EFFECTS OF A METABOTROPIC GLUTAMATE RECEPTOR 5 POSITIVE ALLOSTERIC MODULATOR, CDPPB, ON SPATIAL LEARNING IN RODENTS

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LIST OF ABBREVIATIONS

N-methyl-D-aspartate (NMDA)

α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)

kainic acid (KA)

NMDA receptors (NMDARs)

long term potentiation (LTP)

long term depression (LTD)

metabotropic glutamate receptors (mGlus)

G-protein coupled receptors (GPCRs)

phosphinositide (PI)

phospholipase C (PLC)

phosphatidylinositol 4,5-bisphosphate (PIP2)

messengers diacylglycerol (DAG)

protein kinase C (PKC)

inositol 1,4,5-trisphosphate (IP3)

calcium (Ca2+)

knockout (KO)

evoked postsynaptic potentials (EPSPs)

2-methyl-6-(phenylethynyle)pyridine (MPEP)
conditioned taste aversion (CTA)

Morris Water Maze (MWM)

positive allosteric modulator (PAM)

novel object recognition (NOR)

3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB)
ABSTRACT

Metabotropic glutamate receptor 5 (mGlu5) has been implicated in a variety of learning processes and is important for aversive learning tasks. The present studies used an mGlu5 receptor positive allosteric modulator, 2-cyano-N-(1,3 diphenyl-1H-hyrazol-5-yl)benzamide (CDPPB) to characterize the importance of mGlu5 receptors in aversively- and appetitively-motivated spatial learning. CDPPB, administered prior to 5 daily training sessions in the Barnes maze (Experiment 1) did not significantly enhance acquisition of the task. However, in a second experiment CDPPB (30 mg/kg) significantly enhanced performance compared to vehicle-treated controls during 3 days of reversal learning and had a significant effect on proportion search strategy used. Additionally, CDPPB (30 mg/kg), delivered 20 min prior to 5 daily training sessions (of Experiment 3) enhanced the delay rats were able to withstand in the appetitively-motivated delayed alternation version of the T-maze. The present results emphasize the role of mGlu5 receptors in spatial learning tasks, and demonstrate mGlu5 receptors are important for learning in appetitive, as well as aversive, tasks.
INTRODUCTION

Glutamate is the major excitatory neurotransmitter in the adult central nervous system and acts primarily on two different types of receptors: ionotropic and metabotropic glutamate receptors (Cleva & Olive, 2011). Ionotropic glutamate receptors (iGlus) are ligand-gated channels that open when glutamate is present to allow ions to directly enter the cell. Three distinct types of iGlus have been identified and are named based on the agonists that activate them: N-methyl-D-aspartate (NMDA) receptor, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainic acid (KA) receptor. iGlu receptors affect relatively fast changes in neurons and participate in the generation of excitatory postsynaptic potentials. NMDA receptors (NMDARs) are known to be critical for the formation of most types of long term potentiation (LTP) and long term depression (LTD), which are thought to be the cellular substrates for the formation of long term memories (Bliss & Collingridge, 1993).

The second type of glutamate receptors, metabotropic glutamate receptors (mGlus), are G-protein coupled receptors (GPCRs) that cause slower changes in the cell and provide a mechanism through which glutamate can modulate activity at the same synapses where fast transmission occurs (Hermans & Challiss, 2001). MGlus are linked to intracellular signaling cascades, which help modulate synaptic activity by influencing changes in the resting potential, threshold potential, and firing characteristics (Schoepp,
Metabotropic glutamate receptors are classified into three distinct groups (mGlus: group I, mGlu1 and mGlu5; group II, mGlu2 and mGlu3; group III, mGlu4, mGlu6, mGlu7 and mGlu8) based on sequence homology, signal transduction mechanism, and receptor pharmacology (Conn & Pin, 1997). Considerable differences exist between groups of mGlus, with only 35% sequence homology between receptors of different groups. The low sequence homology between mGlu groups makes these receptors attractive for targeted drug therapies because the differences allow for drug specificity (Nicoletti et al., 2011).

**mGlu5 and NMDA receptors have a functional interaction**

mGlu5 and NMDA receptors are physically linked through anchoring proteins (Fagni, Ango, Perroy, & Bockaert, 2004). Recent studies suggest that group I mGlu receptors bind directly with Homer, and Homer interacts with Shank-GKAP-PSD-95-NMDA receptor complex, strongly suggesting that mGlu5 and NMDA receptors functionally interact to during normal neuronal signaling (Ehlers, 1999; Naisbitt et al., 1999; Tu et al., 1999). In fact, mGlu5 and NMDA receptors were shown to have a reciprocal interaction in which potentiation of one receptor type acts to regulate activity of the other (Alagarsamy et al., 2002; Alagarsamy, Sorensen, & Conn, 2001; Koros, Rosenbrock, Birk, Weiss, & Sams-Dodd, 2007).

In another study, mGlu5 receptor agonists were shown to increase NMDA-receptor mediated responses in mouse cortical neurons (Attucci, Carla, Mannaioni, & Moroni, 2001). Additionally, NMDA has also been shown to enhance mGlu5 receptor
activity by reversing receptor desensitization through dephosphorylation at a specific serine/threonine site (Alagarsamy et al., 1999). mGlu5 receptors have been shown to modulate NMDA-dependent LTP, and were shown to be particularly important for the induction of LTP during the initial stages of memory formation, which suggests that normal functioning of both receptors is required for induction of hippocampal synaptic plasticity (Balschun et al., 1999; Collingridge & Bliss, 1995; Riedel, 1996; Riedel & Reymann, 1996).

**mGlu5 receptor function is necessary for learning and memory**

Group I mGlu receptor activation is primarily coupled to the phosphinositide (PI) second messenger pathway, although it has been linked to other second messenger pathways as well, depending on the brain area of interest and whether the receptor is located pre or post-synaptically (Ritzen, Mathiesen, & Thomsen, 2005). In the PI pathway, phospholipase C (PLC) hydrolyzes a phosphodiester bond in phosphatidylinositol 4,5-bisphosphate (PIP₂), leading to the formation of second messengers diacylglycerol (DAG), which can activate protein kinase C (PKC), and inositol 1,4,5-trisphosphate (IP₃), which stimulates release of calcium (Ca²⁺) from intracellular stores (Abe et al., 1992; Conn & Pin, 1997). Calcium is known to be important in the processes underlying associative learning and the induction of LTP (Lynch, 2004).

Group I mGlus, specifically mGlu5, are located in regions known to be important for learning and memory such as the hippocampus, striatum, frontal cortex, amygdala, and nucleus accumbens (Niswender & Conn, 2010). mGlu5 has been found to be critical
for strengthening of neural connections during associative learning tasks as well as the
induction of NMDA-dependent and NMDA-independent types of synaptic plasticity
(Ayala et al., 2009; Liu et al., 2008).

In vivo, mGlu5 receptor activity has been shown to be important for normal
induction of LTP and LTD and normal performance in a variety of tasks. MGlue receptors
knockout (KO) mice show decreased LTP induction in the CA1 and dentate gyrus regions
of the hippocampus and show greatly reduced evoked postsynaptic potentials (EPSPs),
which provides further evidence of the importance of mGlu5 in modulating NMDA
receptor-dependent LTP (Lu et al., 1997). Similarly, mGlu5 receptor activation has also
been shown to modulate LTD in the hippocampus (Naie & Manahan-Vaughan, 2005).
Furthermore, mGlu5 receptor activation has been shown to induce NMDAR-indensitive forms of LTP and LTD in the Schaffer Collateral-CA1 pyramidal cell synapse
in the hippocampus (Bellone, Luscher & Mameli, 2008; Kullman & Lamsa, 2008; Nicoletti
et al., 2011; Sourdet, Russier, Daoudal, Ankri & Debanne, 2003).

