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Metabotropic glutamate receptor subtype 5 antagonism in learning and memory

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Summary

The role of the metabotropic glutamate receptor 5 (mGlu₅ receptor) in learning and memory and other behaviors are reviewed by examining the influence of selective antagonists and genetic knockout on performance. This receptor is involved in spatial learning, contextual fear conditioning, inhibitory avoidance, fear potentiated startle, and conditioned taste aversion. However, mGlu₅ receptor antagonists have proven to be ineffective in other learning tasks, such as the delayed-match-to-position test and a three-hole spatial learning task. Locomotion is often decreased by mGlu₅ receptor antagonists; and other behaviors such as social interaction and consummatory responses can also be affected. In mGlu₅ receptor knockout mice, performance in contextual fear conditioning and spatial water maze tasks is impaired. Although the available evidence is suggestive of an important contribution of mGlu₅ receptors to cognitive functions, further studies are needed, particularly those with in vivo evaluation of the role of mGlu₅ receptors in selective brain regions in different stages of memory formation.

Keywords

metabotropic glutamate receptor 5; spatial learning; fear conditioning; avoidance learning; MPEP; MTEP; rat; mouse; locomotion

1. Introduction

Metabotropic glutamate receptor 5 (mGlu₅ receptor), a subtype in the group I mGlu receptors, was cloned in 1992 (Abe et al., 1992) and, like the other mGlu receptor subtypes, it is a member of the family 3/C G-protein coupled receptors. The structure and the function of mGlu₅ receptors have been reviewed in several articles (see Conn et al., 2005; Hermans and Challis, 2001; Pin and Acher, 2002; Spooren et al., 2003). In this introduction, we will briefly describe some basic features of mGlu₅ receptors. Their roles in learning and memory processes will be reviewed in detail.

The mGlu₅ receptor has a large N-terminal domain, which contains the glutamate binding side, seven transmembrane-spanning regions that are connected by three extracellular and

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three intracellular loops, a cysteine-rich domain and an intracellular C-terminal domain (Hermans and Challis, 2001). The receptor is a covalently bound homodimer and interacts with a variety of cytoskeletal, scaffolding and signaling proteins (Fagni et al., 2004; Ferraguti and Shigemoto, 2006; Romano et al., 1996b). Although mGlu₅ receptor activation primarily couples to the stimulation of phospholipase C, which leads to the release of calcium from intracellular sources and protein kinase C activation, the signaling cascades of the mGlu₅ receptor are much more complex. Multiple mGlu₅ receptor-associated signaling pathways, both G-protein-dependent and -independent, have been identified (Gerber et al., 2006; Hermans and Challis, 2001). Some of these have been linked to mGlu₅ receptor-dependent forms of synaptic plasticity (Simonyi et al., 2005). However, their contribution to distinct memory stages (acquisition, consolidation, retrieval, extinction, reconsolidation) in different brain regions has yet to be elucidated. In this review, we will summarize psychopharmacological studies using mGlu₅ receptor antagonists and knockout animals, and, in doing so, summarize the presently available data on the role of mGlu₅ receptors in learning and memory.

2. Distribution of mGlu₅ receptors in the central nervous system

mGlu₅ receptors are expressed in two splice variants (mGlu_{5a} and mGlu_{5b}). They differ by a 32-amino acid insert in the intracellular C-terminal domain of mGlu_{5b} receptor (Minakami et al., 1995). The two splice variants are co-expressed in most brain areas, but the mGlu_{5b} receptor predominates in the adult brain (Joly et al., 1995; Romano et al., 1996a). mGlu₅ receptors are mainly localized in postsynaptic elements, and highly targeted to perisynaptic regions (Lujan et al., 1997; Shigemoto et al., 1997). In addition to neurons, mGlu₅ receptors are also found in astrocytes and microglia (Biber et al., 1999; Ciccarelli et al., 1997). In situ hybridization and immunohistochemical studies have demonstrated that mGlu₅ receptors are widely distributed in brain regions implicated in learning and memory such as the cerebral cortex, hippocampus, amygdala, cortical areas (subiculum, entorhinal, cingulate and piriform cortices) and basal ganglia (striatum, nucleus accumbens). Some regions, such as the septum and olfactory bulb, show intense labeling for mGlu₅ receptors; while others, such as brainstem, hypothalamus and cerebellum exhibit low staining (Shigemoto and Mizuno, 2000). mGlu₅ receptors are also expressed in the thalamus and spinal cord (see Ferraguti and Shigemoto, 2006 for a review).

3. mGlu₅ receptor antagonists

The first selective antagonists of mGlu₅ receptors were 6-methyl-2-(phenylazo)-pyridinol (SIB-1757) and (E)-2-methyl-6-(phenylethenyl)pyridine (SIB-1893) (Varney et al., 1999). Their discovery led to the development of the systemically active, non-competitive antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) (Gasparini et al., 1999). The IC₅₀ value of MPEP is 36 nM. Intraperitoneal injection of a 10 mg/kg dose can reach 100% receptor occupancy in an hour; then returns to the baseline over a 4-h period (Anderson et al., 2003). However, in mice, the decline is much faster than in rats (Anderson et al., 2003). Oral administrations have shown a dose linearity and the brain concentration was 1.67 ng/g after a 10 mg/kg dose (Ballard et al., 2005). Although MPEP shows excellent selectivity for mGlu₅ receptors over all other mGlu receptors, it has a weak positive allosteric action at the mGlu₄ receptor (Mathiesen et al., 2003). At high concentrations (above 10 μ M), it blocks NMDA receptors and is an inhibitor of the noradrenaline transporter with an IC₅₀ of 2.8 μ M (Heidbreder et al., 2003; O'Leary et al., 2000).

