CDPPB REVERSES AN NMDA RECEPTOR ANTAGONIST-INDUCED DEFICIT IN INHIBITORY AVOIDANCE AND CONDITIONED TASTE AVERSION LEARNING IN RATS

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

CDPPB REVERSES AN NMDA RECEPTOR ANTAGONIST-INDUCED DEFICIT IN INHIBITORY AVOIDANCE AND CONDITIONED TASTE AVERSION LEARNING IN RATS

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LIST OF ABBREVIATIONS

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

CPP— Conditioned Place Preference

CTA— Conditioned Taste Aversion

DAG— Diacylglyceral

DFB—3,3′-difluorobenzaldazine

DHPG—(S)-3,5-dihydroxyphenylglycine

EPM—Elevated Plus Maze

iGluRs— Ionotropic Glutamate Receptors

LiCl— Lithium Chloride

LTD—Long Term Depression

LTP— Long Term Potentiation

IP_3— Inositol 1,4,5-triphosphate

mGluRs—Metabotropic Glutamate Receptors

mGluR5—Metabotropic Glutamate Receptor 5

MK-801—Dizocilpine Maleate

MPEP—6-methyl-2-(phenylethynyl)pyridine

MWM—Morris Water Maze

NMDA—*N*-methyl-*D*-aspartate

PAM— Positive Allosteric Modulator

PFC—Prefontal Cortex

PIP_2— Phosphatidylinositol 4,5-triphosphate

PLC—Phospholipase C

ABSTRACT

Rats administered a metabotropic glutamate receptor 5 (mGluR5) positive allosteric modulator (PAM), CDPPB, and/or an NMDA receptor antagonist, MK-801, were given inhibitory avoidance or conditioned taste aversion training. Three mg/kg CDPPB, delivered 20 min before the conditioning trial, immediately after MK-801 injection, reversed the antagonist-induced deficit in both tasks. These results are consistent with findings that mGluR5 PAMs reverse the effects of NMDA receptor antagonism and represent a novel class of potential pharmacotherapies for diseases such as schizophrenia.

INTRODUCTION

Glutamate is the major excitatory neurotransmitter in the central nervous system and acts primarily on two different types of receptors. Ionotropic glutamate receptors (iGluRs) are ligand-gated channels that open when glutamate is present to allow Ca^{2+}, Na^{+} and K^{+} ions to directly enter the cell. There are three types of iGluRs: AMPA, NMDA, and kainate receptors, named according to the agonists that activate them (e.g., Masu, Tanabe, Tsuchida, Shigemoto, & Nakanishi, 1991). iGluRs, which are primarily located post-synaptically, effect relatively fast changes in neurons and the generation of EPSPs.

Properties of Metabotropic Glutamate Receptors

For decades, scientists thought that glutamate only affected ligand-gated ion channels; but in the mid-1980's it was discovered that some glutamate receptors were coupled to second messenger systems (see Conn & Pin, 1997). Sladeczek, Pin, Recasens, Bockaert and Weiss (1985), and Sugiyama, Ito and Hirono, (1987) discovered glutamate receptors that were coupled to phosphoinositide hydrolysis, which revealed a mechanism through which glutamate could modulate activity at the same synapses where fast transmission occurred. Glutamate is always excitatory for iGluRs, but it modulates mGluRs through either an excitatory or inhibitory function, which allows mGluRs a greater role in modulating synaptic plasticity. mGluRs cause slower changes in the cell, affecting signal transduction pathways, and play an important role in long term potentiation (LTP) and long term depression (LTD) (Conn & Pin, 1997).

Based on their sequence homology, metabotropic glutamate receptors (mGluRs) belong to Family C of the G-protein coupled receptors, which are unique in that the

endogenous agonist does not bind to the 7 transmembrane domain (Ritzen, Mathiesen, & Thomsen, 2005). These Gq-coupled mGluRs, which are significantly larger than other G-protein coupled receptors, are linked to intracellular signaling cascades, which help modulate synaptic activity by influencing changes in the resting potential, threshold potential, and action potential firing characteristics (Schoepp, Jane, & Monn, 1999). However, it is recognized that these receptors can also couple to other G-proteins (Gi/o, Gs) and proteins independent of G-proteins.

mGluRs have 7 putative membrane spanning segments and a carboxy-terminal domain of variable length (Abe, Sugihara, Nawa, Shigemoto, Mizuno, & Nakanishi, 1992). The C-terminus of the second intracellular loop is critical for G-protein coupling specificity in mGluRs, whereas the third loop is important for most other G-protein linked receptors. mGluRs exist as dimers, linked by disulfide bonds between the cysteines in the N-terminal domain. They also have an unusually large extracellular binding domain on the N-terminus, which is believed to be the glutamate binding site (Romano, Yang, & O'Malley, 1996). Glutamate binding at this site increases the probability of a conformational change, activating a G-protein, and generating the second messenger cascade (Kunishima, Shimada, Tsuji, Sato, Yamamoto, et al., 2000).

Through DNA cloning and functional assays, eight distinct mGluRs have been characterized and named (mGluR 1-8) (Conn & Pin, 1997). These eight mGluRs can be classified into three separate groups based on sequence homology, pharmacology, and the signal transduction mechanism used (Schoepp et al., 1990). Group I includes mGluR1 and 5; Group II includes mGluR2 and 3; and Group III includes mGluR4 and 6-8. Considerable differences exist between groups of mGluRs, with only about 35 percent

homology between them; but mGluRs within a group are strikingly similar with nearly 70 percent sequence homology (Conn & Pin, 1997). The low sequence homology between mGluRs of different groups makes mGluRs attractive for targeted drug therapies because the differences allow for drug specificity, so that the action occurs at one receptor (or one group of receptors) but not others. However, the sequence homology between groups of mGluRs makes it difficult to synthesize compounds selective for only one subtype of receptor (Lindsley, Wisnoski, Leister, O'Brien, Lemaire, et al., 2004). Splice variants (versions with a slightly different RNA sequence) have been identified for mGluRs 1, 4, and 5, 6, 7, 8 although these variants have not been studied in detail. Cloned mGluRs in humans are 93-96 percent identical to those cloned in rats (Conn & Pin, 1997), which validates the use of rats in research seeking to identify the function and process of mGluRs, and to test possible drug therapies for treating human diseases such as schizophrenia and Parkinson's disease (e.g., Chavez-Noriega, et al., 2002; Conn, 1999; Dolen & Bear, 2008; Javitt, 2007; Morin, et al., 2010).

Group 1 mGluRs and Metabotropic Glutamate Receptor 5 (mGluR5)

Group 1 receptor activation is coupled to the phosphoinositide second messenger system. In this pathway, phospholipase C (PLC) hydrolyzes a phosphodiester bond in phosphatidylinositol 4,5-bisphosphate (PIP_2), leading to the formation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3), which are second messengers. DAG remains in the membrane and acts as a cofactor to activate protein kinase C, which can phosphorylate many target proteins leading to amplification and distribution of a signal. IP_3 binds to its receptor and stimulates the release of Ca^{2+} from the endoplasmic reticulum (Ritzen et al., 2005; Abe et al., 1992).

mGluR5 is primarily located postsynaptically (Abe et al., 1992). mGluR1 was the first receptor to be characterized, thus much of the earlier research focused on it (Conn & Pin, 1997). However, in recent years, research interests have shifted to mGluR5 given its implications for learning and memory. The mRNA expression of mGlu5 receptors are highest in the striatum, but are also found prominently in the telencephalic regions: cerebral cortex, hippocampus, subculiculum, internal granual layer of the olfactory bulb, lateral septal nucleus, and nucleus accumbens (Testa et al., 2004); and because of the learning processes believed to be governed by these structures, group 1 mGluRs are thought to be particularly important in modulating LTP and LTD as well as types of learning such as spatial and working memory (Balschun, Zuschratter, & Wetzel, 2006). Additionally, mGluR5 expression significantly overlaps with mGluR1 expression in the dentate gyrus and CA2-4 region of the hippocampus, suggesting both receptors may play a vital role in LTP and spatial memory (Testa et al., 2004).

Interestingly, mGluR5 and NMDA receptors are highly co-localized in the rat brain, especially in the CA1 and CA3 regions of the hippocampus, and are thought to influence one another through interactions with scaffolding proteins (Alagarsamy, S., Rouse, S.T., Junge, C., Hubert, G.W., Gutman, D., Smith, Y. & Conn, P.J., 2002). This process involves group I mGluRs binding with Homer, and the interaction of Homer with the Shank-GKAP-PSD-95-NMDA receptor complex (Alagarsamy, Sorensen, & Conn, 2001). The close association between the two receptor types suggests that the interaction between the two receptors may play an important role in LTP in learning and memory (e.g. Alagarsamy et al., 2001; Alagarsamy et al., 2002; Mannaioni et al., 2001).

Importance of mGluR5 in Learning and Memory

mGluR5 has been implicated in a variety of memory processes including spatial learning, object recognition, inhibitory avoidance, conditioned fear, and conditioned taste aversion (e.g. Ayala, Chen, Banko, Sheffler, Williams, et al., 2009; Schachtman et al., 2003; Simonyi et al., 2005; Xu, Zhu, Contractor & Heinemann, 2009). The importance of mGluR5 in these processes has been investigated using agonists and antagonists, but until recently targeted receptor modulators were not available to increase receptor activity, and enhance learning, without the risk of neurotoxicity.

Conditioned taste aversion is a classical conditioning procedure that allows researchers to study learning and memory by pairing a novel flavor, the conditioned stimulus, with a malaise-inducing drug (usually LiCl), the unconditioned stimulus. When a flavor is paired with the malaise-inducing drug, animals form an association between the two stimuli and consume little of the flavor when it is presented in the future. Conditioned taste aversions typically develop quickly, after a single trial, and last for several days or weeks, allowing researchers to investigate acquisition and extinction.

Schachtman et al. (2003) found rats injected with MPEP, an mGluR5 antagonist, before presentation of a novel flavor saccharin (Sac), and followed by LiCl, consumed more Sac on test trials than control animals, indicating the importance of mGluR5 in taste learning. To further investigate the importance of mGluR5 in taste memory, Bills et al. (2005) administered MPEP prior to each of two taste pre-exposures (a latent inhibition treatment). Rats were then given a flavor solution followed by LiCl. On test trials, researchers found that MPEP during flavor pre-exposure significantly attenuated latent inhibition.

