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ROLE OF GROUP II METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 2 (MGLUR2) IN APPETITIVE AND CONSUMMATORY ASPECTS OF ETHANOL REINFORCEMENT

For the degree of Doctor of Philosophy

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ROLE OF GROUP II METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 2 (MGLUR2) IN APPETITIVE AND CONSUMMATORY ASPECTS OF ETHANOL REINFORCEMENT

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of

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Kyle Allyson Windisch

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For my grandfather

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ABSTRACT

Windisch, Kyle A. Ph.D., Purdue University, December 2014. Role of Group II Metabotropic Glutamate Receptor Subtype 2 (mGluR2) in Appetitive and Consummatory Aspects of Ethanol Reinforcement. Major Professor: Cristine Czachowski.

Background: Group II metabotropic glutamate receptors (mGluR2/3) are predominately presynaptically located $G_{i/o}$ coupled receptors that are highly expressed in the cortex, nucleus accumbens, amygdala, and hippocampus. Previous studies suggest that group II mGluRs are involved in regulating ethanol (EtOH) consumption and seeking following extinction (Backstrom and Hyytia, 2005; Kufahl, et al., 2011). The sipper tube model, which allows for procedural separation of seeking and consumption, was used to further clarify the role of mGluR2/3 in EtOH-seeking and consumption. The non-selective group II mGluR agonist LY379268 (LY37) and selective mGluR2 positive allosteric modulator (PAM) BINA were used to determine the relative contribution of mGlu2 and mGlu3 receptors on EtOH seeking and consumption. Following characterization of the agonist and PAM on EtOH reinforcement, a microinjection study was performed examining the effect of blockade of nucleus accumbens core mGluR2/3 on systemic agonist induced suppression of EtOH-seeking.

Methods: For the systemic agonist/PAM experiments, separate groups of male Wistar rats [n=8-9 group; LY37 (0-2.0 mg/kg) and BINA (0-20 mg/kg)] were trained to complete a

response requirement (RR) of 10 lever presses that resulted in access to 10% EtOH or 2% sucrose (in separate groups) for a 20-minute drinking period. For consummatory testing, animals received weekly drug injections with a RR1. The RR was then increased over sessions to a RR20. For appetitive testing, animals received weekly drug injections followed by a non-reinforced extinction session. To determine effects of blockade of NAc core mGluR2/3 receptors on agonist-induced suppression of EtOH-seeking, a separate group of male Wistar rats (n=15) was trained to complete a RR10 for access to 10% EtOH. Animals were surgically implanted with bilateral guide cannulae terminating 1mm above the NAc core. Following recovery, animals received four sets of microinjections in a balanced design (systemic vehicle + core vehicle, systemic LY37 + core vehicle, systemic LY37 + core LY34, and systemic vehicle + core LY34). A final non-balanced microinjection of LY37 was then performed.

Results and Conclusions: Systemic administration of the mGluR2/3 agonist LY37 significantly reduced EtOH- and sucrose- seeking with no systematic effect on locomotion. Systemic administration of the selective mGluR2 PAM BINA had no significant effect on either seeking or consumption. These findings suggest that modulation of glutamatergic neurotransmission by a systemic mGluR2/3 agonist, but not allosteric modulation of mGluR2, significantly reduces reinforcer seeking. Intra-accumbens core administration of LY37 significantly reduced EtOH-seeking, suggesting a role of NAc core mGluR2/3 modulation in EtOH-seeking during maintenance drinking. Systemic administration of LY37 was also found to significantly reduce sucrose consumption and body weight 24-hours following systemic administration, meriting further examination of the role of mGluR2/3 receptors on feeding behavior.

CHAPTER 1. INTRODUCTION

1.1 Introduction

Drug addiction has a dramatic and wide reaching impact on society. Currently few options are available for the treatment of addiction. The glutamatergic system is a growing area of interest in addiction research as a point for pharmacological intervention for the treatment of drug addiction.

1.2 Effect of EtOH Administration on Glutamate

Ethanol (EtOH) has been shown to influence glutamate following both acute and chronic administration. Acute administration of low to moderate EtOH doses (0.5 – 1 g/kg) has been shown to increase extracellular glutamate concentrations in the ventral tegmental area (VTA) (Ding, et al., 2012), nucleus Accumbens (NAc) (Selim and Bradberry, 1996), and hippocampus (Moghaddam and Bolinao, 1994). Elevated extracellular glutamate concentrations have also been shown during EtOH withdrawal. In particular, elevated levels of glutamate were observed in the NAc following repeated EtOH administration (1 g/kg/day for seven days) (Melendez, et al., 2005). Elevated extracellular glutamate has also been shown in the hippocampus during withdrawal from 4-weeks of chronic ethanol vapor treatment (Dahchour and De Witte, 1999; Dahchour and De Witte, 2003) and in the dorsal hippocampus during withdrawal following six days

of systemic EtOH administration (3.4 g/kg/day) (Chefer, et al., 2011). As well, elevated basal extracellular glutamate levels were observed in both the NAc shell and posterior VTA of rats following voluntary two-bottle choice home cage drinking for eight weeks compared to water drinking control (Ding, et al., 2013). This suggests that acute and chronic EtOH affects glutamatergic signaling particularly within the hippocampus, NAc, and VTA.

The influence of EtOH on glutamatergic neurotransmission was further characterized by Meinhardt, et al. (2013). Following two weeks of EtOH withdrawal after dependence induction using chronic intermittent vapor exposure (seven weeks with 5 cycles of 14 hour EtOH vapor exposure), a marked decrease in transcription of metabotropic glutamate receptor subtype 2 (mGlu2) was noted in the infralimbic cortex (IL), with no change in subtype 3 (mGlu3) transcription, in "post-dependent" Wistar rats. Escalated EtOH self-administration and cue-induced reinstatement of EtOH-seeking was also observed in these "post-dependent" animals. Increasing IL mGlu2 receptor expression in the "post-dependent" animals via lentiviral transfection resulted in a reduction of cue-induced reinstatement of EtOH seeking to that of control animals. This suggests that protracted EtOH exposure influences not only glutamate release, but also glutamate receptor expression. As well, these findings suggest that modulation of glutamate receptors can influence EtOH-seeking and consumption.

1.3 Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors (GPCR) activated by glutamate that consist of seven transmembrane regions with three

extracellular loops and two intracellular loops. Eight different mGluR subtypes have been identified (mGLuR1-8) and are separated into three groups based on their sequence homology, molecular structure, and signal transduction properties (Conn and Pin, 1995). The group I family of mGluRs consist of mGlu1 and mGlu5 receptors. The group II family of mGluRs consist of mGlu2 and mGlu3 receptors. And the group III family of mGluRs consist of mGlu4, mGlu6, mGlu7, and mGlu8 receptors. Group I receptors are predominately postsynaptically located G_q coupled receptors that lead to enhanced neuronal excitation when activated (Cartmell and Schoepp, 2000). Both Group II and Group III mGluR are associated with Gi/o G-proteins and decrease adenylyl cyclase (Olive, 2009). Due to the development of selective agonists and antagonists for group I and II mGluRs, these groups have been the focus of recent investigation, particularly in regard to their role in regulating various aspects of drug reinforcement.

1.4 Group II mGluR

The Group II mGluR family consists of the mGlu2 and mGlu3 receptor subtypes. mGlu2 receptors have been shown to be predominately presynaptically located while mGlu3 receptors are expressed pre- and postsynapically as well as on glial (Ohishi, et al., 1998; Tamaru, et al., 2001; Xi, et al., 2002). Both mGlu2 and mGlu3 receptors are highly expressed in the cortex, NAc, striatum, amygdala, and hippocampus. Presynaptically, group II mGluRs are predominately expressed outside of the synaptic cleft on axons and negatively regulate synaptic glutamate release (Anwyl, 1999; Scanziani, et al., 1997; Xi, et al., 2002). Given their expression in brain regions associated with drug reinforcement

and regulation of excitatory neurotransmission, group II mGluR agonists have been examined for possible involvement in regulating drug reinforcement.

1.5 Group II mGluR Agonists Effect on Reinstatement, Relapse, And Self-Administration

Several studies have examined the effect of systemic administration of non-selective Group II mGluR agonists on reinstatement, relapse, and self-administration for various drugs of abuse. As each of these models address separate aspects of drug reinforcement, they will be considered individually.

1.5.1 Group II mGLuR Agonists Effect on Reinstatement

Reinstatement models of drug-seeking involve the resumption of responding for a previously extinguished reinforcer following exposure to either previously paired drug stimuli (cue-induced reinstatement), presentation of a mild stressor (stress-induced reinstatement), or non-contingent exposure to the reinforcer (drug-primed reinstatement) (See Le and Shaham, 2002 for a review of reinstatement models). In these experiments animals are first trained to respond (typically using a fixed ratio schedule) for access to the reinforcer. Once stable responding is acquired the response behavior is then extinguished either to a set criteria or duration. Following extinction, the animals have a test session in which they are exposed to previously paired stimuli, a stressor, or non-contingent reinforcer presentation prior to an extinction session. Animals can emit two forms of behavioral responses during this test session (active and inactive lever/nose-

poke response). Active lever responding is thought to suggest reinstatement of drug-seeking while inactive lever responding reflects nonspecific activity.

1.5.1.1 Group II mGLuR Agonists Effect on Cue-Induced Reinstatement

Several studies have examined the effect of systemic administration of mGluR2/3 agonists on cue-induced reinstatement of drug-seeking. The non-selective group II mGluR agonist LY379268 (LY37) has been shown at 1 and 3 mg/kg doses to decrease cue-induced reinstatement for heroin in male Long Evans rats initially trained to self-administer heroin (0.05-0.1 mg/kg/infusion, iv) on a fixed ratio 1 (FR1) schedule (Bossert, et al., 2004 and Bossert, et al., 2005). LY37 has also been demonstrated to reduce nicotine seeking at 1 and 3 mg/kg in male Wistar rats trained to administer nicotine (0.03 mg/kg/infusion; iv) on a FR5 schedule (Liechti, et al., et al., 2007). Baptista, et al. (2004) similarly demonstrated that in Wistar rats trained for ten 2-hr operant sessions to self-administer cocaine (0.25 mg/0.1 mL; iv) LY37 (1 and 3 mg/kg) significantly decreased cue-induced reinstatement of cocaine-seeking. Cue-induced reinstatement of EtOH seeking has also been shown to be reduced following systemic administration of LY37 (1 and 3 mg/kg dose) in male Wistar rats initially trained to self-administer EtOH (0.1 mL 10% EtOH following saccharin fade; oral consumption) on a FR1 schedule. This effect of LY37 on cue-induced reinstatement was further demonstrated by Kufahl, et al. (2011). In this study, male Wistar rats were initially trained to self-administer oral EtOH [0.1 mL 10% EtOH following “super sac” (solution of 3% glucose and 0.125% saccharin) fade] then underwent chronic intermittent vapor exposure for either a single withdrawal (12

consecutive days vapor exposure) or repeated withdrawals (four cycles of three days of 14-hr on/10-hr off with five days of withdrawal between cycles). Systemic administration of LY37 (1 and 3 mg/kg) in air exposure control rats significantly decreased cue-induced reinstatement to EtOH seeking. For animals exposed to either a single withdrawal or repeated withdrawals, a more profound reduction in cue-induced reinstatement was observed. In these withdrawal animals, systemic administration of 0.3 mg/kg LY37 (in addition to the 1 and 3 mg/kg doses) significantly reduced EtOH seeking. Overall, LY37 has been shown to reduce cue-induced restatement of drug seeking for drugs of abuse with different mechanisms of action (nicotine, heroin, cocaine, and EtOH) and different routes of administration (iv versus oral) as well as in different breeds of rats (Long Evans and Wistar).

1.5.1.2 Group II mGLuR Agonists Effect on Drug-Primed Reinstatement

Drug-primed reinstatement of seeking involves non-contingent presentation of the reinforcer following extinction training and can result in resumption of drug-seeking. Peters and Kalivas (2006) observed a substantial increase in seeking responding following non-contingent priming injections of cocaine (10 mg/kg, ip) in food deprived male Sprague-Dawley rats that were trained to lever respond on a FR5 schedule for access to a cocaine reinforcer (0.3 mg/kg) prior to extinction. Systemic administration of LY37 (0.3, 1, and 3 mg/kg) dose-dependently reduced cocaine-primed reinstatement of cocaine-seeking. This suggests an involvement of group II mGluRs in regulating cocaine-primed reinstatement of cocaine-seeking.

1.5.1.3 Group II mGLuR Agonists Effect on Stress-Induced Reinstatement

Stress-induced reinstatement of seeking involves the presentation of a mild stressor (e.g., foot-shock) following extinction training, typically resulting in an escalation of reinforcer seeking. Zhou. et al. (2006) demonstrated that mild foot-shock significantly increased EtOH-seeking in male Wistar rats previously trained to self-administer EtOH on a FR1 schedule (0.1 mL 10% EtOH following saccharin fade). Systemic administration of LY37 (1 and 3 mg/kg) was shown to significantly decrease this stress-induced reinstatement of EtOH-seeking. Similarly, Sidhpura, et al. (2010) demonstrated that LY37 (1 and 3 mg/kg) decreased foot-shock induced reinstatement of EtOH-seeking in non-dependent male Wistar rats. Sidhpura and colleagues also found that 0.3 – 3 mg/kg LY37 was effective in decreasing foot-shock induced reinstatement of EtOH-seeking in "postdependent" rats (dependence induced by intragastric EtOH administration four times daily for five consecutive days, followed by 2-weeks withdrawal). Together these findings suggest that agonism of mGlu2/3 receptors by LY37 decreases stress-induced reinstatement of drug seeking.

1.5.2 Group II mGLuR Agonists Effect on Relapse

Relapse is the phenomenon in which reinforcer seeking is augmented when animals are returned to the drug-paired context following a period of forced abstinence without undergoing extinction learning. Lu, et al. (2007) observed a significant increase in non-reinforced (i.e., extinction responding) following a 21-day abstinence period from cocaine but not for a 3-day abstinence period in male Long Evans rats previously trained to operantly self-administer cocaine (0.75 mg/kg/infusion, iv) on a FR1 reinforcement

schedule for ten sessions. Systemic LY37 administration (1.5 and 3 mg/kg) significantly decreased relapse responding in animals with 21-day abstinence period, but there was no effect of LY37 on relapse responding in animals with a 3-day abstinence period. Cannella, et al. (2013) observed a similar escalation in relapse responding for cocaine following a forced 30-day abstinence period in male Sprague-Dawley rats previously trained to operantly self-administer cocaine (0.8 mg/kg/infusion, iv) on a FR5 schedule. Following the protracted self-administration, Cannella scored the rats for addiction-like criteria (motivation for cocaine-taking measured using progressive ratio, persistence of cocaine seeking measured during single extinction session, resistance to punishment measured using a single session with foot-shock paired with reinforcer administration). Following scoring, rats negative for all criteria ("non-addict-like") and positive for all criteria ("addict-like") underwent a forced 30-day abstinence period. Systemic administration of LY37 (0.3 and 3 mg/kg) following the forced abstinence period significantly reduced relapse responding in both the "addict-like" and "non-addict-like" groups. These studies suggest that activation of group II mGluRs via systemic agonist administration diminishes the expression of incubated cocaine craving.

1.5.3 Group II mGLuR Agonists Effect on Drug Self-Administration

In addition to affecting reinforcer seeking, administration of the non-selective mGLuR2/3 agonist LY37 has also been shown to decrease operant self-administration of several drugs of abuse. Systemic administration of LY37 reduced nicotine self-administration (1 and 3 mg/kg dose) in male Wistar rats trained to administer nicotine (0.03 mg/kg/infusion; iv) on a FR5 schedule (Liechti, et al., 2007). Jin et al. (2010)

observed that 3 and 6 mg/kg LY37 decreased cocaine self-administration in male Wistar rats trained to administer cocaine (0.5 mg/kg/infusion) on a FR1 schedule. Sidhpura, et al. (2010) demonstrated that LY37 (3 mg/kg) decreased EtOH self-administration in non-dependent male Wistar rats trained to administer EtOH on a FR1 schedule. In "postdependent" rats (dependence induced by intragastric EtOH administration four times daily for five consecutive days, followed by 2-weeks withdrawal) 0.3 – 3 mg/kg LY37 was effective in decreasing EtOH self-administration. 5 mg/kg LY37, but not 1 or 3 mg/kg, has been shown to decreased EtOH self-administration in male Long Evans rats trained to administer EtOH (0.1 mL 10% EtOH following saccharin fade) on a FR1 schedule (Backstrom and Hyytia, 2005). Similarly, systemic administration of 1 and 3 mg/kg of LY37 had no effect on heroin self-administration in male Long Evans rats trained to self-administer heroin (0.05-0.1 mg/kg/infusion, iv) on a fixed ratio 1 (FR1) schedule (Bossert, et al., 2005). Overall, these findings suggest that systemic administration of the mGluR2/3 agonist LY37 reduces operant self-administration of nicotine, cocaine, and EtOH.

1.5.4 Group II mGLuR Agonists Effect on Alternative Reinforcers and Locomotion

Ideally, a pharmacological treatment for drug dependence should selectively affect drug reinforcement (e.g., EtOH-seeking) without affecting seeking and consumption of natural reinforcers (e.g., sucrose) or locomotor behavior. Several studies have examined the effect of systemic LY37 administration on alternative reinforcers and spontaneous locomotor activity. Systemic LY37 was shown to decrease food-primed reinstatement of food seeking in food deprived male Wistar rats at the highest dose tested

(3 mg/kg) (Peters and Kalivas, 2006). Jin et al. (2010) observed that 6 mg/kg LY37 decreased food self-administration in non-deprived male Wistar rats trained to administer food (45 mg food pellets) on a FR1 schedule. Similarly, systemic administration of LY37 reduced food self-administration (3 mg/kg dose) in food-deprived male Wistar rats trained to administer food (45 mg food pellets) on a FR5 schedule (Liechti, et al., et al., 2007). As well, Kufahl et al. (2011) demonstrated that systemic administration of LY37 (3 mg/kg) reduced self-administration in non-deprived male Wistar rats trained to administer super sac (3% glucose and 0.125% saccharin) on a FR1 schedule. Overall, systemic LY37 administration (3-6 mg/kg) appears to significantly decrease seeking and consumption of food reinforcers.

The doses of LY37 shown to reduce seeking and consumption of food reinforcers have also been shown to have a general effect on locomotion. Systemic administration of LY37 was shown to reduce spontaneous locomotor behavior at 3 mg/kg in male Wistar rats (Kufahl, et al., 2011) and 5 mg/kg in male Long Evans rats (Backstrom and Hyytia, 2005). As the previously observed suppression in alternative reinforcer self-administration is at doses that have also been shown to reduce locomotor behavior, the effect of systemic administration of LY37 on alternative reinforcer seeking and consumption may potentially be due to the non-specific effects of LY37 on motor behavior.

