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M_1 Muscarinic Receptors Modulate Fear-Related Inputs to the Prefrontal Cortex: Implications for Novel Treatments of Posttraumatic Stress Disorder

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ABSTRACT

BACKGROUND: The prefrontal cortex (PFC) integrates information from multiple inputs to exert top-down control allowing for appropriate responses in a given context. In psychiatric disorders such as posttraumatic stress disorder, PFC hyperactivity is associated with inappropriate fear in safe situations. We previously reported a form of muscarinic acetylcholine receptor (mAChR)–dependent long-term depression in the PFC that we hypothesize is involved in appropriate fear responding and could serve to reduce cortical hyperactivity following stress. However, it is unknown whether this long-term depression occurs at fear-related inputs.

METHODS: Using optogenetics with extracellular and whole-cell electrophysiology, we assessed the effect of mAChR activation on the synaptic strength of specific PFC inputs. We used selective pharmacological tools to assess the involvement of M_1 mAChRs in conditioned fear extinction in control mice and in the stress-enhanced fear-learning model.

RESULTS: M_1 mAChR activation induced long-term depression at inputs from the ventral hippocampus and basolateral amygdala but not from the mediodorsal nucleus of the thalamus. We found that systemic M_1 mAChR antagonism impaired contextual fear extinction. Treatment with an M_1 positive allosteric modulator enhanced contextual fear extinction consolidation in stress-enhanced fear learning–conditioned mice.

CONCLUSIONS: M_1 mAChRs dynamically modulate synaptic transmission at two PFC inputs whose activity is necessary for fear extinction, and M_1 mAChR function is required for proper contextual fear extinction. Furthermore, an M_1 positive allosteric modulator enhanced the consolidation of fear extinction in the stress-enhanced fear-learning model, suggesting that M_1 positive allosteric modulators may provide a novel treatment strategy to facilitate exposure therapy in the clinic for the treatment of posttraumatic stress disorder.

Keywords: Fear extinction, M_1 muscarinic receptor, Positive allosteric modulator, Posttraumatic stress disorder, Prefrontal cortex, Synaptic plasticity

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The prefrontal cortex (PFC) integrates information from a diverse set of cortical and subcortical sources (1,2) and is a central structure involved in higher-order cognitive functions (3,4). Normal function of the PFC is critical for top-down processing of internal and external signals to inhibit inappropriate thoughts, emotions, and actions, and it allows for relevant behavioral responses in appropriate contexts (5–7). To properly integrate synaptic information and facilitate executive functions, input to the PFC undergoes dynamic regulation via mechanisms of synaptic plasticity, including long-term potentiation and long-term depression (LTD) of synaptic strength. These forms of synaptic plasticity are commonly considered the molecular correlates of learning and memory (8–10) and are critical in directing PFC activity to guide emotional and behavioral responses (5,11).

The PFC plays a critical role in extinction of fear conditioning by integrating information from the ventral hippocampus (vHipp) and the basolateral amygdala (BLA), key regions for encoding conditioned fear and regulating emotional responses to fearful stimuli (11,12). Interestingly, multiple studies suggest that exposure to acute or repeated stress can induce disruptions in PFC function (6,7) and can dramatically inhibit normal fear extinction (13). Stress-induced loss of fear extinction can impair recovery from trauma and is thought to play a critical role in sustaining pathological fear in persons with posttraumatic stress disorder (PTSD) (13).

Preclinical and clinical studies suggest that cholinergic projections to the PFC from the basal forebrain play important roles in the extinction of fear learning (14). Acetylcholine acts in large part through the five subtypes of muscarinic

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acetylcholine receptors (mAChRs), M_1 to M_5, of which the primarily $G\alpha_q$-coupled M_1 and $G\alpha_i$-coupled M_4 subtypes are the most abundant in the PFC (15). mAChRs are involved in working memory (16) and attention (17), as well as appropriate fear (18) and emotional responses (19). These roles of mAChRs in the PFC have been studied primarily using nonselective pan-mAChR antagonists such as scopolamine (16,18,19), but the relative contribution of each subtype to cognitive and affective functions has remained elusive, in part because of a dearth of subtype-selective compounds. We and others have recently developed selective ligands for mAChR subtypes, including highly selective agonists (20), antagonists (21), and positive allosteric modulators (PAMs) (9,22–26) for the M_1 mAChR. Using these new tools, along with genetic manipulations (27,28), we recently reported that M_1 mAChR activation induces a form of LTD in the rodent prelimbic PFC (PL) (9). This finding is especially interesting in light of studies suggesting that PFC neurons display robust firing during states of high fear and that depression of excitatory inputs to the PFC may be important for fear extinction learning (29–31). This raises the possibility that M_1 LTD could play a role in mAChR regulation of fear extinction learning. If so, this could provide important new insights that are relevant for the treatment of PTSD and other disorders in which fear extinction learning is disrupted. However, the PFC receives input from multiple subcortical areas (1,32), and it is not known whether M_1 LTD is expressed at synapses in the vHipp-PFC-BLA circuit that have been implicated in fear conditioning and extinction learning.

We now report a series of studies in which we found that M_1 mAChR activation induces LTD at the vHipp-PFC and BLA-PFC synapses but not at synapses from the mediodorsal nucleus of the thalamus (MDT). Further studies utilizing viral-mediated deletion of M_1 from pyramidal cells revealed that vHipp-PFC mAChR LTD requires postsynaptic M_1 in PFC pyramidal neurons. Interestingly, selective blockade of M_1 impaired contextual fear extinction. Finally, we found that an M_1 PAM was able to reverse deficits in contextual fear extinction in a rodent model of PTSD, implying that M_1 PAMs may have clinical efficacy as an adjunct to exposure therapy. These results are especially exciting in light of the development of M_1 PAMs as potential therapeutics for psychiatric and neurodegenerative disorders.

METHODS AND MATERIALS

Animal Use

C57BL/6J mice were acquired from Jackson Laboratories (Bar Harbor, ME). *Chrm1*$^{loxP/loxP}$ mice (28) were bred in-house. Experiments were performed in group-housed mice 8 to 12 weeks of age (2–5 per cage) on a 12-hour light/dark cycle (lights on at 6:00 AM) and given access to food and water ad libitum. All experimental protocols were approved by the Vanderbilt Institutional Animal Care and Use Committee. Viral injections were performed as previously described (10,33) with adeno-associated virus 5 (AAV5)–α-calcium/calmodulin-dependent protein kinase II (αCaMKII)–channelrhodopsin-2 (ChR2)–enhanced yellow fluorescent protein (eYFP), AAV5–αCaMKII–Cre–mCherry, and AAV5–αCaMKII–mCherry (UNC Viral Core, Chapel Hill, NC).

Electrophysiology

Extracellular field and whole-cell recordings were performed as previously described (9,10). PFC slices were prepared using an *N*-methyl-D-glucamine–based cutting and recovery solution (34) and transferred to artificial cerebrospinal fluid (aCSF) (126 mmol/L sodium chloride, 2.5 mmol/L potassium chloride, 1.25 mmol/L anhydrous monosodium phosphate, 26 mmol/L sodium bicarbonate, 10 mmol/L glucose, 2 mmol/L calcium dichloride, 1 mmol/L magnesium sulfate) supplemented with 500 μmol/L ascorbate for 1 hour. The recording chamber was perfused with aCSF (31°C ± 1°C) at a rate of 2 mL/min. For field recordings, recording electrodes filled with aCSF were placed in PL layer V. For whole-cell recordings voltage-clamped at −70 mV, mCherry-positive neurons in PL layer V were filled with a potassium-based internal solution (125 mmol/L potassium gluconate, 4 mmol/L sodium chloride, 10 mmol/L HEPES, 4 mmol/L adenosine 5′-triphosphate magnesium salt, 0.3 mmol/L guanosine 5′-triphosphate sodium salt hydrate, 10 mmol/L Tris-phosphocreatine). Local glutamate release was elicited with 470-nm light to activate ChR2 or via a concentric bipolar stimulating electrode in layer II/III at a rate of 0.05 Hz for field and 0.1 Hz for whole-cell recordings.

Behavior-Cued and Contextual Fear Extinction

Fear conditioning was performed as described in Supplemental Materials and Methods. Mice were handled for 2 days prior to fear conditioning. Percent time spent freezing was used as a measure of learned fear. The stress-enhanced fear learning (SEFL) model involved an initial day of 10 random footshocks delivered over 1 hour in a distinct context.

Compounds

Oxotremorine-M (Oxo-M) was obtained from Tocris (Bio-Techne, Minneapolis, MN). VU0255035, VU0364572, and VU0453595 were synthesized in-house. For electrophysiology, stock solutions were prepared in deionized water or dimethyl sulfoxide and diluted to working concentrations in aCSF (≤0.1% dimethyl sulfoxide). For behavior, compounds were prepared in 20% β-cyclodextrin and administered via intraperitoneal injection.

Data Analysis

Data are presented as mean ± standard error. Statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA). A paired or unpaired two-tailed Student's *t* test, one- or two-way analysis of variance, or repeated-measures two-way analysis of variance with Bonferroni's post hoc test were used where appropriate. Results of statistical analyses are presented in the figure legends.