Inhibitory avoidance is a well known procedure for studying learning and

memory involving an aversive stimulus. Two inhibitory avoidance paradigms, step-through avoidance and step-down avoidance, are frequently used to study learning in rodents. In step-down avoidance, the shuttle box consists of a small platform and a grid floor. The animal is initially placed on a small platform, and when it steps off the platform onto the grid floor it receives a foot shock and is returned to its home cage. On the test trial, the animal is again placed on the platform, and its latency to step off the platform is measured and considered an index of learning. Step-down and step-through avoidance tasks are procedures in which learning can occur in one trial, which creates a memory that lasts weeks to months. They are desirable learning paradigms for rodent research because they allow researchers to control stimuli precisely, with respect to the time that drug administration occurs relative to the conditioning trial (a benefit of single-trial learning), and therefore allow researchers to investigate the different forms of information processing (e.g., acquisition, consolidation, and retrieval) during aversive learning.

The results of Simonyi et al. (2007) found that infusing MPEP into the hippocampal CA1 region immediately after training resulted in a dose-dependent decrease in inhibitory avoidance retention. Similarly, Genkova-Papazova, Petkova, Stankova, Ossowska et al. (2007) found administering MPEP immediately after or 30 min after training impaired avoidance learning. These studies demonstrate the importance of mGlu5 receptor activity in consolidation and retention of avoidance learning. Together, these studies suggest mGluR5 is vital for learning and memory in a variety of tasks, including aversively motivated tasks such as inhibitory avoidance and conditioned taste aversion.

Positive Allosteric Modulators and Agonists

Modulators are allosteric ligands that bind to a different topographic site on the receptor than the endogenous ligand binding site, and are only active in the presence of an endogenous or orthosteric ligand, such as glutamate. Positive allosteric modulators (PAMs) potentiate the receptor response to the endogenous ligand (e.g., glutamate) by increasing the activity of the receptor, but do not bind at the endogenous receptor site (Lindsley et al., 2004). To date, only four selective mGluR5 positive allosteric modulators have been described in the literature: DFB, CPPHA, CDPPB and ADX47273 (O'Brien, Lemaire, Chen, Chang, Jacobson, et al., 2003; O'Brien, Lemaire, Wittman, Jacobson, Ha, et al., 2004; Lindsley et al., 2004; de Paulis, Hemstapat, Chen, Zhang, Saleh, et al., 2006).

PAMs do not require the presence of amino acid moieties in their structure, which are a component of virtually all endogenous ligands. Such moieties are polar, charged, and must be actively transported across the blood-brain barrier (Ritzen et al., 2005). PAMs are relatively lipophilic and uncharged, so they can pass through the blood-brain barrier relatively quickly via passive diffusion (Ritzen et al., 2005). This characteristic allows the exploration of systemic effects of PAMs via subcutaneous or intraperitoneal injection.

Balschun, Zuschratter, and Wetzel (2006) used a Y maze spatial-alternation task to investigate the effects of mGluR5 on memory consolidation and retention. They administered DFB, a PAM, into the cerebrospinal fluid after training. In the first of two experiments, rats received 40 training trials with an intertrial interval of 1 min. At test, 24 h later, they found animals treated with DFB made fewer errors than control animals.

In the second experiment, the task was made more difficult by removing the rat from the goal arm after 20 trials (half-way through the experiment) and placing it into the arm that had formerly been the wrong alley, which served as the start arm for the remainder of the experiment. The enhancement of spatial memory increased with the difficulty of the test, from experiment 1 to experiment 2, which supports the role of mGluR5 in memory consolidation.

Gass and Olive (2009) investigated the effect of CDPPB [3-cyano-N-(1, 3-diphenyl-1H-pyrazol-5-yl)benzamide] an extinction memory in a conditioned place preference (CPP) procedure. Researchers found that animals treated with 30mg/kg (s.c.) before extinction trials required significantly fewer trials to reach the extinction criteria than control animals.

CDPPB is the compound used in the present experiments. CDPPB binding affinity is not affected by mGluR5 agonists and it does not affect agonist binding, so the positive modulatory effects of CDPPB can be studied without disrupting normal endogenous ligand binding. Receptor agonists can cause excitotoxicity if given in too high doses; however, PAMs do not bind to the glutamate binding site, so they can be administered in relatively high doses without the risk of triggering excitotoxicity (Kinney et al., 2005).

CDPPB, an mGluR5 PAM

CDPPB is a centrally active pyrazole amide that is a positive allosteric modulator of mGluR5 in both rats and humans (Lindsley et al., 2004). These researchers concluded that CDPPB binds to an allosteric site distinct from the endogenous glutamate-binding site on the receptor (de Paulis et al., 2006 & Lindsley et al., 2004). Kinney et al. (2005)

found that CDPPB has *in vitro* activity in Chinese hamster ovary (CHO) cells, stably expressing human mGlu5 receptors. Using FLIPR (Flourometric Imaging Plate Reader) analysis, Kinney et al. found CDPPB caused a concentration-dependent increase in response of CHO cells in low glutamate conditions with a 7-fold increase in activity, while Lindsley et al. (2004) found a 4-fold increase in activity using the same procedure.

To determine the half-life and bioavailability of CDPPB, Kinney et al. (2005) injected groups of rats with 2 mg/kg CDPPB (i.v.) into the jugular vein and also administered the drug orally to another group of rats at a concentration of 10 mg/kg. After washing out CDPPB from cells, the activity was almost completely removed, indicating CDPPB binding is reversible. Metabolite profiling revealed that CDPPB has a half-life of 4.4 hours when administered intravenously in rats, but that it has low bioavailability when administered orally. Kinney et al. also found that CDPPB is able to cross the blood-brain barrier when injected s.c. These findings indicate that injecting CDPPB s.c. 20 min prior to training will allow enough time for the drug to cross the blood brain barrier and bind at mGlu5 receptors. The drug half-life also indicates that CDPPB will remain in the rats' system during training (acquisition) and consolidation (post-training), but will no longer be present when testing occurs 48 hours later. This allows assessment of the effects of CDPPB on acquisition and consolidation without affecting retrieval or extinction.

Lecourtier, Homayoun, Tamagnan, and Moghaddam (2006) investigated the effect of CDPPB on spontaneous firing of prefrontal cortex (PFC) neurons and dopamine release in the nucleus accumbens. Researchers found that CDPPB dose dependently increased excitatory responses and the average firing rate in PFC neurons of awake,

unrestricted rats. They also found pretreatment with CDPPB blocked alteration of neuronal firing caused by the NMDA antagonist, MK-801. These results suggest mGluR5 PAMs, such as CDPPB, may represent a new class of drugs to treat cognitive disorders such as schizophrenia by modulating neuronal firing.

Interactions of mGluR5 and NMDA Receptors in Learning, Memory & Performance

Homayoun and colleagues (2004) investigated the interaction of mGluR5 and NMDA receptors in four-arm radial maze performance using the NMDA receptor antagonist, dizocilpine maleate (MK-801), and the mGluR5 antagonist, MPEP. These researchers found MK-801 (0.1 mg/kg) significantly inhibited performance compared to control animals, as did a high dose of MPEP (10 mg/kg). More importantly, when MPEP and MK-801 were co-administered (at low doses which, when administered alone, were ineffective), the combination significantly impaired performance. This study supports the results of an earlier *in vitro* study (Mannioni et al., 2001) which found the group I mGluR agonist, DHPG, potentiated NMDA currents in CA1 pyramidal neurons, supporting the theory that mGluR5 and NMDA receptors interact to affect LTP and learning. The interaction of mGluR5 and NMDA receptors in learning and memory is further supported by evidence showing low doses of NMDA potentiate mGluR5 function; while higher doses lead to an increase in phosphorylation by PKC which results in a decrease in mGluR5 function (Alagarsamy et al., 2002).

Gravius and colleagues (2006) studied the interaction of mGluR5 and NMDA receptors in passive avoidance learning. Researchers administered MK-801 (0.2 mg/kg, i.p.) 30 min before training, and tested retention 24 h later. They found that MK-801

impaired passive avoidance learning compared to controls. Researchers then studied the interaction of mGluR5 and NMDA receptors by co-administering MK-801 (0.1 mg/kg) and MTEP (5 mg/kg) at doses that were previously found to be ineffective at altering passive avoidance learning when administered alone. Co-administration of both drugs significantly impaired passive avoidance learning compared to vehicle treated animals and animals who only received one of the two drugs (MK-801 or MTEP). These results, which are similar to those of Homayoun and colleagues (2004) provide strong evidence that the interaction of mGluR5 and NMDA receptors is important for learning and memory.

Uslaner, Parmentier-Batteur, Flick, Surles, Lam, et al. (2009) investigated the effect of increased mGluR5 activation, using CDPPB, on novel-object recognition in impaired and unimpaired rats. They found an inverted-U-shaped dose response curve, with lower CDPPB doses (10 mg/kg) increasing novel-object recognition in (unimpaired) rats and higher doses (30 mg/kg) having no effect compared to control animals. Uslaner et al. also investigated whether CDPPB could reverse a MK-801-induced deficit in novel object recognition. The researchers found 3 mg/kg CDPPB attenuated the MK-801-induced deficit, but 10 and 30 mg/kg CDPPB had no effect (animals treated with higher doses did not significantly differ in performance from animals treated with only MK-801). CDPPB is not the only PAM found to affect recognition memory. Liu, Grauer, Kelley, Navarra, Graf, et al. (2008) investigated the effects of ADX47273, an mGluR5 PAM, on novel object recognition and found that administering the drug 30 min prior to training resulted in an increase of novel object exploration at a 48 h test.

CDPPB Reverses Drug-Induced Alterations in Activity

Not only do NMDA receptor antagonists impair learning and memory, they have also been found to cause increased locomotor activity and stereotypy. Homayoun and colleagues (2004) investigated the effect of an NMDA receptor antagonist on locomotor activity and stereotypy, and found treatment with MK-801 significantly increased both locomotion and stereotypic behaviors compared with control animals.