1.5.5 Group II mGLuR Agonists Conclusions

Overall, previous experiments using systemic administration of the non-specific group II mGluR agonist LY37 suggest that group II mGluRs play a role in regulating

drug seeking during protracted withdrawal or following extinction. The role of group II mGluRs in drug consumption are more difficult to interpret. Although systemic administration of the mGluR2/3 agonist LY37 has been shown to reduce nicotine, cocaine, and EtOH self-administration (Backstrom and Hyytia, 2005; Baptista, et al., 2004; Liechti, et al., 2007; Jin, et al., 2010; Sidhpura, et al., 2010), these studies used a FR schedule of reinforcement (FR1-FR5) which requires animals to engage in a seeking response prior to consumption of a small dose of the reinforcer across the duration of the session. This results in a mixture of seeking and consumption that does not allow one to observe the effect of treatment on consumption specifically. Use of an alternative model, such as the sipper tube model (further discussed in section 1.4: Sipper Tube Model), that allows for procedural separation of seeking from consumption would better allow for analysis of the effects of pharmacological treatment on consumption. As well, the sipper tube model allows for the examination of seeking behavior during maintenance drinking without prior extinction training or protracted forced abstinence.

1.5.6 Role of mGluR2 versus mGluR3

Growing evidence suggests that the preclinical effects of systemic administration of Group II mGluR agonists, such as LY37, on decreasing self-administration and reinstatement of drug-seeking are not due to equal contributions of mGlu2 and mGlu3 receptor modulation. Rather, recent studies suggest that mGlu2 receptors, but not mGlu3 receptors, are responsible for the observed regulation in drug-seeking and consumption. While mGlu3$^{-/-}$ receptor knockout mice display normal cocaine self-administration, extinction, and reinstatement responding compared to C57/BL6J mice (Cannella, et al.,

2013), mGlu2$^{-/-}$ receptor knockout mice display significantly increased conditioned place preference (CPP) for cocaine (Morishima, et al., 2005). mGlu2$^{-/-}$ receptor knockout mice also demonstrate an increased preference for and consumption of EtOH without a significant difference in saccharin or quinine consumption (Zhou, et al., 2013). This suggests that loss of functional mGlu3 receptors does not affect drug seeking or self-administration; however, loss of mGlu2 receptors increases preference for and consumption of drugs of abuse.

The Indiana alcohol-preferring (P) rat was recently shown to be homozygous for a missense mutation in the coding region of the mGlu2 receptor (*Grm2**407) that results in a loss of receptor functionality (Zhou, et al., 2013). Similar to P rats, the selected Roman High-Avoidance (RHA) rats consume moderately elevated levels of EtOH (3.5 g/kg/day) (Fernandez-Teruel, et al., 2002) and have a significant reduction in mGluR2 expression in the frontal cortex compared to Roman Low-Avoidance (RLA) rats (Klein, et al., 2014). It is possible, though speculative, that the escalated EtOH consumption observed with RHA and P rats is due to the decrease in/lack of function mGlu2 receptors.

Increased sequence homology between mGlu2 and mGlu3 receptors has made generation of group II mGluR subtype specific orthosteric agonists/antagonists elusive. However, several subtype-specific positive allosteric modulators (PAMs) of mGlu2 receptors have been generated (Galici, et al., 2006). Of these, biphenyl indanone-A (BINA) has shown promise for reducing escalated drug consumption. Using male Wistar rats with either short (1 hr) or long (6 hr) daily operant sessions, Jin et al., (2010) demonstrated that systemic administration of BINA (20 and 40 mg/kg) suppresses cocaine self-administration without an effect on food self-administration. Similarly,

Dhanya et al. (2011) demonstrated that a modified version of BINA (substitution of chlorine and benzisothiazolone) decreased cocaine self-administration at doses of 20 and 40 mg/kg. However, the effect of positive allosteric modulation of mGluR2 on ethanol seeking and consumption has yet to be examined.

1.6 Sipper Tube Model

The previous studies examining the effect of systemic group II mGluR agonists and PAM administration on consumption of drugs of abuse used operant fixed ratio schedules (Backstrom and Hyytia, 2005; Bossert, et al., 2005; Jin, et al., 2010; Liechti, et al., 2007; Sidhpura, et al., 2010). The use of a FR schedule results in the animals emitting a seeking response for an incremental unit of the reinforcer, yielding a mixture of seeking and consumption. Therefore, an FR schedule does not allow one to specifically address the effect of the agonist on consumption.

In contrast to FR schedules, the sipper tube model allows for discrete procedural separation of seeking from consumption. In this model, animals are allowed 20-minutes to emit a set number of responses (response requirement). Once this response requirement (RR) is met, the lever is retracted and a sipper tube is inserted into the chamber. The animal then has 20 minutes of unrestricted access to the reinforcer. Consumption is assessed during weekly test sessions during which the RR is set to one lever press so that minimal effort is required to gain access to the reinforcer but a response is still required. Separately, seeking is tested during weekly extinction sessions during which the animal does not gain access to the reinforcer regardless of the number

of lever presses emitted. The sipper tube model allows for the separate analyses of pharmacological manipulation on reinforcer seeking and consumption.

1.7 Rationale

The purpose of the first set of experiments was to examine the effect of systemic administration of the non-selective Group II mGluR agonist LY37 on EtOH seeking and consumption during maintenance drinking using the sipper tube model. As there is increasing evidence that the effect of non-selective group II mGluR agonists on EtOH reinforcement is predominately due to agonist activation of mGlu2 receptors, the contribution of modulation of mGluR2 specifically on seeking and consumption was also performed. For this the mGluR2 positive allosteric modulator biphenyl indanone-A (BINA) was used to assess the effect of mGluR2 modulation on EtOH-seeking and consumption during maintenance drinking. Based on the previous findings using systemic LY37 and BINA administration, we hypothesized that systemic modulation of group II mGluRs, by either orthosteric agonist or positive allosteric modulation of mGluR2 specifically, would significantly reduce EtOH seeking with no effect on sucrose seeking in non-deprived Wistar rats during maintenance drinking using a range of doses below those (3-6 mg/kg) previously shown to affect locomotion and alternative reinforcer seeking and consumption. As well, we hypothesized that neither systemic LY37 nor BINA would significantly reduce consumption of either EtOH or sucrose in the sipper tube model. Furthermore, given the increasing evidence that the reduced EtOH-seeking observed with systemic LY37 is due to the agonist's action at mGlu2 but not mGlu3 receptors, we hypothesized that modulation of mGlu2 receptors via systemic

administration of the selective mGluR2 PAM BINA would result in a similar reduction in EtOH-seeking as observed with the mGluR2/3 agonist LY37. A subsequent experiment was performed to begin to examine the neurocircuitry involved in group II mGluR modulation of EtOH-seeking.

CHAPTER 2. EXPERIMENT 1: SYSTEMIC GROUP II MGLURS AGONISTS AND MGLUR2 PAM

2.1 Materials and Methods

2.1.1 Animals

Thirty-six male Wistar rats, weighing 169 - 207 g at the beginning of the experiment, were single housed on a 12-hour light/dark cycle (lights on at 0500). Animals had ad libitum access to both food and water except for a mild water restriction during the first week of operant training. Animal care and procedures were in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee (IACUC).

2.1.2 Apparatus

Sessions were conducted daily (5 days/week) in operant chambers (30x30x24.5 cm; Med-Associates, St Albans, VT). Chambers were located in sound attenuated enclosures with exhaust fans to mask external noise. The chambers were equipped with a house light, a single retractable lever, and a single retractable graduated sipper tube located on the wall opposite the lever. The sipper tube consisted of a graduated cylinder tube with a rubber stopper and stainless steel tube with two ball bearings to prevent leakage. Med-Associates software was used to control input and output from each chamber.

2.1.3 Training

Upon arrival, animals were weighed and handled twice during the week preceding initial training. Sessions were conducted at the same time daily (starting at 0900) during the lights on portion of the light/dark cycle. During initial training, animals underwent a brief (14-18 hr) water deprivation prior to the first training session, followed by a mild 2-4 day water restriction to facilitate acquisition of lever-press responding. Food and water were available ad libitum for the remainder of the testing.

Separate groups of rats (n=17-18; LY37 and BINA) were initially trained to lever press on a FR1 schedule for 15 seconds of access to a 10% oral sucrose reinforcer (See Figure 2.1: Overview of Initial Training). Once lever press was acquired (1-3 sessions), the schedule was increased gradually over sessions to a final FR4 schedule while the sucrose was gradually reduced using a modified sucrose-fade procedure (Samson, 1986). For the sucrose-fade, over a 3-week period, the sucrose concentration was gradually reduced over sessions while EtOH was gradually faded into the solution (for EtOH groups). Final reinforcer concentrations were 2% sucrose (sucrose groups) and 10% EtOH (EtOH groups).

The FR4 schedule was then discontinued and a RR was implemented allowing for procedural separation of seeking from consumption. For this, animals had 20 minutes to complete the RR (initially 4 lever presses). Once the RR was met, the lever was retracted and the sipper tube was inserted into the chamber. Animals then had 20 minutes of unrestricted access to the reinforcer. The RR was gradually increased over sessions to a final RR of 10 lever presses.

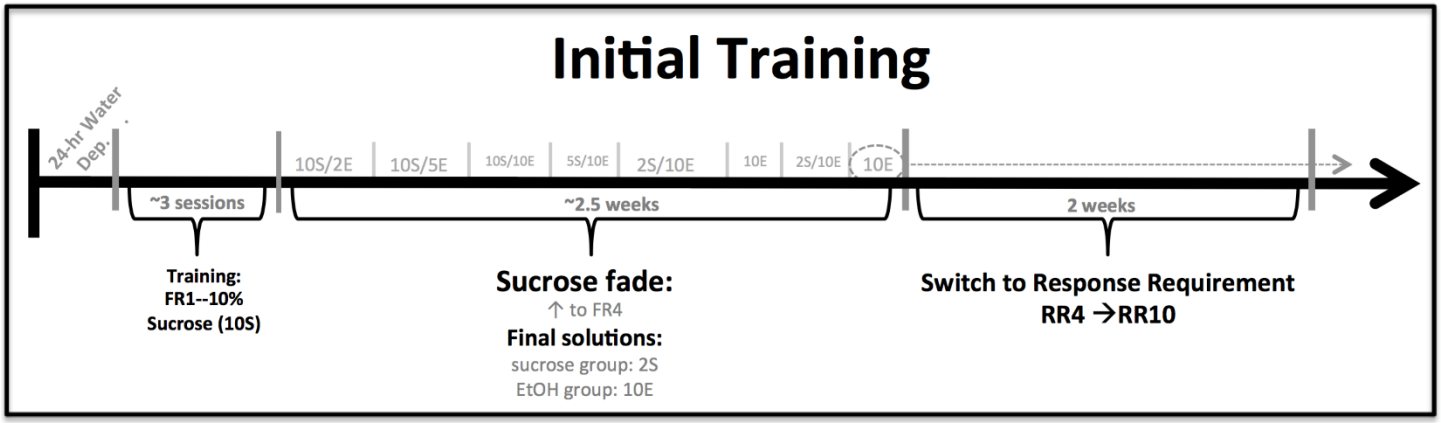

Figure 2.1: Overview of Initial Training

2.1.4 Drinking Test Phase

Following training, animals underwent a six-week Drinking Test Phase. Animals had once weekly test sessions on Thursday with a RR of 1 lever press so that minimal effort was required to gain access to the reinforcer (See Figure 2.2: Overview Drinking and Seeking Test Phases). The other four sessions were non-injection sessions with a RR of 10. Animals were first habituated to the test procedure with a systemic vehicle injection. Animals then received IP drug injections (0.0, 0.3, 1.0, 1.5, and 2.0 mg/kg LY37; 0, 5, 10, and 20 mg/kg BINA) in a balanced design. Following the drinking test, phase animals had a three-week period during which no drugs were administered and the RR was gradually increased from 10 to 20 lever presses.

2.1.5 Seeking Test Phase

Animals then underwent a six-week Seeking Test Phase using the same vehicle habituation, followed by weekly drug injections with doses administered in a balanced design. During the weekly test session, systemic drug injection was followed by a non-reinforced extinction session. During the extinction session, animals had 20 minutes of access to the lever, but did not gain access to the reinforcer. To control for possible scent cues, filled bottles were placed on the retracted holders. Animals had weekly reinforced vehicle injection sessions (on Tuesdays) to reduce the likelihood of systemic injection predicting an extinction session. The other three sessions were normal reinforced sessions.

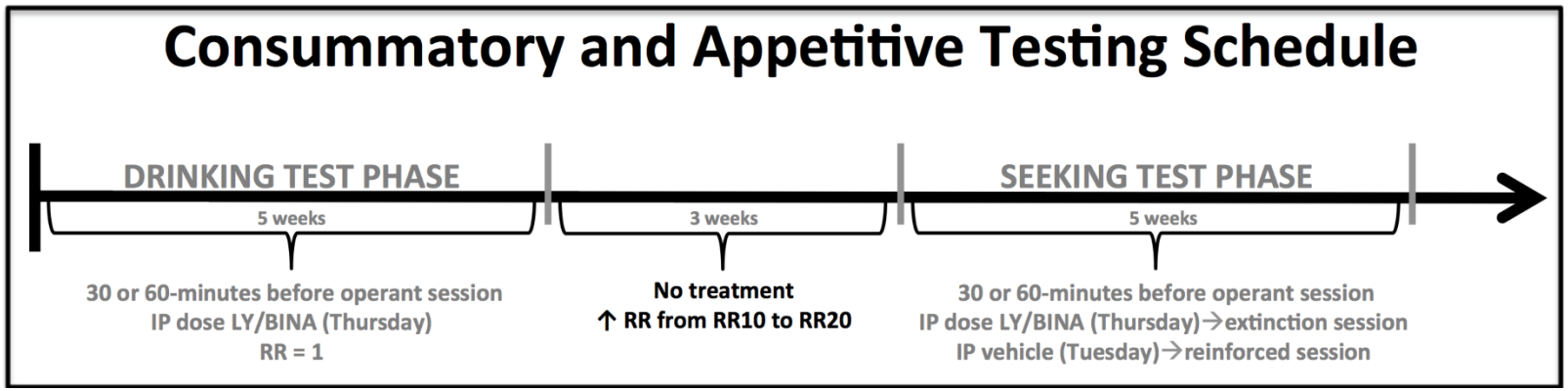

Figure 2.2: Overview of Drinking and Seeking Test Phases

2.1.6 Drugs

Ethanol solutions were prepared volume/volume in water using 95% ethanol. Sucrose and sucrose/ethanol solutions were prepared weight/volume. The non-selective group II mGluR agonist LY379268 [(1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid] (Santa Cruz Biotechnology, Inc., Dallas, TX) was dissolved in sterile 0.9% saline and injected at a volume of 1.0 mL/kg body weight (BW). The selective mGluR2 positive allosteric modulator BINA [Biphenyl-indanone A (3'-[[(2-Cyclopentyl-2,3-dihydro-6,7-dimethyl-1-oxo-1*H*-inden-5-yl)oxy]methyl]-[1,1'-biphenyl]-4-carboxylic acid)] (Santa Cruz Biotechnology, Inc., Dallas, TX; Tocris Bioscience, Minneapolis, MN) was dissolved in 0.5% dimethyl sulphoxide (DMSO) and 1% sodium hydroxide (NaOH) diluted with sterile water and titrated to a final pH of 7.4 using 1% lactic acid and injected at a volume of 5 mL/kg BW. Sterile saline was used as vehicle treatment for LY37 and injected at a volume of 1.0 mL/kg BW. Sterile water plus 0.5% DMSO and 1% NaOH titrated to a final pH of 7.4 using 1% lactic acid was used as vehicle treatment for BINA and administered at a volume of 5.0 mL/kg BW. LY37 (0-2.0 mg/kg) and BINA (0-20 mg/kg) were administered intraperitoneally (ip) 30 and 60 minutes prior to the operant session, respectively. Pretreatment times, drug doses, and injection volumes, and route of administration for each drug were based on previously studied efficacious methods (Ahnaou et al., 2009; Backstrom and Hyytia, 2005; Benneyworth et al., 2007; Cannady et al., 2011; Galici et al., 2006; Hackler et al., 2010; Kufahl et al., 2011).

2.1.7 Data Analysis and Statistics

Daily session intakes of EtOH and sucrose were determined from the change in volume in the sipper tube (mL). EtOH intake (g/kg) and sucrose intake (mL/kg) were calculated from session intake and daily BW measures. Total lever presses and latency to first lick (in seconds) were recorded for each session. EtOH and sucrose consumption data were analyzed separately using one-way within-subject repeated measures analysis of variance (RM ANOVA). Post-hoc comparisons were performed using Student-Newman-Keuls test ($p<0.05$). Appetitive responding, lick latencies, and body weight differences (BW 24-hrs post-injection minus BW 1 hr prior to injection) were analyzed using two-way RM ANOVAs with dose and reinforcer as factors. The effect of systemic treatment on reinforcer intake (comparing intake 24 hrs following systemic administration to intake 24 hrs prior to systemic administration) for each reinforcer was analyzed using two-way RM ANOVAs with dose and day as factors. Post-hoc comparisons were performed using Student-Newman-Keuls test ($p<0.05$). All analyses were conducted using SigmaStat3.5 (Systat Software, Inc., Chicago, IL).

2.2 Results

One animal in the LY37 EtOH group had poor behavioral performance during both testing phases and was removed from analysis. Prior to drug injection, EtOH-reinforced animals consumed a mean of 0.64 ± 0.06 g/kg of EtOH for LY37 and 0.67 ± 0.04 g/kg for BINA.

2.2.1 Consumption

For the Drinking Test Phase, a significant effect of LY37 treatment on sucrose intake (mL/kg) was observed [$F(4, 32) = 12.887$, $p<0.001$] with post hoc analyses indicating a significant decrease in sucrose consumption at the 1.5 and 2.0 mg/kg dose ($p<0.01$) compared to LY37 vehicle administration (See Figure 2.3: Effect of Systemic LY379268 on Sucrose Consumption). No effect of LY37 on EtOH consumption was observed [$F(4, 28) = 1.65$, $p=0.19$] (See Figure 2.4: Effect of Systemic LY379268 on Ethanol Consumption). No effect on either sucrose [$F(3, 24) =0.418$, $p=0.74$] or EtOH consumption [$F(3, 24) =1.34$, $p=0.28$] was observed with BINA administration (See Figure 2.5: Effect of Systemic BINA on Sucrose and Figure 2.6: Effect of Systemic BINA on Ethanol Consumption).

2.2.2 Appetitive Responding

Rats made roughly 70 responses during the vehicle extinction session. A significant main effect of LY37 on appetitive responding was observed [$F(4, 60) =30.33$, $p<0.001$]. Post hoc analyses indicate that LY37 significantly ($p<0.001$) decreased seeking at the 1.0, 1.5, and 2.0 mg/kg LY37 doses (See Figure 2.7: Effect of Systemic LY379268 on Sucrose- and Ethanol-Seeking.). No interaction of dose x reinforcer was observed [$F(4, 60) =1.682$, $p=0.17$]. A main effect of BINA treatment [$F(3, 48) =3.1587$, $p=0.03$] on seeking was observed (figure 2.8: Effect of Systemic BINA on Sucrose- and Ethanol-Seeking). Post hoc analyses indicate that the effect was due to a significant difference between the 5 mg/kg and 20 mg/kg dose ($p=0.03$) and a decrease in seeking from baseline at the 20 mg/kg dose ($p = 0.055$).

