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ARTICLE

Frontal cortex genetic ablation of metabotropic glutamate receptor subtype 3 ($mGlu_3$) impairs postsynaptic plasticity and modulates affective behaviors

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Clinical and translational studies suggest that prefrontal cortex (PFC) dysregulation is a hallmark feature of several affective disorders. Thus, investigating the mechanisms involved in the regulation of PFC function and synaptic plasticity could aid in developing new medications. In recent years, the $mGlu_2$ and $mGlu_3$ subtypes of metabotropic glutamate (mGlu) receptors have emerged as exciting potential targets for the treatment of affective disorders, as $mGlu_{2/3}$ antagonists exert antidepressant-like effects across many rodent models. Several recent studies suggest that presynaptic $mGlu_2$ receptors may contribute to these effects by regulating excitatory transmission at synapses from the thalamus to the PFC. Interestingly, we found that $mGlu_3$ receptors also inhibit excitatory drive to the PFC but act by inducing long-term depression (LTD) at amygdala-PFC synapses. It remains unclear, however, whether blockade of presynaptic, postsynaptic, or glial $mGlu_3$ receptors contribute to long-term effects on PFC circuit function and antidepressant-like effects of $mGlu_{2/3}$ antagonists. To address these outstanding questions, we leveraged transgenic *Grm3*$^{fl/fl}$ mice and viral-mediated gene transfer to genetically ablate $mGlu_3$ receptors from pyramidal cells in the frontal cortex of adult mice of all sexes. Consistent with a role for $mGlu_3$ in PFC pyramidal cells, $mGlu_3$-dependent amygdala-cortical LTD was eliminated following $mGlu_3$ receptor knockdown. Furthermore, knockdown mice displayed a modest, task-specific anxiolytic phenotype and decreased passive coping behaviors. These studies reveal that postsynaptic $mGlu_3$ receptors are critical for $mGlu_3$-dependent LTD and provide convergent genetic evidence suggesting that modulating cortical $mGlu_3$ receptors may provide a promising new approach for the treatment of mood disorders.

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INTRODUCTION

Available medications for mood disorders suffer from several major limitations. Conventional antidepressants require several weeks of treatment, display high rates of discontinuation, and do not improve symptoms in nearly one-third of patients [1–4]. The unsatisfactory nature of antidepressant and anxiolytic treatments contributes to the large socioeconomic burden and negative impact on quality of life imparted by affective disorders [5]. Thus, continued translational research to scrutinize novel treatment approaches is imperative to alleviate individual suffering and societal burdens stemming from the psychiatric disease.

Prefrontal cortex (PFC) dysregulation is a hallmark feature of several affective disorders [6, 7]. Alterations to synaptic proteins and glutamate signaling have been widely implicated in human studies and in animal models of chronic stress [7–10]. Thus, molecules that regulate PFC glutamate transmission, such as metabotropic glutamate (mGlu) receptors, have emerged as exciting targets for the development of novel psychiatric medications. Extensive preclinical studies have demonstrated that non-selective antagonists for the $mGlu_2$ and $mGlu_3$ receptor subtypes exert antidepressant-like effects by enhancing PFC glutamate transmission [11–15]. Several studies suggested that PFC $mGlu_2$ and $mGlu_3$ receptors might serve as presynaptic autoreceptors, as acute administration of $mGlu_{2/3}$ agonists and antagonists modulates parameters that often reflect glutamate release probability [16–20]. However, recent findings using contemporary, selective pharmacological tools suggest these acute presynaptic effects may be strictly attributable to $mGlu_2$ receptors [21]. By contrast, studies from our laboratory and others indicate that mGlu receptor long-term depression (LTD) of excitatory transmission is blocked by $mGlu_3$ negative allosteric modulators (NAMs), which involves postsynaptic signaling cascades, and is manifested via AMPA receptor internalization [22–26]. Thus, $mGlu_2$ receptors transiently depress glutamate release while $mGlu_3$ receptors facilitate persistent postsynaptic LTD. However, $mGlu_3$ receptors are expressed across several PFC cell types [27, 28] and it remains unclear whether presynaptic, postsynaptic, or glial $mGlu_3$ receptors confer long-term effects on PFC circuit function.

Based on previous pharmacological studies, we hypothesized that postsynaptic $mGlu_3$ receptors modulate synaptic physiology and affective behaviors. To test this hypothesis, we leveraged

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newly developed transgenic *Grm3*$^{fl/fl}$ mice and viral-mediated gene transfer to genetically delete $mGlu_3$ receptor expression from pyramidal cells in the frontal cortex of adult female mice and male mice. While $mGlu_3$ receptor ablation had modest effects on pyramidal cell physiology, postsynaptic $mGlu_3$ receptors were critical for $mGlu_3$-dependent LTD, particularly at synapses arising from the basolateral amygdala (BLA). Furthermore, cortical $mGlu_3$ receptor knockdown mice displayed decreased passive coping behavior in multiple assays.

MATERIALS AND METHODS

Animals

Adult (>10 weeks old) mice were used for all experiments and were not excluded by sex. No differences related to external genitalia were detected. *Grm3*$^{fl/fl}$ mice were developed as described [29]. In brief, loxP recombination sites were introduced to flank the third exon within the *Grm3* gene (*Grm3*$^{fl/fl}$). Cre-mediated recombination, therefore, generates several frameshift mutations and ablates $mGlu_3$ receptor protein. Upon arrival, *Grm3*$^{fl/wt}$ mice on a C57BL/6N background were crossed once with C57BL/6J mice. That generation was interbred, and mice were maintained on the mixed F2 C57BL/6N ×6J background. All experiments were conducted with litter- and cage-matched controls. Mice were group-housed (3–5 per cage) on a 12-h light cycle (lights on at 6:00 a.m.) with all experiments conducted during the light phase across multiple days. Food and water were available *ad libitum*. All experimental protocols were approved by the Vanderbilt Institutional Animal Care and Use Committee. We performed behavioral studies across three cohorts. A total of 18 GFP mice (10 ♀, 8 ♂) and 16 Cre mice (8 ♀, 8 ♂) were used. Two cohorts underwent the open field test before further testing. All three cohorts underwent the elevated plus maze, light-dark box (LDB), tail suspension test (TST), and forced swim test (FST), in that order. Only one cohort underwent the second FST. Mice used for biochemistry and electrophysiology were not included in behavioral experiments.