In another locomotor activity experiment, Kinney et al. (2005) studied the ability of a PAM to attenuate an amphetamine induced increase in locomotor activity. Researchers injected rats subcutaneously with vehicle, 10, or 30 mg/kg CDPPB. Animals were pretreated with drug or vehicle 20 min prior to amphetamine administration and training. They found CDPPB treatment suppressed an amphetamine-induced increase in locomotor activity and also reversed the enhancement of prepulse inhibition (PPI) caused by amphetamine treatment. Similarly, Lindsley et al. (2004) found that CDPPB given in doses of 3, 10, or 30 mg/kg (s.c.) 10 min prior to test reversed amphetamine-induced disruption of prepulse inhibition, but found no effect on open-field activity or startle amplitude.

Liu et al. (2008) also found administering the mGluR5 PAM, ADX47273, attenuated amphetamine-induced locomotor activity in mice. This finding is consistent with the previously described studies (Kinney et al., 2005; & Lindsley et al., 2004), which found the mGluR5 PAM, CDPPB, reversed increases in locomotor activity caused by amphetamine or an NMDA receptor antagonist.

The particular role of mGluR5, as well as the interaction of mGluR5 and NMDA receptors, in learning and memory is still not well understood. Few studies have been conducted to investigate the effects of mGluR5 PAMs on various behavioral tasks; and

presently, there are no published studies investigating the effects of CDPPB on inhibitory avoidance or conditioned taste aversion. The present study investigated the effects of CDPPB on acquisition of conditioned taste aversion and inhibitory avoidance learning. It investigated whether MK-801, and NMDA antagonist, attenuates learning and whether CDPPB can reverse a potential MK-801-induced deficit in inhibitory avoidance and taste aversion learning. It was hypothesized that CDPPB alone may increase mGluR5 function resulting in enhanced learning of both tasks. Furthermore, enhancement of mGluR5 activity was expected to reverse the MK-801-induced deficit in both inhibitory avoidance and taste aversion learning.

EXPERIMENTAL DESIGN

CDPPB is a relatively novel drug that remains untested in a variety of learning tasks. Researchers (e.g. Ayala et al., 2009; Kinney et al., 2005; Uslaner et al, 2009) have shown that CDPPB enhances learning in spatial and fear conditioning tasks, but its effects on inhibitory avoidance are not well understood. The purpose of Experiments 1 and 2 was to determine whether CDPPB has an effect on inhibitory avoidance learning. A low dose (3 mg/kg) and a higher dose of (10 mg/kg) were used because the existing literature on CDPPB suggests that the drug has an inverted-U shape dose-response curve with intermediate doses enhancing learning and high doses attenuating learning (Kinney, et al., 2005; Uslaner et al, 2009).

NMDA receptor antagonists, such as MK-801, are often used to model diseases in animals, such as schizophrenia. CDPPB and other mGluR5 PAMs may represent a novel class of drug treatments for these types of diseases, so it is important to know whether the CDPPB by itself induces changes in locomotor activity; and whether, by potentiating mGluR5, it is possible to reverse alterations in activity induced by NMDA receptor blockade.

The purpose of Experiment 3 was to determine whether CDPPB by itself alters locomotor activity. Experiment 4 determined whether CDPPB could reverse an increase in locomotor activity induced by MK-801. Rosenbrock et al. (2010) found pretreatment with ADX47273 30 min prior to ketamine administration attenuated the increase in

locomotor activity induced by ketamine administration, but the effect of CDPPB after ketamine administration was not tested.

The purpose of Experiments 5 and 6 was to determine the effects of CDPPB on learning of a conditioned taste aversion. The role of mGluR5 in CTA has been well documented (Bills et al., 2005; Schachtman et al., 2003; Simonyi et al., 2009; Simonyi et al., 2005), but the effect of CDPPB and other PAMs on CTA has yet to be investigated. It is important to investigate how PAM administration affects a variety of learning tasks in order to better understand the role of mGluR5 in learning and memory.

Several studies have established that administration of NMDA antagonists, such as MK-801, disrupts learning of a taste memory (Escobar, Chao, & Bermudez-Rattoni, 1998; Golden & Houpt, 2007; Traverso, Ruiz, & De la Casa, 2003; Vales, Zach & Bielavska, 2006). The purpose of Experiment 7 was to determine whether CDPPB can attenuate an MK-801-induced deficit in taste aversion learning, and whether the same CDPPB dose-response relationship that is observed in other learning tasks also occurs in conditioned taste aversion.

EXPERIMENT 1: EFFECT OF CDPPB ON INHIBITORY AVOIDANCE LEARNING

The purpose of Experiment 1 was to determine whether 3 or 10 mg/kg CDPPB could enhance learning of inhibitory avoidance. Rats were administered CDPPB 20 min before inhibitory avoidance training, and testing occurred 48 h later.

Materials and Methods

Subjects

Thirty naïve, male, Sprague-Dawley rats (Harlan, Indianapolis, IN), approximately 60 days old, with a body weight range of approximately 220-240 g were used. Animals were group-housed in pairs or two or three. They had access to food and water *ad libitum* and were maintained on a 12 h light/12 h dark cycle. In all experiments animals were randomly assigned to groups, except for counterbalancing by body weight.

Apparatus

A shuttle box (Med Associates, St. Albans, VT) containing a 2.5 cm high, 8 cm wide platform (ENV-010 MSD, Med Associates) and a grid floor connected to a scrambled shock generator was used. The detection system consisted of six pairs of photobeams, located 3.5 cm above the floor and was remotely controlled through an interface connected to an IBM-PC operating Med Associates software (version SOF-700RA-11).

Design and Procedure

Conditioning procedures similar to those used by Simonyi et al. (2007) were used, and consisted of one conditioning trial in which animals were placed on the platform with their nose facing the back corner of the shuttle box. Upon stepping off the platform,

animals received a 0.4 mA, 0.5 s footshock and were immediately removed from the shuttle box and returned to the homecage. Latency to step down was measured. The first retention test occurred 48 h and 14 days after training and utilized the same procedure as conditioning but with the omission of the footshock. Animals were removed from the shuttle box and returned to the homecage immediately after stepping down from the platform. A maximum latency of 180 s was allowed (i.e. subjects were assigned this score if they did not step down from the platform in 3 min), and this measure was used as an indication of learning. The shuttle box was cleaned with 10% ethanol solution between each trial by each subject.

Animals were handled for one week prior to training and testing. Animals were allowed to acclimate to the testing room for 60 min before experimental procedures began. On the conditioning day, animals received an injection (s.c.) of vehicle (n = 10), 3 (n=10) or 10 mg/kg (n=10) CDPPB 20 min before the conditioning trial. This dose was chosen based on effective doses used in other behavioral studies, which ranged from 2-30 mg/kg (Kinney et al., 2005; Lindsley et al., 2004). CDPPB (custom synthesized by IQsynthesis, St. Louis, MO), according to the procedures in Lindsley et al. (2004), and dissolved in phosphate buffered saline (10% Tween-80, v/v) was used.

Habituation to a context has been shown to facilitate learning. Roesler, Vianna, Sant'Anna, Kuyven, Kruel, Quevedo, and Ferreira (1998) found that both pre-training with a low footshock or pre-exposure to the inhibitory avoidance box before training prevented an NMDA receptor antagonist-induced memory impairment. To control for this effect, animals were not habituated to the shuttle box, and animals were tested 48 h after training.

Group	20 min prior to Training	Test
Group No Drug	Vehicle	No Drug
Group Dose 3	3 mg/kg CDPPB	No Drug
Group Dose 10	10 mg/kg CDPPB	No Drug

Table 1. Effect of CDPPB on inhibitory avoidance. Rats were administered (s.c.) vehicle, 3 or 10mg/kg CDPPB 20 min prior to inhibitory avoidance training. No CDPPB was administered at the time of test, 48 h after training.

Data Analysis

Step-down latencies are not normally distributed, so a logarithmic (base 10) transformation was applied to normalize latencies to permit parametric analyses. Data were analyzed in accordance with previously published work using either a one-way between-subjects analysis of variance (ANOVA) or by two-way repeated measures ANOVA, followed by pairwise comparisons using Bonferroni's test. P values of <0.05 were considered statistically significant

Results

A one-way ANOVA revealed no significant difference in latencies between groups on the training day, $F < 1$ (Figure 1). There were no significant differences between groups on test day, $F(2, 27) = 1.14$, $p > .05$ (Figure 2), indicating that CDPPB does not significantly influence learning of inhibitory avoidance in normal, non-drug treated rats compared to a control group. A 14-day retention test revealed no significant difference between groups, $F(2, 27) = 1.23$, $p > .05$ (Figure 3), indicating rats that received CDPPB did not retain inhibitory avoidance learning significantly better than

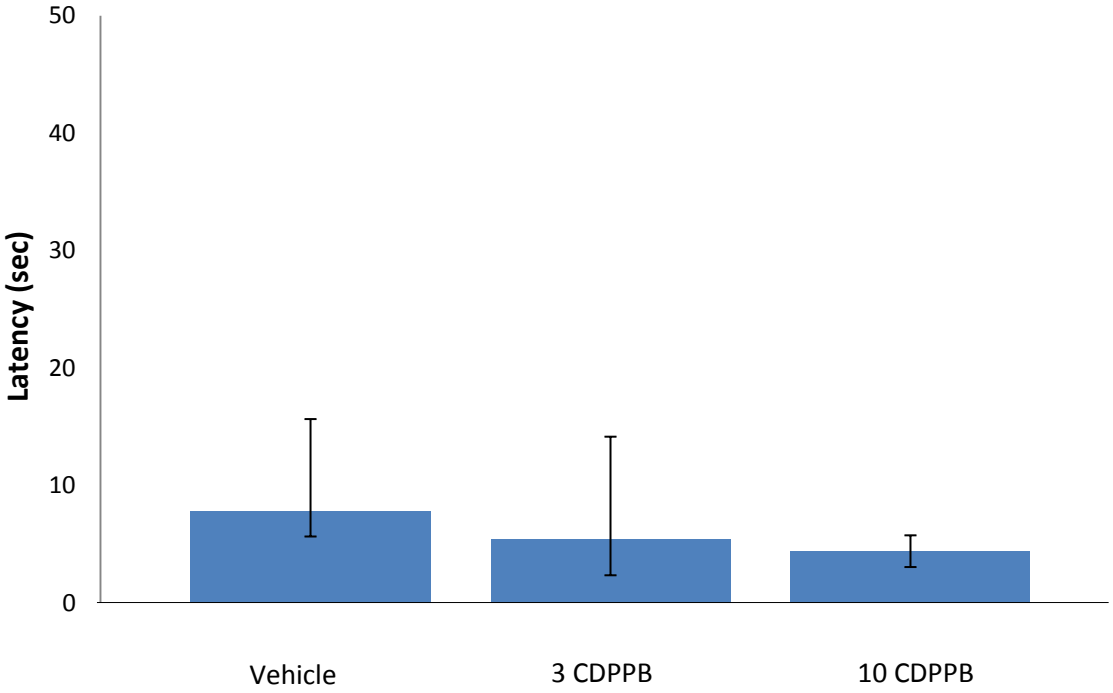

Figure 1. Training day median latencies as a function of CDPPB dose. Error bars represent interquartile range. There was no significant difference in step-down latencies between groups on training day (ns = 10 rats/group).