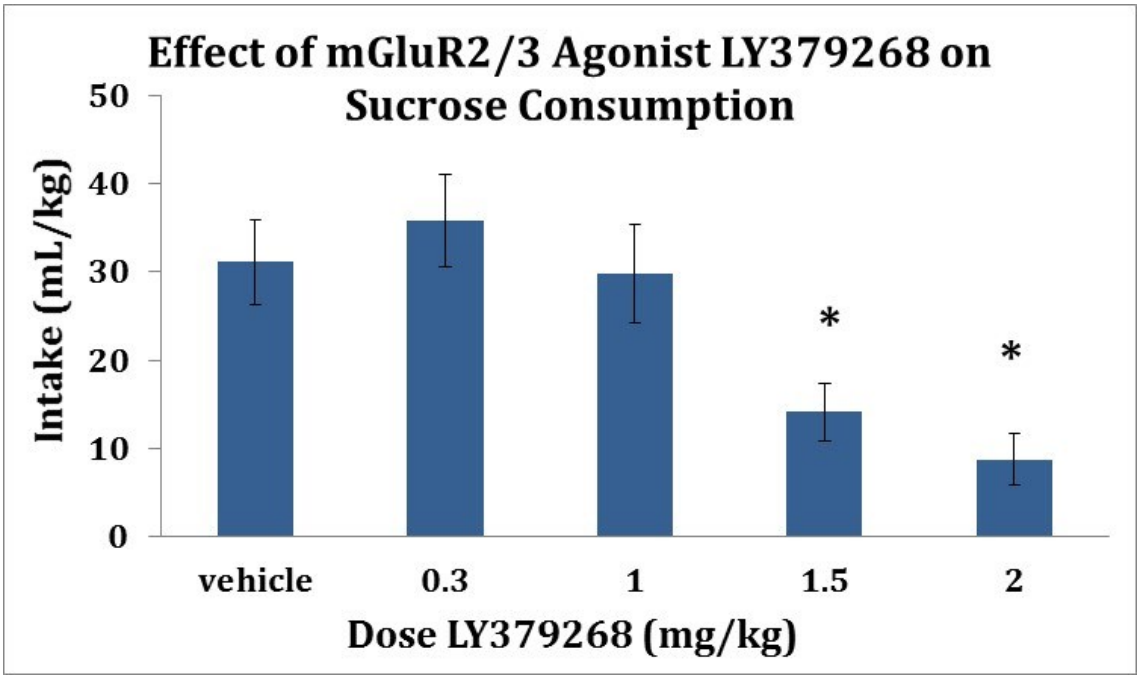

Figure 2.3: Effect of Systemic LY379268 on Sucrose Consumption

Sucrose consumption following weekly systemic injection of the non-selective group II mGluR agonist LY379268 (n=9). A significant reduction in sucrose consumption was observed at the 1.5 and 2.0 mg/kg doses of LY379268. (* $p<0.05$)

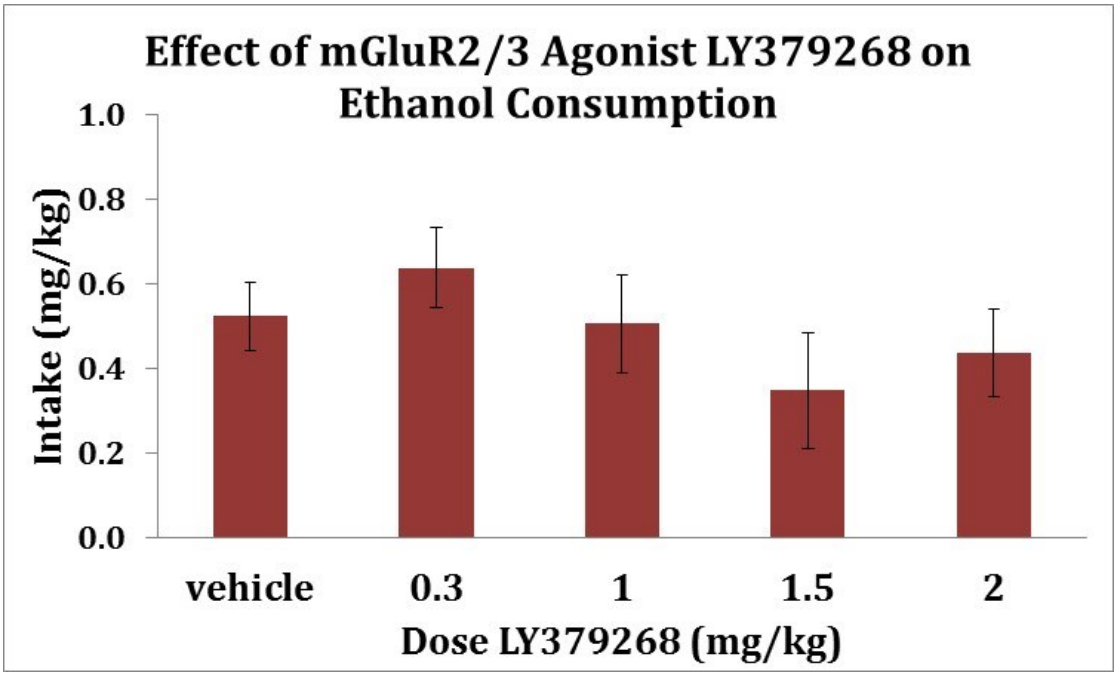

Figure 2.4: Effect of Systemic LY379268 on Ethanol Consumption

EtOH consumption following weekly systemic injection of the non-selective group II mGluR agonist LY379268 (n=8). No significant effect of systemic administration was observed for any dose tested.

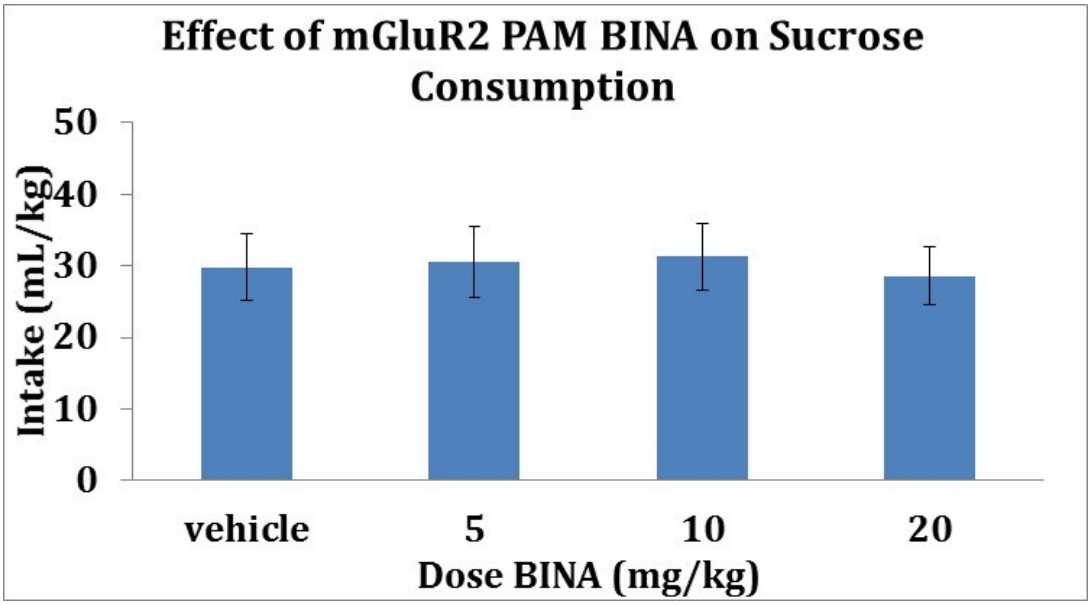

Figure 2.5: Effect of Systemic BINA on Sucrose Consumption

Sucrose consumption following weekly systemic injection of the selective mGluR2 PAM BINA (n=9). No significant effect of systemic administration was observed for any dose tested.

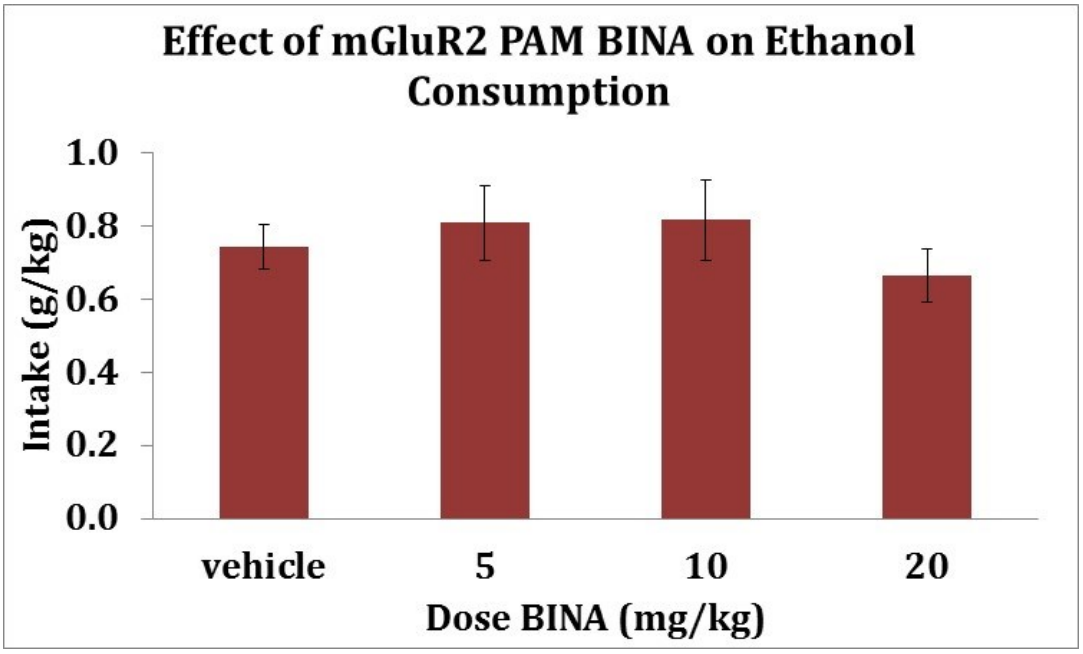

Figure 2.6: Effect of Systemic BINA on Ethanol Consumption

EtOH consumption following weekly systemic injection of the selective mGluR2 PAM BINA (n=9). No significant effect of systemic administration was observed for any dose tested.

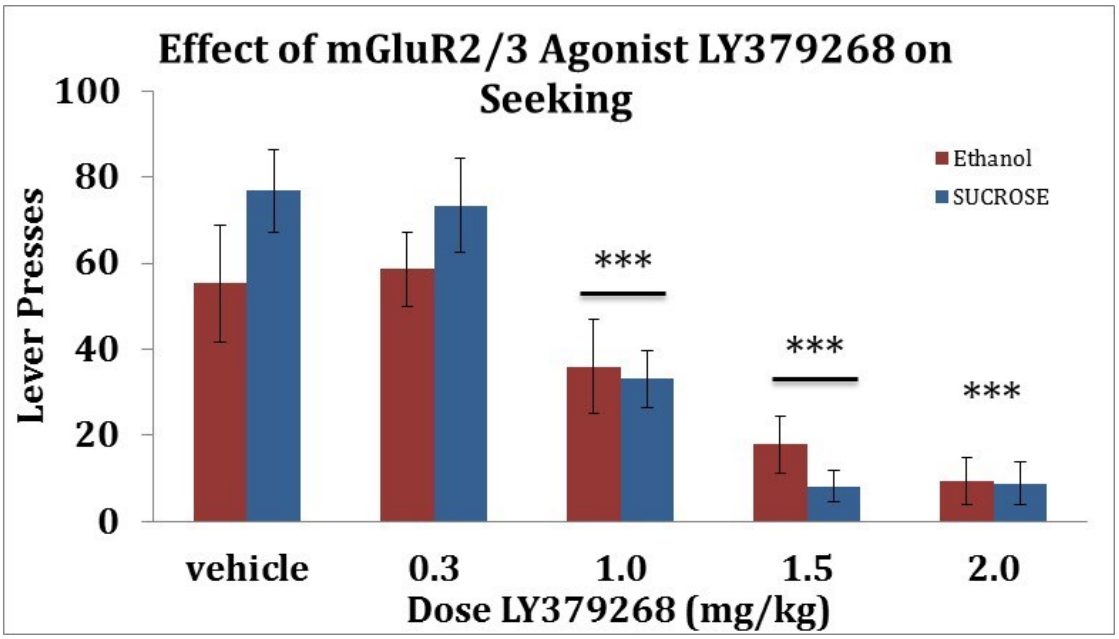

Figure 2.7: Effect of Systemic LY379268 on Sucrose- and Ethanol-Seeking

Appetitive responding for EtOH and sucrose following weekly systemic injection of the non-selective group II mGluR agonist LY379268 (n=9/group). A significant reduction in reinforcer seeking was observed at the 1.0, 1.5, and 2.0 mg/kg doses of LY379268. (*** $p<0.001$)

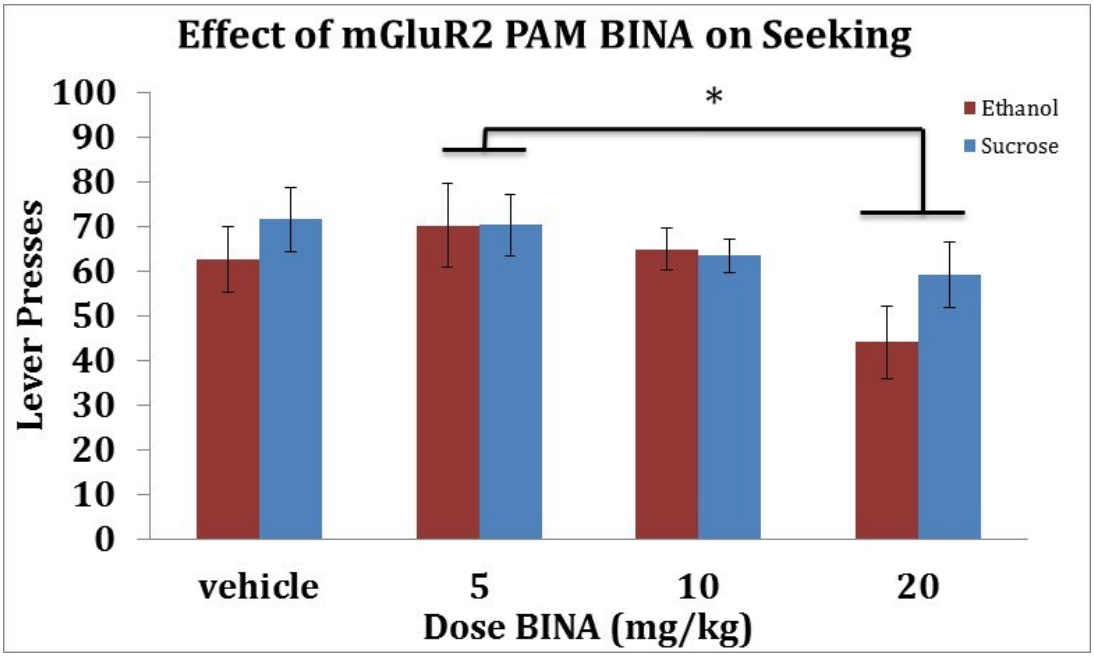

Figure 2.8: Effect of Systemic BINA on Sucrose- and Ethanol-Seeking

Appetitive responding for EtOH and sucrose following weekly systemic injection of the selective mGluR2 PAM BINA (n=9/group). No difference from baseline responding was observed for appetitive responding for any dose of BINA tested. (* $p<0.05$)

2.2.3 Latency to First Lick

To determine possible effects of systemic LY37 and BINA administration on locomotion, the latency to first lick during the drinking test phase was examined. The latency to first lick is the time (in seconds) following successful completion of the lever press response requirement (RR1) for the animal to turn, traverse the chamber, and make initial contact with the sipper tube. Average lick latency for vehicle administration was 2.35 ± 0.59 seconds for LY37 and 1.61 ± 0.13 seconds for BINA. A significant main effect of LY37 dose for latency to first lick was observed [$F(4, 54) = 3.39$, $p = 0.015$] (See Figure 2.9: Effect of Systemic LY379268 on Latency of First Lick). However, post hoc analysis revealed that this effect was due to a within dose difference in first lick latency (between 0.3 and 1.5 mg/kg as well as 1.0 and 1.5 mg/kg doses). One animal was observed to have a lick latency greater than two standard deviations from the mean for the 1.5 mg/kg LY37 dose. However, with this data point removed from the analysis a significant main effect of LY37 on lick latency was still observed [$F(4, 53) = 3.84$, $p = 0.08$] with post hoc analysis revealing a significant difference between the 0.3 and 1.0 mg/kg doses and the 1.5 mg/kg dose. No effect of BINA administration was observed for latency to first lick [$F(3, 48) = 1.63$, $p = 0.20$] (See Figure 2.10: Effect of Systemic BINA on Latency to First Lick). Overall, no dose of either the non-specific mGluR2/3 agonist LY37 or the mGluR2 PAM BINA significantly increased the latency to first lick compared to the vehicle baseline latency suggesting no effect on locomotion for the drug doses tested.

