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Advances in translating $mGlu_2$ and $mGlu_3$ receptor selective allosteric modulators as breakthrough treatments for affective disorders and alcohol use disorder

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Abstract

Metabotropic glutamate (mGlu) receptors are promising targets for the treatment of affective disorders and alcohol use disorder (AUD). Nonspecific ligands for Group II ($mGlu_2$ and $mGlu_3$) mGlu receptors have demonstrated consistent therapeutic potential for affective disorders in preclinical models. Disentangling the specific roles of $mGlu_2$ versus $mGlu_3$ receptors in these effects has persisted as a major challenge, in part due to pharmacological limitations. However, the recent development of highly specific allosteric modulators for both $mGlu_2$ and $mGlu_3$ receptors have enabled straightforward and rigorous investigations into the specific function of each receptor. Here, we review recent experiments using these compounds that have demonstrated both similar and distinct receptor functions in behavioral, molecular, and electrophysiological measures associated with basal function and preclinical models of affective disorders. Studies using these selective drugs have demonstrated that $mGlu_2$ is the predominant receptor subclass involved in presynaptic neurotransmitter release in prefrontal cortex. By contrast, the activation of postsynaptic $mGlu_3$ receptors induces a cascade of cellular changes that results in AMPA receptor internalization, producing long-term depression and diminishing excitatory drive. Acute stress decreases the $mGlu_3$ receptor function and dynamically alters transcript expression for both $mGlu_2$ (*Grm2*) and $mGlu_3$ (*Grm3*) receptors throughout stress- and reward-related brain areas. Accordingly, both $mGlu_2$ and $mGlu_3$ negative allosteric modulators show acute antidepressant-like effects and potential prophylactic effects against acute and traumatic stressors. The wide array of effects displayed by these new allosteric modulators of $mGlu_2$ and $mGlu_3$ receptors suggest that these drugs may act through improving endophenotypes of symptoms observed across several neuropsychiatric disorders. Therefore, recently developed allosteric modulators selective

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Conflict of Interest

The authors declare no potential conflicts of interest.

for $mGlu_2$ or $mGlu_3$ receptors show promise as potential therapeutics for affective disorders and AUD.

Keywords

synaptic plasticity; G protein-coupled receptor; drug discovery; prelimbic cortex; gene expression; electrophysiology

Introduction

Mood disorders, including major depressive disorder (MDD) and anxiety disorders are a broad range of neuropsychiatric disorders characterized by symptoms relating to an individual's emotional state. The 12-month prevalence for mood disorders and anxiety disorders are approximately 9.5% and 18.1%, respectively [1]. These disorders also display high comorbidity with other psychiatric diseases, notably including substance and alcohol use disorder (AUD) [2]. AUD is characterized by excessive and problematic drinking, and many individuals with AUD display protracted affective symptoms during abstinence. Mood disorders and AUD alike suffer from unsatisfactory treatment options and high rates of recurrence or relapse. Small molecules directed at metabotropic glutamate (mGlu) receptors have garnered significant interest as targets for developing new treatments for affective disorders and AUD [3–6]. The family of mGlu receptors consist of eight receptor subtypes that are classified into three groups (Group I – $mGlu_1$ and $mGlu_5$; Group II – $mGlu_2$ and $mGlu_3$; Group III – $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) [7]. In the early 1990s, rotationally constrained glutamate analogs were rapidly developed, optimized, and evaluated in rodent models relevant to several psychiatric diseases [4, 8, 9]. While these studies yielded considerable and consistent findings for the therapeutic potential of several classes of mGlu receptor ligands (reviewed in [4, 5]), early compounds suffered from poor receptor subtype selectivity because they targeted the highly conserved glutamate-binding orthosteric site. The first-in-class $mGlu_2/mGlu_3$ receptor ligands are non-selective, showing comparable activity at both receptor subtypes [9]. Over the last two decades, however, many drug discovery programs have pivoted their primary screens from radioligand displacement to functional assessments of receptor activity (e.g. calcium mobilization, cAMP production, potassium channel activation) [10, 11]. This technical innovation enabled the detection of molecules that interact with mGlu receptors not only near the glutamate-binding domain but also at other sites (i.e. "allosteric) that are less conserved between related receptor subtypes [12]. Through this approach, subtype-specific positive and negative allosteric modulators (PAMs and NAMs) have been identified for $mGlu_2$ and $mGlu_3$ receptors, including highly potent molecules suitable for systemic administration (Table 1). These groundbreaking compounds have enabled advances in the specific functional properties of mGlu receptor subtypes, while also providing significant evidence for the therapeutic potential of subtype-specific mGlu receptor modulators.

The $mGlu_2$ and $mGlu_3$ receptor subtypes are exclusively categorized as Group II mGlu receptors, evolutionarily related by high sequency homology and therefore shared orthosteric pharmacology. Canonical $mGlu_2$ and $mGlu_3$ receptor signaling proceeds through G_i protein-

coupled effectors [7, 10]. Thus, receptor activation facilitates the inhibition of adenylate cyclase activity and cyclic adenosine monophosphate (cAMP) production, as well as the modulation of potassium and calcium channels. These and other signaling cascades affect a host of processes that regulate brain function and behavior, including neuronal intrinsic properties, synaptic plasticity mechanisms, and glial function [13]. $mGlu_2$ and $mGlu_3$ receptors are both expressed at presynaptic terminals, but $mGlu_3$ receptors are also highly expressed at postsynaptic locations and on glia [14–16]. Group II mGlu receptors can form functional homodimers with themselves (ex. $mGlu_2$-$mGlu_2$ or $mGlu_3$-$mGlu_3$) or form heterodimers with the other subunit ($mGlu_2$-$mGlu_3$) [17–19]. The ability to pharmacologically target homodimers or heterodimers holds significant promise to modulate discrete neural circuits. While some allosteric modulators and nanobodies have been developed for $mGlu_2$ homomers [20], we are not aware of the existence of similar tools for $mGlu_2$-$mGlu_3$ heterodimers or $mGlu_3$ homomers. Similarly, the development of biased PAMs and/or NAMs that are biased towards specific effectors (as observed with $mGlu_5$ PAMs [21]) could provide opportunities to minimize potential off target effects while retaining efficacy. More research needs to be carried out to characterize potential signaling bias of existing Group II compounds and to discover new chemical entities with these properties. For more details regarding $mGlu_2$ and $mGlu_3$ receptor signaling mechanisms, genetics and protein structure, or expression and localization, we direct the reader to comprehensive reviews [6, 7, 22, 23].

