaling independently may affect the expression of the extinction responses during training but are not required for the consolidation of the extinction memory.

Discussion

Our results demonstrate that a stimulus associated with a positive outcome can serve as a potent modulator of fear learning through dopamine-dependent regulation of fear circuitry. We find that the effectiveness of a positive stimulus enhancing fear extinction is dependent on when the CS_{Rew}+ is presented, indicating that precise timing is critical. Mechanistically, we demonstrate that generalized fear responses are associated with non-discriminatory encoding in the CeA. Precisely timed transient increases in dopamine within the CeA can facilitate the reversal of generalized fear behavior and non-discriminatory encoding, and the same is true for the facilitation of extinction. The release of dopamine in the CeA in response to both the reward US and fear CS is consistent with this neurotransmitter encoding salience within the structure[14,19,44].

Within the CeA, *Drd2* expression is most strongly co-localized to *Penk* (>90% of *Drd2*$^{+}$ neurons are *Penk* expressing) neurons[27,35-37]. Consistent with our observations that inactivation of *Penk* results in persistent generalized fear following reconditioning with stimulation of dopamine terminals or following reconditioning with CS_{Rew}+ presentations, reductions in *Penk* mRNA levels in the CeA are associated with reduced freezing during conditioning and decreased anxiety in rats[40]. In addition to co-expression with *Penk*, *Drd2* is also co-localized with *Prkcd* (PKCδ) expressing neurons[27,35-37]. PKCδ neurons within the CeA have been defined as Fear-Off neurons (inhibited by CS_{Fear}+)[32], and we find significantly fewer CeA inhibited cells following *Th* mutagenesis in the VTA, suggesting that dopamine acting on D2 receptors may be a key mediator of these observed inhibitions. In addition to their responsiveness to fear-associated stimuli[32,45], PKCδ neurons are also responsive to satiety-related signals[46] and numerous cells within the CeA have been shown to be responsive to both appetitive and aversive stimuli[44,47,48]. Like the reward consumption-related responses we observed in *Penk*-CeA neurons, *Penk*-

expressing neurons of the nucleus accumbens have been shown to be responsive during reward consumption[49], suggesting a likely broader role for this endogenous opioid in the regulation of food reinforcement. We observed that dopamine release to food reward becomes more inhibited as Pavlovian reward conditioning progresses. In contrast, *Penk*-CeA neurons become progressively more activated, consistent with an inhibitory role for dopamine release on these cells.

Drd1 is most strongly localized to the medial subdivision of the CeA (CeM), where its expression overlaps with *Tac2*, *Nts*, and *Sst*[35]. The CeM is associated with driving conditioned fear responses[25], and *Tac2* neurons have been shown to be important for fear memory consolidation[50], as has dopamine more broadly[51]. Consistent with recent reports that *Sst*-CeA neurons respond to both food and foot shock[44], we find that *Drd1*-CeA neurons develop conditioned responses to food reward following Pavlovian conditioning and show increasing responses to reward consumption and foot shock; however, these cells do not develop robust conditioned responses to the CS_{Fear}+.

We find that inactivation of *Drd1* or *Drd2* in CeA GABA neurons has distinct effects on fear related behaviors. Reduced D1 receptors resulted in the facilitated reduction in freezing during extinction training with VTA dopamine terminal stimulation in the CeA, suggesting that D1 receptor activation promotes the expression of fear memory. In contrast, reduced D2 receptors resulted in persistent freezing during extinction training with VTA dopamine terminal stimulation in the CeA, suggesting that D2 receptor activation may be required for adjusting the certainty of the CS prediction of US delivery in real-time. Inactivation of either receptor did not affect the expression of the extinction memory, suggesting that dopamine signaling in the CeA may not be important for the consolidation of the extinction memory. Instead, dopamine

signaling in other regions linked to fear extinction[52] may be critical for consolidation, such as the medial prefrontal cortex[53,54], the nucleus accumbens[55,56] or tail of the striatum[57,58].

In conclusion, we find that the CeA is a crucial site for integrating information associated with different valences. Stimuli associated with positive reinforcement are an effective means to modify the encoding of CeA neurons and fear-related behavior. Manipulating the salience encoding of dopamine, either naturally or artificially, has a potent influence over discriminatory learning, but the timing is critical.

