THE GROUP II METABOTROPIC GLUTAMATE AGONIST, LY379268, DECREASES
METHAMPHETAMINE SELF-ADMINISTRATION IN RATS

By

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LIST OF ABBREVIATIONS

ANOVA – Analysis of Variance
D1,2,3 – Dopamine Receptor subtypes
DA – Dopamine
DAT – Dopamine Transporter
DAWN – Drug Abuse Warning Network
DSM-IV – Diagnostic and Statistic Manual of Mental Disorders, Fourth Edition
FR1, FR3 – Fixed Ratio Schedules of Reinforcement
GABA – γ-Aminobutyric Acid
i.p. – Intraperitoneal
i.v. – Intravenous
LgA – Long Access
LY379268, LY – (–)-2-oxa-4-aminobicyclohexane-4,6-dicarboxylic acid
METH – Methamphetamine
mGluR – Metabotropic Glutamate Receptor
NAc – Nucleus Accumbens
NET – Norepinephrine Transporter
NIDA – National Institute on Drug Abuse
NSDUH – National Survey on Drug Use and Health
PFC – Prefrontal Cortex
PR – Progressive Ratio Schedule of Reinforcement
SERT – Serotonin Transporter
ShA – Short Access
TEDS – Treatment Episode Data Set
Veh – Vehicle (0.9% physiological saline)
VMAT – Vesicular Monoamine Transporter
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ABSTRACT

Rationale: Methamphetamine (METH) abuse is a growing problem worldwide, for which there is no effective pharmacotherapy. Group II metabotropic glutamate receptor (mGluR) agonists are proposed to serve as an addiction treatment and have been shown to reduce the reinforcing properties of cocaine, but their efficacy has yet to be tested in METH. Objectives: The present study investigated the efficacy of a selective group II mGluR agonist (LY379268) in reducing METH self-administration in a well-established animal model of drug abuse. Methods: The effects of LY379268 on progressive ratio breakpoints for METH or food were assessed. Rats were trained to self-administer either METH or a control reinforcer (food) on a progressive ratio schedule. Rats were pretreated with vehicle (Veh) or LY379268 (0.01, 0.1, 0.3, 1.0 mg/kg, i.p.) 30 minutes before the behavioral session. Additionally, METH trained rats underwent two weeks of 6 hour METH access, after which the efficacy of LY379268 in reducing METH self-administration was assessed. Results: Rats increased their daily METH intake over the course of 14 days of exposure to 6 hour sessions. Systemic pretreatment with LY379268 dose-dependently inhibited METH self-administration in animals with limited and extended METH histories but had no effect on food self-administration at the doses tested. There was no significant difference in sensitivity to the effects of LY379268 before and after escalation. Conclusions: These results indicate a role for group II mGluR in mediating the reinforcing effects of METH. Furthermore, they support the notion that group II mGluR agonists may be potential pharmacotherapies for METH dependence.
INTRODUCTION
The abuse of the N-methyl amphetamine analog methamphetamine (METH) is linked to significant health problems including memory loss, psychotic behavior, cardiac damage, dental damage, and increased risk for transmission of HIV (NIDA Research Report, 2006). Its illegal synthesis continues to grow, particularly in the Western United States and Southeast Asia (Lineberry and Bostwick, 2006), and recent reports suggest that over 1.2 million persons used METH in the past year in the U.S. alone (NSDUH, 2009). Of particular concern are emergency room reports which show a 50% increase in METH related emergency room visits between 1995 and 2002 (DAWN, SAMHSA, 2006) and 95% of all stimulant admissions involved METH (TEDS, SAMHSA, 2007). This increase in METH-related hospital visits may be due to the pattern in which METH is abused. Gonzalez-Castro et al. (2000) found that METH users reported significantly less time between first use and regular use, as well as less time between first use and treatment when compared to cocaine users. Thus, METH users are at greater risk for developing dependence and need treatment sooner than other psychostimulant abusers. However, an effective pharmacotherapy for METH does not currently exist. In order to effectively treat METH abuse, a therapy must attempt to curb one or more of the behaviors associated with drug intake. Some of the key characteristics of addiction as defined by the DSM-IV are a compulsion to take drugs, a loss of control in limiting intake, and a gradual increase in the amount of drug taken per use episode (DSM-IV, APA, 2000).

