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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 ($mGlu_2$ and $mGlu_3$) 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 $mGlu_3$ induces long-term depression (LTD) of excitatory transmission in the PFC at inputs from the basolateral amygdala. Our data suggest $mGlu_3$-LTD is mediated by postsynaptic AMPAR internalization in PFC pyramidal cells, and we observed a profound impairment in $mGlu_3$-LTD following a single, 20-min restraint stress exposure. Finally, blocking $mGlu_3$ activation *in vivo* prevented the stress-induced maladaptive changes to amygdalo-cortical physiology and motivated behavior. These data demonstrate that $mGlu_3$ mediates stress-induced physiological and behavioral impairments and further support the potential for $mGlu_3$ 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-

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Conflict of Interest

C.W.L. has been funded by the NIH, Johnson and Johnson, Bristol-Myers Squibb, AstraZeneca, Michael J. Fox Foundation, as well as Seaside Therapeutics. He has consulted for AbbVie and received compensation. P.J.C. has been funded by NIH, AstraZeneca, Bristol-Myers Squibb, Michael J. Fox Foundation, Dystonia Medical Research Foundation, CHDI Foundation, and Thome Memorial Foundation. Over the past three years he has served on the Scientific Advisory Boards for Michael J. Fox Foundation, Stanley Center for Psychiatric Research Broad Institute, Karuna Pharmaceuticals, Lieber Institute for Brain Development, Clinical Mechanism and Proof of Concept Consortium, and Neurobiology Foundation for Schizophrenia and Bipolar Disorder. C.W.L. and P.J.C. are inventors on patents that protect different classes of metabotropic glutamate allosteric modulators. M.E.J., C.I.S., and J.L.E. declare no potential conflicts of interest.

dependent motivational tasks[1–4]. Patients with major depressive disorder (MDD) exhibit reduced total PFC volume[5], impaired PFC activation during cognitive performance[6], and loss of dendritic branching of pyramidal cells[7,8]. 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[9–11].

Along with intra-cortical glutamate signaling, the PFC receives substantial excitatory input from the basolateral amygdala (BLA) and ventral hippocampus (VH)[12]. These afferents are thought to convey distinct components of motivation; specifically, BLA activity is associated with emotional state[13,14] and VH activity regulates the expression and retrieval of previous memories[15,16]. 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[17,18]. 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[19].

In recent years, metabotropic glutamate (mGlu) receptor subtype 3 ($mGlu_3$) has emerged as a promising target for modulating glutamatergic transmission in the PFC[9,20]. Loss-of-function mutations in *GRM3* are associated with PFC-related behavioral deficits in schizophrenia patients and healthy volunteers[21,22]. Conversely, recent studies suggest that activation of $mGlu_3$ plays important roles in PFC-dependent working memory[23,24] and extinction learning[25]. These data suggest that $mGlu_3$ likely regulates key aspects of PFC function. Recent studies from our lab and others suggest that $mGlu_3$ is postsynaptically localized in PFC pyramidal cells in both rodents and primates, where it modulates calcium and cAMP signaling[23,25]. Furthermore, activation of $mGlu_3$ induces robust long-term depression (LTD) of excitatory transmission onto PFC pyramidal cells[25–27]. Each of these actions is likely to underlie the ability of $mGlu_3$ to regulate PFC-mediated responses.

We now present a series of studies in which we found that $mGlu_3$-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 $mGlu_3$-LTD is selectively expressed at amygdalar but not hippocampal inputs. Remarkably, we found that a single, acute stressor impairs the induction of $mGlu_3$-LTD, and that blocking $mGlu_3$ 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 $mGlu_3$ 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 $mGlu_3$ modulators may provide therapeutic benefits for the treatment of stress-related psychiatric disorders.

Materials and Methods

Animals

Adult (>8week), 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[28]. 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, 2.5$CaCl_2$, 1.3MgCl2, 1NaH_2PO_4, 11glucose, and 26$NaHCO_3$. The recording chamber was perfused with warm (30±1°C), oxygenated (95/5%O_2/CO_2) 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[29]. 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 (PPPQVPSRPNRAPPG) 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 Bonferonni post-tests were used as appropriate. Post-hoc power analyses ensured a sufficient number of cells and mice were used.