Recent studies have revealed that $mGlu_2$ and $mGlu_3$ receptors serve both similar and distinct roles across several neural circuits and behavioral outcomes involved in adaptations to stress and models of affective disorders. In this review, we first overview new pharmacological tools available to assess the function of $mGlu_2$ and $mGlu_3$ receptor sub-types. We then describe the current understanding of how each $mGlu_2$ and $mGlu_3$ receptor subtype regulates the physiology of the prefrontal cortex, hippocampus, striatum, and amygdala. We then summarize the acute antidepressant and anxiolytic effects of $mGlu_2$ and $mGlu_3$ drugs. Next, we summarize recent advances in understanding how both receptor subtypes are involved in adaptations to stress in preclinical models. Finally, we discuss recent findings of $mGlu_2$ and $mGlu_3$ receptors on alcohol-related behaviors. We conclude with a brief discussion of the clinical literature around $mGlu_2$ and $mGlu_3$ receptors and clinical trials that have been conducted using selective allosteric modulators of $mGlu_2$ or $mGlu_3$.

Pharmacology

In the mid-1990’s several agonists and antagonists targeting the Group II mGlu receptors were developed, paving the way for physiological and behavioral studies evaluating their basic function and therapeutic potential. The systemically active orthosteric agonists LY354740 and LY379268 (518 PubMed-indexed publications since 1997), along with the related antagonist LY341495 (454 publications since 1997), are among the most widely used mGlu receptor tool compounds in preclinical studies. Reviewed well by others [4, 5], these and related molecules have been used to build significant evidence that $mGlu_{2/3}$ agonists can attenuate anxiety-like behavior and $mGlu_{2/3}$ antagonists can deliver antidepressant-like effects. However, until recently, the relative contribution of $mGlu_2$ vs $mGlu_3$ receptors in these effects has not been well understood.

While the development of subtype-specific transgenic mouse lines has helped separate some functions of the distinct Group II mGlu receptors, compensatory adaptations have interfered with clear interpretations in some cases. With that in mind, the development of selective pharmacological tools to modulate only one receptor subtype has been invaluable in helping to understand the individual contributions of $mGlu_2$ or $mGlu_3$ receptors in affective behaviors and neural circuitry. The first series of Group II selective compounds were $mGlu_2$ PAMs (originally termed 'potentiators'). These $mGlu_2$ PAMs (LY181837, LY487379, AZD8529 and Biphenyl-indanone A (BINA)) provided the unprecedented ability to ask questions about the specific role of a single Group II mGlu receptor subtype. More recently, highly selective and centrally penetrant NAMs have been developed for both $mGlu_2$ and $mGlu_3$ receptors [24]. These classes of compounds, notably including the $mGlu_2$-selective NAM VU6001966 [25] and the $mGlu_3$-selective NAMs VU0650786 [26] and VU6010572 [27], have enabled thorough and mechanistic preclinical studies that detail how $mGlu_2$ and $mGlu_3$ receptors separably influence neurophysiology (Table 1) and affective behaviors (Table 2).

Function and Physiology

Prefrontal cortex

The prefrontal cortex (PFC) regulates the direction and vigor of cognitive and motivated behaviors by computing information related to internal state, environmental cues, and long-term goals [28]. Human studies have consistently detected population-level associations in PFC function with affective disorders, and both human and preclinical studies indicate the PFC is exquisitely sensitive to stressors of all magnitudes and modalities [29]. The PFC can be broadly split into orbitofrontal, ventral, dorsal, and cingulate regions, which roughly correspond to the orbitofrontal (OFC), infralimbic (ILC), prelimbic (PLC), and anterior cingulate (ACC) cortices. In general, the function of the anterior and ventral regions (OFC/ILC) is associated with autonomic and emotional responses, whereas the posterior and dorsal regions (PLC/ACC) are more involved in higher-order planning [30]. Preclinical physiology and plasticity studies have primarily focused on understanding how $mGlu_2$ and $mGlu_3$ receptors regulate PLC function and this section will be accordingly biased.

In rodent PFC, $mGlu_{2/3}$ agonists rapidly attenuate glutamate release probability and induce sustained long-term depression (LTD) of excitatory transmission [31, 32]. Until recently, it was unclear whether these actions were related to each other and if either is mediated by one specific mGlu receptor subtype. Substantial evidence now indicates that presynaptic $mGlu_2$ receptors regulate acute glutamate release probability. $mGlu_2$ PAMs, like BINA, attenuate increases in glutamate release triggered by serotonergic activation in PLC slices [33, 34]. In addition, incubating PLC slices with the selective $mGlu_2$ NAM VU6001966 increases glutamatergic tone and increases indices of presynaptic release probability onto pyramidal cells [35]. Similar findings have been obtained from synaptosomes prepared from whole cortex: both BINA and another $mGlu_2$ PAM, LY566332, facilitated the inhibition of glutamate exocytosis from cortical synaptosomes [36]. In those studies, two $mGlu_3$ NAMs also partially attenuated the effect of an $mGlu_{2/3}$ agonist, suggesting $mGlu_{2/3}$ heterodimers may assemble at presynaptic terminals in PFC, consistent with molecular

studies [17–19]. Finally, acute presynaptic inhibition of glutamate release has also been observed in pyramidal cells within layers 2/3 of human cortex [37], demonstrating important conservation of $mGlu_2$ receptor function in cortex across species.

