Metabotropic glutamate receptor subtype 3 gates acute stress-induced dysregulation of amygdalo-cortical function

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Abstract

Stress can precipitate or worsen symptoms of many psychiatric disorders by dysregulating glutamatergic function within the prefrontal cortex (PFC). Previous studies suggest that antagonists of group II metabotropic glutamate (mGlu) receptors (mGlu₂ and mGlu₃) reduce stress-induced anhedonia through actions in the PFC, but the mechanisms by which these receptors act are not known. We now report that activation of mGlu₃ induces long-term depression (LTD) of excitatory transmission in the PFC at inputs from the basolateral amygdala. Our data suggest mGlu₃-LTD is mediated by postsynaptic AMPAR internalization in PFC pyramidal cells, and we observed a profound impairment in mGlu₃-LTD following a single, 20-min restraint stress exposure. Finally, blocking mGlu₃ activation in vivo prevented the stress-induced maladaptive changes to amygdalo-cortical physiology and motivated behavior. These data demonstrate that mGlu₃ mediates stress-induced physiological and behavioral impairments and further support the potential for mGlu₃ modulation as a treatment for stress-related psychiatric disorders.

Introduction

Stress, which is known to cause or exacerbate symptoms of mood disorders, alters synaptic function in the prefrontal cortex (PFC) and induces coincidental impairments to PFC-
dependent motivational tasks. Patients with major depressive disorder (MDD) exhibit reduced total PFC volume, impaired PFC activation during cognitive performance, and loss of dendritic branching of pyramidal cells. Furthermore, findings from preclinical studies in rodent models align with clinical studies and suggest that dysfunction of PFC glutamatergic neurotransmission is a key substrate underlying the cognitive and motivational effects of stress exposure.

Along with intra-cortical glutamate signaling, the PFC receives substantial excitatory input from the basolateral amygdala (BLA) and ventral hippocampus (VH). These afferents are thought to convey distinct components of motivation; specifically, BLA activity is associated with emotional state and VH activity regulates the expression and retrieval of previous memories. This convergent excitatory signaling is processed, and Layer 5 pyramidal neurons provide the primary PFC output that encodes for the selection and execution of complex, goal-directed tasks. Despite the accepted role of this motivational circuit, it is unknown to what extent physiological differences between the long-range glutamatergic afferents to the PFC may exist. A better understanding of these mechanisms is essential for the development of novel treatments for mental illnesses associated with motivational deficits.

In recent years, metabotropic glutamate (mGlu) receptor subtype 3 (mGlu3) has emerged as a promising target for modulating glutamatergic transmission in the PFC. Loss-of-function mutations in GRM3 are associated with PFC-related behavioral deficits in schizophrenia patients and healthy volunteers. Conversely, recent studies suggest that activation of mGlu3 plays important roles in PFC-dependent working memory and extinction learning. These data suggest that mGlu3 likely regulates key aspects of PFC function. Recent studies from our lab and others suggest that mGlu3 is postsynaptically localized in PFC pyramidal cells in both rodents and primates, where it modulates calcium and cAMP signaling. Furthermore, activation of mGlu3 induces robust long-term depression (LTD) of excitatory transmission onto PFC pyramidal cells. Each of these actions is likely to underlie the ability of mGlu3 to regulate PFC-mediated responses.

We now present a series of studies in which we found that mGlu3-LTD is restricted to excitatory transmission on pyramidal cells and the mechanism is consistent with postsynaptic AMPAR internalization. Furthermore, using projection-specific optogenetic techniques, we found that PFC mGlu3-LTD is selectively expressed at amygdalar but not hippocampal inputs. Remarkably, we found that a single, acute stressor impairs the induction of mGlu3-LTD, and that blocking mGlu3 activity in vivo prevents stress-induced perturbations in amygdalo-cortical function and motivated behavior. Together, these findings show that stress dysregulates postsynaptic synaptic plasticity in the amygdalar input to the PFC and that this can be prevented by administration of a selective mGlu3 NAM. These preclinical studies increase our understanding of the initial stress-induced physiological changes, and may provide mechanistic insights into changes in PFC function observed in patients. Furthermore, these studies raise the exciting possibility that mGlu3 modulators may provide therapeutic benefits for the treatment of stress-related psychiatric disorders.
Materials and Methods

Animals

Adult (>8 week), male, C57Bl6/J mice, group-housed (2–5/cage) on a 12-hour cycle (lights on at 06:00), were used for all experiments. Food and water were available ad libitum. All protocols were approved by the Vanderbilt Institutional Animal Care and Use Committee. VU0650786 was administered via intraperitoneal (i.p.) injections in 10% Tween-80 vehicle (10μL/g).