Results

Specific expression of $mGlu_3$-LTD by excitatory transmission on PFC pyramidal cells

LTD induced by the activation of $mGlu_{2/3}$ is often assumed to involve a reduction in presynaptic release probability. However, data from our lab and others suggest that $mGlu_3$ has direct effects on excitability of PFC pyramidal cells[25,30] and $mGlu_3$-LTD in the PFC may involve postsynaptic signaling mechanisms[23,25,30,31]. 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 $mGlu_{2/3}$ 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 $mGlu_3$ and not $mGlu_2$[25]. Furthermore, we found that $mGlu_3$ activation modulates calcium signaling in PFC pyramidal cells[25], suggesting that $mGlu_3$ may act postsynaptically on pyramidal cells to induce LTD. To confirm that $mGlu_3$ mediates LTD in whole-cell configuration, we used the $mGlu_3$ NAM VU0650786, which exhibits no off target activity at any other mGlu receptor[32] (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 $mGlu_3$ signaling. Pretreatment with the $mGlu_{2/3}$ antagonist LY341495 blocked both the initial depression and LTD (Figure S2), suggesting that $mGlu_2$ mediates the transient decrease in EPSC amplitude. Taken together, these data suggest that $mGlu_3$-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 $mGlu_3$ in inducing PFC LTD.

PFC $mGlu_3$-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[33]. We performed several analyses to identify which of these factors underlie $mGlu_3$ 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 $mGlu_3$-LTD expression. The paired-pulse ratio is thought to be related to release probability and not the number of active synapses. While 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 $mGlu_3$-LTD (Figure 2b). The discrepancy between these two analyses is consistent with a rapid decrease in active synapse number underlying $mGlu_3$-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 $mGlu_3$-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 $mGlu_3$-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^{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[34] and nucleus accumbens[35], mGlu-LTD requires the internalization of AMPA receptors through dynamin-dependent endocytosis Therefore, we assessed the involvement of endocytotic machinery in $mGlu_3$-LTD by using a well-characterized dominant negative peptide that blocks the interaction between dynamin and adapter proteins (D15)[34]. Inclusion of D15 in the patch pipette blocked $mGlu_3$-LTD (Figure 2h), indicating that dynamin-dependent endocytosis is required for $mGlu_3$-LTD. Together, these data strongly suggest that postsynaptic $mGlu_3$ induces LTD in the PFC through AMPAR internalization.

Synapses from amygdalar, but not ventral hippocampal, afferents express $mGlu_3$-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 $mGlu_3$-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 $mGlu_3$-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 $mGlu_{2/3}$ activation (Figure 3e& 3g). To confirm that BLA LTD is also mediated by $mGlu_3$, we returned to VU0650786. As expected the $mGlu_3$ NAM blocked LTD at the amygdalo-cortical pathway, whereas the $mGlu_2$ NAM MRK-8-29[25] had no effect (Figure 3h), corroborating the $mGlu_3$-dependent mechanism at the BLA-PFC synapse.

Single exposure to restraint stress rapidly impairs PFC $mGlu_3$-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[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[3,4,36,37], therefore we aimed to assess whether acute stress modulates the induction of $mGlu_3$ 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 $mGlu_3$-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 $mGlu_3$ or a downstream signaling partner is likely responsible.

$mGlu_3$ NAM administration prevents stress-induced deficits in BLA-PFC $mGlu_3$-LTD and motivation

Having demonstrated that acute stress dysregulated $mGlu_3$ plasticity, we hypothesized that blocking $mGlu_3$ 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[32]. 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 $mGlu_3$-LTD at BLA-PFC synapses (Figure 5d). Pretreatment with VU0650786 prevented the stress-induced impairment (Figures 5e & 5f), suggesting that $mGlu_3$ 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[2,11,38,39]. 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[40]. 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 $mGlu_3$ NAM VU0650786 in a dose-dependent manner, at doses consistent with the pharmacodynamic/pharmacokinetic relationship of this $mGlu_3$ NAM[32]. These data indicate that blocking $mGlu_3$ 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 $mGlu_3$-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 $mGlu_3$ 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 $mGlu_3$ in regulating PFC function in non-human primates[23,30], and with clinical studies on the impact of *GRM3* mutations[21,22] and stress on PFC function[3,4]. 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 $mGlu_3$ NAMs as treatments for stress-related disorders.

