Glutamate hyperexcitability and aggression during withdrawal from escalated ethanol consumption in outbred male mice.

Anna J. Nathanson, Lara S. Hwa, Emily Newman, Joseph F. DeBold, and Klaus A. Miczek

Senior Honors Thesis

Psychology Department, Tufts University, 2012

Abstract:

Ethanol withdrawal syndrome, characterized by glutamatergic hyperexcitability in the brain, is a defining characteristic of alcohol dependence. Glutamate hyperexcitability and an upregulation of NMDA receptors have been observed in the brains of mice in withdrawal from ethanol consumption. The present study examined the effects of the uncompetitive NMDA receptor antagonist memantine on aggression during withdrawal from ethanol. Outbred Carworth Farm Webster (CFW) male mice were given intermittent, 2-bottle choice access to 20% w/v ethanol and water for ten weeks. CFW males voluntarily consumed ethanol, ranging from 5-20 grams/kilogram (g/kg) bodyweight in 24 hours over the 10 weeks of intermittent access. To measure alcohol withdrawal severity, handling-induced convulsion (HIC) scores were assessed every two hours during the ethanol withdrawal period. Outbred mice exhibited significantly greater HIC severity than water controls. In the same individuals, aggression during withdrawal was probed with a memantine or ketamine challenge to assess glutamate excitability. Resident CFW mice were injected with memantine (0, 3, 5, 10, or 30 mg/kg, i.p.) or ketamine (0, 3, 5, or 10 mg/kg, i.p.) and tested for aggression against an intruder at eight hours into withdrawal. Memantine significantly increased aggression in CFW mice during the withdrawal period, specifically at the 5 mg/kg dose, while ketamine did not significantly affect measures of aggression during ethanol withdrawal. These findings suggest that the uncompetitive NMDA receptor antagonist memantine, but not ketamine, biphasically increased withdrawal-related aggression in outbred CFW mice. Future studies will use microdialysis to examine glutamate levels in the mouse brain throughout the ethanol withdrawal period and will measure NMDA receptor regulation in the ethanol-withdrawn brain using immunohistological techniques.

Alcoholism is one of the most common psychiatric disorders, with about 8 million people in the United States meeting the criteria for alcohol dependence (Grant et al., 2004). Withdrawal is a defining characteristic of ethanol dependence, and at its most extreme is typified by increased anxiety, tremors, and seizures (Dahchour & DeWitte, 2003). In order to avoid these symptoms, both humans and animals experiencing ethanol withdrawal will increase their ethanol intake (Brown, et al., 1998; (DeWitte, Pinto, Ansseau, & Verbanck, 2003; Spanagel & Kiefer, 2008), making withdrawal a potent negative reinforcer for alcohol use. The neurobiological basis of ethanol withdrawal has yet to be fully elucidated, although one important mechanism is thought to be the upregulation of glutamatergic receptors in the brain during withdrawal (Dahchour & DeWitte, 1999; Dahchour & DeWitte, 2003; Follesa & Ticku, 1996; Grant, Valverius, Hudspith, & Tabakoff, 1990; Kumari & Ticku, 1998). The aim of the current work is to explore the role of the glutamate receptor NMDA-mediated hyperexcitability in the withdrawal phase in an animal model of ethanol dependence.

Alcohol and Society

Humans have imbibed alcohol since the prehistoric era (Rehm et al., 2009). Although most adults abstain from regular alcohol use (Rehm et al., 2009), according to the World Health Organization, about 55% of adults have consumed alcohol, with alcohol abuse creating significant public health and safety problems the world over (WHO, 2011). Alcohol consumption is responsible for 2.5 million deaths worldwide, and is the third highest risk factor for disease burden (WHO, 2011). 3.6% of people in the world have an alcohol-use disorder, with men still being far more likely to consume more alcohol and develop an alcohol-use disorder than women (Rehm et al., 2009). The alcohol-related economic cost varies among countries, with alcohol consumption leading to productivity loss, health-care costs, and law enforcement costs (Rehm et

al., 2009). Overall, it is clear that alcohol abuse has a significant detrimental impact on society. Discovering the neurobiological mechanism of alcohol and the biological basis of alcoholism could help differentiate social alcohol use from problematic use, both of which have serious societal consequences.

Animal Models of Alcohol Use

Creating translational animal models of alcohol use allows better study of the neurobiological mechanisms associated with alcoholism, given the ethical constraints associated with performing tightly controlled, drug-related experiments on human subjects. In addition, animal models allow for attempts to understand alcoholism at many levels of analysis, from behavioral to molecular, and provides a way to discover targets for new pharmacological substances that could possibly treat alcoholism (Kamdar et al., 2007)

A variety of different types of animal models of alcohol drinking and alcohol dependence have been developed over the past century (Spanagel, 2000). The following table represents a summary of the major animal models, with their respective advantages and disadvantages with regard to mimicking human drinking behavior.

Method	**Description**	**Reference**	**Advantages**	**Disadvantages**	**Blood Ethanol Concentration**
One-bottle/Ethanol diet	One bottle with an ethanol solution (w/v %)	(Lieber & DeCarli, 1982)	Animals drink elevated ethanol levels	Forced drinking; Ethanol is animal's only source of calories/nutrients	100-150 mg/dl ((Lieber & DeCarli, 1982)
Sucrose fading	Ethanol solution mixed with saccharin, saccharin concentration is progressively	(Samson, 1986; Tolliver, Sadeghi, & Samson, 1988)	Animals develop ethanol preference as the taste of the solution is not initially	Does not consider the taste aversion of ethanol; Ethanol is not naturally sweet; Sucrose is calorically rich	40-110 mg/dl (Matthews, Overstreet, Rezvani, Devaud, & Morrow, 2001)

	decreased		aversive		
Ethanol vapor chamber	Animals breathe in vaporized ethanol solution	(Rogers, Wiener, & Bloom, 1979)	Renders physical dependence on ethanol	Forced; Ethanol is typically consumed orally in nature, not via inhalation	200-300 mg/dl (Griffin, III, Lopez, & Becker, 2009)
Drinking in the dark	20% ethanol solution at 2 or 4 hours into the dark cycle	(Rhodes, Best, Belknap, Finn, & Crabbe, 2005)	Oral consumption; Model of "binge-like" ethanol consumption	Access to ethanol is time-limited; Does not induce ethanol dependence	0-250 mg/dl (Rhodes et al., 2005)
Two-bottle choice/Intermittent Access	Choice between water and ethanol solution every other day	(Wise, 1973; Hwa et al., 2011)	Leads to high voluntary ethanol consumption Represents cyclical nature of human alcohol drinking (alternating access and deprivation)	Difficult to assess motivation to drink ethanol	79.58-166.93 mg/dl (Hwa et al., 2011)

Intermittent Access Procedure.

All the ethanol-drinking procedures outlined above lead to high levels of ethanol consumption and represent attempts to mimic human alcoholic drinking behavior, each with its own limitations. Thus far, no animal model has fully captured all of the complex sociocultural and behavioral factors that are involved in human alcoholism. Some models, however, have more potential for translation to humans than others. The key features of an externally valid animal models would include oral ethanol consumption and free-choice, and preferential

drinking of ethanol over other liquids leading to ethanol dependence (Dole, Ho, & Gentry, 1985; Lester & Freed, 1973).

In addition, studies have suggested that human alcoholism results from a specific pattern of drinking behavior: intermittent heavy drinking, or "binge," episodes followed by periods of deprivation (Breese et al., 2005). These cycles of high ethanol consumption and abstinence can lead to a "kindling" effect in the brain, similar to electrophysiological kindling (Ballenger & Post, 1978). In electrophysiological kindling, the brain becomes sensitized to excitatory electrical impulses (Goddard, McIntyre, & Leech, 1969). In alcohol dependence, ethanol withdrawal is theorized to lead to a hyperexcitable state due to the upregulation of glutamatergic activity in the brain (Chefer et al., 2011; Dahchour & DeWitte, 1999; Dahchour & DeWitte, 2003; Rossetti, Carboni, & Fadda, 1999). Thus, each episode of ethanol withdrawal may serve as an excitatory stimulus, leading to a kindling-like process in which the nervous system becomes sensitized to the effects of ethanol withdrawal (Ballenger & Post, 1978).

Thus, prior experience with ethanol withdrawal may lead to sensitization during subsequent withdrawal episodes. Ethanol withdrawal is typically accompanied by a number of aversive effects in humans and animals, including anxiety and seizures (Dahchour & DeWitte, 2003). Individuals will often increase ethanol intake after experiencing withdrawal (Heyser, Schulteis, & Koob, 1997), perhaps to relieve withdrawal's negative symptoms (Dahchour & DeWitte, 2003). Repeated episodes of ethanol withdrawal have been shown to augment the intensity of aversive events, such as seizures (Becker & Hale, 1993; Hwa et al., 2011), and animals subjected to intermittent periods of ethanol access and withdrawal increase their ethanol intake accordingly, to alcoholic-like levels (Becker & Lopez, 2004; Hwa et al., 2011). The intermittent access procedure, in which animals are presented with the option to drink ethanol or

water every other day, with the removal of ethanol solution occurring on alternate days (Hwa et al., 2011), thus generates a promising animal model for alcohol dependence by changing neurotransmitter activity.

Neurobiological Mechanisms of Alcohol and Alcohol Withdrawal

Alcohol intake affects a variety of neurotransmitter systems in the brain via action on receptors, including the dopaminergic, serotonergic, opioidergic, GABAergic, and glutamatergic systems (Gass & Olive, 2008; Koob, 1992; Spanagel, 2009). The current work will focus on alcohol's effects on glutamate, in order to better understand alcohol withdrawal's effect on the glutamatergic system.

Alcohol and the NMDA Receptor.

Glutamatergic neurotransmission accounts for 70% of syntaptic transmission in the brain (Gass & Olive, 2008). The *N*-methyl-D-aspartate receptor (NMDAR) is an ionotropic, ligand-gated glutamate receptor that allows sodium and calcium influx and potassium efflux upon the binding of glutamate and glycine (Gass & Olive, 2008; Kew & Kemp, 2005). The NMDAR is heterotetrameric in structure, typically composed of two NR1 subunits and two NR2 subunits (Dingledine, Borges, Bowie, & Traynelis, 1999; Monyer et al., 1992). In addition, the NMDAR is voltage-dependent, due to a magnesium ion that blocks the ion channel of the receptor that is only removed with a change in membrane voltage (Gass & Olive, 2008; Kew & Kemp, 2005). NMDA receptors are present throughout the brain (Gass & Olive, 2008), and are important for a number of phenomena. For instance, NMDAR activation has been linked to learning and memory, and has been shown to mediate long-term potentiation (Watt, Sjostrom, Hausser, Nelson, & Turrigiano, 2004). On the other hand, excessive calcium influx mediated by NMDA

receptors is responsible for excitotoxic cell death, and may underlie forms of brain damage and diseases of age (Choi, 1992).