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Figures and legends

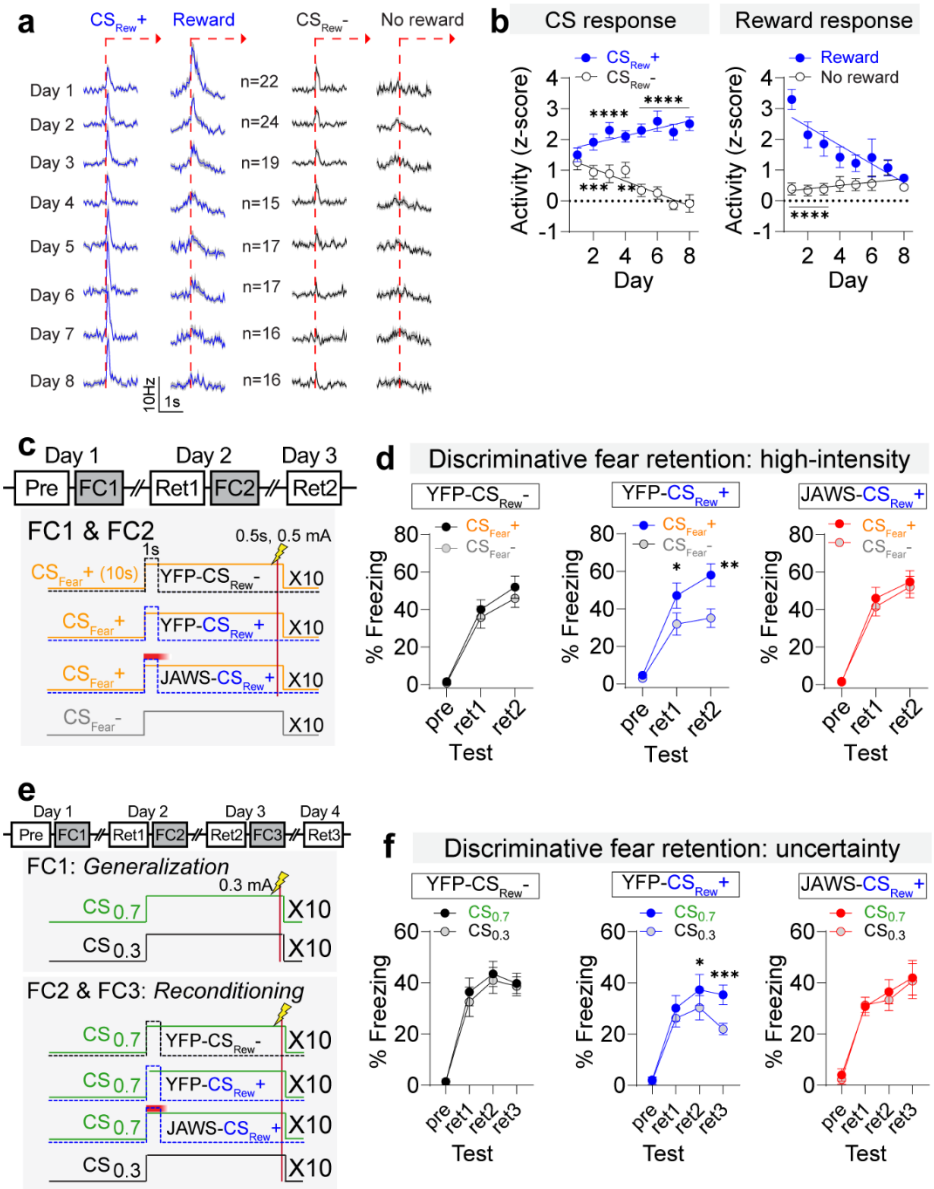

Figure 1. Impact of CS_{Rew}+ evoked dopamine on discriminatory fear. (**a**) Average dopaminergic responses to CSs and head entries for reward consumption (total 146 cells from 7 mice). (**b**) Normalized responses to CS response (CS_{Rew}+ and CS_{Rew}-) and Reward response(reward retrieval and no reward response after CS_{Rew}-), relative to baseline firing before each CS. CS_{Rew}+ and CS_{Rew}- responses were positively and negatively correlated with training days, respectively (Pearson's correlation, CS_{Rew}+, r = 0.26, P = 0.002 and negatively CS_{Rew}-, r = -0.43, P < 0.001). Reward responses were negatively correlated in CS_{Rew}+ trials (r = -0.4, P <

0.001). Significant differences between CS_{Rew}+ versus CS_{Rew}-, and Reward versus No reward following the CS_{Rew}- were observed (**P < 0.01, ***P < 0.001, ****P < 0.0001). (**c**) Schematic of timing sequences for CS_{Rew}+ and CS_{Rew}- presentations and JAWS-mediated inhibition during fear conditioning. (**d**) Freezing responses to the CS_{Fear}+ and CS_{Fear}- during the pre-test, retention tests 1 and 2. CS_{Rew}+ mice showed significant discriminatory freezing (*P < 0.05, **P < 0.01, YFP-CS_{Rew}+ = 10 mice, YFP-CS_{Rew}- = 10 mice, and JAWS- CS_{Rew}+ = 10 mice). (**e**) Schematic of probabilistic fear conditioning paradigm to induce fear generalization (FC1) and CS_{Rew}+ reversal (FC2 and FC3). (**f**) Following the induction of a generalized threat response, reconditioning with the CS_{Rew}+ facilitated discrimination between the $CS_{0.7}$ and $CS_{0.3}$ that was not observed with co-presentation of the CS_{Rew}- or when dopamine neurons were inhibited by JAWS during the CS_{Rew}+ presentation (*P < 0.05, ***P < 0.001, YFP-CS_{Rew}+ = 10 mice, YFP-CS_{Rew}- = 10 mice, and JAWS- CS_{Rew}+ = 10 mice). All data presented as mean ± S.E.M. Detailed information about statistical results is provided in Extended Data Table 1.