Stereotaxic injections

At 4–5 weeks of age, *Grm3*$^{fl/fl}$ mice underwent surgery under 1.5–2% isoflurane anesthesia for viral-mediated expression of green fluorescent protein (GFP) or Cre recombinase (800 nL, AAV5-CaMKII-GFP or AAV5-CaMKII-Cre-GFP, $\geq 1 \times 10^{11}$ vg/mL, UNC Viral Vector Core) in the PFC [M/L: ±0.3, A/P: +1.9, D/V: −2.3]. In some experiments, the red-shifted opsin Chrimson (400 nL, AAV5-Syn-ChrimsonR-tdTomato, $\geq 7 \times 10^{12}$ vg/mL, Addgene viral prep # 59171-AAV5) was expressed in the BLA [M/L: −3.2, A/P: −1.6, D/V: −4.4] during the same procedure as PFC virus delivery. Viruses were infused at 100 nL/min. AAV5-Syn-ChrimsonR-tdTomato was a gift from Edward Boyden. Experiments were conducted at least 6 weeks following viral-mediated Cre expression.

Synaptosome preparation and SDS-PAGE Western blotting

The PFC was microdissected and synaptosome preparations were prepared as described [30]. Briefly, brain tissue samples were homogenized in ice-cold buffer (320 mM sucrose, 4.2 mM HEPES, pH 7.4) with Roche cOmplete™ EDTA-free protease inhibitor cocktail (Sigma) using a glass homogenizer (Wheaton). After homogenization, samples were centrifuged at 1000 g for 5 min at 4 °C and the resulting supernatant was subsequently centrifuged at 12,000 g for 15 min. The P2 pellet was resuspended in radioimmunoprecipitation assay buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.1% sodium dodecyl sulfate, 1% Triton X-100, and 1% deoxycholate) (Sigma) containing protease inhibitors Roche cOmplete™ EDTA-free and phenylmethylsulfonyl fluoride, and phosphatase inhibitors cocktails 1 and 2 (Sigma). Protein concentration was determined using a bicinchoninic acid protein assay (Pierce). As previously described [31], 50 µg of total protein was electrophoretically separated using a 4–20% SDS polyacrylamide gel and transferred onto a nitrocellulose membrane (iBlot2, ThermoFisher). Membranes were blocked in TBS Odyssey blocking buffer (LI-COR) for 1 hr at room temperature. Membranes were probed with primary antibodies overnight at 4 °C: rabbit anti-$mGlu_3$ (1:1000, Alomone, AGC_012) and mouse anti-Gapdh (1:1000, ThermoFisher, MA5-15738), followed by the fluorescent secondary antibodies: goat anti-rabbit (800 nm, 1:5000, LI-COR) and goat anti-mouse (680 nm, 1:10,000, LI-COR). Fluorescence was then quantified using the Image Studio Lite software (LI-COR). Values were normalized to GAPDH and compared relative to controls (*Grm3*$^{fl/fl}$ GFP).

Whole-cell electrophysiology

Slices were prepared and recordings made as described [32]. Mice were anesthetized with isoflurane and decapitated. Coronal slices (300 µm) were prepared using an *N*-Methyl-D-glucamine-based cutting and recovery solution. The artificial cerebrospinal fluid solute concentrations were (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 1 NaH_2PO_4, 11 glucose, and 26 $NaHCO_3$. Recordings were performed in a heated (30 ± 1°C), oxygenated (95% O_2/5% CO_2) bath, perfused at 2 mL/min. Pyramidal cells in layer 5 were filled with a potassium-based solution (in mM): 125 K-gluconate, 4 NaCl, 10 HEPES, 4 MgATP, 0.3 NaGTP, 10 Tris-phosphocreatine. Electrical or optical excitatory postsynaptic currents (EPSCs or op-EPSCs) were elicited by 0.1–0.2 ms, 5–50 µA electrical stimulation within layer 5, or by 1–3 ms, 620 nm light stimulation passed through the microscope objective. Recordings were made at −80 mV and LTD was induced by applying 200 nM LY379268 (Abcam) for 10 min [22, 24].

Open-field test

The Activity Monitor Program was used to track mouse movement and activity within an open field chamber housed inside a sound-attenuating cabinet (ENV-510 and MED-OFA-022, MedAssociates). Experiments were conducted over 90 min without any prior habituation to the chamber.

Elevated zero-maze (EZM)

The EZM was performed as previously described [33]. ANYmaze software tracked mouse movement on the elevated zero maze apparatus. Percent time in open arms, open arm entries and distance traveled were analyzed.

Light-dark box (LDB)

The LDB was performed for 10 min using a tinted insert that covers half of an open field chamber (ENV-511, MedAssociates). A zoning analysis was conducted to assess the amount of time spent on the light side and the number of entries/crossings into the light side.

Tail suspension test (TST)

Mice were suspended by their tails onto a computer-monitored load cell. The Tail Suspension Program in conjunction with the Tail Suspension Starter Package (MED-TSS-MS, MedAssociates) was used to detect movement, and immobility was defined as the lack of movements below a set threshold. Each session lasted 6 min and the total time of immobility and the latency to enter the first 10-s immobile bout were recorded.

Forced swim test (FST)

Mice were placed in a Plexiglas cylinder (diameter 22 cm, height 26 cm) with ~10 cm of water (23 ± 1 °C). Six-minute swim sessions were manually scored by a blinded observer. Total time of immobility and latency to enter the first 10-s immobile bout was recorded. A subset of mice underwent a second forced swim one day after the initial test.

Statistics

The number of cells and mice for each experiment is denoted by "*n*" and "*N*", respectively. No overt sex differences were observed

so all data were pooled and analyzed together. Data are presented as mean ± standard error. For individual data points in behavioral experiments, circles indicate data obtained from female mice, and squares indicate values from male mice. Analyses were performed using GraphPad Prism. Statistical outliers were detected and removed using the ROUT method and a maximum false discovery rate of 5%. Two-tailed Student's *t*-test, two-way ANOVA with Sidak post-tests, or the Log-rank (Mantel–Cox) test were used to detect significance.

RESULTS

Frontal cortex $mGlu_3$ receptor ablation

Acute inhibition of $mGlu_3$ receptors generates antidepressant-like effects in animal models [34–36], but the requisite cell types mediating the responses remain unclear. Here, we leveraged *Grm3*$^{fl/fl}$ transgenic mice recently developed by our laboratory [29] to assess how $mGlu_3$ receptors modulate PFC function. We implemented a viral approach to regionally express Cre recombinase in pyramidal cells in adult mice (Figs. 1a and S1).