Ethanol has been shown to acutely inhibit the function of NMDARs (Lovinger, White, & Weight, 1989), although the precise mechanism of its antagonism is still unclear. Given that glutamate is the brain's primary excitatory neurotransmitter, ethanol's antagonism of the NMDAR produces a number of effects, including perhaps the general sedative effect seen in organisms that consume high levels of ethanol (Tsai & Coyle, 1998).

Alcohol Withdrawal and the NMDAR.

Alcohol withdrawal is characterized by an upregulation of glutamatergic activity in the brain, leading to a general state of hyperexcitability (Dahchour & DeWitte, 1999; Dahchour & DeWitte, 2003; Follesa & Ticku, 1996; Grant et al., 1990; Kumari & Ticku, 1998). Chronic ethanol exposure, with its accompanying persistent antagonism of NMDARs and activation of the inhibitory γ-Aminobutyric acid-A receptors ($GABA_AR$) (Mehta & Ticku, 1988; Nakahiro, Arakawa, & Narahashi, 1991), leads to homeostatic changes in the subunit expression of the NMDAR, and expression of this receptor overall increases (Follesa & Ticku, 1996; Grant et al., 1990; Hendricson et al., 2007; Kumari & Ticku, 1998), while $GABA_A$ R expression decreases (Kumar et al., 2010). The absence of ethanol, as during alcohol withdrawal, manifests itself as an imbalance between excitatory and inhibitory neurotransmitters. Microdialysis studies have shown increased levels of glutamate and decreased levels of GABA in the hippocampus and striatum during ethanol withdrawal, particularly after multiple episodes of ethanol consumption and withdrawal (Chefer et al., 2011; Dahchour & DeWitte, 1999; Dahchour & DeWitte, 2003).

Rossetti and colleagues (1999) found that application of NMDA—an NMDAR agonist—to ethanol-withdrawn rats increased striatal glutamate levels significantly above

baseline, compared to control sucrose-treated animals treated with NMDA. These data suggest that NMDAR upregulation during ethanol withdrawal may mediate a general increase in glutamatergic neurotransmission, perhaps leading to the central nervous system hyperexcitability that characterizes ethanol withdrawal. The effects of this hyperexcitability include many of the main symptoms of alcohol withdrawal, including seizures and anxiety (Grant et al., 1990), and can even lead to excitotoxic cell death (Idrus, McGough, Riley, & Thomas, 2011; Ikonomidou et al., 2000). Findings by Grant and colleagues (1990) also support the NMDA-mediated hyperexcitability hypothesis. Administration of NMDA in ethanol-dependent mice during ethanol withdrawal exacerbated handling-induced seizures, suggesting that NMDAR upregulation may be responsible for this common symptom of ethanol withdrawal.

In addition, application of NMDAR antagonists has been shown to ameliorate the symptoms of ethanol withdrawal. Administration of the non-competitive NMDAR antagonist MK-801 reduced handling-induced seizures in ethanol-withdrawn mice (Grant et al., 1990), as did uncompetitive NMDAR antagonist MRZ 2/579 (Bienkowski et al., 2001). The noncompetitive NMDAR antagonist dextromethorphan has been shown to reduce hyperlocomotion and audiogenic seizures in ethanol-withdrawn rats (Erden et al., 1999). Acamprosate is a partial co-agonist of the NMDAR, inhibiting receptor function at high concentrations when receptor concentration is also high (Littleton, 2007; Mason & Heyser, 2010; al, Bouchenafa, & Littleton, 1998). Acamprosate is an FDA-approved drug for the treatment of relapse and cravings in alcoholics, has also been tested for effects on ethanol withdrawal symptoms. Acamprosate produces anxiolytic behavior in ethanol-withdrawn rats on the elevated-plus maze (Kotlinska & Bochenski, 2008) and inhibits ethanol-withdrawal induced calcium ion entry and neurotoxicity (Mayer et al., 2002). Taken together, all these data strongly suggest that

the NDMAR plays an important role in mediating the hyperexcitable effects of ethanol withdrawal.

Memantine

Memantine hydrochloride is an uncompetitive NMDA receptor antagonist (Kew & Kemp, 2005; Johnson & Kotermanski, 2006) currently approved by the FDA for treatment of moderate to severe Alzheimer's disease (Kavirajan, 2009). It, like many other NMDAR antagonists, has been shown to ameliorate ethanol-withdrawal induced seizures in animals (Stepanyan et al., 2008) and even protect against ethanol-withdrawal induced excitotoxic cell death (Idrus et al., 2011; Stepanyan et al., 2008). In humans, memantine can reduce craving for ethanol in moderate alcohol users and alcohol-dependent subjects (Bisaga & Evans, 2004; Krupitsky et al., 2007).

Memantine's specific pharmacokinetic properties may be responsible for its favorable clinical profile, an unusual feature among NMDAR antagonists (Kavirajan, 2009). The NMDAR is important for many basic brain functions (Johnson & Kotermanski, 2006), thus many NMDAR antagonists may produce aversive central nervous system side-effects in humans, including schizophrenia-like psychosis (Bisaga, Popik, Bespalov, & Danysz, 2000; Krystal et al., 2003a). Memantine acts to antagonize the NMDAR by blocking the receptor channel after binding of an NMDAR agonist and channel opening (Blanpied, Boeckman, Aizenman, & Johnson, 1997; Parsons, Panchenko, Pinchenko, Tsyndrenko, & Krishtal, 1996), similarly to other, less clinically well-tolerated drugs, including ketamine and phencyclidine (Macdonald et al., 1991). However, memantine has faster on/off receptor kinetics than most NMDAR antagonists (Parsons, Gruner, Rozental, Millar, & Lodge, 1993). In addition, memantine acts as a 'partial-trapper,' rather than a 'full-trapper,' in that not all molecules of memantine bound to

NMDAR channels become trapped after the removal of the NMDAR agonist and channel closure (Blanpied et al., 1997; Kotermanski, Wood, & Johnson, 2009).

Kotermanski and colleagues (2009) found that memantine may actually antagonize the NMDAR via two different sites on the NMDAR: a superficial 'non-trapping' site that is accessible whether the channel is open or closed and a deep 'trapping' site that is available only when the channel is open. When memantine binds to the deep site, it can be trapped by the closing of the channel. But when memantine binds to the superficial site, it is not trapped by the closing of the channel, and can thus readily dissociate, causing the partial-trapping phenomenon. Partial-trapping may be very important to memantine's clinical utility, as incomplete trapping could augment the number of NMDARs that are available for normal synaptic functioning, decreasing the amount of aversive side-effects experienced (Johnson & Kotermanski, 2006; Mealing, Lanthorn, Murray, Small, & Morley, 1999) and making memantine a valuable potential treatment for the symptoms of ethanol withdrawal.

Ketamine

Ketamine, like memantine, is an uncompetitive NMDAR antagonist that binds to and blocks the NMDAR channel only when an agonist is bound and the channel is open (Macdonald et al., 1991; Parsons et al., 1995). Ketamine is currently used as a veterinary anesthetic (Bednarski et al., 2011), but has been found to dose-dependently substitute for ethanol in discriminative stimulus effect tasks in pigeons and mice (Grant, Knisely, Tabakoff, Barrett, & Balster, 1991). In addition, ketamine retards the development of tolerance to chronic ethanol administration in rats (Khanna, Shah, Weiner, Wu, & Kalant, 1993). In one study, ketamine blocked cellular calcium ion accumulation and neurotoxicity associated with application of NMDA in rat cortical cells (Takadera, Suzuki, & Mohri, 1990). In humans, ketamine has been

shown to produce ethanol-like subjective effects in recovering ethanol-dependent patients (Krystal et al., 2003b).

Ketamine, however, has different pharmacokinetics than memantine. Ketamine does not bind to the superficial NMDAR site and is thus a 'full-trapper,' in that all molecules of ketamine become trapped in the NMDAR channel after channel closure (Kotermanski et al., 2009). These pharmacokinetic differences may result in ketamine's unfavorable clinical profile. Although memantine is clinically well-tolerated in human patients (Kavirajan, 2009), ketamine produces significantly disturbing side-effects in patients (Krystal et al., 2003a) making it more unsuitable for human clinical use.

Alcohol and Aggression

The Social Cost.

In some individuals, alcohol use and dependence can lead to atypical aggression, causing problems for individuals and society at large. Alcohol and increased aggression are linked throughout the world's cultures (WHO, 2006). Alcohol is associated with two-thirds of violent actions in humans (Krug et al., 2002) and high-levels of alcohol consumption are a significant risk-factor for child abuse, intimate partner abuse, elder abuse, and sexual violence (WHO, 2006). Although alcohol does not produce violence in most individuals (Higley, 2001; Miczek, 1987), it is clear that for some alcohol consumption can have a particularly destructive effect. Scientific research into the alcohol-aggression link could help society understand who is at risk for alcohol-heightened aggression and possibly diminish alcohol's violent effects in communities.

Animal Models.