MGlue5 KO mice show deficits in a variety of tasks including Morris water maze,
fear conditioning, and inhibitory learning (Lu et al., 1997; Olsen, Childs, Stanwood, &
Winder, 2010; Xu, Zhu, Contractor, & Heinemann, 2009). In addition to knockout
studies, mGlu5 antagonists provide valuable insights into the importance of mGlu5
function in learning and memory. The mGlu5 receptor antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP) dose dependently impairs performance in operant
learning, spatial learning, and working memory (Homayoun & Moghaddam, 2010).
Administering MPEP to the medial prefrontal cortex of behaving rats was found to
impair burst activity in nearly half of pyramidal neurons, which provides evidence that
tonic mGlu5 activity is important for maintenance of proper neuronal firing rate and
pattern (Homayoun & Moghaddam, 2010). It is interesting to note that, in the
aforementioned study, MPEP preferentially inhibited neurons with higher baseline firing
rates, which the authors suggest is due to the increased constitutive activity of these
receptors, which makes them more susceptible to mGlu5 antagonism. In another study,
MPEP prior to tetanization in freely moving rats resulted in significantly earlier decay of
potentiation in the dentate gyrus and CA1 region of the hippocampus compared to
controls (Balschun & Wetzel, 2002). A review by Simonyi, Schachtman and
Christofferson (2010) describes findings that show treatment with MPEP reduced
evidence of LTP in rats examined 24 hours after tetanization, indicating mGlu5 function
is necessary for normal induction of LTP. MTEP, a more selective mGlu5 receptor
antagonist with fewer side effects than MPEP, administered prior to conditioning, was
found to impair performance in a fear potentiated startle experiment when rats were
tested 24 h later; and in a related study, MPEP, administered prior to training, dose-
dependently blocked the acquisition of fear potentiated startle (Gravius, Pietraszek,
Schafer, Schmidt, & Danysz, 2005; Schulz et al., 2001). Similarly, MPEP infused into the
basolateral amygdala impaired performance in auditory and contextual fear
conditioning tests, and MPEP infused into the lateral nucleus of the amygdala prior to
conditioning attenuated fear-potentiated startle (Fendt & Schmid, 2002; Rodrigues,
Bauer, Farb, Schafe, & LeDoux, 2002). In a recent fear conditioning experiment, mGlu5
receptors were hyper-expressed in the CA1 region of the hippocampus one day after
conditioning and in the CA3 region 10 days after conditioning, providing some evidence that mGlu5 activity might be differentially important for early vs. late LTP (for a review, see Simonyi et al., 2005).

In conditioned taste aversion (CTA), MPEP was shown to dose-dependently attenuate the formation of CTA without affecting saccharin consumption on the conditioning trial (Schachtman et al., 2003). Additionally, systemic MPEP administration prior to taste pre-exposure caused disruption of latent inhibition in a dose-dependent manner (Bills et al., 2005). In another aversively motivated task, inhibitory avoidance, post-training MPEP infusion into the hippocampus attenuated long term retention (Simonyi et al., 2007).

These studies indicate that proper mGlu5 function is important for learning in a wide variety of tasks; although some research indicates that mGlu5 may be more important for learning in aversively motivated tasks compared to appetitively motivated ones (Simonyi, Schachtman, & Christoffersen, 2005). Administering MPEP prior to training in an appetitive 3-choice task does not significantly affect performance (Petersen, Bomme, Baastrup, Kemp, & Christoffersen, 2002). Additionally, MPEP failed to impair performance in a delayed non-match-to-position radial maze task as well as a delayed-match-to-position lever pressing task (Ballard et al., 2005; Campbell et al., 2004). These tasks may lend credence to the idea that mGlu5 activity is essential for aversively-motivated learning, but less-so for appetitively motivated tasks (Petersen et al., 2002).
mGlu5 receptors are particularly important for hippocampal-dependent learning

It is well known that the hippocampus is critical for spatial learning and memory performance, and mGlu5 receptors have been shown to be particularly important in a variety of these tasks. They have been shown to help regulate LTP induction, maintenance and reference memory formation; and the extent of mGlu5 receptor expression has been shown to be a key determinant of spatial learning ability in rats (Manahan-Vaughan & Braunewell, 2005; Naie & Manahan-Vaughan, 2005a, 2005b). MPEP, administered prior to training, produces a significant decrease in retention of the difficult (but not easy) version of the shock-motivated Y-maze spatial alternation task (Balschun & Wetzel, 2002). In another hippocampal-dependent spatial task, mGlu5 KO mice displayed deficits in Morris Water Maze (MWM) acquisition compared to controls (Lu et al., 1997).

In addition to aversive tasks, several researchers have found mGlu5 receptor function is important for performance in appetitively-motivated tasks as well. MPEP, administered systemically as well as infused bilaterally into the prelimbic cortex, was also found to impair short-term memory in the cross maze and object discrimination tasks (Christoffersen et al., 2008). In that study MPEP (i.p.) impaired long-term retention in the cross maze and significantly reduced exploration, as well as impaired spontaneous alternation behavior during maze exploration. MGlu5 receptor function has been shown to be important for learning in other spatial tasks, such as the 4 and 8-arm radial mazes. MPEP infused into the lateral cerebral ventricle prior to training significantly attenuated working and reference memory in the 8-arm radial maze (Naie
& Manahan-Vaughan, 2004). In a related study, MTEP was found to impair reference memory in the same maze (Gravius et al., 2008).

Balschun, et al. (1999) stated that mGlu5 receptors may be particularly important for learning and memory when cellular resources (such as calcium) are limited during LTP induction and in instances of difficult learning tasks, including tasks that involve conflicting information. This assertion is supported by evidence showing mGlu5 activation resulted in an increase in intracellular calcium concentration in the hippocampus (likely a result of release from the endoplasmic reticulum) and also that mGlu5 activity contributes to persistent bursts of spiking, which are thought to participate in the maintenance of cognitive functions through prolonged excitatory activity (Homayoun & Moghaddam, 2010; Lee, Wong, Chuang, Shin, & Bianchi, 2002; Mannaioni, Marino, Valenti, Traynelis, & Conn, 2001). Further support is provided by evidence showing mGlu5 antagonists impair performance in a difficult version of the Y-maze, and that the mGlu5 positive allosteric modulator (PAM), DFB, increases performance in that version of that maze (Balschun & Wetzel, 2002; Balschun, Zuschratter, & Wetzel, 2006). Additionally, acquisition and reacquisition MWM learning is inhibited by mGlu5 antagonist administration (Steckler et al., 2005). The authors found performance in the high spatial-demand version of the maze was more affected by antagonist administration than was performance in the low spatial-demand version, providing additional support for the idea that mGlu5 receptors are particularly critical during difficult tasks or those involving conflicting information—such as reversal training. Additionally, Steckler et al. (2005) found evidence that group I receptor
antagonists impair acquisition of new information in the water maze, but may leave already-learned information relatively unaffected. MPEP, administered prior to the probe trial, only partially inhibited performance; whereas performance was significantly impaired when the drug was administered prior to training. Similarly, fenobam impaired acquisition in the MWM, but did not affect retrieval (Jacob et al., 2009). In a novel object recognition (NOR) test, rats administered MPEP prior to initial exposure to the objects explored both the novel and familiar object equal proportions of time, indicating a deficit in familiarity discrimination (Christoffersen et al., 2008). In a related study, MPEP and LY341495 (a group II antagonist), when administered together prior to object exposure, produced a profound deficit in familiarity discrimination at 24 h, but not 15 min, delay (Barker, Bashir, Brown, & Warburton, 2006). However, the group found that blocking only one group of these two receptor types did not affect performance in the task.