RESULTS

Muscarinic LTD in the PFC Is Input Specific

Our lab and others previously reported that the cholinergic agonist carbachol induces LTD of extracellular field excitatory postsynaptic potentials (fEPSPs) recorded in layer V in response to electrical stimulation of layer II/III in PL slices (9,35). We first confirmed that this LTD is induced by the

mAChR-selective agonist Oxo-M (36) in acute slices of the mouse PFC. Bath application of Oxo-M (10 μmol/L) induced a robust LTD of electrically evoked fEPSPs measured after drug washout (Figure 1A, E), a finding that is consistent with our previous carbachol data and confirms that LTD in the PFC can be induced by a more selective mAChR agonist.

We then determined whether Oxo-M would induce LTD at distinct subcortical inputs to the PFC. We used an optogenetic approach whereby we injected mice with virus encoding the expression of eYFP-tagged ChR2 into the afferent region of interest and prepared acute PFC slices 3 to 4 weeks later. Corroborating previous reports (32), we detected terminals from the vHipp, the BLA, and the MDT throughout the PFC (Supplemental Figure S1). After establishing a stable baseline of optically evoked fEPSPs (ofEPSPs), bath application of Oxo-M (10 μmol/L) induced LTD of vHipp-evoked ofEPSPs (Figure 1B, E) and of BLA-evoked ofEPSPs (Figure 1C, E) but not of MDT-evoked ofEPSPs (Figure 1D, E). The LTD of electrically evoked fEPSPs and vHipp- and BLA-evoked ofEPSPs were of similar magnitude but were all significantly different from the MDT input (Figure 1F). Together, these data suggest that mAChR LTD of glutamatergic transmission in the PFC exhibits input specificity and is observed at specific inputs from the BLA and vHipp.

Input-Specific mAChR LTD Is Mediated by M_1 Receptors

Next, we assessed whether M_1 mediates mAChR LTD at vHipp-PFC and BLA-PFC synapses. Consistent with prior studies using electrical stimulation (9), Oxo-M-induced LTD at the vHipp input was blocked in the constant presence of the M_1 antagonist VU0255035 (10 μmol/L) at a concentration selective for M_1 over other mAChR subtypes (9,21,37,38) (Figure 2A, C). Furthermore, we found that bath application of the selective M_1 allosteric agonist VU0364572 (20) (30 μmol/L) was sufficient to induce LTD at the vHipp-PFC synapse (Figure 2B, C). Similarly, BLA-PFC mAChR LTD was significantly attenuated by VU0255035 (Figure 2D, F) and was induced by the allosteric agonist VU0364572 (Figure 2E, F). This finding is consistent with the role of M_1 in mediating mAChR LTD of electrically evoked fEPSPs and confirms that M_1 is the subtype mediating mAChR LTD at inputs from the vHipp and BLA to the PFC.

vHipp-PFC mAChR LTD Requires Postsynaptic M_1 Receptors

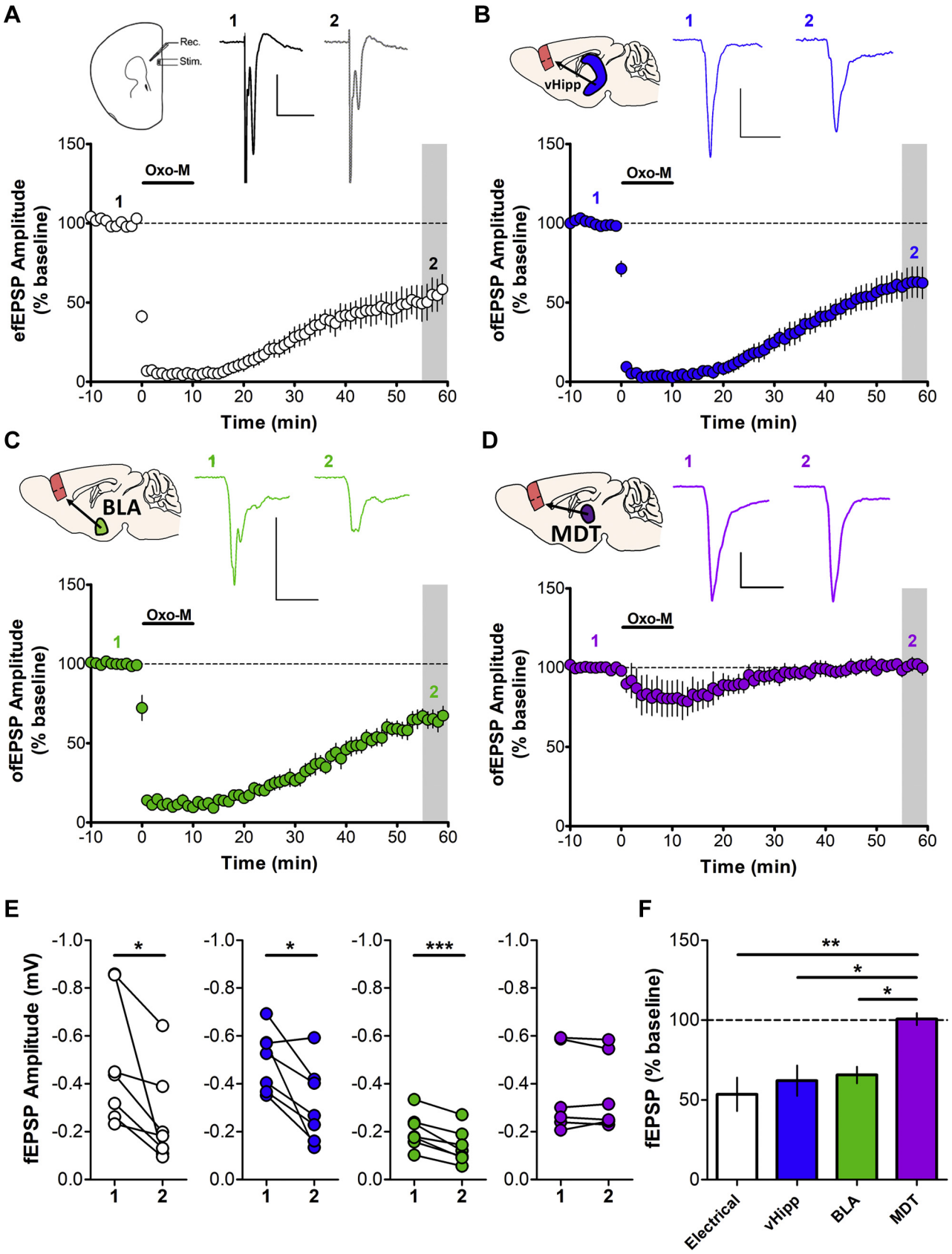
Previously, we reported that mAChR LTD of electrically evoked fEPSPs correlated with increased inhibition onto layer V pyramidal neurons and that this may contribute to M_1 LTD (33). As M_1 is expressed on both PFC glutamatergic pyramidal neurons and gamma-aminobutyric acidergic interneurons (39–41), this finding brings into question the localization of M_1 involved in M_1 LTD. To address this, we used a viral-mediated knockdown approach allowing for selective deletion of M_1 receptors from glutamatergic pyramidal neurons in the PFC (Figure 3A). Five to 6 weeks postinjection, we prepared slices to confirm viral expression and observed cell bodies labeled with mCherry and terminals positive for eYFP throughout the PFC (Supplemental Figure S2).

Using whole-cell electrophysiology in acute slices, we confirmed the genetic deletion of M_1 by monitoring the depolarizing inward current induced by a cholinergic agonist, previously shown to be dependent on postsynaptic M_1 receptors (42). In mCherry-positive neurons from αCaMKII–mCherry–infected mice, Oxo-M (10 μM) induced a depolarizing inward current, while in mCherry-positive neurons from αCaMKII–Cre–mCherry–infected mice, Oxo-M did not cause any change in the holding current (Figure 3B). Oxo-M caused a significant increase in the frequency of spontaneous excitatory postsynaptic currents (EPSCs) during Oxo-M add that returned to baseline levels on washout (Figure 3C) in control-infected cells. In Cre-infected cells, the Oxo-M-induced increase in spontaneous EPSC frequency was abolished and, interestingly, we observed a significant decrease in spontaneous EPSC frequency that persisted following drug washout (Figure 3D), a finding that might be due to activation of other, inhibitory mAChRs (15). These data functionally confirm deletion of M_1 from PFC pyramidal cells, validating our genetic approach.

Having confirmed deletion of M_1 from pyramidal cells, we then determined whether postsynaptic M_1 receptors were required for mAChR LTD at the vHipp-PFC synapse. We selected the vHipp-PFC input based on the complete blockade of LTD by the M_1 antagonist (Figure 2A) compared with the significant but incomplete block of BLA-PFC LTD (Figure 2D). Furthermore, to control for the effects of incomplete viral infection on extracellular field recordings (Supplemental Figure S3), we used whole-cell patch clamp recordings to measure optically evoked EPSCs from vHipp terminals. In mice infected with control virus, Oxo-M (10 μmol/L) induced an LTD of optically evoked EPSCs (Figure 3E, G). Compared with that in control mice, LTD induced by Oxo-M in mice infected with Cre virus was significantly attenuated (Figure 3F, H, I). These data indicate that postsynaptic M_1 mediates mAChR LTD at vHipp-PFC synapses.