Optogenetics

Channelrhodopsin-2 (ChR2) was virally expressed in glutamatergic neurons as described. Mice were anesthetized with isoflurane and 250–400nL virus (AAV5-CaMKII-ChR2-EYFP, UNC) was delivered to: (mm) BLA (ML: −2.9, AP: −1.4, DV: −4.7) and VH (ML: −3.6, AP: −3.4, DV: −4.0).

Whole-cell electrophysiology

Mice were anesthetized with isoflurane and decapitated. Coronal slices (300μM) were prepared with NMDG-based cutting/recovery solution. Holding and recording chambers contained artificial cerebrospinal fluid (aCSF): (mM) 119NaCl, 2.5KCl, 2.5CaCl2, 1.3MgCl2, 1NaH2PO4, 11glucose, and 26NaHCO3. The recording chamber was perfused with warm (30±1°C), oxygenated (95/5%O2/CO2) aCSF at 2ml/min. Layer 5 prelimbic PFC neurons were filled with potassium-based internal solution: (mM) 125K-gluconate, 4NaCl, 10HEPES, 4MgATP, 0.3NaGTP, 10Tris-phosphocreatine. Local glutamate release was elicited at 0.1Hz with 0.1–0.15ms electrical stimulation from a concentric bipolar electrode in Layer 5. In ChR2-expressing slices, input-specific glutamate release was evoked with light stimulation (1–4ms, 470nm, LEDD1B, Thor labs). To preclude recording inhibitory currents, recordings were made at −70mV. Control recordings were interleaved with recordings under each experimental condition at an approximate ratio of one control cell per three experimental cells.

Progressive ratio task

Mice were trained on a progressive ratio schedule of reinforcement as described. Assessments of drug and stress action occurred in a pseudo-random, counterbalanced, within-subjects design. Test day performance was normalized to the previous two sessions. Mice were not food-restricted and the experimenter was blind to all drug treatments.

Drugs

LY379268 and tetrodotoxin were purchased from Abcam. LY341495 and CNQX were purchased from Tocris. VU0650786 and MRK-8-29 were synthesized in-house. The D15 peptide (PPPQVPSRPNNAPP) was prepared by Bio-Synthesis.

Statistics

The number of cells in each experiment is denoted by “n” and the mice by “N”. Data are presented as mean±SEM. Analyses were performed using GraphPad Prism. Two-tailed
Student’s t-test and one-way ANOVA with Bonferroni post-tests were used as appropriate. Post-hoc power analyses ensured a sufficient number of cells and mice were used.

Results

Specific expression of mGlu3-LTD by excitatory transmission on PFC pyramidal cells

LTD induced by the activation of mGlu2/3 is often assumed to involve a reduction in presynaptic release probability. However, data from our lab and others suggest that mGlu3 has direct effects on excitability of PFC pyramidal cells. To test this hypothesis, we utilized whole-cell patch-clamp electrophysiology. Single neurons in Layer 5 of the prelimbic PFC were classified by their firing properties. Regular-spiking neurons were identified by characteristic spike-firing adaptation (Figure 1a) in contrast to the rapid patterns of fast-spiking interneurons (Figure 1b). As reported previously, bath application of the mGlu2/3 receptor agonist LY379268 induced LTD of excitatory synaptic transmission on putative pyramidal cells (Figure 1a & 1c). Strikingly, LY379268 did not induce LTD at excitatory synapses onto fast-spiking interneurons (Figure 1b & 1d). In addition, LY379268 did not exert any effect on inhibitory postsynaptic currents (Figure S1). Together, these data are consistent with a postsynaptic locus of LTD specific to excitatory transmission onto pyramidal cells.