Due to the obvious ethical constraints in performing tightly controlled experiments concerning alcohol's effect on aggression in human subjects, animal models of alcohol-heightened aggression are useful in trying to understand the neurobiological mechanisms mediating the phenomenon (Higley, 2001). In particular, mouse aggression can be readily observed using the resident-intruder aggression paradigm, in which a male "resident" mouse defends his territory against an unfamiliar "intruder" mouse that is placed inside the resident's cage (Miczek & O'Donnell, 1978a). Miczek and colleagues found that only 27% of ethanol-intoxicated pair-housed outbred male mice showed reliable heightening of aggression against an intruder mouse (alcohol-heightened aggression, or AHA), while 64% showed no reliable changes in aggression (alcohol-non-heightened aggression, or ANA) and 9% actually showed decreases in aggressive behavior (alcohol-suppressed aggression, or ASA) (Miczek, Barros, Sakoda, & Weerts, 1998). Below, these subgroups are displayed as the change in standard deviations from control attack bite frequency:

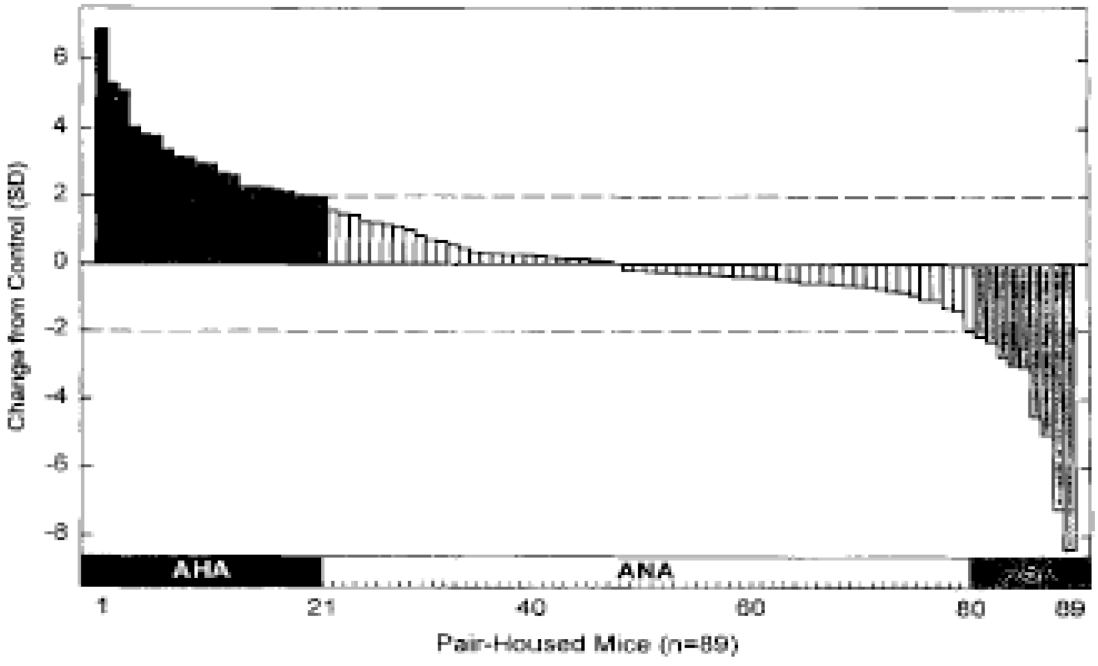


Figure 1. Individual differences in aggressive behavior following ethanol consumption by pair-housed male mice. Change in attack bite frequency after consumption of 1.0 g/kg of ethanol compared to consumption of an equal volume of water. From Miczek et al., 1998

Experiments with single-housed male "resident" mice produced similar results, with 35% of the intoxicated residents exhibiting AHA, 48% exhibiting ANA, and 16% exhibiting ASA:

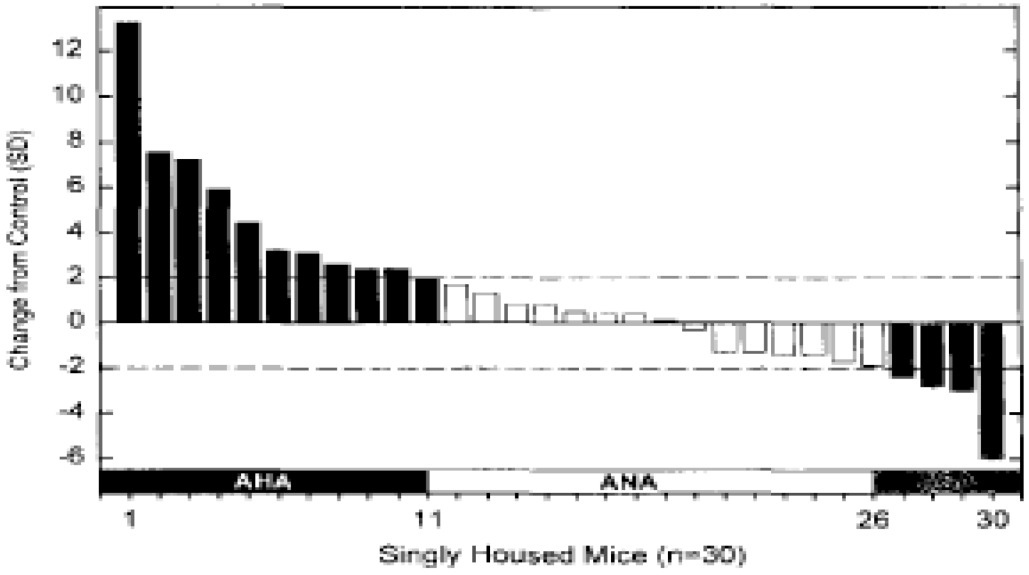


Figure 2. Individual differences in aggression behavior following ethanol consumption in single-housed male mice. Change in attack bite frequency after consumption of 1.0 g/kg of ethanol compared to consumption of an equal volume of water. From Miczek et al., 1998

The subjects in the above experiments did not show significant changes in other behaviors, such as locomotion, after ethanol intoxication, suggesting that AHA is not the product of a general dysinhibition of behavior, but a specific effect on a specific subset of behavior (Miczek et al., 1998). The above data also allowed subsequent experimenters to utilize the identification of the different aggressive subgroups of mice (AHA, ANA, ASA) to discover the possible neurobiological and/or genetic differences that mediate AHA.

Neurobiological Mechanisms of Aggression.

A number of neural structures and neurotransmitter systems have been implicated in aggressive behavior. For instance, Flynn (Flynn, 1967) found that differential stimulation of the ventromedial and lateral hypothalamus in cats led to affective defense and quiet biting attack, respectively, showing that different brain areas mediate different aspects of the aggressive response. In more recent studies, species atypical aggression, including AHA, has been examined. In particular, the prefrontal cortex (PFC) has been studied as a key modulator of aggression. The PFC is thought to underlie impulse control (Amat et al., 2005), the dysregulation of which could lead to inappropriate aggressive behavior. Consistent with this hypothesis, human subjects with frontal lobe damage showed increased aggressiveness (Anderson, Bechara, Damasio, Tranel, & Damasio, 1999). In addition, PET studies have correlated aggressive behavior with decreased metabolism in the PFC (New et al., 2004). Alcohol use has been experimentally associated with similar PFC dysfunction (Easdon & Vogel-Sprott, 2000; Mulvihill, Skilling, & Vogel-Sprott, 1997; Peterson, Rothfleisch, Zelazo, & Pihl, 1990; Schweizer, Vogel-Sprott, Dixon, & Jolicoeur, 2005) and an electrophysiological study showed that ingestion of alcohol in humans reduces activity in the PFC (Ridderinkhof et al., 2002).

In terms of neurotransmitters, the serotonin (5-HT), GABA, dopamine (DA), and glutamate systems have all been implicated in aggressive behavior (Celada, Puig, Casanovas, Guillazo, & Artigas, 2001; Heinz, Beck, Meyer-Lindenberg, Sterzer, & Heinz, 2011). The present paper will focus on the 5-HT and glutamate systems to explore the effect of ethanol withdrawal-induced increased glutamatergic tone on aggression in mice.

Serotonin and Aggression.

5-HT is considered to be of great importance in potential neural circuits for impulsive types of aggression (Miczek, Hussain, & Faccidomo, 1998; Takahashi, Quadros, de Almeida, & Miczek, 2011). In general, low levels of 5-HT or its metabolites have been associated with higher levels of aggressive behavior (Brown, Goodwin, Ballenger, Goyer, & Major, 1979; Heinz et al., 2011; Miczek, Fish, DeBold, & de Almeida, 2002), although in some studies this correlation is absent (Miczek et al., 2002). However, AHA mice do show decreased serotonin receptor expression (Chiavegatto, Quadros, Ambar, & Miczek, 2010) and a microdialysis study revealed a decrease in 5-HT levels in the PFC during and after an aggressive encounter between a resident rat and an intruder (Van Erp & Miczek, 2000). In addition, a variety of serotonergic drugs have been shown to lower aggressive behavior. For example, agonists of the 5-HT_{1a} autoreceptor, including 8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin (8-OH-DPAT) and alnespirone, reduced aggression in male resident mice (Miczek, Faccidomo, Fish, & DeBold, 2007; Takahashi et al., 2011). Antagonists of 5-HT_2 receptors like 2,5-dimethoxy-4-iodoamphetamine (DOI) and 3-Trifluoromethylphenylpiperazine (TFMPP) reduce aggression at doses lower than those that affect general locomotor behavior (Miczek et al., 2007). Finally, selective serotonin reuptake inhibitors (SSRIs) like fluoxetine, which act to increase 5-HT neurotransmission (Stahl, 1998), also reduce aggressive behavior (Coccaro & Kavoussi, 1997; Miczek et al., 2007) and even increase PFC metabolism in human subjects with impulsive aggression problems (New et al., 2004).

Although the manner in which 5-HT mediates aggression is not yet fully understood, some progress has been made with regards to the possible complex neural circuitry. Celada and colleagues (2001) reported that stimulation of the PFC regulates 5-HT activity in the dorsal raphe

nucleus (DRN) via descending excitatory afferents acting on postsynaptic NMDARs and other ionotropic glutamate receptors and via indirect activation of inhibitory receptors such as the 5-HT1a autoreceptor and the GABA-A receptor. These excitatory and inhibitory pathways lead to the secretion of 5-HT or GABA, respectively, which act on other 5-HT neurons in the DRN to inhibit 5-HT activity overall (Celada et al., 2001). In addition, ascending 5-HT pathways from the DRN to the PFC may constitute a feedback loop that controls the activity of the PFC's descending pathways via postsynaptic 5-HT1a receptors (Celada et al., 2001). Alcohol could affect this pathway through inhibition of the PFC or action on GABA-A receptors, leading to dysregulation of 5-HT levels and increased aggression.

5-HT, however, is clearly not the only neurotransmitter involved in aggressive behavior. In particular, glutamatergic neurotransmission has been implicated in aggression, although its exact role remains unclear (Miczek & Fish, 2005). One early hypothesis proposed that aggression results from an imbalance between glutamate and GABA (Mandel, Mack, & Kempf, 1979). In support of this hypothesis, aggressive animals have been found to have increased levels of glutamate and decreased levels of GABA in their brains compared to nonaggressive animals (Miczek et al., 2007). Lowering glutamate levels and increasing GABA levels decreases aggressive behavior (Miczek et al., 2007). Animals that have experienced electrical kindling in amygdala and hippocampal sites exhibit increased reactivity to possible threats (i.e. presence of an intruder animal) (Kalynchuk, Pinel, & Treit, 1999), suggesting that inducing hyperexcitability can change aggressive responses.