M_1 Receptor Function Is Necessary for Contextual but Not Cued Fear Extinction

Together, these data show that M_1 is poised to regulate synaptic transmission at two long-range inputs to the PFC. Given the established role of mAChRs and inputs from the BLA and vHipp in extinction of fear conditioning, we hypothesized that the in vivo relevance of this input-specific modulation may relate to fear extinction. We implemented a 5-day fear conditioning protocol to assess the effects of M_1 antagonism on both auditory-cued and contextual fear extinction (Figure 4A). Mice were conditioned on day 1. During cued extinction on day 2 and context extinction on day 4, mice were administered vehicle (20% β-cyclodextrin) or 3, 10, or 30 mg/kg VU0255035 via intraperitoneal injection 30 minutes prior to being placed into the extinction context. There was no significant effect of M_1 antagonism within the cued fear extinction session (Figure 4B) nor on cued extinction recall on day 3 (Figure 4B). Interestingly, there was a significant effect of M_1 antagonism on within-session contextual fear extinction (Figure 4C), and mice administered 30 mg/kg VU0255035 prior to contextual fear extinction on day 4 displayed significantly higher freezing to the context on recall day 5 compared with vehicle-treated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mice (Figure 4C). Importantly, the maximal dose of 30 mg/kg VU0255035 did not affect freezing in animals that were not exposed to footshocks on day 1 (Supplemental Figure S4). Overall, these data suggest that M_1 activation is not required for auditory-cued fear extinction but is required for contextual fear extinction.

M_1 Potentiation Enhances Fear Extinction in a Model of PTSD

Impaired fear extinction is a hallmark of anxiety-related disorders, including PTSD, which is estimated to affect 3.5% of the United States population annually (43). Exposure therapy is one of the most common treatment paradigms for PTSD, and it shares many similarities with pavlovian fear extinction used in rodents (44). Pharmacological manipulations that enhance the acquisition and/or consolidation of fear extinction therefore may be beneficial for use in conjunction with exposure therapy. Based on our findings, we hypothesized that enhancing M_1 function with a PAM may enhance contextual fear extinction in a rodent model of PTSD.

To test this hypothesis, we used the extensively validated SEFL model, which produces phenotypes in rodents that mimic PTSD symptoms in the clinical population (43) (Figure 5A). On day 1, mice underwent SEFL conditioning and exhibited greater freezing during fear acquisition in a novel context (context B) on day 2 (Figure 5B) and when exposed to context B on day 3 (Figure 5C). SEFL-conditioned mice then received either vehicle (20% β-cyclodextrin) or the M_1 PAM VU0453595 (10 mg/kg) prior to contextual fear extinction in context B on day 3. Pretreatment with VU0453595 had no effect on the expression of contextual fear assessed during the first 3 minutes in context B and no effect on within-session extinction (Figure 5D). When mice were tested for the consolidation of extinction on day 4, PAM-treated mice spent significantly less time freezing than vehicle-treated mice (Figure 5D), indicating that VU0453595 enhanced the consolidation of contextual fear extinction in SEFL-conditioned mice.

DISCUSSION

In these studies, we found that mAChR activation induces LTD at synapses onto PL layer V from the vHipp and BLA inputs but not from the MDT. Furthermore, we confirmed that M_1 mediates LTD at both inputs and that postsynaptic M_1 is required for LTD at the vHipp-PFC synapse. This suggests that M_1 activation modulates fear-related inputs to the PFC in an input-selective manner. Based on the roles of the vHipp, BLA, and PFC in fear extinction, we further identified M_1 as necessary for contextual fear extinction. Finally, we demonstrated that M_1 potentiation enhances fear extinction in a rodent model of PTSD, suggesting that M_1 PAMs have potential clinical utility in the treatment of PTSD and stress-related disorders.

Dysregulated connectivity of subcortical regions to the PFC is present in multiple psychiatric disorders (45–47). Understanding the functional consequences of this finding has been a major focus of psychiatric-related research, and it has been aided by novel circuit-based techniques, including optogenetics (48). There have been tremendous advances in establishing the circuitry underlying specific behaviors and how these circuits might be perturbed in psychiatric disorders. However, there is a critical need to identify circuit-specific targets to translate preclinical observations into clinically effective treatments (49). We took advantage of these circuit-based approaches and found that activation of M_1 selectively induces LTD at the vHipp and BLA inputs to the PFC, identifying M_1 as a potential therapeutic target to modulate these circuits.

Intact communication between the hippocampus, amygdala, and PFC is essential for proper fear extinction in both humans (50) and rodents (51) and is dysregulated in anxiety-related disorders such as PTSD (52). In animal models, BLA and vHipp inputs to the PFC are involved in anxiety-related behaviors (48,53,54), and inactivation studies demonstrate that the vHipp, BLA, and PFC are all required for fear extinction (51) while the vHipp-PFC pathway gates fear after extinction learning (29). Thus, it is clear that the vHipp and BLA inputs to the PFC are important for fear extinction and may be disrupted in anxiety-disorder models.

Our observation that M_1 activation induces LTD at the vHipp-PFC and BLA-PFC synapses along with work demonstrating that PFC mAChRs are required for fear extinction (18) suggested that these two phenomena are related. Consistently, we found that M_1 antagonism impaired contextual fear extinction but had no effect on the extinction of auditory-cued fear. Our data do not definitively identify M_1 in the PFC as the mediator of these behavioral effects because of technical limitations including that muscarinic LTD measured

Figure 1. Muscarinic long-term depression (LTD) in the prefrontal cortex is input specific. Acute slices of the mouse prefrontal cortex were prepared 3 to 4 weeks after adeno-associated virus 5–α-calcium/calmodulin-dependent protein kinase II–channelrhodopsin-2 (ChR2)–enhanced yellow fluorescent protein was injected into the ventral hippocampus (vHipp) (blue), basolateral amygdala (BLA) (green), or mediodorsal nucleus of the thalamus (MDT) (purple). **(A)** Electrical stimulation of prelimbic prefrontal cortex layer II/III evoked field excitatory postsynaptic potentials (efEPSPs) recorded in layer V (inset, sample traces). Application of 10 μmol/L oxotremorine-M (Oxo-M) induced an acute depression followed by LTD of fEPSPs measured 55–59 minutes post drug add (53.49% ± 10.47%; 7 slices). **(B)** Optical stimulation of afferents from vHipp-ChR2–injected mice with paired pulses of 470-nm blue light (1-ms pulse duration; 50-ms interpulse interval) elicited optically evoked fEPSPs (ofEPSPs) that also underwent induction of LTD following bath application of Oxo-M (10 μmol/L) (62.01% ± 9.50%; 7 slices). **(C)** ofEPSPs evoked from stimulation of BLA-ChR2 afferents were also sensitive to Oxo-M (10 μmol/L) and expressed LTD (65.61% ± 5.28%; 7 slices). **(D)** ofEPSPs evoked in MDT-ChR2–injected mice exhibited a small acute depression in the presence of Oxo-M (10 μmol/L) but rapidly returned to baseline, not expressing LTD (100.6% ± 3.72%; 6 slices). Sample traces for panels **(A–D)** correspond to the baseline (1) and the gray-shaded area (2) (scale bars = 0.2 mV and 20 ms). **(E)** Summary data of change in fEPSP amplitude for each input; (1) baseline amplitude, (2) amplitude at 55–59 minutes post drug add corresponding to the gray-shaded regions in panels **(A–D)**. Paired Student's *t* test: Electrical, vHipp $p < .05$, BLA $p < .001$, MDT $p = .778$. **(F)** Summary data of fEPSP amplitude corresponding to gray-shaded regions expressed as a percent of baseline compared across inputs. One-way analysis of variance: $F_{3,23} = 6.228$, $p = .003$. Bonferroni's post hoc test: Electrical vs. MDT: $p < .01$, vHipp vs. MDT and BLA vs. MDT: $p < .05$, Electrical vs. vHipp: $p > .05$; Electrical vs. BLA: $p > .05$; BLA vs. vHipp: $p > .05$. $^*p < .05$; $^{**}p < .01$; $^{***}p < .001$. Rec., recording electrode; Stim., stimulating electrode.