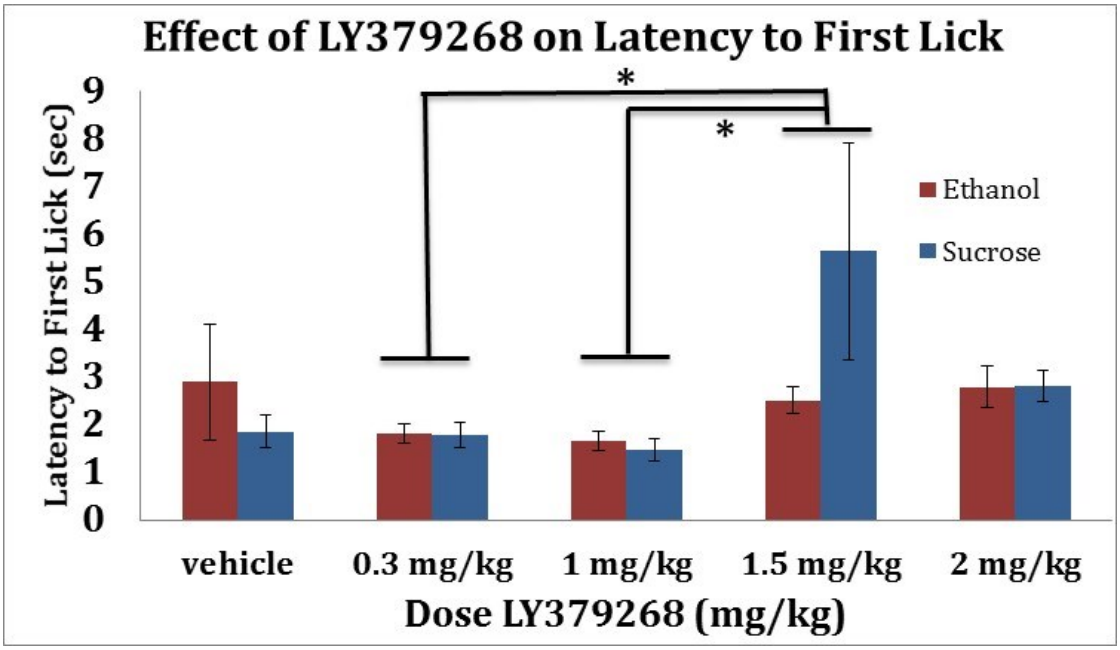

Figure 2.9: Effect of Systemic LY379268 on Latency to First Lick

Effect of LY379268 on latency to first lick. Latency to first lick is the duration of time (in seconds) for the animal to traverse the chamber and make initial contact with the sipper tube following completion of the RR1 lever response. No effect of systemic administration of LY379268 compared to vehicle baseline latency was observed for latency to first lick. (* $p<0.05$)

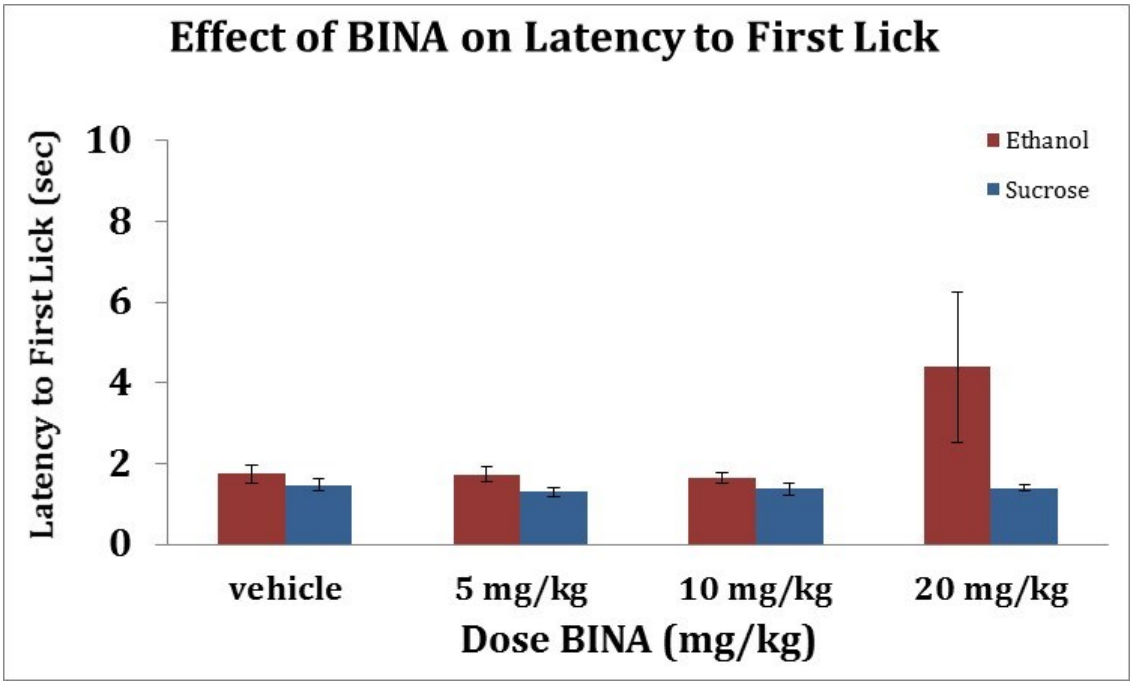

Figure 2.10: Effect of Systemic BINA on Latency to First Lick

Effect of BINA on latency to first lick. Latency to first lick is the duration of time (in seconds) for the animal to traverse the chamber and make initial contact with the sipper tube following completion of the RR1 lever response. No effect of systemic administration of BINA was observed for latency to first lick.

2.2.4 Systemic Agonist and PAM Effect on Body Weight

The difference in body weight between injection session and subsequent session (roughly 24 hours post-injection) during the drinking and seeking test phases were computed to examine possible effects of systemic LY37 and BINA administration on body weight. A significant main effect of LY37 on body weight during the drinking test phase was observed [$F(4, 64) = 17.99$, $p<0.001$]. Post hoc analyses indicate that LY37 significantly ($p<0.01$) decreased body weight 24 hours following systemic injection at the 1.0, 1.5, and 2.0 mg/kg LY37 doses (See Figure 2.11: Effect of Systemic LY379268 on Body Weight during Drinking Test Phase). No interaction of dose x reinforcer was observed [$F(4, 64) = 1.35$, $p=0.26$]. Similarly, a significant main effect of LY37 on body weight during the seeking test phase was observed [$F(4, 64) = 10.271$, $p<0.001$]. Post hoc analyses indicate a significant ($p<0.01$) reduction in body weight 24 hours following systemic LY37 administration at the 1.0, 1.5, and 2.0 mg/kg doses (See Figure 2.12: Effect of Systemic LY379268 on Body Weight during Seeking Test Phase). No effect of systemic BINA administration was observed for body weight during either the drinking test phase [$F(3, 48) = 0.56$, $p=0.65$] (See Figure 2.13: Effect of Systemic BINA on Body Weight During Drinking Test Phase) or seeking test phase [$F(3, 48) = 1.337$, $p=0.27$] (See Figure 2.14: Effect of Systemic BINA on Body Weight During Seeking Test Phase). This suggests that systemic administration of the non-specific mGluR2/3 agonist LY37, but not the mGluR2 PAM BINA, significantly decreases body weight 24-hours following systemic administration.

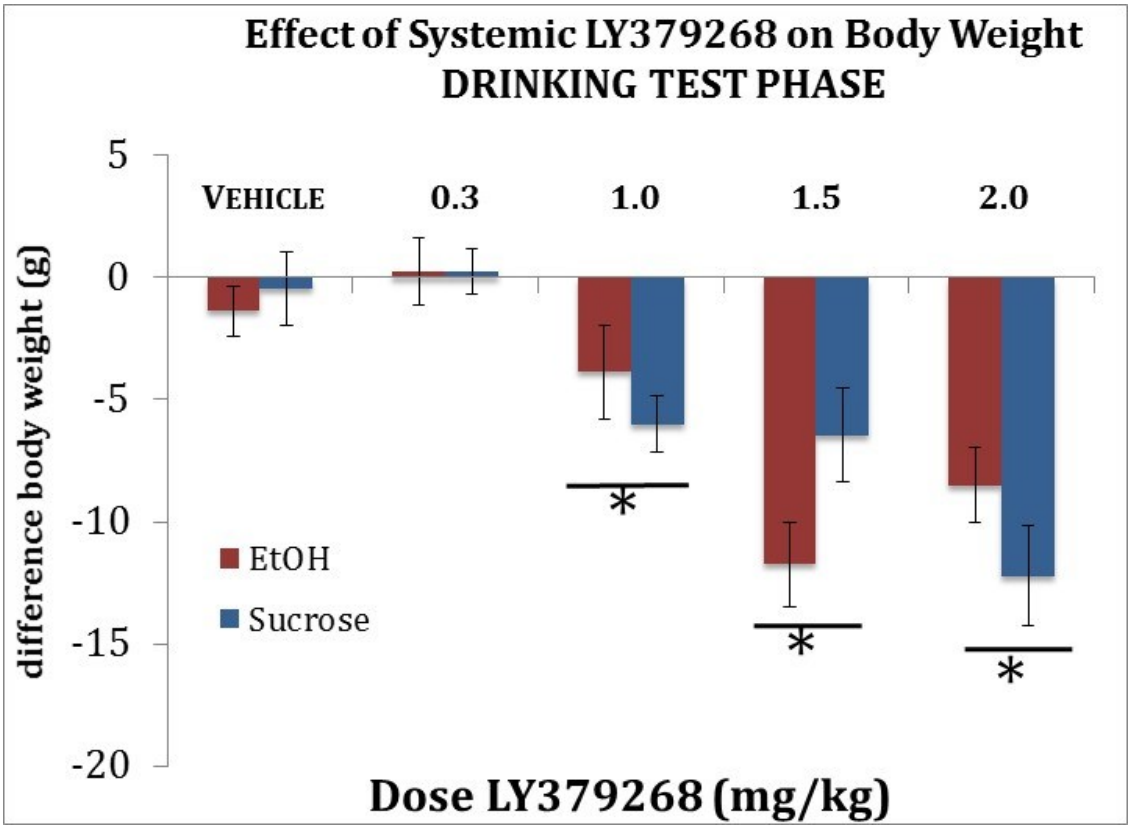

Figure 2.11: Effect of Systemic LY379268 on Body Weight during Drinking Test Phase

Differences in body weight between injection session and subsequent session (roughly 24 hours post-injection) during the drinking test phase for systemic LY379268. A significant reduction in body weight was observed at the 1.0, 1.5, and 2.0 mg/kg doses of LY379268. (* $p<0.05$)

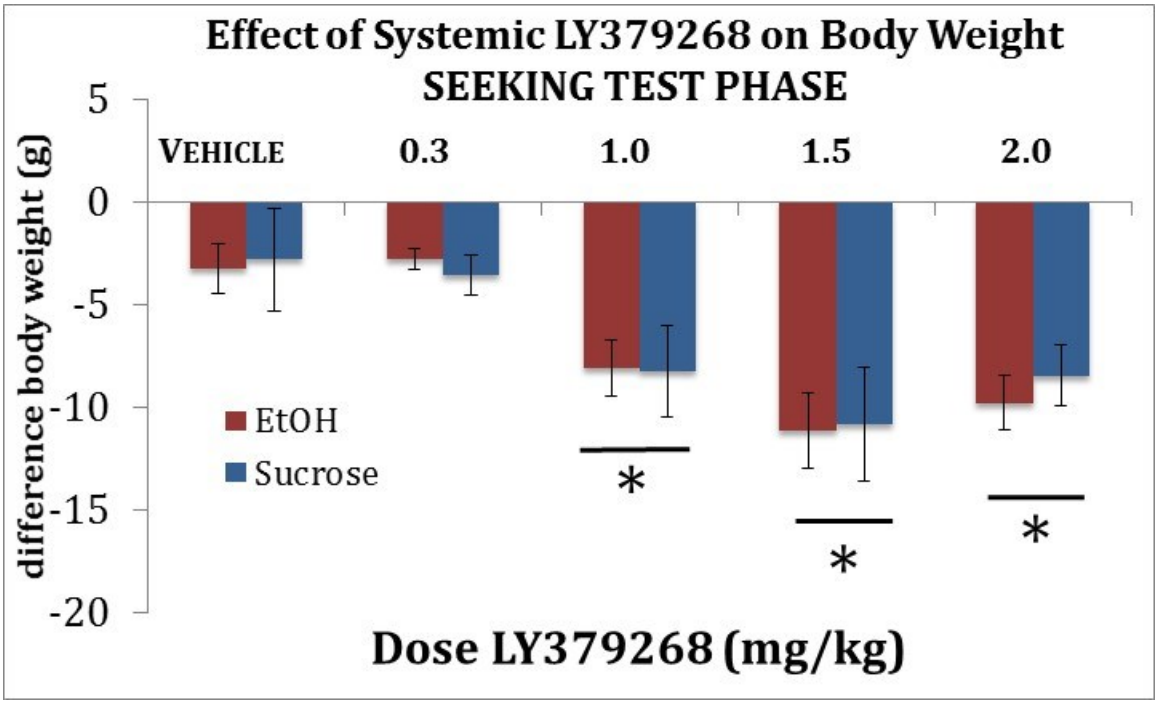

Figure 2.12: Effect of Systemic LY379268 on Body Weight during Seeking Test Phase

Differences in body weight between injection session and subsequent session (roughly 24 hours post-injection) during the seeking test phase for systemic LY379268. A significant reduction in body weight was observed at the 1.0, 1.5, and 2.0 mg/kg doses of LY379268. (* $p<0.05$)

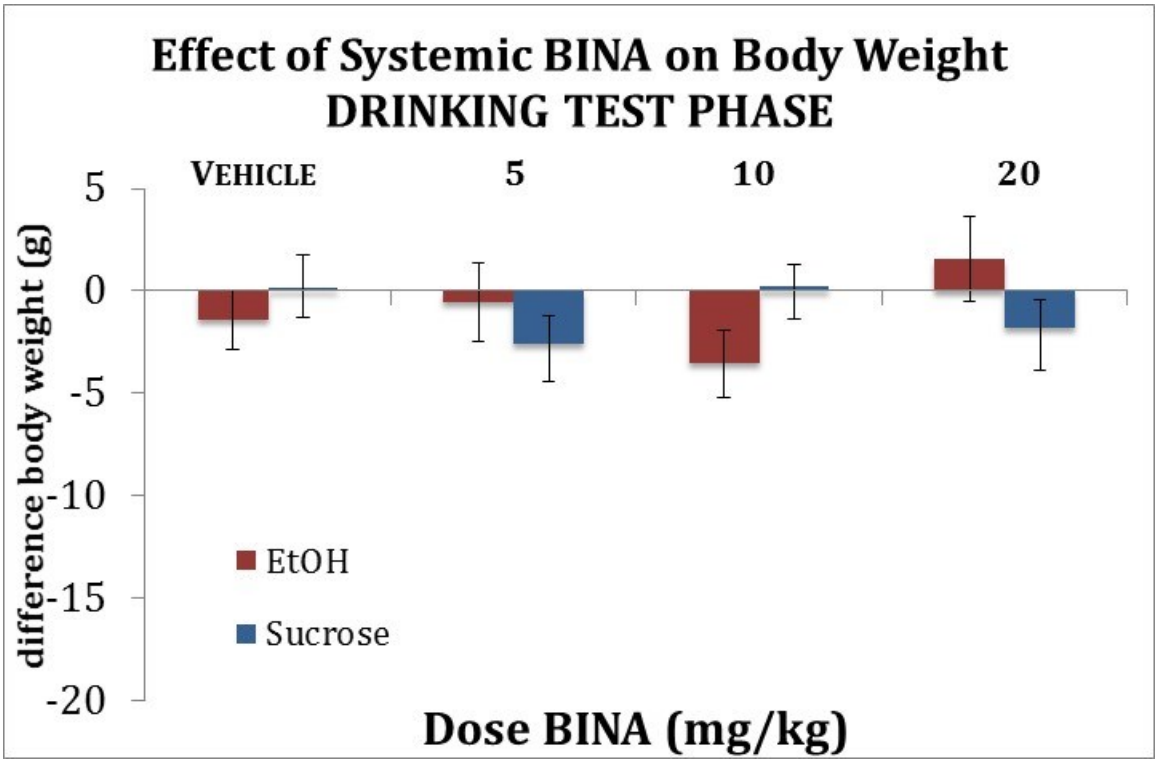

Figure 2.13: Effect of Systemic BINA on Body Weight during Drinking Test Phase

Differences in body weight between injection session and subsequent session (roughly 24 hours post-injection) during the drinking test phase for systemic BINA. No significant effect of systemic administration was observed for any dose tested.

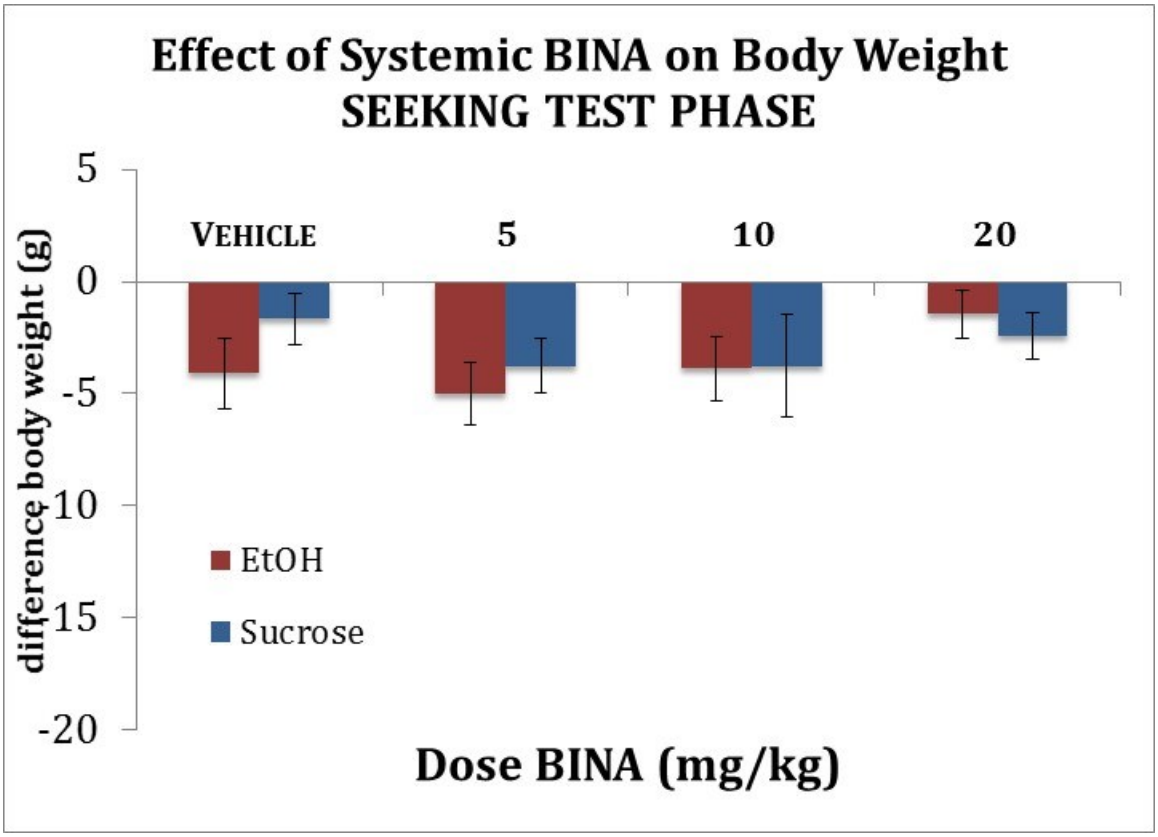

Figure 2.14 : Effect of Systemic BINA on Body Weight during Seeking Test Phase

Differences in body weight between injection session and subsequent session (roughly 24 hours post-injection) during the seeking test phase for systemic BINA. No significant effect of systemic administration was observed for any dose tested.

2.2.5 Systemic Agonist and PAM Effect on Next Day drinking

Given the effect of systemic LY37 administration on body weight 24 hour following administration, the difference in reinforcer consumption between the Wednesday reinforced session (24 hours prior to systemic administration) and Friday reinforced session (24 hours post-systemic administration) was compared to examine for a possible compensatory increase in reinforcer consumption following systemic agonist and PAM treatment. No effect on either sucrose [$F(4, 32) = 2.309$, $p=0.08$] or EtOH consumption [$F(4, 28) = 1.975$, $p=0.13$] was observed with systemic LY37 administration (See Figure 2.15: Effect of Systemic LY379268 on Sucrose Intake during Drinking Test Phase and Figures 2.16: Effect of Systemic LY379268 on Ethanol Intake during Drinking Test Phase). As well, no effect on either sucrose [$F(3, 24) = 0.254$, $p=0.86$] or EtOH consumption [$F(3, 24) = 1.154$, $p=0.35$] was observed with systemic BINA administration (See Figure 2.17: Effect of Systemic BINA on Sucrose Intake during Drinking Test Phase and Figure 2.18: Effect of Systemic BINA on Ethanol Intake during Drinking Test Phase).

2.3 Discussion

Overall, systemic administration of the non-selective group II mGluR agonist LY37 significantly decreased reinforcer seeking and selectively decreased sucrose consumption at doses not shown to affect locomotion. As well, systemic LY37 administration was noted to decrease body weight 24-hours following administration. Systemic administration of the mGluR2 PAM BINA had no effect on reinforcer consumption and no statistically meaningful effect on reinforcer-seeking. Systemic administration of BINA was also shown to have no effect on body weight.