In PLC slices, $mGlu_{2/3}$ agonists also mediate LTD of excitatory transmission that persists for >30 minutes following agonist wash-out [38–40]. This LTD is *almost* entirely dependent on $mGlu_3$ receptors (see next paragraph for exception). With respect to field potentials or excitatory postsynaptic currents evoked with electrical stimulation, selective $mGlu_3$ NAMs completely block PFC LTD, while $mGlu_2$ NAMs do not [38–40]. Similar findings have been observed in $mGlu_3$ knockout mice but not $mGlu_2$ knockout mice [38, 41]. Electron microscopy studies display high subcellular localization of $mGlu_3$ receptors in dendrites and other postsynaptic compartments in monkey PFC [42], and several studies implicate the necessary participation of postsynaptic signaling cascades and the internalization of AMPA receptors as the final step in LTD expression. LTD was associated with decreased amplitude of spontaneous excitatory postsynaptic currents, did not affect NMDA receptor responses, and was blocked by a dynamin inhibitory peptide that disrupts endocytosis within the postsynaptic neuron [39]. LTD was not dependent on the mobilization of intracellular calcium stores but did require activation of the PI3K/Akt signaling pathway [40]. We also found that other molecules associated with postsynaptic signaling, including $mGlu_5$ receptors, Glycogen Synthase Kinase 3, and Homer proteins, are critically involved in $mGlu_3$-LTD [40]. Thus, cortical $mGlu_3$ receptor activation initiates a cascade of intracellular signaling events that culminates in AMPA receptor internalization and reduced excitatory drive onto pyramidal cells.

The PFC receives glutamatergic input from a variety of cortical and subcortical afferents. Studies using optogenetics have identified that $mGlu_2$ and $mGlu_3$ receptors are differentially involved in regulating the strength of glutamate transmission at distinct inputs. We discovered that $mGlu_3$ receptors induce LTD at isolated inputs from the basolateral amygdala (BLA), whereas transmission from the ventral hippocampus was not affected by an $mGlu_{2/3}$ agonist [39]. Furthermore, by leveraging a combinatorial viral strategy to selectively express channelrhodopsin in the BLA and genetically reduce $mGlu_3$ receptor expression in PLC, we demonstrated that $mGlu_3$ receptors expressed in postsynaptic sites *themselves* are required for LTD. By contrast, PLC synapses arising from the mediodorsal thalamus (MDT) express LTD mediated by either $mGlu_2$ or $mGlu_3$ [43]. LTD inducted by an $mGlu_{2/3}$ agonist was only partially blocked by either the $mGlu_3$ NAM VU0650786 or two structurally distinct $mGlu_2$ NAMs. Considering that $mGlu_2$ NAMs increase measurements of glutamate release probability but $mGlu_3$ NAMs do not, these data suggest that $mGlu_2$ receptors mediate presynaptic LTD at MDT→PLC synapses. MDT-arising synapses are also regulated by $mGlu_2/mGlu_4$ receptor heterodimers, while BLA and other inputs to PLC are not [34]. Taken together, these findings indicate that synapses from the BLA and MDT both participate in postsynaptic $mGlu_3$ LTD, and synapses from the MDT also engage in presynaptic $mGlu_2$-dependent LTD, similar to phenomena reported at thalamic terminals in striatum [44].

Hippocampus

The hippocampus is a cortical brain area intimately linked with memory formation and retrieval. One of its key functions is converting short-term memories into long-term memories; thus, it is not surprising that hippocampus function has been associated with the development of PTSD and disorders associated with chronic stressful experiences [45]. The hippocampus has been one of the best-studied brain regions in preclinical electrophysiology and synaptic plasticity research, largely due to its defined, laminar structure and prominent location. The canonical path of information flow through the hippocampus proceeds through a glutamatergic tri-synaptic circuit: perforant path inputs from entorhinal cortex enter the hippocampus within the dentate gyrus; the mossy fiber carries dentate gyrus granule cell projections to CA3; the Schaffer collateral describes synapses from CA3 pyramidal cells to CA1; CA1 and subiculum pyramidal cells then project to a variety of cortical and subcortical structures [46].

$mGlu_2$ and $mGlu_3$ receptors are expressed throughout the hippocampus. $mGlu_2$ receptors are expressed in the lateral perforant path, where they attenuate glutamate release probability onto granule cells in the dentate gyrus [47, 48]. Cells within the dentate gyrus display the highest levels of *Grm2* transcript within the hippocampus [49], and, accordingly, mossy fiber synapses terminating in CA3 are also regulated by $mGlu_2$ receptors [20]. Interestingly, recent studies using allosteric nanobodies and novel small molecules indicate that $mGlu_2$ receptors assemble as distinct dimers across hippocampal synapses, with $mGlu_{2/2}$ homodimers [20] regulating glutamate release at the mossy fiber synapses while $mGlu_{2/4}$ heterodimers function within the performant path [47]. Historically, $mGlu_2$ receptors were not thought to regulate glutamate release within the CA1 region, but recent studies have identified that transmission along the temporo-ammonic path (the direct input from entorhinal cortex to CA1) is sensitive to $mGlu_2$ receptor modulation [50].

Grm3 transcript (encodes $mGlu_3$) is broadly expressed throughout hippocampal subregions, but $mGlu_3$ receptor functions have been described best within CA1. Despite being similarly expressed in post/peri-synaptic sites and in glia, hippocampal $mGlu_3$ receptors regulate glutamate transmission in a distinct manner from their cortical counterparts. Recent research has identified that activation of $mGlu_3$ receptors promotes the LTP induction at the Schaeffer collateral synapse [51, 52]. Subthreshold activation of $mGlu_3$ receptors facilitates the induction of tetanus-induced LTP and saturating agonist application can increase glutamate transmission on its own. Like $mGlu_3$-LTD in frontal cortex, $mGlu_3$-LTP in CA1 is dependent on the coordinated activation of $mGlu_5$ receptors [51], however $mGlu_3$-LTP is also dependent on the activation of NMDA receptors and the mobilization of intracellular Ca^{2+} [52]. Studies using cell type-specific transgenic mice demonstrated that neuronal expression of $mGlu_3$ and $mGlu_5$ receptors is necessary for LY379268 to potentiate LTP and the restoration of deficits in trace fear learning [51]. Taken together, these studies indicate that neuronal $mGlu_3$ receptors play an important role in regulating the strength of transmission through hippocampal circuits as well as trace fear learning.