This progressive increase in intake over time has been successfully modeled in animals self-administering METH, as well as other drugs of abuse. Preclinical intravenous self-administration models which result in increased daily drug intake (escalation) serve as a behavioral framework in which the neurobiological mechanisms underlying excessive drug intake may be examined. The original escalation model exposed rats to either 1-hour (Short
access, ShA) or 6-hour (Long access, LgA) daily access to cocaine and found that rats with longer access (LgA) increased their daily intake significantly over the course of three weeks, especially within the first hour of each session (Ahmed and Koob, 1998). In contrast, rats with limited exposure (ShA) to cocaine did not escalate their intake, but maintained a steady level of responding (Ahmed and Koob, 1998). This extended access escalation model has been replicated with a wide range of drugs of abuse (Ahmed and Koob, 1998; Ahmed et al., 2000) and more recently has been tested in animals responding for METH. Kitamura and colleagues found that, similar to cocaine and heroin, LgA rats escalate their daily intake of METH over the course of a few weeks while ShA rats maintain a steady level of low responding (Kitamura et al., 2006). Because behavioral measures (e.g. PR breakpoints) can be repeated at multiple time points within this paradigm, it is well suited to test the effects of potential therapeutic compounds, particularly with respect to whether or not chronic exposure to a drug of abuse decreases sensitivity to a given compound.

Previous preclinical pharmacotherapy studies for METH abuse have selected dopaminergic targets, as increasing extracellular dopamine (DA) is one of the main effects of psychostimulants (Wee et al., 2007; Orio et al., 2010; Higley et al., 2010). METH increases DA release (Fibiger and McGeer, 1971; Sieden et al., 1976; Ricaurte et al., 1980; Bustamante et al., 2002) and prevents DA reuptake by reversing the flow of the vesicular monoamine transporter (VMAT2) within the synaptic bouton (Pifl et al., 1995; Wilhelm et al., 2004) as well as the dopamine, serotonin, and norepinephrine transporters (DAT, SERT, NET) within the synaptic membrane (Kuczenski and Segal, 1994; Eshleman et al., 1999). Thus, pharmacological manipulations of the DA system which result in decreased DA release could attenuate some of the behavioral effects of METH. There is concern, however, that treating psychostimulant addiction with dopaminergic agonists may result in substitution for the drug of abuse, whereas a
full antagonist may interfere with ongoing natural reward processing. Three recent studies have investigated the effects of partial agonists at D2 and D3 receptors as well as a D3 antagonist, and reported reductions in responding for METH self-administration, particularly in LgA animals, suggesting a shift in sensitivity to certain dopaminergic compounds as a result of escalation (Wee et al., 2007; Orio et al., 2010; Higley et al., 2010). It should be noted that the partial agonists tested in the above studies are also active at serotonergic targets, thus the exact pharmacological mechanisms by which these ligands reduced METH intake are unclear.

While one of the primary reinforcing effects of METH is due to its effects within dopaminergic system, there is increasing evidence for a role for the glutamatergic system in mediating the behavioral effects of METH, especially with respect to relapse. Microdialysis work done by Nash and Yamamoto revealed that systemic METH treatment resulted in a significant increase in extracellular glutamate within the anteromedial striatum. This increase in extracellular glutamate was followed by a decrease in striatal DA content found 7 days post METH administration but not after MDMA administration, suggesting a link between METH induced glutamate rise and alterations within the dopaminergic system (Nash and Yamamoto, 1992). Further behavioral work investigating other psychostimulants revealed that stimulating ionotrophic glutamate receptors (NMDA and AMPA) within the nucleus accumbens (NAc) augmented the reinforcing effects of cocaine, and implying a role for glutamate transmission in the relapse to drug-seeking behaviors (Cornish et al., 1999). McFarland and colleagues observed simultaneous elevations of DA and glutamate within the NAc following a priming injection of cocaine in animals whose responding was extinguished. They then showed that increased extracellular glutamate within the NAc during reinstatement can be traced back to glutamatergic afferents that project from the prefrontal cortex (PFC) into the NAc, as inhibiting these pathways prevented the predicted rise in NAc glutamate (McFarland et al., 2003). The
effect of METH on this pathway was more thoroughly explored in the study by Mark et al. (2004), which showed METH increased D1-mediated striatonigral GABAergic transmission, activating GABA-A receptors within the substantia nigra. This activation at inhibitory synapses lead to a decrease in nigrothalamic inhibition which resulted in increased corticostriatal glutamate release and depletion of striatal DA (Mark et al., 2004).