Additionally, activating mGlu5 receptors has been found to increase performance in difficult, but not easy, tasks. For example, mGlu5 PAMs were found to increase performance in a NOR task and decreased premature responding in the 5-choice serial reaction time test, as well as increase performance in the difficult version of an alternating Y-maze task (Balschun et al., 2006 (Balschun et al., 2006; Liu et al., 2008; Uslaner et al., 2009). Moreover, MPEP was found to block the induction of late LTP; and the mGlu5 PAM, ADX47273, was recently found to increase late (but not early) LTP formation in the hippocampus, indicating mGlu5 receptor activity is important for protein synthesis-dependent LTP (Francesconi, Cammalleri, & Sanna, 2004; Kroker, Rast,
& Rosenbrock, 2011). If mGlu5 receptors are differentially important for difficult vs. easy learning tasks, this might account for the discrepancy in the literature regarding effects of mGlu5 receptor agonists/antagonists in appetitively vs. aversively motivated tasks.

_Barnes maze assesses hippocampal-dependent spatial learning and memory in rodents_

The Barnes maze is a hippocampal-dependent spatial learning task that is primarily used to assess reference memory in rodents. The maze consists of an elevated circular platform with round holes cut around the perimeter. One of the holes, the “escape hole” contains a small black box which the animal can crawl into. The design takes advantage of a rodent’s natural preference for a dark environment, as it is assumed that preference will motivate the rodent to navigate the brightly lit maze, using visible spatial cues, and enter the escape box (Pompl, Mullan, Bjugstad, & Arendash, 1999).

The Barnes maze was originally designed to provide an alternative to the MWM and involves a circular maze which does not contain water or require the rodent to swim (Barnes, 1979). Thus, the Barnes maze also prevents excessive stress associated with food deprivation, which can be required for other appetitive tasks (Kennard & Woodruff-Pak, 2011). This procedure avoids potential experimental confounds associated with the MWM, such as differences in swimming ability (which is especially a concern with aged or disease-model animals) and excessive stress to the animal (Koopmans, Blokland, van Nieuwenhuijzen, & Prickaerts, 2003).
Performance in the Barnes maze is highly dependent on intact hippocampal functioning (Kennard & Woodruff-Pak, 2011). Animals with cognitive impairments, either due to normal aging or disease pathology, have been shown to exhibit impaired performance, indicated by increased error rate and latency to find the platform, compared to control animals (Barreto, Huang, & Giffard, 2010; Huang & Kandel, 1995; Pompl et al., 1999). Additionally, aged animals also show impairments in reversal learning compared to young controls (McLay, Freeman, Harlan, Kastin, & Zadina, 1999). Because the task has been shown to be sensitive to hippocampal dysfunction and because the paradigm frequently avoids ceiling effects in performance, it is expected to also be sensitive to improvements in performance which might be induced by cognitive enhancing drugs, such as CDPPB.

One issue that may arise with the use of the Barnes maze for mice is that mice tend to hesitate near the escape hole. Additionally, they may be more likely than rats to explore other holes instead of entering the target hole, even when the location of escape hole has been learned (Grootendorst, de Kloet, Vossen, Dalm, & Oitzl, 2001). This tendency produces inflated latency and errors that can mask true learning differences between groups. One strategy that has been adopted by many researchers is to calculate latency and error rate to the first encounter with the correct target hole, instead of using the total latency and errors until the animal actually enters the hole. This procedure seems to provide a better indication of when the animal has learned the task and can capture differences in groups not discernible by measuring total latency,
distance traveled and errors (Harrison, Reiserer, Tomarken, & McDonald, 2006; Patil, Sunyer, Hoger, & Lubec, 2009).

*T-Maze assesses hippocampal and PFC-dependent spatial learning and memory*

The T-maze is commonly used to assess hippocampal-dependent spatial learning and memory in rodents, and is particularly sensitive to cognitive dysfunction caused by disease pathology, pharmaceutical intervention or normal aging (Deacon & Rawlins, 2006). One advantage of the T-maze is that it allows researchers to assess both reference and working memory utilizing the same apparatus. Reference memory refers to information which is useful across all trials of a task, whereas working memory refers to information that changes across trials. Rats with hippocampal lesions still perform well on measures of reference memory in the T-maze, unless the task involves a strong spatial component (Deacon, Bannerman, & Rawlins, 2001). For this reason, most T-maze studies assess working memory, which requires normal hippocampal and frontal cortex functioning, instead of reference memory function. Spowart-Manning and van der Staay (2004) found that C57Bl/6 mice performed above chance on a continuous alternation version of the task, suggesting that the C57 background may be more suited for testing in the T-maze than HsdWin:CFW1 129S6 backgrounds, which performed at or below chance.

The T-maze is shaped like a “T” with a choice point at the top where animals can either enter the right or left goal arm. This protocol, which requires the use of working memory, capitalizes on an animal’s natural tendency to explore its environment to
locate food and water resources (Deacon & Rawlins, 2006). An alternation protocol is commonly employed where the animal must enter into alternate sides of the maze on subsequent trials to obtain a food reward (Tsaltas, Kyriazi, Pouloupolou, Kontis, & Maillis, 2007). This protocol takes advantage of the natural tendency for animals to enter the less recently visited arm, implying the need to remember which arm was visited last (Sharma, Rakocy, & Brown-Borg, 2010). The alternation procedure consists of 2 runs (sample and choice) per trial. On the sample run, rodents are placed in the maze and allowed to navigate to the choice point (at the top of the “T”) and enter either the right or left goal arm (either only one arm is available or the subject gets to choose, depending on the procedure used). Animals are then removed from the maze for a period of time (the delay period), which can be increased to make the maze more difficult and subsequently placed back at the base of the “T”. Animals are again allowed to navigate to the choice point and enter either the right or left arm. Good performance means that on the choice run, animals will enter the arm not previously chosen on the sample run.

Delayed alternation tasks are particularly sensitive to disturbances in hippocampal and prefrontal cortex function in mammals and is also sensitive to enhancement of memory by nootropic drugs (Ito & Canseliet, 2010; Markowitsch & Pritzel, 1977; Pierard et al., 2007). Franowicz et al. (2002) used a version of the delayed alternation procedure they found to be most sensitive to differences in performance in drug-treated versus control mice. The researchers trained mice to a 70% correct criterion prior to drug treatment, and then calculated the delay animals were able to
withstand once treatment began. Additionally, researchers decided, a priori, which
trials would be analyzed for difference in performance. This procedure reduces
differences in performance at the start of drug-treatment, which can make
interpretation of results more difficult.

*mGlu5 PAMs represent a way to safely modulate glutamate receptor activity*

Recently, PAMs of mGlu receptors have emerged as a potentially viable way to
modulate receptor function without the risk of excitotoxicity. PAMs potentiate the
receptor response to endogenous glutamate, but do not directly cause receptor
activation; thus avoiding receptor desensitization as well as the excitotoxicity caused by
agonists (for a review see Niswender & Conn, 2010). Recent research has provided
evidence to support this hypothesis. Several researchers have found that enhancing
mGlu5 receptor function with PAMs increases performance on a variety of spatial and
working memory tasks including: Morris water maze, Y-maze, and novel object
recognition tasks (Ayala et al., 2009; Balschun et al., 2006; Liu et al., 2008; Olive, 2009).