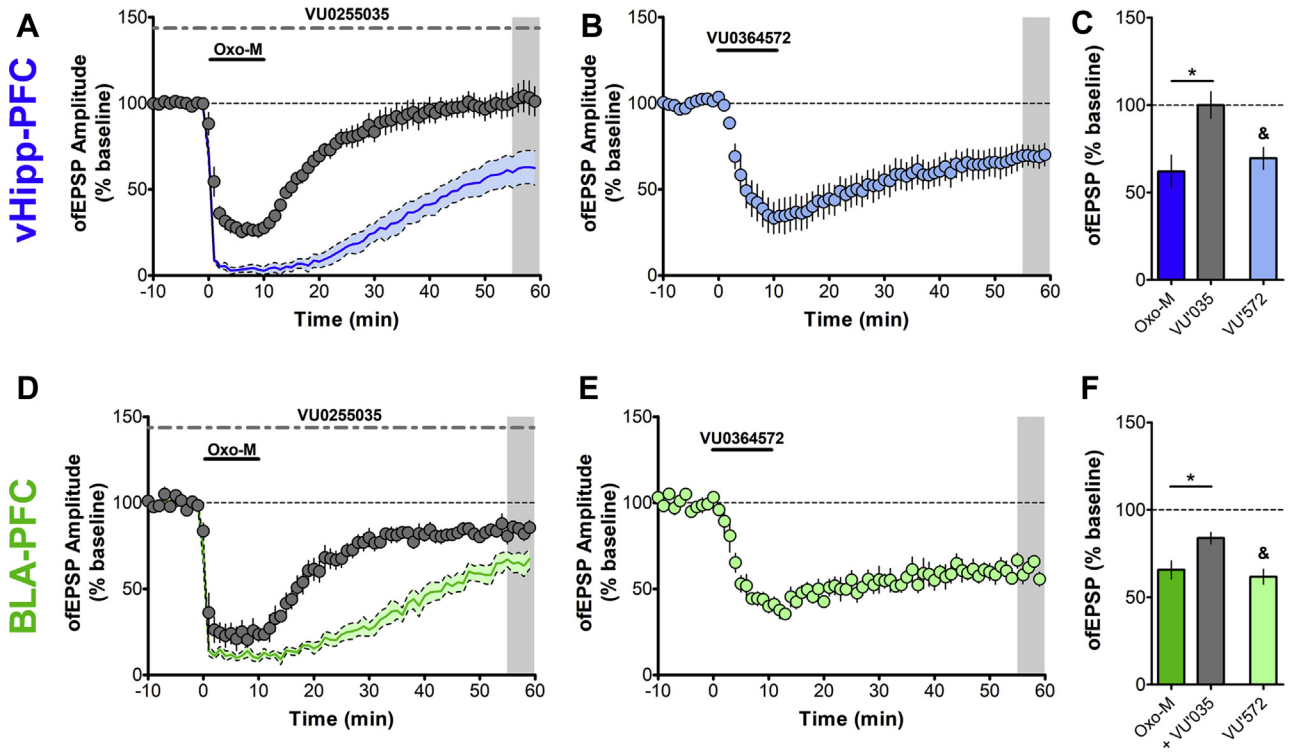

Figure 2. Input-specific muscarinic acetylcholine receptor long-term depression (LTD) is mediated by M_1 receptors. Recordings from ventral hippocampus (vHipp)-channelrhodopsin-2–injected or basolateral amygdala (BLA)–channelrhodopsin-2–injected mice. **(A)** In the constant presence of the selective M_1 antagonist VU0255035 (VU'035) (10 μmol/L), oxotremorine-M (Oxo-M) (10 μmol/L) induced an acute depression of optically evoked field excitatory postsynaptic potentials (ofEPSPs) in prelimbic prefrontal cortex (PFC) layer V evoked from vHipp afferents, but muscarinic acetylcholine receptor LTD was blocked (99.96% ± 7.67%; 5 slices). **(B)** Bath application of the selective M_1 allosteric agonist VU0364572 (VU'572, 30 μmol/L) for 10 minutes also induced LTD of ofEPSPs elicited from vHipp afferent stimulation (69.48% ± 6.38%; 5 slices). **(C)** Summary data for vHipp ofEPSP amplitude 55–59 minutes post drug add. Unpaired Student's *t* test, Oxo-M vs. Oxo-M + VU'035: $p < .05$; paired Student's *t* test comparing baseline to shaded area in panel **(B)**: $p < .05$. **(D)** LTD of ofEPSPs evoked from BLA–channelrhodopsin-2–expressing afferents in response to Oxo-M (10 μmol/L) was also blocked in the constant presence of VU'035 (83.86% ± 3.34%; 6 slices). **(E)** Bath application of VU'572 for 10 minutes also induced LTD of ofEPSPs elicited from BLA afferent stimulation (61.71% ± 4.24%; 5 slices). **(F)** Summary data for BLA ofEPSP amplitude 55–59 minutes post drug add. Unpaired Student's *t* test, Oxo-M vs. Oxo-M + VU'035: $p < .05$; paired Student's *t* test comparing baseline to shaded area in panel **(E)**: $p < .05$. Shaded time courses in panels **(A)** and **(D)** correspond to Oxo-M alone from Figure 1. In panels **(A)** and **(D),** the solid, colored line represents the mean ofEPSP amplitude and the gray-shaded region around the line shows ±SEM. *Unpaired Student's *t* test $p < .05$, &paired Student's *t* test $p < .05$.

extracellularly was still intact in Cre-injected *Chrm1*$^{loxP/loxP}$ mice. This finding suggests incomplete viral knockdown of M_1; therefore, testing the necessity of PFC M_1 for the observed behavioral effects remains elusive. Nonetheless, our approach identified the involvement of M_1 in fear extinction and that an M_1 PAM could enhance fear extinction in a model of PTSD, thus translating circuit-based neuroscience to a potential therapeutic mechanism.

Concerning the potential mechanism, the hippocampus communicates contextual information to the PFC via monosynaptic connections from the ventral pole (55). M_1 LTD at the vHipp-PFC synapse may therefore reflect a modulation of contextual information flowing into the PFC and may be more related to regulation of contextual aspects of fear rather than to nonspatial cued fear (56). This finding is consistent with our observation that M_1 antagonism blocks LTD at the vHipp-PFC synapse and impairs contextual fear extinction. Furthermore, single-unit recordings in PL indicate that decreased activity of PL pyramidal neurons corresponds with reduced fear responses (29), consistent with a reduced afferent drive into the PL via an LTD-like mechanism. M_1 LTD of vHipp-PL transmission could be required to reduce fear responses during contextual fear extinction by reducing vHipp-mediated excitation of PL neurons. While we identified postsynaptic M_1 as necessary for mAChR LTD at the vHipp-PFC synapse, the molecular mechanisms mediating vHipp-PFC M_1 LTD are still unknown. Future work investigating signaling downstream of M_1 that is necessary for the induction, expression, and maintenance mechanisms will be instrumental to investigate how this plasticity changes after fear extinction and will identify targets and mechanisms that could improve the treatment of disorders with dysfunctional vHipp-PFC connectivity. M_1 also enhances the output of infralimbic (IL) cortex pyramidal neurons, and fear extinction correlates with enhanced activity of IL neurons (18,57); thus, M_1 PAMs might enhance fear extinction via actions in the IL in addition to LTD in the PL. It is possible that both mechanisms contribute to extinction, and investigating the differential involvement of M_1 in the PL and IL to fear extinction is an interesting future direction.

