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REVIEW

$mGlu_5$: A double-edged sword for aversive learning related therapeutics

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Abstract

Aversive memories underlie many types of anxiety disorders. One area of research to more effectively treat anxiety disorders has therefore been identifying pharmacological targets to affect memory processes. Among these targets, the metabotropic glutamate 5 receptor ($mGlu_5$) has received attention due to the availability of drugs to utilize its role in learning and memory. In this review, we highlight preclinical studies examining the role of $mGlu_5$ at various stages of aversive learning and its inhibition via extinction in order to gain a better understanding of its therapeutic potential. We suggest that $mGlu_5$ has distinct roles at different stages of memory that not only makes it a tricky target, but a double-edged sword as a therapeutic. However, the selective involvement of $mGlu_5$ in different memory stages allows for certain precision that could be harnessed clinically. We therefore suggest potential applications, limitations, and pitfalls when considering use of mGlu5 modulators as therapeutics. In addition, we recommend future studies to address important gaps in this literature, such as sex and age factors in light of anxiety disorders being more prevalent in those demographics.

Key words: $mGlu_5$; Learning and Memory; Aversive Learning; Fear Conditioning; Neuropharmacology

1. Introduction

Learning and memory are crucial for survival. In particular, aversive learning allows prevention and avoidance of detrimental outcomes (e.g., injury, predator etc.) [1]. When expressed pervasively, however, the memory of an aversive event can lead to symptoms such as heightened fear, avoidance, etc. that can interfere with necessary activities, resulting in anxiety disorders. Anxiety disorders are highly prevalent and are among the biggest causes of health burden worldwide [2]. Yet, current therapeutics often face issues with efficacy and relapse [3–6]. This has led researchers to seek novel pharmacotherapies to affect aversive memory to treat anxiety disorders [7–9].

1.1. Aversive learning and memory

Aversive memory is widely studied in the laboratory through Pavlovian conditioning paradigm, in which an initially neutral conditioned stimulus (CS) is paired with an intrinsically aversive unconditioned stimulus (US). The CS is typically a discrete cue such as a tone or a light. Additionally, the context in which the US takes place can serve as a type of CS that can be associated with the US. After such pairings, presentation of the CS by itself can elicit a range of behaviors related to the US, such as defensive action associated with fear (e.g., immobility) [10]. Notably, Pavlovian conditioning is the process whereby the occurrence of either the CS or the US is not necessarily dependent on the behavior of the animal [11].

In contrast, instrumental conditioning refers to the learning of an action-outcome relationship that requires the animal to perform a specific behavior for the US to occur [12]. In aversive instrumental learning, an animal may move away from its current location to escape discomfort or pain [13]. Although rarely treated as such, the Morris water maze is an example of aversive instrumental learning [14]. It involves an animal swimming to find a submerged platform using distal and/or proximal spatial cues to escape the water. Alternatively, it could involve an animal avoiding a context or even some flavors. For example, in passive or in-

hibitory avoidance tasks, an animal is conditioned to avoid certain areas of a maze after being exposed to an aversive stimulus there (e.g. footshock, cat urine, etc.) [15, 16]. Similarly in conditioned taste aversion tasks, animals are conditioned to avoid and/or show disgust to a flavor (usually done through injection of LiCl to cause toxicosis in the lab [17]. Importantly, Pavlovian conditioning can be incorporated into instrumental conditioning as powerful mediators of behavior [18]. Specifically, an instrumental response can be followed by both the CS and the US. Subsequently, CS can initiate the instrumental response by itself [11, 19, 20]. Behaviors arising from Pavlovian and/or instrumental conditioning are referred to as conditioned responses (CRs).

Aversive memories can amplify the excessive worry/stress in anxiety disorders [21]. Consistent with this idea, one treatment approach that has received significant attention in the last two decades is exposure therapy, which often forms a part of cognitive behavior therapy [7, 8, 22–27]. It typically involves repeatedly exposing a patient to the stimulus that elicits fear in the absence of any danger. Exposure therapy is based on the process of extinction, which is the decrease in CR following presentations of the CS without the US. In instrumental learning, the CR can also be extinguished when the US no longer follows the CR. Furthermore, repeated presentations of the CS alone without explicit extinction training of the action-outcome contingency can also significantly reduce instrumental CR [19, 28] demonstrating the potency of the CS in influencing action-outcome behaviors.