We previously demonstrated that this LTD is lost following genetic deletion of mGlu3 and not mGlu2. Furthermore, we found that mGlu3 activation modulates calcium signaling in PFC pyramidal cells, suggesting that mGlu3 may act postsynaptically on pyramidal cells to induce LTD. To confirm that mGlu3 mediates LTD in whole-cell configuration, we used the mGlu3 NAM VU0650786, which exhibits no off target activity at any other mGlu receptor (Figure 1e). Moreover, restriction of VU0650786 to the patch pipette was also sufficient to block LTD (Figure 1f), consistent with a postsynaptic site of action for mGlu3 signaling. Pretreatment with the mGlu2/3 antagonist LY341495 blocked both the initial depression and LTD (Figure S2), suggesting that mGlu3 mediates the transient decrease in EPSC amplitude. Taken together, these data suggest that mGlu3-LTD in the PFC is mediated by postsynaptic mGlu receptors located on pyramidal cells. We next performed studies to further understand the mechanism of action of mGlu3 in inducing PFC LTD.

PFC mGlu3-LTD is mediated by AMPAR internalization

In general, synaptic strength can be related to quantal size, neurotransmitter release probability, and/or the number of synapses. We performed several analyses to identify which of these factors underlie mGlu3 LTD. The coefficient of variation of the EPSC is inversely proportional to both release probability and synapse number. For each control recording, we normalized the change in the coefficient of variation with the magnitude of LTD (Figure 2a). This analysis revealed a positive correlation, indicating that either a decrease in active synapse number and/or release probability is related to mGlu3-LTD expression. The paired-pulse ratio is thought to be related to release probability and not the number of active synapses. We observed a trend towards a positive correlation between the change in paired-pulse ratio and the amount of LTD, the slope was significantly
less than for the coefficient of variation analysis. We also sampled several additional interstimulus intervals and found no change in paired-pulse ratio following mGlu3-LTD (Figure 2b). The discrepancy between these two analyses is consistent with a rapid decrease in active synapse number underlying mGlu3-LTD.

Miniature EPSC (mEPSC) amplitude and frequency are commonly evaluated to measure changes in quantal size, release probability, and synapse number (Figure 2c). The mEPSC amplitude generally reflects quantal size, whereas mEPSC frequency is related to synapse number and release probability. Cells that underwent mGlu3-LTD exhibited a reduction in both mEPSC amplitude and frequency (Figure 2d/e/f). Furthermore, we observed a time-dependent decrease in spontaneous EPSC amplitude and frequency (Figure S3). These data suggest that mGlu3-LTD is mediated by rapid AMPAR internalization, culminating in a decrease in active synapse number. To further test this hypothesis, we isolated NMDA receptor (NMDAR) currents by removing Mg\textsuperscript{2+} from the aCSF to reduce the voltage-dependent block. The contribution of AMPAR currents was prevented with the antagonist CNQX. LY379268 induced a transient depression of the NMDAR current, however the response returned to baseline (Figure 2g), demonstrating that only AMPAR-mediated responses undergo LTD. In many brain regions, including the hippocampus\textsuperscript{34} and nucleus accumbens\textsuperscript{35}, mGlu-LTD requires the internalization of AMPA receptors through dynamin-dependent endocytosis. Therefore, we assessed the involvement of endocytotic machinery in mGlu3-LTD by using a well-characterized dominant negative peptide that blocks the interaction between dynamin and adapter proteins (D15)\textsuperscript{34}. Inclusion of D15 in the patch pipette blocked mGlu3-LTD (Figure 2h), indicating that dynamin-dependent endocytosis is required for mGlu3-LTD. Together, these data strongly suggest that postsynaptic mGlu3 induces LTD in the PFC through AMPAR internalization.

**Synapses from amygdalar, but not ventral hippocampal, afferents express mGlu3-LTD**

The PFC receives excitatory input from several limbic structures involved in stress-related adaptations, notably including the BLA and VH (Figure 3a). To examine whether mGlu3-LTD is expressed at these long-range projections, we took a viral approach to exogenously express ChR2 in a regionally-specific manner. Monosynaptic optical EPSCs were evoked with light stimulation. We observed no difference in the coefficient of variation or paired-pulse ratio between BLA-PFC and VH-PFC synapses (Figure 3b & 3c) and examined mGlu3-LTD at each synapse. Consistent with electrical stimulation, the optically-activated BLA input underwent LTD following bath application of LY379268 (Figure 3d & 3g). In contrast, the VH-PFC synapse resisted both the initial and long-term depressions of synaptic transmission induced by mGlu2/3 activation (Figure 3e& 3g). To confirm that BLA LTD is also mediated by mGlu3, we returned to VU0650786. As expected the mGlu3 NAM blocked LTD at the amygdalo-cortical pathway, whereas the mGlu2 NAM MRK-8-29\textsuperscript{25} had no effect (Figure 3h), corroborating the mGlu3-dependent mechanism at the BLA-PFC synapse.