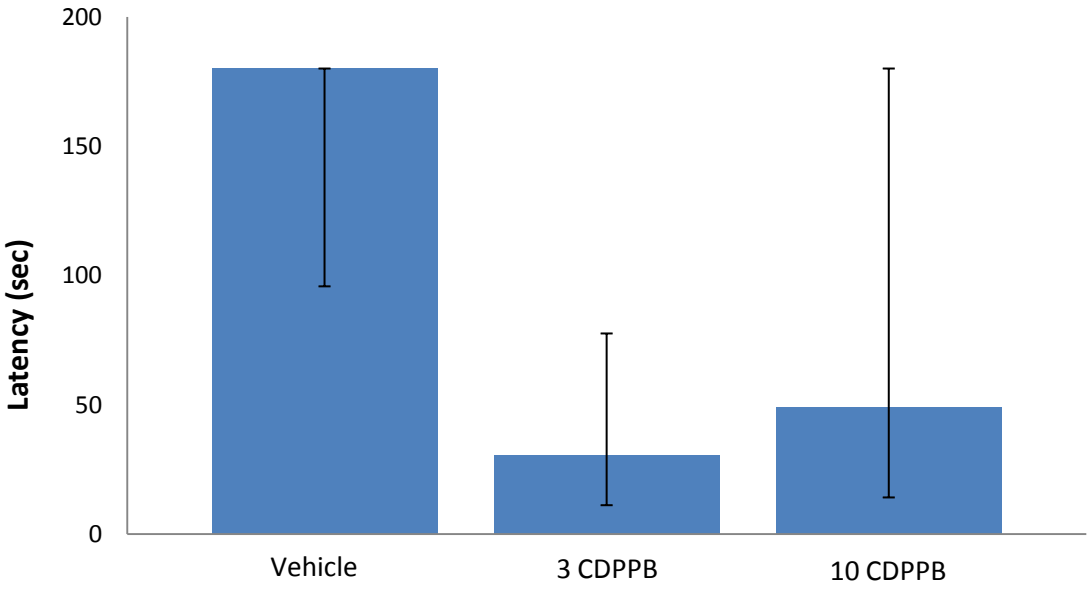

Figure 2. Median latencies as a function of drug dose at the 48 h test in an inhibitory avoidance task. Error bars represent interquartile range. There were no significant differences in step-down latencies among the groups on test day, indicating that CDPPB does not significantly enhance learning of an inhibitory avoidance task (ns = 10 rats/group).

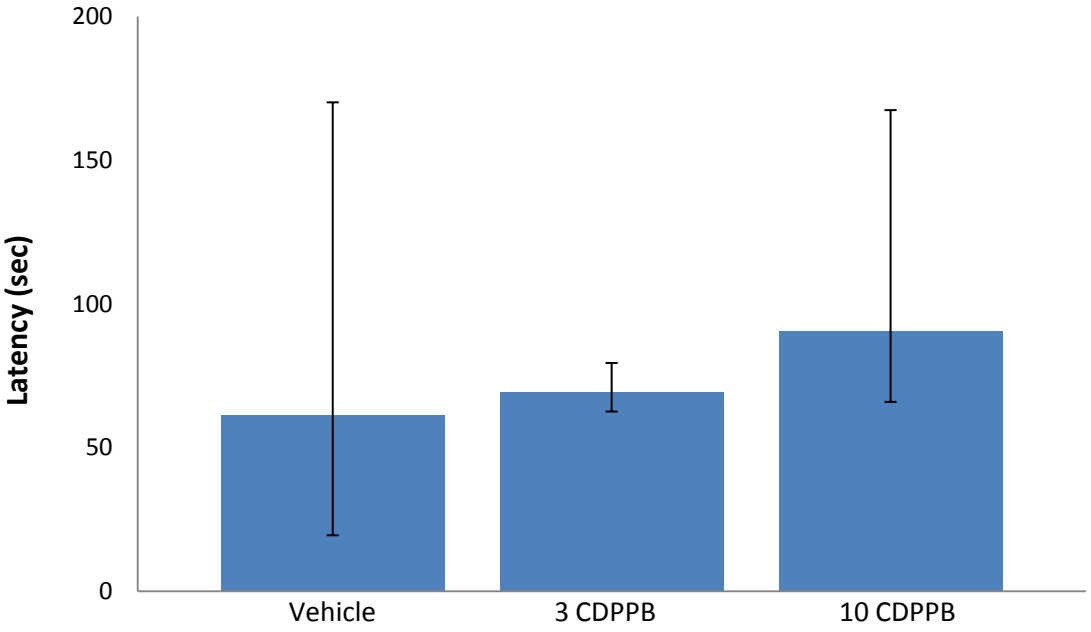

Figure 3. Median latencies as a function of drug dose at the 14-day retest. Error bars represent interquartile range. There was no significant difference in step-down latencies between groups at 14 day retest, indicating that CDPPB does not significantly enhance retention of an inhibitory avoidance task (ns = 10 rats/group).

control animals. Together, these results indicate that CDPPB does not significantly enhance learning of an inhibitory avoidance task in normal, non-drug treated rats.

Experiment 1 indicates that an mGluR5 positive allosteric modulator, CDPPB, does not enhance learning of an inhibitory avoidance task compared to control animals. Research suggests that mGluR5 and NMDA receptors interact to affect functioning of the other receptor, so it is possible that potentiation of mGluR5 by CDPPB, although having no effect on its own, may reverse a deficit in learning caused by an NMDA receptor antagonist.

EXPERIMENT 2: EFFECT OF CDPPB ON AN MK-801-INDUCED DEFICIT IN INHIBITORY AVOIDANCE LEARNING

The purpose of Experiment 2 was to determine if the mGluR5 PAM, CDPPB, could attenuate a learning deficit caused by NMDA receptor blockade. Research suggests that NMDA receptors and mGluR5s may co-regulate one another (e.g., Homayoun, et al., 2004; Mannaioni, et al., 2001; Uslaner, et al., 2009), so it is reasonable to hypothesize that potentiating mGluR5 function may reverse a deficit in inhibitory avoidance learning induced by NMDA receptor antagonism.

Materials and Methods

Subjects

All animal and colony procedures were identical to those described in Experiment 1. Forty-five animals were handled for one week prior to training and testing.

Materials

Experiment 2 utilized the same shuttle box as Experiment 1. The NMDA receptor antagonist, MK-801 was purchased from Ascent Scientific (Princeton, NY), dissolved in PBS. All other drugs and chemicals were acquired as described earlier.

Design and Procedure

The procedure for Experiment 2 was identical to that of Experiment 1 except that 0.2 mg/kg MK-801 or PBS was administered (i.p.) immediately before CDPPB (0=vehicle=PBS:Tween-80, 9:1, v/v, 3, or 10 mg/kg). The following treatment groups were used: PBS/vehicle (n = 12), MK/vehicle (n = 11), MK801/3 mg/kg CDPPB (n = 10), MK-801/10 mg/kg CDPPB (n = 11).

Group	20 min prior to training	Test
No Drug	0 mg/kg MK-801 0mg/kg CDPPB	No Drug
MK-801 Vehicle	MK-801 0mg/kg CDPPB	No Drug
MK-801 3 mg/kg CDPPB	MK-801 3mg/kg CDPPB	No Drug
MK-801 10 mg/kg CDPPB	MK-801 10mg/kg CDPPB	No Drug

Table 2. Effect of MK-801 and CDPPB on inhibitory avoidance. Rats were administered (i.p.) MK-801 (0 or 0.2 mg/kg) and (s.c.) CDPPB (0, 3, or 10 mg/kg 20 min prior to inhibitory avoidance training. No drugs were administered at the time of test, 48 h after training.

Data analysis

The same data analysis procedures used in Experiment 1 were used in Experiment 2.

Results

One-way ANOVA revealed a significant difference in group latencies on the conditioning day $F(3, 40) = 6.85$, $p < .008$ (Figure 4). Comparisons using Bonferroni's test revealed a significant difference between the PBS/Vehicle and MK-801/Vehicle groups, $p < .001$, which suggests that animals receiving MK-801 alone had a different conditioning experience than animals receiving PBS/Vehicle or those receiving MK-801 and CDPPB.