3-[2-methyl-1,3-thiazol-4yl]ethynyl]pyridine (MTEP) is a highly selective, potent non-competitive mGlu₅ receptor antagonist lacking the off-target effects of MPEP (Cosford et al., 2003). It readily enters the brain and has an IC₅₀ value of 5 nM in a calcium-flux assay.

Full receptor occupancy was achieved with a dose of 10 mg/kg (i.p., one h after injection) in rats, and 30 mg/kg (s.c., 20 min after injection) in mice (Busse et al., 2004). After a dose of 5 mg/kg (i.p.), MTEP concentration can reach a level of 5000 nM in the rat brain (Loscher et al., 2006).

Fenobam ([N-(3-chlorophenyl)-N'-(4,5-dihydro-1-methyl-4-oxo-1H-imidazole-2-yl)urea]) was originally a non-benzodiazepine anxiolytic compound with an unknown molecular target (Porter et al., 2005). Fenobam is a potent, selective, non-competitive mGlu₅ receptor antagonist with an inverse agonist activity (Porter et al., 2005). Fenobam has lower affinity to the mGlu₅ receptor than MPEP but it is a similarly selective antagonist with an IC₅₀ value of 58 nM and it acts via the same allosteric binding site as MPEP and MTEP (Malherbe et al., 2006). Oral administration of 30 mg/kg to rats resulted in a brain concentration of 600 nM in 40 min (Jacob et al., 2009). These mGlu₅ receptor antagonists are intensively metabolized so drug discovery research has been expanded for potent, metabolically stable and safe antagonists. Although several other mGlu₅ receptor antagonists have been identified (see Gasparini et al., 2008 and Jaeschke et al., 2008 for recent reviews), they have not been tested in animal models of learning and memory.

4. Studies with mGlu₅ receptor antagonists and knockout mice

4.1. mGlu₅ receptors in spatial learning

Spatial learning requires memory processing in the hippocampus, a brain region with high levels of mGlu₅ receptors. The first evidence for the role of mGlu₅ receptors in spatial learning comes from studies with mGlu₅ receptor knockout animals using the Morris water maze (Lu et al., 1997). In this task, knockout mice showed impeded acquisition and poor long-term retention. Similar results were found recently with a newly generated null mutant (Xu et al., 2009). However, mGlu₅ receptor antagonism has produced other results on this task. Using a fixed location, the hidden platform version of the Morris water maze, MPEP (up to 30 mg/kg, p.o.) did not affect the distance travelled to find the submerged platform throughout 8 training days (Ballard et al., 2005). Twenty-four hours after the 8th day of training even the 30 mg/kg condition still showed significant preference for the platform quadrant – although this preference was significantly smaller than in the control group of rats – but barely, thereby suggesting a small effect of MPEP (Ballard et al., 2005). Relatively small effects were seen in the water maze when MPEP was injected using mice (Steckler et al., 2006). Only the highest dose tested (10 mg/kg, s.c.) impaired acquisition during training but a decrease in locomotor activity was also found on the probe trial (Steckler et al., 2006). In a recent study, fenobam (30 mg/kg, p.o.) impaired acquisition in the Morris water maze but did not affect retention (Jacob et al., 2009).

In the radial arm maze, reference memory (memory for information that does not change from session to session) has been impaired by 5 mg/kg (i.p.) MTEP (Gravius et al., 2008); and rats trained in the 8-arm radial maze and injected in the lateral cerebral ventricle with MPEP (1.8 µg) have shown attenuation of both reference and working memory (memory for information that is specifically relevant to the current trial) (Naie and Manahan-Vaughan, 2004). A subsequent study showed differences between results derived from two rat strains (Manahan-Vaughan and Braunewell, 2005). Wistar rats showed greater between-session acquisition of reference memory information than Hooded Lister rats. However, daily intracerebral MPEP reduced working and reference memory in Wistar rats, while Lister rats showed MPEP-resistance with respect to reference memory – although MPEP did impair working memory (Manahan-Vaughan and Braunewell, 2005). In the 4-arm radial maze (non-elevated cross maze) i.p. injections of 5- or 10 mg/kg and prelimbic injections of 5- or 10 µg per side MPEP did produce significantly impaired appetitive spatial conditioning in rats (Christoffersen et al., 2008). The same two treatments also inhibited spatial working

memory accuracy in the cross maze (*ibid.*) and 10 mg/kg MPEP (i.p.) showed a similar effect in the earlier work by Homayoun and colleagues (Homayoun et al., 2004). In a Y-maze alternation test, i.c.v. injections (13.8 µg) of MPEP significantly impaired 24-h retention when injected 30 min before training; but they had no effect when administered immediately after the training session (Balschun and Wetzel, 2002). However, i.c.v. injections of the mGlu₅ receptor positive allosteric modulator, DFB (3,3'-difluorobenzaldazine), immediately after Y-maze spatial alternation training, did have a significant promnesic effect 24 h later (Balschun et al., 2006). In an object-place association task, MPEP at high doses (6, 12, 24 mg/kg, i.p.) impaired visuo-spatial discrimination in mice; but lower doses (1.5, 3 mg/kg, i.p.) had no effect (De Leonibus et al., 2009). These results indicate an involvement of mGlu₅ receptors in both spatial conditioning and spatial working/reference memory: mGlu₅ receptor antagonists can impair spatial learning and memory.