Extinction is readily observed across species, and due to its high clinical relevance, extinction is the most commonly utilized paradigm to study how the expression of an aversive memory can be reduced [21, 22, 29–31]. The decrease in CR is due to the animal learning that the CR or the CS no longer predicts the US. The dominant theory is that extinction involves acquisition of CS-no US and/or CR-no US memory that inhibits the original aversive memory [21, 29], although there is also evidence for erasure of aversive memory due to extinction [4, 32–34].

Another commonly studied memory process in the context of anxiety disorders is reconsolidation, for which a previous consolidated memory destabilizes and becomes labile through its reactivation/recall [35, 36] Once recalled, the previously consolidated memory requires reconsolidation in order to stabilize again, failing which, the memory would not be retained [37, 38]. This reconsolidation window therefore allows consolidated memories to be reinforced or altered, making it an attractive target for therapeutics aiming to alter memories. A focus of contemporary research has been to target receptors involved in acquisition, extinction, or reconsolidation of aversive memories with the aim to either reduce initial threat learning or facilitate extinction to ultimately promote adaptive behaviors in people with anxiety disorders.

1.2 Glutamate and metabotropic glutamate 5 receptors

The widely accepted putative neural mechanism for learning and memory is synaptic plasticity, which refers to when the strength of synaptic transmission is either upregulated or downregulated [39, 40]. Hebb [41, pp. xix, 335-xix, 335] was the first to describe a process in which synaptic changes are observed when either a cell excites another cell repeatedly or is consistently involved in its excitability. This process causes synaptic changes so that the first cell's efficiency in activating the second cell is increased. At present, the most studied form of synaptic plasticity is long-term potentiation (LTP) that is found in the hippocampus, prefrontal cortex, and the amygdala [42, 43], neural structures critical for aversive learning and memory [44–48]. LTP is a long term enhancement in synaptic excitability resulting from coincident activity of pre- and post-synaptic elements [49] and is a putative mechanism for learning and memory [50].

Glutamate plays an important role in LTP [51]. L-glutamate is the major excitatory neurotransmitter in the central nervous system. It acts on ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). iGluRs are ligand gated channels, namely N-methyl-D-aspartate (NMDA) receptors, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate receptors [52]. Typically, excitatory transmission happens when glutamate is released into the synapse and acts on AMPA receptors, causing an influx of depolarizing ions. This depolarization can then activate NMDA receptors, which function as coincidence detectors that are critical for LTP as well as learning [53]. For example, antagonism of NMDA receptors, can block LTP in the hippocampus in vivo [54]. Correspondingly, animals administered with the NMDA antagonists (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid or MK-801 show impaired acquisition of spatial learning [55, 56].

Modulating NMDA receptors directly can however be tricky as NMDA antagonist and agonists are highly associated with cell toxicity [57, 58], while the efficacy of safer partial agonists like d-cycloserine has not been supported [59]. Overall, this creates a need for other targets affecting learning and memory. mGluRs presents a way to modulate NMDA transmission in a more controlled manner – a fine tuning mechanism of sorts. The metabotropic glutamate 5 receptor ($mGlu_5$), in particular, has received significant attention as a potential therapeutic target. Compared to other mGluRs, $mGlu_5$ is highly expressed in the amygdala [60, 61], a central structure for fear learning and memory. Furthermore, compared to other mGluRs, $mGlu_5$ is densely expressed in the cortical brain regions in the first 3 weeks of development [60, 62, 63], which highlights its potential involvement in emotional learning and memory involved in anxiety disorders which typically starts in childhood/adolescence [64]. $mGlu_5$ is a seven transmembrane G protein-coupled receptor belonging to Group I mGluRs predominantly expressed postsynaptically (typically extrasynaptic). When activated by the neurotransmitter glutamate, they cause a cascade of chemical changes with leads to an influx of Ca²⁺ via the inositol 1,4,5-triphosphate and diacyl-glycerol pathway, which cause further downstream effects [65, 66]. Importantly, $mGlu_5$ is co-localized and interacts with N-methyl-D-aspartate (NMDA) receptors [67]. For example, low concentrations of NMDA are able to reverse desensitization of signaling caused by phosphorylation of specific serine/threonine molecules in $mGlu_5$, whilst high NMDA concentrations can inhibit $mGlu_5$ [68]. Further, $mGlu_5$ positive allosteric modulators (PAM) are also able to potentiate the activation of NMDA receptor activation and reverse inhibition by the NMDA antagonist D-APV [69].