$mGlu_3$ receptors are the primary, if not only, mGlu receptor subtype expressed in astrocytes in adulthood [53]. Glial $mGlu_3$ receptors were first implicated in regulating hippocampal plasticity by a series of early studies demonstrating Group II mGlu receptors can inhibit

adrenergic facilitation of LTP [54, 55]. Recent studies using selective NAMs, knockout mice, and glial toxins, indicate these effects are exclusively mediated by $mGlu_3$ receptors and require intact glia [56]. Coordinated activation of $mGlu_3$ receptors and β receptors results in a large increase in cyclic AMP, release of adenosine, and activation of presynaptic A1 adenosine receptors to block the induction of LTP. Overall, the recent literature indicates that glial and neuronal $mGlu_3$ receptors regulate hippocampal metaplasticity through divergent mechanisms and result in distinct circuit-level outcomes. As microglia have also been implicated in stress-related disorders, the ability of $mGlu_3$ receptors to regulate microglia function [57] also merits further investigation.

Striatum and amygdala

While less is known regarding their signaling mechanisms or ability to participate in synaptic plasticity, recent studies using contemporary tools have investigated $mGlu_2$ and $mGlu_3$ receptors in other regions. The dorsal and ventral striatum are widely implicated in reward learning and mood disorders [58]. Within dorsal striatum, $mGlu_2$ receptors, but not $mGlu_3$ receptors attenuate glutamate and dopamine release probabilities [44, 59]. This plasticity appears to be related to the inhibition of presynaptic Ca^{2+} entry through P/Q-type channels [60]. In related findings, $mGlu_2$, but not $mGlu_3$, receptor knockout mice displayed enhanced novelty-induced locomotor activity and reduced hyperactivity following methamphetamine administration [61], suggesting $mGlu_2$ receptor function has clear relevance for behaviors dependent on striatal function. Nonselective $mGlu_{2/3}$ agonists have long been known to induce LTD in the nucleus accumbens [62], but the relative contribution of each receptor subtype has yet to be established.

$mGlu_{2/3}$ agonists also attenuate glutamate transmission and induce LTD within several nuclei of the extended amygdala, including the BLA [63], the lateral amygdala [64], the central nucleus [65], and the bed nucleus of the stria terminalis [66]. These extended amygdala regions play an important role in mood disorders and AUD [67]. In most of these nuclei, the individual contribution of $mGlu_2$ or $mGlu_3$ receptors to LTD is unknown. In the lateral amygdala, studies using knockout rats, knockout mice, and the mixed compound LY541850 demonstrated that both $mGlu_2$ and $mGlu_3$ receptors can induce LTD [64]. In the BLA, studies have shown that an $mGlu_2$ positive allosteric modulator can attenuate glutamate release [68] but it is not clear whether $mGlu_3$ receptors may regulate plasticity as well.

Effects of Stress Exposure

Receptor expression

Stress is a major factor in the etiology of many affective disorders. Relatively few studies have directly assessed how chronic stress affects the function of $mGlu_2$ and $mGlu_3$ receptors. Studies examining chronic social isolation in male mice have related decreased prefrontal cortex *Grm2* (encodes $mGlu_2$) expression with depressive-like outcomes [69]. We have also demonstrated decreased *Grm2* expression in ILC four weeks after an acute exposure to a predator odor stressor in male, Long-Evans rats [70]. In a related study, decreased *Grm2* and $mGlu_2$ protein expression was observed in the BLA in panic-prone

rats [68]. Similarly, in the dentate gyrus, protein and transcript levels for $mGlu_2$ receptors were decreased in mice following three weeks chronic restraint stress [71]. The same authors also found decreased $mGlu_2$ receptor protein expression in hippocampus and PFC in mice that were susceptible to four weeks chronic unpredictable stress, whereas resilient mice displayed decreased PFC $mGlu_2$ receptor expression, but intact hippocampal expression relative to controls [72]. These studies collectively suggest that $mGlu_2$ receptor expression levels, across multiple brain regions, may be inversely related to stress exposure and depressive-like behaviors. Consistent with that hypothesis, epigenetic increases in *Grm2* expression have been linked with antidepressant drug treatment. Studies from the Nicoletti lab have shown that the well-tolerated drug L-acetylcarnitine epigenetically increases hippocampal and PFC *Grm2* expression in Flinders Sensitive Line rats and in mice exposed to chronic unpredictable stress [73], suggesting a bidirectional relationship between cortical $mGlu_2$ receptor function and affective behaviors. Based on this literature, potentiating $mGlu_2$ receptor function, via PAMs or alternative mechanisms, may be able to reverse stress-induced adaptations and related affective disturbances.

In addition to the effects of stress on $mGlu_2$ receptors, stress also impacts $mGlu_3$ expression (gene: *Grm3*). Our lab has found acute exposure to a predator odor stressor in rats downregulates *Grm3* levels in the PLC and dorsal hippocampus two days following the stressor. By contrast, we detected upregulated *Grm3* in the nucleus accumbens at the same timepoint, indicating that changes in regulation of $mGlu_3$ receptor expression following traumatic stress are dependent on brain region. In addition, the time elapsed following predator odor is a crucial variable that regulates $mGlu_3$ mRNA levels, as we found that *Grm3* was upregulated in the insular cortex two weeks following stress exposure. [70]. Overall, traumatic stress dynamically alters $mGlu_3$ receptor expression in cortex and striatum at early timepoints, and the durable effects in the insular cortex may have important ramifications for developing new treatments for PTSD. Nonetheless, more research will be needed to further identify the cell types involved in these phenomena (e.g. neurons vs. glia) and to better understand the effects of mild and/or chronic stress on $mGlu_3$ receptor expression.