Ayala (2009) found that potentiating mGlu5 receptor function with PAMs
ADX47273 and 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) enhanced
performance in the MWM, indicating that enhancing mGlu5 function can facilitate
learning in spatial tasks. The mGlu5 PAM, CDPPB, was also found to facilitate extinction
learning in a conditioned place preference procedure, but this effect was blocked by
administration of MK-801 (a NMDAR antagonist) or MTEP (Gass & Olive, 2009).
The aforementioned results provide behavioral data supporting the importance of mGlu5 receptors in learning and memory; and they suggest that mGlu5 PAMs act as cognitive enhancers, at least in some tasks. However, more research is needed to fully understand the role of mGlu5 receptors in many of these tasks, particularly appetitively-motivated tasks. Additionally, few research studies exist which have examined the role of mGlu5 receptors in the Barnes or T-maze learning. Therefore, the present experiments aimed to investigate the impact of potentiating mGlu5 receptor function in the Barnes and T mazes. CDPPB, administered prior to 5 daily training sessions in the Barnes maze (Experiment 1) did not significantly enhance acquisition of the task. However, in a second experiment CDPPB (10 or 30 mg/kg) significantly enhanced performance compared to vehicle-treated controls during 3 days of reversal learning. CDPPB also enhanced performance in the appetitively-motivated delayed alternation version of the T-maze. CDPPB (10 or 30 mg/kg), delivered 20 min prior to 5 daily training sessions enhanced the delay rats were able to withstand. The present results emphasize the role of mGlu5 receptors in spatial learning tasks, and demonstrate mGlu5 receptors are important for learning in appetitive, as well as aversive, tasks.
EXPERIMENT 1: EFFECT OF CDPPB ON HIPPOCAMPAL DEPENDENT SPATIAL LEARNING IN THE BARNES MAZE

Group I mGlu receptors are known to be important in synaptic plasticity and long lasting learning (Anwyl, 1999; Mannaioni et al., 2001). Thus, increasing the activity of these receptors using agonists or PAMs has recently begun receiving attention as a way to potentially increase performance on cognitive tasks. PAMs of mGlu5 may be particularly well-suited for this purpose, as mentioned, since they increase receptor activity with a much lower risk of excitotoxicity than traditional agonists (Kinney et al., 2005). Additionally, they have been shown to not alter the balance of LTP/LTD in the hippocampus, indicating they may represent a way to increase receptor activity while largely avoiding increases in LTD, which can lead to seizure activity which has been associated with the use of mGlu5 receptor agonists (Ayala et al., 2009).

To date, relatively few studies have investigated the effect of mGlu5 PAMs on spatial learning and memory, and none have specifically studied whether CDPPB administration increases performance in the Barnes maze. The purpose of Experiment 1, then, was to determine whether administration of CDPPB prior to acquisition training in the Barnes maze would enhance acquisition learning or retention after a 14-day interval. Mice were administered a relatively low dose of CDPPB (10 mg/kg) or vehicle (i.p.) 20 min prior each of five daily training sessions, and errors and latency to locate
the escape hole were measured.

Method

Subjects

Koopmans et al. (2003) assessed the performance of different mouse strains in the maze, and C57Bl mice demonstrated the greatest improvement in performance over time compared with other mouse strains, suggesting that these mice are ideal for training in the task. Based on this suggestion, we selected C57Bl/6 mice for use in our Barnes maze studies.

Nineteen, male C57Bl/6 mice (Harlan, Indianapolis, IN), fourteen weeks old, with a body weight range of 23-26 g were group housed in Plexiglas cages containing cotton nesting material and mouse houses. They were maintained on a 12 h light/12 h dark cycle and were handled for one week prior to the start of the experiment. Animals had access to food and water *ad libitum* throughout the experiment. All procedures were approved by the institutional Animal Care and Use Committee (Protocol #6460), and were conducted in accordance with established animal care guidelines. In all experiments animals were randomly assigned to groups except for counterbalancing by body weight.

Apparatus

The maze consisted of a grey circular platform 75cm in diameter with 20 holes measuring 5 cm in diameter evenly spaced around the perimeter of the maze (Fig 1). The Barnes maze was brightly lit from above with three 150 W lights encased in
aluminum shells. The bright lighting was used to create a slightly aversive environment to motivate the mice to escape into the dark “safety” of the box.

Figure 1. Arial view of the Barnes Maze. The maze was located in the corner of a behavioral testing room and surrounded by black curtains to limit available extra-maze spatial cues.

The maze was elevated 56.5 cm above the floor and surrounded by a 28 cm high black wall, which contained spatial cues (four basic shapes: triangle, square, circle, and vertical rectangle) at evenly spaced intervals around the inside of the wall. The floor and walls surrounding the maze were covered with black fabric to ensure the appearance of the maze remained consistent across days and to prevent the use of any extra-maze spatial cues. Research has indicated that mice show a strong preference for using these cues, even when proximal cues are present (Harrison et al., 2006), and proximal cues are more likely to remain constant over the course of training. The triangular escape box (17.8x5.1x10.2x7 cm) containing a small ramp was secured beneath the designated escape hole for each individual mouse.
Design and Procedure

The procedure used was the same as that of Walker et al. (2011). Briefly, mice were acclimated to the maze during two shaping trials which occurred prior to the first day of acquisition training. Shaping procedures were identical to those used during training; however, shaping trial data was not analyzed and is not included in the results described below. During training mice received 2 acquisition trials per day for five days with a 5 min intertrial interval. Each mouse was assigned an escape hole number. Hole numbers were alternated by 90 degrees (i.e. holes 5, 10, 15, 20) across different mice for counterbalancing, and the escape box location remained constant for a mouse over all trials. To eliminate odor cues, the maze and all holes were wiped down with a 20% ethanol solution between each trial.

Animals were allowed to acclimate to the testing room for 30 min each day prior to the start of training. A trial began by placing the mouse under a black starting box positioned in the center of the platform. After 30 sec, the box was lifted and the mouse was allowed to search for the goal box. Latency and total errors (nose-pokes into non-escape holes) were recorded. Time spent in the goal quadrant and total distance traveled were also calculated. A trial terminated when the subject entered the goal box or failed to enter the goal box in 5 min (at which time the subject was gently guided to the goal box). Animals were retested 14 days after the final acquisition trial to assess memory retention. Four retention trials were conducted with an intertrial interval of 5 minutes.
**Data Analysis**

The mean acquisition latency and error count for each set of two daily training trials were computed to provide an index of daily performance. Mean Acquisition latency and error data were analyzed using two-way repeated measures ANOVA, and Bonferroni comparisons where appropriate. Graphical depiction of the results is presented as mean ± SEM for each treatment group.

Latency and error count for each of the four retention trials were analyzed using a two-way repeated measures ANOVA; and, as with acquisition performance, graphical representations are presented as mean ± SEM for each treatment group.

**Results**

Repeated measures ANOVA of training latencies produced a significant effect of day, indicating that performance increased across training days, $F(4, 17) = 19.46, p < 0.001$. However, no difference between drug treatment groups was indicated, $F < 1, p > .05$ (Fig 2). Analysis of errors committed during acquisition produced similar results. Briefly, a significant effect of day was present, indicating animals committed fewer errors as training progressed, $F(4, 17) = 25.35, p < 0.001$; but no significant effect of drug treatment was present, $F < 1, p > 0.05$ (Fig. 2).
Figure 2. CDPPB administration prior to training does not affect latency during acquisition trials in the Barnes maze. Average daily training latency for 0 and 10 mg/kg CDPPB groups across 5 days of acquisition training. Mice who received 10 mg/kg CDPPB (n = 10) did not perform significantly better than mice who received a vehicle injection (n = 9).