We have previously shown that genetic CaMKII-Cre expression reduces *Grm3* expression in hippocampal pyramidal cells but not in glia [29]. To validate that viral-mediated expression of Cre recombinase ablates $mGlu_3$ receptors, we isolated crude synaptosomes and performed Western blots to assess protein expression. *Grm3*$^{fl/fl}$ mice displayed comparable basal levels of synaptic $mGlu_3$ receptor protein to congenic C57BL/6J mice (94.9 ± 7.5% C57BL/6J, n.s., $t_6 = 0.6212$, $N = 4$ mice per group, data not shown). As expected, cortical synaptosomes from Cre-expressing tissues displayed decreased $mGlu_3$ receptor protein relative to matched controls (Fig. 1b).

As synaptosomes contain pre- and postsynaptic membranes, receptor location cannot be inferred from these data alone. In addition, residual $mGlu_3$ expression in Cre mice might result from (1) presynaptic receptors from long-range afferents (e.g., BLA), (2) receptors not yet degraded following *Grm3* excision, (3) receptors on PFC cell types not targeted by the virus (e.g., glial receptors that may have contaminated the crude synaptosome preparation), and/or (4) receptors on PFC pyramidal cells outside the range of virus spread. Based on these limitations, we functionally assessed how reduced $mGlu_3$ expression affects the physiology of PFC pyramidal cells. Guided by GFP fluorescence, we targeted Cre-expressing neurons for whole-cell patch-clamp electrophysiology in acute PFC slices. All fluorescent cells ($n = 56$) exhibited the characteristic firing properties of pyramidal cells, including large capacitance, spike-firing adaptation, and hyperpolarization rebound sag. The rebound sag in PFC pyramidal cells is mediated by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels [37, 38], which are activated by cAMP and other related molecules (Fig. 1c). $mGlu_3$ receptors canonically couple with G_{ai} proteins [39]; therefore, we predicted that reduced $mGlu_3$ receptor expression would elevate basal cAMP levels and facilitate HCN channel function. Indeed, Cre-expressing pyramidal cells displayed enhanced hyperpolarization sag relative to GFP controls, consistent with reduced $mGlu_3$ receptor expression (Fig. 1d, e). Otherwise, Cre-expressing neurons exhibited comparable intrinsic physiology relative to GFP controls (Fig. S2a–c), with the sole exception of the reduced medium afterhyperpolarization (Fig. S2d).

Pyramidal cell expression of $mGlu_3$ gates synaptic plasticity in the mouse PFC

We next aimed to assess how genetic ablation of neuronal $mGlu_3$ receptors affects synaptic physiology and plasticity. Cre- and GFP-expressing pyramidal cells did not differ regarding basal excitatory synaptic strength (Fig. S2e, f) and we proceeded to assess synaptic plasticity. $mGlu_3$ receptor activation induces postsynaptically-maintained LTD of excitatory transmission in the mouse PFC

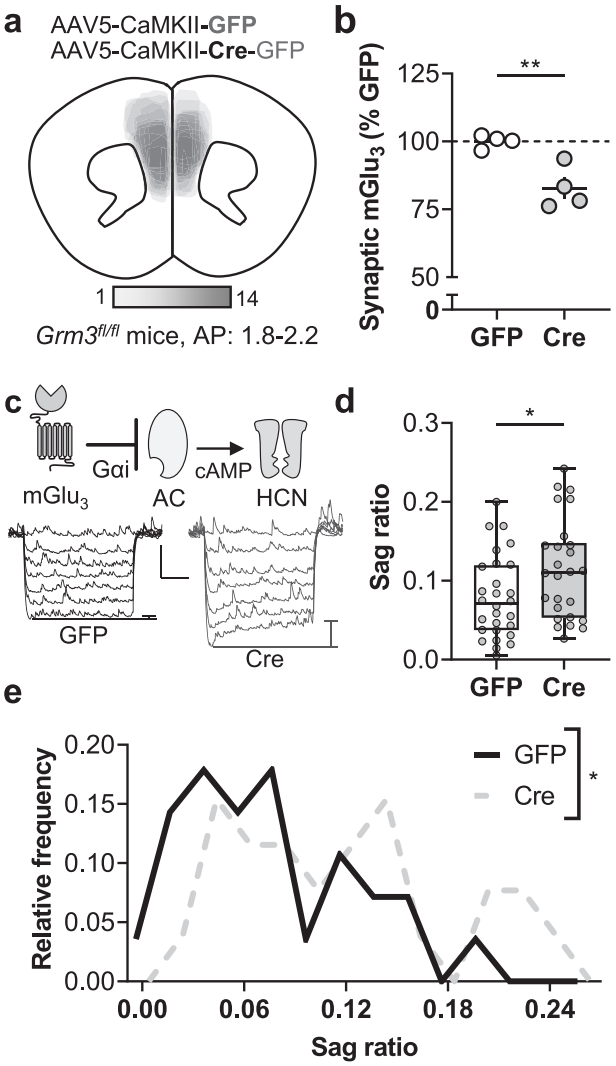

Fig. 1 Viral-mediated Cre expression in *Grm3*$^{f/f}$ mouse frontal cortex attenuates neuronal $mGlu_3$ expression and function. a The adeno-associated virus was delivered to express Cre recombinase or GFP alone as a control. The illustration depicts the location of fluorophore expression in the mouse frontal cortex from the Cre-expressing mice used in the behavioral experiments (Figs. 4 and 5). **b** Crude synaptosomes were prepared from the frontal cortices of mice that underwent viral-mediated Cre expression. $mGlu_3$ receptor expression was assessed via Western blot. Cre samples contained less $mGlu_3$ receptor protein than GFP controls (82.8 ± 3.9% GFP, $^{**}p < 0.01$, $t_6 = 4.196$, $N = 4$ mice per group). **c** Hyperpolarization sag ratio was assessed as a downstream measurement related to $mGlu_3$ receptor signaling. In layer 5 prelimbic pyramidal cells, hyperpolarization-activated cation (HCN) currents are regulated by intracellular cAMP and adenylate cyclase (AC) activity, processes inhibited by G_{ai} protein signaling. The representative traces depict how HCN channel currents are detected as upward, depolarizing rebounds following negative current injections (−150…0, Δ25 pA). Scale bars indicate 5 mV, 250 ms. **d** Cre expression increased the hyperpolarization sag ratio in layer 5 pyramidal cells (0.114 ± 0.012 Cre vs 0.079 ± 0.010 GFP, $^{*}p < 0.05$, $t_{52} = 2.256$, $n/N = 26$–28 cells from $N = 12$–13 mice per group). **e** Layer 5 pyramidal cells displayed a broad, biphasic distribution in sag ratio. The Cre-expressing neuron sag ratio distribution was significantly different than GFP controls ($^{*}p < 0.05$, Log-rank test).