Canonical plasticity induced by $mGlu_{2/3}$ involves a decrease in presynaptic release probability[41–45], and recent publications have shown that activation of $mGlu_{2/3}$ can modify postsynaptic NMDAR receptor function under some circumstances[46,47]. However, the present data provide direct evidence that activation of $mGlu_3$ induces LTD by postsynaptic AMPAR internalization in PFC pyramidal cells, similar to the mechanism by which $mGlu_5$-LTD occurs in the hippocampus and nucleus accumbens[48,49]. These results raise the possibility that $mGlu_3$ may regulate postsynaptic glutamatergic signaling in other brain regions and disease states[50,51].

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[3,4,37]. Following chronic stress, profound reductions in AMPA receptor function are known to occur[2,10,52]. Having revealed the mechanism of $mGlu_3$-LTD to involve AMPAR internalization and a reduction in active synapse number, we predicted that stress may impair $mGlu_3$-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[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 $mGlu_3$ and/or its downstream signaling partners.

In general, stress-induced PFC impairments are thought to result from excessive glutamate signaling during stress exposure[54,55]. In this light, the loss of $mGlu_3$-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 $mGlu_3$ 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 $mGlu_{2/3}$ antagonists act as rapid-acting antidepressants in animal models of chronic stress exposure[20,56–58]. The efficacy of $mGlu_{2/3}$ antagonists is thought to result from mechanisms like those of ketamine, involving a rapid re-potentiation of PFC glutamate transmission[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[10,52], an $mGlu_3$ 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[61,62], recent studies suggest that the two regions provide different contributions towards stress-related behavior[63–65]. We found that $mGlu_3$ activation induced synaptic plasticity only at BLA-PFC synapses, and these exciting data suggest that $mGlu_3$ may regulate emotional and motivational responses to stress exposure, while leaving memory-related components of stress experience intact. Consistent with that idea, inhibition of $mGlu_3$ function modulates escape behavior in the forced swim test[32], but does not disrupt the acquisition of conditioned freezing[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[18,66,67]. Because $mGlu_3$-LTD occurred in every control cell examined, we find it unlikely that basal tract-specific differences in $mGlu_3$ function exist. However, based on the variability observed following stress, $mGlu_3$-LTD may be impaired in discrete pyramidal cell sub-populations, similar to reported tract-specific changes in spine morphology[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 $mGlu_3$ 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[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[71], sustained attention[72], and executive function[11]. Impairments in executive function can be caused by increases in rigid, perseverative behaviors. PFC $mGlu_3$-LTD may comprise one mechanism that permits behavioral flexibility in the face of changing response contingencies or requirements[40]. Consistent with this notion, decreased expression of cortical $mGlu_{2/3}$ has been observed in subjects with major depressive disorder[73], loss of function mutations in *GRM3* are associated with schizophrenia and low cognition in healthy controls[21,22], and $mGlu_3$ inhibition disrupts extinction learning in rodents[25]. Additionally, in a model of cocaine abuse, only rats that exhibited addiction-like drug-seeking displayed a loss of PFC $mGlu_3$-LTD[74]. As such, $mGlu_3$-LTD may function as a biological substrate that underlies comorbidities between stress, substance use disorders, and potentially schizophrenia[75]. Impairments in $mGlu_3$-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 $mGlu_3$ function may be one such approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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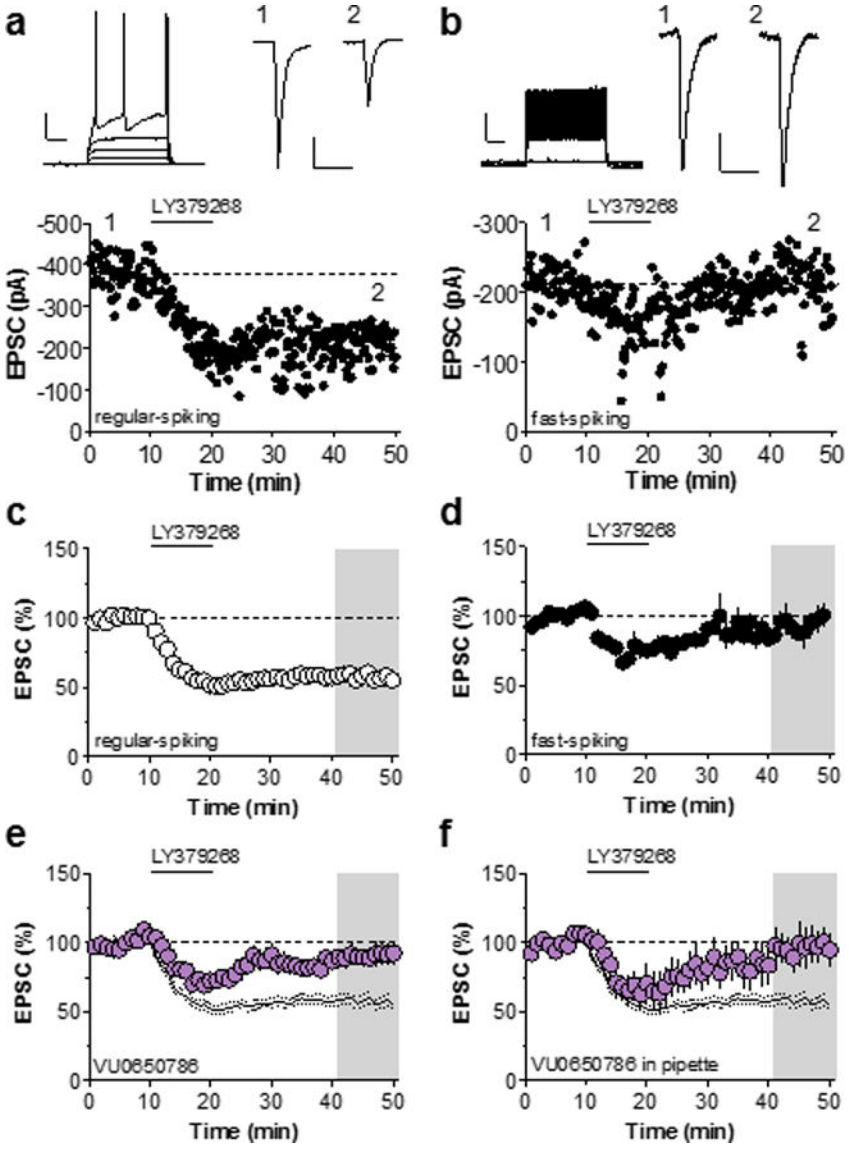