Figure 3. CDPPB administration prior to training does not affect number of errors committed during acquisition training in the Barnes maze. Average number of errors committed per day for 0 and 10 mg/kg CDPPB groups across 5 days of acquisition training. Mice who received 10 mg/kg CDPPB (n = 10) did not perform significantly better than mice who received vehicle (n = 9).
Analysis of long term retention, measured 14 d after the last acquisition trial, indicated that there was no difference in latency ($F < 1, p > 0.05$) or number of errors committed between groups ($F < 1, p < 0.05$), indicating drug treatment had no effect on long term retention in the Barnes maze (Fig. 3). The first retention trial is most indicative of memory recall, so further analysis was conducted to determine whether there was a difference between treatment groups on this trial. Comparison of 0 and 10 mg/kg CDPPB groups revealed no significant difference in latency ($t$-test, $t = 1.52, p = 0.14$) or errors ($t$-test, $t = 1.22, p = 0.24$) committed during the first trial.

![Graph](image)

Figure 4. Drug treatment does not affect performance on a 14 d retention test in the Barnes maze. Latency on each of 4 retention tests, conducted 14 d after the last acquisition trial, for 0 and 10 mg/kg CDPPB groups. Drug treated animals ($n = 10$) did not show enhanced long-term memory compared with control animals ($n = 9$).
Figure 5. Drug treatment does not significantly affect number of errors committed on retention trials in the Barnes maze. Errors committed on each of 4 retention tests, conducted 14 d after the last acquisition trial, for 0 and 10 mg/kg CDPPB groups. Drug treated animals (n = 10) did not show enhanced long term memory compared with control animals (n = 9).

Overtraining in the Barnes maze can result in longer latencies and more errors during subsequent testing (as discussed earlier on pp. 10-11), so retention data were analyzed to assess whether the second two retention trials could be omitted on subsequent experiments to decrease the number of times animals were exposed to the maze. If no difference in performance was apparent between the first half of the retention trials (block 1) and the second half (block 2), then retention testing would be decreased to 2 trials in the subsequent experiment. Two-way repeated measures ANOVA revealed no significant difference between blocks of trials, F(1, 17) = 0.30, p > 0.05, indicating that continued testing didn’t produce improved recall in animals (Fig.6). Hence, the retest trials were decreased to 2 in the next experiment to help prevent over
exposure to the maze, which can serve to habituate animals to the bright lights and decrease averseness of the maze.

Figure 6. Recall performance was not improved between the first and second blocks of trials during retention testing in the Barnes maze. Latency (A) and error (B) data for each of the 4 retention tests are depicted on the left side of the figure, while the right side represents the data blocked into groups of 2 trials. No difference between block 1 and 2 was detected, indicating animals did not improve their recall with the addition of more trials. In the subsequent experiment, the second block of trials will be omitted to prevent over training.

Discussion

Several studies have reported that systemic administration of mGlu5 PAMs, such as CDPPB, improved performance in a variety of tasks, including Y-maze, novel object recognition, and Morris water maze (Ayala et al., 2009; Balschun et al., 2006; Liu et al., 2008; Uslaner et al., 2009); although that result was not confirmed in the present experiment. It has been suggested that mGlu5 receptors are particularly important for
formation of LTP and learning in difficult tasks or those that involve conflicting information. Experiment 1 may not have detected an effect of CDPPB treatment because the task was not difficult enough and/or did not contain conflicting information, or alternatively, that a ceiling effect in performance prevented the ability to detect differences.

Additionally, many of the previously-mentioned experiments used higher doses of CDPPB than the dose used here. It is possible that the dose chosen for this experiment was too low to elicit a behavioral response, so a second dose (30 mg/kg) was included in Experiment 2. Furthermore, motor speed and activity of mice was not assessed so we cannot rule out the possibility that drug treatment affected ambulation. Due to lack of computerized software during Experiment 1, time spent in each quadrant, use of different search strategies and motor speed while exploring the maze was not assessed or compared between groups. A second Barnes maze experiment was conducted (Experiment 2) to address these questions and determine whether CDPPB enhances performance in a more difficult version of this task.
EXPERIMENT 2: EFFECT OF CDPPB ON REVERSAL LEARNING IN THE BARNES MAZE

MGlu5 receptors have been suggested to be of particular importance for learning during difficult tasks or instances of conflicting information (Balschun et al., 1999), so in Experiment 2 a reversal learning procedure was used and the effects of CDPPB on reversal learning was examined. The reversal learning procedure allows researchers to assess an animal's ability to discard a previously learned rule (or set of rules) and acquire a new one in order to complete the task.

Experiment 1 could not rule out differences in ambulation, which could confound interpretation of such a result, so in Experiment 2 mean speed while exploring the maze was computed for each group of animals using a video tracking system, and statistical software was used to compare mean speed among treatment groups. Additionally, one of the over-head lights was removed to decrease the brightness of the maze, in an attempt to elicit a slight decrease in performance (evidenced by increased latency) and facilitate detection of between-group differences (by avoiding a floor effect for latency).

Method

Subjects

Twenty-six, male C57Bl/6 mice (Harlan, Indianapolis, IN), fourteen weeks old, with a body weight range of 24-30 g were group housed in Plexiglas cages containing
cotton nesting material and mouse houses. All procedures were approved by the institutional Animal Care and Use Committee (Protocol #6460), and were conducted in accordance with established animal care guidelines. Other details regarding maintenance of the mice were the same as in Experiment 1. In all experiments animals were randomly assigned to groups based on their performance during the retention tests, and counterbalanced for body weight.

Apparatus

The maze was the same as that used in Experiment 1 except for the following changes. One of the overhead lights was removed, leaving only two 150 watt lights to illuminate the maze and provide the aversive stimulus. Additionally, in Experiment 2, performance was recorded using a camera mounted above the maze (Logitech, Fremont, CA). Animal movements were tracked and analyzed using ANY-Maze video tracking system (Stoelting Co., Wood Dale, IL).

Design and Procedure

The procedure used was similar to that described above except for the following changes. First, CDPPB was not administered until after the first phase of training. An additional CDPPB dose (30 mg/kg) was added to increase the chances of detecting an effect of the drug. The retention interval was decreased from 14 to 10 days, and animals only received 2 retention trials instead of 4. There was not a significant difference in performance between the first 2 and the last 2 retention trials in Experiment 1 ($F(1, 17) < 1, p > 0.05$), indicating animals nor experimental sensitivity benefitted, in terms of performance, from the two additional trials. In light of this
evidence, the number of retention trials in Experiment 2 was decreased to minimize overtraining and overexposure to the maze.

Finally, a reversal training phase was added after the retention interval test as mentioned, and animals did not receive drug treatment until they began this phase. Animals were randomly assigned to treatment groups based on their performance during the retention tests and were also counterbalanced for body weight. Reversal training consisted of 2 trials per day for 3 days, with an intertrial interval of 5 minutes. Prior to the start of reversal training, each mouse was assigned a new escape hole, which was on the opposite side of the maze from the previously assigned hole (Fig. 7). Animals received 0, 10 or 30 mg/kg CDPPB (i.p.) daily 20 min prior to the first reversal training trial.

Figure 7. During reversal training in the Barnes maze, the escape hole is relocated across the maze from its initial position. An aerial view of the Barnes maze with the initial escape hole (marked by a star) and the new escape hole (marked by an “X”) shown.
Data Analysis

The mean latency and error count for each set of two daily reversal trials was computed to provide an index of daily performance. Mean reversal latency and error data were analyzed using two-way repeated measures ANOVA, and Bonferroni comparisons where appropriate. Search strategies used during reversal trials were categorized into three groups: random, serial and spatial as described by (Jasarevic et al., 2011). Briefly, searches were classified as random when localized searches of holes were interrupted by center crosses or when no pattern was discernible. Serial searches were defined as searches of consecutive holes around the maze, and spatial searches were defined as searches that followed a direct path to the hole (Fig 11). Each search strategy was coded as a number (random = 1; serial = 2; and spatial = 3) so statistical analysis could be conducted using the Chi Square (for comparison between groups on day 2) and two-way repeated measures ANOVA (for comparison of strategy use across days) tests.