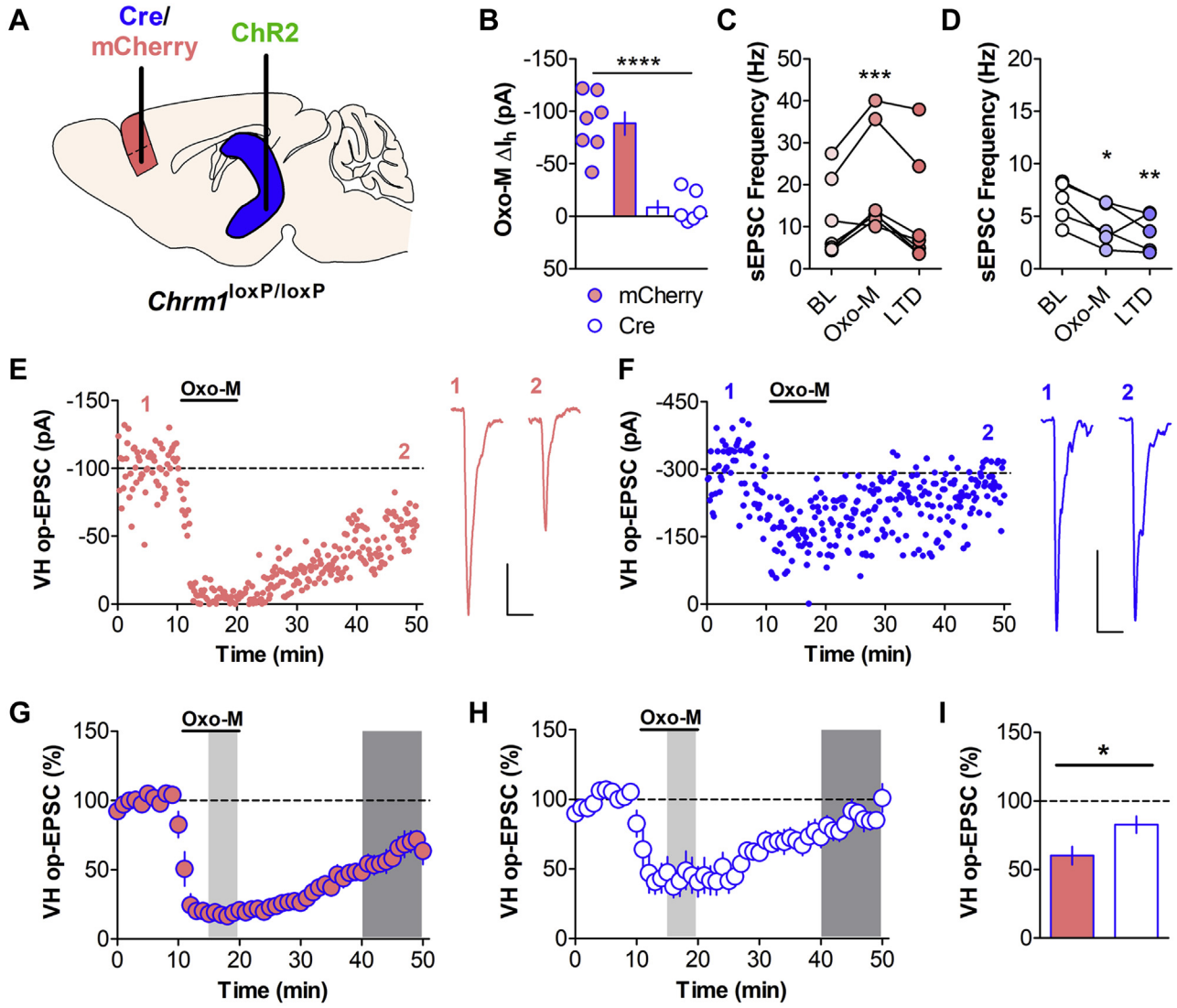

Figure 3. Ventral hippocampus–prefrontal cortex muscarinic acetylcholine receptor long-term depression (LTD) requires postsynaptic M_1 receptors. **(A)** *Chrm1*$^{loxP/loxP}$ mice were injected with adeno-associated virus 5–α-calcium/calmodulin-dependent protein kinase II (αCaMKII)–Cre–mCherry (white with blue outline throughout) or adeno-associated virus 5–αCaMKII–mCherry (red with blue outline throughout) into the prefrontal cortex and coinjected with adeno-associated virus 5–αCaMKII–channelrhodopsin-2 (ChR2)–enhanced yellow fluorescent protein into the ventral hippocampus. Recordings were performed 5–6 weeks post injection. **(B)** Oxotremorine-M (Oxo-M) (10 μmol/L) induced an inward current in neurons from control mCherry-infected mice (−88.55 ± 10.92 pA; 7 cells) but failed to elicit an inward current in neurons from Cre-mCherry–infected mice (−8.651 ± 6.06 pA; 6 cells) (Student's *t* test, mCherry vs. Cre $p <$ .0001). **(C)** Oxo-M (10 μmol/L) induced a significant increase in spontaneous excitatory postsynaptic current (sEPSC) frequency recorded before optical stimulation in mCherry neurons. (One-way repeated-measures analysis of variance, $F_{2,6} = 13.52$, $p < .001$, Bonferroni's post hoc test $p < .001$ baseline [BL] vs. Oxo-M, 7 cells). **(D)** Conversely, Oxo-M induced a significant decrease in sEPSC frequency in Cre-mCherry neurons. (One-way repeated-measures analysis of variance, $F_{2,4} = 11.49$, $p < .01$, Bonferroni's post hoc test $p < .05$ BL vs. Oxo-M, $p < .01$ BL vs. LTD, 5 cells). **(E)** A representative experiment for an mCherry-infected neuron (scale bar = 25 pA and 25 ms) and **(F)** a Cre-infected neuron (scale bar = 100 pA and 25 ms). **(G)** Summary time course for control mCherry muscarinic acetylcholine receptor LTD experiments. Bath application of Oxo-M (10 μmol/L) induced an LTD of optically evoked EPSCs (op-EPSCs) evoked from ventral hippocampus-ChR2 terminals in mCherry-infected neurons (60.15% ± 6.67%; 7 cells). **(H)** Summary time course for Cre LTD experiments. LTD of op-EPSCs was attenuated in Cre-mCherry–infected neurons (82.66% ± 6.13%; 6 cells). In both panel **(G)** and panel **(H)**, light shaded areas correspond to the time at which Oxo-M sEPSC measurements were taken for panels **(C)** and **(D)**. Dark shaded areas correspond to the time at which LTD sEPSC measurements were taken for panels **(C)** and **(D)** and for quantification in panel **(I)**. **(I)** Summary data for op-EPSC amplitude 40–49 minutes post Oxo-M add. Unpaired Student's *t* test, $p < .05$. *$p < .05$; **$p < .01$; ***$p < .001$; ****$p < .0001$. ΔI_h, change in holding current; VH, ventral hippocampus.

vHipp afferents increase feedforward inhibition (FFI), contributing to the decreased activity of PL pyramidal neurons during reduced fear responding during extinction (29). We found that M_1 LTD at the vHipp-PFC synapse occurs at excitatory inputs onto PL pyramidal neurons recorded under whole-cell conditions where the contribution of inhibition is negligible. Therefore, vHipp-PL LTD may occur simultaneously with enhanced vHipp-mediated FFI to synergistically reduce