NMDAR antagonists have mixed effects on aggressive behavior. PCP and MK-801 increase aggression in male mice with little previous fighting experience (Burkhalter & Balster, 1979; Rewerski, Kostowski, Piechocki, & Rylski, 1971; Wilmot, Vander Wende, & Spoerlein,

1987), while they decrease aggression in mice with much previous aggressive experience (Miczek & Haney, 1994; Tyler & Miczek, 1982). One study found that PCP, MK-801, and memantine inhibit aggression in isolated male mice only at doses that also produce ataxia (Belozertseva & Bespalov, 1999). On the other hand, Sukhotina and Bespalov (Sukhotina & Bespalov, 2000) showed that memantine and MRZ 2/579 inhibit aggression induced by morphine withdrawal in male mice. In another study, memantine and neramexane have been found to dose-dependently increase aggressive behavior in male mice that had self-administered 1 g/kg of ethanol prior to the aggressive encounter, while ketamine dose-dependently increased aggression only in mice that had previously ingested water (Newman et al., 2012 *in press*).

Objective

Many studies have examined the influence of ethanol consumption on aggressive behavior, but none so far have looked at aggressive behavior during ethanol withdrawal. The purpose of the present study was to use aggression in male mice exposed to ethanol via the intermittent access paradigm as a possible measure of central nervous system changes during ethanol withdrawal. Specifically, the study aimed to investigate if glutamate and NMDARs in particular played a role in mediating aggression that occurred during withdrawal from alcohol, via intraperitoneal injections of various doses of the NMDAR antagonists memantine and ketamine at the peak of ethanol withdrawal symptoms. As ethanol withdrawal is thought to be accompanied by an upregulation of NMDARs and increased glutamatergic neurotransmission, we would expect that NMDAR antagonism would restore normal glutamate activity, thereby decreasing aggression. An alternate hypothesis would be that administration of NDMAR

antagonist would increase aggressive behavior in ethanol-withdrawn mice, perhaps by affecting the PFC-DRN circuitry.

Materials and Methods

Subjects.

Seventy-two ethanol-naïve Swiss-derived Carworth Farm Webster (CFW) mice were received from Charles River Laboratories International Inc. in Wilmington, MA) and were randomly assigned into three cohorts. Ten other CFW mice were assigned to be water controls. All of these resident mice were eight weeks old upon arrival and were initially group-housed for three days in groups of six before being housed individually in polycarbonate cages (28 x 17 x 12 cm) with stainless steel wire mesh lids and pine shavings on the floor. Intruder mice were housed in groups of eight to ten in 46 x 24 x 16 cm polycarbonate cages with stainless steel wire mesh lids and corn cob shavings used as bedding. Bedding in resident and intruder cages was changed once a week, at least 24 hours before any behavioral testing was done. All animals were given one week to habituate to laboratory conditions on an inverse 12-hour light/dark cycle (lights off at 6 AM) with constant temperature (21 ± 2º Celsius) and humidity (25%). Subjects had unrestricted access to food (LabDiet 5001 Rodent Diet, PMI Nutrition International, Brentwood, MO) and water. All experimental procedures were approved by the Tufts University Institutional Animal Care and Use Committee and complied by the NIH Guide for Care and Use of Laboratory Animals.

Ethanol Intake.

For three days prior to the beginning of experimentation, resident animals were provided with two 50 mL tubes (Nalgene) with no. 5 rubber stoppers (Fisher Scientific, Agawam, MA) containing stainless steel ball-bearing sippers (Ancare Corp., Bellmore, NY) filled with water on

their cage lids so as to acclimate to drinking from sipper nozzles. 20% w/v ethanol solutions were prepared in tap water from 95% ethyl alcohol (Pharmaco-AAPER, Brookfield, CT) and were administered in the same tubes used for acclimation. Drinking tubes were held in the wire mesh cage lid and given to mice 3 hours into their dark cycle (9 AM). The tubes were weighed to the nearest hundredth of a gram 24 hours after fluid presentation. Weekly 'drip' averages (loss of fluid in a cage not containing an animal) were calculated and subtracted from each animal's fluid intake to control for accidental spillage or evaporation (Hwa et al., 2011). Animals were weighed to the nearest tenth of a gram before each ethanol drinking session in order to calculate the grams of ethanol consumed per kilogram body weight (g/kg ethanol intake).

A procedure similar to the one outlined above was followed for water control mice as well. However, water control mice only had access to two bottles of water, and bottles were not weighed to measure fluid intake. Water control mice were weighed once a week to ascertain body weight.

Intermittent Access to Ethanol.

Subjects were given intermittent access to a 20% ethanol solution according to the procedure used in inbred C57BL/6J mice (Hwa et al., 2011). Animals were given 20% w/v ethanol solution in one tube and tap water in the other each Monday, Wednesday, and Friday. Animals had access to both tubes for 24 hours. On Tuesdays, Thursdays, and Saturdays, drinking tubes were removed and weighed, and were then thoroughly cleansed of ethanol and filled with water. Mice had access to the two water tubes until the next presentation of ethanol. The placement of ethanol- and water-containing tubes on the wire mesh cage was alternated every drinking session to avoid the development of side preferences. This procedure continued

until mice drank ethanol at stable levels, defined as less than 15% variability in intake across three ethanol drinking sessions, which took about eight weeks. Animals were assigned three groups based on g/kg ethanol intake: the high-drinking group (drank >10 g/kg ethanol consistently), the on/off group (drank >10 g/kg ethanol at least once a week), and the low drinking group (drank <10 g/kg ethanol consistently).

The procedure for water control mice was the same as above, except these mice had access to two bottles of water all for the entire experimental procedure.

Aggression Procedures.

Before the first presentation of ethanol, forty-eight ethanol-consuming subjects (IAA mice) and the ten water control subjects were initially screened for aggressive behavior according the resident-intruder protocol (Miczek & O'Donnell, 1978). The experimental subjects (resident mice) were first presented in their home cage with a group-housed animal inside of a perforated bottle for five minutes in order to socially instigate the experimental mice (Fish, Faccidomo, & Miczek, 1999). After presentation of the instigator, the resident mice were presented with smaller, younger, group-housed, unfamiliar intruder mice in the resident home cage. Aggressive encounters began when the intruders were placed into the resident's cage and ended five minutes after the first attack bite. During the initial screens for aggression, only attack latency (time to first attack bite) and number of attack bites were measured. Each resident was screened at least five times to establish aggression as part of its behavioral repertoire.

Aggression during Ethanol Withdrawal.

After four weeks of intermittent access to ethanol, twenty-four IAA mice and nine water control mice were tested for aggression at eight and twenty-three hours into the withdrawal periods. One water control mouse escaped from its cage before aggression testing could

commence. The eight-hour time-point has been found to be the peak of withdrawal behaviors in C57BL/6J mice (Hwa et al., 2011), and the twenty-three hour time-point was used as a baseline measurement, as it occurs past the peak of ethanol withdrawal. The aggression protocol employed was the same as that used for aggression screening. The number of attack bites and the attack latency was measured for each aggressive encounter, and the session was recorded with a JVC Everio GZ-MG670 digital camera for later analysis of other aggressive behaviors, such as sideways threat, tail rattling, biting, and pursuit, and non-aggressive behaviors, such as anonasal contact, self-grooming, rearing, and walking. Analysis was completed using the Observer XT 9.0 software (Noldus; Wageningen, The Netherlands) by a trained observer (intra-observer reliability: $r>.90$). After eight weeks of intermittent access to ethanol, mice were tested once again at eight hours into ethanol withdrawal using the same procedures as above.

Handling-Induced Convulsions.

After withdrawal aggression testing was completed, twenty-four IAA mice and eight water control mice were tested for alcohol dependence by assessing physical reactions to ethanol withdrawal (Goldstein & Pal, 1971). One water control mouse died before HIC scoring could be completed. The handling-induced convulsion (HIC) method is described by Goldstein (Goldstein, 1972): on a 0 to 4 scale (0 = no withdrawal signs; 1 = tonic convulsion when the mouse is lifted and given a gentle 180° turn; 2 = tonic-clonic convulsion elicited by the gentle spin, or tonic convulsion when lifted without turning; 3 = tonic-clonic convulsion not requiring any spin; 4 = violent tonic-clonic convulsion, often continuing after release of the mouse). HIC measurements were taken from each mouse by the investigator every 2 hours during the ethanol withdrawal period, from 0 hours—when access to ethanol ended—to 10 hours.

Pharmacological Manipulations.

Memantine and ketamine were purchased from Sigma-Aldrich (St. Louis, MO) and diluted with dH_2O and saline, respectively, for dose concentrations. Animals were habituated to i.p. injections with saline and dH_2O vehicle for four days prior to the beginning of experimental sessions. On test days, twenty-four IAA animals and seven water control animals were weighed and injected i.p. with dH_2O vehicle or memantine at various doses (3.0, 5.0, 10.0, 30.0 mg/kg). One water control mouse died before memantine treatment began. Twenty-four other animals were weighed and injected i.p. with saline vehicle or ketamine at various doses (3.0, 5.0, 10.0 mg/kg). All drugs were delivered at .1 mL of drug per 10 grams of body weight. Aggression at eight hours into withdrawal was assessed 20 minutes following injection for memantine. For ketamine, aggression at eight hours into withdrawal was evaluated at 10 minutes following injection. Behavioral testing occurred in counterbalanced order, the highest dose being administered last for all subjects.

Blood Ethanol Concentration.

Blood was taken from 24 mice after they had completed 10 weeks of ethanol self-administration on the intermittent access procedure. On the day of blood collection, mice were given access to one bottle of water and one bottle of 20% ethanol for one hour. After that hour, fluid bottles were removed and weighed to determine water and ethanol consumption. Blood was taken from each mouse via the submandibular vein. Blood samples were then centrifuged at 4°C for 5 minutes at 5,000 rpm to distill plasma. Blood ethanol concentrations (BEC, mg/dl) were assessed with an enzyme-linked colorimetric ethanol assay kit that read at 570 wavelengths (Biovision Inc., Mountain View, VA) and absorbance was analyzed with a SmartSpec 3000 spectrophotometer (Bio-Rad, Hercules, CA).

Statistical Analysis.

Statistical analyses were performed with SigmaStat 11.0 (Systat Software, San Jose, CA). Multiple one-way, repeated measures ANOVAs were done to compare differences in weekly ethanol consumption (g/kg) and weekly ethanol preference ratios within the ethanol consumption groups (High, On/Off, and Low) over the 10 weeks of intermittent access to ethanol. Post-hoc Bonferroni t-testing was used if any main effects or interactions were found to be significant ($p<.05$).