Such relationship between NMDA receptors and $mGlu_5$ is believed to affect long-term potentiation (LTP). For example, Lu *et al.* [70] was the first to show that $mGlu_5$ knockout (KO) mutant mice had reduced LTP in CA1 and DG region of the dorsal hippocampus. Early phase LTP seems to be dependent on NMDA receptors and not on $mGlu_5$ [71]. This is further evidenced by Gerstein *et al.* [118], who showed that late- but not early-phase LTP is impaired in hippocampal slices of mice lacking Homer1c (a scaffolding protein associated with $mGlu_5$). We do note that the purpose of the review is not to compare and contrast NMDA vs $mGlu_5$ but to understand the role of $mGlu_5$ in regard to behavior. In addition, $mGlu_5$ on its own has been shown to be necessary for LTP [72]. Notably, $mGlu_5$ signaling has also been shown to be crucial for NMDA-independent long-term depression (LTD) and depotentiation [73], which are also synaptic plasticity mechanisms involved in extinction more than conditioning [74–77]. Therefore, $mGlu_5$ may particularly be suited to modulating extinction processes that occur in exposure therapy.

In addition, $mGlu_5$ are densely expressed in structures important for learning and memory such as the hippocampus, amygdala, striatum and nucleus accumbens [78, 79]. Considering that reduction of memory expression following extinction involves the formation of a new inhibitory memory, $mGlu_5$ manipulation to

reduce or enhance emotional memory is in fact a "double-edged sword". That is, attempts to enhance extinction may enhance conditioning processes, whereas disrupting conditioning may also disrupt extinction processes. A clear understanding of the role of $mGlu_5$ signaling in conditioning and extinction is necessary to exploit $mGlu_5$ as a therapeutic target. In this mini-review, we describe and assess the role of $mGlu_5$ in the various stages of acquisition and extinction of aversive memories based on extensive rodent literature, in order to gain a better understanding of how to target memory processes using $mGlu_5$ modulators as potential therapy for anxiety disorders.

2 Metabotropic glutamate 5 receptor in acquisition and maintenance of aversive memories

2.1 Acquisition

The role of $mGlu_5$ in learning and memory has been demonstrated firstly using the Morris water maze [70]. While this task is not typically studied in the context of aversive learning, it requires the animal's motivation to escape an aversive situation using spatial memory. Systemic injections of $mGlu_5$ PAMs CDPPB (10 mg/kg) or ADX47273 (10 mg/kg) once before each day of Morris water maze trials enhanced the acquisition of learning in mice, as indicated by fewer number of days to reach criterion [80]. Although all mice were trained to criterion, drug-free probe trial showed that mice previously injected with CDPPB or ADX47273 spent more time in the target quadrant compared to the vehicle group [80], which highlights that the effects of $mGlu_5$ PAMs on acquisition of learning is long-lasting and may indicate stronger memory overall (Table 1).