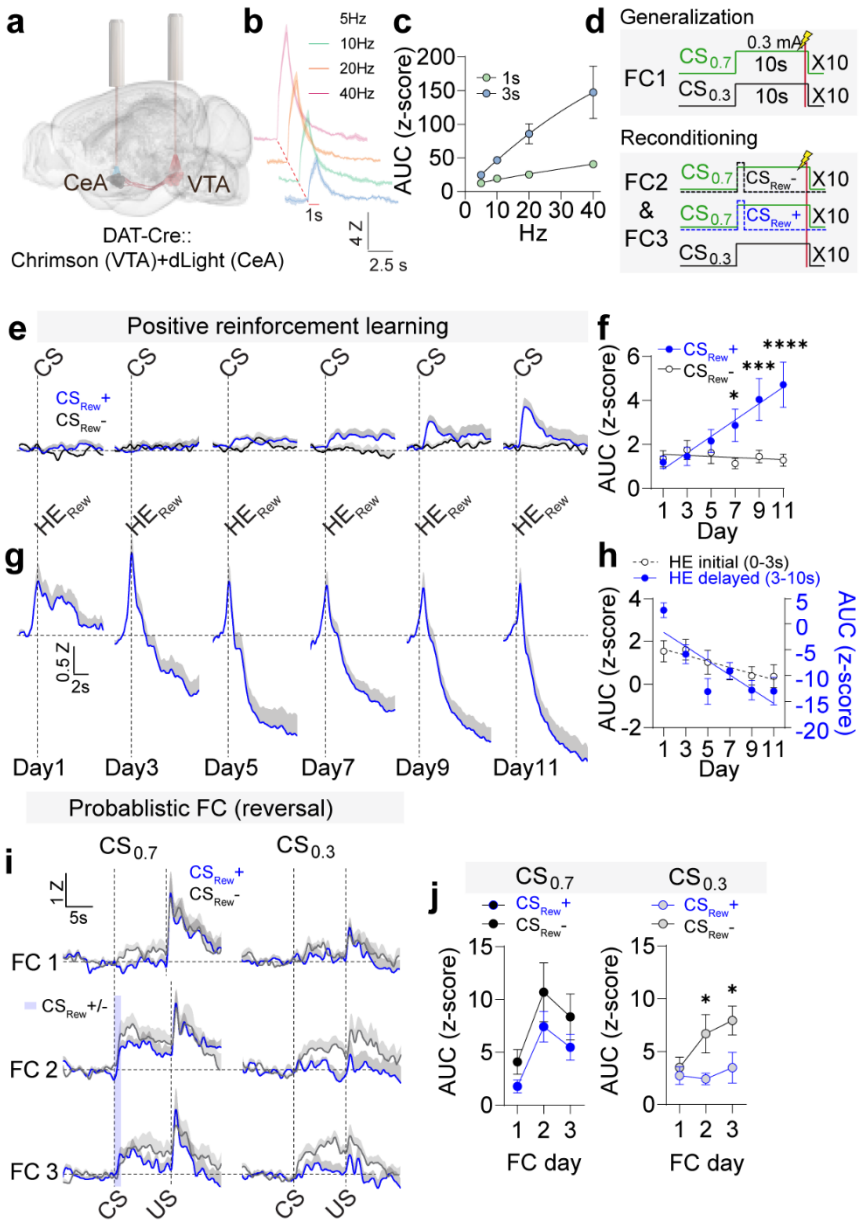

Figure 2. Impact of CS_{Rew}+ on fear-evoked dopamine in the CeA. (**a**) Schematic illustrating expression of Chrimson-tdTomato in the VTA and dLight1.3b in the CeA. (**b**) Mean light-evoked dopamine signals in the CeA following VTA stimulation at different frequencies. (**c**) Area under curve (AUC) of dopamine signals to VTA stimulations. (**d**) Schematic of probabilistic fear conditioning paradigm to induce fear generalization and CS_{Rew}+ reversal. (**e**) Average traces showing increased dopamine release in the CeA during positive reinforcement

learning to the CS_{Rew}+ but not to the CS_{Rew}- (N = 19 mice). (**f**) Average AUC for CS_{Rew}+ and CS_{Rew}- (**P* < 0.05, ****P* < 0.001, *****P* < 0.001). (**g**) Averaged CeA dopamine signals in response to head entry for reward retrieval (HE_{Rew}). (**h**) AUC during head entry for reward retrieval segregated into the initial response (0-3 s, R^2 = 0.04941, *P* = 0.0147) and secondary response (3-10 s, R^2 = 0.1953, *P* < 0.0001). (**i**) Dopamine signal in the CeA during fear conditioning with the $CS_{0.7}$ and $CS_{0.3}$ (FC1) and during reconditioning (FC2 and FC3) with the CS_{Rew}+ (N = 9) or CS_{Rew}- (N = 10). (**j**) Mean AUC of the z-scored dopamine signals in the CeA to the $CS_{0.7}$ and $CS_{0.3}$ during fear conditioning and reconditioning. Co-presentation of the CS_{Rew}+ prevented the increase in dopamine release in the CeA in response to the $CS_{0.3}$ (**P* < 0.05). All data presented as mean ± S.E.M. Detailed information about statistical results is provided in Extended Data Table 1.