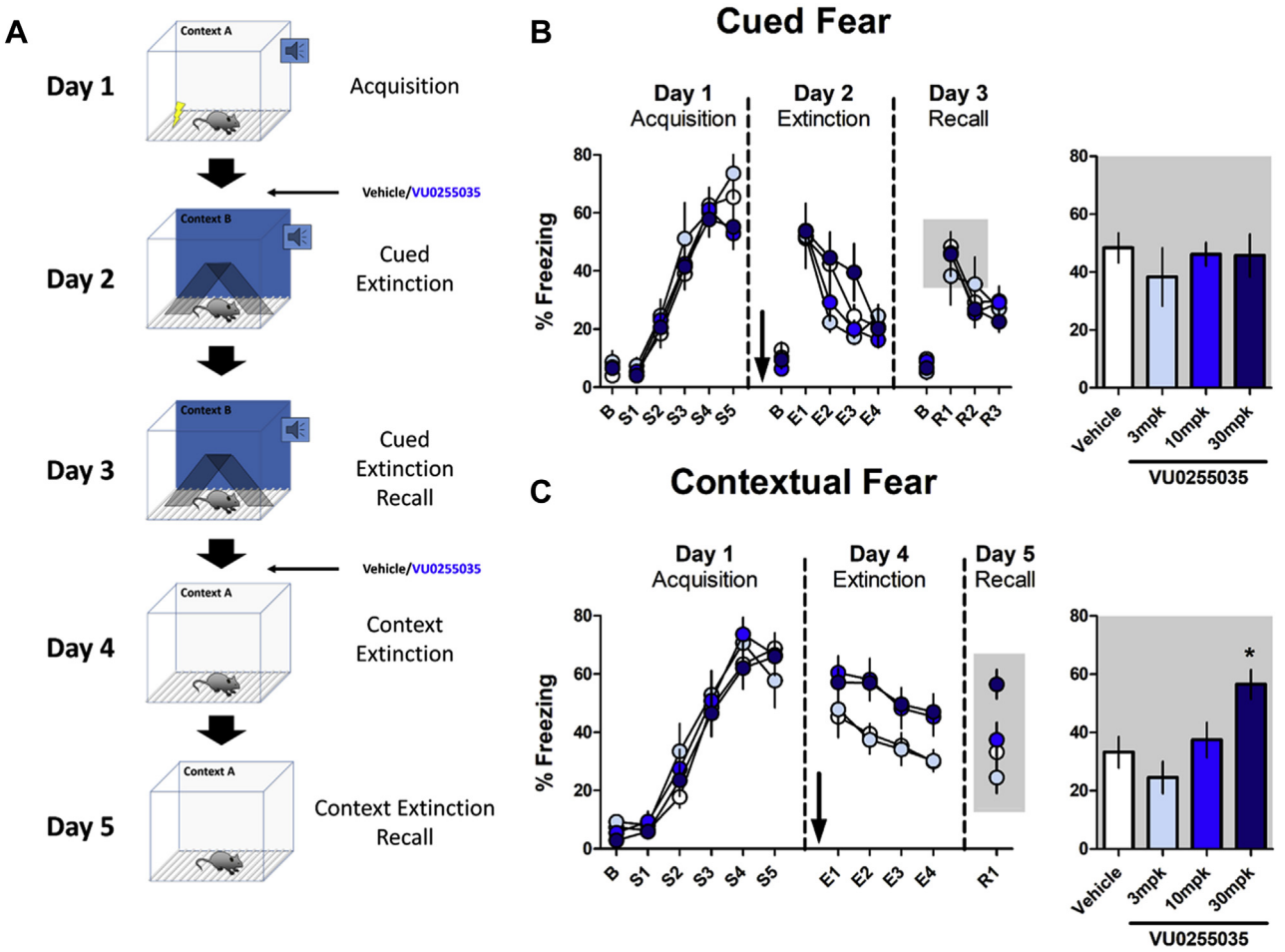

Figure 4. M_1 receptor function is necessary for contextual but not cued fear extinction. **(A)** Schematic depicting the training and testing procedure used. Mice were conditioned in context A with five mild footshocks, each preceded by a 30-second tone. On day 2, mice were administered the M_1 antagonist VU0255035 (3, 10, or 30 mg/kg [mpk], via intraperitoneal injection) or vehicle (20% β-cyclodextrin) 30 minutes before being exposed to a series of 12 tones in a novel context B to assess extinction of auditory cued fear. On day 3, mice were placed back in context B and exposed to nine tones to assess consolidation of cued fear extinction. On day 4, mice were again administered VU0255035 or vehicle and placed in context A for 12 minutes to assess contextual fear extinction. On day 5, mice were placed back in context A for 3 minutes to assess contextual fear extinction consolidation. **(B)** At all doses, VU0255035 had no effect on auditory cued fear extinction on extinction day 2 (two-way repeated-measures analysis of variance (ANOVA), effect of drug: $F_{3,35} = 0.960$, $p = .423$; effect of tone block: $F_{3,35} = 36.00$, $p < .0001$; interaction, $F_{9,35} = 1.787$, $p = .079$) or on recall day 3 (one-way ANOVA, $F_{3,35} = 0.350$, $p = .789$). Data for days 2 and 3 are binned by three tones, and mice were excluded from analysis if baseline freezing was >30%. The bar graph depicts average percent freezing to the first three tones on recall day 3, corresponding to the gray-shaded box ($n_{Vehicle} = 13$, $n_{3\ mg/kg} = 5$, $n_{10\ mg/kg} = 12$, $n_{30\ mg/kg} = 9$). **(C)** Systemic M_1 antagonism impaired within-session contextual fear extinction (two-way repeated-measures ANOVA; effect of drug: $F_{3,39} = 3.663$, $p = .020$; effect of time block: $F_{3,39} = 12.56$, $p < .0001$; interaction: $F_{3,39} = 0.317$, $p = .968$), and 30 mg/kg VU0255035 significantly impaired contextual extinction recall on day 5 (one-way ANOVA, $F_{3,39} = 5.177$, $p < .01$; Bonferroni's post hoc test, vehicle vs. 30 mg/kg $p < .05$). Extinction on days 4 and 5 are depicted as 3-minute bins. The bar graph depicts R1 ($n_{Vehicle} = 14$, $n_{3\ mg/kg} = 7$, $n_{10\ mg/kg} = 11$, $n_{30\ mg/kg} = 11$). *$p < .05$. B, baseline; E, extinction bin; R, recall bin; S, shock.

the activity of PL pyramidal neurons. M_1 activation enhances PFC interneuron activity (41), and an M_1-driven increase in FFI may also contribute to fear extinction. Our previous finding that M_1 LTD of electrically evoked fEPSPs correlates with enhanced inhibition onto PL pyramidal neurons (33) may suggest this, and the contribution of muscarinic modulation of inhibition to fear extinction is an interesting future direction, as our results do not rule out contributions of both enhanced FFI and M_1 LTD mechanisms to fear extinction. M_1 is expressed in pyramidal neurons in the human cortex (58–60), but M_1 in gamma-aminobutyric acidergic interneurons has been demonstrated only in rodent (40,41) and nonhuman primate (61) cortices. Therefore, while our results pertaining to M_1 in PFC pyramidal neurons are likely relevant to humans, the clinical implications of M_1 modulation of inhibitory transmission are unknown and would require identification of M_1 in human cortical interneurons.

Systemic and intracortical delivery of the pan-muscarinic antagonist scopolamine impairs the consolidation of cued fear extinction in rats (18). In contrast to these findings, the M_1 antagonist VU0255035 did not impair cued fear extinction in the present studies. While M_1 mAChR activation promotes cued fear consolidation (62), our findings suggest that M_1 is not necessary for cued fear extinction, and other muscarinic

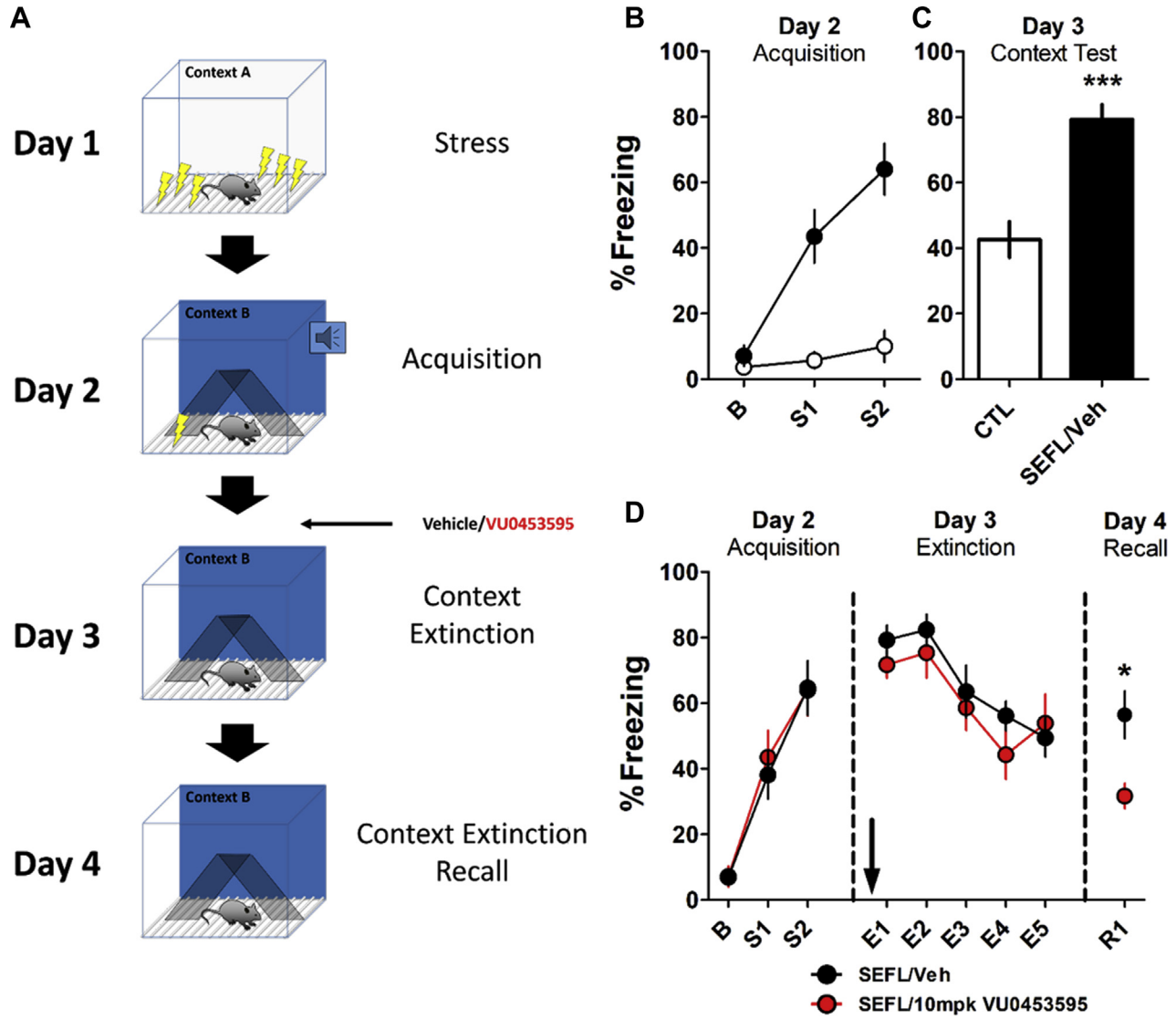

Figure 5. M_1 potentiation enhances fear extinction in a model of posttraumatic stress disorder. **(A)** Schematic illustrating the stress-enhanced fear learning (SEFL) model and experimental design. On day 1, mice underwent SEFL conditioning in context A, where they received 10 footshocks at random intervals over 1 hour. Control (CTL) mice were placed in context A for 1 hour. Days 2, 3, and 4 were performed in a novel context B. On day 2, mice were conditioned with two mild footshocks in context B. On day 3, SEFL-conditioned mice were administered vehicle (Veh) (20% β-cyclodextrin) or 10 mg/kg (mpk) VU0453595 via intraperitoneal injection 15 minutes before being placed back in context B, where they underwent a 15-minute context extinction session. On day 4, mice were placed back in context B for 3 minutes to assess context extinction consolidation. Mice that received SEFL on day 1 froze significantly more **(B)** on day 2 during acquisition and **(C)** on day 3. The bar graph depicts the first 3 minutes in context B on day 3 (unpaired Student's *t* test, $p < .001$; $n_{CTL} = 8$, $n_{SEFL/Veh} = 10$). **(D)** Administration of 10 mg/kg VU0453595 had no effect on within-session extinction on day 3 (two-way repeated-measures analysis of variance: effect of drug: $F_{1,18} = 5.033$, $p = .440$; effect of time block: $F_{4,18} = 15.15$, $p < .0001$; interaction: $F_{4,18} = 0.782$, $p = .541$) but enhanced consolidation of contextual fear extinction measured on day 4 (unpaired Student's *t* test, $p < .05$; $n_{SEFL/Veh} = 10$, $n_{SEFL/10\ mg/kg\ VU0453595} = 10$). *$p < .05$; ***$p < .001$. B, baseline; E, extinction bin; R, recall bin; S, shock.

subtypes may contribute to extinction of cued fear. Our present studies provide insight into this hypothesis. M_1 antagonism or genetic deletion does not impair the acute depression of fEPSPs at vHipp/BLA-PFC synapses. This transient depression may be permissive for cued, but not contextual, fear extinction. Additionally, although the M_1 antagonist attenuated mAChR LTD at the BLA-PFC synapse, we did not observe a complete block. Other muscarinic receptors, such as M_4, likely contribute to mAChR LTD at the BLA-PFC synapse, and M_1-independent depression may be sufficient for cued fear extinction. The involvement of M_4 in fear extinction is an intriguing future direction given the aforementioned scopolamine effect and the relatively high expression of M_4 in the PFC.