Physiology and synaptic plasticity

Studies have begun to address how stressful experiences alter the separable physiological functions of $mGlu_2$ and $mGlu_3$ receptor signaling. In mice, we have found that a single exposure to 20 minutes of immobilization stress, 30 minutes before sacrifice, impairs $mGlu_3$-LTD within PLC [39, 40]. Stress also impaired $mGlu_3$-LTD when animals were sacrificed one day later, and the plasticity recovered to comparable levels as controls following three days. Systemic administration with the $mGlu_3$ NAM VU0650786 (30 mg/kg) 15 minutes prior to, or immediately following, 20 minutes restraint stress exposure restored the ability to induce $mGlu_3$-LTD *ex vivo* [39], indicating that receptor activation during stress is necessary for the physiological changes in plasticity to occur. In addition, the stress-induced impairment in $mGlu_3$-LTD was rescued via *ex vivo* application of the $mGlu_5$ PAM VU0409551 [40]. Notably, several alternative $mGlu_5$-dependent functions were intact following acute stress, indicating a selective alteration in signaling related to $mGlu_3$-LTD. These findings raise the possibility that $mGlu_3$ NAMs may be useful in preventing the

consolidation of stress-induced adaptations to PFC function. Another exciting hypothesis for future studies is that $mGlu_3$ PAMs or potentiators may be efficacious in ameliorating stress disturbances that have been consolidated or established. Unfortunately, the limited availability of selective $mGlu_3$ PAMs has hindered efforts in clearly testing this hypothesis. In addition, whether acute and chronic stress affects $mGlu_2$ and $mGlu_3$ receptor-dependent plasticity in other cortical and subcortical areas remains an open area of inquiry.

Behavior

Newly developed $mGlu_2$ and $mGlu_3$ NAMs have shown promising results in preclinical animal experiments for their role in alleviating stress-induced adaptations in depressive-like behaviors. For example, an $mGlu_3$ NAM (VU0650786, 10-30 mg/kg) dose-dependently blocked restraint stress-induced changes in motivation to work for palatable food on a progressive ratio schedule of reinforcement in male mice when administered 15-min before restraint stress [39]. In another study modeling chronic stress effects on reward behavior, both chronic corticosterone (CORT) exposure in the drinking water and chronic variable stress decreased sucrose preference. Treatment with either the $mGlu_2$ NAM VU6001966 (10 mg/kg) or the $mGlu_3$ NAM VU0650786 (30 mg/kg) one day before the sucrose preference test reversed the stress-induced anhedonia-like behavior [43]. Importantly, future studies should also assess how to best prevent or mitigate potential side effects related to $mGlu_2$ or $mGlu_3$ receptor inhibition, considering observed effects on cognitive functions including working memory [42] and extinction learning [38].

Similar to studies examining non-conditioned behaviors, studies assessing stress-induced behaviors in $mGlu_2$ and $mGlu_3$ knockout mice have yielded mixed results. In studies using mice on a CD1 background, $mGlu_2$ knockouts showed resilience to the development of escape deficits induced by inescapable shock stress, while $mGlu_3$ knockouts did not [74]. Furthermore, $mGlu_2$ knockouts did not exhibit decreased escape behavior following CORT administration or anhedonia-like behavior induced by chronic social defeat stress [74]. By contrast, other knockout studies in mice on a C57BL/6J background have reached the opposite conclusion, that *Grm2* genetic deletion enhances susceptibility to stress. Nasca et al. found that after four weeks of chronic unpredictable stress, $mGlu_2$ knockout mice displayed increased immobility on the forced swim, decreased body weight, and greater fur coat deterioration relative to matched C57BL/6J controls [72]. Together, these data show that while $mGlu_2$ receptor NAMs demonstrate therapeutic potential for treating anhedonia in MDD, additional mechanistic preclinical neuroscience research is needed to reconcile inconsistencies related to rodent genetic background.

Behavioral studies in traumatic stress models have revealed prophylactic anti-stress effects of $mGlu_3$ NAM administration. Pretreatment with the $mGlu_3$ NAM VU6010572 (3 mg/kg) prior to exposure to an acute predator odor stressor did not affect the engagement in stress-reactivity behavior during the stressor, but blocked freezing behavior when rats were re-exposed to the stressor context two weeks later [75]. As exposure to the scent of a predator has been used to model aspects of a traumatic stress experience [70, 76, 77], an intriguing hypothesis for future studies is that signaling triggered by $mGlu_3$ receptors could be recruited during traumatic stressors and contribute to long-term adaptations related

to PTSD. Consistent with that notion, $mGlu_3$ receptor activation was found to block the reconsolidation of fear learning [56]. Molecularly, VU6010572 blocked increased *Grin3B* upregulation by predator odor exposure in the insular cortex and bed nucleus of the stria terminalis [75]. *Grin3B* is the gene that encodes for the NMDA receptor subunit GluN3B and has recently been identified as a predictive blood biomarker of PTSD symptomology following a traumatic experience [78]. Based on this, *Grin3B* expression represents an exciting potential biomarker to be deployed in future studies assessing the therapeutic potential for $mGlu_3$ NAMs in treating PTSD and/or other affective disorders.

Antidepressant- and Anxiolytic-like Behavioral Effects

Passive coping behaviors

Their abilities to modulate adaptations to stress, and neural circuits related to anxiety, affect, and reward in general, suggest that $mGlu_2$ and $mGlu_3$ receptors are poised to regulate motivated behaviors [79]. Indeed, as discussed, systemic administration of $mGlu_2$ and $mGlu_3$ NAMs confers rapid antidepressant-like effects in rodent models. Several studies have shown that $mGlu_3$ NAMs decrease passive coping behavior in acute rodent models. The $mGlu_3$ NAM VU0650786 decreased time spent immobile in male mice in a forced swim test (56.6 mg/kg, 30-min pretreatment; all compounds administered *i.p.* unless otherwise noted) [26]. The structurally distinct $mGlu_3$ NAM VU6010572 (3 mg/kg, 15-min pretreatment) also demonstrated antidepressant-like effects in a tail-suspension test in male mice [27], consistent with on-target $mGlu_3$ receptor inhibition driving effects on passive coping behavior. In two studies that performed a head-to-head comparison, the $mGlu_3$ NAMs VU0650786 (30 mg/kg) and VU6010572 (1.8-3 mg/kg), but not the $mGlu_2$ NAM VU6001966 (10-30 mg/kg), increased the latency to immobility and decreased the total time spent immobile in the tail suspension test [27, 43]. Interestingly, both the $mGlu_2$ NAM VU6001966 (10 mg/kg) and the $mGlu_3$ NAM VU0650786 (30 mg/kg) administered 45 minutes before a forced swim test in male C57BL/6J mice decreased latency to float immobile and decreased total time immobile [43], suggesting that $mGlu_2$ NAM effects on passive coping behavior may not generalize to all behavioral tasks. Chemogenetic inhibition of MDT→PFC circuitry blocked the behavioral effect of both the $mGlu_2$ and $mGlu_3$ NAMs in the forced swim test [43]. This finding suggests that, despite the differences in synaptic mechanisms, MDT→PFC circuitry is similarly involved in the antidepressant-like effects of both $mGlu_2$ and $mGlu_3$ NAMs.