There is increasing evidence that the group II metabotropic glutamate receptor family may be an effective target for psychostimulant pharmacotherapy. This family is comprised of two G, coupled receptor subtypes: mGluR2 and mGluR3. mGluR2 are primarily presynaptic, functioning as autoreceptors to decrease neurotransmitter release (Conn and Pin, 1997; Xi et al., 2002a), while mGluR3 are more widely distributed across pre- and post-synaptic elements as well as on glial cells, and their functions are more varied (Schoepp, 2001). Several preclinical models have confirmed the ability of group II mGluRs to regulate both reward processing and drug seeking, primarily via agonism at group II mGluR to control release of both glutamate and, indirectly, DA (Hu et al., 1999; Karasawa et al., 2006; Moussawi and Kalivas, 2010). The efficacy of the highly selective group II agonist, LY379268 [(-)-2-oxa-4-aminobicyclo hexane-4,6-dicarboxylic acid, Monn et al., 1999], 80-fold selectivity over Group I and Group III mGluR (Tocris Bioscience), in reducing behaviors associated with drugs of abuse has been examined in a variety experimental paradigms. LY379268 has been shown to inhibit cue-induced reinstatement of cocaine-seeking (Baptista et al., 2004), cue and context-induced reinstatement of heroin-seeking (Bosser et al., 2005; 2006), and cocaine-induced reinstatement of cocaine-seeking (Peters and Kalivas, 2006), as well as cocaine self-administration (Adewale et al., 2006) and alcohol self-administration (Bäckström and Hyytiä, 2005). Despite the strong evidence for glutamatergic involvement in regulating the effects of METH, agonism at group II mGluRs has yet to be investigated as a potential pharmacotherapy for METH.
The aim of the present study, therefore, was to examine the effects of systemic LY379268 on intravenous (i.v.) METH self-administration in rats. Specifically, we tested the effect of LY379268 on METH maintained behavior under a progressive ratio of reinforcement. Additionally, we also examined the effect of LY379268 on non-drug reward using a food self-administration paradigm.
METHODS

Animals, Surgery, and Housing

All experiments were conducted using male, Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing approximately 350 g at the beginning of the experiment. Animals were given a week to acclimate to the laboratory environment. Animals were then anesthetized using a combination of ketamine (100 mg/kg; i.p.) and xylazine (8 mg/kg; i.p.), and a chronic, indwelling Silastic cannula was implanted into the right jugular vein that exited through the back of the animal in the region of the scapulae. Ketoprofen (5 mg/kg; i.p.) was used as a post-operative analgesic, and animals were allowed to recover from surgery for a minimum of 3 days. Following surgery, animals were housed in 30 x 30 x 30-cm operant chambers under a reversed 12-h light/dark cycle (lights on at 1500 hours) with ad libitum food and water access. All procedures were conducted in concordance with the Wake Forest University Animal Care and Use Committee guidelines.

Methamphetamine self-administration

The beginning of the self-administration session was indicated by the extension of the lever into the self-administration chamber, which began 6 hours into the dark cycle (0900 hours). A single response on the lever resulted in an infusion of methamphetamine (0.05 mg/kg) over a 2 sec period, which was paired with a 2 second LED illumination. All subjects were trained on an FR1 schedule until the maximum number of infusions (20) per session was self-administered for 3 consecutive days. The sessions occurred 7 days/week, and lasted 6 hours, or until 20 infusions had been achieved. After completion of the FR1 training criteria, all rats were switched to a 6 hour progressive ratio schedule (PR) (Richardson and Roberts, 1996) for a minimum of 3 days. Some subjects (n=11) were then placed into an escalation paradigm (Kitamura et al., 2006),
which consisted of 14 days of 6 hour access to METH on an FR1 schedule (0.05 mg/kg/inf, no
time out, no maximum number of infusions). Additional subjects (n=9) were used to generate a
PR dose-response curve, described below.