**Single exposure to restraint stress rapidly impairs PFC mGlu3-LTD**

While many psychiatric disorders are associated with long-term and/or intense stress exposure, mild stressors occur on a day-to-day basis and affect motivated decision-making within the general population\textsuperscript{36}. Moreover, acute stress can instigate relapse events in
individuals with remitted psychiatric disorders. Human neuroimaging and mechanistic rodent studies have related these effects to impairments in PFC function, therefore we aimed to assess whether acute stress modulates the induction of mGlur3 synaptic plasticity in the PFC. Mice were sacrificed 30 minutes after the termination of 20-minutes restraint stress (Figure 4a). Restraint stress did not affect excitability or basal membrane properties (Figure 4b), and the acute inhibition of EPSCs induced by LY379268 remained intact (Figure 4c & 4d). However, mice exposed to acute stress displayed a significant impairment of mGlur3-mediated LTD (Figure 4d & 4e). This impairment persisted for one day, but not three days, after stress exposure (Figure S4).

Loss of synaptic plasticity can generally be attributed to an impairment in the induction mechanism or to occlusion (i.e. a floor effect). To address this, we measured mEPSCs in pyramidal cells from control slices and slices from stressed mice, and found that stress exposure did not affect mEPSC amplitude or frequency (Figures 4f & 4g). Moreover, the baseline coefficient of variation and paired-pulse ratio were not affected by stress (data not shown). These data suggest that the stress-induced LTD impairment was not caused by occlusion, and that a loss of function in mGlur3 or a downstream signaling partner is likely responsible.

**mGlur3 NAM administration prevents stress-induced deficits in BLA-PFC mGlur3-LTD and motivation**

Having demonstrated that acute stress dysregulated mGlur3 plasticity, we hypothesized that blocking mGlur3 activation during exposure to stress in vivo would prevent the maladaptive changes in PFC physiology and function. To test this hypothesis, we administered VU0650786 or vehicle 15 minutes prior to the acute stress (Figure 5a). In addition to high selectivity against other mGlu subtypes, VU0650786 exhibited no off-target activity in a screen against 68 clinically-relevant drug targets. The basal synaptic properties of amygdalo-cortical transmission taken from vehicle- and VU0650786-pretreated, stressed mice were not different from each other, or from naïve control mice (Figures 5b & 5c). As observed with electrical stimulation, acute stress disrupted mGlur3-LTD at BLA-PFC synapses (Figure 5d). Pretreatment with VU0650786 prevented the stress-induced impairment (Figures 5e & 5f), suggesting that mGlur3 activity in vivo is necessary for this stress-induced change to occur. Interestingly, treatment with VU0650786 immediately after stress exposure also prevented the deficits in ex vivo LTD (Figure S5).

PFC function regulates motivated behaviors and stress impairs motivation in clinical populations and animal models. We sought to examine motivation by training mice to respond in an operant apparatus for delivery of a liquid reinforcer. Mice were then switched to a progressive ratio (PR) schedule, where the number of hole pokes required to earn a reinforcer increases exponentially during the task. The number of reinforcers earned on the PR schedule is decreased by lesions of the PFC but not of other cortical structures. Once performance stabilized, acute restraint sessions were administered to mice immediately prior to the task (Figure 5g). Same-day acute restraint stress significantly decreased the number of reinforcers earned and the holepokes executed, consistent with a decreased motivational state. To measure the effects of VU0650786 on this stress-induced
behavioral deficit, performance during restraint stress test sessions was normalized to the average of the two preceding baseline days. During control (no stress) test sessions, vehicle-treated mice displayed identical performance relative to baseline, and increasing doses of VU0650786 had no effect (Figure 5h & 5i, top). In contrast, during test sessions that immediately followed stress exposure, vehicle-treated mice recapitulated the deficit in PR performance (Figure 5h & 5i, bottom). This stress-induced deficit was prevented by pretreatment with the mGlu₃ NAM VU0650786 in a dose-dependent manner, at doses consistent with the pharmacodynamic/pharmacokinetic relationship of this mGlu₃ NAM. These data indicate that blocking mGlu₃ activation in vivo is sufficient to prevent decreased motivation following acute stress, and suggest that intact amygdalo-cortical neuroplasticity may underlie this phenomenon.