The rodent PL shares connectivity and anatomical similarities to the human dorsal anterior cingulate cortex (dACC), and thus the LTD we observed could relate to decreased activity of the human dACC observed during fear extinction (63). In a

functional magnetic resonance imaging study, PTSD patients exhibited dACC and amygdala hyperactivity and hippocampal hypoactivity compared with that of control subjects during a fear extinction task (52). Hyperactivity of the dACC and amygdala might reflect a deficit in mechanisms similar to mAChR LTD, while reductions in hippocampal activity could relate to deficits in a previously described hippocampal LTD-to–long-term potentiation switch (64), a reduction in vHipp-mediated FFI, and/or a deficit in vHipp-PFC M_1 LTD. The aforementioned functions of M_1 suggest that it could be a valid therapeutic target to rescue deficient extinction in PTSD and imply with M_1 expression in human cortex that our findings have translational relevance to humans.

M_1 potentiation could possibly reduce dACC hyperactivity in PTSD patients via LTD of hyperactive amygdala inputs and shifting vHipp input toward inhibition via enhanced FFI and LTD of excitatory transmission. This hypothesis is consistent with our finding that the M_1 PAM VU0453595 enhances contextual fear extinction in the SEFL model. Mimicking the disrupted circuitry in PTSD, rodents exposed to stressors including SEFL exhibit hyperactivity of the PL and BLA and hypoactivity of the hippocampus (13), suggesting that these models exhibit excellent face validity with respect to the human disorder. Treatment with an M_1 PAM before extinction enhanced the consolidation and recall of contextual fear extinction, suggesting that M_1 PAMs may be effective therapeutics to enhance exposure therapy in the clinic. Dysfunctional connectivity between the hippocampus, amygdala, and PFC (46,65) and impaired fear extinction (66) are present in many psychiatric disorders; therefore, these results and potential translatability may be relevant to disorders other than PTSD. This is especially exciting as M_1 PAMs have entered or completed phase I trials (see ClinicalTrials.gov Identifiers NCT03220295 and NCT02769065) with schizophrenia and Alzheimer's disease as intended therapeutic indications. Excitingly, our findings suggest that PTSD might be another promising therapeutic area for these novel drugs.

In conclusion, we report that activation of M_1 induces LTD of fear-related inputs from the vHipp and BLA to the PFC. This finding is consistent with those of previous studies demonstrating mAChR LTD at hippocampal inputs to the PFC (33,67), and it further identifies the BLA, but not the MDT, as another input that expresses this form of synaptic plasticity. We also show that M_1 activation is required for contextual fear extinction and that potentiating M_1 in vivo with a PAM enhances contextual fear extinction in the SEFL model of PTSD. Our results add M_1 LTD at the vHipp and BLA inputs to the extensively studied functions of M_1 in the PFC; however, future studies are necessary to determine the role of M_1-dependent input-specific modulation in other PFC-dependent processes. Overall, these results demonstrate that M_1 is poised to regulate fear-related information processing and suggest that M_1 PAMs could modulate aberrant limbic inputs to the PFC and could be useful as adjunct therapeutics to facilitate exposure therapy for PTSD in the clinic.

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JM and PJC designed the study and wrote the manuscript. JM, MEJ, and SPM designed and performed the electrophysiology experiments. JM and BJS designed and JM and BL performed the behavioral experiments. JM and BL performed the immunofluorescence experiments. KT, DWE, and CWL developed and synthesized VU0255035, VU0364572, and VU0453595. JJL provided the *Chrm1*$^{loxP/loxP}$ mice. All authors contributed to the preparation of the manuscript.

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DWE is an inventor on patents that protect different classes of metabotropic glutamate and muscarinic allosteric modulators. CWL has been funded by the National Institutes of Health, Johnson and Johnson, Bristol-Myers Squibb, AstraZeneca, Michael J. Fox Foundation, and Seaside Therapeutics. He has consulted for AbbVie and received compensation. He is an inventor on patents that protect different classes of metabotropic glutamate and muscarinic receptor allosteric modulators. PJC has been funded by the National Institutes of Health, Michael J. Fox Foundation, Dystonia Medical Research Foundation, CHDI Foundation, and others. Over the past 3 years, he has served on the Scientific Advisory Boards for Michael J. Fox Foundation, Stanley Center for Psychiatric Research Broad Institute (Massachusetts Institute of Technology/Harvard), Karuna Pharmaceuticals, Lieber Institute for Brain Development, Proof of Clinical Mechanism (POCM) and Proof of Concept (POC) Consortium, and Neurobiology Foundation for Schizophrenia and Bipolar Disorder. He is an inventor on patents that protect different classes of metabotropic glutamate and muscarinic receptor allosteric modulators. All other authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

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REFERENCES

1. Hoover WB, Vertes RP (2007): Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149–179.
2. Vertes RP (2006): Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience 142:1–20.
3. Goldman-Rakic PS (1995): Cellular basis of working memory. Neuron 14:477–485.
4. Gazzaley A, Nobre AC (2012): Top-down modulation: Bridging selective attention and working memory. Trends Cogn Sci 16:129–135.
5. Miller EK, Cohen JD (2001): An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167–202.
6. Arnsten AFT (2009): Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci 10:410–422.
7. Arnsten AFT, Jin LE (2014): Molecular influences on working memory circuits in dorsolateral prefrontal cortex. Prog Mol Biol Transl Sci 122:211–231.