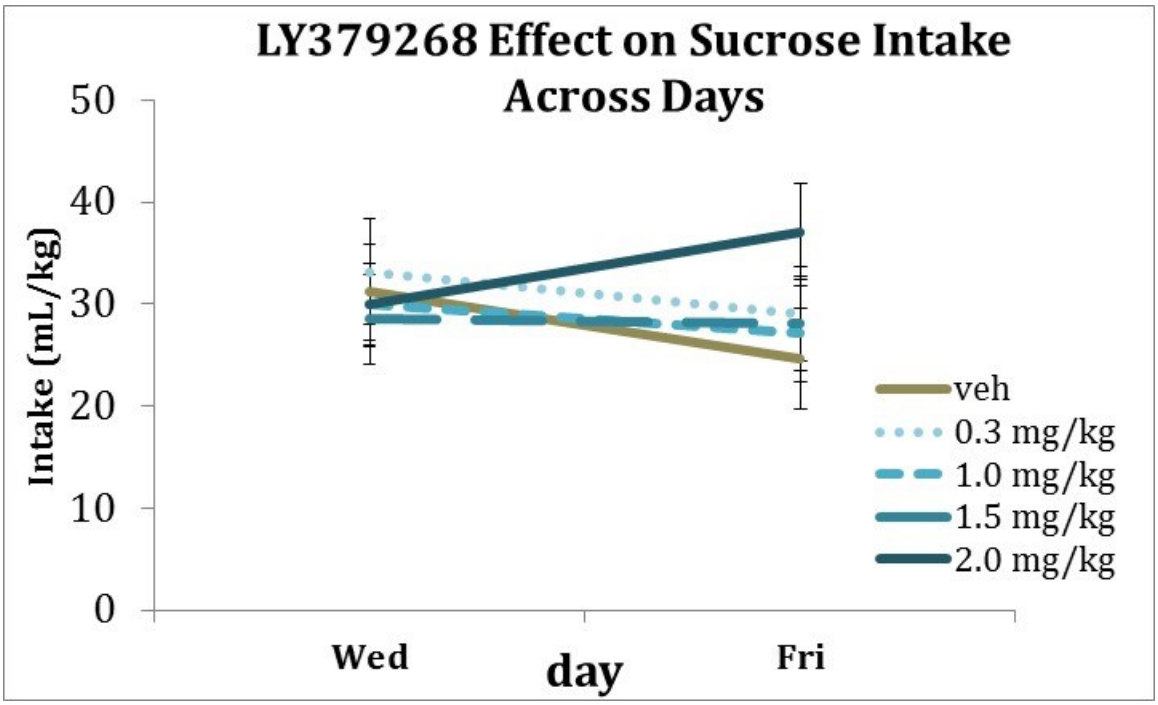

Figure 2.15: Effect of Systemic LY379268 on Sucrose Intake during Drinking Test Phase

Comparison of session intake of sucrose (in mL/kg) between session before and after systemic injection (roughly 24 hours pre- and post-injection) during the drinking test phase for systemic LY379268. No significant effect of systemic administration was observed for any dose tested.

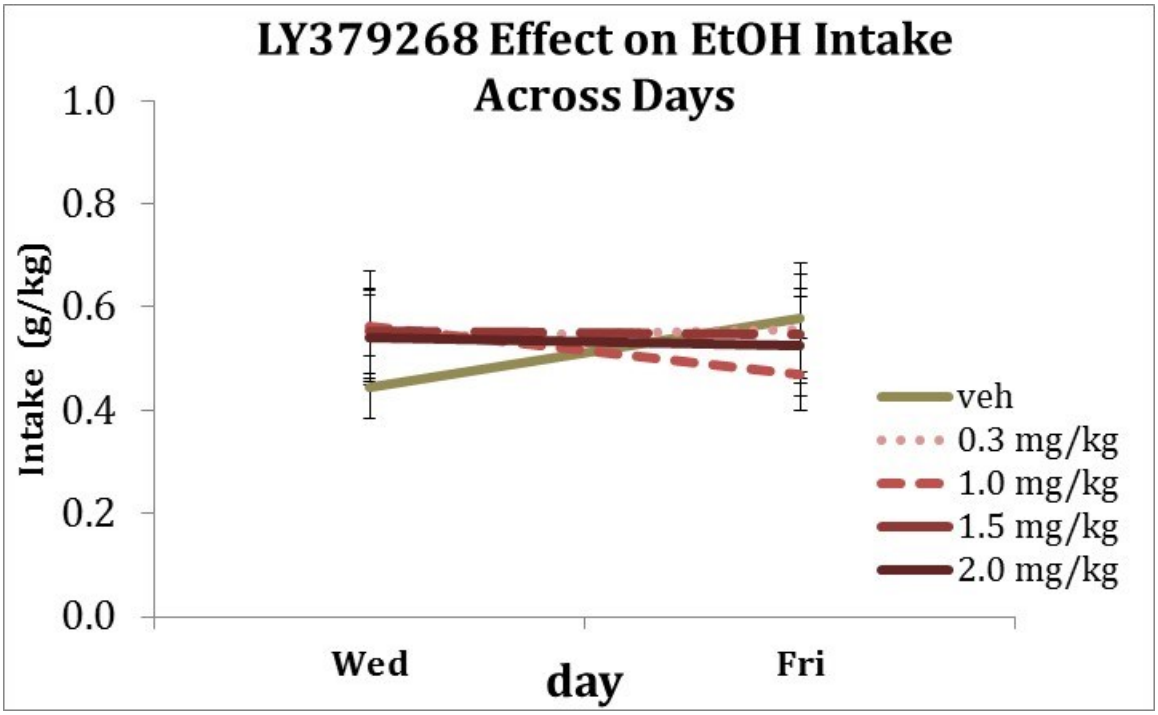

Figure 2.16: Effect of Systemic LY379268 on Ethanol Intake during Drinking Test Phase

Comparison of session intake of ethanol (in g/kg) between session before and after systemic injection (roughly 24 hours pre- and post-injection) during the drinking test phase for systemic LY379268. No significant effect of systemic administration was observed for any dose tested.

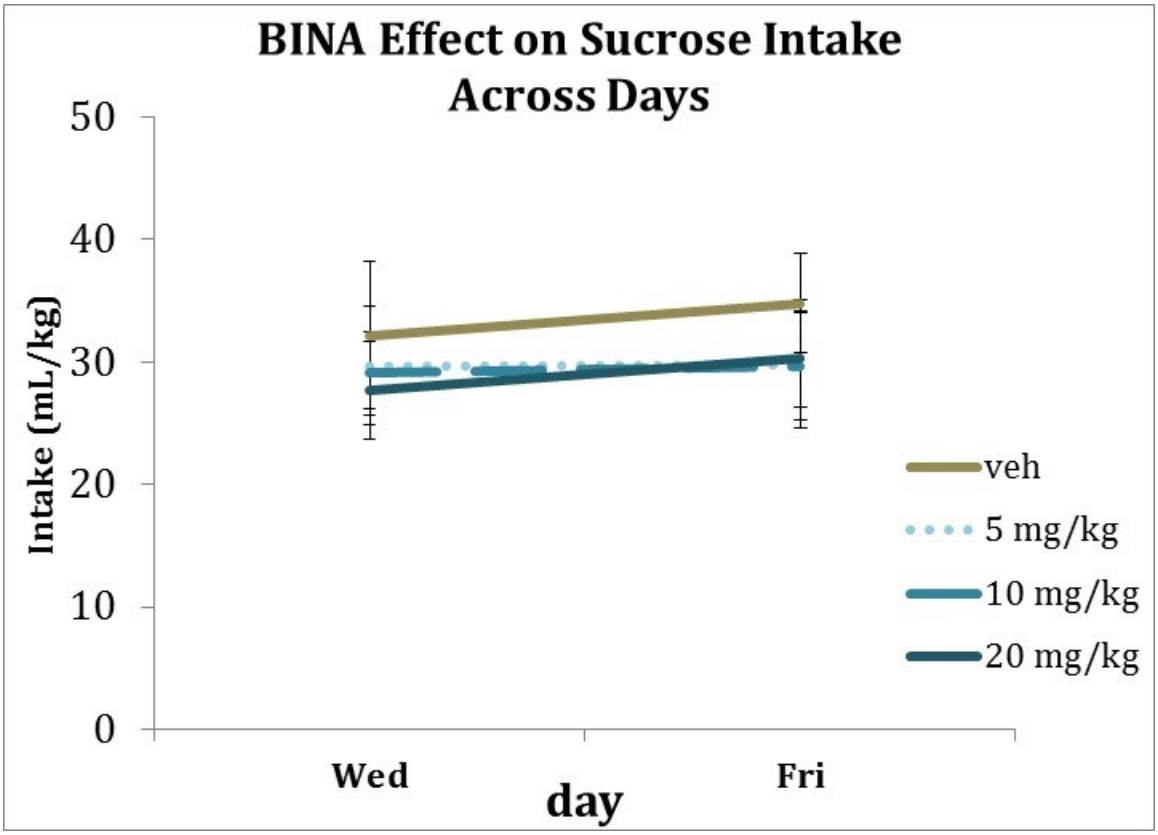

Figure 2.17: Effect of Systemic BINA on Sucrose Intake during Drinking Test Phase

Comparison of session intake of sucrose (in mL/kg) between session before and after systemic injection (roughly 24 hours pre- and post-injection) during the drinking test phase for systemic BINA. No significant effect of systemic administration was observed for any dose tested.

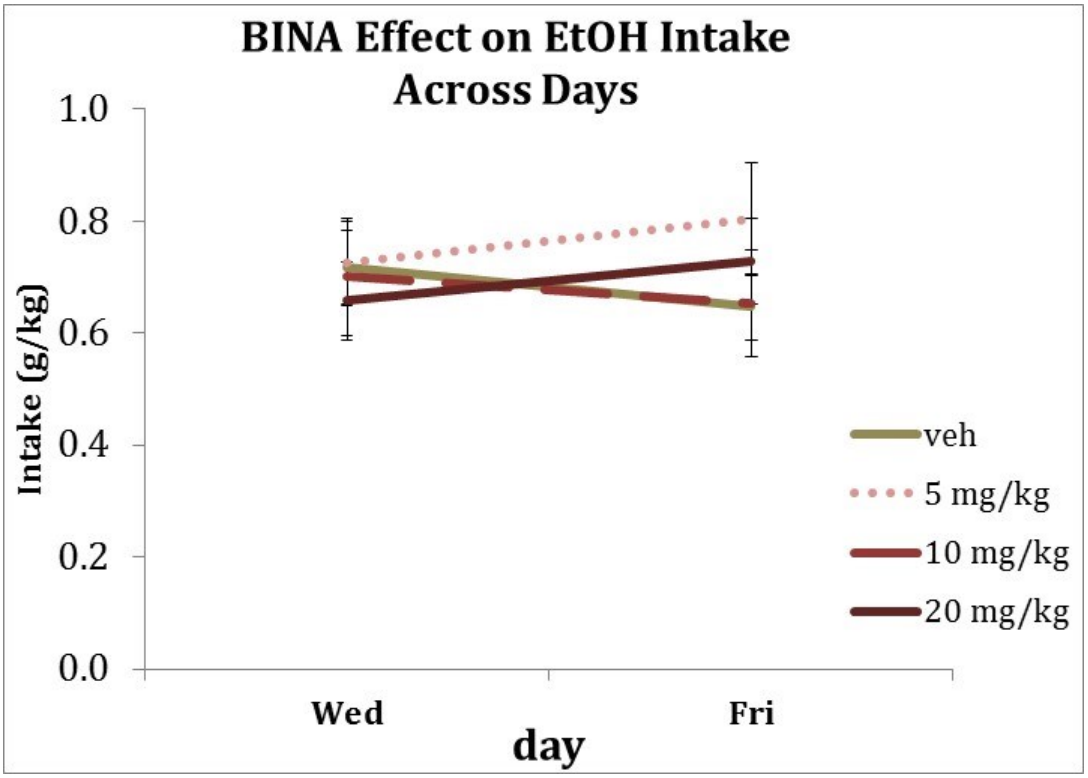

Figure 2.18: Effect of Systemic BINA on Ethanol Intake during Drinking Test Phase

Comparison of session intake of ethanol (in g/kg) between session before and after systemic injection (roughly 24 hours pre- and post-injection) during the drinking test phase for systemic BINA. No significant effect of systemic administration was observed for any dose tested.

2.3.1 Effect LY37 on Appetitive Responding

Similar to previous studies, systemic administration of the non-selective group II agonist LY37 was shown to decrease EtOH-seeking. This finding extends the previous literature as it demonstrates a reduction in EtOH-seeking in non-food or water-deprived animals during maintenance drinking. Previously, Baptista, et al. (2004) showed a decrease in cue-induced sweetened condensed milk seeking following systemic LY37 administration (3 mg/kg, but not 0.3 or 1.0 mg/kg) in non-food-deprived Wistar rats. Similarly, Peters and Kalivas (2006) showed a decrease in cue-induced food-seeking with systemic LY37 administration (3 mg/kg, but not 0.3 or 1.0 mg/kg) in food-restricted Sprague-Dawley rats. However, as this dose of LY37 (3 mg/kg) has been shown to decrease spontaneous locomotion (Kufahl, et al., 2011), previous findings on decreased alternative reinforcer intake were attributed to non-specific locomotor effects of LY37 rather than an effect on consumption of the alternative reinforcer. In this study, systemic administration of LY37 was found to significantly reduce sucrose-seeking Wistar rats at LY37 doses not shown to result in a significant change in locomotion from that of baseline. This suggests that in non-deprived animals, LY37 influences reinforcer seeking generally.

2.3.2 Effect LY37 on EtOH Consumption

Systemic administration of the non-selective group II agonist LY37 did not significantly affect EtOH consumption in non-deprived Wistar rats at the doses tested. Previous studies examining the effect of LY37 on reinforcer self-administration have utilized FR responding which can be better characterized as a mixture of reinforcer-

seeking and consumption across the operant session (Backstrom and Hyytia, 2005; Bossert, et al., 2005; Jin, et al., 2010; Liechti, et al., 2007; Sidhpura, et al., 2010). Here, I demonstrate that when seeking and consumption are teased apart using the sipper tube model, LY37 does not significantly affect EtOH consumption. This suggests that activation of mGlu2/3 receptors modulates the appetitive, but not consummatory, aspects of EtOH reinforcement.

2.3.3 Effect LY37 on Sucrose Consumption and Body Weight

A significant decrease in sucrose consumption was observed at the 1.5 and 2.0 mg/kg doses of LY37 in non-deprived Wistar rats. As neither the 1.5 nor 2.0 mg/kg LY37 were demonstrated to have a meaningful effect on latency to first lick, the effect of these doses of LY37 is most likely not do to a non-specific effect of LY37 on locomotion. Systemic administration of LY37 was also shown to significantly decrease body weight 24 hours following systemic administration. The observed effect on diminished sucrose consumption and body weight following systemic administration suggest a possible effect of mGluR2/3 activation on feeding behavior. This potential effect of systemic LY37 on feeding and satiety could be due to the regulation of serotonin (5-HT) release by group II mGluRs.

In addition to negatively regulating glutamate release, group II mGluRs have been shown to regulate release of other neurotransmitters. Specifically, group II mGluRs have been shown to regulate 5-HT release in the medial prefrontal cortex (mPFC) (Cartmell, et al., 2001). LY37 was shown to substantially increase extracellular 5-HT concentration in the mPFC 30-minutes following systemic administration with extracellular 5-HT levels

remaining elevated for the 4 hour microdialysis experiment. Several studies have demonstrated that increasing 5-HT neurotransmission leads to decreases in food intake (Fletcher and Burton, 1984; Lucki, et al., 1988; Pollock and Rowland, 1981; Schreiber, et al., 2000; Willner, et al., 1990). However, pharmacological manipulations that result in increased serotonergic neurotransmission (administration of 5-HT, 5-HT precursors, 5-HT reuptake inhibitors, and 5-HT releasers) have consistently demonstrated a reduction in EtOH consumption in rats (see LeMarquand, et al., 1994 for review). Therefore, if the increased level of extracellular 5-HT in the mPFC following systemic LY37 administration is the primary factor in decreasing sucrose consumption, then a suppression in EtOH consumption would also be expected. However, as there was no significant effect of systemic LY37 administration on EtOH consumption, the observed suppression in sucrose consumption is not fully explained by the increased serotoninergic neurotransmission following systemic LY37 administration. The observed reduction in sucrose intake, but not in EtOH intake, may instead be potentially due to a qualitative difference between the sucrose and EtOH reinforcers in non-deprived animals or the immediate onset of effects following sucrose consumption compared to the temporally delayed onset of pharmacological effect of orally consumed EtOH.

2.3.4 Effect BINA on Seeking and Consumption

Systemic administration of the selective mGluR2 PAM BINA (0-20 mg/kg, ip) did not significantly affect seeking or consumption of either EtOH or sucrose. Previously, BINA was shown to decrease both cocaine self-administration (20 and 40 mg/kg) and cue-induced reinstatement of cocaine seeking (10, 20, and 40 mg/kg) with no effect on

food self-administration or cue-induced reinstatement of food-seeking (Jin, et al., 2010). The lack of a significant effect of systemic administration of the mGluR2 selective PAM BINA on EtOH seeking may suggest that the decreased reinforcer seeking observed following systemic administration of the non-selective mGluR2/3 agonist LY37 is driven by agonist action at mGlu3 receptors. However, interpretation of the separate roles of mGlu2 and mGlu3 receptors on reinforcer seeking by systemic LY37 agonist administration using the effect of BINA on seeking behavior is tenuous at best due to the differing mechanisms of action of agonists compared to PAMs.

2.3.4.1 Orthosteric versus Allosteric Modulation

The agonist LY37 interacts with mGlu2 and mGlu3 receptors at the orthosteric binding site in the extracellular venus flytrap domain (Doumazane, et al., 2013). Agonist binding triggers closure of the venus flytrap leading to activation of the receptor. As a PAM, BINA interacts with the mGlu2 receptor at allosteric sites within the transmembrane domain of the receptor (Doumazane, et al., 2013), which increases G-protein coupling to mGlu2 receptors, resulting in enhanced potency/efficacy of agonists (Conn, et al., 2009). Therefore, systemic administration of BINA increases the likelihood of mGlu2 receptors activation by orthosteric ligands; however, BINA alone does not activate mGlu2 receptors. Conversely, the orthosteric agonist LY37 can activate mGlu2 and mGlu3 receptors alone without need for other ligands binding to the receptor (Doumazane, et al., 2013). Thus, the action of BINA and LY37 on mGlu2 receptors is different with BINA increasing the probability of receptor activation while LY37 alone

can active the receptor. Therefore, as BINA requires the presence of endogenous ligands for activation of mGlu2 receptors, the lack of suppression of EtOH-seeking following BINA administration suggests that either a higher dose of BINA is needed for activation of the receptors or that there is insufficient endogenous mGluR2 ligands present during seeking to result in BINA facilitated activation of mGlu2 receptors. However, the lack of suppression of EtOH seeking with BINA does not allow for analysis of the role of group II mGluR subtypes in the LY37 induced suppression of EtOH seeking.