Results

Performance during acquisition and retention testing were recorded and used to randomize animals into drug-treatment groups. This procedure ensured treatment groups were at a similar mean performance at the start of reversal training. Initial acquisition training and 10-day retest occurred without CDPPB, therefore this experiment examined the effect of drug on reversal learning.
Figure 8. 10-day retention testing, prior to drug administration, indicates that all animals were at the same performance level in the Barnes Maze prior to reversal training, when CDPPB was administered. There was not a significant difference in Latency (A) or Errors (B) between groups at the end of retention testing (trial 2), indicating all groups were at a similar performance level at the beginning of reversal training. Animals were assigned to treatment groups based on their performance during retention testing to ensure that all treatment groups had a similar mean performance level at the start of reversal training. One-way ANOVA confirmed no difference in latency (panel A), $F(2, 23) < 1$, $p > 0.05$, or errors committed (panel B), $F(23) = 1.11$, $p > 0.05$, between groups. Graphical depiction of the results is presented as mean ± SEM for each treatment group.

Analysis of the latency data across 3 days of reversal testing revealed a significant main effect of day (two-way repeated measures ANOVA, $F(2, 23) = 14.30$, $p < 0.01$), indicating performance for all groups increased as training progressed (Fig. 9). Additionally, there was a significant main effect of drug treatment (two-way repeated measures ANOVA, $F(2, 23) = 3.72$, $p < 0.05$). Post hoc analysis determined that animals treated with CDPPB performed significantly better than control subjects on Day 2 (Bonferroni comparison, $p < 0.05$), although it should be noted that there was no difference in performance between the two drug-treated groups (Fig 9, inset).
Figure 9. Treatment with CDPPB decreases latency to find the escape hole during reversal training in the Barnes maze. Average daily training latency for 0, 10 and 30 mg/kg CDPPB groups across 3 days of reversal training. Mice who received 30 mg/kg CDPPB (n = 8) had significantly shorter escape latencies on day 2 of reversal training than mice who received vehicle (n = 9). * indicates significance at $p < 0.05$ level.

Analysis of the number of errors committed during reversal training paralleled that of latency data. Briefly, there was a significant main effect of day (two-way repeated measures ANOVA, $F(2, 23) = 19.31, p < 0.05$) and also a significant main effect of drug treatment, ($F(2, 23) = 11.01, p < 0.001$); although, as with the latency data, no significant interaction was present (Fig 9). Post-hoc analysis determined there was a significant difference between the vehicle and 30 mg/kg CDPPB treatment group on day 2 of training (Bonferroni comparison test, $p < 0.05$, Fig 9, inset).
Animals tend to alter their search strategy from random search, used at the beginning of training, to a more efficient spatial search as training progresses (Harrison et al., 2006; Jasarevic et al., 2011). Considering this information and the fact that 30 mg/kg CDPPB treatment resulted in significantly shorter latency and decreased error rate on day 2 of reversal training, we analyzed search strategies used across days of training to discern whether drug treatment also affected search strategy. Proportion of random, serial and spatial search strategies used during each day of training was assessed as described above and compared among treatment groups. Repeated
measures ANOVA revealed a significant main effect of drug ($F(2, 23) = 3.45, p < 0.05$), indicating that drug treatment effected the proportion of strategy used (Fig 12). As with latency and error rate on day 2, a significant difference was found in search strategy used between 0 and 30 mg/kg CDPPB treatment groups on day 2 of reversal training ($X^2 = 9.34, p < 0.01$, Fig 13) with animals in the 30 mg/kg CDPPB group using more spatial search than vehicle-treated animals.

![Image](image-url)

**Figure 11.** Representative images of search strategies used in the Barnes maze. Typical exploration patterns of (A) random search—no discernible pattern (B) serial search—consecutive holes were visited in a clockwise or counterclockwise direction (C) spatial search—direct path to hole.

![Image](image-url)

**Figure 12.** Proportion search strategy used across trials for 0 and 30 mg/kg CDPPB treatment groups in the Barnes maze. The 30 mg/kg CDPPB treatment group uses a significantly higher proportion of spatial search across trials compared to the control.
group. Two-way ANOVA indicates a significant main effect of drug treatment across the 6 reversal training trials ($F(2, 23) = 3.45, \ p < 0.05$).

![Graph showing the effect of CDPPB dose on strategy use.](image)

Figure 13. CDPPB administration affects search strategy used during day 2 of reversal learning in the Barnes maze. Animals treated with 30 mg/kg CDPPB use a higher proportion of spatial search compared with vehicle-treated controls. * indicates significance from control at $p < 0.05$ level.

Analysis of average speed using two-way ANOVA revealed no significant effect of drug ($F(2, 23) = 0.25, \ p > 0.05$) or trial ($F(2, 23) = 0.39 \ p > 0.05$), indicating that treatment with CDPPB did not cause differences in activity levels of mice while exploring the maze.
Drug treatment does not affect activity level. No significant difference between drug treatment groups was detected in average speed during 3 days of reversal training.

Discussion

The present experiment found CDPPB increases performance during the second trial of reversal training, measured by number of errors committed and latency to reach the escape hole. This finding compliments other studies which have found that modulating mGlu5 receptor activity with PAMs such as CDPPB increases performance in hippocampal-dependent tasks (Ayala et al., 2009; Balschun et al., 2006; Darrah, Stefani, & Moghaddam, 2008; Uslaner et al., 2009).

As expected, no difference in groups was detected during the first day of reversal training indicating each treatment group was able to determine the escape hole had been relocated and discover the new location with similar efficiency. On day 2, the 30 mg/kg CDPPB treatment group was able to locate the hole more quickly and efficiently than the vehicle treated group. Xu et al. (2009) found mGlu5 receptors are necessary for reversal learning in the MWM. MGlu5 KO mice showed severe deficits in MWM.
reversal learning, although their overall spatial learning was intact and they showed only mild deficits in acquisition learning. Several studies have also found mGlu5 receptors are important for the translation of information from short-term to long-term storage. Additionally, mGlu5 receptors have been shown to mediate metaplasticity (for example, mRNA translation in dendritic spines), and so it is important, but not surprising that an increase in mGlu5 receptor activity results in better learning (Abraham, 2008; Bikbaev et al., 2008; Weiler et al., 1997).

There was no difference in mean speed during training trials among drug treated and control groups, so it is not likely that increased performance was a result of animals traveling around the maze faster. It is also noteworthy to mention that mGlu5 receptor activity has been associated with anxiety, and mGlu5 receptor antagonists and negative allosteric modulators have been shown to have anxiolytic effects (Christoffersen et al., 2008; Pietraszek et al., 2005). The question could be raised, then, whether mGlu5 PAMs might have the opposite effect and increase anxiety, perhaps making the maze even more aversive and encouraging animals to locate the escape hole more quickly. Currently, no researchers report anxiogenic effects of mGlu5 PAMs, although increasing mGlu5 receptor activity via agonists has been suggested to promote fear extinction by increasing LTD in the CA1 region of the hippocampus (Camodeca, Breakwell, Rowan, & Anwyl, 1999; Popkirov & Manahan-Vaughan, 2011). However, CDPPB has been shown not to alter the balance of LTP/LTD in the hippocampus, so it is unlikely that these considerations significantly contributed to the enhanced performance observed here (Ayala et al., 2009).
By day 3 the vehicle group’s performance had reached that of the drug-treated groups, suggesting that like in Experiment 2, a “ceiling effect” was reached whereby both groups had adequately acquired the task by day 3 so as no further difference in performance could be detected. A third experiment was conducted using another task, the T-maze. The procedure used with the T-maze allowed the difficulty of the task to be further increased, avoiding a ceiling effect between groups.
EXPERIMENT 3: EFFECT OF CDPPB ON T-MAZE LEARNING

The purpose of Experiment 3 was to determine whether modulation of mGlu5 receptor activity using 10 or 30 mg/kg CDPPB would enhance performance in the delayed alternation T-maze. In this task, each trial consisted of two parts, the “sample” and “choice.” At the start of a trial, the animal was placed in the start box at the base of the “T” and allowed to navigate to the choice point and enter either the right or left goal. The animal was then removed from the maze and placed back in the base of the T. For the second portion of the trial, the “choice” portion, the animal was allowed to navigate to the choice point and choose either the right or left arm. To correctly complete the task, on the choice portion of a trial, the animal should enter the arm not previously chosen during the sample portion of the trial.