While such study using PAMs suggests that $mGlu_5$ signaling is *sufficient* to acquire aversion-motivated spatial memory, whether $mGlu_5$ signaling is *necessary* for acquisition of spatial memory is less clear. Ballard *et al.* [81] showed that intraperitoneal (i.p.) injection of $mGlu_5$ negative allosteric modulator (NAM) MPEP (3, 10 and 30 mg/kg) in rats once before each day of Morris water maze trials had no effects on acquisition (Table 2). Car *et al.* [82] also showed in rats that MPEP (1 mg/kg) administered intravenously once before each day of Morris water maze trials had no effects compared to vehicle injections on acquisition. This discrepancy between the effects of PAMs vs. NAMs may be related to limitations with pharmacological approaches, including how allosteric modulators allow continued orthosteric binding of glutamate. On the other hand, $mGlu_5$ KO mice implicate the function of $mGlu_5$ at a global and chronic level. Indeed, $mGlu_5$ KO mice are significantly impaired in acquisition of Morris water maze task compared to wildtype mice [70, 83]. A limitation in interpreting such finding is that germline KO mice may experience developmental differences/compensation compared to their wildtype littermates. In addition, while these studies highlight the hippocampus as the likely locus of $mGlu_5$ effects, deletion of $mGlu_5$ is not anatomically specific in germline KO mice. Tan *et al.*[84] addressed some of these limitations by knocking down $mGlu_5$ selectively in the dorsal hippocampus (dHPC) during adulthood using $mGlu_5$ floxed mice. Significant acquisition deficits in the Morris water maze were observed in that study, providing causal evidence for hippocampus $mGlu_5$ involvement in the acquisition of aversion-motivated spatial learning.

Consistent with findings using Morris water maze, systemic injection of the $mGlu_5$ NAM MPEP (0.3-30 mg/kg) before fear conditioning has been shown to dose-dependently block acquisition of conditioned fear-potentiated startle to a light CS in rats [85]. Similarly, systemic injection of MTEP (3-30 mg/kg), a $mGlu_5$ NAM with ten-fold greater selectivity than MPEP, prior to fear conditioning also dose-dependently blocked acquisition of conditioned fear to both context and tone CS in mice [86]. MTEP also impaired acquistion in a passive avoidance task and conditioned fear potentiated startle [87]. Although Lu *et al.* [70] showed no difference in post-shock freezing between $mGlu_5$ KO and wildtype mice following a single tone-footshock pairing, with multiple tone-footshock pairings, Xu *et al.* [88] showed impaired post-shock freezing in $mGlu_5$ KO mice to both tone and context. In terms of studies examining positive modulation of $mGlu_5$, the $mGlu_5$ PAM CDPPB had no effects when administed pre-training for a single-trial step-down inhibitory avaoidance learning task and conditioned taste aversion [89]. Taken together, while these findings generally highlight that $mGlu_5$ is important for the acquistion of aversive learning, more work is needed to understand the nuances of their effects in different tasks, and the difference between positive and negative modulation.

2.2 Consolidation and retrieval

While $mGlu_5$ plays a major role in acquisition of aversive learning, it does not appear to be necessary for the consolidation of aversive memory. Administration of the $mGlu_5$ agonist CHPG or NAM MPEP immediately following fear conditioning did not produce any effects [90]. Similarly, MTEP given post-conditioning did not affect conditioned fear to context nor tone CS at test [86]. The lack of involvement of $mGlu_5$ in consolidation of contextual fear memory is surprising given the critical role of $mGlu_5$ in hippocampal LTD [91], which has been shown to be important for consolidation of spatial memory [92]. More work, especially using the selective NAM MTEP following Morris water maze training, would be helpful to delineate whether $mGlu_5$ is involved in consolidation of spatial memory.

Retrieval of Morris water maze memory does not appear to require $mGlu_5$. Following Morris water maze training, Lu *et al.* [70] showed that $mGlu_5$ KO mice were impaired in probe trial suggesting a possible impairment in retrieval of memory. However, Xu *et al.* [88] showed no effect of genotype during the probe trial. It is likely that the impairment seen in Lu *et al.* [70] is due to the pre-existing differences in acquisition. Specifically, $mGlu_5$ KO mice had significantly higher latency to platform at last acquistion trial in Lu *et al.* [70] , whereas there were no genotype differences by the end of acquisition in Xu *et al.* [88]. Similarly, Tan *et al.* [84] noted no effect of dHPC specific $mGlu_5$ knockdown during probe trial of Morris water maze. One study did report that 30 mg/kg of MPEP, but not 3 nor 10 mg/kg, given prior to probe trial had a small but statistically significant reduction in proportional distance travelled in platform quadrant [81]. In that study, however, the platform location was cued and visible.