2.3.4.2 Role of Heterodimers

mGluRs have been shown to predominately exist as dimers (Brock, et al., 2007; Kniazeff, et al., 2004). In particular, mGlu2 receptors have been shown to exist as homodimers (Moustaine, et al., 2012), with growing evidence suggesting that mGlu2 receptors form heterodimers with other GPCRs (Doumazane, et al., 2011; Kammermeier, 2012). In particular, mGlu2 receptors have been shown to form a heterodimer with mGlu4 receptors (mGlu2-mGlu4) (Kammermeier, 2012). In superior cervical ganglion (SCG) neurons expressing mGlu2 receptors under conditions that favor mGlu2 dimer formation, application of the selective mGlu2 PAM BINA was shown to potentiate inhibition of calcium currents following glutamate application (Kammermeier, 2012). However, in SCG neurons expressing mGlu2 and mGlu4 receptors under in vitro conditions that favor formation of mGlu2-mGlu4 heterdimers, BINA had no effect on inhibition of calcium currents by glutamate. This suggests that allosteric modulation of mGlu2 by BINA does not affect mGlu2-mGlu4 heterodimers and, potentially, other

heterdimers containing mGlu2 receptors. The effect of LY37 on reinforcer seeking may potentially be due LY37 acting upon mGlu2 heterodimers that are not affected by administration of allosteric modulators, such as BINA.

2.3.5 Conclusions

Overall, this study demonstrated that systemic administration of the mGluR2/3 agonist LY37 decreases sucrose consumption and both EtOH- and sucrose-seeking at doses not shown to result in a significant sedation compared to baseline. Systemic LY37 also resulted in a significant reduction in body weight 24 hours following administration. Selective modulation of mGlu2 receptors using the mGlu2 PAM BINA did not significantly affect seeking or consumption of EtOH or sucrose. However, due to differences in how the non-selective agonist and PAM associate with mGluRs, a definitive conclusion on role of mGlu2 receptors in EtOH reinforcement cannot be determined from these findings.

CHAPTER 3. EXPERIMENT 2: INVOLVEMENT OF NUCLEUS ACCUMBENS CORE GROUP II MGLURS IN ETHANOL-SEEKING

3.1 Introduction

In Experiment 1, systemic administration of the non-selective group II mGluR agonist LY37, but not mGluR2 selective PAM BINA, was shown to significantly reduce appetitive responding for EtOH, suggesting a role of group II mGluRs in regulating EtOH-seeking. As LY37 was administered systemically, the specific loci of action of LY37 in brain cannot be inferred from these data. In this experiment we began the examination of the neurocircuitry involved in the agonist induced suppression of EtOH-seeking.

Several key brain regions have been implicated in EtOH reinforcement, including the NAc, VTA, PFC, hippocampus, and amygdala. Increased extracellular glutamate release in the NAc has been observed following both experimenter-delivered moderate doses of EtOH (1 mg/kg; Melendez et al., 2005) and protracted EtOH self-administration (Ding, et al., 2013). Administration of both non-specific glutamate receptor antagonists and NMDA specific antagonists into the NAc has been shown to decrease ethanol self-administration (Rassnick, et al., 1992a; Rassnick, et al., 1992b). The increased glutamate release within the NAc in response to EtOH and decreased self-administration following glutamate receptor blockade suggests that glutamatergic signaling within the NAc may be involved in EtOH reinforcement. As activation of group II mGluRs results in decreased

glutamatergic signaling, NAc mGluR2/3 may be involved in the suppressed EtOH-seeking following systemic administration of the mGLuR2/3 agonist LY37.

3.1.1 NAc Microinjections Group II mGluR Agonists

The NAc receives glutamatergic input from the PFC, thalamus, basolateral amygdala (BLA), and hippocampus (Brog, et al., 1993; Groenewegen, et al., 1999; Heimer, et al., 1997). Intra-accumbens administration of the non-selective group II mGluR agonist (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC) has been shown to decrease NAc glutamate release (Xi, et al., 2002). Several studies have examined the effect of intra-accumbens administration of non-selective group II mGluR agonists on EtOH consumption. Intra-accumbens administration of LY37 in "post-dependent" C57BL/6J mice was shown to reduced 2-hour limited access home cage EtOH drinking to that of the non-dependent air exposed control mice (Griffin III et al., 2014). Kapasova and Szumlinski (2008) found that intra-accumbens administration of the non-selective group II mGluR agonist APDC decreased 4-bottle choice EtOH drinking in both C57BL/6J and DBA mice. In rats, intra-accumbens administration of LY37 has been shown to reduce EtOH self-administration in P rats but also produced nonspecific reductions in locomotor activity (Besheer, et al., 2010). Overall, these findings suggest the involvement of group II mGluRs in the NAc in regulating EtOH reinforcement.

The NAc is composed of two heterogeneous subregions (core and shell). Several regions have afferent projections to both the shell and core regions (orbital cortex, entorhinal cortex, BLA, hippocampus, thalamus, and raphe nuclei); however, several structures preferentially project to either shell or core (Shell: peduncular and IL; Core:

dorsal and ventral prelimbic cortex (PrL), and perirhinal cortex) (Borg, et al., 1993). In addition to receiving signals from different brain regions, the NAc subregions appear to serve different roles in regulating EtOH seeking. In particular, inactivation of the NAc core, but not NAc shell, reduced responding to an EtOH-conditioned stimulus in a novel context (Chaudhri, et al., 2010). Inactivation of the NAc core prior to the first extinction session in animals trained to operantly respond for access to a cocaine reinforcer resulted in a reduction in extinction responding while inactivation of IL, PrL, and NAc shell had no effect on elevated responding during the first extinction session (Peters, et al., 2008). These findings suggest that the NAc core may be involved in EtOH seeking. Given the potential involvement of NAc core glutamatergic neurotransmission in EtOH seeking, the purpose of the second experiment was to examine if NAc core mGluR2/3 are involved in the regulation of EtOH-seeking.

3.1.2 NAc Microinjections Group II mGluR Antagonist LY341495

Intra-accumbens (non-specific core versus shell) administration of LY37 has been shown to reduce EtOH self-administration (0.17 μg/side), but also produced nonspecific reductions in locomotor activity (Besheer, et al., 2010). The non-specific effect of NAc core LY37 administration on locomotion confounds the interpretation of the effect of LY37 activation of NAc core mGluR2/3 on EtOH self-administration. However, microinjection of the non-selective group II antagonist LY341495 (LY34) into NAc core (0.1, 10, and 100 μg/0.5 μL/side) has been shown to have no effect on locomotor behavior (Chi, et al., 2006). Therefore to minimize a possible locomotor confound of LY37 microinjection into NAc core, for this experiment NAc core blockade of mGlu2/3

receptors using the non-selective group II antagonist LY34 following systemic administration of the mGluR2/3 agonist LY37 was performed. Attenuation of the suppressive effects of systemic LY37 administration on EtOH-seeking would suggest the involvement of NAc core mGluR2/3 in the regulation of EtOH-seeking. Given the previous findings of NAc core glutamatergic contribution to reinforcer seeking, we hypothesized that NAc core mGlu2/3 receptors are involved in regulating synaptic glutamatergic signaling involved in EtOH-seeking. Therefore, we predicted that intraaccumbens core blockade of mGluR2/3 would attenuate the systemic mGluR2/3 agonist LY37 induced suppression of EtOH-seeking.

3.2 Materials and Methods

3.2.1 Animals

Fifteen male Wistar rats, weighing 176 - 203 g at the beginning of the experiment, were single housed on a 12-hour light/dark cycle (lights on at 0500). Animals had ad libitum access to both food and water except for a mild water restriction during the first week of operant training. Animal care and procedures were in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee (IACUC).

3.2.2 Apparatus and Training

The apparatus and training were the identical to those of the first experiment (see Chapter 2: Apparatus and Training).

3.2.3 Surgery

Following training, animals were surgically implanted with bilateral guide cannulae directed towards the NAc core. Thirty minutes prior to surgery, the non-steroidal anti-inflammatory drug (NSAID) carprofen was administered subcutaneously (5 mg/kg) for pain relief. Rats were anesthetized with sodium pentobarbital (60 mg/kg, ip), the top of the head shaved, and the rat placed in the stereotaxic apparatus (Benchmark Digital Stereotaxic; myNeurolab, St. Louis, MO) with incisor bar set at 3.3 mm below the interaural line. Stainless steel guide cannulae (13 mm; 26 gauge) were implanted bilaterally terminating 1 mm dorsal to the NAc core using bregma, midline, and dura surface as reference (AP +1.6, ML ±1.6, DV -6.0; Paxinos and Watson, 1998). To limit obstruction of the guide cannulae and maintain patency, removable wire obturators (13mm length; 33 gauge) were placed into the guide cannulae. Following surgery, animals had a minimum of two days to recover prior to resuming operant sessions. Animals were checked daily to ensure proper wound healing and lack of infection.

3.2.4 Microinfusions

NAc core blockade of systemic mGluR2/3 agonist (LY37 1.5 mg/kg dose) suppression of EtOH-seeking was performed using the non-selective group II mGluR antagonist LY34 (1 μg/side) (See Figure 3.1: Overview of Microinjection Experiment). For this, each animal received the following treatment sets in a balanced design: systemic vehicle + NAc core vehicle, systemic LY 37 + NAc core vehicle, systemic LY37 + NAc core LY34, and systemic vehicle + NAc core (See Figure 3.2: Overview Microinjection Sets). To prevent an association of the microinjection procedure with the extinction

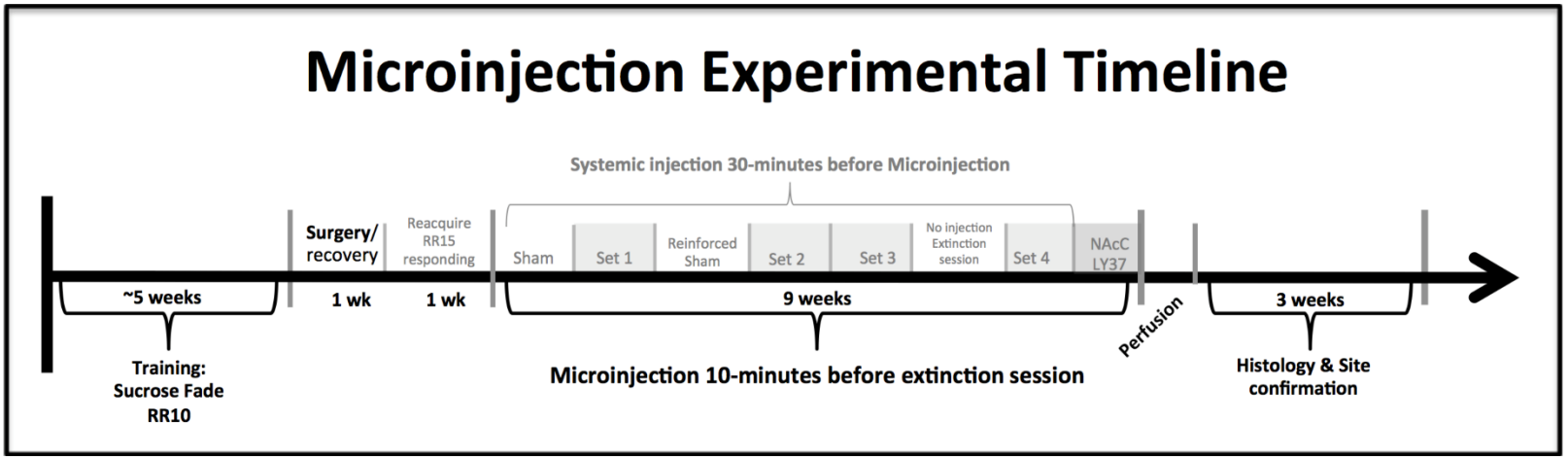

Figure 3.1: Overview of Microinjection Experiment

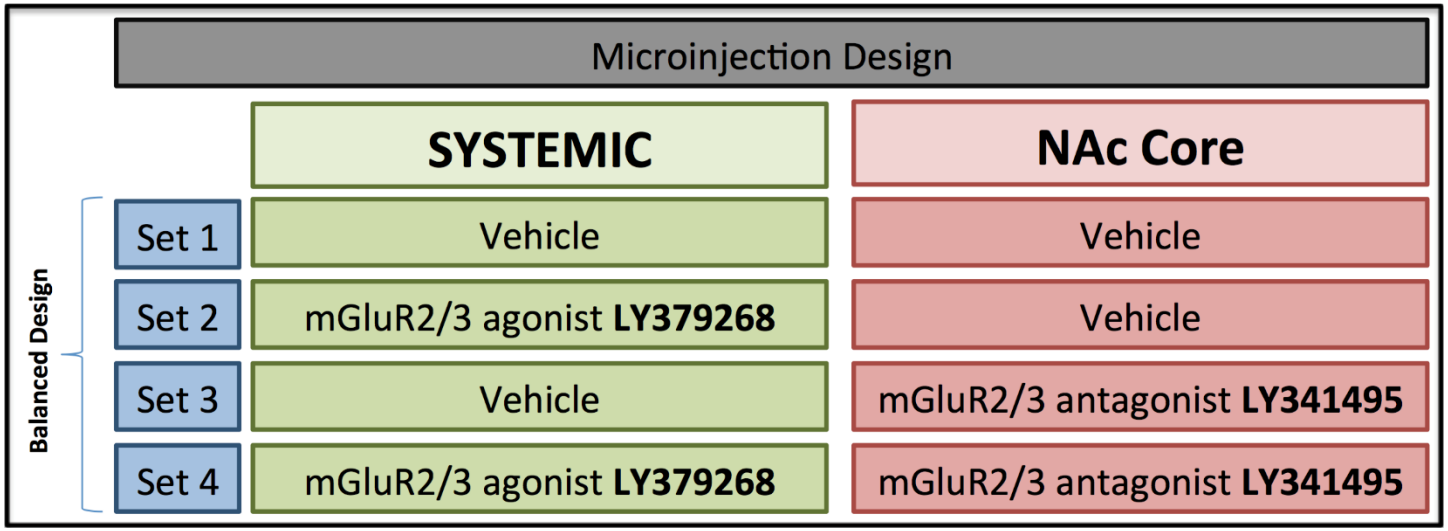

Figure 3.2: Overview Microinjection Sets

session, a reinforced sham session (<10mm long injectors placed in cannulae with no fluid administered) and no injection extinction session occurred the week following the first and third sets of microinjections, respectively. For microinjections, rats were gently restrained in a small holding tub (27 x 17 x 12 cm). Each obturator was removed and replaced with a stainless steel injector (33 gauge) that extended 1 mm beyond the end of the guide cannulae. Drug solutions were delivered bilaterally in a volume of 0.5 μg/side over a one minute period using 25.0 μL Hamilton syringes and KD Scientific Infusion Pumps (Model 101; KD Scientific Inc., Holliston, MA). The drug was then allowed 30 seconds to diffuse prior to removal of the injector. Following injection, obturators were replaced and the animal was returned to the animal carrier prior to the operant session.

3.2.5 Drug-Testing on Appetitive Responding

Following surgery, animals were allowed to reacquire lever press responding with the RR gradually increased over sessions to a final response requirement of 15. Animals then had weekly microinjection extinction test sessions on Thursdays with "normal" reinforcer sessions the remaining four days (Monday-Wednesday and Friday). For test sessions, animals received a systemic drug injection 30 minutes prior to a bilateral NAc core microinjection. Ten minutes following the microinjection, animals were placed into the operant chambers for a non-reinforced extinction session identical to extinction sessions during Seeking Test Phase of Experiment 1 (20 minutes of access to the lever with the sipper tube retracted from the chamber and filled sipper tube placed on the retractable holder to control for scent cues). Animals were initially habituated to the microinjection procedure with a systemic vehicle injection plus sham microinjection (<10

mm injectors placed into guide cannulae with no fluid administered) followed by an extinction session. Animals then received each of four sets of systemic injection plus NAc core microinjection in a balanced design. After the final set of systemic injection plus NAc core microinjections, animals received an additional microinjection of LY37 (0.5 μg/side with injection volume of 0.5 μL/side) without systemic injection to determine the effects of agonist administration into the NAc core.

3.2.6 Histology

Following the completion of the final operant session, the animals were deeply anesthetized with sodium pentobarbital (120 mg/kg, ip) and transcardially perfused with phosphate buffered saline (PBS) then 10% formalin. The brains were removed and stored in 10% formalin for 14 days. The brains were then sliced (60 μm sections) using a cryostat (Leica CM1950, Leica Microsystems Inc., Buffalo Grove, IL), mounted, and stained using cresyl violet. Site verification was performed using a light microscope.

3.2.7 Drugs

Ethanol solutions were prepared volume/volume in water using 95% ethanol. Sucrose/ethanol solutions were prepared weight/volume. The non-selective group II mGluR agonist LY379268 [(1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid] (Santa Cruz Biotechnology, Inc., Dallas, TX) was dissolved in sterile 0.9% saline and injected at a volume of 1.0 mL/kg BW for systemic injection. LY379268 was dissolved in artificial cerebrospinal fluid (aCSF; Harvard Apparatus, Holliston, MA) and injected at a volume of 0.5 μL/side for microinjection. The non-selective group II

mGluR antagonist LY341495 [(2S)-2-Amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid] (Santa Cruz Biotechnology, Inc., Dallas, TX) was dissolved in 20% dimethyl sulphoxide (DMSO), then diluted with aCSF and injected at a volume of 5 µL/side. Sterile saline was used as the vehicle treatment for systemic LY37 and injected at a volume of 1.0 mL/kg BW. Sterile aCSF and 20% DMSO was used as the vehicle treatment for LY341495 and administered at a volume of 5 µL/side. LY37 (1.5 mg/kg) was administered ip 30 minutes prior to the microinjection. LY34 (1.0 µg/side) was administered 10 minutes prior to the start of the operant session. Pretreatment times, drug doses, and injection volumes, and route of administration for each drug were based on previously studied efficacious methods (Backstrom and Hyytia, 2005; Cannady et al., 2011; David, and Abraini, 2003; Kufahl et al., 2011; Moussawi, et al., 2011; Xi, et al., 2006).

3.2.8 Data Analysis and Statistics

Daily session intake of EtOH was determined from the change in volume in the sipper tube (mL). EtOH intake (g/kg) was calculated from session intake and daily BW measure. Latency to first lever presses (in seconds) was recorded for each session. Appetitive responding, lever press latencies, and BW differences (BW 24-hrs post-injection minus BW 1 hr prior to injection) were analyzed using one-way within-subject RM ANOVA. Post-hoc comparisons were performed using Student-Newman-Keuls test ($p<0.05$). As the LY37 microinjection was not balanced across sessions, the systemic vehicle plus NAc core vehicle and NAc core LY37 were compared using a t-test. All analyses were conducted using SigmaStat3.5 (Systat Software, Inc., Chicago, IL).

3.3 Results

Four subjects were removed from the experiment prior to the start of the microinjection testing due to poor behavioral performance. Of the remaining subjects, six subjects were confirmed to have bilateral cannulae placement with injection into the NAc core (n=6). During the week prior to the sham habituation injection, animals consumed a mean of 0.44 ± 0.05 g/kg EtOH. EtOH intake was slightly lower than that observed during the systemic administration experiments; however, this decrement in EtOH intake has been observed previously following the cannulation surgery and microinjection procedure.