In contrast, several investigations reported no effect of mGlu₅ receptor antagonism on spatial learning tasks. The first was an appetitive spatial conditioning task: a three-hole task in which the choice of a correct hole was rewarded by juice (Petersen et al., 2002). Here, neither i.p. nor i.v. applications of MPEP (up to 10 mg/kg) affected long-term acquisition of associative information that is used across many trials. In a delayed non-match-to-position radial maze task, MPEP (up to 10 mg/kg, i.p.) had no effect (Campbell et al., 2004). MPEP (up to 30 mg/kg, p.o.) has also failed to affect choice accuracy in a delayed-match-to-position lever pressing task in most conditions – only 100 mg/kg induced a reduction of working memory accuracy at the longest delays (24 sec) (Ballard et al., 2005). The 5-choice serial reaction time task has shown that MPEP (maximum of 9 mg/kg, i.p.) had no significant effect on choice accuracy (Semenova and Markou, 2007), but did affect other measures of memory performance at the highest dose. At the moment it is not possible to provide firm conclusions regarding the conflicting findings of mGlu₅ receptor antagonists on spatial learning (no effect versus disruption). Therefore, the effects of selective blockade of mGlu₅ receptors in spatial memory await further investigation.

4.2. mGlu₅ receptors in fear conditioning

The hippocampus and amygdala are both involved in contextual fear conditioning whereas only the amygdala is involved in cue conditioning (Kim and Fanselow, 1992; Philips and LeDoux, 1992); and different neural mechanisms underlie contextual and discrete cue conditioning (Philips and LeDoux, 1992; Rodrigues et al., 2004). As with spatial learning, the first evidence for the role of mGlu₅ receptors in fear conditioning stems from work using mGlu₅ receptor knockout mice. Lu et al. (1997) administered a single conditioning trial in which a salient tone (43 dB intensity above a 52 dB background) was paired with a 2 sec, 0.75 mA footshock. A test occurred 24 h later in which a 5-min context fear test was given first, and then the next day the tone was tested using a single 3-min tone exposure. The knockout mice showed less fear conditioning to the context — especially during the first two min of the test. There was no attenuation in cue conditioning to the tone for the knockout mice. The poor context fear, and a lack of difference for tone conditioning, by the knockout mice suggests hippocampal involvement of mGlu₅ receptors in such fear conditioning. Xu et al. (2009) used a newly generated mGlu₅ receptor knockout mouse when examining fear conditioning to a tone and also assessed contextual fear. Several complications (e.g., differences between knockout and control mice in postshock freezing and differences in pretone freezing responses) make interpretation of these results difficult; and group differences on initial days in the experiment make it hard to draw conclusions about later phases of the study. However, the authors reported that, like Lu et al. (1997), mice lacking mGlu₅ receptor showed less fear to the contextual cues on a test given 24 h after conditioning. Yet, there were no differences in fear to the tone on test presentations in a

separate (test) context when the test was given 48 h after tone-footshock pairings. The results are consistent with the conclusion (and with much of the evidence described later in this section) that the mGlu₅ receptor is important for fear conditioning to contextual clues.

Rodrigues et al. (2002) was the first to study the role of the amygdalar mGlu₅ receptors in fear conditioning. They found that 0.15 µg and 1.5 µg MPEP administered into each lateral amygdala 20–30 min prior to fear conditioning (5 pairings of a tone with a 0.5 sec, 0.5 mA footshock) disrupted performance when tested 1 h after training (short-term retention), and when tested 24 h after training (long-term retention). Fear conditioning to a discrete cue and contextual fear conditioning was disrupted. MPEP administered immediately after training or prior to testing had no effect on short-term memory or on performance 24 h after training for cue or for contextual fear conditioning. The authors concluded that the activation of amygdalar mGlu₅ receptors is required for acquisition of fear conditioning. Maciejak et al. (2003) gave contextual fear conditioning such that rats received three footshocks (0.7 mA for —150/300 ms) in a single session, and administered MPEP (2.5 or 51 nM) into the dentate gyrus post-training (3 min after the last shock). They found no effect on contextual fear conditioning when measured 24 h later during a 10-min test. These results suggest that activation of mGlu₅ receptors is needed at the time of the fear conditioning trials. Indeed, Jacob et al. (2009) administered fenobam (10, 30 and 100 mg/kg, p.o.) 60 min before the conditioning session and found that all three doses reduced contextual fear conditioning. Similarly, Gravius et al. (2008) found that 5 mg/kg of MTEP (i.p.) administered 30 min before conditioning produced an attenuation of context fear conditioning. They also used a sub-chronic MTEP pretreatment and found that its effects on memory as well as its anxiolytic effects do not show any evidence of tolerance within the range of treatment given in their study (see also Pilc et al., 2002).