In retrieval of conditioned fear memory, $mGlu_5$ KO mice were impaired in freezing to the conditioned context but not to the tone [70, 88]. However, Xu *et al.* [88] suggested that this was an effect on acquisition rather than expression of once-memorized fear response, suggesting no effect on retrieval of memory. It is indeed difficult to assess retrieval effects using $mGlu_5$ KO mice following impairments in acquisition – genotype effects could be due to carry-over from differences at acquisition. Interestingly, 30 mg/kg MPEP, but not 0.3 or 3.0 mg/kg, administered 60 min before retrieval test reduced expression of conditioned fear measured by potentiated startle [85]. At this dose, the authors noted that MPEP may be having a broadly anxiolytic effect rather than affecting memory retrieval. It remains equivocal whether $mGlu_5$ is involved in retrieval of aversive memories.

3 Metabotropic glutamate 5 receptor in aversive memory extinction and reconsolidation

Adaptive learning and behavioral flexibility are highly important in response to an ever-changing environment. Importantly, it has

Table 1. Studies using positive allosteric modulators (PAM) or agonists of $mGlu_5$ cited in this paper

	Reference	Task	Drug	Route	Time Injected	Outcome
Acquisition	Ayala *et al.* 2009	MWM	CDPPB	Systemic (I.P.)	Pre-acquisition	Enhanced Acquisition
	Ayala *et al.* 2009	MWM	ADX47273	Systemic (I.P.)	Pre-acquisition	Enhanced Acquisition
	Fowler *et al.* 2011	Inhibitory Avoidance	CDPPB	Systemic (S.C.)	Pre-acquisition	No effect
	Fowler *et al.* 2011	Conditioned Taste Aversion	CDPPB	Systemic (S.C.)	Pre-acquisition	No effect
	Sethna *et al.* 2014	Contextual Fear Conditioning	CDPPB	Systemic (I.P.)	Pre-acquisition	Enhanced Consolidation (no effect on Acquisition, Increase freezing during test)
Consolidation & Retrieval	Maciejak *et al.* 2003	Contextual Fear Conditioning	CHPG	Hippocampus microinfusion	Post-acquisition	No effect
	Sethna *et al.* 2014	Contextual Fear Conditioning	CDPPB	Systemic (I.P.)	24h Post-acquisition/24h pre-test	No effect
Extinction & Reconsolidation	Xu *et al.* 2013	Context and Tone Fear Conditioning	ADX47273	Systemic (I.P.)	Pre-extinction	No effect
	Sethna *et al.* 2014	Contextual Fear Conditioning	CDPPB	Systemic I.P.)	Pre-extinction	Enhanced extinction acquisition
	Ganella *et al.* 2016	Tone Fear Conditioning	CDPPB	Systemic (S.C.)	Pre-extinction	Enhanced consolidation of extinction at P17 but not P24 or adult
	Xu *et al.* 2013	Contextual Fear Conditioning	ADX47273	Systemic (I.P.)	Multi Session Pre-extinction	No effect
	Xu *et al.* 2013	Contextual Fear Conditioning	ADX47273	Systemic (I.P.)	Multi Session Post-extinction	No effect
	Xu *et al.* 2013	Tone Fear Conditioning	ADX47273	Systemic (I.P.)	retrieval–reconsolidation window (30-45min after single tone retrieval)	No difference in extinction day, lowered freezing day after; no effect on spontaneous recovery; lowered freezing in renewal

implications on treatment of pervasively expressed memory disorders – the ability to respond differently to cues with established associations is crucial to the success of treatment. This can be modelled through extinction and reconsolidation.

3.1 Extinction

Similar to conditioning, extinction is largely a new memory that involves acquisition, consolidation, and retrieval [9], which strongly suggests that the role of $mGlu_5$ signaling in acquisition of conditioning may also apply for acquisition of extinction. Indeed, Sethna & Wang [93] showed that pre-extinction systemic injection of $mGlu_5$ PAM CDPPB facilitated acquisition of extinction, and further significantly reduced freezing at test the next day. This suggests either an effect on acquisition of extinction alone, or an effect on both acquisition and consolidation of extinction that resulted in reduced freezing. In contrast, an i.p. injection of $mGlu_5$ NAM MPEP before extinction did not affect extinction acquisition but significantly increased freezing at test the next day [94]. This effect was replicated when MPEP was injected into the infralimbic cortex (IL), a part of the prefrontal cortex that is critical for consolidation of extinction [47, 94, 95]. Those results suggest MPEP effects on extinction consolidation rather than acquisition.