**Drug Treatment**
LY379268 was given as point body weight intraperitoneally (i.p.) 30 minutes before behavioral
testing (food or METH self-administration). Doses for LY379268 were chosen based on existing
literature (Baptista et al., 2004; Peters and Kalivas, 2006). Each rat received each LY dose once
and responding was allowed to recover for at least two sessions before the next dose was
administered. Doses were given in a randomized order.

**Progressive Ratio Dose-response curve**
The standard dose of METH chosen in our escalation paradigm was based on a progressive ratio
(PR) dose-response curve for various doses of METH (see Richardson and Roberts, 1996 for PR
details). Briefly, PR measures the relative reinforcing properties of a drug. The number of lever
presses required by a rat to receive a single injection of METH increases along an exponential
function after each infusion. A ‘break point’ in responding can be taken as a measure of effort
given for a particular dose. The dose-response curve was generated in rats that had just
completed training (n=9) using concentrations of METH along a logarithmic scale (0.017, 0.03,
0.056 and 0.1 mg/kg/inf). Doses were based on animal body weight and were administered in a
within-subject Latin square design. Sessions lasted 6 hours and all subjects received each dose
twice, but never the same dose sequentially.

**Food self-administration**
Experimentally naïve rats (n=8) were 22-hours food deprived and trained to administer sucrose pellets (45 mg, Research Diets, Inc., New Brunswick, NJ) on an FR1 schedule, which gradually increased to FR3. Once lever training was complete, rats were switched to a PR schedule, which was maintained throughout drug testing. Each PR session lasted 30 minutes and was followed by an hour of ad libitum food. Water was available ad libitum throughout the experiment. LY379268 was administered 30 minutes before the start of food self-administration (0.01, 0.1, 0.3, 1.0 mg/kg, i.p.). Rats were weighed before the start of each daily session and after the hour of ad lib food access.

**Data collection and storage**

Two IBM-compatible computers were used to control sixteen self-administration chambers. Programs for controlling the equipment, storing the data and analyzing the results were written in Borland Developer Suite. Every event related to the physical equipment and the experimental session (lever up, lever down, pump on/off, session start time, etc.) was entered into a data stream with a time stamp in milliseconds. One MS-DOS computer controlled four operant chambers in the food study.

**Drugs**

(+) Methamphetamine hydrochloride (NIDA Drug Supply Program, Research Triangle Park, NC) was dissolved in physiological saline (0.9%). All experiments used a standard methamphetamine dose of 0.05 mg/kg/infusion, except the dose response curve, which used four doses on a logarithmic scale (0.017, 0.03, 0.056, and 0.1 mg/kg). This dose was selected based on previous literature (Kitamura et al., 2006; Wee et al., 2007) as well as a progressive ratio dose-response curve (see below). LY379268 (Tocris Bioscience, Ellisville, MO) was dissolved in physiological saline to yield the following concentrations: 1.0 mg/ml, 0.3 mg/ml, 0.1 mg/ml, and 0.01 mg/ml.
and was administered in a 1 ml/kg volume. All drug doses are expressed as the weight of the salt.

**Data analysis** All statistics were performed using SigmaPlot v.11.
RESULTS

A total of 38 rats were used in the studies described below. Most animals completed training requirements (20 infusions taken on FR1 schedule for three consecutive days) within the first week of lever exposure. The effect of LY379268 was initially examined in 6 rats before significant methamphetamine exposure. Eleven animals were placed into extended access, of which one displayed a loss of patency and was excluded. The ten remaining animals were used to examine the effect of LY379268 on PR responding after escalation, but data from three rats were excluded due to a loss of patency. Nine animals were used to generate a PR dose-response curve for METH. Eight animals were used to examine the effect of LY379268 on food self-administration.