HIC scores for IAA and water control mice were reported in median values and interquartile ranges for each 2-hour assessment. The trapezoidal method was used to calculate area-under-the-curve (AUC) for withdrawal severity over time for both groups. T-tests were used to compare the mean AUC values for IAA and water control mice and to compare the mean AUC values between the ethanol consumption groups.

Multiple one-way repeated measures ANOVAs were used to examine differences between withdrawal testing time points (4 weeks IAA: 23 and 8 hours, 8 weeks IAA: 8 hours) on aggressive and non-aggressive behaviors in both IAA and water control mice. These ANOVAs were followed by post-hoc Bonferroni tests if significant main effects of withdrawal time were found ($p<.05$). A two-way repeated measures ANOVA was performed to analyze any potential differences in aggressive and non-aggressive behaviors between IAA and water control mice in these pre-drug trials. Post-hoc Holm-Sidak tests were used if main effects or interactions were found ($p<.05$).

The effect of different doses of memantine or ketamine on aggressive and non-aggressive behaviors were also assessed using multiple one-way repeated measures ANOVAs. These ANOVAs were followed by post-hoc Bonferroni tests if significant main effects of drug

treatment were found ($p<.05$). Two-way repeated measures ANOVAs were used to examine the effect of memantine or ketamine treatment on differences in aggressive and non-aggressive behaviors between ethanol consumption groups. Post-hoc Holm-Sidak tests were used if main effects or interactions were found ($p<.05$).Multiple two-way repeated measures ANOVAs were employed to compare the IAA mice treated and water control mice treated with memantine on aggressive and non-aggressive behaviors. Post-hoc Holm-Sidak tests were used if main effects or interactions were found ($p<.05$). In addition, mice treated with ketamine were divided into two groups—one that experienced an increase in attack bite frequency (n=11) after drug treatment and one that experienced a decrease (n=10)—and compared using two-way repeated measures ANOVAs.

Results

Ethanol Drinking and Ethanol Withdrawal

Acquisition and Maintenance of Intermittent Access.

Adult male CFW mice (n=72) were given intermittent access to 20% ethanol for ten weeks. All but one mouse (n=71) completed the drinking procedure. The intermittent access procedure induced stable ethanol drinking behavior (Figure 3a) at variable levels (Figure 3b). Multiple one-way repeated measures ANOVAs were done to examine the effect of time on ethanol consumption in each of the three ethanol consumption groups (High, On/Off, and Low). For the High ethanol consumption group, the ANOVA showed a significant main effect of time ($F(27,279)=9.47$, $p<.001$), with post-hoc Bonferroni t-tests revealing that ethanol consumption within this group was significantly higher on Weeks 4-10 of the intermittent access procedure compared to ethanol consumption the first week. In the On/Off group, a significant main effect of time was also found ($F(31,319)=4.5$, $p<.001$). In this group, however, post-hoc Bonferroni t-

tests showed that compared to the first week of ethanol access, ethanol consumption was significantly higher only on Weeks 6, 8, and 9. In the Low group, the ANOVA did not find a significant main effect of time.

The preference ratio for alcohol was calculated for 20% ethanol versus water. The formula for this calculation was ethanol intake (ml) divided by total fluid intake (ml). The intermittent access procedure led to stable levels of ethanol preference ratios (Figure 4a) at variable levels (Figure 4b). Only High-ethanol consuming mice reached a .5 (or 50%) preference for ethanol. Multiple one-way repeated measures ANOVAs were done to examine the effect of time on preference ratios in each of the three ethanol consumption groups (High, On/Off, and Low). In the High group, the ANOVA found a significant main effect of time ($F(27,279)=8.336$, $p<.001$). Post-hoc Bonferroni t-tests showed that preference ratios in Weeks 4-10 were significantly greater than that in the first week of the intermittent access procedure. In the On/Off group, there was also a significant main effect of time ($F(31,319)=6.817$, $p<.001$), with Bonferroni t-tests showing that preference ratios in Weeks 6-10 of the intermittent access procedure were significant greater than that in Week 1. In the Low group, there was a significant main effect of time ($F(10,109)=2.03$, $p<.05$); however, Bonferroni t-tests showed that preference ratio throughout the intermittent access procedure did not significantly change compared to the preference in the first week.

Handling-induced Convulsion (HIC).

HIC scores were performed for 71 male CFW mice undergoing the intermittent access procedure (IAA mice) and for 8 male CFW water control mice. Median HIC scores for the IAA mice increased over time, with 8-10 hours into ethanol withdrawal having the highest median

HIC scores (Figure 5a). Median HIC scores for water control mice remained stable over the period of assessment.

A t-test comparing AUC for IAA and water control mice revealed that IAA mice had significantly greater HIC score AUC than water control mice (T=3.148, p<.01), indicating that IAA mice were experiencing symptoms of ethanol withdrawal (Figure 5b). T-tests comparing the mean AUCs of the High, On/Off, and Low ethanol consumption groups found no significant differences between the groups (Figure 5c).

Blood Ethanol Concentration.

Blood ethanol concentration (BEC) was analyzed for a set of individual mice (n=24) that had completed 10 weeks of intermittent access to ethanol. BEC values ranged from 2.25 to 6.03 mg/dl, with the mean BEC being 3.16±.17 mg/dl. There was a significant positive correlation between each animal's BEC value and the g/kg amount of ethanol they had consumed in the one hour prior to blood collection (r=.789, p<.001) (Figure 6).

Aggression During Ethanol Withdrawal

Aggressive Behaviors.

48 IAA mice and 7 water control mice were assessed for aggressive and non-aggressive behaviors 4 weeks into the IAA procedure at 8 and 23 hours into ethanol withdrawal and 8 weeks into the IAA procedure at 8 hours into ethanol withdrawal. Two-way repeated measures ANOVAs examining the differences between test session and the differences between IAA and water control mice found no significant main effect of test session on either attack bite frequency or sideways threat frequency, and no significant main effect of drinking condition on either of these aggressive behaviors (Figures 7a and 7b).

Non-aggressive Behaviors.

During the 23 hour and two 8 hour withdrawal aggression sessions, non-aggressive behavior such as walking duration, rearing duration, and self-grooming duration were also analyzed (Figures 8a-c). Multiple two-way repeated measures ANOVAs comparing the differences between test session and the differences between IAA and water control mice found no significant main effect for either test session or drinking condition for any of the non-aggressive behaviors.

Aggression During Ethanol Withdrawal: Drug Treatment

Memantine: Aggressive Behaviors.

24 IAA mice and 7 water control mice were assessed for aggression at 8 hours into ethanol withdrawal after i.p. injections of memantine. 3 IAA mice were excluded from analysis as they never exhibited aggressive behavior. In IAA mice, a one-way repeated measures ANOVA showed a main effect of memantine treatment on attack bite frequency ($F(20,103)=13.593$, $p<.001$) (Figure 9a). Post-hoc Bonferroni t-tests revealed that only the 5 mg/kg memantine dose augmented attack bite frequency significantly compared to vehicle ($T=4.933$, $p<.001$). In water control mice, memantine also significantly altered attack bite frequency ($F(6,34)=14.147$, $p<.001$), with post-hoc Bonferroni t-tests showing that the 3 mg/kg dose significantly increased attack bite frequency ($T=4.002$, $p<.01$), while the 30 mg/kg dose significantly decreased it ($T=3.159$, $p<.05$). A two-way repeated measures ANOVA comparing attack bite frequencies across memantine dosages for IAA and water control groups revealed main effects for both drinking condition ($F(1,138)=7.376$, $p<.05$) and memantine dose ($F(1,138)=9.208$, $p<.001$), as well as an interaction between the two ($F(1,138)=3.915$, $p<.01$).

Post-hoc Holm-Sidak tests showed that the attack bite frequency was significantly different between the two experimental conditions only at the 5 mg/kg dose (T=4.468, p<.001).

The frequency of sideways threats was also assessed for IAA and water control mice during memantine-treated aggressive encounters (Figure 9b). There were no significant differences in sideways threat frequency between memantine doses within the IAA group. In the water control group, there was a main effect of memantine treatment on sideways threat frequency (F(6,34)=5.016, p<.01). Post-hoc Bonferroni t-tests revealed that the 30 mg/kg dose significantly decreased sideways threat frequency in water control mice (T=2.706, p<.05). A two-way repeated measures ANOVA comparing sideways threat frequency in IAA and water control mice showed a main effect for both drinking condition (F(1,138)=5.192, p<.05) and memantine dose (F(1,138)=2.556, p<.05), but no significant interaction between the two. Post-hoc Holm Sidak tests revealed that the water control group had a significantly lower number of sideways threats overall compared to the IAA group at the 5 and 30 mg/kg doses (T=2.092, p<.05; T=2.559, p<.05).

The effect of memantine treatment on the frequency of aggressive behaviors was compared between the High (n=5), On/Off (n=9), and Low (n=5) ethanol consumption groups (Figures 10a and 10b). A two-way repeated measures ANOVA comparing attack bite frequency between the three groups showed a main effect of memantine dose (F(2,93)=10.724, p<.001) but no main effect of consumption group. Multiple one-way repeated measures ANOVAs found a significant main effect of memantine treatment on attack bite frequency in the High (F(4,24)=16.268, p<.001) and On/Off groups (F(8,43)=7.749, p<.001), but not in the Low group. Post-hoc Bonferroni tests showed that within the High consumption group, memantine significantly increased attack bite frequency compared to vehicle at the 5 mg/kg and 10 mg/kg

doses (T=4.552, p<.001; T=3.125, p<.05). Within the On/Off group, memantine was shown to significantly increase attack bites compared to vehicle only at the 5 mg/kg dose (T=4.104, p<.001). For sideways threat frequency, a two-way repeated measures ANOVA revealed no significant main effects of either consumption group or memantine dose.

Memantine: Non-aggressive Behaviors.

During aggressive encounters, non-aggressive behaviors, including the duration of walking (Figure 11a), rearing (Figure 11b), and self-grooming (Figure 11c), were also analyzed. In IAA mice, a one-way ANOVA showed a main effect of memantine treatment on walking behavior (F(20,103)=4.377, p<.01), but post-hoc Bonferroni t-tests revealed no significant differences between memantine-treated walking duration and vehicle. A one-way ANOVA examining the effect of memantine treatment on rearing behavior in IAA mice had a main effect of memantine (F(20,103)=3.386, p<.05), but once again post-hoc Bonferroni t-tests showed no significant differences in rearing duration between memantine trials and vehicle. Finally, a one-way ANOVA looking at memantine's effect on self-grooming duration had a main effect of memantine treatment (F(20,103)=3.072, p<.05). Post-hoc Bonferroni t-tests showed that the 30 mg/kg memantine dose significantly decreased self-grooming duration in IAA mice (T=3.491, p<.01).