Gravius et al. (2006b) administered MTEP (1.25, 2.5, 5 mg/kg, i.p.) 30 min prior to tone conditioning (four presentations of 30-sec, 18-dB above background tone followed by a one sec, 1 mA scrambled footshock). Testing of the tone occurred 24 h later in a context other than where conditioning occurred. The authors found no effect of MTEP on conditioning to the tone. However, MTEP at the two higher doses (2.5 and 5 mg/kg) given 60 min before the test resulted in reduced fear to the tone at the time of fear expression. Context conditioning (MTEP doses were 0.625, 1.25, 2.5, 5, 10 mg/kg) involved 3 presentations of a 1 sec, 0.45 mA footshock and testing occurred 24 h later. MTEP at doses of 2.5 mg/kg and greater all resulted in less fear to contextual cues when administered prior to conditioning. However, the two largest doses (5 and 10 mg/kg) produced a preshock freezing response, thereby making interpretation of the conditioning effect as a result of these doses less clear. Three doses of MTEP (0.3, 1 and 3 mg/kg) were applied 60 min before the context fear assessment. The highest dose of MTEP caused reduced freezing at test to the contextual cues revealing that there is an effect of the drug at the time of behavioral expression.

Further evidence of mGlu₅ receptor involvement in fear conditioning comes from studies using a fear-potentiated startle procedure. Fear-potentiated startle is a classical conditioning procedure in which a conditioned stimulus (e.g., a light) is paired with an aversive event (typically footshock). Then a target stimulus (a noise) is presented and the startle response to this noise is measured in the presence or the absence of the conditioned stimulus (the light). Schulz et al. (2001) used systemic administration of MPEP (0.3, 3.0, or 30 mg/kg, p.o.) 60 min before light-shock conditioning (10 trials in which a light was paired with a 0.6 mA, 0.5 sec footshock). Other rats received MPEP just before testing or before training and testing. MPEP reduced fear-potentiated startle (i.e., light-shock conditioning was likely disrupted) when it was administered at doses of 3 or 30 mg/kg before conditioning. The highest dose also reduced fear-potentiated startle when given before testing. A state dependency effect

was ruled out in that the group given 3 mg/kg at both training and testing showed attenuated fear-potentiated startle despite a lack of a possible state change.

Fendt and Schmid (2002) infused MPEP into the lateral nucleus of the amygdala and examined its effects on fear-potentiated startle two days after conditioning. MPEP administered before conditioning (ten pairings of a light with a 0.5 sec, 0.6 mA footshock) attenuated fear-potentiated startle. MPEP administered after conditioning or prior to testing had no effect, thereby revealing no influence on consolidation or expression. Gravius et al. (2005) administered MTEP (1.25, 2.5, and 5 mg/kg, i.p.) 30 min prior to conditioning in which 15 pairings of an 8-W light with a 0.5 sec, 0.6 mA scrambled footshock were given. The two highest doses reduced fear-potentiated startle when the rats were tested 24 h later.

Many researchers have applied MPEP or MTEP at the time of testing for fear conditioning and fear-potentiated startle; the results show a strong claim (cf. Fendt and Schmid, 2002) that such drugs attenuate fear at test (Brodkin et al., 2002; Busse et al., 2004; Gravius et al., 2006b; Schulz et al., 2001). A few of these studies (Fendt and Schmid, 2002; Gravius et al., 2006b) have already been briefly mentioned above. The anxiolytic effects of MTEP and MPEP are well-known (see Palucha and Pilc, 2007 for a current review) and such results can be explained in these terms. Pietraszek et al. (2005b) administered MTEP (0.6, 1.25, 2.5, 5.0 mg/kg, i.p.) 30 min prior to a fear-potentiated startle test trial and found a decrease in startle at the two highest doses. Their effects of MTEP at test on contextual fear conditioning were more surprising in that the dose (among those listed immediately above) that caused the greatest attenuation of freezing was the moderate dose (1.25 mg/kg), producing a U-shaped curve for the range of doses used.

In summary, mGlu₅ receptors are involved in contextual fear conditioning in that mGlu₅ receptor antagonists disrupt such learning. The data are more equivocal with regard to the role of these receptors in fear conditioning of a discrete conditioned stimulus. However, we know that fear conditioning *does* cause an upregulation of mGlu₅ receptor protein levels in the hippocampus (Riedel et al., 2000). MGLu₅ receptor antagonists, when they do attenuate fear conditioning, are only effective when applied before conditioning, rather than after the conditioning session. Also, mGlu₅ receptors, although their role in fear conditioning using an auditory or visual cue per se is not clear, do exert an influence on fear-potentiated startle, which is a procedure that involves administering the drug at the time of conditioned stimulus-unconditioned stimulus pairings (thereby looking at the effect of the drug on such conditioning); but – rather than testing on the conditioned stimulus directly as with fear conditioning – the conditioned stimulus is used to influence responding to a target startle stimulus. Hence, both tasks are classical conditioning procedures using a discrete cue, so the disparate results are presently inexplicable (see Gravius et al., 2006b for a discussion), and future research is needed to explore the difference between these two tasks.