Figure 1A depicts the progressive increase in total METH intake after access to 0.05 mg/kg/inf METH was increased to 6 hours per day. Rats (n=10) progressively increased their daily METH intake compared with their first session intake. A one-way repeated measures ANOVA revealed that there was a main effect of Session Day ($F_{13,117}=16.924$, $p<0.001$). Post hoc comparisons revealed that METH intake significantly increased above first session intake from the fourth session onward (Student-Newman-Keuls, $p<0.05$). Figure 1B depicts a further analysis of escalation, METH intake within the first hour of each daily session. A one-way repeated measures ANOVA revealed that there was a main effect of Session Day ($F_{13,117}= 6.215$, $p<0.001$) with post-hoc analyses revealing significant differences between sessions from the eighth session onward (Student-Newman-Keuls, $p<0.05$).
Figure 1. Escalation of METH intake during 6 hour access. Figure 1A: Data points indicate mean daily METH intake over the course of 14 consecutive days (Mean±SEM, n=10). Figure 1B: Data points indicate mean intake within the first hour of a 6 hour session (Mean±SEM, n=10). Rats significantly increased their daily and first hour METH intake over their first session intake when given consecutive days of extended access to METH on an FR1 schedule. Asterisks (*) indicate a significant increase in intake over intake within the first session (p<0.05).
Figure 2 depicts a progressive ratio dose response curve for METH. Responding for various doses of METH on a progressive ratio schedule of reinforcement was recorded. Doses of METH were selected along a logarithmic scale and given in a randomized, Latin-square design. Each subject (n=9) received each dose twice, but never the same dose consecutively. A two-way repeated measures ANOVA failed to find a significant effect of Dose ($F_{3,24}=2.403$, $p=0.092$), Order ($F_{1,24}=0.0319$, $p=0.863$) or a Dose x Order interaction ($F_{3,24}=0.617$, $p=0.611$).

**FIG 2.**

![Progressive ratio dose-response curve](image)

Figure 2. Progressive ratio dose-response curve. Data points indicate PR breakpoints achieved for four different concentrations of METH (Mean±SEM, n=9). Doses were based on body weight measured before the first dose and infusion volume was held constant. Doses were given in a randomized, Latin-square design over the course of eight days (each dose given to each animal twice).

Figure 3 shows the effect of LY379268 on breakpoints obtained under the progressive ratio schedule. LY379268 was given administered (i.p.) 30 minutes before behavioral sessions in
escalated (n=7) and non-escalated animals (n=6). Doses were selected from previous literature (Baptista et al., 2004; Peters and Kalivas, 2006) and given in a randomized order. A two-way repeated measures ANOVA revealed a significant main effect of Dose ($F_{2,18}=36.603$, $p<0.001$). Post hoc analyses indicated that doses of 0.3, and 1.0 mg/kg significantly inhibited METH self-administration (Student-Newman-Keuls, $p<0.05$) compared to Vehicle pretreatment.

**FIG 3.**

Figure 3. LY379268 dose-dependently decreased METH self-administration. Data points indicate PR breakpoints achieved for METH (0.05 mg/kg/inf) after pretreatment with LY379268 (Mean±SEM, n=13 for Baseline and 0.3 mg/kg, n=8 for 1.0 mg/kg). Systemic treatment (i.p.) with two doses of LY379268 (0.3 and 1.0 mg/kg) decreased progressive ratio breakpoints for METH. The efficacy of LY379268 in decreasing METH self-administration was tested in escalated and non-escalated animals (n=7 for escalated, n=6 for non-escalated). There was no significant difference in the effect of LY379268 between groups. Asterisks (*) indicate a significant decrease in responding when compared to baseline ($p<0.05$).
To further analyze how LY379268 affected responding for METH on a progressive ratio schedule of reinforcement, two cumulative records from a representative rat are shown (Figure 4). Figure 4 shows cumulative responses for METH within a 6 hour PR session in an animal treated with Veh (A) and 0.3 mg/kg LY379268 (i.p.) (B). Responding in rats pretreated with LY379268 was typically suppressed when compared to Veh treated animals and resulted in lower breakpoints.