Analysis of the test data 48 h after conditioning revealed a statistically significant difference in test latency among groups $F(3, 40) = 12.03$, $p < .001$ (Figure 5). Pairwise comparisons revealed significant differences between the PBS/Vehicle and MK-801/Vehicle groups, $p < .001$, indicating that (to the extent that conditioning day latencies did not confound group mean test differences) the NMDA antagonist, MK-801,

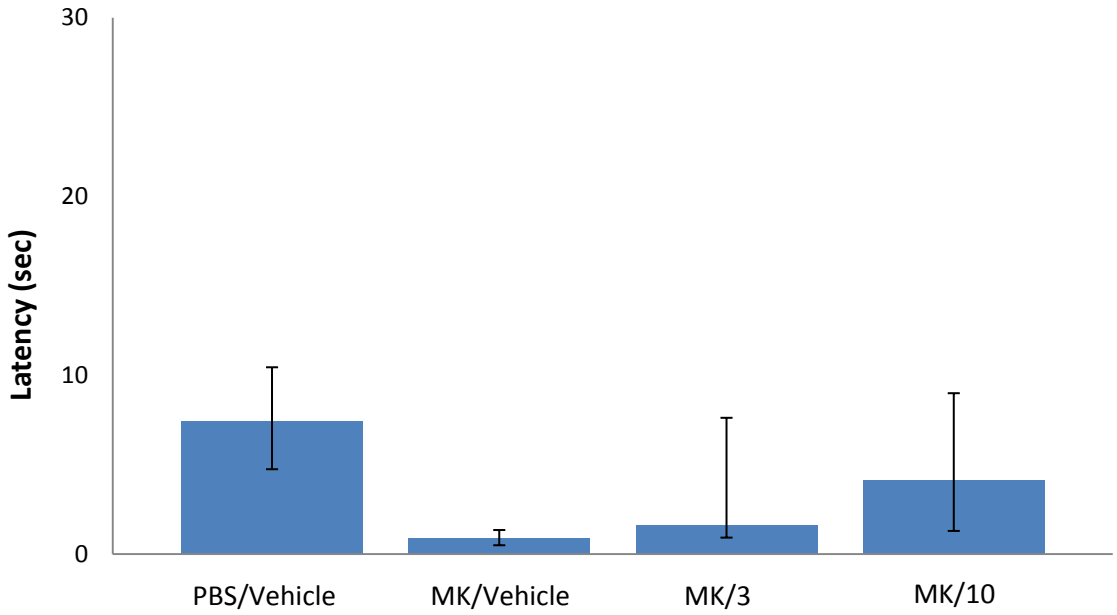

Figure 4. Median latencies on the conditioning trial as a function of drug dose. Rats who received MK-801 alone had a significantly shorter training step-down latency than control animals, indicating they potentially had had a different conditioning experience (ns = 10-12 rats/group).

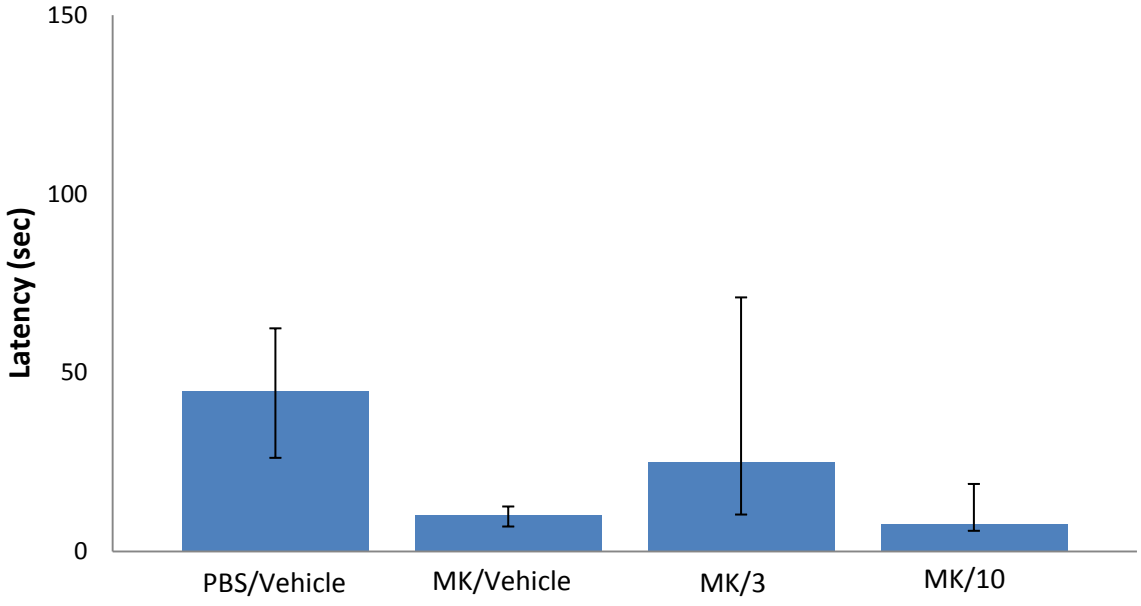

Figure 5. Median test day latency as a function of drug dose. 3mg/kg CDPPB reversed the NMDA antagonist-induced memory impairment but 10mg/kg CDPPB did not (ns = 10-12 rats/group).

significantly impaired learning of an inhibitory avoidance. The MK-801/10 mg/kg CDPPB group had a significantly shorter latency than the control group, $p < .001$, indicating that 10 mg/kg CDPPB does not attenuate the deficit in inhibitory avoidance learning caused by MK-801. The MK-801/3 mg/kg CDPPB group had significantly longer step-down latencies on test day than the MK-801/Vehicle group, $p < .03$, indicating that 3 mg/kg CDPPB can attenuate the learning deficit caused by the NMDA receptor antagonist, MK-801. These two groups did not differ in performance on the conditioning trial, indicating both received similar conditioning experiences. The MK-801/3 mg/kg CDPPB group also had significantly longer step-down latencies than the MK-801/10 mg/kg CDPB group, $p < .02$ — two other conditions that did not differ on the conditioning trial. These results indicate that 3 mg/kg CDPPB reversed an MK-801-induced deficit in inhibitory avoidance learning, but 10 mg/kg did not. These results are consistent with those of Uslaner et al. (2009) and Kinney et al. (2005) which found an inverted-U dose response curve for CDPPB where low doses of the drug attenuated the effects of MK-801.

EXPERIMENT 3: EFFECT OF CDPPB ON LOCOMOTOR ACTIVITY

Experiment 3 sought to investigate whether CDPPB caused any changes in locomotor activity levels. To assess this possibility, locomotor activity was assessed for 30 min in an open field test.

Subjects

Twenty-one animals were handled daily for one week prior to the start of the experiment. The source and maintenance of the animals were identical to those described in Experiment 1.

Apparatus

Activity was automatically assessed using Med Associates (Georgia VT) Open Field Test Environments (ENV-515). The activity chamber was a clear acrylic cage (43.2 × 43.2 × 30.5 cm), containing a 16 x 16 horizontal grid of infrared sensors and a bank of 16 vertical sensors. Each activity chamber was housed in a large sound-resistant cubicle (ENV-017M). Med Associates' Open Field Activity Software (SOF-811) was used to measure the total distance traveled (cm) in 5-min blocks by recording the number of sensor breaks.

Design and Procedure

On test day, animals were injected (s.c.) with 3 or 10 mg/kg CDPPB (ns = 7 rats/group) 20 min before being placed in the activity chamber. Activity was recorded for 30 min.

Data Analysis

Total distance traveled data were analyzed by a one-way ANOVA. Graphical depiction of the results is presented as mean ± SEM.

Results

ANOVA revealed no significant difference in activity between groups, $F < 1$, $p > .05$, indicating that CDPPB did not significantly affect spontaneous activity in an open field which is consistent with earlier published findings (Kinney et al., 2005; Uslaner et al., 2009). This result is also consistent with the previous inhibitory avoidance experiment [Experiment 1] which found that CDPPB alone did not affect learning of an aversively motivated task.

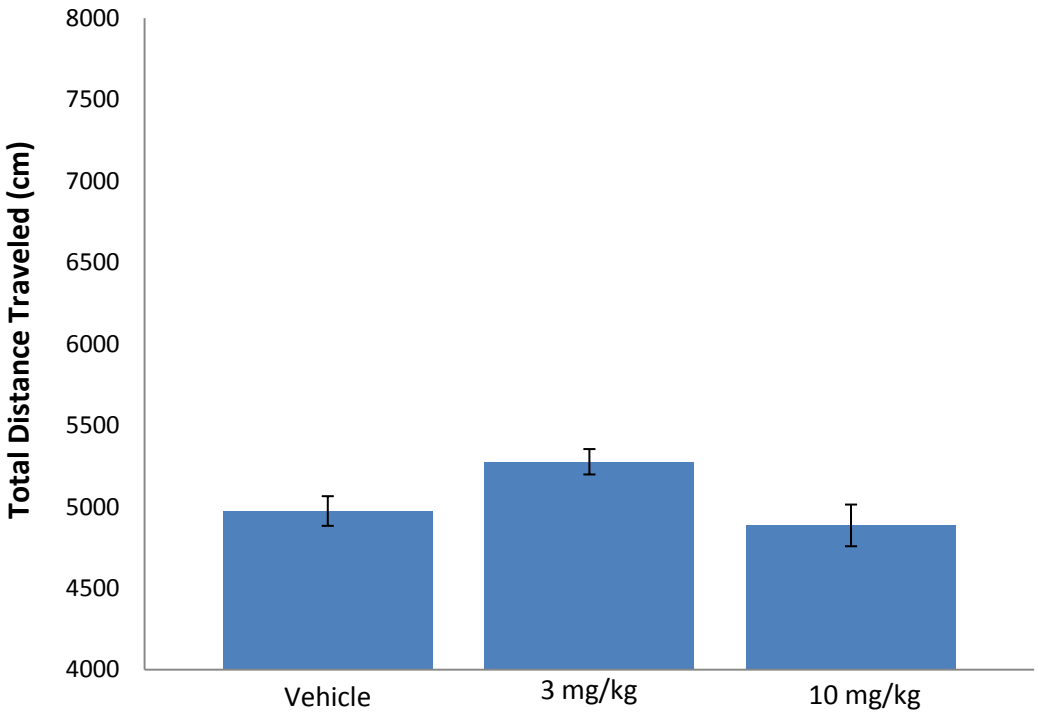

Figure 6. Total distance traveled for each CDPPB dose (ns = 7 rats/group). There was no significant difference in distance traveled between groups.