4.3. mGlu₅ receptors in avoidance learning

Inhibitory or passive avoidance learning (actually, a punishment procedure) is a hippocampus-dependent associative learning task which is a widely used model to study memory processes (Gold, 1986; Izquierdo and McGaugh, 2000). In rodents, two forms of inhibitory avoidance are frequently used: the step-down and the step-through avoidance tasks. In the step-down inhibitory avoidance, the animal is placed on a platform and receives a shock when it steps off the platform. Memory for the shock is measured as an increased latency to step off the platform on subsequent trials. In the step-through inhibitory avoidance, animals learn to avoid an otherwise preferred dark compartment because of an aversive experience (mild footshock) in that part of the chamber. Memory is tested by measuring the latency to enter the dark compartment. In both avoidance tasks, one or a few

trials can induce memory lasting for a long period of time, allowing good stimulus control, and permitting the separation of drug-induced effects on different memory stages.

Several studies have provided evidence that mGlu₅ receptors are involved in inhibitory avoidance (Genkova-Papazova et al., 2007; Gravius et al., 2005; Jacob et al., 2009; Simonyi et al., 2005, 2007). MTEP (2.5, 5, or 10 mg/kg, i.p.) was given prior to the training trial using a step-through avoidance procedure and the highest dose was found to impair long-term retention measured 24 h later (Gravius et al., 2005). Post-training and pre-test administration of MTEP (10 mg/kg, i.p.) did not influence performance (Gravius et al., 2005). Similarly, fenobam (10, 30, or 100 mg/kg, p.o.) administered 60 min before training caused a dose-dependent decrease in latencies (i.e., poorer performance) to enter the dark compartment 24 h later (Jacob et al., 2009). Using a single-trial step-down inhibitory avoidance procedure, MPEP (3 or 10 mg/kg, i.p.) given before training significantly reduced latencies at the higher dose 24 h after training without influencing step-down latencies during training (Simonyi et al., 2005). Another study investigated the effects of MPEP administered to the dorsal hippocampus on the consolidation and extinction of memory for inhibitory avoidance learning. MPEP (1.5 or 5.0 µg/side) or saline were infused bilaterally into the CA1 region immediately after training or immediately after the first retention test (Simonyi et al., 2007). Rats receiving MPEP infusion after training exhibited a dose-dependent decrease in retention when tested 24 h later indicating the importance of mGlu₅ receptors in long-term memory consolidation. MPEP injected after an initial extinction trial which also served as a retention test (24 h after training) had no effect on subsequent extinction test trials (Simonyi et al., 2007). In a multiple-trial step-down avoidance task, both pre- and post-training administration of MPEP (5 or 10 mg/kg, i.p.) resulted in decreased retention 24 h and 7 days later (Genkova-Papazova et al., 2007). It is important to mention that a shock titration experiment showed that mGlu₅ receptor antagonism has no effect on responsivity to electric foot shocks (Gravius et al., 2006a).

Inhibitory avoidance has also been used with 1-day-old chicks (Matsushima et al., 2003). Chicks explore their environment by pecking and rapidly learn to distinguish between edible and distasteful objects. If a chick is presented with a bead coated with a bitter-tasting substance such as methylanthranilate, it will peck once, show a characteristic disgust response, and subsequently avoid a similar but dry bead presented later (Gibbs and Ng, 1977). Injection of MPEP (4.5 µg/side) into the intermediate medial mesopallium either before or after training reduced retention 24 h later (Salinska et al., 2006). When chicks were given a reminder (a dry bead) 2 h after the initial training and injected with MPEP immediately after the reminder, a decrease in avoidance was found up to 4 h later; but there were no significant differences between MPEP and control groups 24 h later. These results show that mGlu₅ receptors play an important role in both memory consolidation and reconsolidation (Salinska, 2006).

Active avoidance is an associative learning task in which the animal learns to avoid a foot shock upon the presentation of a specific stimulus by moving to a different compartment of the chamber. Memory is assessed by measuring the number of successful avoidance responses and response latencies. When rats were trained in a single session (50 trials) with the combined presentation of a tone and light, MPEP injection (5 or 10 mg/kg, i.p.) either before or after training had no effect on the number of avoidance responses tested 7 days later (Genkova-Papazova et al., 2007).

4.4. mGlu₅ receptors in taste memories

Conditioned taste aversion is a form of aversive classical conditioning in which a taste or flavored substance (the conditioned stimulus) is paired with a drug or experience that produces internal malaise (the unconditioned stimulus), and this pairing results in the

conditioned response — the subjects avoid the substance on a test trial (Bures et al., 1998). Conditioned taste aversion is subserved by specific brain regions including the insular cortex and the amygdala although their precise role in such learning is still unclear (Lamprecht and Dudai, 2000; Reilly and Schachtman, 2009; Yamamoto et al., 1994). A study using systemic administration of MPEP (6 or 12 mg/kg, i.p.) before conditioning demonstrated that activation of mGlu₅ receptors is required for taste aversion learning (Schachtman et al., 2003). MPEP attenuated learning following a saccharin-LiCl pairing resulting in a weak conditioned response but had no effect when injected before test (Bills et al., 2005; Schachtman et al., 2003). mGlu₅ receptors also exert an important role in latent inhibition using a conditioned taste aversion procedure. Latent inhibition is a phenomenon by which pre-exposure of a conditioned stimulus prior to the conditioned stimulus-unconditioned stimulus pairings retards conditioned responding as a result of those pairings. MPEP (3, 6 or 12 mg/kg, i.p.) administration before taste pre-exposure caused a disruption in latent inhibition in a dose-dependent manner (Bills et al., 2005). A recent study examined the role of mGlu₅ receptors in the encoding of the taste memory by using microinjection of MTEP into the insular cortex and basolateral amygdala in rats (Simonyi et al., 2009). MTEP (5.0 µg/side) injection into the insular cortex had no effect on taste aversion learning, although it can influence saccharin intake per se. MTEP injection into the basolateral amygdala resulted in normal conditioned taste aversion on the initial test trial, but slowed extinction; that is, MTEP enhanced conditioned taste aversion since strong associations are slow to extinguish. These results indicate that mGlu₅ receptors are involved in taste memories in a region-specific manner (Simonyi et al., 2009).