[22–24]. This LTD is blocked by bath application of highly selective $mGlu_3$ NAMs but the cellular location of receptor expression is not known. We evoked EPSCs with electrical stimulation of medial layer 5 (Fig. 2a, b). In the PFC, $mGlu_3$ receptors gate postsynaptically-

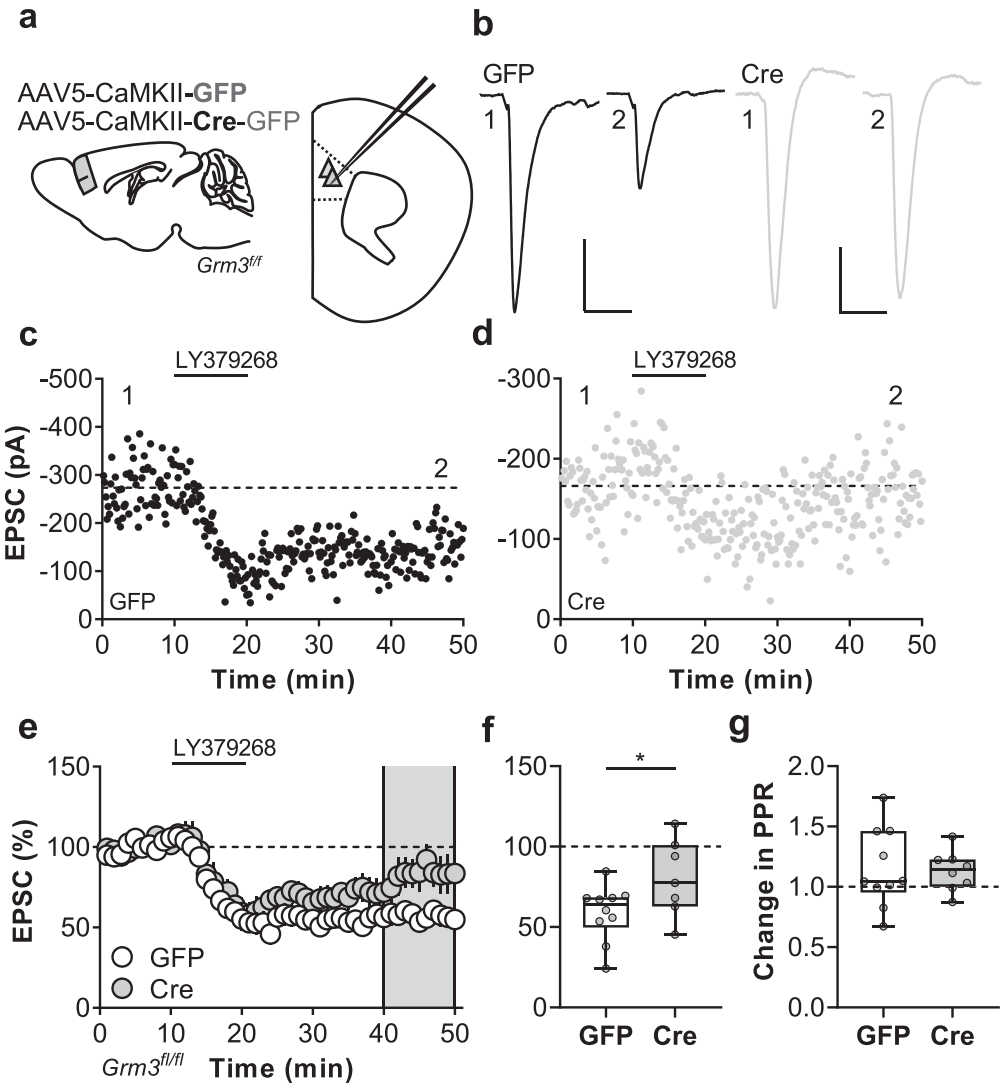

Fig. 2 Neuronal $mGlu_3$ receptors mediate synaptic plasticity in the prefrontal cortex (PFC). a Schematic depicting viral-mediated expression of Cre recombinase in mouse PFC. At least four weeks following the procedure, acute slices containing PFC were prepared and whole-cell patch-clamp recordings were made from fluorescent pyramidal cells in layer 5 prelimbic cortex. **b** Excitatory postsynaptic currents (EPSCs) were elicited via local electrical stimulation and long-term depression (LTD) of synaptic transmission was evoked by bath applying the $mGlu_{2/3}$ agonist LY379268 (200 nm, 10 min). Representative traces depict EPSCs during baseline (1) and after LTD (2) from neurons expressing GFP alone (left, black) or Cre (right, green). Scale bars indicate 100/50 pA, 25 ms. **c** Representative LTD experiment from GFP control pyramidal cell. **d** Representative LTD experiment from Cre-expressing pyramidal cell. **e** Summarized time-courses from multiple LTD experiments. The EPSC amplitude in each experiment is normalized to its baseline value ($n/N = 7–10$ cells from 5–6 mice per group). **f** Average relative change in EPSC amplitude following LTD. Expression of Cre recombinase attenuated the induction of LTD (80.3 ± 9.1% Cre vs 58.9 ± 5.5% GFP, $^*p < 0.05$, $t_{15} = 2.153$). **g** LTD was not associated with a change in the paired-pulse ratio (PPR) in either GFP or Cre cells.

maintained synaptic plasticity, while $mGlu_2$ receptors modulate presynaptic glutamate release at thalamocortical synapses [34]. Thus, non-selective $mGlu_{2/3}$ agonists exert several effects on PFC neurotransmission. As reported previously, bath application of the $mGlu_{2/3}$ receptor agonist LY379268 (200 nM) generated an acute depression and a persistent LTD in control pyramidal cells (Fig. 2c, e, f). We also observed the acute depression of synaptic transmission in Cre-expressing cells, consistent with the retained function of presynaptic $mGlu_2$ receptors (Fig. 2d, e). By contrast, Cre-expressing neurons displayed an impairment of LTD (Fig. 2e, f), indicating that $mGlu_3$ receptors expressed in pyramidal cells are required for this form of PFC plasticity. Consistent with the presynaptic expression of $mGlu_2$ receptors, LY379268 transiently increased the paired-pulse ratio (PPR) in GFP- and Cre-expressing mice (Fig. S3) without having any long-term effect (Fig. 2g), consistent with a postsynaptic locus for LTD expression.