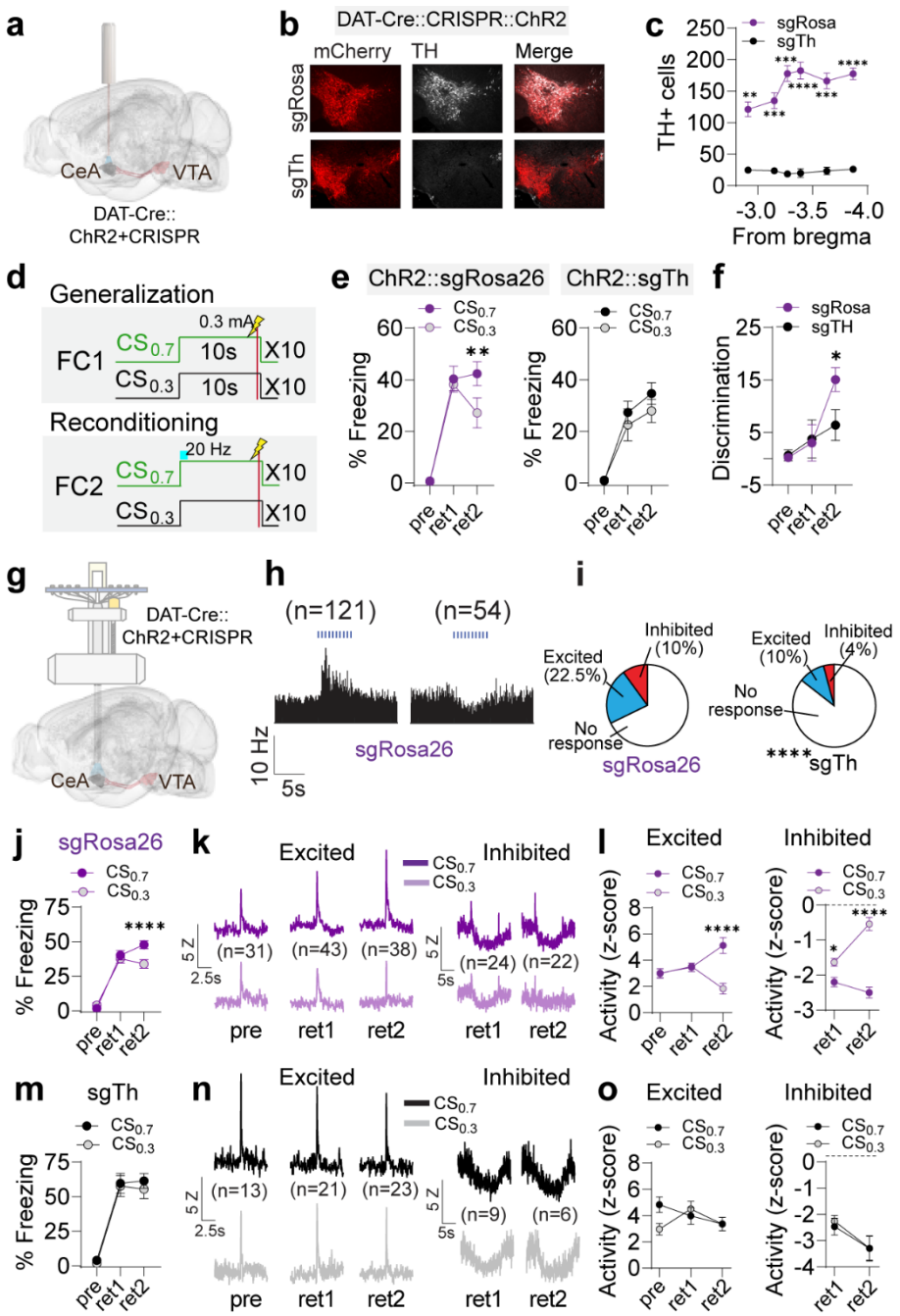

Figure 3. Dopamine release in the CeA is critical for reversing generalization. (**a**) Schematic of AAV-FLEX-ChR2-mCherry and AAV-FLEX-SaCas9-u6-sg*Th* injected into the VTA of DAT-Cre mice and optical fiber placement over the CeA. (**b**) Histological validation of *Th* mutation. (**c**) Quantitative analysis of reduced TH levels in the VTA of sg*Rosa26* control (N = 6) and sg*Th* (N = 5) mice (***P* < 0.01, ****P* < 0.001, *****P* < 0.001, 6 sections/mice). (**d**)

Schematic of probabilistic fear conditioning paradigm and optogenetic stimulation during reconditioning. (**e**) Following the induction of generalized fear response, reconditioning with the optical stimulation of dopamine terminals for 1s facilitated discrimination between the $CS_{0.7}$ and $CS_{0.3}$ that was not observed in *Th* mutagenized mice ($^{**}P < 0.01$; sg*Rosa26* = 10 mice and sg*Th* = 10 mice). (**f**) Comparison of fear discrimination between groups ($^{*}P < 0.05$). (**g**) Schematic of AAV-FLEX-ChR2-mCherry and AAV-FLEX-SaCas9-u6-sg*Th* injected into the VTA of DAT-Cre mice and optrode implant for analysis of dopamine neuron activity. (**h**) Examples of excitatory and inhibitory responses to dopamine terminal stimulation from sg*Rosa26* mice. (**i**) Responses of CeA neurons to dopamine terminal stimulation showing reduced excitatory and inhibitory responses in sg*Th* compared to sg*Rosa26* mice. (**j**) Freezing to the $CS_{0.7}$ and $CS_{0.3}$ in sg*Rosa26* mice with CeA recordings during preconditioning, retention test 1, and retention test 2 ($^{****}P < 0.0001$; sg*Rosa26*= 12 mice). (**k**) Average firing to $CS_{0.7}$ and $CS_{0.3}$ in sg*Rosa26* mice during pre-test, retention test 1, and retention test 2. (**l**) Normalized responses to $CS_{0.7}$ and $CS_{0.3}$ (relative to baseline firing before each CS) in sg*Rosa26* mice. During pre-test and retention test 1, there was no discriminatory encoding in excited cells. Following reconditioning with dopamine terminal stimulation, discriminatory encoding was significantly enhanced ($^{*}P < 0.05$, $^{****}P < 0.0001$). In inhibited cells, a small but significant discrimination between the encoding of the $CS_{0.7}$ and $CS_{0.3}$ was observed during retention test 1, but this was greatly enhanced following reconditioning with dopamine terminal stimulation. (**m**) Freezing to the $CS_{0.7}$ and $CS_{0.3}$ in sg*Th* mice with CeA recordings during pre-test, retention test 1, and retention test 2 (sg*Th* = 6 mice). (**n**) Average firing to $CS_{0.7}$ and $CS_{0.3}$ in sg*Th* mice during pre-test, retention test 1, and retention test 2. (**o**) Normalized responses to $CS_{0.7}$ and $CS_{0.3}$ (relative to baseline firing before each CS) in sg*Th* mice. During pre-test, retention test 1 and retention test 2, there was no discriminatory