4.5. mGlu₅ receptors in other learning tasks

Effects of MPEP (up to 10 mg/kg, i.p.) have been investigated in the object recognition task. The first investigation reported no effect of MPEP given before training — neither on object retention after a 15 min retention interval nor a 24 h retention interval (Barker et al., 2006). However, a later study (Christoffersen et al., 2008) found that retention 5 min after training was inhibited by pretraining i.p. injections (5 or 10 mg/kg). Methodological differences, including experimental procedure, animal strain and time of day (dark versus light cycle), may have had a role in the different results. The later study also found that MPEP impaired object exploration which may have influenced object discrimination. However, prelimbic applications of MPEP (5 or 10 µg per side) before training impaired retention comparably to i.p. administrations without any significant effects on exploration during sampling (Christoffersen et al., 2008). The study by Barker and colleagues (2006) also showed that MPEP given together with a group II mGlu receptor antagonist impaired object discrimination (familiar versus novel) after a long-term delay, and this effect was mediated by the perirhinal cortex.

In a color — key response association task, MPEP (1–10 mg/kg, i.p.) failed to affect both within-session and between-session learning in rats (Campbell et al., 2004). In an appetitive light-nosepoke association task for rats, acquisition between daily training sessions was significantly impaired by 10 mg/kg MPEP (but not by 3 mg/kg) after i.p. injections (Homayoun et al., 2004).

4.6. mGlu₅ receptors in social interaction

Koros et al. (2006) found a MTEP-induced (3 and 10 but not 1 mg/kg, i.p.) reduction in social interaction in male rats. The effects of MTEP were assessed after sub-chronic administration (5 daily injections) of such doses or after a single, acute injection prior to testing; and both regimens produced the same effect on social interaction. Social interaction was defined as the duration with a maximal distance of 20 cm between the rats and included behaviors like sniffing, following or grooming the other rat during the 10-min test in a novel

arena. Navarro et al. (2006) administered MPEP (5–25 mg/kg doses, i.p.) to male mice 30 min before a social interaction test, and found a decrease in time spent engaging in threat and attack behaviors at all doses. Social investigatory behaviors (e.g., following, crawl near, sniffing) were increased by all doses. Of course, these two measures are related in that if an animal engages less in one category (threat and attack) it will have more time to engage in other behaviors.

4.7. mGlu₅ receptors in locomotor activity

A large amount of research has been devoted to examining the role of mGlu₅ receptors on locomotion and exploration. Studies have reported either no effect of MPEP or MTEP on locomotion (Belozertseva et al., 2007; Henry et al., 2002; Homayoun et al., 2004; Kinney et al., 2003; Li et al., 2006; Naie and Manahan-Vaughan, 2004; Tatarczynska et al., 2001) or a decrease in locomotion produced by these antagonists (Christoffersen et al., 2008; Koros et al., 2007; Nicolas et al., 2007; Pietraszek et al., 2005a; Spooren et al., 2000a, b; Varty et al., 2005; Zhu et al., 2004). Jacob et al. (2009) examined the effects of fenobam in rats and found that the drug (10, 30, and 100 mg/kg, p.o.) given 60 min before the assessment did not affect horizontal locomotion, but rearing behavior was reduced at all three doses tested.

Exceptions to the usual decrease in exploration or no effect by these antagonists should be noted. Navarro et al. (2006) obtained an increase in exploration in mice by all doses of MPEP used (5, 10, 15, 20, and 25 mg/kg, i.p.), and their assessment of non-social exploration and social investigation (in which both categories resulted in a MPEP-induced increase) included many different behaviors. Similarly, McGeehan et al. (2004) showed an increase in locomotor activity in mice at MPEP doses of 5 and 20 mg/kg (i.p.). Montana et al. (2009) found that 30 mg/kg (i.p.) of fenobam given to mice 30 min prior to the assessment produced an increase in locomotion (distance travelled). In summary, as mentioned, many findings reveal, if anything, a decrease in locomotion produced by mGlu₅ receptor antagonists, but some increases in movement have been found.

Christoffersen et al. (2008) reported that MPEP (e.g., with i.p. administration) may have its greatest effect when locomotion is tested immediately after introduction into a new environment. Similar observation was made after p.o. treatment with MTEP (Nicolas et al., 2007). Christoffersen et al. (2008) found that MPEP given i.p. to rats in doses of 5 and 10 mg/kg significantly inhibits the initial surge of exploratory locomotion that appears immediately after such placement. This early exploratory locomotion was reduced both in the open field test and in the cross-maze - in fact, within the first 5 min in the open field, locomotion was reduced by 74% at 10 mg/kg compared to the control group. Christoffersen et al. (2008) suggested that exploration might be reduced by mGlu₅ receptor antagonism through impaired working memory function (memory for previously-visited locations), and emphasized the importance of distinguishing between locomotor effects observed before and after familiarization with novel stimuli.