$mGlu_5$ KO mice also showed impairments in between-session extinction to context and cue [88], suggesting $mGlu_5$'s role in extinction consolidation. In contrast, $mGlu_5$ PAM ADX47273 systemically injected prior to either context or tone extinction session had no effects during extinction or test, though the lack of ADX47273 effects may be due to insufficient dosing, or due to a floor effect with the vehicle group freezing very low at test [95]. Interestingly, the role of $mGlu_5$ on extinction consolidation may be age-dependent. CDPPB or MTEP injection before extinction facilitated or impaired consolidation of extinction, respectively, in postnatal day 17 (P17) juvenile rats without affecting P24 or adult rats [96]. The authors proposed that their findings were due to rodent $mGlu_5$ having an unusual neurodevelopmental profile compared to other mGluRs, with a high expression at birth that steadily decreases from 3rd week into adulthood [78].

3.2 Reconsolidation

A relatively modern approach to "remove" aversive memories has been to target reconsolidation [97–101]. This works on the basis that memories become labile following reactivation – referred to as a reconsolidation window [37]. Therefore, the short reconsolidation period following reactivation may be vulnerable to therapeutics to disrupt aversive memory. For example, Monfils *et al.* [99] showed that extinction 10 min or 1 hr following fear memory reactivation (by a single CS presentation) significantly reduced spontaneous recovery of fear compared to extinction that was not followed by memory reactivation.

Xu *et al.* [95] aimed to test whether $mGlu_5$ signaling plays a

Table 2. Studies using negative allosteric modulators (NAM) of $mGlu_5$ cited in this paper

	Reference	Task	Drug	Route	Time Injected	Outcome
Acquisition	Schulz *et al.* 2001	Fear Conditioning	MPEP	Systemic (I.P.)	Pre-acquisition	Impaired acquisition
	Ballard *et al.* 2005	MWM	MPEP	Systemic (I.P.)	Pre-acquisition	No difference in Acquisition nor probe
	Gravius *et al.* 2005	Passive Avoidance Response	MTEP	Systemic (I.P.)	Pre-acquisition	Impaired acquisition
	Gravius *et al.* 2005	Conditioned Fear Potentiated Startle	MTEP	Systemic (I.P.)	Pre-acquisition	Impaired acquisition
	Car *et al.* 2007	MWM	MPEP	Systemic (I.V.)	Pre-acquisition	No difference in Acquisition, nor probe
	Handford *et al.* 2014	Fear Conditioning	MTEP	Systemic (I.P.)	Pre-acquisition	Impaired acquisition
Consolidation & Retrieval	Handford *et al.* 2014	Fear Conditioning	MTEP	Systemic (I.P.)	Post-acquisition	No effect
	Maciejak *et al.* 2003	Contextual Fear Conditioning	MPEP	Hippocampus microinfusion	Post-acquisition	No effect
	Gravius *et al.* 2005	Passive Avoidance Response	MTEP	Systemic (I.P.)	Post-acquisition	No effect
Extinction & Reconsolidation	Fontanez-Nuin *et al.* 2011	Tone Fear Conditioning	MPEP	Systemic (I.P.)	Pre-extinction	Normal extinction, impaired recall 24h later
	Fontanez-Nuin *et al.* 2011	Tone Fear Conditioning	MPEP	IL microinfusion	Pre-extinction	Normal extinction, impaired recall 24h later
	Ganella *et al.* 2016	Tone Fear Conditioning	MPEP	Systemic (S.C.)	Pre-extinction	Impaired consolidation of extinction at P17 but not P24 or adult