Postsynaptic $mGlu_3$ receptors are essential for PFC synaptic plasticity

The previous experiments clearly implicated pyramidal cell $mGlu_3$ receptors as necessary for the expression of $mGlu_3$-dependent LTD, yet a non-negligible LTD remained. This finding suggests that the residual component is mediated by: (1) incomplete ablation of $mGlu_3$ protein and/or (2) presynaptic $mGlu_2$ receptors (likely at thalamic terminals). Moreover, the experiments using electrical stimulation did not definitively implicate postsynaptic $mGlu_3$ receptors, as LTD could potentially involve $mGlu_3$ receptors expressed in presynaptic terminals from superficial cortical layers [27, 28]. To address all these outstanding questions, we targeted long-range afferents arising from the BLA, which display $mGlu_3$-LTD without detectable effects following $mGlu_2$ receptor activation [24]. In these studies, we virally expressed Chrimson to optogenetically isolate BLA-PFC synapses in GFP- and

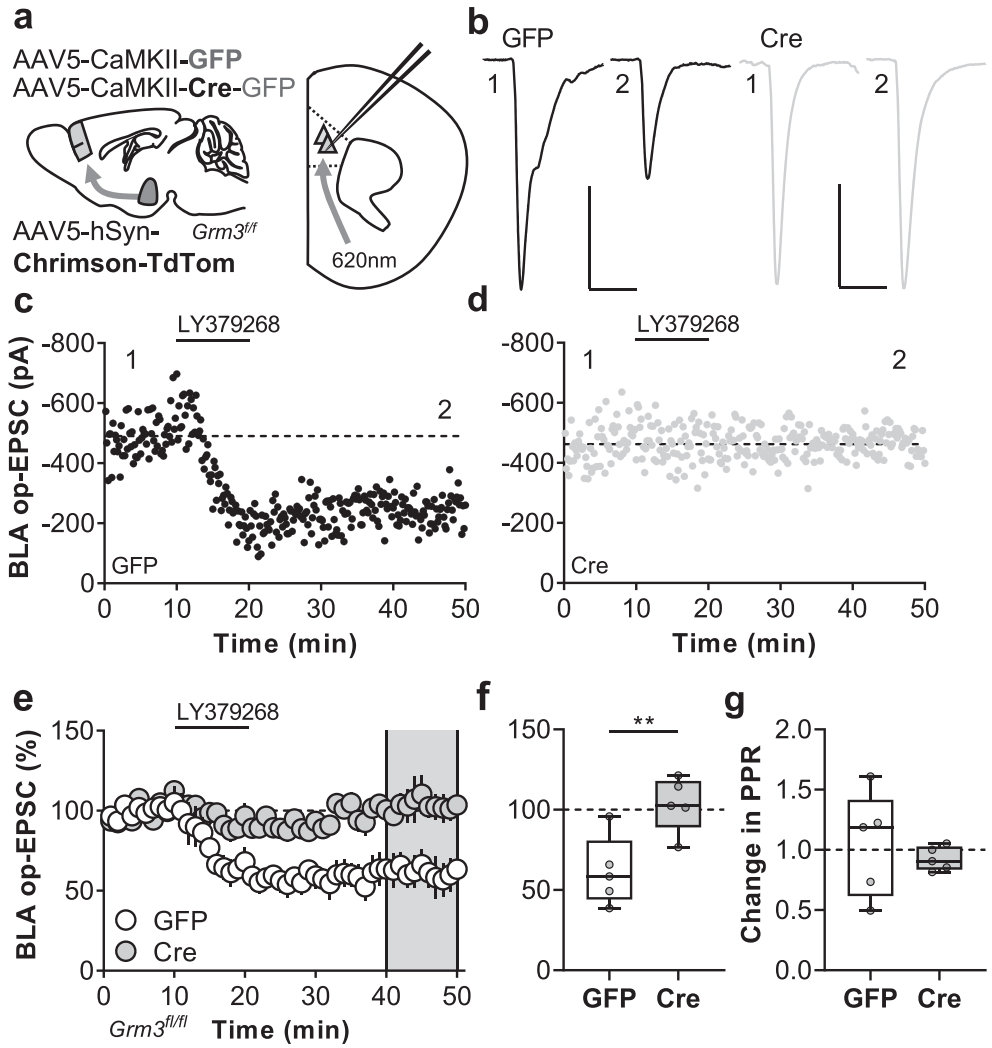

Fig. 3 Postsynaptic $mGlu_3$ receptors are indispensable for synaptic plasticity at amygdalo-cortical synapses. a Schematic depicting viral-mediated expression of Cre recombinase in mouse prefrontal cortex (PFC) and Chrimson expression in mouse basolateral amygdala (BLA). Acute slices containing PFC were prepared and whole-cell patch-clamp recordings were made from fluorescent pyramidal cells in layer 5 prelimbic cortex. **b** Optical excitatory postsynaptic currents (op-EPSCs) were elicited via red light stimulation (620 nm, 1–3 ms) and long-term depression (LTD) of synaptic transmission was evoked with the $mGlu_{2/3}$ agonist LY379268 (200 nm, 10 min). Representative traces depict op-EPSCs during baseline (1) and after LTD (2) from neurons expressing GFP alone (left, black) or Cre (right, green). Scale bars indicate 200 pA, 25 ms. **c** Representative LTD experiment from GFP control pyramidal cell. **d** Representative LTD experiment from Cre-expressing pyramidal cell. **e** Summarized time-courses from multiple LTD experiments where the op-EPSC amplitude in each experiment is normalized to its baseline value ($n/N = 5$ cells from 2–4 mice per group). **f** Average relative change in op-EPSC amplitude following LTD. Expression of Cre recombinase attenuated the induction of LTD (103.3 ± 7.6% Cre vs 61.6 ± 9.7% GFP, $^{**}p < 0.01$, $t_8 = 3.382$). **g** LTD was not associated with a change in the paired-pulse ratio (PPR) in either GFP or Cre cells.