**FIG 4.**

![Graph showing cumulative responses for METH](image)

Figure 4. Effect of LY379268 on PR responding. Two cumulative records from a representative rat pretreated with Vehicle (A, Final ratio of 118) or 0.3 mg/kg LY379268 (B, Final ratio of 50) 30 minutes before responding on a progressive ratio schedule.

Figure 5 depicts the effect of LY379268 on progressive ratio breakpoints in rats responding for food (sucrose pellets, n=8) and METH (n=6). All subjects were treated with Vehicle as well as four doses of LY379268 (0.01, 0.1, 0.3, 1.0 mg/kg, i.p.) given in a randomized
order. A two-way repeated measures ANOVA revealed a significant main effect of Dose ($F_{4,48}=6.699$, $p<0.001$) and a significant Group x Dose interaction ($F_{4,48}=15.333$, $p<0.001$) with post-hoc analyses revealing no effect of LY379268 dose within the food group but significant inhibition of METH self-administration at all doses tested (Student-Newman-Keuls, $p<0.05$).

**FIG 5.**

Figure 5. LY379268 does not affect food responding. Data points indicate PR breakpoints achieved for food (45 mg sucrose pellet) or METH (0.05 mg/kg/inf) after systemic treatment (i.p.) with four doses of LY379268 (0.01, 0.1, 0.3, 1.0 mg/kg) (Mean±SEM, $n=8$ for food, $n=6$ for METH). Pretreatment with LY379268 dose-dependently decreased progressive ratio breakpoints for METH, but had no effect on breakpoints for food. Asterisks (*) denote a significant difference in final ratio from baseline responding and from food responding at the same dose.
DISCUSSION

The present study investigated the effects of a highly selective group II mGluR agonist (LY379268) on METH self-administration in rats. When daily access to METH was increased to 6 hours on an FR1 schedule, rats increased their first-hour and total METH intake significantly by the fourth session when compared to their first session. When given systemically 30 minutes prior to a behavioral session, LY379268 dose-dependently decreased responding on a progressive ratio schedule of reinforcement, regardless of whether the animal had had extended access to METH or not. LY379268 did not have a significant effect on responding for a natural food reinforcer at the doses tested, suggesting a lack of a non-specific effect on reward processing or suppression of general motor behavior.

Extending daily access to a drug has been shown previously to cause a progressive increase in daily intake in rats self-administering cocaine, heroin, methylphenidate, and methamphetamine on similar schedules (Ahmed and Koob, 1998; Ahmed et al., 2000; Kitamura et al., 2006; Schwendt et al., 2009; Krasnova et al., 2010; Marusich et al., 2010; Hadamitzky et al., 2011). Importantly, one of the key features of human addiction is a steady increase in drug intake over time (DSM-IV, American Psychiatric Association, 2000). Escalation may play a significant role in the etiology of METH addiction, as Gonzalez-Castro et al. (2000) found that METH users progress to regular use and loss of control over intake more quickly than cocaine users. Since this extended access animal model replicates one of the major facets of human addiction, it is possible that it also induces similar neuroadaptations as a result of chronic drug exposure (Ahmed and Koob, 2005).

Interest in manipulations of glutamatergic neurotransmission as a therapeutic strategy stems from previously observed neurochemical changes due to drug abuse within the prefrontal
cortex and the involvement of these neuroadaptations in cognition. Drug addiction is often put forth as a cognitive disorder characterized by poor decision-making and dysfunctional motivational circuits (Kalivas and Volkow, 2005), factors which can result in the compulsive use of drugs and loss of control over drug intake (Koob and LeMoal, 2001). These behavioral and biological deficits are thought to be the result of neuroadaptations, specifically reductions in a number of regulatory receptor populations and neurotransmitter release within the orbitofrontal glutamatergic projections to the NAc, caused by extended, repetitive exposure to drugs of abuse (Kalivas and Volkow, 2005). Work by Hyman et al. (2006) reinforces these ideas, suggesting that compulsive drug use is driven by changes in the molecular and cellular mechanisms involved in the forebrain storage and processing of associative memories, particularly the prefrontal cortex and striatum, which receive input from the mesolimbic dopamine neurons. The glutamate homeostasis theory (Kalivas, 2009) suggests that some of these neuroadaptations within the PFC lead to an imbalance between synaptic and non-synaptic glutamate, which in turn dysregulates communication into the NAc. Thus, a current therapeutic strategy may be to target existing receptors and pathways which control glutamate release.