role in reconsolidation. Mice first received three tone-footshock pairings. The next day, mice received a single but prolonged tone CS trial, to which they showed high levels of freezing indicating memory reactivation. Within 45 minutes of this reactivation trial, mice were given either the $mGlu_5$ PAM ADX47273 or vehicle, and then received CS extinction. ADX47273 showed no effects during extinction. At test the next day, however, ADX47273 group showed reduced freezing compared to vehicle group. The authors suggested that increased $mGlu_5$ signaling during the reconsolidation window disrupted the original memory. However, it appears that $mGlu_5$ PAM simply facilitated CS extinction because a critical control group (i.e., extinction without reactivation) was missing. It may well be the case that PAM facilitated CS extinction even without any reactivation. Hence this finding may not be indicative of affecting any reconsolidation process. Future studies can utilize $mGlu_5$ PAM or NAM following reactivation without any extinction, to really determine whether $mGlu_5$ is involved in reconsolidation. Specifically, if $mGlu_5$ signaling is necessary and/or sufficient for reconsolidation, then NAM will disrupt CS memory and/or PAM will enhance CS memory when given following reactivation.

Overall, $mGlu_5$ appears to have distinct roles in acquisition and inhibition of aversive memories. While there still are inconsistencies between studies, the overall conclusion, taking into considerations limitations of each study, is that $mGlu_5$ is both sufficient and necessary for acquisition but not consolidation of aversive memories. While $mGlu_5$ does not seem to play a role in retrieval of aversive memories, studies examining this are limited, and more work would be necessary to rule out $mGlu_5$'s role in aversive memory retrieval. Importantly, $mGlu_5$ appears to play a role in acquisition *and* consolidation of extinction memory, which has major implications in the modulation of $mGlu_5$ as a pharmacotherapeutic target – a topic we will cover in the next section.

4. Metabotropic glutamate 5 receptor as a potential pharmacotherapy

Learning and memory clearly involve $mGlu_5$ signaling, highlighting its powerful potential as a target for anxiety disorder therapeutics. Yet its distinct roles at different stages of memory make it not only a tricky target, but a double-edged sword as a therapeutic. For acquisition of aversive learning, $mGlu_5$ signaling is likely necessary and sufficient (Table 1, 2). Consolidation of conditioned fear or Morris water maze learning appears $mGlu_5$ independent. Retrieval also is unlikely to involve $mGlu_5$ signaling, with studies attributing any effects to anxiolysis or pre-existing differences in acquisition. Therapeutically, $mGlu_5$ not being involved in consolidation of fear memory allows for certain precision – there is then reduced concern of increasing consolidation of a previous stressful or traumatic event. This, however, also means that $mGlu_5$ antagonist are probably not the most useful therapeutics for lowering the impact of recent traumatic memories.

Extinction is $mGlu_5$-dependent, with more evidence for its sufficiency/necessity during consolidation than acquisition (Table 1, 2). Together with the fact that $mGlu_5$ is unlikely to affect retrieval of memory, increasing $mGlu_5$ signaling using PAMs may be an exciting psychological adjunct to strengthen exposure therapy. Whether taken during or post-therapy, it would not unnecessarily increase the recall of aversive memory, which is a perceived risk by clinicians during exposure therapies [102]. However, strong conclusions cannot be drawn without assessing $mGlu_5$'s role in extinction recall. Exposure therapy typically require repeat sessions, and it would be important to first understand how $mGlu_5$ agents may affect extinction recall in subsequent sessions. It would also be a risk in case of new trauma, with the effects of $mGlu_5$ agonism showing to enhance aversive memory acquisition.

The use of $mGlu_5$ PAM during retrieval-reconsolidation window to disrupt the memory process is also an interesting avenue to explore, however, the study on $mGlu_5$ modulation during reconsolidation is too limited. Furthermore, techniques that manipu-

late memories during the retrieval-reconsolidation window work within a narrow window of time [103] and if not handled properly, could lead to exacerbation of the problem (multiple consolidations would serve to reinforce rather than extinguish the original memory [119]). These issues will only increase with the addition of pharmacotherapeutics like $mGlu_5$ modulators. Overall, better understanding of $mGlu_5$ modulators on reconsolidation is needed to ascertain the efficacy of such an intervention.