Figure 1. Specific expression of mGlu$_3$-LTD by PFC pyramidal cells

(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; $mGlu_3$, metabotropic glutamate receptor subtype 3; NAM, negative allosteric modulator; PFC, prefrontal cortex.

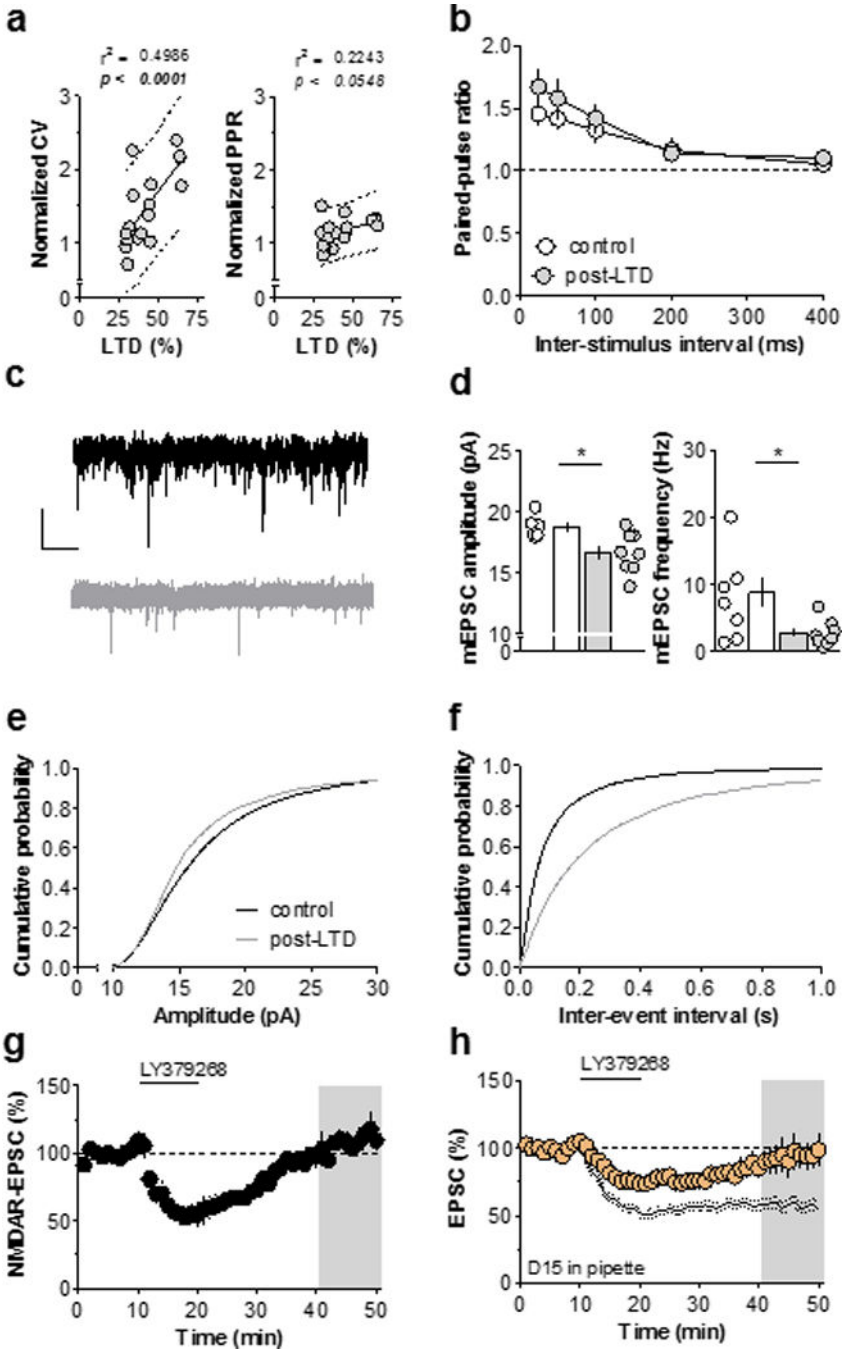

Figure 2. mGlu$_3$-LTD is mediated by AMPAR internalization

(a) Left, significant positive correlation between the change in CV and magnitude of mGlu$_3$-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 mGlu$_3$-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 mGlu$_3$-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 $mGlu_3$-LTD. **(f)** Cumulative probability distribution of mEPSC interevent interval for control cells and cells that underwent $mGlu_3$-LTD. (g) Activation of $mGlu_3$ 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 $mGlu_3$-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; $mGlu_3$, metabotropic glutamate receptor subtype 3; NMDAR, N-methyl-D-aspartate receptor; PPR, paired-pulse ratio; sEPSC, spontaneous EPSC.