encoding in excited cells. In inhibited cells, no discrimination between the encoding of the $CS_{0.7}$ and $CS_{0.3}$ was observed during retention test 1 or retention test 2. All data presented as mean ± S.E.M. Detailed information about statistical results is provided in Extended Data Table 1.

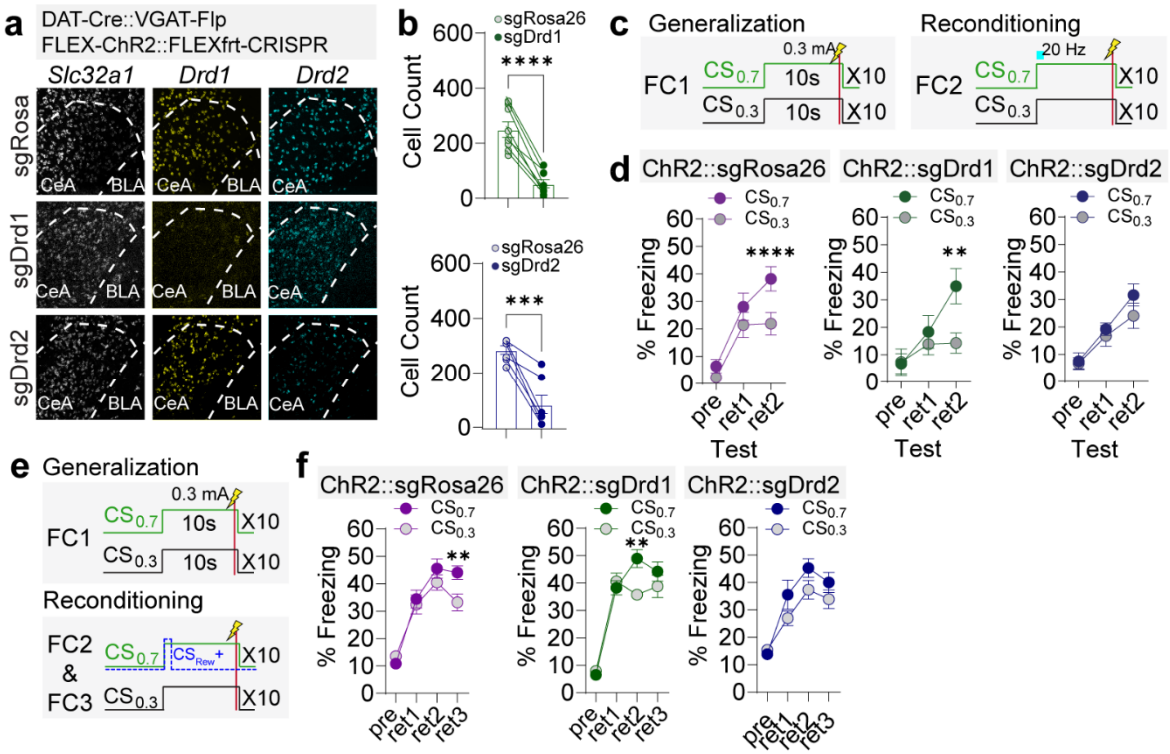

Figure 4. Differential impacts of *Drd1* and *Drd*2 mutagenesis on reversal of generalization. (**a**) RNAscope validation of *Drd1* and *Drd2* mRNA levels in the CeA following mutagenesis. (**b**) Quantitative of reduced mRNA levels associated with nonsense mediated mRNA decay following CRISPR mutagenesis (****P* < 0.001, *****P* < 0.0001; sg*Rosa26*/sg*Drd1* = 8 sections from 4 mice and sg*Rosa26*/sg*Drd2* = 7 sections from 4 mice). (**c**) Schematic of probabilistic fear conditioning paradigm and optogenetic stimulation during reconditioning. (**d**) Following the induction of generalized fear response, reconditioning with the optical stimulation of dopamine terminals for 1s facilitated discrimination between the $CS_{0.7}$ and $CS_{0.3}$ in sg*Rosa26* and sg*Drd1* mice that was not observed in *Drd2* mutagenized mice (***P* < 0.01, *****P* < 0.0001, sg*Rosa26* = 15 mice, sg*Drd1* = 10 mice, and sg*Drd2* =16 mice). (**e**) Schematic of probabilistic fear conditioning paradigm to induce fear generalization and CS_{Rew}+ reversal. (**f**) Following the induction of generalized fear response, reconditioning with the CS_{Rew}+ facilitated discrimination between the $CS_{0.7}$ and $CS_{0.3}$ in sg*Rosa26* and sg*Drd1* mice that was not observed in *Drd2*

mutagenized mice (**$P < 0.01$, sg*Rosa26* = 16 mice, sg*Drd1* = 9 mice, and sg*Drd2* =14 mice). All data presented as mean ± S.E.M. Detailed information about statistical results is provided in Extended Data Table 1.