Discussion

Here we report a novel mechanism by which acute stress dysregulates PFC function in rodents. We found that mGlu₃-LTD is specific to pyramidal cells and proceeds through a postsynaptic site of action. Moreover, the plasticity exists at long-range inputs from the BLA, but not VH, and is impaired by a single stress exposure. Finally, inhibiting mGlu₃ function in vivo prevented the stress-induced deficit in BLA-PFC LTD as well as a motivational impairment in food-reinforced behavior. While the present studies are restricted to rodents, they are well-aligned with recent work describing a role for mGlu₃ in regulating PFC function in non-human primates, and with clinical studies on the impact of GRM3 mutations and stress on PFC function. The present studies provide a strong mechanistic basis to guide future human studies aimed at evaluating how stress affects amygdalo-cortical function and the potential utility of mGlu₃ NAMs as treatments for stress-related disorders.

Canonical plasticity induced by mGlu₂/₃ involves a decrease in presynaptic release probability, and recent publications have shown that activation of mGlu₂/₃ can modify postsynaptic NMDAR receptor function under some circumstances. However, the present data provide direct evidence that activation of mGlu₃ induces LTD by postsynaptic AMPAR internalization in PFC pyramidal cells, similar to the mechanism by which mGlu₅-LTD occurs in the hippocampus and nucleus accumbens. These results raise the possibility that mGlu₃ may regulate postsynaptic glutamatergic signaling in other brain regions and disease states.

While changes in the hippocampus and other areas take several days or weeks to occur, stress-induced changes in dorsolateral/prelimbic PFC physiology can be observed following a single stress event. Following chronic stress, profound reductions in AMPA receptor function are known to occur. Having revealed the mechanism of mGlu₃-LTD to involve AMPAR internalization and a reduction in active synapse number, we predicted that stress may impair mGlu₃-LTD induction by occlusion (i.e. that stress usurps similar signaling mechanisms and initiates an LTD-like process in vivo). Based on that hypothesis, we expected to observe baseline differences in mEPSC frequency and amplitude, and were surprised to find no differences in pyramidal cell physiology between the control and stress groups. In contrast to our findings, a previous study found that acute stress enhanced...
AMPAR and NMDAR function in the PFC\textsuperscript{53}. However, those experiments were performed in prepubertal rats and the physiological changes were measured 4-hours after the stress. The present data suggest that in adult animals, stress rapidly impairs glutamatergic synaptic plasticity in the PFC via desensitization of mGlu\textsubscript{3} and/or its downstream signaling partners.

In general, stress-induced PFC impairments are thought to result from excessive glutamate signaling during stress exposure\textsuperscript{54,55}. In this light, the loss of mGlu\textsubscript{3}-LTD following acute stress may provide a permissive initial step towards further impairments by exacerbating hyperactive glutamate signaling during future stress experiences. Testing whether long-term mGlu\textsubscript{3} inhibition (i.e. pharmacological and/or genetic) prevents the development of chronic stress-related pathophysiology is an intriguing and important future experiment. Along those lines, mixed mGlu\textsubscript{2/3} antagonists act as rapid-acting antidepressants in animal models of chronic stress exposure\textsuperscript{20,56–58}. The efficacy of mGlu\textsubscript{2/3} antagonists is thought to result from mechanisms like those of ketamine, involving a rapid re-potentiation of PFC glutamate transmission\textsuperscript{10,59,60}. While neither ex vivo nor in vivo administration of VU0650786 had a profound effect on basal transmission in the present study, the situation may be quite different following chronic stress exposure. In that condition, where AMPAR function is impaired\textsuperscript{10,52}, an mGlu\textsubscript{3} NAM might exert fast-acting antidepressant actions by potentiating PFC glutamate signaling.