8. Goto Y, Yang CR, Otani S (2010): Functional and dysfunctional synaptic plasticity in prefrontal cortex: Roles in psychiatric disorders. Biol Psychiatry 67:199–207.
9. Ghoshal A, Rook JM, Dickerson JW, Roop GN, Morrison RD, Jalan-Sakrikar N, *et al.* (2016): Potentiation of M_1 muscarinic receptor reverses plasticity deficits and negative and cognitive symptoms in a schizophrenia mouse model. Neuropsychopharmacology 41:598–610.
10. Joffe ME, Santiago CI, Engers JL, Lindsley CW, Conn PJ (2017): Metabotropic glutamate receptor subtype 3 gates acute stress-induced dysregulation of amygdalo-cortical function [published online ahead of print Dec 21]. Mol Psychiatry.
11. Maren S, Phan KL, Liberzon I (2013): The contextual brain: Implications for fear conditioning, extinction and psychopathology. Nat Rev Neurosci 14:417–428.
12. Chattarji S, Tomar A, Suvrathan A, Ghosh S, Rahman MM (2015): Neighborhood matters: Divergent patterns of stress-induced plasticity across the brain. Nat Neurosci 18:1364–1375.
13. Maren S, Holmes A (2016): Stress and fear extinction. Neuropsychopharmacology 41:58–79.
14. Wilson MA, Fadel JR (2017): Cholinergic regulation of fear learning and extinction. J Neurosci Res 95:836–852.
15. Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR (1991): Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 11:3218–3226.
16. Pilcher JJ, Sessions GR, McBride SA (1997): Scopolamine impairs spatial working memory in the radial maze: An analysis by error type and arm choice. Pharmacol Biochem Behav 58:449–459.
17. Mirza NR, Stolerman IP (2000): The role of nicotinic and muscarinic acetylcholine receptors in attention. Psychopharmacology (Berl) 148:243–250.
18. Santini E, Sepulveda-Orengo M, Porter JT (2012): Muscarinic receptors modulate the intrinsic excitability of infralimbic neurons and consolidation of fear extinction. Neuropsychopharmacology 37:2047–2056.
19. Navarria A, Wohleb ES, Voleti B, Ota KT, Dutheil S, Lepack AE, *et al.* (2015): Rapid antidepressant actions of scopolamine: Role of medial prefrontal cortex and M_1-subtype muscarinic acetylcholine receptors. Neurobiol Dis 82:254–261.
20. Digby GJ, Noetzel MJ, Bubser M, Utley TJ, Walker AG, Byun NE, *et al.* (2012): Novel allosteric agonists of M_1 muscarinic acetylcholine receptors induce brain region-specific responses that correspond with behavioral effects in animal models. J Neurosci 32:8532–8544.
21. Sheffler DJ, Williams R, Bridges TM, Xiang Z, Kane AS, Byun NE, *et al.* (2009): A novel selective muscarinic acetylcholine receptor subtype 1 antagonist reduces seizures without impairing hippocampus-dependent learning. Mol Pharmacol 76:356–368.
22. Ma L, Seager MA, Wittmann M, Jacobson M, Bickel D, Burno M, *et al.* (2009): Selective activation of the M_1 muscarinic acetylcholine receptor achieved by allosteric potentiation. Proc Natl Acad Sci U S A 106:15950–15955.
23. Marlo JE, Niswender CM, Days EL, Bridges TM, Xiang Y, Rodriguez AL, *et al.* (2009): Discovery and characterization of novel allosteric potentiators of M_1 muscarinic receptors reveals multiple modes of activity. Mol Pharmacol 75:577–588.
24. Grannan MD, Mielnik CA, Moran SP, Gould RW, Ball J, Lu Z, *et al.* (2016): Prefrontal cortex-mediated impairments in a genetic model of NMDA receptor hypofunction are reversed by the novel M_1 PAM VU6004256. ACS Chem Neurosci 7:1706–1716.
25. Moran SP, Dickerson JW, Cho HP, Xiang Z, Maksymetz J, Remke DH, *et al.* (2018): M_1-positive allosteric modulators lacking agonist activity provide the optimal profile for enhancing cognition. Neuropsychopharmacology 43:1763–1771.
26. Rook JM, Bertron JL, Cho HP, Garcia-Barrantes PM, Moran SP, Maksymetz JT, *et al.* (2018): A novel M_1 PAM VU0486846 exerts efficacy in cognition models without displaying agonist activity or cholinergic toxicity. ACS Chem Neurosci 9:2274–2285.
27. Hamilton SE, Loose MD, Qi M, Levey AI, Hille B, McKnight GS, *et al.* (1997): Disruption of the M_1 receptor gene ablates muscarinic receptor-dependent m current regulation and seizure activity in mice. Proc Natl Acad Sci U S A 94:13311–13316.
28. Kamsler A, McHugh TJ, Gerber D, Huang SY, Tonegawa S (2010): Presynaptic M_1 muscarinic receptors are necessary for mGluR long-term depression in the hippocampus. Proc Natl Acad Sci U S A 107:1618–1623.
29. Sotres-Bayon F, Sierra-Mercado D, Pardilla-Delgado E, Quirk GJ (2012): Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. Neuron 76:804–812.
30. Burgos-Robles A, Vidal-Gonzalez I, Quirk GJ (2009): Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. J Neurosci 29:8474–8482.
31. Walker AG, Wenthur CJ, Xiang Z, Rook JM, Emmitte KA, Niswender CM, *et al.* (2015): Metabotropic glutamate receptor 3 activation is required for long-term depression in medial prefrontal cortex and fear extinction. Proc Natl Acad Sci U S A 112:1196–1201.
32. Little JP, Carter AG (2012): Subcellular synaptic connectivity of layer 2 pyramidal neurons in the medial prefrontal cortex. J Neurosci 32:12808–12819.
33. Ghoshal A, Moran SP, Dickerson JW, Joffe ME, Grueter BA, Xiang Z, *et al.* (2017): Role of mGlu5 receptors and inhibitory neurotransmission in M_1 dependent muscarinic LTD in the prefrontal cortex: Implications in schizophrenia. ACS Chem Neurosci 8:2254–2265.
34. Ting JT, Lee BR, Chong P, Soler-Llavina G, Cobbs C, Koch C, *et al.* (2018): Preparation of acute brain slices using an optimized *N*-methyl-D-glucamine protective recovery method. J Vis Exp e53825.
35. Caruana DA, Warburton EC, Bashir ZI (2011): Induction of activity-dependent LTD requires muscarinic receptor activation in medial prefrontal cortex. J Neurosci 31:18464–18478.
36. Bubser M, Byun N, Wood MR, Jones CK (2012): Muscarinic receptor pharmacology and circuitry for the modulation of cognition. Handb Exp Pharmacol 121–166.
37. Bell LA, Bell KA, McQuiston AR (2013): Synaptic muscarinic response types in hippocampal ca1 interneurons depend on different levels of presynaptic activity and different muscarinic receptor subtypes. Neuropharmacology 73:160–173.
38. Xiang Z, Thompson AD, Jones CK, Lindsley CW, Conn PJ (2012): Roles of the M_1 muscarinic acetylcholine receptor subtype in the regulation of basal ganglia function and implications for the treatment of Parkinson's disease. J Pharmacol Exp Ther 340:595–603.
39. Yamasaki M, Matsui M, Watanabe M (2010): Preferential localization of muscarinic M_1 receptor on dendritic shaft and spine of cortical pyramidal cells and its anatomical evidence for volume transmission. J Neurosci 30:4408–4418.
40. Wohleb ES, Wu M, Gerhard DM, Taylor SR, Picciotto MR, Alreja M, *et al.* (2016): GABA interneurons mediate the rapid antidepressant-like effects of scopolamine. J Clin Invest 126:2482–2494.
41. Yi F, Ball J, Stoll KE, Satpute VC, Mitchell SM, Pauli JL, *et al.* (2014): Direct excitation of parvalbumin-positive interneurons by M_1 muscarinic acetylcholine receptors: Roles in cellular excitability, inhibitory transmission and cognition. J Physiol 592:3463–3494.
42. Shirey JK, Brady AE, Jones PJ, Davis AA, Bridges TM, Kennedy JP, *et al.* (2009): A selective allosteric potentiator of the M_1 muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. J Neurosci 29:14271–14286.
43. Perusini JN, Meyer EM, Long VA, Rau V, Nocera N, Avershal J, *et al.* (2016): Induction and expression of fear sensitization caused by acute traumatic stress. Neuropsychopharmacology 41:45–57.
44. Graham BM, Milad MR (2011): The study of fear extinction: Implications for anxiety disorders. Am. J. Psychiatry 168:1255–1265.
45. Godsil BP, Kiss JP, Spedding M, Jay TM (2013): The hippocampal-prefrontal pathway: The weak link in psychiatric disorders? Eur Neuropsychopharmacol 23:1165–1181.
46. Ghoshal A, Conn PJ (2015): The hippocampo-prefrontal pathway: A possible therapeutic target for negative and cognitive symptoms of schizophrenia. Future Neurol 10:115–128.

47. Rauch SL, Shin LM, Phelps EA (2006): Neurocircuitry models of posttraumatic stress disorder and extinction: Human neuroimaging research—past, present, and future. Biol Psychiatry 60:376–382.
48. Allsop SA, Vander Weele CM, Wichmann R, Tye KM (2014): Optogenetic insights on the relationship between anxiety-related behaviors and social deficits. Front Behav Neurosci 8:241.
49. Gordon JA (2016): On being a circuit psychiatrist. Nat Neurosci 19:1385–1386.
50. Phelps EA, Delgado MR, Nearing KI, LeDoux JE (2004): Extinction learning in humans: Role of the amygdala and VMPFC. Neuron 43:897–905.
51. Sierra-Mercado D, Padilla-Coreano N, Quirk GJ (2011): Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology 36:529–538.
52. Milad MR, Pitman RK, Ellis CB, Gold AL, Shin LM, Lasko NB, *et al.* (2009): Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol Psychiatry 66:1075–1082.
53. Felix-Ortiz AC, Burgos-Robles A, Bhagat ND, Leppla CA, Tye KM (2016): Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. Neuroscience 321:197–209.
54. Padilla-Coreano N, Bolkan SS, Pierce GM, Blackman DR, Hardin WD, Garcia-Garcia AL, *et al.* (2016): Direct ventral hippocampal-prefrontal input is required for anxiety-related neural activity and behavior. Neuron 89:857–866.
55. Sigurdsson T, Duvarci S (2015): Hippocampal-prefrontal interactions in cognition, behavior and psychiatric disease. Front Syst Neurosci 9:190.
56. Tovote P, Fadok JP, Lüthi A (2015): Neuronal circuits for fear and anxiety. Nat Rev Neurosci 16:317–331.
57. Santini E, Quirk GJ, Porter JT (2008): Fear conditioning and extinction differentially modify the intrinsic excitability of infralimbic neurons. J Neurosci 28:4028–4036.
58. Scarr E, Hopper S, Vos V, Seo MS, Everall IP, Aumann TD, *et al.* (2018): Low levels of muscarinic M_1 receptor-positive neurons in cortical layers iii and v in Brodmann areas 9 and 17 from individuals with schizophrenia. J Psychiatry Neurosci 43:338–346.
59. Scarr E, Seo MS, Aumann TD, Chana G, Everall IP, Dean B (2016): The distribution of muscarinic M_1 receptors in the human hippocampus. J Chem Neuroanat 77:187–192.
60. Levey AI (1996): Muscarinic acetylcholine receptor expression in memory circuits: Implications for treatment of Alzheimer disease. Proc Natl Acad Sci U S A 93:13541–13546.
61. Disney AA, Domakonda KV, Aoki C (2006): Differential expression of muscarinic acetylcholine receptors across excitatory and inhibitory cells in visual cortical areas v1 and v2 of the macaque monkey. J Comp Neurol 499:49–63.
62. Young MB, Thomas SA (2014): M_1-muscarinic receptors promote fear memory consolidation via phospholipase c and the m-current. J Neurosci 34:1570–1578.
63. Milad MR, Quirk GJ, Pitman RK, Orr SP, Fischl B, Rauch SL (2007): A role for the human dorsal anterior cingulate cortex in fear expression. Biol Psychiatry 62:1191–1194.
64. Stansley BJ, Fisher NM, Gogliotti RG, Lindsley CW, Conn PJ, Niswender CM (2018): Contextual fear extinction induces hippocampal metaplasticity mediated by metabotropic glutamate receptor 5. Cereb Cortex 28:4291–4304.
65. Park J, Chun J-W, Park H-J, Kim E, Kim J-J (2018): Involvement of amygdala-prefrontal dysfunction in the influence of negative emotion on the resolution of cognitive conflict in patients with schizophrenia. Brain Behav 8:e01064.
66. Holt DJ, Lebron-Milad K, Milad MR, Rauch SL, Pitman RK, Orr SP, *et al.* (2009): Extinction memory is impaired in schizophrenia. Biol Psychiatry 65:455–463.
67. Wang L, Yuan L-L (2009): Activation of M_2 muscarinic receptors leads to sustained suppression of hippocampal transmission in the medial prefrontal cortex. J Physiol 587(pt 21):5139–5147.