EXPERIMENT 4: EFFECT OF CDPPB AND MK-801 ON LOCOMOTOR ACTIVITY

Previous research has indicated that NMDA receptor blockade by antagonists such as MK-801 induces hyperactivity (Homayoun et al., 2004), which could account for differences in group latencies observed at conditioning (i.e. differences in step-down latencies prior to receiving footshock). To investigate this possibility, locomotor activity was monitored and total distance traveled in 30 min was recorded.

Subjects

Twenty-six animals were handled daily for one week prior to the start of the experiment. The source and maintenance of the animals were identical to those described in Experiment 1.

Design and Procedure

The apparatus and procedure were identical to that of Experiment 3 except that animals were administered either 0.2 mg/kg MK-801 or PBS before CDPPB administration. The following groups were used: PBS/Vehicle (n = 7), MK-801/Vehicle (n = 6), MK-801/3 mg/kg CDPPB (n = 6), MK-801/10 mg/kg CDPPB (n = 7).

Data Analysis

Data analysis procedures were identical to those used in Experiment 3.

Results

ANOVA revealed a difference in activity between groups, $F(3, 25) = 8.56$, $p < .05$, indicating there was a significant difference in total distance traveled between drug treatment groups.

Planned comparisons revealed significant differences in distance traveled between

the MK-801/Vehicle and PBS/Vehicle group (p <.001), indicating that the NMDA receptor antagonist, MK-801, caused hyperactivity. The MK-801/Vehicle group was also significantly more active than the MK-801/3 mg/kg CDPPB group (p < .01), showing that 3 mg/kg CDPPB attenuated MK-801-induced hyperactivity. Planned comparisons also revealed a significant difference in locomotor activity between the MK-801/10 mg/kg CDPPB group and the PBS/Vehicle group (p < .01), showing that 10 mg/kg did not attenuate an increase in locomotor activity induced by MK-801.

Together, the results of Experiments 3 and 4 indicate that CDPPB alone does not increase locomotor activity, which is consistent with earlier published findings (Kinney et al., 2005; Uslaner et al, 2009). They also indicate that MK-801 induces hyperactivity when injected 20 min prior to testing. Three mg/kg CDPPB, when administered at the same time as MK-801, reverses the hyperactivity induced by the NMDA receptor antagonist. However, 10 mg/kg CDPPB does not attenuate MK-801-induced hyperactivity in an open field test. The open-field test data—as a function of drug dose—parallel the inhibitory avoidance data in Experiments 1 and 2.

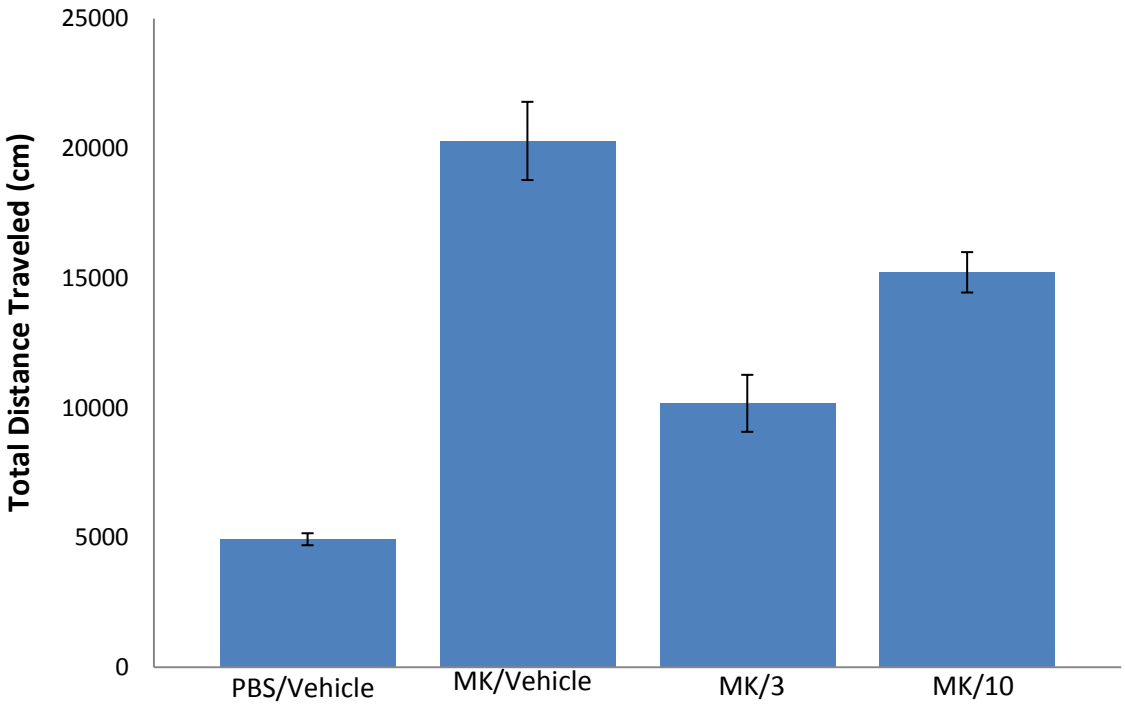

Figure 7. Total distance traveled according to drug group (ns = 6-7 rats/group). Rats who received MK/Vehicle were significantly more active than rats who received PBS/Vehicle or MK/3, indicating that 3 mg/kg CDPPB can reverse MK-801 induced hyperactivity in the open field.

EXPERIMENT 5: EFFECT OF CDPPB ON CONDITIONED TASTE AVERSION LEARNING

Earlier published work examining the effects of mGluR5 PAMs, such as CDPPB, focused primarily on spatial and object recognition tasks (e.g. Ayala et al., 2009; Kinney et al., 2005; Uslaner et al, 2009), although there is strong evidence that mGluR5 activity modulates other types of learning tasks as well (e.g. Bills et al, 2005; Schachtman et al.2003; Simonyi et al., 2005; Vardigan et al., 2010). The primary objective of Experiment 5 was to assess the effect of CDPPB on conditioned taste aversion learning, a non-spatial learning task.

Subjects

Animals were housed individually in stainless steel, wire-mesh hanging cages and had access to food *ad libitum* but controlled water access. They were maintained on a 16 h light/8 h dark cycle. Twenty-one animals were handled for three days prior to conditioning and testing. Animals were water deprived for 24 hours. The animals were then acclimated to drinking from the drinking tubes for 4 days to obtain their daily water within 15 minutes, and water consumption was measured. Throughout the experiment, access to solutions occurred in the home cage during the light portion of the light/dark cycle.

Materials

Solutions were delivered through an inverted plastic centrifuge tube fitted with a rubber stopper and lick tube attached. The amount of solution consumed was measured in milliliters by weighing the tubes before and after consumption. Saccharin (Sigma Chemical, St. Louis, MO) was dissolved in water. LiCl (Sigma Chemical, St. Louis,

MO) was dissolved in water and autoclaved. All other drugs were obtained and prepared as described in the previous experiments.

Design and Procedure

Water consumption was measured for 4 days prior to the beginning of the experiment to ensure animals were habituated to the new drinking tubes and would consume a sufficient amount of solution. On the conditioning day, animals received an injection (s.c.) of 3 mg/kg CDPPB, 10mg/kg CDPPB, or vehicle 20 min before conditioning. Animals then received 15 min access to 7 ml of the 0.1% saccharin solution delivered through an inverted plastic drinking bottle with a lick tube attached, and the amount of saccharin consumed was measured. Animals were injected with 0.15M LiCl at 1.33% body weight (i.p.) immediately after the presentation of saccharin. The first test day occurred 48 h after conditioning, and saccharin consumption was measured for 4 days as an index of taste aversion learning.

Group	20 min prior to conditioning	Immediately after Sac presentation	Test
Group No Drug	Vehicle	.15M LiCl @1.33% bw	No Drug
Group Dose 3	3mg/kg CDPPB	.15M LiCl @1.33% bw	No Drug
Group Dose 10	10mg/kg CDPPB	.15M LiCl @1.33% bw	No Drug

Table 3. Effect of CDPPB on conditioned taste aversion. Rats were administered (s.c.) CDPPB (0, 3, or 10 mg/kg) 20 min prior to conditioning. Thirsty rats received a 15 min presentation of a saccharin flavor. Immediately after saccharin presentation, they were injected (i.p.) with LiCl (.15M at 1.33% b.w.). No CDPPB was administered at the time of test, 48 h after conditioning.

Data Analysis

Saccharin consumption data are normally distributed so no logarithmic

transformation is necessary with taste aversion experiments. Training and test day 1 data were analyzed by one-way ANOVA. Data from test days 1-4 were analyzed by a one-way between–groups repeated measures ANOVA. Data are represented graphically as mean ± standard error of the mean (SEM).

Results

There was no significant difference in sac consumption between groups on the conditioning day, $F < 1$. (Figure 7), indicating that CDPPB did not affect consumption of a novel flavor. There was also no significant effect of CDPPB dose on sac consumption on the first test day, $F < 1$, demonstrating that CDPPB by itself did not significantly influence learning of a conditioned taste aversion.

The effect of CDPPB across the four days of testing was examined by a 3x4 (Drug Dose [0, 3, 10] x Day ANOVA. The main effect of Drug Dose was not significant $F < 1$. There was a significant main effect of Day, $F(3, 48) = 77.69$, $p < .001$ (Figure 8), but there was no interaction between Drug Dose and Day $F(6, 48) = .53$, $p > .05$, indicating that the taste aversion became weaker across test trials but was not impacted by the CDPPB dose. These results indicate that CDPPB by itself does not influence learning of a conditioned taste aversion; however, it is possible that enhanced learning might be observed with a weaker degree of conditioned taste aversion.