While evidence suggests that both amygdalar and hippocampal afferents to the PFC promote anxiety- and depressive-like behavior\textsuperscript{61,62}, recent studies suggest that the two regions provide different contributions towards stress-related behavior\textsuperscript{63–65}. We found that mGlu\textsubscript{3} activation induced synaptic plasticity only at BLA-PFC synapses, and these exciting data suggest that mGlu\textsubscript{3} may regulate emotional and motivational responses to stress exposure, while leaving memory-related components of stress experience intact. Consistent with that idea, inhibition of mGlu\textsubscript{3} function modulates escape behavior in the forced swim test\textsuperscript{32}, but does not disrupt the acquisition of conditioned freezing\textsuperscript{25}. These findings raise many interesting questions related to the functional roles of the distinct limbic inputs into the PFC and whether specific neurotransmitter receptor signaling pathways may be exploited to have tailored therapeutic outcomes.

In addition to the varied sources of glutamate received by the PFC, the divergent flow of information out of the structure may provide a means of crafting nuanced treatment approaches. Pyramidal cells can be readily demarcated by their anatomical projection target and robust differences between these populations have been reported\textsuperscript{18,66,67}. Because mGlu\textsubscript{3}-LTD occurred in every control cell examined, we find it unlikely that basal tract-specific differences in mGlu\textsubscript{3} function exist. However, based on the variability observed following stress, mGlu\textsubscript{3}-LTD may be impaired in discrete pyramidal cell sub-populations, similar to reported tract-specific changes in spine morphology\textsuperscript{67}. For example, it is conceivable that LTD could be differentially impacted in pyramidal cells projecting to stress or anxiety-related brain structures relative to neurons projecting to areas that promote motivated behavior. Additionally, PFC pyramidal neurons have been sub-classified based on the expression of cell surface receptors and mGlu\textsubscript{3} function might be differentially dysregulated across those populations. For example, pyramidal cells expressing dopamine receptor subtypes D1 and D2 exert distinct effects on decision-making\textsuperscript{68–70}. These and other
genetically-classified pyramidal cell populations warrant further examination in the context of stress and mGlu function.

Alongside changes in motivated behavior, stress is known to disrupt several cognitive functions that require intact PFC function, such as working memory\textsuperscript{71}, sustained attention\textsuperscript{72}, and executive function\textsuperscript{11}. Impairments in executive function can be caused by increases in rigid, perseverative behaviors. PFC mGlu\textsubscript{3}-LTD may comprise one mechanism that permits behavioral flexibility in the face of changing response contingencies or requirements\textsuperscript{40}. Consistent with this notion, decreased expression of cortical mGlu\textsubscript{2/3} has been observed in subjects with major depressive disorder\textsuperscript{73}, loss of function mutations in \textit{GRM3} are associated with schizophrenia and low cognition in healthy controls\textsuperscript{21,22}, and mGlu\textsubscript{3} inhibition disrupts extinction learning in rodents\textsuperscript{25}. Additionally, in a model of cocaine abuse, only rats that exhibited addiction-like drug-seeking displayed a loss of PFC mGlu\textsubscript{3}-LTD\textsuperscript{74}. As such, mGlu\textsubscript{3}-LTD may function as a biological substrate that underlies comorbidities between stress, substance use disorders, and potentially schizophrenia\textsuperscript{75}. Impairments in mGlu\textsubscript{3}-LTD might therefore comprise one mechanism by which dysregulated top-down control increases the likelihood of a stress-induced relapse event or psychotic episode. Further research into circuit-specific changes in PFC physiology will enhance our understanding of the behavioral ramifications of stress experience in the context of specific disease states. Clearly, continued holistic efforts to understand the molecular, circuit-level, and behavioral mechanisms underlying PFC dysfunction will be essential in efforts to translate novel preclinical mechanisms into efficacious therapies for stress-related psychiatric disorders. The data presented here reinforce that modulating mGlu\textsubscript{3} function may be one such approach.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Specific expression of mGlu3-LTD by PFC pyramidal cells
(a) Top left, representative input-output curve displaying characteristic spike-firing adaptation. Scale bars denote 250 ms and 20 mV. Top right, EPSCs recorded during baseline and after LTD induction. Scale bars denote 50 ms and 100 pA. Bottom, representative LTD time course in regular-spiking pyramidal cell. (b) Top right, representative input-output curve displaying fast-spiking properties characteristic of interneurons. Scale bars denote 250 ms and 20 mV. Top right, EPSCs recorded during baseline and after LTD induction. Scale bars denote 50 ms and 50 pA. Bottom, representative LTD time course in fast-spiking interneuron. (c) Summary of control time courses. Application of LY379268 induces LTD of EPSCs on PFC pyramidal cells (55 ± 3 % baseline, n/N = 17/14 cells/mice). (d) Summary of time course experiments in fast-spiking interneurons. LY379268 transiently depresses EPSCs on fast-spiking interneurons but does not induce LTD (93 ± 8 % baseline, n/N = 5/5). Black lines denote control LTD from panel C. (e) Bath application of the mGlu3 NAM VU0650786 blocks LTD induced by LY379268 (90 ± 7 % baseline, n/N = 9/8). (f)
Restriction of VU0650786 to the patch pipette is sufficient to block mGlu$_3$-LTD (96 ± 12% baseline, n/N = 5/3). EPSC, excitatory postsynaptic current; LTD, long-term depression; mGlus, metabotropic glutamate receptor subtype 3; NAM, negative allosteric modulator; PFC, prefrontal cortex.
Figure 2. mGlu3-LTD is mediated by AMPAR internalization