Similar studies using genetic manipulations have yielded more mixed results than those using selective pharmacology. In a series of studies using male mice on a C57BL/6J background, $mGlu_3$ knockouts displayed decreased immobility in the forced swim test [80]. In studies from another group, $mGlu_2$ knockouts on a C57BL/6J background had no baseline differences in behavior in the forced swim test relative to controls [72]. By contrast, a recent set of studies using CD1 background knockout mice found that $mGlu_2$ but not $mGlu_3$ knockout mice exhibited reduced immobility in the forced swim test and fewer escape failures in a learned helplessness assay [74]. Thus, some evidence from knockout mouse suggests that both $mGlu_2$ and $mGlu_3$ NAMs have antidepressant-like potential, however these findings may critically depend based on genetic background. Finally, we observed that

selective genetic knockdown of $mGlu_3$ receptors within the PFC led to decreased immobility in both the forced swim and tail suspension tests in both male and female mice on a hybrid C57BL/6N x 6J background [41]. These studies provide evidence that $mGlu_3$ receptor inhibition exerts antidepressant-like effects in both male and female rodents and implicate PFC circuits in mediating behavioral responses to $mGlu_3$ receptor modulators.

Anxiety-like behaviors

A few studies assessing anxiety-like behaviors have produced evidence that selective inhibition of $mGlu_3$ receptors may attenuate anxiety; however, to our knowledge selective $mGlu_2$ NAMs have not been examined in similar studies. The $mGlu_3$ NAM VU0650786 decreased marble burying behavior (10-56.5 mg/kg; 15-min pretreatment) in male mice [26], consistent with a potential decrease in anxiety-like behavior. In separate studies in rats, a single administration of $mGlu_3$ NAM VU6010572 (3 mg/kg) increased time spent in the open arms of an elevated zero maze test when tested 2 weeks after treatment, demonstrating that this drug may have prophylactic effects in addition to its acute effects [75]. Studies using knockout mice corroborate the recent pharmacological studies to some extent. A modest anxiolytic-like effect was observed in male $mGlu_3$ knockout mice on some but not all endpoints in the light-dark transition [80]. In a more comprehensive study with respect to anxiety-like behaviors, male $mGlu_3$ receptor knockout mice were found to exhibit a trend increase in open arm time on the elevated plus maze, a shorter latency to enter the open arms, and a shorter latency to eat novel food in a hypophagia task [81]. By contrast, $mGlu_2$ knockout mice in the same study were not different than controls on any measure of anxiety-like behavior. Finally, consistent with the modest anxiolytic-like effect observed in global knockouts, $mGlu_3$ receptor knockdown in the mouse PFC also increased time spent in the open arms of the elevated zero maze but had no effect on the light-dark box test [41]. These convergent data from multiple laboratories suggest that $mGlu_3$ NAMs have the potential to be developed as novel anxiolytic medications, but enthusiasm should be tempered due to the limited breadth across preclinical models related to anxiety. Some evidence from preclinical models also suggests that $mGlu_2$ PAMs may have potential anxiolytic activity [82, 83].

Alcohol behaviors

Nonselective $mGlu_{2/3}$ agonists have repeatedly been shown to reduce alcohol intake behaviors, including operant self-administration and cue-induced reinstatement, but the relative contribution of $mGlu_2$ vs $mGlu_3$ receptors in these effects remains considerably unclear [3, 6, 84, 85]. Interestingly, the $mGlu_{2/3}$ agonist LY379268 (3 mg/kg, *subcutaneous*) was found to be more effective in reducing ethanol self-administration and reinstatement in dependent Wistar rats relative to non-dependent controls [85], suggesting that chronic ethanol exposure may upregulate $mGlu_{2/3}$ receptor function. Consistent with this hypothesis, we recently discovered that prior exposure to intermittent drinking enhanced the ability of LY379268 to attenuate presynaptic glutamate release probability on intratelencephalic neurons within mouse PLC [86]. Considering this function is mediated by $mGlu_2$ and not $mGlu_3$ receptors [43], these findings provide a potential neurobiological substrate through which $mGlu_2$-directed compounds could alter ethanol-related behaviors. Furthermore, intermittent alcohol vapor exposure decreases $mGlu_2$ receptor expression in ILC pyramidal

neurons and resulted in insensitivity to $mGlu_{2/3}$ agonist-induced decreases in extracellular glutamate in the nucleus accumbens shell [87]. Viral-mediated restoration of $mGlu_2$ receptor expression in the ILC reversed escalated alcohol-seeking.

Studies in rats have also demonstrated the potential for $mGlu_2$ receptor potentiation to attenuated maladaptive drinking. The $mGlu_2$-selective PAM, AZD8529 (20-40 mg/kg, *subcutaneous*), modestly decreased operant alcohol (20%) self-administration in male, Wistar rats [88]. More robustly, AZD8529, blocked cue-induced alcohol seeking in the same rats. This effect was not observed in alcohol preferring (P rats) rats, which lack expression of $mGlu_2$ receptors [89], demonstrating the functional role of $mGlu_2$ in the effect on cue-induced alcohol seeking [88]. Another $mGlu_2$ PAM BINA (20 mg/kg), failed to show these effects on self-administration and cue-induced reinstatement, demonstrating that this effect may be dependent on the $mGlu_2$ PAM used or other technical parameters between investigators [90]. Once selective $mGlu_3$ PAMs are widely available, assessing whether these compounds can recapitulate the broad ability of $mGlu_{2/3}$ agonists to reduce motivation to drink in preclinical models will be an important series of studies. Alternatively, it is possible that potentiation of receptor activity is insufficient to reduce drinking and we may find that agonist activity at $mGlu_2$ or $mGlu_3$ receptors is essential.