Rats were administered CDPPB (s.c.) 20 min prior to each of five daily training sessions and the average delay animals were able to withstand between sample and choice portions of each trial were assessed and considered a measure of working memory.

Method

Subjects

Twenty-four, male, Sprague-Dawley rats (Harlan, Indianapolis, IN), with a body weight range of 300-340 g were individually housed in wire-mesh cages with a Plexiglas
platform covering half of the cage floor and an environmental enrichment toy (PVC tube) in their cage. They were maintained on a 16 h light/8 h dark cycle and were handled for one week prior to the start of the experiment. Animals had access to food and water *ad libitum* prior to the beginning of the experiment, but were maintained on a restricted feeding schedule at 85% of their free feeding body weight during experimental procedures. All procedures were approved by the institutional Animal Care and Use Committee (Protocol #6858), and were conducted in accordance with established animal care guidelines. In all experiments animals were counterbalanced by body weight and performance (number of days required to reach initial learning criterion) during initial T-maze learning phase.

**Apparatus**

The T-maze (60cm X 45 cm X 10cm) was constructed of wood and painted dark grey. It was placed on a table 1 m high, and all trials were recorded using a video camera above the T-maze. Food wells (clear plastic bottle caps) were secured 5 cm from the end of each arm. The purpose of the food well was to hide the food reward so when standing at the choice point, the animal could not visually assess which arm contained the reward. Additionally, to prevent the use of scent cues in determining which arm contains the food reward during the “choice” portion of each trial, a cereal food (Honey Nut Cheerio, General Mills, Minneapolis, MN) was secured (but inaccessible to the rats) on the outside upper wall of the arm which did not contain the food reward. The Cheerio scent was available to the rat through a small mesh-covered hole in the upper wall of the arm, but was not visible during the trial.
Design and Procedure

Animals were initially exposed to the reward in their home cages during two separate instances. Rats received 10 cheerios, placed on the Plexiglas floor of their cage, for two days prior to habituation to the apparatus. During habituation, animals received one 3-min-long habituation trial per day in the T-maze for 4 days prior to the start of training. During the habituation trials, cheerios were placed throughout the maze and replenished as needed. Following habituation trials, animals began initial training in the T-maze, which consisted of 10 trials per day with an intertrial interval of 10 min.

The basic T-Maze procedure was as follows: Each trial consisted of two parts, the “sample” and “choice.” Both goal arms were baited with one half a cheerio placed in each food well before the trial began. At the start of the trial, the animal was placed in the start box at the base of the “T” and allowed to navigate to the choice point and enter either the right or left goal arm and consume the food reward. This will be referred to as the “sample” portion of the trial, and was not counted, except to note which arm was chosen. If an animal choose one goal arm three consecutive times, that arm was blocked on the following trial (forcing the animal to enter the other goal arm) to prevent the development of an arm preference. Immediately after consuming the reward, the animal was removed from the goal arm, the choice point was wiped with 20% ethanol solution, and then the animal was returned to the start box (facing the back wall). The animal was again allowed to navigate to the choice point and choose either the right or left arm. This portion of the trial will be referred to as the “choice”
portion. To consume the reward, the animal must choose the arm not chosen in the previous trial (Fig. 12). To eliminate scent cues, the maze was cleaned between trials using a 20% ethanol solution.

![Arial view of T-Maze.](image)

Figure 15. Arial view of T-Maze. Animals were initially trained to a criterion of 70% trials correct for one day without any drug administration and then they moved onto the Delay Phase. During the Delay Phase, animals received 0, 10 or 30 mg/kg CDPPB 20 min prior to each of 5 daily training sessions.

During the initial maze acquisition period, animals were trained to a performance criterion of 70% correct trials for one day, and no drug was administered during this time. Once an animal achieved the 70% correct performance criterion, it began the second phase of the experiment. During phase 2, animals received CDPPB (0 10 or 30 mg/kg, s.c.) 20 min prior to each daily testing session. Drug administration/delay testing occurred daily for 5 days. To assess working memory performance, the delay between the “sample” and “choice” portions of the trial increased in 5 sec intervals each time an animal made 4 out of 5 correct arm choices (4/5). The 4/5 criterion was calculated in a cascading trial fashion, such that any 4 correct choices in a series of 5 trials was sufficient to meet the criterion (e.g. trials were not strictly divided into blocks of 5 trials during testing). The delay an animal was able to withstand was used to assess memory performance. It should be noted that since an
animal must attain 4/5 correct arm choices to receive a 5 sec increase in delay, results
are only calculated and presented for blocks of 5 trials (e.g. trial 5, 10, 15...50) since a
block of 5 trials contains the minimum amount of trials required to progress to the next
delay. Additionally, because all animals begin at the same delay (5 sec), it was expected
that group differences would not emerge until later in testing (as delays lengthen, drug-
treated animals would reach criteria more quickly than vehicle-treated animals). Thus,
although the average delay was calculated for every 5 trials throughout the experiment,
only trials 40, 45 and 50 were analyzed for between group differences.

Data Analysis

Mean delay on trials 40, 45 and 50 was analyzed for between group differences
using a two-way repeated measures ANOVA and Bonferroni Multiple Comparisons
where appropriate.

Results

Analysis of 0 and 30 mg/kg CDPPB groups on trials 40, 45 and 50 revealed that
animals significantly increased the length of delay they were able to attain as training
progressed, indicated by a significant main effect of trial ($F(2, 28) = 53.61, p < 0.001$, Fig.
13). Additionally, a significant main effect of drug was present ($F(1, 28) = 4.74, p <
0.05$), and Bonferroni comparison revealed 30 mg/kg CDPPB-treated animals were able
to withstand a longer delay on trial 45 than vehicle-treated controls ($p < 0.05$).
Figure 16. The length of delay animals were able to successfully withstand in the T-Maze increased over time. Trials 40, 45 and 50 (marked by grey box) were chosen a priori for analysis of between group differences. *** indicates significance of main effect of trial at the $p < 0.001$ level.