(a) Left, significant positive correlation between the change in CV and magnitude of mGlu3-LTD ($r^2 = 0.4986$, $p < 0.0015$, n/N = 17/14 cells/mice). Dotted lines signify linear regression and 95% prediction limits. Right, trend towards a positive correlation between the change in PPR (50 ms ISI) and magnitude of mGlu3-LTD ($r^2 = 0.2243$, $p < 0.0548$, n/N = 17/14). (b) No difference in PPR was observed across wide range of ISIs (n/N = 11/6, 9/5). (c) Representative traces of mEPSC currents. Scale bars denote 500 ms and 20 pA. (d) Expression of mGlu3-LTD is associated with a persistent decrease in mEPSC amplitude.
(left) and frequency (right) (n/N = 6/4, 8/4, *: p < 0.05, t-test). (e) Cumulative probability distribution of mEPSC amplitude for control cells and cells that underwent mGlu₃-LTD. (f) Cumulative probability distribution of mEPSC interevent interval for control cells and cells that underwent mGlu₃-LTD. (g) Activation of mGlu₃ does not induce a long-term change in amplitude of NMDAR EPSCs (108 ± 5% baseline, n/N = 4/3). (h) Inclusion of a dynamin dominant negative peptide, D15, in the patch pipette blocks mGlu₃-LTD (99 ± 11% baseline, n/N = 5/3). Black lines denote control LTD from panel 1C. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CV, coefficient of variation; EPSC, excitatory postsynaptic current; ISI, interstimulus interval; LTD, long-term depression; mGlu₃, metabotropic glutamate receptor subtype 3; NMDAR, N-methyl-D-aspartate receptor; PPR, paired-pulse ratio; sEPSC, spontaneous EPSC.
Figure 3. BLA, but not VH, afferents to PFC express mGlu$_3$-LTD

(a) Schematic displaying region-specific approach. AAV-CaMKII-ChR2 was injected into the BLA or VH of young mice and slice recordings were prepared 3–5 weeks later. (b) No difference in the baseline CV was observed across BLA or VH inputs (n/N = 13/7, 10/4 cells/mice). (c) No difference in PPR (50 ms ISI) was observed across inputs (n/N = 13/7, 7/4 cells/mice). (d) Summary time course of BLA-PFC recordings. LY379286 application induced LTD (56 ± 6 % baseline, n/N = 10/7). (e) LY3792678 did not depress excitatory transmission at VH-PFC synapses (94 ± 9 % baseline, n/N = 7/4). (f) Pharmacological confirmation of mGlu$_3$-LTD at BLA-PFC input. The mGlu$_3$ NAM VU0650786 blocked BLA-PFC LTD (96 ± 8 % baseline, n/N = 4/3) whereas LTD remained in the presence of the mGlu$_2$ NAM MRK-8-29 (43 ± 9 % baseline, n/N = 5/4). (g) Summary of last 10 minutes of LTD across inputs (**: p < 0.01, t-test). (h) Summary of last 10 minutes of BLA-PFC pharmacological experiments (**: p < 0.01). BLA, basolateral amygdala; ISI, interstimulus interval; CV, coefficient of variation; mGlu, metabotropic glutamate receptor; op-EPSC, optical excitatory postsynaptic current; PFC, prefrontal cortex; PPR, paired-pulse ratio; VH, ventral hippocampus.
Figure 4. Acute restraint stress rapidly impairs PFC mGlu₃-LTD