Several lines of convergent evidence indicate that $mGlu_3$ receptors may be a promising target for AUD treatment development. Genetic knock out of $mGlu_3$ receptors in mice blocked the conditioned place preference of alcohol [91], suggesting that $mGlu_3$ receptors may be involved in the rewarding and/or interoceptive effects of alcohol. Both $mGlu_{2/3}$ agonists and antagonists attenuate the interoceptive stimulus effects (i.e., the subjective effects) of alcohol in rats [92, 93]. In recent studies using the newly developed selective NAMs, both the $mGlu_2$ NAM VU6001966 (6 mg/kg) and the $mGlu_3$ NAM VU6010572 (12 mg/kg) attenuate the interoceptive stimulus effects of alcohol in rats (2 g/kg, *intragastric*) [94]. These data raise the possibility that these receptors are both involved in the interoceptive effects of alcohol, and/or their effects on cellular physiology can blunt the interoceptive effects of alcohol. Taken together, the ability of both $mGlu_2$ and $mGlu_3$ NAMs to attenuate the interoceptive effects of alcohol, produce acute and lasting antidepressant-like effects, and reverse stress-induced adaptations, may make these drugs ideal candidates for potentially treating affective disorders and AUD.

Clinical studies and looking ahead

While limited in number and interpretation, some findings from post-mortem studies have implicated $mGlu_2$ and $mGlu_3$ receptor function in affective disorders. One post-mortem study found decreased binding of the $mGlu_{2/3}$ antagonist [^{3}H]LY341495 in the anterior cingulate cortex of subjects that had MDD [101]. Another post-mortem study also found decreased $mGlu_{2/3}$ receptor immunoreactivity in the PFC from individuals with MDD compared to controls [102]. Together, these studies indicate that depression is associated with decreased expression of Group II mGlu receptors in the brain, which are consistent with the effects of stress and depressive-like behavior in rodents. Given their divergence in regulating synaptic plasticity, glial function, and other neurobiological actions, future post-mortem studies should be designed to disentangle $mGlu_2$ versus $mGlu_3$ receptor expression,

should assess cellular and subcellular location of the receptors, and should consider using more selective radioligands and/or assessing receptor expression at the transcript level. Future studies should also assess potential changes in $mGlu_2$ and $mGlu_3$ receptor expression in anxiety disorders and AUD.

Agonists of $mGlu_{2/3}$ receptors have undergone clinical trials for several neuropsychiatric disorders. One clinical trial investigated the efficacy of LY354740 (an $mGlu_{2/3}$ agonist) and LY544344 (an LY354740 prodrug) for generalized anxiety disorder. Patients treated with LY544344 showed significant improvements compared to baseline in Hamilton Anxiety and Clinical Global Impression—Improvement scores. Unfortunately, this trial was discontinued due to findings of convulsions in animal studies, despite the lack of any similar adverse events in humans [103]. One the other hand, while results have not yet been made public, two phase 1 trials recently assessed the Group II mGlu receptor antagonists BCI-838 and BCI-632/MGS0039 (NCT01546051 and NCT01548703). Similarly, results from a phase 2 trial for adjunct treatment in MDD with the Group II NAM decoglurant/RO4995819 (NCT01457677) were recently disclosed indicating no separation from a relatively large placebo response [95]. While clinical trials employing nonselective Group II compounds have yielded disappointing results, additional studies examining the antagonist TS-161 [96] are underway (NCT04821271), and findings from trials investigating selective Group II modulators give reason for more optimism. Treatment with the $mGlu_2$ PAM JNJ-40411813/ADX71149 improved panic disorder symptoms in a small group of patients [68]. A larger trial assessing the $mGlu_2$ PAM as an adjunct treatment in high-anxiety MDD was discontinued due to lack of separation from placebo [97], but the compound is currently enrolled in a large Phase 2 trial for adjunct treatment in epilepsy (NCT04836559) that will provide additional safety and tolerability data. Moving forward, trials employing new selective and potent NAMs, ideally as standalone treatments, would be ideal to test whether selective $mGlu_2$ or $mGlu_3$ inhibition can confer antidepressant and other therapeutic effects in the clinic.

The $mGlu_2$ PAM AZD8529 has been evaluated in clinical trials of schizophrenia [98, 99] and smoking cessation (NCT02401022), but not for AUD. To our knowledge, the primary outcomes of the Phase 2 smoking cessation study have not yet been fully disclosed. In the schizophrenia trials, AZD8529 did not affect primary outcome measurements, including negative symptoms [99], but the compound did increase activation of the anterior cingulate cortex and striatum during a working memory task [98]. This finding demonstrates important proof-of-principle that $mGlu_2$ PAMs can modulate reward circuitry in humans and may pave the way for the development of biomarkers for further trials. While efforts in developing suitable radiotracers are underway, until those tools can be widely deployed, functional biomarkers will be essential for assessing target engagement and potential utility in broad clinical populations. Considering the breadth and depth of preclinical literature supporting the potential utility of selective $mGlu_2$ and $mGlu_3$ allosteric modulators for treating mood disorders and AUD, we hope to see more clinical trials on the horizon.