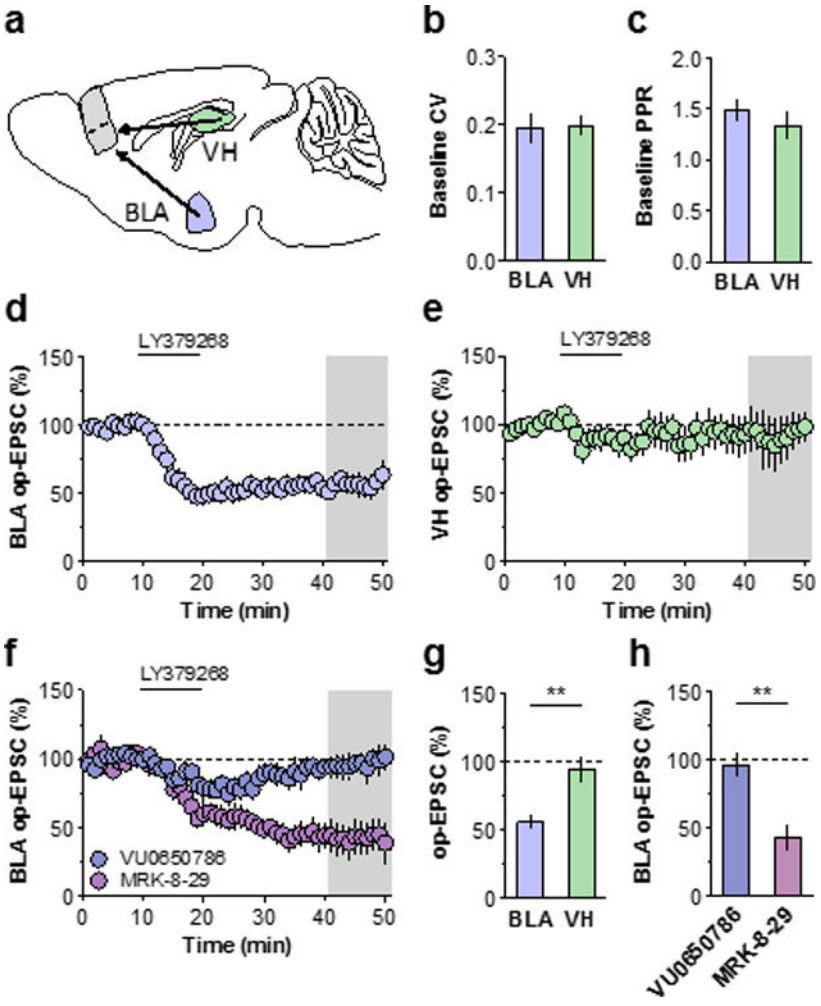

Figure 3. BLA, but not VH, afferents to PFC express $mGlu_3$-LTD

(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.

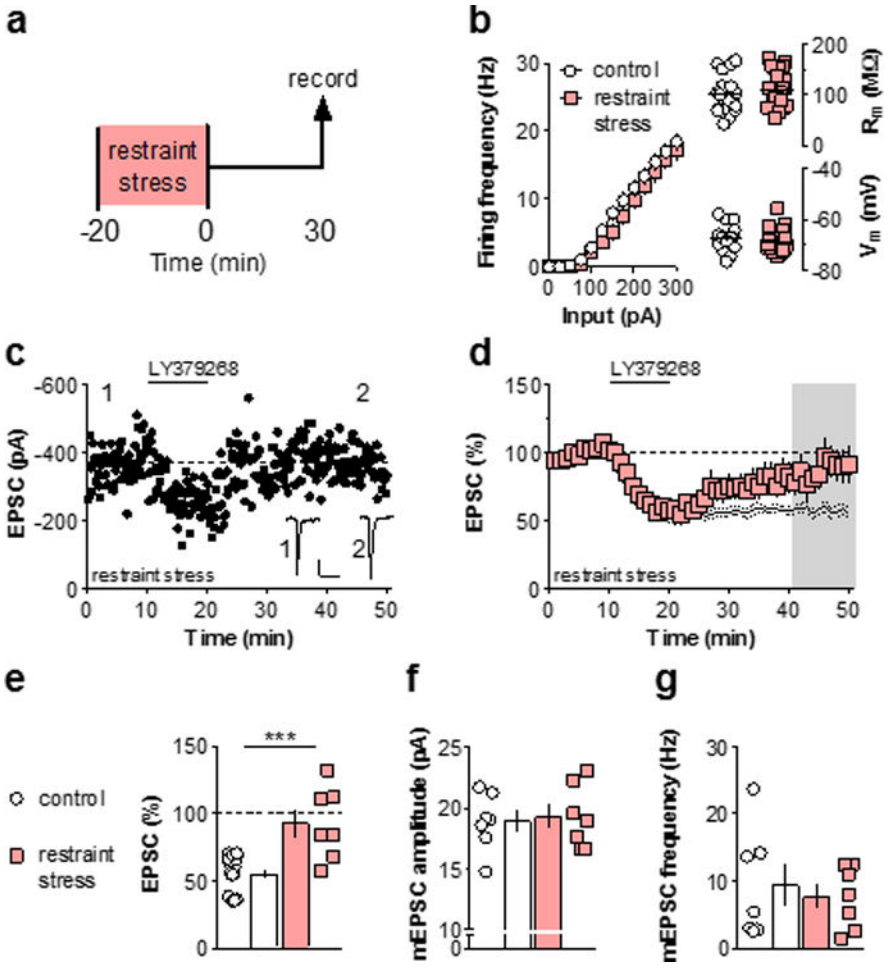

Figure 4. Acute restraint stress rapidly impairs PFC $mGlu_3$-LTD

(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; $mGlu_3$, metabotropic glutamate receptor subtype 3; mEPSC, miniature excitatory postsynaptic current; PFC, prefrontal cortex; R_m, membrane/input resistance; sac, sacrifice; V_m, resting membrane potential.