3.3.1 Appetitive Responding

Rats made roughly 50 responses during the non-injection extinction session. Responding during the non-injection extinction and systemic vehicle plus NAc core vehicle sessions were not significantly different [$t(5) = -2.21$, $p=0.08$]. For systemic plus NAc core mGluR2/3 modulation, a significant main effect of treatment on appetitive responding was observed [$F(4, 20) = 12.58$, $p<0.001$]. Post hoc analyses indicate that systemic LY37 plus NAc core vehicle significantly ($p<0.01$) decreased seeking compared to systemic vehicle plus NAc core vehicle (See Figure 3.3: Effect of Intraaccumbens Core Antagonist on Systemic Agonist Induced Suppression of Ethanol-Seeking). Systemic LY37 plus NAc core LY34 was shown to significantly ($p=0.016$) decrease seeking compared to systemic vehicle plus NAc core vehicle. However, appetitive responding following systemic LY37 plus NAc core LY34 was not significantly different ($p=0.56$) from responding during systemic LY37 plus NAc core vehicle. Administration

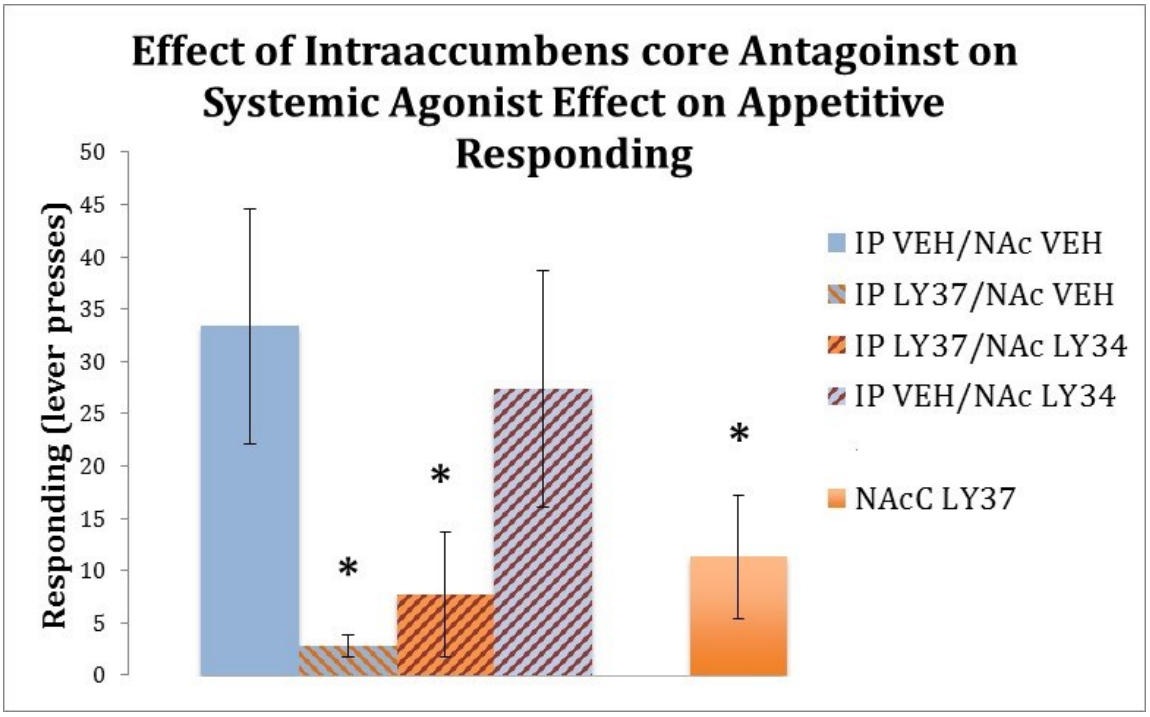

Figure 3.3: Effect of Intraaccumbens Core Antagonist on Systemic Agonist Induced Suppression of Ethanol-Seeking

Appetitive responding for EtOH following systemic injection of the non-selective group II mGluR agonist LY379268 (1.5 mg/kg) or vehicle followed by intra-accumbens core administration of non-selective group II mGluR antagonist LY341495 (1.0 µg/side) or vehicle (n=6/group). Additional microinjection of LY379268 (1.0 µg/side) without systemic injection was also performed. A significant reduction in EtOH-seeking was observed with systemic LY37 plus NAc core vehicle, systemic LY37 plus NAc core LY34 compared to systemic vehicle plus NAc core vehicle. EtOH-seeking was also reduced following NAc core administration of LY37 compared to systemic vehicle plus NAc core vehicle. (* $p<0.05$)

of mGluR2/3 antagonist LY34 following systemic vehicle administration did not significantly decrease seeking compared to systemic vehicle plus NAc core vehicle ($p=0.85$). Given the findings of a lack of attenuation of systemic mGluR2/3 agonist LY37 suppression of EtOH-seeking following NAc core mGluR2/3 antagonist LY34 administration, LY37 was microinjected into the NAc core to clarify if NAc core group II mGluRs are involved in the regulation of EtOH seeking. As this injection was not counterbalanced across animals, the data were analyzed using a pair-samples t-test (NAc core LY37 vs systemic vehicle plus NAc core vehicle). Appetitive responding following NAc core LY37 administration was significantly decreased compared to systemic vehicle plus NAc core vehicle [$t(5)=2.58$, $p=0.05$].

3.3.2 Latency to First Lever Press

To determine possible effects of NAc core microinjection of the mGluR2/3 antagonist LY34 and agonist LY37 on locomotion, the latency to first lever press was examined. Examination of lever press latency can be confounded for sessions in which the animal does not emit a lever press response as this could indicate either a suppression of locomotor behavior or lack of reinforcer seeking. Therefore, analysis of lever press latency was performed using both a conservative approach (non-response characterized as reflecting seeking behavior and trials excluded from analysis) and liberal approach [non-response characterized as reflecting diminished locomotion and maximum latency (1200 seconds) used]. Neither the conservative [$F(3,11)=0.355$, $p=0.79$] nor liberal [$F(3,15)=0.314$, $p=0.82$] analyses were significant [see Figure 3.4: Effect of Systemic LY379268 and Intraaccumbens Core LY341495 on Latency to First Lever Press (Non-

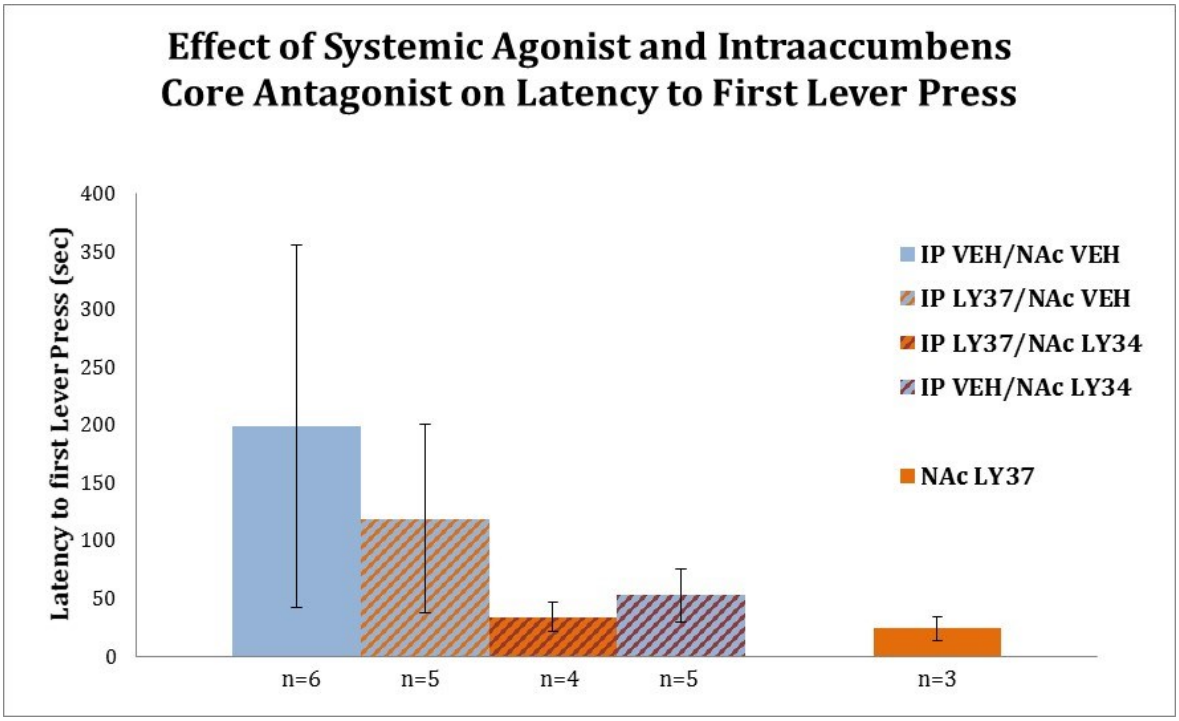

Figure 3.4: Effect of Systemic LY379268 and Intraaccumbens Core LY341495 on Latency to First Lever Press (non-response excluded)

Latency to first lever press following systemic injection (with non-response sessions excluded from analysis) of the non-selective group II mGluR agonist LY379268 (1.5 mg/kg) or vehicle followed by intra-accumbens core administration of non-selective group II mGluR antagonist LY341495 (1.0 µg/side) or vehicle. Additional microinjection of LY379268 (1.0 µg/side) without systemic injection was also performed. No significant effect of systemic plus NAC core administration or NAc core LY37 was observed for latency to first lever press.

Response Excluded) and Figure 3.5: Effect of Systemic LY379268 and Intraaccumbens Core LY341495 on Latency to First Lever Press (Non-Response as Maximum Latency)]. The effect of NAc core LY37 administration was analyzed separately using a pair-samples t-test (NAc core LY37 vs systemic vehicle plus NAc core vehicle). Neither the conservative [$t(2) = 0.81$, $p = 0.51$] nor liberal [$t(5) = -1.74$, $p = 0.14$] analyses were significant. Overall, the latency to first lever press results suggest that NAc core administration of neither the mGluR2/3 antagonist LY34 nor the mGluR2/3 agonist LY37 had a significant effect on locomotion.

3.3.3 Systemic Agonist Effect on Body Weight

The difference in body weight between injection session and subsequent session (roughly 24 hours post-injection) was computed to examine possible effects of systemic LY37 administration and microinjection on body weight. A significant main effect of systemic treatment on body weight during drinking test phase was observed [$F(1, 10) = 8.87$, $p = 0.01$]. Post hoc analyses indicate that systemic LY37 administration significantly ($p = 0.01$) decreased body weight 24 hours following systemic injection (See Figure 3.6: Effect of Systemic LY379268 on Body Weight). The average body weight difference following systemic LY37 administration was -21.4 ± 2.1 g.

3.4 Discussion

The observed suppression of appetitive responding following systemic LY37 plus NAc core vehicle replicates the findings of reduced EtOH-seeking following systemic administration of LY37 observed in the first experiment. No effect of systemic LY37,

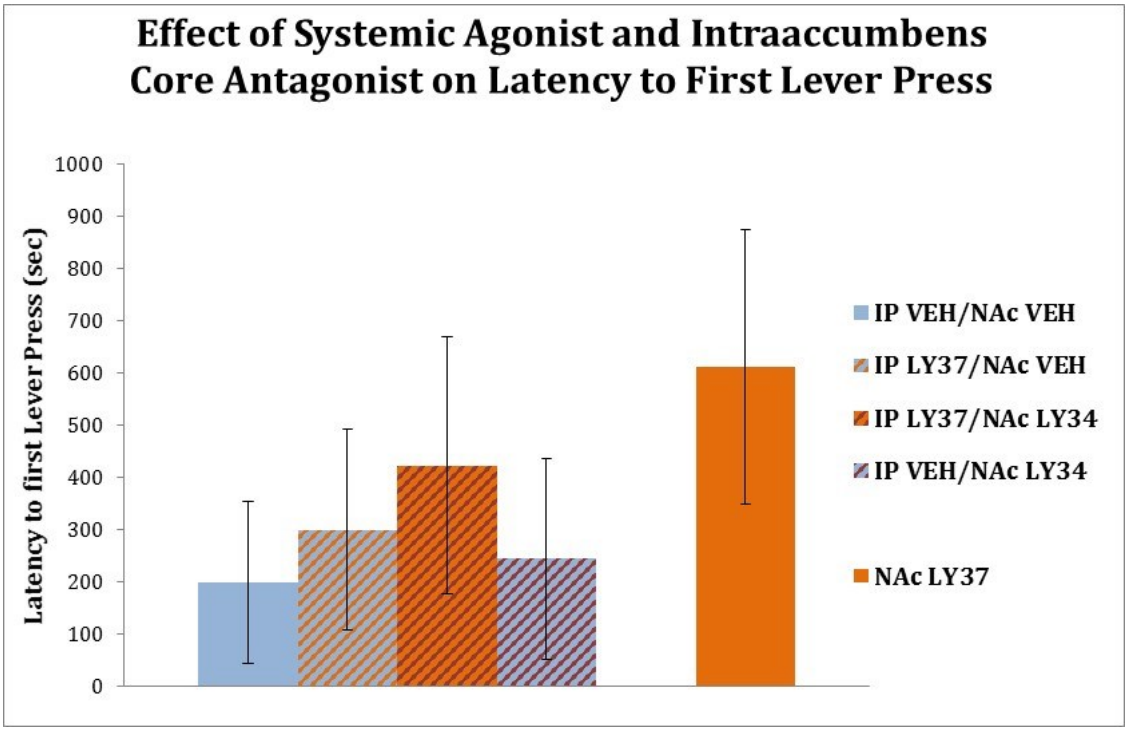

Figure 3.5: Effect of Systemic LY379268 and Intraaccumbens Core LY341495 on Latency to First Lever Press (Non-Response as Maximum Latency)

Latency to first lever press following systemic injection (with non-response sessions analyzed with maximum latency) of the non-selective group II mGluR agonist LY379268 (1.5 mg/kg) or vehicle followed by intra-accumbens core administration of non-selective group II mGluR antagonist LY341495 (1.0 µg/side) or vehicle (n=6/group). Additional microinjection of LY379268 (1.0 µg/side) without systemic injection was also performed. No significant effect of systemic plus NAC core administration or NAc core LY37 was observed for latency to first lever press.

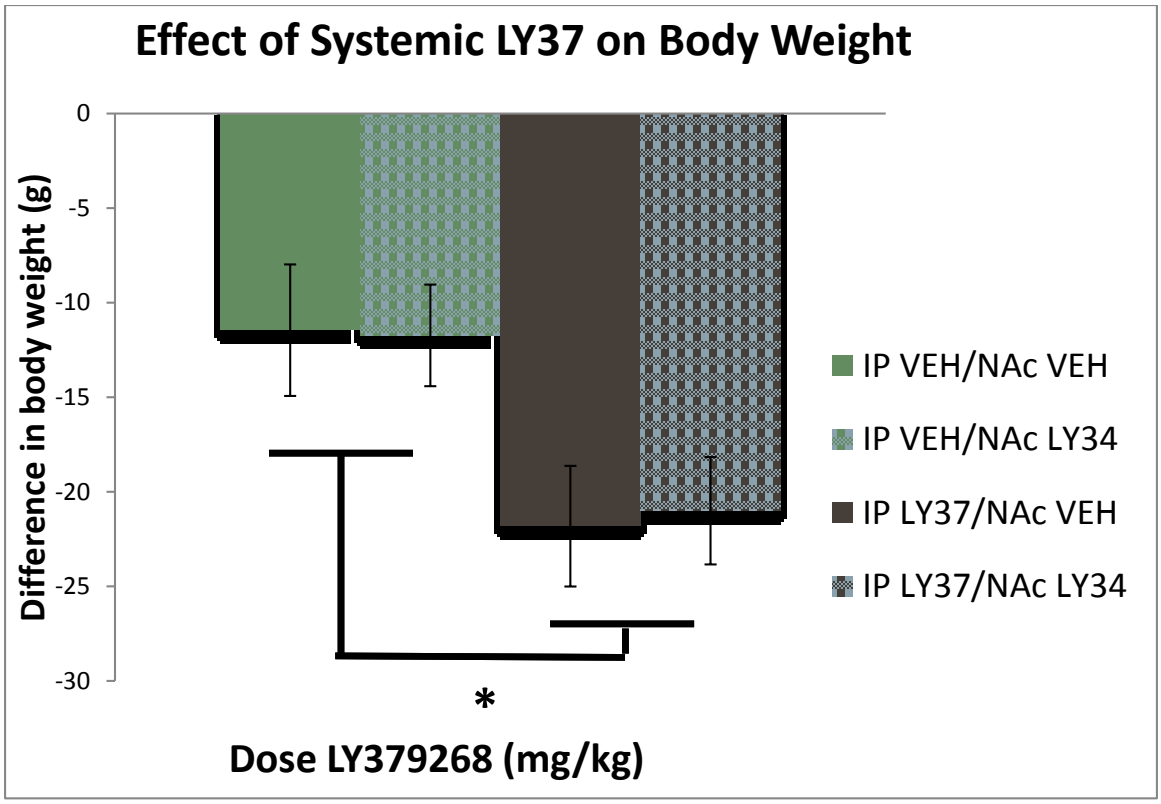

Figure 3.6: Effect of Systemic LY379268 on Body Weight

Differences in body weight between injection session and subsequent session (roughly24 hours post-injection) following systemic injection of the non-selective group II mGluR agonist LY379268 (1.5 mg/kg) or vehicle followed by intra-accumbens core administration of non-selective group II mGluR antagonist LY341495 (1.0 µg/side) or vehicle (n=6/group). A significant reduction in body weight was observed for systemic LY379268 administration. (* $p<0.05$)

intra-accumbens core LY34, or intra-accumbens core LY37 on latency to first lever press was observed. This suggests that intra-accumbens core administration of either the mGluR2/3 antagonist LY34 or agonist LY37 did not result in a significant effect on locomotion and the observed effects of LY34 and LY37 on appetitive responding are due to their effect on seeking behavior rather than a general effect on locomotion. Similar to the first experiment, the non-selective group II mGluR agonist LY37 was found to significantly decrease body weight 24-hours following systemic administration. The precise mechanism of action for the observed reduction in body weight is not clear, though may be due in part to a mGluR2/3 agonist induced increase mPFC 5-HT.

3.4.1 Effect of Intra-Accumbens Core Antagonist on Systemic Agonist Induced Suppression of Appetitive Responding

No effect of intra-accumbens core LY34 administration on EtOH-seeking was observed following systemic vehicle injection. As group II mGluRs are located presynaptically outside of the synapse (Ohishi, et al., 1998; Tamaru, et al., 2001), they are activated during periods of increased glutamatergic transmission when glutamate spills from the synapse. mGluR2/3 receptors negatively regulate glutamate release resulting in reduced glutamatergic neurotransmission. Antagonism of mGluR2/3 does not further augment glutamatergic signaling, rather blockade of mGlu2/3 receptors maintains glutamatergic signaling. Therefore, NAc core administration of the mGluR2/3 antagonist LY34 should not affect EtOH-seeking as we observed.