(a) Schematic displaying stress exposure paradigm. Mice were sacrificed for electrophysiology 30 minutes after the termination of 20-minutes immobilization stress. (b) Acute stress did not affect the basal membrane properties of PFC pyramidal cells (n/N = 23/12, 18/6 cells/mice). (c) Representative experiment displaying loss of LTD following restraint stress. Scale bars denote 100 pA, 50 ms. (d) Summary time course of long-term recordings following stress. While the acute depression during drug application remained intact, LTD did not occur following stress (93 ± 10 % baseline, n/N = 7/5). Black lines denote control data from figure 1C. (e) Summary of last 10 minutes of long-term recordings. Acute restraint stress impairs induction of LTD ex vivo (***, p < 0.001, t-test). (f) mEPSC amplitude does not differ between the restraint stress group and controls (n/N = 7/4, 7/3). (g) mEPSC frequency does not differ between the restraint stress group and controls (n/N = 7/4, 7/3). EPSC, excitatory postsynaptic current; LTD, long-term depression; mGlu₃, metabotropic glutamate receptor subtype 3; mEPSC, miniature excitatory postsynaptic current; PFC, prefrontal cortex; Rₘ, membrane/input resistance; sac, sacrifice; Vₘ, resting membrane potential.
Figure 5. Blocking mGlu3 activation in vivo prevents stress-induced deficits to BLA-PFC mGlu3-LTD and motivation
(a) Schematic displaying stress exposure paradigm. Mice were pretreated with i.p. injections of the mGlu3 NAM VU0650786 or vehicle 15 minutes prior to immobilization stress. Slices were prepared for electrophysiology 30 minutes after the stress ended. (b) Acute stress does not affect BLA-PFC CV. Control value taken from figure 3B. (c) Acute stress does not affect BLA-PFC PPR (50 ms ISI). Control value taken from figure 3C. (d) BLA-PFC LTD is impaired in cells from vehicle-treated mice exposed to restraint stress (89 ± 4 % baseline, n/N = 7/4 cells/mice). Blue lines denote control data from figure 3D. (e) Systemic pretreatment with the mGlu3 NAM rescues the stress-induced deficit in mGlu3-LTD (47
± 10% baseline, n/N = 7/3). (f) Summary of last 10 minutes of LTD recordings. (**: p < 0.01). (g) Top, schematic. Operant responding for liquid food was assessed on a PR schedule of reinforcement. Bottom, stress impaired performance on the PR schedule as assessed by the number of reinforcers earned and the number of holepokes elicited (N = 10, *: p < 0.05, Bonferroni post-tests vs. BL1, BL2, and post). (h) Effects of stress, VU0650786, and combination, on PR performance. The number of reinforcers earned on the test day is expressed as a percentage change relative to the two preceding baseline days. Top, injections of vehicle and VU0650786 did not alter the number of reinforcers earned on the test day in control mice. Bottom, vehicle-treated mice exposed to restraint stress exhibited a decrease in the number of reinforcers earned relative to baseline (N = 10, #: p < 0.05, one-sample t-test). Pretreatment with VU0650786 generated a dose-dependent reversal of the stress-induced impairment (N = 10, **: p < 0.01, Bonferroni post-test vs. veh). (i) PR performance in all conditions as measured by the number of holepokes elicited (N = 10, #: p < 0.05, $: p < 0.10, one-sample t-test; **: p < 0.01, Bonferroni post-test vs. veh). BL, baseline; BLA, basolateral amygdala; CV, coefficient of variation; EPSC, excitatory postsynaptic current; i.p., intraperitoneal; ISI, inter-stimulus interval; LTD, long-term depression; mGlu3, metabotropic glutamate receptor subtype 3; mEPSC, miniature excitatory postsynaptic current; NAM, negative allosteric modulator; PFC, prefrontal cortex; PPR, paired-pulse ratio; PR, progressive ratio schedule of reinforcement; sac, sacrifice; veh, vehicle.