In water control mice, a one-way ANOVA showed a main effect of memantine treatment on walking duration (F(6,34)=6.314, p=.001). Bonferroni post-hoc tests revealed that the 30 mg/kg dose significantly decreased walking duration (T=2.914, p<.05). There was no significant effect of memantine treatment on rearing duration or self-grooming duration in water control mice.

Two-way repeated measures ANOVAs were performed to compare the duration of non-aggressive behaviors between IAA and water control mice. For walking duration, there was a main effect of both drinking condition ($F(1,138)=8.875$, $p<.01$) and memantine treatment ($F(1,138)=5.983$, $p<.001$), with no significant interaction between the two. Post-hoc Holm-Sidak testing showed that, overall, water control mice displayed significantly shorter durations of walking behavior than IAA mice ($T=2.985$, $p<.01$). For rearing duration, there was no significant main effect of either drinking condition or memantine treatment. The ANOVA done for self-grooming duration showed no main effect of either drinking condition or memantine treatment, but did reveal a significant interaction between the two factors ($F(1,138)=5.083$, $p<.001$). Post-hoc Holm-Sidak tests showed that at the 30 mg/kg memantine dose, the water control group exhibited significantly greater durations of self-grooming than IAA mice ($T=3.909$, $p<.001$).

The effect of memantine treatment on the duration of non-aggressive behaviors was compared between the High (n=5), On/Off (n=9), and Low (n=5) ethanol consumption groups (Figures 12a-12c). A two-way repeated measures ANOVA comparing the groups on walking duration found no significant main effects of either memantine treatment or consumption group. Another two-way repeated measures ANOVA done for rearing duration, however, showed a significant main effect of memantine dose ($F(2,93)=4.225$, $p<.01$) but not consumption group, and no significant interaction between the two factors. Post-hoc Holm-Sidak tests revealed that only animals within the Low ethanol consumption group exhibited an effect of memantine on rearing behavior; specifically, within the Low group rearing duration significantly decreased compared to vehicle at the 30 mg/kg dose ($T=3.146$, $p<.05$). For self-grooming duration, a two-way repeated measures ANOVA showed a significant main effect of memantine dose ($F(2,93)=3.838$, $p<.01$), but no significant main effect of consumption group nor a significant

interaction between the two factors. Post-hoc Holm Sidak tests revealed that memantine only had a significant effect on self-grooming duration within the Low consumption group, with animals in that group experiencing a significant decrease in self-grooming behavior compared to vehicle at the 5 mg/kg, 10 mg/kg, and 30 mg/kg doses (T=3.118, p<.05; T=3.784, p<.01; T=4.349, p<.001). Additionally, post-hoc tests showed that within the vehicle dose of memantine, the High and On/Off groups showed significantly less self-grooming behavior than the Low group (T=2.835, p<.05; T=2.577, p<.05).

Ketamine: Aggressive Behaviors.

24 IAA mice were tested for aggressive and non-aggressive behaviors following i.p. injections of ketamine at eight hours into ethanol withdrawal (Figures 13a and 13b). 1 IAA mouse was excluded from analysis as it never exhibited aggressive behavior. One-way ANOVAs examining the effect of ketamine treatment on attack bite frequency and sideways threat frequency found no main effect of ketamine for either aggressive behavior.

The effect of ketamine treatment on aggressive behaviors was also analyzed and compared between the High (n=14), On/Off (n=9), and Low (n=1) ethanol consumption groups (Figures 14a and 14b). A two-way repeated measures ANOVA examining attack bite frequency found a significant main effect of ethanol consumption group (F(2,91)=4.698), p<.05), but no significant main effect of ketamine dose nor a significant interaction between the two factors. Post-hoc Holm-Sidak tests revealed that within the vehicle dose, the High group displayed significantly more attack bites than the On/Off group (T=2.493, p<.05). A two-way repeated measures ANOVA examining sideways threat frequency found a significant effect of ethanol consumption group (F(2,91)=3.636, p<.05), and no significant main effect of ketamine dose nor an interaction between the two factors. Post-hoc Holm-Sidak tests showed that within the 5

mg/kg ketamine dose, the High group exhibited significantly more sideways threats than the On/Off group (T=2.54, p<.05).

Mice treated with ketamine showed individual variability in their response to the drug, with some exhibiting reliable increases in attack bite frequency (the Increase group, n=11) and others showing no response (the Non-increase group, n=10). The aggressive behavior of these two groups were compared (Figures 15a and 15b). A two-way repeated measures ANOVA comparing these two groups on attack bite frequency showed no significant main effect of either group or ketamine dose, but did show a significant interaction between the two factors ($F(1,91)=8.599$, p<.001). Post-hoc Holm-Sidak tests revealed that animals within the Increase group displayed significantly more attack bites at the 3 mg/kg and 5 mg/kg doses compared to vehicle (T=4.087, p<.001; T=4.021, p<.001), while those within the Non-increase group, as expected, did not show significant alterations in attack bites compared to vehicle. In addition, the Increase group exhibited significantly more attack bites than the Non-increase group at the 3 mg/kg and 5 mg/kg doses (T=2.689, p<.01; T=2.987, p<.01).

A two-way repeated measures ANOVA comparing the two groups on sideways threat frequency found no significant main effects of either group or ketamine dose, but did find a significant interaction between the two ($F(1, 91)=6.776$, p<.001). Post-hoc Holm-Sidak tests revealed that ketamine only significantly altered sideways threat behavior within the Increase group, with this group displaying significantly more sideways threats at the 3 mg/kg, 5 mg/kg, and 10 mg/kg doses compared to vehicle (T=*3.491,* p<.01; T=2.722, p<.05; T=2.568, p<.05). Furthermore, the Increase group showed significantly more sideways threats than the Non-increase group at the 3 mg/kg dose (T=2.355, p<.05).

Ketamine: Non-aggressive Behaviors

One-way ANOVAs examining the effect of ketamine treatment on walking, rearing, and self-grooming durations (Figures 16a-c) found no significant main effect of ketamine for any of these behaviors. However, effect of ketamine treatment on rearing duration was almost significant ($F(22,91)=2.629$, $p=.057$).

The effect of ketamine treatment on non-aggressive behaviors was also analyzed and compared between the High, On/Off, and Low ethanol consumption groups (Figures 17a-c). Multiple two-way repeated measures ANOVAs examining walking, rearing, and self-grooming duration found no significant main effects of ketamine dose or ethanol consumption group on any of these non-aggressive behaviors.

Mice were also compared for non-aggressive behavior on the basis of the Increase and Non-increase groups (Figures 18a-c). A two-way repeated measures ANOVA examining walking duration found no significant main effects of either group or ketamine dose, but did find a significant interaction between the two factors ($F(1,91)=4.074$, $p<.05$). Post-hoc Holm-Sidak tests showed that animals within the Increase group exhibited significantly increased walking behavior compared to vehicle at the 3 mg/kg and 5 mg/kg doses ($T=3.003$, $p<.05$; $T=3.271$, $p<.05$). There were no significant differences in walking behavior between the two groups. Two-way repeated measures ANOVAs analyzing rearing and self-grooming duration between the two groups found no significant main effects of group or ketamine dose on either of the behaviors.

Discussion

The intermittent access procedure that Hwa and colleagues (2011) employed to induce escalated levels of ethanol consumption in inbred C57BL/6J mice also brought about increased ethanol drinking in outbred CFW mice. A large proportion of CFW mice in the present study,

designated as the High consuming group, drank on average up to 21 g/kg of ethanol per 24 hours. However, not all mice achieved escalated levels of ethanol consumption, some—the Low consuming group—drinking only very small amounts of ethanol without significant change for the entire ten week of the intermittent access procedure. And mice in the On/Off consumption group never drank ethanol stably, vacillating between higher and lower g/kg ethanol intake. This difference may reflect the increased genetic diversity of this outbred strain of mice. In addition, no group of mice attained preference for ethanol over water; those mice that consumed high amounts of ethanol still drank large quantities of water. Still, the data in the present study suggest that it is possible to induce high levels of voluntary ethanol consumption in this strain via intermittent access. Importantly, it seems that the mice in the present study experienced withdrawal when denied access to ethanol, as indicated by their significant seizure activity during the withdrawal period. This suggests that these mice did become dependent on ethanol. However, it must be noted that the ethanol consumption groups did not differ in their severity of ethanol withdrawal, suggesting that animals that drank more ethanol did not in fact experience more severe ethanol withdrawal than those animals that consumed much less.

With regard to the main behavioral outcome examined in the current study—aggression—ethanol-withdrawn animals exhibited higher levels of aggressive behavior than water controls, albeit only during drug testing. In pre-drug aggression tests ethanol-withdrawn animals did not display significantly more aggression than water controls. This finding, however, may be due to the small number of water control animals and their high variability in aggressive behavior. Adding additional animals to the water control group could resolve this issue. Nevertheless, ethanol-withdrawn animals did exhibit higher levels of aggression during drug testing; additionally, ethanol-withdrawn animals showed higher levels of locomotor behavior

than water controls during both pre-drug and drug-treatment aggression tests. These data suggest that the glutamatergic hyperexcitability that accompanies ethanol withdrawal (Chefer et al., 2011; Dahchour & DeWitte, 2003; Follessa & Ticku, 1996; Grant et al., 1990; Hendricson et al., 2007; Kumari & Ticku, 1998; Rossetti et al., 1999) does in fact lead to measurable increases in aggressive and non-aggressive behaviors.

Memantine, but not ketamine, increased aggressive behavior in both ethanol-withdrawn and water control mice, albeit at different doses—5 mg/kg and 3 mg/kg, respectively. It is interesting to note that within the ethanol-withdrawn mice, memantine only produced this aggression heightening effect in those mice that drank high levels of ethanol, either reliably or unstably. Animals that drank low levels of ethanol were not significantly affected by memantine, although there was a trend towards a significant increase in aggressive behavior at the 5 mg/kg dose. This suggests that the amount of ethanol previously consumed could affect memantine's action on aggression. Neither of the doses that affected aggression significantly affected non-aggressive behaviors, suggesting that the effect on aggressive behavior is not the result of gross changes in locomotor behavior. Since animals in ethanol-withdrawal would be expected to have higher levels of glutamate and NMDARs than water controls (Chefer et al., 2011; Dahchour & DeWitte, 2003; Follessa & Ticku, 1996; Grant et al., 1990; Hendricson et al., 2007; Kumari & Ticku, 1998; Rossetti et al., 1999) it is expected that larger doses of an NMDAR antagonist like memantine would be required to affect behavior. This would also explain why only water control animals experienced significant decreases in aggressive behavior at the highest dose of memantine, although ethanol-withdrawn animals did show observable motor impairment at the 30 mg/kg dose.