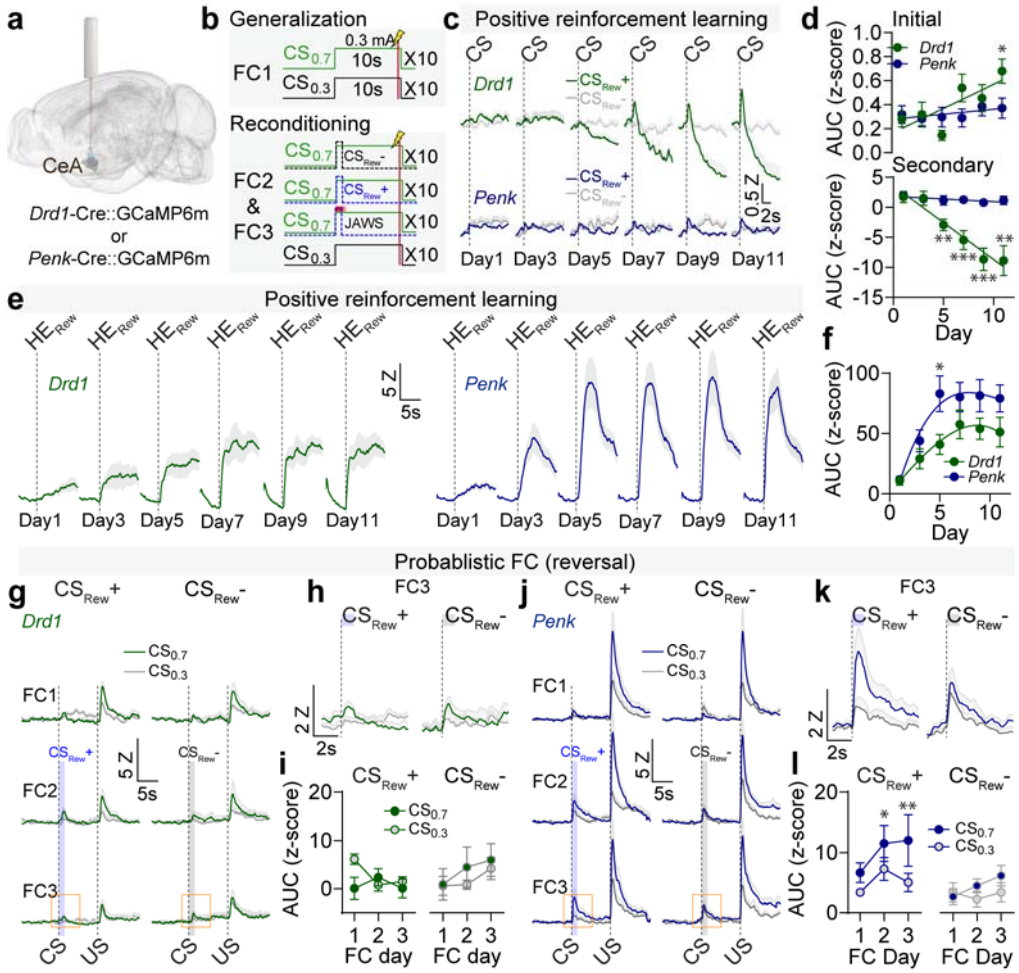

Figure 5. Distinct encoding patterns in *Drd1* and *Penk* CeA neurons during Pavlovian reinforcement learning and reversal of fear generalization. (**a**) Schematic of AAV-FLEX-GCaMP6m injection into the CeA of Drd1-Cre or Penk-Cre mice, with an optical fiber positioned over the CeA. (**b**) Schematic of probabilistic fear conditioning paradigm to induce fear generalization and CS_{Rew}+ reversal. (**c**) Average calcium signals from *Drd1* and *Penk* CeA neurons exhibited differential responses to CS_{Rew}+ and CS_{Rew}-during positive reinforcement learning. (**d**) Area under curve (AUC) for the CS segregated into the initial response (*Drd1*: 0-1 s, $R^2 = 0.1150$, $P = 0.0007$) and secondary response (*Drd1*: 2-10 s, $R^2 = 0.3097$, $P < 0.0001$). (**e**) Averaged calcium traces of *Drd1* and *Penk* CeA neurons in response to head entry for reward retrieval (HE_{Rew}). (**f**) AUC during head entry for reward retrieval (*$P < 0.05$). (**g**) Activity of