4.8. Non-mnemonic side effects

Tests of drugs that affect memory are usually supplemented with investigations of side-effects that might affect performance in learning tasks without interfering directly with memory processes. Such side-effects could involve impaired motor or sensory capacities, altered levels of wakefulness or attention, or changed emotional states. Tests used to assess side-effects may include: rotarod, elevated plus maze, shock sensitivity, locomotor activity in an open field and/or in mazes, and swim speed in a water-maze. We included a description of some of the side effects of mGlu₅ receptor antagonism in other sections of this review; therefore, here, we are going to briefly mention some other important findings.

Motor functions—See Section 4.7. for studies examining locomotor activity. Experiments using rotarod have found that MPEP (doses up to 300 mg/kg, p.o.) and MTEP (5 mg/kg, i.p.) had no significant effect on rats (Spooren et al., 2000a). However, at a dose of 30 mg/kg and above, MPEP and MTEP given i.p. impaired rotarod performance (Varty et al., 2005).

Anxiety and stress—For studies on anxiety and stress, see Palucha and Pilc (2007) and Durand et al. (2008) for reviews. Due to the anxiolytic effects of mGlu₅ receptor antagonists, it seems likely that in learning tasks involving aversive unconditioned stimuli, the anxiety-inducing effects of such stimuli may have been ameliorated by the presence of the antagonist during training. This could, in turn, impair subsequent processing and account for at least part of the performance impairments. Nevertheless, based on the large variety of studies included in this review, it seems clear that mGlu₅ receptor antagonism can also directly interfere with learning processes.

Hypothermia—MPEP and MTEP (10 mg/kg, i.p.) can significantly lower the body temperature of rats (Varty et al., 2005) and this hypothermia might influence activity levels.

Consumption—mGlu₅ receptor antagonism can affect the response to reward in appetitive tasks. mGlu₅ receptors modulate central reward pathways; and accordingly, intake of juice reward in a cross-maze was reduced by MPEP (Christoffersen et al., 2008). Similarly, MPEP was found to reduce water and flavored solution consumption (Bills et al., 2005). Intake of food by mice and rats has been reduced by MTEP (up to 10 mg/kg, s.c.) and MPEP (9 mg/kg, i.p.) (Bradbury et al., 2005; Semenova and Markou, 2007). Reduced consumption of appetitive rewards (used as unconditioned stimuli in some learning tasks) may have indirectly influenced mGlu₅ receptor antagonist-induced memory impairments in appetitive learning tasks.

5. Concluding remarks

In the past several years, significant progress in behavioral pharmacology of mGlu₅ receptor has been made. Data suggest that the involvement of mGlu₅ receptor in learning and memory is task specific (Table 1). The mGlu₅ receptor has a pivotal role in aversive learning and it is especially important for memory acquisition in avoidance and fear learning tasks. However, there is also some suggestion of a role of the mGlu₅ receptor in the consolidation and expression of fear memories. In other learning tasks, selective blockade of the mGlu₅ receptor have usually produced an impairment in performance when given pre-training. It remains to be determined, however, whether and how the mGlu₅ receptor influences other memory phases such as extinction and/or reconsolidation.

Studies consistently revealed that the mGlu₅ receptor participates in long-term memory formation. Hippocampal mGlu₅ receptor has a unique role in memory processing as suggested by studies showing the modification of function and/or expression of mGlu₅ receptor after fear conditioning, eye-blink conditioning and maze learning. In addition, mGlu₅ receptors in the lateral amygdala make an important contribution to fear conditioning and conditioned taste aversion. Yet, further studies are necessary to elucidate the involvement of mGlu₅ receptors in selective brain regions in different stages of memory formation using a variety of learning tasks. Recent years have seen the emergence of new, selective and potent antagonists, agonists and positive allosteric modulators of mGlu₅ receptors. The next few years will certainly permit the extensive characterization of these drugs in behavioral models in order to better understand the distinctive role of mGlu₅ receptor in cognitive processes.

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Table 1Effects of mGlu₅ receptor antagonists on learning and memory and behavior