Ketamine seemed to produce variable effects on aggressive and non-aggressive behavior in ethanol-withdrawn animals. Dividing the subjects into groups depending on whether or not ketamine treatment increased or decreased their attack bite frequency allows better examination of these individual differences. Indeed, ketamine did increase aggressive behavior in a subset of ethanol-withdrawn animals at the 3 mg/kg and 5 mg/kg doses. However, unlike in memantine-treated animals, these doses were also accompanied by a significant increase in locomotor activity. Thus, the increase seen in aggression could be due to gross alterations in locomotor behavior. Ketamine's effects did not seem to reliably differ among the ethanol consumption groups, suggesting that the amount of ethanol previously consumed may not influence ketamine's effects on aggressive and non-aggressive behaviors.

The differentiation in the effect of memantine and ketamine on aggressive behavior during ethanol withdrawal is likely due to their dissimilar mechanisms of action. Memantine is particular in its pharmacokinetics, displaying low receptor affinity, rapid on/off kinetics, and partial trapping that allows it to dissociate from receptors in between action potentials (Blanpied et al., 1997; Kotermanski et al., 2009). On the other hand, ketamine has high receptor affinity, slower on/off kinetics, and exhibits full trapping that requires an additional action potential to re-open the NMDAR channel to terminate receptor binding (Blanpied et al., 1997; Kotermanski et al., 2009; Parsons et al., 1995). Thus, ketamine is more likely to prevent normal glutamatergic activity, producing side-effects that could interfere with normal behavior and produce variable effects on aggression. The pharmacokinetics of memantine, however, allow it to block only excessive glutamate activity—such as the like observed during ethanol withdrawal—leaving normal brain processes and behavior intact.

It is interesting to note that, according to the results of the current study, both ethanol withdrawal with its accompanying augmentation of glutamate activity *and* treatment with an NMDAR antagonist that blocks glutamate activity increase aggressive behavior. It is perhaps expected that treatment with an NMDAR antagonist might increase aggression. Ethanol, after all, is known to antagonize the NMDAR (Lovinger et al., 1989) and ethanol consumption produces escalated aggression in a subpopulation of mice (Miczek et al., 1998). One previous study found that memantine dose-dependently increased aggression in mice that had previously consumed 1 g/kg of ethanol (Newman et al., 2012 *in press*). Newman and colleagues suggested that antagonism of the NMDAR, by ethanol or memantine or both, interferes with behavioral inhibition controlled by the PFC via glutamatergic projections to areas such as the DRN (Celada et al., 2001; Lee, Kim, Valentino, & Waterhouse, 2003). Thus, administration of memantine may lead to dysregulation of the PFC-DRN circuit, causing behavioral disinhibition and increased aggressive behavior in both ethanol-withdrawn and water control mice.

On the other hand, the relative increase in aggressive behavior and non-aggressive behavior seen in ethanol-withdrawn mice compared to water controls may be due to the effect of ethanol withdrawal on the brain. As discussed previously, ethanol withdrawal leads to a state of nervous system hyperexcitability (Chefer et al., 2011; Dahchour & DeWitte, 2003; Follessa & Ticku, 1996; Grant et al., 1990; Hendricson et al., 2007; Kumari & Ticku, 1998; Rossetti et al., 1999), resulting in anxiogenesis and increased irritability (Dahchour & DeWitte, 2003; Knapp, Saiers, & Pohorecky, 1999). Simple hyperexcitability could cause the increases in locomotor activity seen in the ethanol-withdrawn mice, while the anxiogenic effects of hyperexcitability could lead to increased aggression (Keele, 2005).

In addition, it is worth noting that ethanol has effects on other neurotransmitter systems that are relevant to the expression of aggressive behavior, such as 5-HT and GABA. Chronic intermittent access to ethanol and ethanol withdrawal could lead to homeostatic changes in these systems, perhaps causing the increased aggressive behavior seen in ethanol-withdrawn animals in the present study. Future studies should employ pharmacological treatments affecting these neurotransmitter systems—for example, the application of 5-HT agonists or antagonists—before aggressive encounters in ethanol-withdrawn mice. Studies of this kind could help to pinpoint the cause of withdrawal-induced increases in aggressive behavior.

Additional studies will be needed to address the limitations of the current studies. For instance, there is no water control group for the IAA animals treated with ketamine; this control should be added in the future. In addition, although the blood ethanol concentrations obtained from mice in the present study did correlate tightly with previous ethanol intake, the mg/dl values obtained do not reflect blood ethanol levels sufficient to induce intoxication. This may be an issue with the ethanol assay used, and blood ethanol concentration will be re-examined using a different assay kit. Furthermore, although the intermittent access paradigm represents a good model of ethanol dependence, it does not measure the animal's motivation to drink. Motivation is considered a key aspect of alcoholism in humans (Rhodes et al., 2007), and future studies may wish to use operant self-administration procedures to better evaluate the motivation of outbred mice to drink ethanol.

Importantly, although animals in the present study exhibit behavioral signs of glutamatergic hyperexcitability, there is no objective measure of glutamate levels or NMDAR expression. Future studies should employ microdialysis to measure glutamate in real-time in ethanol-withdrawn mice throughout the ethanol withdrawal period. NMDAR expression levels in

ethanol withdrawal could be measured using immunohistological techniques. Both of these procedures would provide an objective measure of excessive glutmate activity, and validate the current study's findings of behavioral hyperexcitability. In addition, microinjection of either memantine or ketamine into brain areas known to be involved in aggressive behavior, such as the PFC or DRN, could further elucidate the specific actions these compounds have, or fail to have, on aggression.

Finally, a project concurrent with present paper found that in inbred C57BL/6J mice given intermittent access to ethanol, memantine treatment during ethanol withdrawal caused a dose-dependent decrease in aggressive behavior (Dodman et al., *unpublished)*. These findings clearly contrast with the memantine-induced increase in aggressive behavior in CFW mice seen in the current paper. Future studies should further examine the differences in the two mice strains and use microdialysis and immunohistochemistry to determine any differentiation in brain changes during ethanol withdrawal.

References

Al Qatari, M., Bouchenafa, O., & Littleton, J. (1998). Mechanism of action of acamprosate. Part II. Ethanol dependence modifies effects of acamprosate on NMDA receptor binding in membranes from rat cerebral cortex. *Alcohol Clin Exp.Res., 22,* 810-814.

Amat, J., Baratta, M. V., Paul, E., Bland, S. T., Watkins, L. R., & Maier, S. F. (2005). Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nature Neuroscience, 8,* 365-371.

Anderson, S. W., Bechara, A., Damasio, H., Tranel, D., & Damasio, A. R. (1999). Impairment of social and moral behavior related to early damage in human prefrontal cortex. *Nature Neuroscience, 2,* 1032-1037.

Ballenger, J. C. & Post, R. M. (1978). Kindling as a model for alcohol withdrawal syndromes. *Br.J Psychiatry, 133,* 1-14.

Becker, H. C. & Hale, R. L. (1993). Repeated episodes of ethanol withdrawal potentiate the severity of subsequent withdrawal seizures: an animal model of alcohol withdrawal "kindling". *Alcohol Clin Exp Res, 17,* 94-98.

Becker, H. C. & Lopez, M. F. (2004). Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice. *Alcohol Clin Exp Res, 28,* 1829-1838.

Bednarski, R., Grimm, K., Harvey, R., Lukasik, V. M., Penn, W. S., Sargent, B. et al. (2011). AAHA anesthesia guidelines for dogs and cats. *J Am.Anim Hosp.Assoc., 47,* 377-385.

Belozertseva, I. V. & Bespalov, A. Y. (1999). Effects of NMDA receptor channel blockade on aggression in isolated male mice. *Aggress Behav, 25,* 381-396.

Bienkowski, P., Krzascik, P., Koros, E., Kostowski, W., Scinska, A., & Danysz, W. (2001). Effects of a novel uncompetitive NMDA receptor antagonist, MRZ 2/579 on ethanol self-administration and ethanol withdrawal seizures in the rat. *Eur J Pharmacol, 413,* 81-89.

Bisaga, A. & Evans, S. M. (2004). Acute effects of memantine in combination with alcohol in moderate drinkers. *Psychopharmacology (Berl), 172,* 16-24.

Bisaga, A., Popik, P., Bespalov, A. Y., & Danysz, W. (2000). Therapeutic potential of NMDA receptor antagonists in the treatment of alcohol and substance use disorders. *Expert.Opin.Investig.Drugs, 9,* 2233-2248.

Blanpied, T. A., Boeckman, F. A., Aizenman, E., & Johnson, J. W. (1997). Trapping channel block of NMDA-activated responses by amantadine and memantine. *J Neurophysiol., 77,* 309-323.

Breese, G. R., Chu, K., Dayas, C. V., Funk, D., Knapp, D. J., Koob, G. F. et al. (2005). Stress enhancement of craving during sobriety: A risk for relapse. *Alcoholism-Clinical and Experimental Research, 29,* 185-195.

Brown, G. L., Goodwin, F. K., Ballenger, J. C., Goyer, P. F., & Major, L. F. (1979). Aggression in humans correlates with cerebrospinal fluid amine metabolites. *Psychiatry Research, 1,* 131-139.

Burkhalter, J. E. & Balster, R. L. (1979). The effects of phencyclidine on isolation-induced aggression in mice. *Psychol Rep, 45,* 571-576.

Celada, P., Puig, M. V., Casanovas, J. M., Guillazo, G., & Artigas, F. (2001). Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: Involvement of serotonin-1A, GABA(A), and glutamate receptors. *J.Neurosci., 21,* 9917-9929.

Chefer, V., Meis, J., Wang, G., Kuzmin, A., Bakalkin, G., & Shippenberg, T. (2011). Repeated exposure to moderate doses of ethanol augments hippocampal glutamate neurotransmission by increasing release. *Addict.Biol, 16,* 229-237.