Drd1 CeA neurons during fear conditioning with the $CS_{0.7}$ and $CS_{0.3}$ (FC1) and during reconditioning (FC2 and FC3) with the CS_{Rew}+ (N = 10 mice) or CS_{Rew}- (N = 8 mice). (**h**) Average traces of *Drd1* CeA neurons during the CS presentation in FC3 for CS_{Rew}+ and CS_{Rew}- groups (magnified view from orange squares in **g**). (i) AUC for the CS across FC1, FC2, and FC3. (**j**) Activity of *Penk* CeA neurons during fear conditioning with the $CS_{0.7}$ and $CS_{0.3}$ (FC1) and during reconditioning (FC2 and FC3) with the CS_{Rew}+ (N = 13 mice) or CS_{Rew}- (N = 13 mice). (**k**) Average traces of *Penk* CeA neurons during the CS presentation in FC3 for CS_{Rew}+ and CS_{Rew}- groups (magnified view from orange squares in **j**). (**l**) AUC for the CS across FC1, FC2, and FC3 (* $P < 0.05$, ** $P < 0.01$). All data presented as mean ± S.E.M. Detailed information about statistical results is provided in Extended Data Table 1.

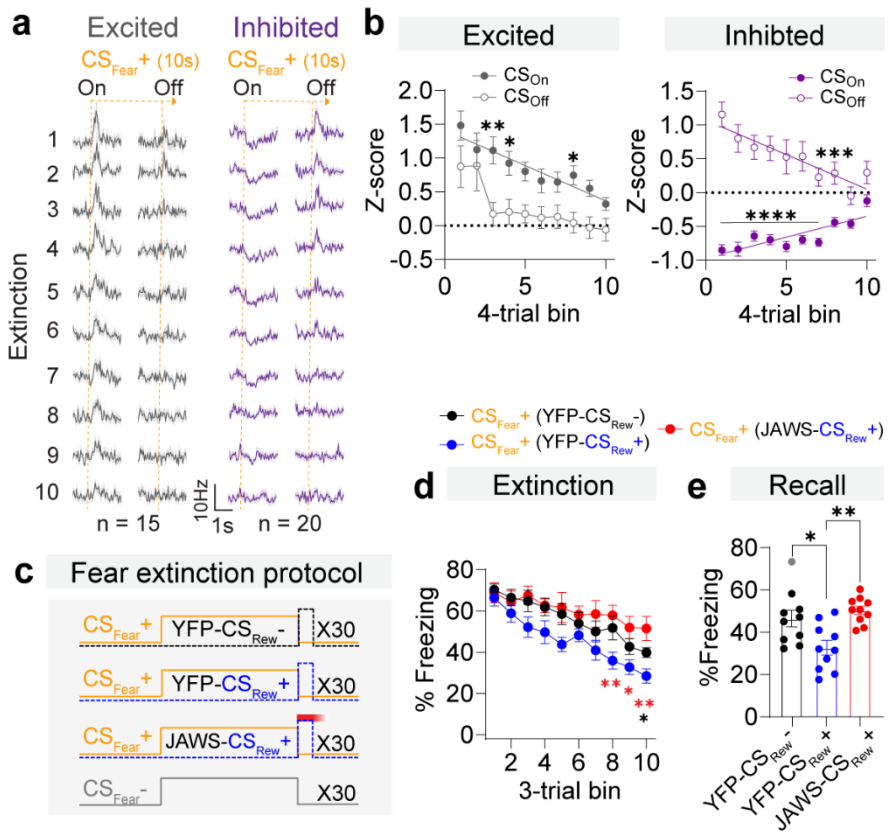

Figure 6. Reward conditioned stimulus enhancement of fear extinction. (**a**) Averaged activity of activated and inhibited neurons extinction trials (3 trial-bin). (**b**) Normalized responses to CS_{Fear}+ onset (CS_{On}) and CS_{Fear}+ offset (CS_{Off}) from CS excited neurons (N = 15 cells; CS_{On}: $R^2 = 0.1747$, $P < 0.0001$, CS_{Off}: $R^2 = 0.1021$, $P < 0.0001$) and CS inhibited neurons (N = 20 cells; CS_{On}: $R^2 = 0.1973$, $P < 0.0001$, CS_{Off}: $R^2 = 0.1138$, $P < 0.0001$). (**c**) Schematic of simple Pavlovian fear conditioning and extinction paradigm with precise timing of CS_{Rew}+, CS_{Rew}-, and JAWS inhibition during extinction training. (**d**) Fear extinction training in all three groups showing enhanced extinction in the YFP-CS_{Rew}+ group (*$P < 0.05$, **$P < 0.01$). (**e**) Extinction memory recall showing enhanced recall in the YFP-CS_{Rew}+ group (*$P < 0.05$, **$P < 0.01$). All data presented as mean ± S.E.M. Detailed information about statistical results is provided in Extended Data Table 1.