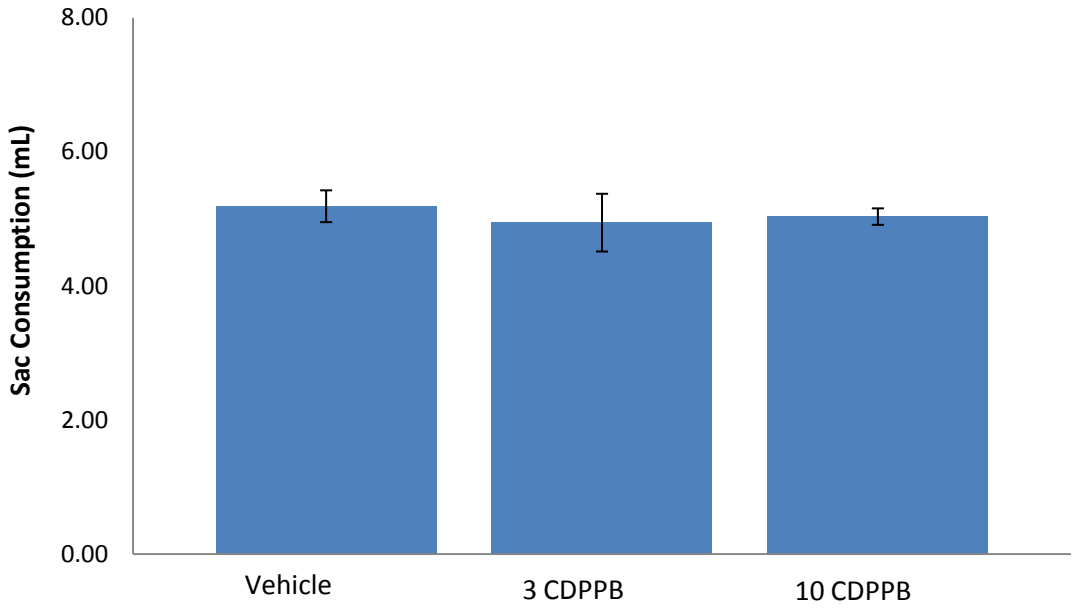

Figure 8. Saccharin consumption on the conditioning day as a function of CDPPB dose. There were no significant differences in consumption. (ns = 6-8 rats/group).

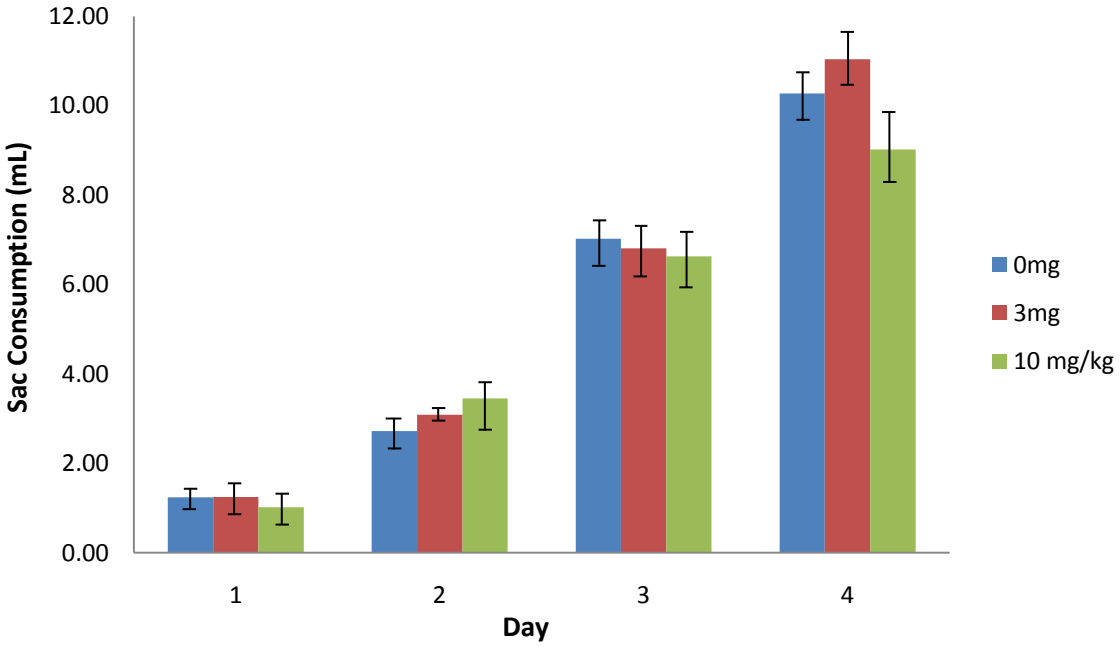

Figure 9. Saccharin consumption across test days according to CDPPB dose. Taste aversion became weaker across test days, as indicated by an increase in saccharin consumption, but was not affected by CDPPB dose (ns = 6-8 rats/group).

EXPERIMENT 6: EFFECT OF CDPPB ON LEARNING OF A WEAK CONDITIONED TASTE AVERSION

Experiment 6 was conducted to verify that CDPPB does not enhance the learning of a conditioned taste aversion. It is possible that in Experiment 5 the taste aversion was so strong that a floor effect for consumption was observed (i.e. the rats learned the association so well that sensitivity to detecting an enhancement of learning was poor). Creating a weaker taste aversion may facilitate observation of a CDPPB-induced enhancement in learning. To test this possibility, Experiment 6 was performed with a lower dose of LiCl which was administered 30 min after conditioning.

Subjects

Twenty-one animals were handled for three days prior to conditioning and testing. All animal and colony procedures were identical to those in Experiment 5.

Materials

All materials and drugs used were purchased and prepared as described in Experiment 5.

Design and Procedure

The procedure for Experiment 6 was identical to that of Experiment 5 except that .075M LiCl at 1.33% body weight was used, and it was injected 30 min after saccharin exposure instead of immediately after exposure as in Experiment 5.

Data Analysis

Data were analyzed according to procedures in Experiment 4.

Group	20 min prior to conditioning	30 min after Sac presentation	Test
Group No Drug	Vehicle	.075M LiCl @1.33% bw	No Drug
Group Dose 3	3mg/kg CDPPB	.075M LiCl @1.33% bw	No Drug
Group Dose 10	10mg/kg CDPPB	.075M LiCl @1.33% bw	No Drug

Table 4. Effect of CDPPB in a weak conditioned taste aversion. Rats were administered (s.c.) CDPPB (0, 3, 10 mg/kg) 20 min prior to conditioning. Thirsty rats received a 15 min presentation of a saccharin flavor. 30 min after saccharin presentation, they were injected (i.p.) with LiCl (.075M at 1.33% b.w.). No CDPPB was administered at the time of test, 48 h after conditioning.

Results

There were no significant differences in saccharin consumption between groups on conditioning day $F < 1$. A one-way within-subject ANOVA conducted on the conditioning and test day 1 revealed a significant difference in consumption between conditioning and test day 1, $F(1, 18) = 5.35$, $p < .05$ (Figure 10), indicating that all groups acquired a taste aversion to saccharin during the conditioning trial. A one-way ANOVA conducted on the saccharin consumption on the first test day revealed no significant effect of CDPPB dose, $F < 1$, indicating that CDPPB did not enhance the learning of a weak conditioned taste aversion.

The effect of CDPPB over the three days of testing was examined by a 3x3 (Drug Dose [0, 3, 10] x Day [1, 2, 3]) analysis of variance (ANOVA). The main effect of Drug Dose was not significant $F < 1$ (Figure 10). There was a significant main effect of Day, *F*

(2, 36) = 8.81, p <.001, but there was no interaction between Drug Dose and Day F < 1. These results, which are similar to Experiment 5, indicate that the taste aversion became weaker across extinction trials but was not impacted by CDPPB.

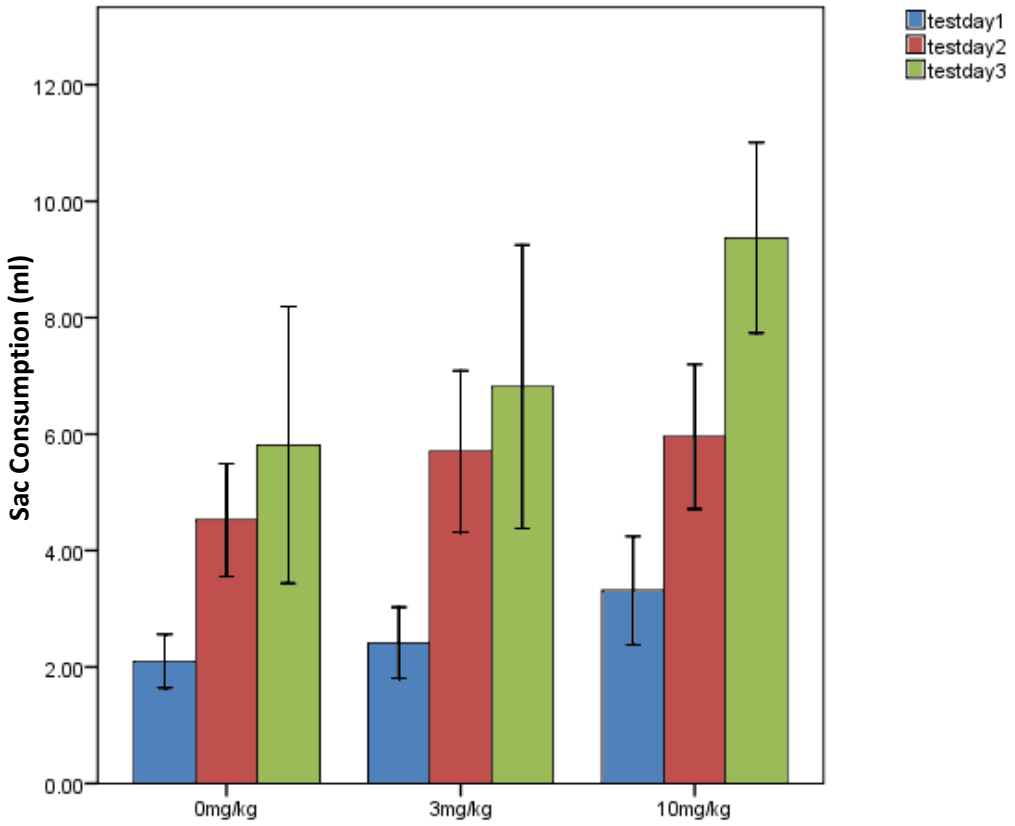

Figure 10. Saccharin consumption as a function of CDPPB dose across days in a weak conditioned taste aversion procedure. Taste aversion became weaker across test days as indicated by more sac consumption but was not affected by CDPPB dose (ns=7 rats/group).