Figure 17. CDPPB treatment significantly increases the delay animals were able to achieve in the delayed alternation version of the T-maze. Bonferroni comparisons revealed a significant difference in delay achieved on trial 45 between 30 mg/kg CDPPB-treated animals and controls ($n = 8-9$). * indicates significant difference from control at $p < 0.05$ level.
Discussion

The present experiment found that treatment with 30 mg/kg CDPPB significantly increased performance on trial 45 of the delayed alternation version of the T-maze. Although it appears that differences in performance began to emerge around trial 30, only the final three blocks of trials (40, 45 and 50) were analyzed for differences between groups since those were selected a priori as the trials most likely to show a divergence in performance levels. As expected, CDPPB treatment enhanced rats’ ability to withstand delays placed between the sample and choice portions of each trial. Unexpectedly, there was no difference in performance between the 10 and 30 mg/kg groups. Among the wide range of CDPPB dosages cited in the literature, 10 mg/kg represents a relatively low dose and 30 mg/kg representing a commonly used moderate dose, so it is possible that a higher dose might be needed to observe additional improvements in performance. It is also possible, and perhaps more probable, that there is a limit to the working memory ability of rats, and the animals in our experiment were approaching that upper limit. Thus, even with increased drug concentration, they may not be able to withstand a significantly longer delay.
GENERAL DISCUSSION

MGLu5 receptors are known to be important for hippocampal-dependent, as well as hippocampal independent tasks (Christoffersen et al., 2008). The present work shows that potentiating receptors with the mGlu5 PAM CDPPB enhances reversal learning performance in the Barnes maze and increases the delay animals are able to withstand in the delayed alternation version of the T-maze. The highest dose (30 mg/kg) CDPPB produced an enhancement in learning compared to vehicle treated controls, but we found no difference in performance between the low (i.e. 10 mg/kg) and high CDPPB treatment groups. Xu et al. (2009) recently found mGlu5 receptor function is required for normal reversal learning in the MWM. In the study, mGlu5 KO mice showed only mild deficits in acquisition performance, but displayed severe deficits when the hidden platform was moved to a new location (during reversal learning). The researchers note that mGlu5 KO mice spent more time in the previous target quadrant than wildtype mice throughout reversal training; and additionally, KO mice made more entries into the previous target quadrant than wildtype mice, indicating mGlu5 receptors are required for suppressing previously acquired information in order to adapt to a new situation. The current experiments compliment previous findings and emphasize the importance of mGlu5 receptor function in this process, as potentiating receptor function with 30 mg/kg CDPPB was found to enhance performance during
reversal learning. In the Barnes maze, animals treated with 30 mg/kg CDPPB prior to reversal training had significantly shorter latencies and committed fewer errors during the second day of reversal training compared to vehicle-treated controls. There was no evidence of a performance difference between groups on the first day of reversal learning, indicating all groups were able to identify the new location of the escape hole. However, drug-treated animals performed better during the second day of reversal learning, suggesting that potentiation of mGlu5 receptor function produces better learning in this task. Moreover, assessment of search strategy indicated that animals treated with the highest CDPPB dose (30 mg/kg) used significantly more spatial search on day 2 compared with vehicle-treated controls.

Interestingly, in previous studies we found 10 mg/kg CDPPB (s.c.) was not sufficient to increase performance of rats in two non-spatial learning tasks, inhibitory avoidance and conditioned taste aversion learning (Fowler et al., 2010). This seeming discrepancy between mGlu5 PAM effects is likely due to the difference in tasks as the role of mGlu5 receptors in learning have been noted to be largely task-dependent (Simonyi et al., 2005). This argument is bolstered by the fact that the previous CTA work used both a strong (0.15 M LiCl, immediately after flavor) and weak (0.075 M LiCl, 30 min after flavor) conditioning procedure and yielded null results in both cases, suggesting that lack of results weren’t due to floor or ceiling effects.

It is not likely that the present results are attributable to non-specific drug effects because we found no difference in mean speed while exploring between drug-treated and control mice in the Barnes maze. Additionally, we have previously showed
that 10 mg/kg CDPPB does not affect locomotor activity in the open field in rats (Fowler et al., 2010). Other mGlu5 PAMs, such as ADX47273, do not affect activity in the open field mice (Liu et al., 2008); however, no data on the effect of the specific mGlu5 PAM, CDPPB, in mice was available. Although the effect of a higher dose of CDPPB (i.e. 30 mg/kg) was not assessed in the open field in mice, based on mean speed while in the maze as well as information available in the literature, it is expected that 30 mg/kg did not have a significant effect on activity levels in the animals tested. MGlu5 receptor activity has been associated with anxiety; and mGlu5 receptor antagonists and negative allosteric modulators have been shown to have anxiolytic effects (Christoffersen et al., 2008; Pietraszek et al., 2005). However, no researchers currently report anxiogenic effects of mGlus PAMs so it is likely that anxiety was not a significant contributing factor to the present experimental results.

Chronic administration (7 days) of 30 mg/kg CDPPB (i.p.) has been shown to cause an 18% decrease in mGlu5 receptor density in the frontal cortex compared with rats who received an acute dose. It also caused an increase in time spent awake, which unsurprisingly coincided with decreased Delta waves and time spent in REM sleep on day 1, but by the 7th day of drug administration all sleep changes were absent (Parmentier-Batteur et al., 2010). Repeated drug-dosing is virtually unavoidable in spatial tasks due to the number of trials required for animals to learn the task. However, our T-maze study was designed with the results of Parmentier-Batteur et al. (2010) in mind (keeping training to less than 7 days) to avoid this confound as much as possible. Training animals to a 70% criterion prior to drug administration ensured
consistency of task performance at the start of drug treatment. It also allowed us to avoid administering CDPPB for more days than necessary as a way to minimize the effects on receptor density. Additionally, in their study Parmentier-Batteur et al. administered CDPPB i.p. whereas we administered the drug s.c. It is unclear whether administering the drug s.c. could avoid effects on receptor density, but it is well known that different routes of administration can produce different effects on behavior. For example, i.c.v. administration of MPEP was shown to impair spatial learning (Balschun & Wetzel, 2002), but systemic administration was ineffective (Petersen et al., 2002).

It is worthy of mentioning that, although Parmentier-Batteur and colleagues did find a change in receptor density in the frontal cortex, they report that receptor levels were unchanged in the striatum so the effect may be specific to individual brain areas. MGl u5 receptors in other brain areas, such as the hippocampus and striatum are important for activation of immediate early genes and induction of synaptic plasticity, (Ferre et al., 2002; Parelkar & Wang, 2004; Pintor, Pezzola, Reggio, Quarta, & Popoli, 2000), so it is likely that decreased receptor density in the PFC would not greatly affect overall learning in this circuit if other brain areas were unaffected. In fact, the researchers report that the change in receptor density did not affect behavioral response to drug-administration, which supports the idea that overall learning may not be greatly affected by a change in receptor density in only one area of a much larger circuit. It is also important to note that another study assessing effects of repeated CDPPB administration in mice, found six days of treatment with 30 mg/kg CDPPB (i.p.) had no effect on receptor density in the frontal cortex (Pacey, Tharmalingam, &
Hampson, 2011). This result is noteworthy with respect to our present experiment since it suggests that receptor density changes due to repeated CDPPB administration might be specific to rats. Based on the results of Pacey and colleagues, we don’t find it likely that the enhancement in learning we found was significantly affected by changes to receptor density.

The findings that potentiating mGlu5 receptor activity with the PAM CDPPB enhances performance in the Barnes and T-mazes compliments and expands the existing body of research indicating mGlu5 receptors are important for a variety of spatial learning tasks. Simonyi et al. (2005, 2010) claimed that mGlu5 receptors have a large role in aversive tasks with a lesser role in appetitive tasks. Although their reviews mentioned that results stemming from the use of mGlu5 antagonists, such as MPEP or MTEP drugs and radial arm maze show that these receptors can exert an influence in such tasks, the receptors have been claimed to primarily impact aversive tasks. The present T-maze results emphasize the role the mGlu5 receptors have on appetitive tasks; and as mentioned, may shift the focus to task difficulty per se.
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VITA

I am primarily engaged in animal research utilizing behavioral paradigms to investigate neurodegenerative diseases and neuronal signaling involved in learning and memory. I have a specific interest in receptor interactions, such as those between ionotropic and metabotropic receptors. In particular, I am currently studying the interaction of NMDA and mGlu5 receptors known for having substantive effects on learning and anxiety. More recently, I have focused on investigating the efficacy of mGlu5 PAMs as potential pharmacotherapies for schizophrenia. I also participate in research involving TgCRND8 mice as a murine animal model of Alzheimer’s disease.