Cre-expressing slices (Fig. 3a, b). Consistent with previous studies, control neurons displayed slow-onset BLA-PFC LTD following LY379268 application (Fig. 3c, e, f). Cre-expressing cells, however, exhibited no LTD whatsoever (Fig. 3d–f). As expected, changes in BLA op-EPSC PPR were not observed at any timepoint in GFP- or Cre-expressing cells (Figs. S3 and 3g). While an occlusion effect in Cre-expressing cells is not inconsistent with the present findings, these data indicate that postsynaptic $mGlu_3$ receptors mediate synaptic plasticity in PFC.

Cortical ablation of $mGlu_3$ receptors induces a modest anxiolytic phenotype

Acute inhibition of $mGlu_3$ receptors attenuates behavioral responses to stress and alters cortical synaptic plasticity [24]. Based on this, we predicted that cortical $mGlu_3$ receptor downregulation might confer anxiolytic-like effects in rodent models. Prior to examining affective behaviors in specialized tasks, we performed an open field test in GFP and Cre mice to test whether genetic deletion of cortical $mGlu_3$ receptors overtly alters locomotor activity. Similar to previous studies in global $Grm3^{-/-}$ mice [40–42], we observed comparable patterns of locomotor activity in a novel environment (Fig S4) and therefore felt confident in our ability to interpret data from studies that involve mouse locomotion.

We assessed anxiety-like behavior in two tasks that take advantage of approach-avoidance conflicts. The EZM consists of a platform with brightly lit open arms and sheltered closed arms flanked by raised walls. A rodent's innate tendency to explore a new environment is counteracted by its instinct to avoid exposed spaces, such that anxiolytic manipulations generally increase the amount of time spent in the open arms. Cre mice displayed more time in the open arms of the EZM than GFP littermate controls (Fig. 4a), consistent with a potential anxiolytic phenotype following $mGlu_3$ receptor knockdown. No differences were observed in the number of arm crossings (Fig. 4b), suggesting

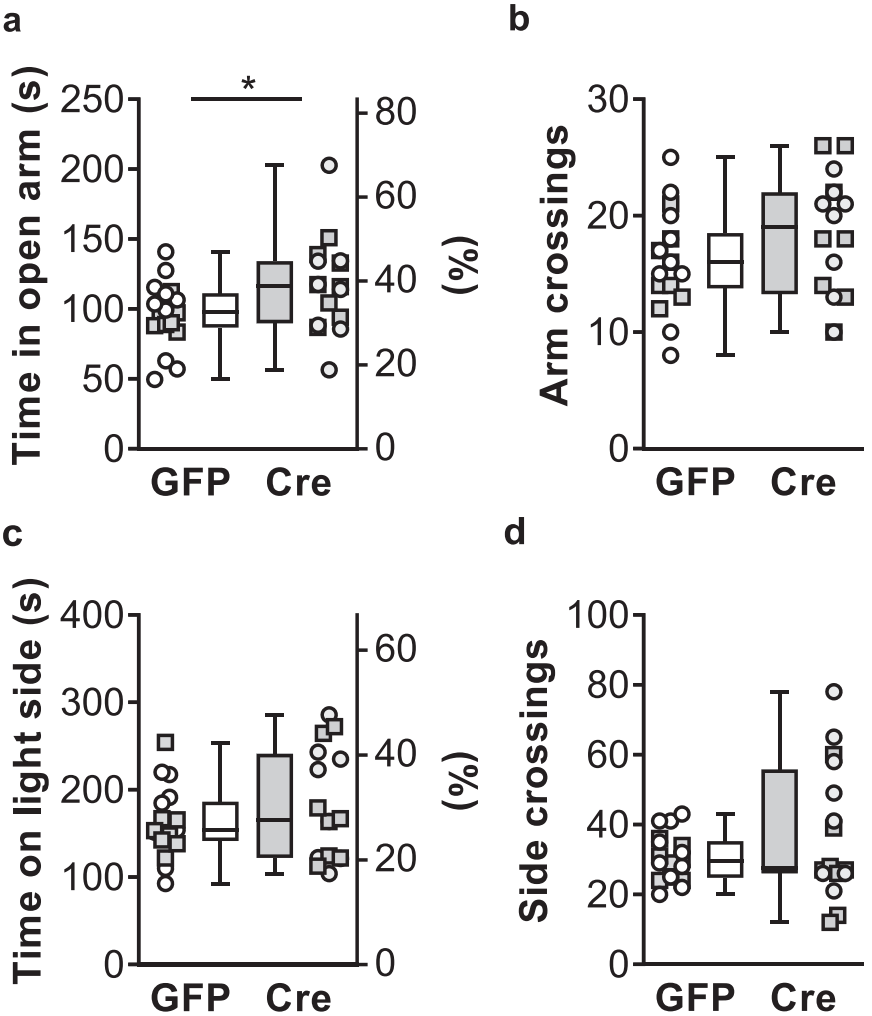

Fig. 4 Frontal cortex $mGlu_3$ receptor knockdown induces a modest anxiolytic phenotype. a Cre-expressing mice (green circles ♀, green squares ♂) spent more time in the open components of the elevated-zero maze than GFP controls (white circles ♀, gray squares ♂) (117.2 ± 8.4 s Cre vs 96.4 ± 5.5 s GFP, $^*p < 0.05$, $t_{32} = 2.115$, $N = 16–18$ mice/group). **b** No difference in the total number of arm crossings between GFP and Cre mice. **c** No difference in the proportion of time spent in the brightly lit side of a light-dark box between GFP and Cre mice. **d** No group difference in the number of side crossings.

that changes in locomotor activity did not contribute to the amount of time spent in the open arms. We also assessed anxiety-like behavior using the LDB. The LDB is operationally similar to the EZM, in that mice face a conflict to approach the novel area but avoid the light-exposed environment. In this task, no differences were observed with respect to the total time spent in the light zone (Fig. 4c) or the number of zone crossings (Fig. 4d). In addition, GFP- and Cre-expressing mice displayed comparable center time when placed in a novel open field (Fig. S4). Collectively, these data suggest that cortical $mGlu_3$ knockdown may generate a modest and/or task-dependent anxiolytic phenotype in mice. These data are consistent with previous studies [40, 41] that showed modest differences in a subset of anxiety-like in global *Grm3*$^{-/-}$ mice.