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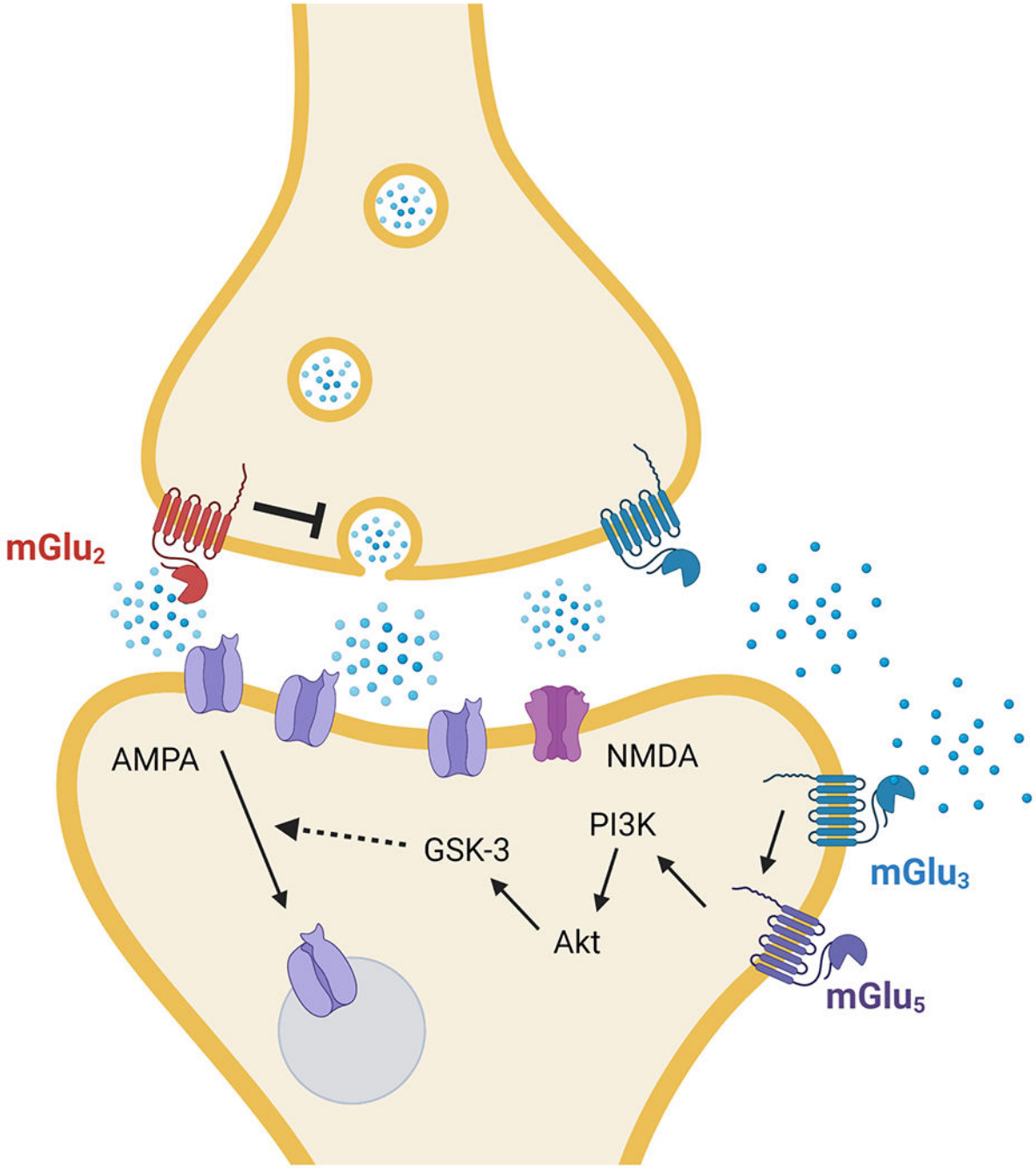

Figure 1. Synaptic locus dissociation of $mGlu_2$ and $mGlu_3$ receptor functions in mouse prefrontal cortex.

Presynaptic $mGlu_2$ receptors gate glutamate release probability in PFC. At synapses arising from the MDT, presynaptic $mGlu_2$ receptors can induce a long-term depression (LTD) of excitatory transmission. By contrast, neuronal $mGlu_3$ receptors mediate a postsynaptic form of LTD. Postsynaptic $mGlu_3$ LTD proceeds through the coordinated signaling of $mGlu_5$ receptors, phosphoinositide 3-kinase (PI3K), Akt, glycogen synthase kinase-3 (GSK-3), and the internalization of AMPA receptors. $mGlu_3$ LTD is also differentially expressed across

long-range inputs to PFC, having been observed at synapses arising from the MDT and BLA, but not the ventral hippocampus.

Table 1.

Effects of $mGlu_2$ and $mGlu_3$ receptors across brain circuits involved in mood disorders and alcohol use disorder

Brain Region	Synaptic Plasticity
Prelimbic Prefrontal Cortex	▪ $mGlu_{2/3}$ agonism attenuates glutamate release and promotes LTD (31-43) ▪ $mGlu_2$ regulates glutamate release (33-35,43) ▪ $mGlu_3$ mediates postsynaptic LTD via AMPAR internalization (38-43)
Hippocampus	▪ $mGlu_{2/2}$ homodimers regulate glutamate release at mossy fiber (20) ▪ $mGlu_{2/4}$ heterodimers regulate glutamate release in perforant path (46) ▪ $mGlu_3$ activation promotes NMDAR-LTP (50, 51) ▪ Glial $mGlu_3$ regulates adrenergic plasticity (54, 55)
Striatum	▪ $mGlu_2$ regulates glutamate and dopamine release (44, 58) ▪ $mGlu_{2/3}$ agonism induces LTD in the nucleus accumbens (61)
Amygdala	▪ $mGlu_{2/3}$ agonism attenuates glutamate release and induces LTD in BLA (62), LA (63), CEA (64), and BNST (65)

Abbreviations: BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CEA, central nucleus of the amygdala; LA, lateral amygdala; LTD, long-term depression; LTP, long-term potentiation

Table 2.

Physiochemical and behavioral properties of novel $mGlu_2$ and $mGlu_3$ negative allosteric modulators

Compound	Target	IC_{50} (μM)	Brain K_p	$T_{1/2}$ (min)	Dose *i.p.* (mg/kg)	Behavioral Effects	Refence
VU6001966	$mGlu_2$	78	1.9	20	10 – 30	↔ immobility TST ↓ immobility FST	Bollinger et al. *J Med Chem Letters*. 2017
VU0650786	$mGlu_3$	392	1.7	49	10 – 56.5	↓ marble burying ↓ immobility TST and FST reverse anhedonia and amotivation after stressors	Engers et al. *J Med Chem*. 2015
VU6010572	$mGlu_3$	245	1.15	?	1.8 – 3 (mouse/ rat)	↓ immobility TST ↑ open arm time EPM (rat) ↓ habituation to startle (rat) ↓ TMT-induced hyperactivity (rat)	Engers et al. *J Med Chem Letters*. 2017

Note: Findings are from mouse studies unless otherwise specified

Abbreviations: EPM, elevated pus maze; FST, forced swim test; TMT, trimethylthiazoline predator odor; TST, tail suspension test