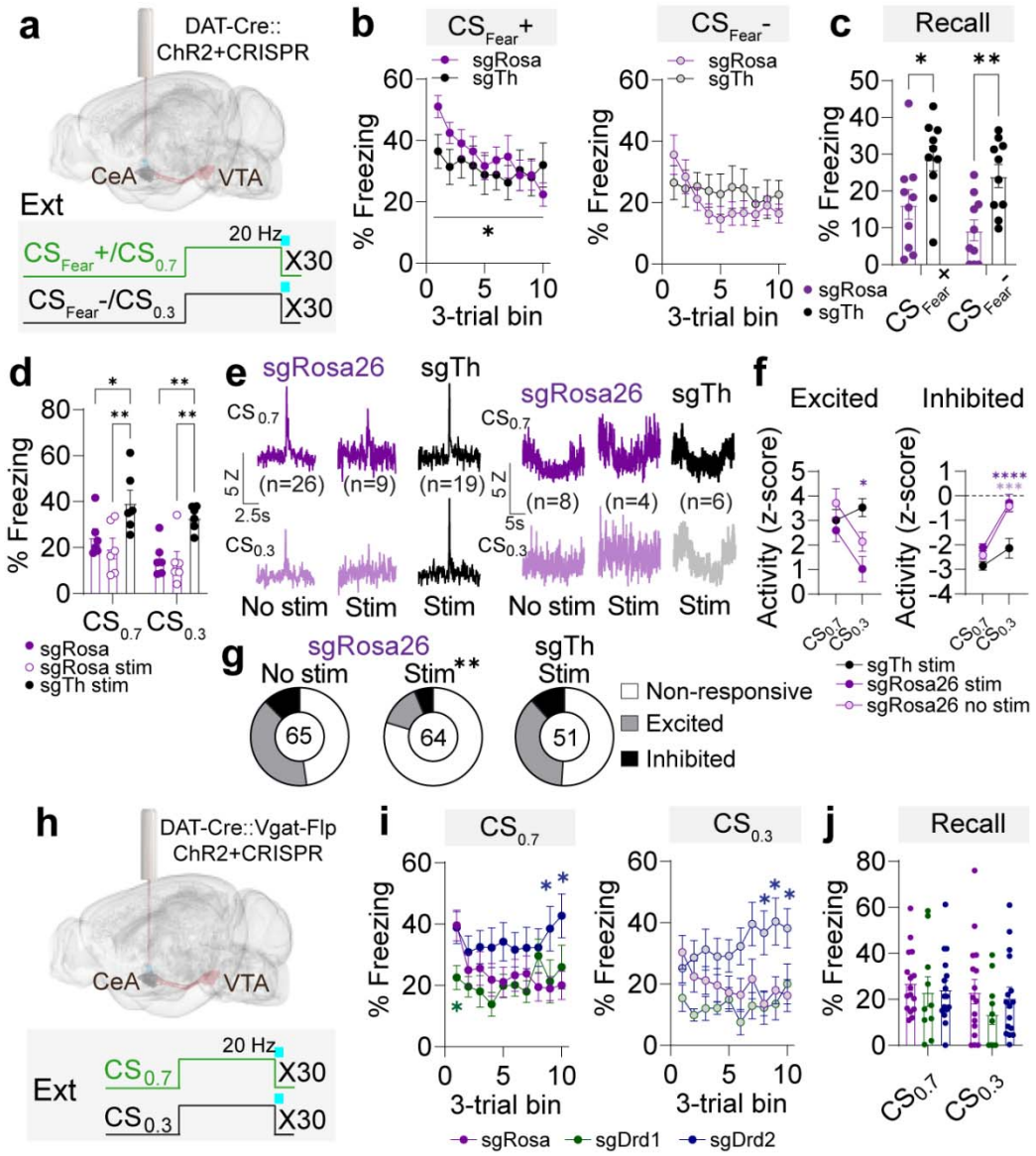

Figure 7. Dopamine dependent extinction of fear encoding in the CeA. (**a**) Schematic of extinction paradigm with precise timing of optical stimulation of dopamine terminals in the CeA during extinction training. **(b**) Fear extinction training in both groups showing enhanced extinction in the sg*Rosa26* stimulated mice (**P* < 0.05, sg*Rosa26* = 10 mice and sg*Th* = 10 mice). (**c**) Extinction memory recall showing enhanced recall in the sg*Rosa26* stimulated mice (**P* < 0.05, ***P* < 0.01). (**d**) Freezing to the $CS_{0.7}$ and $CS_{0.3}$ during extinction recall with CeA recordings in sg*Rosa26* mice with and without dopamine terminal stimulation and in sg*Th* mice

with dopamine terminal stimulation during extinction training (*$P < 0.05$, **$P < 0.01$; sg*Rosa26* = 6 mice, sg*Rosa26* stim = 6 mice and sg*Th* stim = 6 mice). (**e**) Average firing to $CS_{0.7}$ and $CS_{0.3}$ in all groups during extinction recall. (**f**) Normalized responses to $CS_{0.7}$ and $CS_{0.3}$ (relative to baseline firing before each CS) in all groups during extinction recall. There were no differences detected in the excited cells in response to the $CS_{0.7}$, but significant differences were observed in response to the $CS_{0.3}$ (*$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$). (**g**) The proportion of cells either excited or inhibited by the CSs during extinction recall was significantly reduced in the sg*Rosa26* mice with dopamine terminal stimulation during extinction training compared to sg*Rosa26* mice without dopamine terminal stimulation and sg*Th* mice with dopamine terminal stimulation (Fisher's exact test, **$P < 0.01$). (**h**) Schematic of extinction paradigm with precise timing of optical stimulation of dopamine terminals in the CeA during extinction training. (**i**) During fear extinction training, freezing to the $CS_{0.7}$ and $CS_{0.3}$ was reduced early in sg*Drd1* mice and increased late in sg*Drd2* mice relative to sg*Rosa26* control mice (*$P < 0.05$). (**j**) Freezing during extinction memory recall was not different between groups. All data presented as mean ± S.E.M. Detailed information about statistical results is provided in Extended Data Table 1.