Cortical knockdown of $mGlu_3$ decreases passive coping behavior Conventional, novel, and experimental antidepressant treatments, including $mGlu_3$ NAMs [34–36], decrease passive coping behavior in preclinical models. By contrast, studies involving constitutive $mGlu_3$ receptor knockout mice have yielded mixed findings related to escape behavior [11, 42]. We, therefore, performed the TST and FST in mice with $mGlu_3$ receptors ablated from PFC. In these assays, mice initially attempt to free themselves from noxious yet inescapable positions but eventually submit to an immobile posture, reminiscent of defeat. Consistent with an antidepressant-like manipulation, Cre mice displayed a longer latency to immobility than controls in the TST (Fig. 5a), although total immobility did not reach statistical significance between groups (Fig. 5b). We next assessed passive coping behavior in the FST. In this task, Cre mice took longer to first enter an immobile posture than GFP mice (Fig. 5c) and spent more total time immobile throughout the task (Fig. 5d). In a subset of mice, we examined the FST on the second day of testing when rodents spend more time immobile than during their first exposure. Both GFP and Cre mice displayed rapid transitions into immobility on the second day and no difference between groups was observed (Fig. 5e). Nonetheless, Cre mice exhibited decreased total time immobile on day 2 relative to GFP controls (Fig. 5f). We did not find evidence for correlated behaviors across assays in either Cre or GFP mice (Fig. S5). Taken together, these data indicate that decreased expression of cortical $mGlu_3$ receptors decreases passive coping behavior, consistent with an antidepressant-like effect.

DISCUSSION

Acute inhibition of $mGlu_3$ receptors confers antidepressant-like effects in preclinical models [34–36], but the effects of *Grm3*

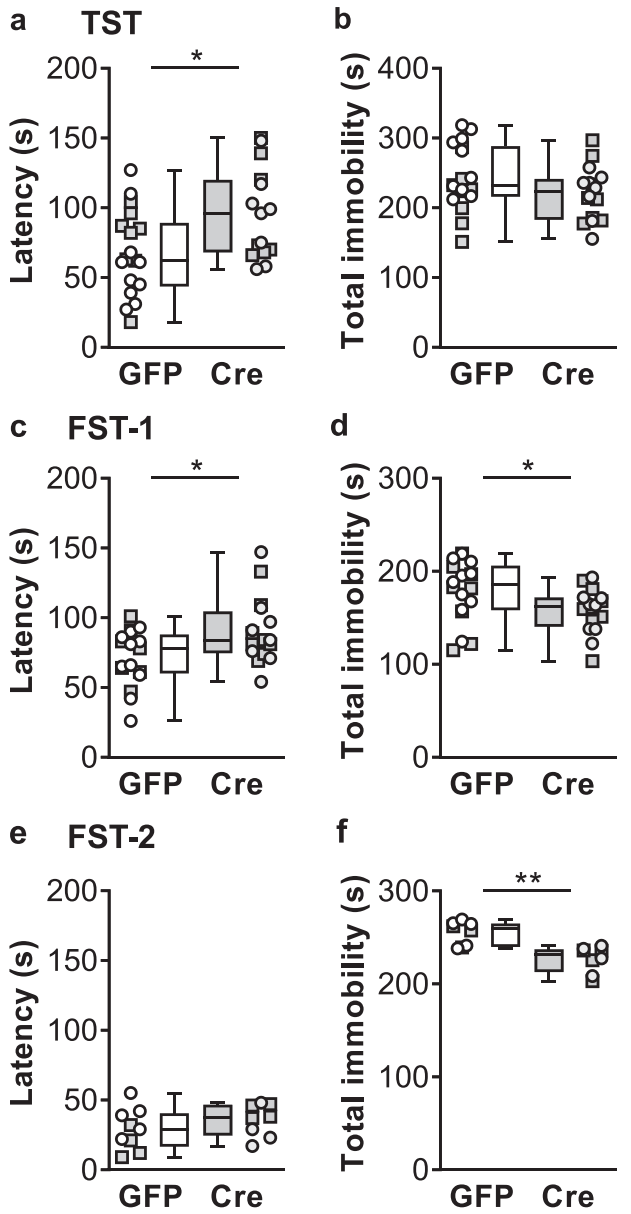

Fig. 5 Frontal cortex $mGlu_3$ receptor knockdown decreases passive coping behaviors. a Cre-expressing mice (green circles ♀, green squares ♂) exhibited increased latency to enter an immobile posture in the tail-suspension test relative to GFP controls (white circles ♀, gray squares ♂) (95.9 ± 8.4 s Cre vs 67.4 ± 7.2 s GFP, $^*p < 0.05$, $t_{31} = 2.589$, $N = 15–18$ mice/group). **b** No difference in the total time of immobility during the tail suspension test between GFP and Cre mice. **c** Cre-expressing mice displayed a longer latency to immobility on the first exposure to the forced swim test (90.4 ± 6.0 s Cre vs 71.5 ± 4.9 s GFP, $^*p < 0.05$, $t_{31} = 2.453$, $N = 16–17$). **d** Decreased total immobility in the first forced to swim in Cre mice relative to GFP controls (157.7 ± 6.0 s Cre vs 179.4 ± 7.8 s GFP, $^*p < 0.05$, $t_{32} = 2.172$, $N = 16–18$). **e** No difference in latency to immobility on the second exposure to a forced swim between GFP- and Cre-expressing mice. **f** Cre expression decreased the total immobility on the second exposure to a forced swim (227.0. ± 5.0 s Cre vs 254.7 ± 4.6 s GFP, $^{**}p < 0.01$, $t_{32} = 4.109$, $N = 8$).

manipulation and the neurocircuits recruited have not been adequately characterized. Here, we leveraged the recent development of transgenic mice to manipulate $mGlu_3$ receptor expression in neocortical neurons. Physiological studies provided the first concrete evidence that $mGlu_3$ receptors expressed at a postsynaptic location are indispensable for synaptic plasticity. Furthermore, the genetic deletion of $mGlu_3$ receptors from adult mice precipitated effective behavioral changes. Together, these studies provide valuable mechanistic insight and translational evidence that bolster the therapeutic potential for $mGlu_3$ receptor inhibition as a novel approach to treat affective disorders.