Appetitive responding following systemic LY37 plus NAc core LY34 was not significantly different from responding during systemic LY37 plus NAc core vehicle. The

inability of NAc core mGluR2/3 blockade to significant attenuate LY34 reduction in appetitive responding may suggest that group II mGluRs in the NAc core may not be involved in the regulation of EtOH-seeking. To examine this possibility, the mGluR2/3 agonist LY37 was microinjected into the NAc core (0.5 μg/side). Intra-accumbens core microinjection of LY37 significantly reduced EtOH-seeking without affecting the latency to first lever press, suggesting that NAc core mGlu2/3 receptors are involved in regulating EtOH-seeking. Therefore, the observed lack of attenuation of LY37 suppression of EtOH-seeking by NAc core LY34 is more likely due to methodological confounds (e.g., LY34 dose selection or systemic administration of agonist prior to microinjection) than NAc core mGluR2/3 not being involved in the regulation of EtOH-seeking.

3.4.2 Conclusions

Overall, systemic administration of the mGluR2/3 agonist LY37 was observed to significant decrease EtOH-seeking. Intra-accumbens core mGluR2/3 blockade had no effect on the systemic LY37 induced suppression of EtOH-seeking. However, intra-accumbens core administration of LY37 significantly reduced EtOH-seeking, suggesting that NAc core mGluR2/3 regulation of glutamatergic neurotransmission is involved in the control of EtOH-seeking.

CHAPTER 4. GENERAL DISCUSSION

There are several important findings from these experiments. Here we observed that systemic administration of the mGluR2/3 agonist LY37 decreased EtOH seeking. This suggests, as previous relapse and reinstatement studies using LY37 have observed, that activation of group II mGluRs results in a reduction in glutamatergic neurotransmission in key brain regions associated with drug seeking observed here during maintenance drinking. Previous studies have also observed a decrease in operant EtOH self-administration using a fixed ratio schedule following systemic LY37 administration (Backstrom and Hyytia, 2005; Jin, et al., 2010; Sidhpura, et al., 2010). Here we observed that systemic LY37 administration does not significantly affect EtOH consumption using the sipper tube model. The lack of LY37 effect on EtOH consumption emphasizes the limitation of fixed ratio schedules as they measure a combination of reinforcer seeking and consumption across the session while the sipper tube model allows for examination of reinforcer consumption specifically without the seeking confound. Together these findings suggest that regulation of neurotransmission via mGluR2/3 activation influences EtOH seeking without affecting EtOH consumption.

More importantly, systemic administration of the group II mGluR agonist LY37 was observed to reduce seeking and consumption of an alternative reinforcer (sucrose) at doses not found to significantly affect locomotion compared to baseline. Few studies

have examined the effect of LY37 on seeking and/or consumption of an alternative reinforcer. In these studies only the highest dose of LY37 tested (3 mg/kg) was shown to significantly reduce alternative reinforcer seeking in food-deprived male Wistar rats (Peters and Kalivas, 2006) and self-administration in food-deprived (Liechti, et al., 2007) and non-deprived (Kufahl, et al., 2011) male Wistar rats. However, the same dose of LY37 (3 mg/kg) shown to reduce seeking and consumption of food reinforcers was also shown to significantly reduce spontaneous locomotor behavior (Kufahl, et al., 2011). This decrease in locomotion suggests that the observed effect of LY37 on alternative reinforcer seeking and consumption was due to the sedative effects of LY37 rather than an effect on reinforcer seeking and consumption. However, here we demonstrated that LY37 does, in fact, reduce not only sucrose seeking but also sucrose consumption and body weight 24-hours following systemic administration at doses not observed to result in a significant change in locomotion from baseline. This finding suggests that modulation of group II mGluRs is not specific to EtOH-seeking, but rather modulation of mGluR2/3 influences reinforcer seeking generally. Though the fact that LY37 reduces seeking of natural reinforcers does not completely rule out the potential clinical utility of mGluR2/3 agonists for the treatment of drug dependence, this finding highlights the need for experimental controls (i.e., alternative reinforcers) when examining potential therapeutics. As well, the reduction in sucrose seeking and consumption may suggest that the reduction in EtOH seeking is not due to a reduction in the incentive salience of EtOH specifically but rather a general reduction in the incentive salience of reinforcers. This general reduction in reinforcer seeking following LY37 administration may be due to the

induction of a malaise or transient drug-induced anhedonic-like state that needs further investigation.

Despite preclinical studies demonstrating a significant reduction in reinstatement of drug seeking for nicotine, cocaine, and ethanol following systemic administration of group II mGluR agonists (Baptista, et al., 2004; Kufahl, et al., 2011; Liechti, et al., 2007), clinical efficacy of group II mGluR agonists in the treatment of drug addiction has yet to be demonstrated. Several studies have examined the effects of group II mGluR agonists for the treatment of schizophrenia, generalized anxiety, and panic disorder in humans. Patil et al. (2007) found a significant decrease in both negative and positive symptoms in schizophrenics (n=98) following four weeks of treatment with LY2140023 (4 mg twice daily), a prodrug of the non-selective group II mGluR agonist LY404039. Dunayevich, et al. (2008) found a significant improvement in anxiety in a small sample of patients with generalized anxiety disorder following eight weeks of treatment with LY5444344 (16 mg twice daily), a prodrug of the non-selective group II mGluR agonist LY354740. However, the study was terminated early due significant adverse event (convulsions) observed in preclinical trials of LY354740. LY354740 failed to show any effect of treatment for panic disorder following nine weeks of chronic administration (100 and 200 mg/day) (Bergink and Westenberg, 2005). Overall from these clinical studies, there is some preliminary indication of utility of mGluR2/3 agonists in the treatment of psychiatric disorders; however, as no follow-up studies with larger patient populations have been subsequently published, the potential clinical utility of the current mGluR2/3 agonists is questionable.

Preclinically, chronic systemic administration of LY37 (1 mg/kg/day) in male Wistar rats trained to self-administer nicotine resulted in the development of tolerance such that nicotine self-administration was not significantly different from control animals after 12 days of systemic administration (Liechti, et al., 2007). This rapid development of tolerance suggests a limited clinical efficacy for the current mGluR2/3 agonists for the treatment of drug dependence. As well, Meinhardt et al. (2013) demonstrated a significant reduction in mGlu2 receptor expression in alcoholics post-mortem. This reduced mGluR2 expression may limit the ability of mGluR2/3 agonists to modulate glutamatergic neurotransmission to suppression drug-seeking. Overall, the clinical utility of the current mGluR2/3 agonists does not seem favorable for the treatment of drug dependency. However, the shortcomings with the current non-selective group II mGluR agonists may be potentially overcome using yet-to-be developed subtype selective group II mGluR agonists or PAMs which may be efficacious in the clinical treatment of drug dependency.

4.1 Future Directions

An interesting and unexpected finding of these experiments was the significant reduction in body weight 24-hours after systemic LY37 administration. A more controlled evaluation of the effect of systemic administration on body weight following acute and chronic administration of group II agonists is merited for the potential use of mGluR2/3 agonists clinically for the treatment of obesity. However, given the rapid development of tolerance observed with nicotine self-administration (Liechti, et al., 2007), it is possible that a similar tolerance to the effect on appetite could rapidly develop

following chronic administration of LY37 thus limiting the clinical utility of LY37 for the treatment of obesity.

Systemic and intra-accumbens core administration of the group II mGluR agonist LY37 was found to decrease EtOH-seeking. This reduction in reinforcer seeking is thought to be related to the reduction in glutamatergic neurotransmission observed following mGluR2/3 activation (as group II mGluRs negatively regulate synaptic glutamate release), a view that is supported by but not definitively demonstrated with the observed suppression of EtOH-seeking following accumbens core LY37 administration. However, as group II mGluRs have been shown to regulate not only glutamate release, but also the release of other neurotransmitters (Xi, et al., 2002), further examination of the mechanism by which activation of mGluR2/3 regulate EtOH-seeking should be performed.

The role of mGlu2 receptors in regulating EtOH seeking could not be addressed due to differences in the mechanisms of action between the mGluR2/3 agonist LY37 and mGluR2 PAM BINA. Further examination of the contribution of mGlu2 receptors on EtOH-seeking is needed. For this, systemic administration of the selective mGluR2 agonist/mGluR3 antagonist LY395756 or a combination of the selective non-competitive mGluR2 antagonist Ro 64-5229 and LY37 using the sipper tube model may further clarify the role of mGlu2 receptors in EtOH seeking.

4.2 Summary

Previous studies examining the role of group II mGluRs have utilized either reinstatement or operant self-administration models to characterize the influence of

mGlu2/3 receptors on EtOH seeking and consumption (Backstrom and Hyytia, 2005; Bossert, et al., 2005; Jin, et al., 2010; Liechti, et al., 2007; Sidhpura, et al., 2010). Generally, reinstatement studies suggest that group II mGluRs are involved in regulating drug seeking during protracted withdrawal or following extinction. Using a behavioral model that allows for discrete separation of reinforcer seeking and consumption (the sipper tube model), systemic administration of the mGluR2/3 agonist LY37 was shown to decrease EtOH-seeking but not consumption. Intra-accumbens core administration of LY37 was also shown to significantly reduced EtOH-seeking, further implicating group II mGluRs in EtOH-seeking. However, systemic administration of the selective mGluR2 PAM BINA had no significant effect on either seeking or consumption of EtOH or sucrose. Systemic administration of LY37 was found to significantly reduce sucrose consumption and body weight 24-hours following systemic administration meriting further examination of the role of mGluR2/3 receptors on feeding. Overall, activation of group II mGluRs by agonist administered either systemically or within the NAc core was shown to significantly reduce reinforcer seeking with the observed suppression in seeking not attributable to general effects on locomotion. This suggests that regulation of glutamatergic transmission by group II mGluRs is involved in the appetitive but not consummatory aspects of reinforcement. Further research is needed to elucidate the role of the mGlu2 and mGlu3 receptors in regulating reinforcer seeking.

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VITA

VITA

Kyle Allyson Windisch

Education and Training

2009 – Present	M.S./Ph.D. program	Addiction Neuroscience Department of Psychology IUPUI, Indianapolis, IN Mentor: Cristine Czachowski, Ph.D.
2009 – 2012	M.S.	Addiction Neuroscience Department of Psychology IUPUI, Indianapolis, IN Mentor: Cristine Czachowski, Ph.D.
Aug 2005-2009	Research Technician	Alcohol Research Laboratory Department of Psychiatry IUSM Indianapolis, IN Supervisor: Sean O'Connor, M.D.
2005	B.S.	Major in Biology and Psychology Indiana University, Bloomington IN
2005	B.A.	Major in Chemistry Indiana University, Bloomington IN

Research Experience

2009-2014 Alcohol Research Laboratory, Psychology, Indiana University
Supervisor: Cristine Czachowski, Ph.D.

Developing a Functional IV Ethanol Self-Administration Method in P Rats (2009-2013). A valid model by which rats will self-administer pharmacologically relevant levels of ethanol by the IV route has remained elusive. This line of research attempts to modify previous IV ethanol self-administration techniques to garner a functional method by which rats will administer pharmacologically relevant levels of ethanol.

Examining the Role of Group II Metabotropic Glutamate Receptors in the Appetitive and Consummatory Aspects of Ethanol and Sucrose Reinforcement (2013-present). Group II metabotropic glutamate receptors (mGluR2/3) are

presynaptically expressed receptors that have been shown to negatively regulate glutamate and dopamine release. Several studies have suggested a role for these receptors in regulating ethanol reinforcement. This line of research examines the influence of systemic modulation of mGluR2/3 receptors on appetitive responding and consumption of ethanol and sucrose. As well it examines the influence of nucleus accumbens core mGluR2/3 receptors in appetitive responding for ethanol.

2005-2009 Alcohol Research Laboratory, Psychology, Indiana University
Supervisor: Sean O'Connor, M.D.

Acute Tolerance to Alcohol and the Familial Risk for Alcoholism (2005-2009). The effect family history of alcohol has on several dependent measures (heart rate, resting EEG, saccade and fixation eye movements, and subjective measures) was examined in non-dependent light social drinking adults (age 21-30). Our preliminary findings were a distinct interaction between family history and individual recent drinking history on subject response. Additional analysis of the data is ongoing.

Human Alcohol Deprivation Effect (2005-2009). The effects of alcohol deprivation in healthy non-dependent heavy social drinkers (age 21-30) were measured. Neurological and physiological tests were performed during two infusion sessions separated by a one-week abstinence period. Reduction and analysis still underway.

2003-2004 Cognitive Neuroscience Laboratory, Psychology, Indiana University
Supervisor: Julie Stout, Ph.D.

Set-shifting mechanisms in Parkinson's disease (2003-2004). I was involved in research that examined the ability to set-shift in Parkinson's patients. We found that Parkinson's patients had significant impairment with set-shifting.

Honors and Awards

Summer 2014	Research Society on Alcoholism Travel Award
Summer 2013	Research Society on Alcoholism Travel Award
Summer 2012	Research Society on Alcoholism Travel Award
Summer 2011	Research Society on Alcoholism Travel Award
2011-present	Predoctoral Trainee; NIAAA sponsored T32 AA007462
Spring 2003	Indiana University Bloomington Dean's List
Fall 2002	Indiana University Bloomington Dean's List
Spring 2002	IUPUI Dean's List
Fall 2001	IUPUI Dean's List
Spring 2000	IUPUI Dean's List
Fall 2000	IUPUI Dean's List
Spring 2001	Hal Tobin Freshman Writing Award, IUPUI
Fall 2000	IUPUI School of Science 'A' Student Award

Professional Affiliations

2010-present Student Member, Research Society on Alcoholism

2007-present	Associate Member, Society for Neuroscience
Spring 2004	Phi Beta Kappa Induction, Indiana University
Spring 2004	Order of Omega Induction, Indiana University
Spring 2003	Golden Key Induction, Indiana University
2002-2005	Student Member, Psi Chi, Indiana University
Fall 2002	Alpha Epsilon Delta Induction, Indiana University
Fall 2002	Alpha Chi Sigma Induction, Epsilon Chapter, Indiana University

Publications

L Wetherill, T Foroud, SL Morzorati, T Darlington, K Windisch, SJ O'Connor. (2012). Subjective Perceptions Associated with the Ascending and Descending Slopes of Breath Alcohol Exposure Vary with Recent Drinking History. *Alcoholism: Clinical and Experimental Research,* 36(6):1050-1057. PMCID: PMC3288407.

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Kosobud, AEK; Wetherill, L; Plawecki, M; Kareken, D; Liang, T; Nurnberger Jr., J; Windisch, K; Xuei, X; Edenberg, H; Foroud, T; O'Connor, S (submitted). Adaptation of Subjective Responses to Alcohol Is Affected By an Interaction of GABRA2 Genotype and Recent Drinking. *Alcoholism: Clinical and Experimental Research*.

Abstracts/Poster Presentations

KA Windisch, B Maynard, W White, IM White. (2008). Amygdala Influence on Amphetamine-Induced Hyperactivity. Presented at Annual Meeting of the Society for Neuroscience, Washington D.C., Program No. 161.14.2008 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2008. Online.

IM White, K Windisch, T Applegate, H.-T. Kim, D.-H. Kwak, H Han, K Lee. (2008). Event Related Potentials During Abstract Rule Shifting in College Students. Program No 682.13.2008 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2008. Online.

K Windisch, M Plawecki, L Flury-Wetherill, J Nurnberger, T Darlington, and S O'Connor. (2008) Rapid Tolerance of Resting EEG to Alcohol is Associated with Familial Alcoholism. *Alcoholism: Clinical and Experimental Research* 32(s1):180A

T Darlington, IM White, K Windisch, M Plawecki, L Flury-Wetherill, and S O'Connor: (2008) Family History of Alcoholism Predicts Cardiac Response and its Rapid Tolerance to Alcohol. *Alcoholism: Clinical and Experimental Research* 32(s1):179A

J Tian, T Blekher, L Flury-Wetherill, T Foroud, RD Yee, TM Darlington, K Windisch, S O'Connor. (2008) Rapid Tolerance of Saccadic Eye Movements to Alcohol is Associated with Familial Alcoholism. *Alcoholism: Clinical and Experimental Research* 32(s1):180A

MJ Walker, CA Cox, V Bragulat, M Dzemidzic, KA Windisch, SJ O'Connor, NJ Grahame, DA Kareken. (2010) Negative Prediction Error to Classically Conditioned

Novel Cues of Alcohol Intoxication. *Alcoholism: Clinical and Experimental Research* 34(s2):163A.

DA Kareken, MJ Walker, M Dzemidzic, CA Cox, V Bragulat, KA Windisch, SJ O'Connor, NJ Grahame. (2010) Regional Brain Responses to Experimentally Classically Conditioned Novel Cues of Alcohol Intoxication. *Alcoholism: Clinical and Experimental Research* 34(s3):129A.

KA Windisch; AEK Kosobud; RA Chambers, A Sentir; S O'Connor; CL Czachowski. (2011) Pharmacologically relevant levels of IV ethanol self-administration in the P rat. *Alcoholism: Clinical and Experimental Research* 35(6s1):121A.

MH Plawecki, P Hazra, K Windisch, V Vitvitskiy, US Zimmermann, A Kosobud, S O'Connor (2011). Design of Progressive Work Paradigms for Intravenous Self-Administration of Ethanol. *Alcoholism: Clinical and Experimental Research* 35(6s1):205A

KA Windisch, AEK Kosobud, CL Czachowski (2012). Revisiting Intravenous Ethanol Self Administration in the P Rat Using A Multiple Schedule of Reinforcement. *Alcoholism: Clinical and Experimental Research*, 36(6s1):110A.

KA Windisch, P Hazra, MH Plawecki, L Wetherill, H Edenberg, DA Kareken, S O'Connor (2012). Acute Sensitivity and Adaptation to Alcohol Associated With Family History of Alcoholism and *GABRG1* Genetic Status Using the Stop Signal Task. *Alcoholism: Clinical and Experimental Research*, 36(6s1):172A.

KA Windisch, CL Czachowski (2013). Effects of CNQX and Tetrodotoxin in the Prefrontal Cortex on Ethanol- and Sucrose-Seeking. Presented at the Annual Meeting of the Research Society on Alcoholism, Orlando, FL. *Alcoholism: Clinical and Experimental Research* 37(s2):232A.

A.E.K. Kosobud; L. Wetherill; M.H. Plawecki; T. Liang; D.A. Kareken; J.L. Nurnberger; K. Windisch; X. Xuei; H. J. Edenberg; T.M. Foroud; S.J. O'Connor. An Interaction of *GABRA2* Genotype and Recent Drinking Modifies Subjective Responses to Alcohol. Presented at the Genes, Brain & Behavior meeting of the International Behavioural and Neural Genetics Society, Chicago, IL, May, 2014.

KA Windisch, CL Czachowski (2014). Role of Group II Metabotropic Glutamate Receptors in Appetitive and Consummatory Aspects of Ethanol and Sucrose Reinforcement. Presented at the Annual Meeting of the Research Society on Alcoholism, Bellevue, WA. *Alcoholism: Clinical and Experimental Research*, 38(s1):166A.

A.E.K. Kosobud; L. Wetherill; M.H. Plawecki; K. Windisch; D.A. Kareken; J.M. Hays; K.E. White; J.L. Nurnberger; T. Liang; X. Xuei; H.J. Edenberg; T.M. Foroud; S.J. O'Connor (2014). An Interaction Between Recent Drinking History and *GABRA2* Genotype Affects Adaptation to the Subjective Effects of Alcohol. Presented at the Annual Meeting of the Research Society on Alcoholism, Bellevue, WA. *Alcoholism: Clinical and Experimental Research*, 38(s1):149A.

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Available upon request.