EXPERIMENT 7: EFFECT OF CDPPB ON AN MK-801-INDUCED DEFICIT IN CONDITIONED TASTE AVERSION LEARNING

Experiment 2 demonstrated that 3 mg/kg CDPPB can reverse a learning deficit caused by an NMDA receptor antagonist in an inhibitory avoidance task, but it was unclear whether the PAM could also attenuate the MK-801-induced deficit in a different type of learning task. Experiment 7 investigated the ability of CDPPB to attenuate a deficit in taste aversion learning caused by an NMDA receptor antagonist, MK-801.

Subjects

Fifty-one animals were handled for three days prior to conditioning and testing. All colony and animal procedures were identical to those used in Experiments 5.

Materials

All materials were identical to those used in Experiments 5 with the addition of MK-801, which was purchased and prepared as described in Experiment 2.

Design and Procedure

The procedure used was identical to that used in Experiment 5 except that 0.2 mg/kg MK-801 was administered (i.p.) immediately before CDPPB administration.

Group	20 min prior to conditioning	Immediately after Sac presentation	Test
No Drug	0 mg/kg MK-801 0mg/kg CDPPB	.15M LiCl @ 1.33% bw	No Drug
MK-801 Vehicle	MK-801 0mg/kg CDPPB	.15M LiCl @ 1.33% bw	No Drug
MK-801 3 mg/kg CDPPB	MK-801 3mg/kg CDPPB	.15M LiCl @ 1.33% bw	No Drug
MK-801 10 mg/kg CDPPB	MK-801 10mg/kg CDPPB	.15M LiCl @ 1.33% bw	No Drug

Table 5. Rats were administered (i.p.) MK-801 (0 or .2 mg/kg) and (s.c.) CDPPB (0, 3, or 10 mg/kg) 20 min prior to conditioning. Thirsty rats received a 15 min presentation of a saccharin flavor. Immediately after saccharin presentation, they were injected (i.p.) with LiCl (.15M at 1.33% b.w.). No drugs were administered at the time of test, 48 h after conditioning.

Data Analysis

Data were analyzed in accordance with previously published work (e.g., Schachtman et al., 2003) using a one-way ANOVA, followed by post hoc analysis using Bonferroni's Pairwise Comparison test. P values of <0.05 were considered statistically significant.

Results

A one-way ANOVA revealed no significant difference in saccharin consumption between groups on the conditioning trial, $F(3, 48) = 2.06$, $p > .05$. A one-way ANOVA of saccharin consumption on test day 1 revealed a significant difference between groups $F(3, 47) = 6.21$, $p < .001$ (Figure 11). Post hoc comparisons with Bonferroni's Pairwise Comparison test indicated MK-801 significantly impaired learning of a conditioned taste aversion compared with the PBS/vehicle treated group $p < .001$. Additionally, there was a significant difference between the MK-801 treated group and the MK-801/3mg/kg/kg

CDPPB group $p < .05$, indicating that 3 mg/kg CDPPB reversed the learning deficit induced by MK-801.

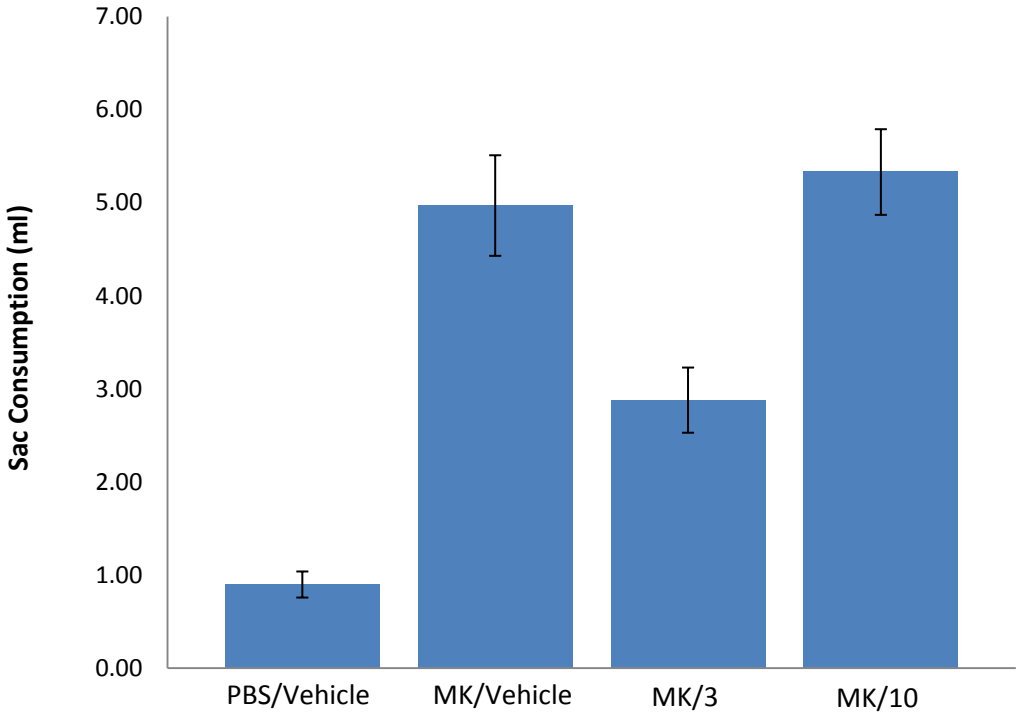

Figure 11. Saccharin consumption as a function of drug dose on test day, 48 h after conditioning. 3mg/kg CDPPB reversed the NMDA antagonist-induced memory impairment but 10mg/kg CDPPB did not. (n = 11-14 rats/group).

DISCUSSION

The goal of the present experiments was to investigate the interaction of mGluR5 and NMDA receptors in learning and memory. The study examined whether the mGluR5 PAM, CDPPB, could reverse a learning deficit induced by the NMDA receptor antagonist, MK-801, in two different aversive learning tasks.

Systemic administration of MK-801 significantly impaired performance in both the inhibitory avoidance and conditioned taste aversion procedures, which is consistent with findings that indicate NMDA receptor activity is necessary for learning in a variety of procedures (e.g. Escobar et al., 1998; Golden & Houpt, 2007; Homayoun et al., 2004; LaLumiere, et al., 2003). Additionally, MK-801 induced hyperactivity in the open field, which is consistent with previous research findings that indicate NMDA antagonists, such as MK-801 and PCP cause hyperactivity and stereotypic behaviors (e.g. Homayoun et al., 2004; Schlumberger, et al., 2009; Uslaner et al., 2009).

CDPPB alone did not significantly enhance performance in the inhibitory avoidance or conditioned taste aversion tasks. However, when CDPPB was co-administered with MK-801, the 3 mg/kg dose reversed the MK-801-induced learning deficit in both tasks. Similarly, Vardigan et al. (2010) found that MK-801 caused a deficit in sucrose preference that was reversed by CDPPB. In the present studies, the 3 mg/kg CDPPB dose also attenuated the MK-801-induced hyperactivity in locomotor activity tests. This result is similar to that of Rosenbrock et al. (2010), which found that the PAM ADX47273 reversed hyperlocomotion induced by the NMDA antagonist ketamine. It is interesting that 3 mg/kg CDPPB was able to attenuate the MK-801 effect,

but the higher dose (10 mg/kg) was ineffective. This result suggests the existence of an inverted-U shaped dose-response curve, which was also found by Uslaner et al. (2009). This type of dose-response curve is not uncommon among cognitive enhancers, and for a specific drug can vary depending on which behavioral task is being used. Uslaner et al. (2009) note that mGluR5 activation produces multiple downstream effects which are not always straightforward, such as influences on LTP and LTD. The fact that mGluR5 activation leads to different downstream effects, depending on which behavioral task is being used and the amount of drug administered may account for the present dose-response relationship.

mGluR5 and NMDA receptors are highly co-localized in regions associated with learning and memory, such as the hippocampus and amygdala (Alagarsamy et al., 2001), and are physically linked through anchoring proteins, which allow the synergistic activation of many proteins such as MAPKs and CREB (Alagarsamy et al., 2002). Co-activation of these receptors is required for learning and memory in a variety of tasks (Gravius et al., 2006; Gravius et al., 2010; Homayoun et al,. 2010), and when the activity of one receptor type is blocked (i.e. by an mGluR5 or NMDA antagonist), learning is poor. Several lines of research suggest that potentiating the activity of one receptor can compensate for hypofunction of the other receptor type (e.g. Lecourtier et al., 2007; Kinney et al., 2005; Vales et al, 2010; Vardigan et al., 2010). These studies have found that increasing mGluR5 activity reverses a learning deficit caused by NMDA receptor hypofunction (typically induced by NMDA receptor antagonist such as MK-801). These studies have important implications for translational research seeking to identify potential drug treatments for a variety of diseases involving glutamate receptor dysfunction.

Researchers hypothesize that the negative symptoms of schizophrenia may be the result of NMDA receptor hypofunction (Chavez-Noriega et al., 2002; Conn & Pinn, 1997; Conn et al., 2009; Olney et al., 1995; Olney et al., 1998; Javitt, 2007). Using agonists to increase receptor function has not been an extremely successful therapeutic technique because agonists pose a high risk of excitotoxicity, which is why selective mGluR5 positive allosteric modulators are receiving so much empirical attention. These compounds potentiate mGluR5 receptors, which provides a way to increase NMDA receptor activity without the risk of excitotoxicity.

Consistent with previous reports, the present research found that MK-801 caused a significant impairment in learning of inhibitory avoidance and a conditioned taste aversion. Additionally, it was found to induce hyperactivity in rats an open field test. The selective mGluR5 positive allosteric modulator, CDPPB, reversed the MK-801-induced memory deficit in both tasks when administered at a dose of 3 mg/kg. The same dose of CDPPB also attenuated MK-801-induced hyperactivity in the open field. These results support the hypothesis that the interaction mGluR5 and NMDA receptors is important in learning and memory. The results also suggest that mGluR5 positive allosteric modulators, such as CDPPB, represent a novel class of pharmacotherapies for treatment of disorders, in which mGluR5 is implicated, such as schizophrenia.

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