Taks	Species	Antagonists, infusion route and time	Effect	References
Spatial learning				
Morris water maze	LH rat	MPEP; p.o.; pre-training	no effect ^a	Ballard et al., 2005
	SD rat	Fenobam; p.o.; pre-training	impairment	Jacob et al., 2009
	C57 mouse	MPEP; s.c.; pre-training	impairment	Steckler et al., 2006
Radial arm maze	SD rat	MTEP; i.p.; pre-training	impairment	Gravius et al., 2008
	W rat	MPEP; i.c.v.; pre-training	impairment	Naie and Manahan-Vaughan, 2004
DMTP Radial arm maze	LE rat	MPEP; i.p.; pre-training	no effect	Campbell et al., 2004
DMTP Lever press	LH rat	MPEP; p.o.; pre-training	no effect ^a	Ballard et al., 2005
Cross maze	SD rat	MPEP; i.p.; PL; pre-training	impairment	Christoffersen et al., 2008
		MPEP; i.p.; pre-training	impairment	Homayoun et al., 2004
Y maze	W rat	MPEP; i.c.v.; pre-training	impairment	Balschun and Wetzel, 2002
		post-training	no effect	
3-hole task	PVG rat	MPEP; i.p.; pre-training	no effect	Petersen et al., 2002
5-choice serial RT task	W rat	MPEP; i.p.; pre-training	no effect ^a	Semanova and Markou, 2006
Object-place association	CD1 mouse	MPEP, i.p.; pre-training	impairment	De Leonibus et al., 2009
Fear conditioning				
Context	W rat	MPEP; DG; post-training	no effect	Maceijak et al., 2003
	SD rat	MTEP; i.p.; pre-training	impairment	Gravius et al., 2006b; 2008
		pre-test	impairment	Gravius et al., 2006b
				Pietraszek et al., 2005b
	SD rat	MPEP; LA; pre-training	impairment	Rodrigues et al., 2002
		post-training	no effect	
		pre-test	no effect	
		Fenobam; p.o.; pre-training	impairment	Jacob et al., 2009
Cue	SD rat	MTEP; i.p.; pre-training	no effect	Gravius et al., 2006b
		pre-test	impairment	
		MPEP; LA; pre-training	impairment	Rodrigues et al., 2002
		post-training	no effect	
Startle	W rat	pre-test	no effect	
		MPEP; i.p.; pre-test	impairment	Brodkin et al., 2002
		MTEP; i.p.; pre-test	impairment	Busse et al., 2004
	SD rat	MPEP; p.o.; pre-training	impairment	Schultz et al., 2001
		pre-test	impairment	
		pre-training+pre-test	impairment	
		MPEP; LA; pre-training	impairment	Fendt and Schmid, 2002
		post-training	no effect	
		pre-test	no effect	
		MTEP; i.p.; pre-training	impairment	Gravius et al., 2005
		MTEP; i.p.; pre-test	impairment	Pietraszek et al., 2005b

Taks	Species	Antagonists, infusion route and time	Effect	References
Avoidance learning				
Bead pecking	Chick	MPEP; IMM; pre-training	impairment	Salinska, 2006
		post-training	impairment	
Step-through	SD rat	MTEP; i.p.; pre-training	impairment	Gravius et al., 2005
		post-training	no effect	
		pre-test	no effect	
		Fenobam; i.p.; pre-training	impairment	Jacob et al., 2009
Step-down	SD rat	MPEP; i.p.; pre-training	impairment	Simonyi et al., 2005
		MPEP; CA1; post-training	impairment	Simonyi et al., 2007
		post-test	no effect	
	W rat	MPEP; i.p.; pre-training	impairment	Genkova-Papazova et al., 2007
		post-training	impairment	
Active avoidance	W rat	MPEP; i.p.; pre-training	no effect	Genkova-Papazova et al., 2007
		post-training	no effect	
Taste memories				
Conditioned taste aversion	SD rat	MPEP; i.p.; pre-training	impairment	Schachtman et al., 2003
		MPEP; i.p.; pre-test	no effect	Bills et al., 2005
		MTEP; IC; pre-training	no effect	Simonyi et al., 2009
		BLA; pre-training	enhancement	
Other learning tasks				
Object recognition	DA rat	MPEP; i.p., pre-training	no effect	Baker et al., 2006
	SD rat	MPEP; i.p.; PL; pre-training	impairment	Christoffersen et al., 2008
Color-key conditioning	LE rat	MPEP; i.p.; pre-training	no effect	Campbell et al., 2004
Light-nosepoke conditioning	SD rat	MPEP; i.p.; pre-training	impairment	Homayoun et al., 2004
Social interaction				
	OF1 mouse	MPEP; i.p.	increase	Navarro et al., 2006
	W rat	MTEP; i.p.	decrease	Koros et al., 2007
Locomotor activity				
	C57 mouse	MPEP, MTEP; i.p.	no effect	Belozertseva et al., 2007
	Swiss mouse	MPEP, MTEP; i.p.	no effect	Li et al., 2006
		Fenobam; i.p.	increase	Montana et al., 2009
	OF1 mouse	MPEP; i.p.	increase	Navarro et al., 2006
		MPEP; p.o.	decrease	Spooren et al., 2000b
	DBA mouse	MPEP; i.p.	increase	McGeehan et al., 2004
	W rat	MPEP; i.p.	no effect	Tatarczynska et al., 2001
		MPEP; p.o.	decrease	Spooren et al., 2000a
		MTEP; i.p.	decrease	Koros et al., 2007
		MPEP; i.c.v.	no effect	Naie and Manahan-Vaughan, 2004
	LH rat	MTEP; p.o.	decrease	Nicolas et al., 2007
	CD rat	MPEP, MTEP; i.p.	decrease	Varty et al., 2005
	SD rat	MPEP; i.p.	no effect	Henry et al., 2002
				Homayoun et al., 2004
				Kinney et al., 2003
			decrease	Christoffersen et al., 2008

Taks	Species	Antagonists, infusion route and time	Effect	References
		MPEP, MTEP; i.p.	decrease	Zhu et al., 2004
		MTEP; i.p.	no effect	Pietraszek et al., 2005a
		Fenobam; p.o.	no effect	Jacob et al., 2009

^a but see further discussion in the text

Abbreviations: DMTP, delayed-match-to-position; DNMTP, delayed non-match-to-position; RT, reaction time LE, Long-Evans; LH, Lister hooded; SD, Sprague-Dawley; W, Wistar BLA, basolateral amygdala; DG, dentate gyrus; IC, insular cortex; IMM, intermediate medial mesopallium; LA, lateral amygdala; PL, prelimbic cortex