One primary therapeutic target within the glutamatergic system are the Group II metabotropic glutamate receptors. Early studies examining the involvement of glutamate in the effects of cocaine found that glutamate release within the NAc mediates cocaine-seeking behavior (Cornish et al., 1999). Further research revealed the source of this increased NAc glutamate to be afferents from the PFC (McFarland et al., 2003). Mark et al. (2004) showed that the effects of METH on dopamine within the striatum lead to similar increases in extracellular glutamate in PFC-NAc synapses. Specifically, increases in nigrostriatal dopamine by D1 activation increases inhibition within the substantia nigra, in turn removing inhibition along nigrothalamic pathways. This removal of inhibition results in an increase in thalamocortical and
corticostriatal glutamate release (Mark et al., 2004). More recent studies have demonstrated that projections into the NAc co-release glutamate and dopamine (Stuber et al., 2010; Tecuapetla et al., 2010). Group II mGluRs act primarily as inhibitory autoreceptors within these pathways to regulate this release of glutamate and dopamine into the NAc (Conn and Pin, 1997; Hu et al., 1999; Karasawa et al., 2006; Xi et al., 2002a). Previous research has established that agonism of presynaptic group II mGluR decreases glutamate release (Dietrich et al., 2002) and that both systemic treatment and direct microinjections of LY379268 into the NAc inhibits cocaine-seeking in rats (Peters and Kalivas, 2006). Additional microdialysis studies have shown that group II mGluR antagonists increase DA release and group II agonists decrease DA release into the NAc (Karasawa et al., 2006). Thus, agonism at group II mGluRs could reduce the rise in glutamate and dopamine into the NAc following METH administration.

The effects of LY379268 on METH self-administration are consistent with previous studies examining the effects of this compound on other drugs of abuse (Baptista et al., 2004, 2005; Bossert et al., 2005, 2006; Adewale et al., 2006; Peters and Kalivas 2006). Systemic treatment with the group II mGluR agonist LY379268 dose-dependently decreased METH self-administration on a progressive ratio schedule of reinforcement. This is the first time this compound has been examined in animals self-administering METH. Most studies have evaluated LY379268’s ability to decrease reinstatement behaviors to drugs, cues, or contexts (Baptista et al., 2004; Peters and Kalivas, 2006; Bossert et al, 2005; 2006). However, Adewale et al. (2006) found that LY379268 did attenuate cocaine self-administration in squirrel monkeys with significant cocaine histories. Additionally, Baptista and colleagues found that LY379268 dose-dependently attenuated cocaine self-administration in escalated rats, but was only effective at the highest dose in rats with low daily access (Baptista et al., 2005). Our finding that LY379268 dose-dependently decreased breakpoints for METH prior to escalation may indicate
that METH-exposed rats are more sensitive to the effects of group II mGluR agonism, or that METH acts more quickly than other drugs of abuse in dysregulating glutamatergic circuitry within the brain. An examination of the cumulative records of responding on the PR schedule reinforces the idea that systemic treatment with LY379268 attenuated the rewarding effects of METH. During a treatment session, rats typically began responding in the normal manner, but stopped at lower breakpoints and did not attempt to resume responding for the remainder of the 6 hour PR session.

Previous studies examining the efficacy of novel pharmacotherapies for METH addiction have noted shifts in sensitivity to following escalation (Wee et al., 2007; Orio et al., 2010). Studies investigating other drugs of abuse have been unclear as to whether repeated exposure to other psychostimulants such as cocaine induces significant reductions in group II mGluR (Xi et al., 2002b), or increases (Beveridge et al., 2011). However, decreases in basal levels of glutamate in NAc and enhanced ability of psychostimulants to increase glutamate and dopamine within the NAc have been observed (Pierce et al., 1996; Xi et al., 2002b). These neuroadaptations could have an effect on the ability of LY379268 to modulate neurotransmitter release and attenuate the reinforcing effects of METH in escalated animals. While we did not observe any behavioral differences between the effects of LY379268 in escalated and non-escalated animals, chronic METH exposure could still have effects on the sensitivity of animals to the compound, via changes in protein expression.