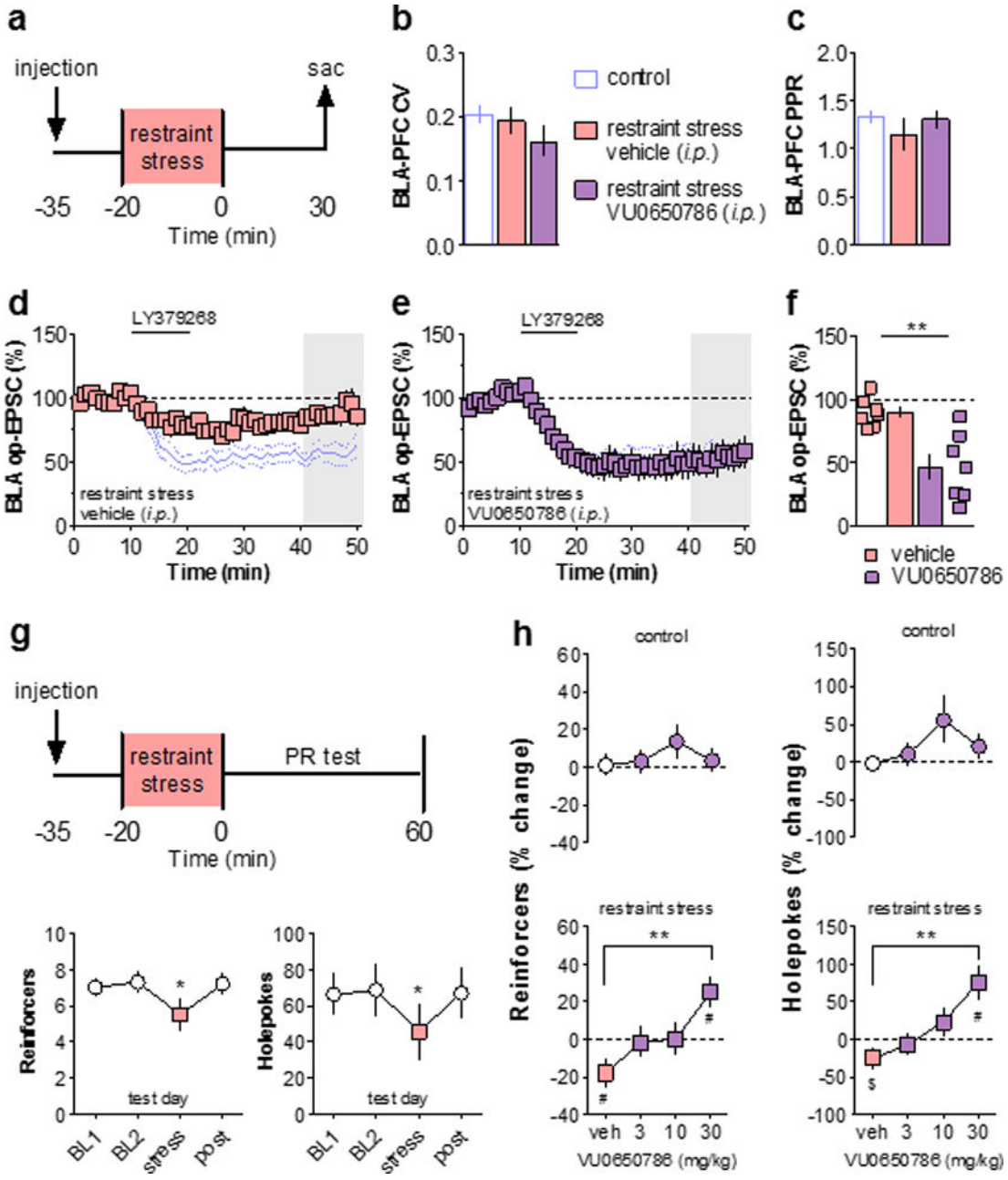

Figure 5. Blocking $mGlu_3$ activation *in vivo* prevents stress-induced deficits to BLA-PFC $mGlu_3$-LTD and motivation

(a) Schematic displaying stress exposure paradigm. Mice were pretreated with *i.p.* injections of the $mGlu_3$ 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 $mGlu_3$ NAM rescues the stress-induced deficit in $mGlu_3$-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, *: $p < 0.05$, Bonferonni 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, **: $p < 0.01$, Bonferonni 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; **: $p < 0.01$, Bonferonni 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; $mGlu_3$, 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.