In summary, $mGlu_5$ is both sufficient and necessary for acquisition but not consolidation of aversive memories. This indicates that giving an antagonist to disrupt the initial aversive memory would be impractical because consolidation does not require $mGlu_5$ signaling. $mGlu_5$ does not seem to play a role in retrieval. $mGlu_5$ appears to play a role in acquisition and consolidation of extinction memory. Therefore, the potentially most efficacious way of applying $mGlu_5$ modulator to alter traumatic memory processes would be to use an agonist before or after acute or chronic exposure therapy.

We do also note that while learnt fear is a well-established model to study processes underlying the treatment of anxiety disorders [9], it by no means fully capture all aspects of anxiety disorders [104]. While beyond the scope of this review, it would be important to consider other $mGlu_5$ studies that assess state or trait anxiety (e.g., elevated plus maze) following stress that may provide additional information relevant towards anxiety disorders [105–107].

5. Conclusions

Future efforts with development of $mGlu_5$ modulators as a therapeutic of aversive learning/memory-based disorders should aim to accurately ascertain effects of $mGlu_5$ PAMs and NAMs on different stages of aversive learning. In particular, the relationship between $mGlu_5$ signaling and extinction retrieval needs more attention. Further complicating the matters, $mGlu_5$ signaling in extinction retrieval has been thoroughly assessed with preclinical studies modelling substance use disorders, with NAMs (rather than PAMs) being promoted because they reduce reinstatement of drug-seeking in rodents [108]. It would be important to determine whether $mGlu_5$'s role in extinction recall is dissociated between aversive vs appetitive memories, given the co-morbidity of anxiety and substance use disorders [109]. Further work examining sex difference should also be considered. Sex-specific $mGlu_5$ expression is unknown [110], with studies examining $mGlu_5$ expression in the brain only using female rats [61–63], or not specific on sex [60]. These studies showed highest $mGlu_5$ expression in the first 3 weeks of postnatal life. Consistent with this observation, highest efficacy in $mGlu_5$ positive or negative allosteric modulation on extinction was observed in P17 male rats relative to older male rats [96], suggesting that the developmental profile of $mGlu_5$ expression in males may be similar to females. Nevertheless, the possibility of differential rate of decline in $mGlu_5$ expression across maturation in males versus females remains.

Lastly, it is striking that every $mGlu_5$ behavioral study described in this review has used males, despite the higher prevalence of anxiety disorders in females over males [111, 112]. In addition, all but one study used adult rodents, when 75% of all anxiety disorders are diagnosed by adolescence [64]. There is clear evidence of age-specific sex differences in aversive learning and memory [113–117]. Given $mGlu_5$'s consistent role in extinction, we hope future research to highlight potential age- and sex-specific mechanisms of how mGluR5 signaling impacts extinction learning and recall, which are cognitive processes critical for treatment of anxiety disorders.

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Editorial Checks

- Plagiarism: Plagiarism detection software found no evidence of plagiarism.
- References: Zotero did not identify any references in the RetractionWatch database.

Peer Review

The review process for this paper was conducted double-blind because one of the authors is a member of the committee of management of the publisher, Episteme Health Inc. During review, neither the authors nor the reviewers were aware of each other's identities.

For the benefit of readers, reviewers are asked to write a public summary of their review to highlight the key strengths and weaknesses of the paper. Signing of reviews is optional.

Reviewer 1 (Anonymous)

he authors discuss a role for $mGlu_5$ in learning and memory processes. The review outlines much of this work, stating that $mGlu_5$ modulators should be used as a therapeutic took to reduced maladaptive responding from aversive learning. However, there are portions of the review that are unclear and topics that should be introduced prior to their discussion.

Reviewer 2 (Anonymous)

This review on the "double-edged sword" of targeting the $mGlu_5$ receptor for anxiety disorders is interesting and well written. However, I feel that this review could be improved further if the researchers touched on more naturalistic models of anxiety. I think it is important that the authors put their work into a larger context outside of fear conditioning, particularly if the focus is on the clinical potential of $mGlu_5$.

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