Pharmacological manipulations that stimulate PFC neurotransmission can exert robust antidepressant activity [43, 44]. Based on this hypothesis, non-selective $mGlu_{2/3}$ antagonists have been proposed as a potential antidepressant approach [12], with conventional wisdom dictating that these compounds act by inhibiting autoreceptors to acutely facilitate glutamate release. Increasing evidence, however, suggests that $mGlu_2$ and $mGlu_3$ receptors serve vastly different roles in the central nervous system. Within the neocortex [21], hippocampus [45], and striatum [46], $mGlu_2$ receptors do, in fact, function as autoreceptors to regulate neurotransmitter release probability. In contrast, $mGlu_3$ receptors have been recently implicated in several forms of postsynaptic plasticity. Within the PFC, $mGlu_3$ receptors gate the internalization of AMPA receptors through a PI3K/Akt-dependent pathway [22, 24]. Despite the clear postsynaptic locus for these downstream signaling cascades, the precise cellular location of the requisite $mGlu_3$ receptor molecules has remained debatable. Now, by regionally demarcating Cre expression within the PFC and opsin expression in a distal glutamate afferent, the present studies provide clear evidence that the postsynaptic $mGlu_3$ receptors guide synaptic plasticity. This critical experiment culminates in a series of recent studies that have fundamentally reshaped our understanding of how $mGlu_3$ receptors regulate central nervous system function.

The prototypical fast-acting antidepressant ketamine attenuates depressive-like behavior in rodent models through cortical disinhibition and exerts efficacy in treatment-resistant clinical populations within hours of administration [47–49]. Unfortunately, ketamine and other fast-acting antidepressants display several limitations that hinder their utility in broad clinical populations. In addition to concerns related to abuse liability and its burdensome route of administration, ketamine displays disconcerting on-target side effects [50–52]. Like other NMDA receptor antagonists, ketamine administration can alter oscillatory activity within the gamma range [53, 54] and induce delirious side effects by inhibiting glutamate receptors expressed on inhibitory interneurons [55]. This disinhibitory motif is also essential for ketamine's antidepressant effects [56], thus presenting challenges in tailoring ketamine-based therapies to retain efficacy while mitigating the risk of disruptive side effects. In contrast, $mGlu_3$ receptors do not appear to regulate gamma power [15], and the present findings suggest that modulating $mGlu_3$ receptor signaling on pyramidal cells is sufficient to confer antidepressant-like effects. By avoiding direct modulation of cortical interneurons, $mGlu_3$ NAMs may confer lower risks of psychotomimetic side effects. Indeed, studies using global knockout mice, $mGlu_{2/3}$ antagonists, and $mGlu_3$ NAMs have revealed minimal evidence of psychotomimetic-like phenotypes [40–42]. On the other hand, $mGlu_3$ NAMs and ketamine may confer antidepressant-like effects through overlapping neurocircuits. Ketamine induces its antidepressant-like effects in mice by disinhibiting BLA-projecting PFC pyramidal cells [57], which receive strong reciprocal glutamate projections from the BLA [58, 59]. As these synapses express $mGlu_3$ receptor-dependent LTD, in vivo $mGlu_3$ NAM administration may therefore potentiate similar PFC circuits as ketamine and other fast-acting antidepressants. Nonetheless, the current studies certainly do not exclude the possibility that $mGlu_3$ NAMs also promote antidepressant-like effects through extracortical loci. Several brain regions involved in affective behaviors and mood disorder etiology, such as the hippocampus and nucleus accumbens, highly express $mGlu_3$ receptors and represent intriguing substrates for continued investigation [60]. Future studies involving chronic pharmacological inhibition will also be needed to translate $mGlu_3$ receptor NAMs as potential breakthrough antidepressants.

In addition to regulating synaptic plasticity, our findings showed that $mGlu_3$ receptors regulate PFC pyramidal cell membrane physiology to some extent. An interesting hypothesis for future studies is that $mGlu_3$ receptors regulate coping and anxiety-like behaviors by potentiating HCN channels, which have been shown to regulate depressive-like behavior in rodent models [37, 61]. The antidepressant-like effects of $mGlu_3$ receptor modulation might also involve changes to other pathways related to cAMP and/or pathways related to crosstalk with $mGlu_5$ receptors [23, 62]. Indeed, considering that $mGlu_5$ receptors inhibit small-conductance potassium channels [22, 63, 64], the decreased mAHP we observed in Cre-expressing cells suggests that $mGlu_3$ receptor ablation affects multiple modes of $mGlu_5$ receptor signaling. Nonetheless, several pieces of evidence suggest the signaling mechanisms involved in $mGlu_3$-LTD are necessary for the concomitant anxiolytic and antidepressant-like behavioral ramifications following receptor inhibition. For one, $mGlu_3$-LTD ultimately proceeds through the internalization of AMPA receptors [24], and PFC infusions of AMPA receptor antagonists block the antidepressant-like effects of $mGlu_{2/3}$ antagonists [13, 14]. These findings suggest that functional changes in PFC AMPA receptors are intimately linked with coping behaviors and other antidepressant-like outcomes. In addition, clinical associations with mood disorders have been observed with several signaling molecules that are critical for $mGlu_3$-LTD, including mGlu receptor subtype 5 (*GRM5*) [65], Homer1 (*HOMER1*) [66], and glycogen synthase kinase 3β (*GSK3B*) [67]. Conversely, manipulations that target these molecules modulate affective behaviors in preclinical models [68–74]. Together, these findings suggest that antidepressant-like manipulations that inhibit cortical $mGlu_3$ receptors exert their efficacy by inhibiting LTD and altering how information from the limbic system is processed within the PFC.

Collectively, the current studies conclusively demonstrate that postsynaptic $mGlu_3$ receptors are essential for LTD. Decreased expression of cortical $mGlu_3$ receptors modulates affective behaviors, an important translational finding considering that the efficacy of many antidepressant and anxiolytic medications wanes following chronic treatment. Means to inhibit cortical $mGlu_3$ receptors thus represent a promising novel approach for the treatment of mood disorders.

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AUTHOR CONTRIBUTIONS

Conceptualization, MEJ and CIS; investigation, MEJ, CIS, SADV, NMF, and SD; supervision, MEJ, CMN, and PJC; writing–original draft, MEJ and CIS; writing–review & editing—all authors.

ADDITIONAL INFORMATION

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