Similar to previous observations by Baptista et al. (2004) in rats responding for sweetened condensed milk, LY379268 did not affect responding for food (sucrose pellets) at the doses tested. This is important because research has shown that LY379268 is associated with a nonspecific motor-depressant effect (Cartmell et al., 1999) and can inhibit responding for
natural rewards at higher doses (3.0 mg/kg; Peters and Kalivas, 2006). While this dose is somewhat higher than those used in the present study, caution against the direct utility of LY379268 as a therapeutic is warranted. Such findings may indicate a relatively narrow therapeutic window in which treatment would be well tolerated.

Future studies should seek to examine the efficacy of chronic treatment with LY379268 in attenuating METH self-administration, as well as the ability of LY379268 to reduce cue-, context-, and drug-induced reinstatement behaviors in METH exposed animals. Additionally, it would be interesting to examine whether chronic METH exposure significantly reduced levels of group II mGluR within the brain, particularly in the NAc.

In summary, systemic pretreatment with the specific group II mGluR agonist LY379268 dose-dependently decreased METH self-administration on a progressive ratio schedule. A significant loss of sensitivity to the compound was not observed in escalated animals, contrary to findings which have evaluated dopaminergic compounds as therapeutics. LY379268 did not affect PR responding for food at the doses tested, suggesting a possible therapeutic window in which it could operate effectively. This study extends the inhibitory effects of LY379268 on self-administration behaviors to METH and reinforces the notion that group II mGluR may be a potential pharmacotherapeutic target.

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DISCLOSURE
The authors declare no conflicts of interest.
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RESEARCH SKILLS:

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Faculty Teaching Award Committee Co-Chair – 2011 – Helped lead a student committee which nominates and determines the recipient of the Neuroscience Faculty Teaching Award.

INVITED ADDRESSES:

Crawford JT. The role of glutamate receptors in methamphetamine self-administration. Talk presented at Wake Forest University School of Medicine, Winston-Salem, NC. Spring 2011.

BIBLIOGRAPHY:

JOURNAL ARTICLES:
Crawford JT, Beveridge TJR, Roberts DCS. The group II metabotropic glutamate agonist, LY379268, decreases methamphetamine self-administration in rats. (in preparation).

**POSTER PRESENTATIONS:**

*Single Exposure Conditioned Place Preference to EtOH in C57BL/6J Mice is Opiod Mediated.* Poster Presentation at 38th meeting of the Society for Neuroscience, November 2008, Washington D.C.

*Differing Sensitivities in Wild-Type C57BL/6J and B-Endorphin Deficient C57BL/6J Mice to Drugs Affecting the GABA-A Receptor are due to Fundamental Differences in GABA-A Receptor Makeup.* Poster Presentation at SYNAPSE conference, College of Charleston, March 2009 and at Furman Engaged conference, April 2009.

*Preferred Dose and Speed of Cocaine or Methamphetamine Revealed by a Novel Self-Administration Procedure.* Poster Presentation at 40th meeting of the Society for Neuroscience, November 2010, San Diego, CA.

*LY379268, a selective group II metabotropic glutamate receptor agonist, dose-dependently decreases methamphetamine self-administration in rats.* Poster Presentation at 73rd meeting of The College on Problems of Drug Dependence, June 2011, Hollywood, FL.

**ABSTRACTS:**


Crawford JT and Roberts DCS. *Rats self-administer high doses of methamphetamine on a novel hold-down procedure.* SfN. 67.11, November 2010.

Crawford JT, Roberts DCS, Beveridge TJR. *LY379268, a selective group II metabotropic glutamate receptor agonist, dose-dependently decreases methamphetamine self-administration in rats.* CPDD. June 2011.

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