Chiavegatto, S., Quadros, I. M., Ambar, G., & Miczek, K. A. (2010). Individual vulnerability to escalated aggressive behavior by a low dose of alcohol: decreased serotonin receptor mRNA in the prefrontal cortex of male mice. *Genes Brain Behav, 9,* 110-119.

Choi, D. W. (1992). Bench to bedside: the glutamate connection. *Science, 258,* 241-243.

Coccaro, E. F. & Kavoussi, R. J. (1997). Fluoxetine and impulsive aggressive behavior in personality-disordered subjects. *Archives of General Psychiatry, 54,* 1081-1088.

Dahchour, A. & DeWitte, P. (1999). Effect of repeated ethanol withdrawal on glutamate microdialysate in the hippocampus. *Alcohol Clin Exp.Res., 23,* 1698-1703.

Dahchour, A. & DeWitte, P. (2003). Excitatory and inhibitory amino acid changes during repeated episodes of ethanol withdrawal: an in vivo microdialysis study. *Eur J Pharmacol, 459,* 171-178.

DeWitte, P., Pinto, E., Ansseau, M., & Verbanck, P. (2003). Alcohol and withdrawal: from animal research to clinical issues. *Neurosci Biobehav Rev, 27,* 189-197.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacol Rev, 51,* 7-61.

Dodman, K., Hwa, L.S., DeBold, J.F., & Miczek, K.A. The role of the NMDA receptor in ethanol withdrawal: As evidenced by aggressive behavior in C57BL/6 mice. Unpublished.

Dole, V. P., Ho, A., & Gentry, R. T. (1985). Toward an analogue of alcoholism in mice: criteria for recognition of pharmacologically motivated drinking. *Proc Natl Acad Sci U S A, 82,* 3469-3471.

Easdon, C. M. & Vogel-Sprott, M. (2000). Alcohol and behavioral control: impaired response inhibition and flexibility in social drinkers. *Exp.Clin Psychopharmacol, 8,* 387-394.

Erden, B. F., Ozdemirci, S., Yildiran, G., Utkan, T., Gacar, N., & Ulak, G. (1999). Dextromethorphan attenuates ethanol withdrawal syndrome in rats. *Pharmacol Biochem Behav, 62,* 537-541.

Fish, E. W., Faccidomo, S., & Miczek, K. A. (1999). Aggression heightened by alcohol or social instigation in mice: reduction by the 5-HT1B receptor agonist CP-94,253. *Psychopharmacology, 146,* 391-399.

Flynn, J. P. (1967). The neural basis of aggression in cats. In D.C.Glass (Ed.), *Neurophysiology and Emotion* (pp. 40-60). New York: Rockefeller University Press.

Follesa, P. & Ticku, M. K. (1996). Chronic ethanol-mediated up-regulation of the N-methyl-D-aspartate receptor polypeptide subunits in mouse cortical neurons in culture. *J Biol Chem, 271,* 13297-13299.

Gass, J. T. & Olive, M. F. (2008). Glutamatergic substrates of drug addiction and alcoholism. *Biochem Pharmacol, 75,* 218-265.

Goddard, G. V., McIntyre, D. C., & Leech, C. K. (1969). A permanent change in brain function resulting from daily electrical stimulation. *Exp.Neurol., 25,* 295-330.

Goldstein, D. B. (1972). Relationship of alcohol dose to intensity of withdrawal signs in mice. *J Pharmacol Exp Ther, 180,* 203-215.

Goldstein, D. B. & Pal, N. (1971). Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. *Science, 172,* 288-290.

Grant, B. F., Stinson, F. S., Dawson, D. A., Chou, S. P., Dufour, M. C., Compton, W. et al. (2004). Prevalence and co-occurrence of substance use disorders and independent mood and anxiety disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Arch.Gen.Psychiatry, 61,* 807-816.

Grant, K. A., Knisely, J. S., Tabakoff, B., Barrett, J. E., & Balster, R. L. (1991). Ethanol-like discriminative stimulus effects of non-competitive n-methyl-d-aspartate antagonists. *Behav Pharmacol, 2,* 87-95.

Grant, K. A., Valverius, P., Hudspith, M., & Tabakoff, B. (1990). Ethanol withdrawal seizures and the NMDA receptor complex. *Eur J Pharmacol, 176,* 289-296.

Griffin, W. C., III, Lopez, M. F., & Becker, H. C. (2009). Intensity and duration of chronic ethanol exposure is critical for subsequent escalation of voluntary ethanol drinking in mice. *Alcohol Clin Exp.Res., 33,* 1893-1900.

Heinz, A. J., Beck, A., Meyer-Lindenberg, A., Sterzer, P., & Heinz, A. (2011). Cognitive and neurobiological mechanisms of alcohol-related aggression. *Nat.Rev Neurosci, 12,* 400-413.

Hendricson, A. W., Maldve, R. E., Salinas, A. G., Theile, J. W., Zhang, T. A., Diaz, L. M. et al. (2007). Aberrant synaptic activation of N-methyl-D-aspartate receptors underlies ethanol withdrawal hyperexcitability. *J Pharmacol Exp.Ther., 321,* 60-72.

Heyser, C. J., Schulteis, G., & Koob, G. F. (1997). Increased ethanol self administration after a period of imposed ethanol deprivation in rats trained in a limited access paradigm. *Alcoholism: Clinical and Experimental Research, 21,* 784-791.

Higley, J. D. (2001). Individual differences in alcohol-induced aggression. A nonhuman- primate model. *Alcohol Res.Health, 25,* 12-19.

Hwa, L. S., Chu, A., Levinson, S. A., Kayyali, T. M., DeBold, J. F., & Miczek, K. A. (2011). Persistent Escalation of Alcohol Drinking in C57BL/6J Mice With Intermittent Access to 20% Ethanol. *Alcohol Clin.Exp.Res.*.

Idrus, N. M., McGough, N. N., Riley, E. P., & Thomas, J. D. (2011). Administration of memantine during ethanol withdrawal in neonatal rats: effects on long-term ethanol-induced motor incoordination and cerebellar Purkinje cell loss. *Alcohol Clin Exp.Res., 35,* 355-364.

Ikonomidou, C., Bittigau, P., Ishimaru, M. J., Wozniak, D. F., Koch, C., Genz, K. et al. (2000). Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science, 287,* 1056-1060.

Johnson, J. W. & Kotermanski, S. E. (2006). Mechanism of action of memantine. *Curr.Opin.Pharmacol, 6,* 61-67.

Kalynchuk, L. E., Pinel, J. P., & Treit, D. (1999). Characterization of the defensive nature of kindling-induced emotionality. *Behav.Neurosci, 113,* 766-775.

Kamdar, N. K., Miller, S. A., Syed, Y. M., Bhayana, R., Gupta, T., & Rhodes, J. S. (2007). Acute effects of naltrexone and GBR 12909 on ethanol drinking-in-the-dark in C57BL/6J mice. *Psychopharmacology (Berl), 192,* 207-217.

Kavirajan, H. (2009). Memantine: a comprehensive review of safety and efficacy. *Expert.Opin.Drug Saf, 8,* 89-109.

Keele, N.B. (2005). The role of serotonin in impulsive and aggressive behaviors associated with epilepsy-like neuronal hyperexcitability in the amygdala. *Epilepsy & Behavior, 7,* 325-335.

Kew, J. N. C. & Kemp, J. A. (2005). Ionotropic and metabotropic glutamate receptor structure and pharmacology (vol 179, pg 4, 2005). *Psychopharmacology, 182,* 320.

Khanna, J. M., Shah, G., Weiner, J., Wu, P. H., & Kalant, H. (1993). Effect of NMDA receptor antagonists on rapid tolerance to ethanol. *European Journal of Pharmacology, 230,* 23-31.

Knapp, D.J., Saiers, J.A., & Pohorecky, L.A. (1993). Observations of novel behaviors as indices of ethanol withdrawal-induced anxiety. *Alcohol Alcohol Suppl., 2,* 489-493.

Koob, G. F. (1992). Dopamine, addiction and reward. *Seminars in the Neurosciences, 4,* 139-148.

Kotermanski, S. E., Wood, J. T., & Johnson, J. W. (2009). Memantine binding to a superficial site on NMDA receptors contributes to partial trapping. *J Physiol, 587,* 4589-4604.

Kotlinska, J. & Bochenski, M. (2008). The influence of various glutamate receptors antagonists on anxiety-like effect of ethanol withdrawal in a plus-maze test in rats. *Eur J Pharmacol, 598,* 57-63.

Krug, E. G., Dahlberg, L. L., Mercy, J. A., Zwi, A. B., Lozito, R., & (eds.) (2002). *World report on violence and health*. Geneva: World Health Organization.

Krupitsky, E. M., Neznanova, O., Masalov, D., Burakov, A. M., Didenko, T., Romanova, T. et al. (2007). Effect of memantine on cue-induced alcohol craving in recovering alcohol-dependent patients. *Am.J Psychiatry, 164,* 519-523.

Krystal, J. H., D'Souza, D. C., Mathalon, D., Perry, E., Belger, A., & Hoffman, R. (2003a). NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmacology (Berl), 169,* 215-233.

Krystal, J. H., Petrakis, I. L., Limoncelli, D., Webb, E., Gueorgueva, R., D'Souza, D. C. et al. (2003b). Altered NMDA glutamate receptor antagonist response in recovering ethanol-dependent patients. *Neuropsychopharmacology, 28,* 2020-2028.

Kumar, S., Suryanarayanan, A., Boyd, K.N., Comerford, C.E., Lai, M.A., Ren, Q.L., & Morrow, A.L. (2010). Ethanol reduces GABA(A) alpha 1 subunit receptor surface expression by a protein kinase C gamma-dependent mechanism in cultured cerebral cortical neurons. *Molecular Pharmacology, 77,* 793-803.

Kumari, M. & Ticku, M. K. (1998). Ethanol and regulation of the NMDA receptor subunits in fetal cortical neurons. *J Neurochem., 70,* 1467-1473.

Lee, H.S., Kim, M.A., Valentino, R.J., & Waterhouse, B.D. (2003). Glutamatergic afferent projections to the dorsal raphe nucleus of the rat. *Brain Res*., *963*, 57-71.

Lester, D. & Freed, E. X. (1973). Criteria for an animal model of alcoholism. *Pharmacol Biochem Behav, 1,* 103-107.

Lieber, C. S. & DeCarli, L. M. (1982). The feeding of alcohol in liquid diets: two decades of applications and 1982 update. *Alcohol Clin Exp Res, 6,* 523-531.

Littleton, J. M. (2007). Acamprosate in alcohol dependence: implications of a unique mechanism of action. *J Addict.Med., 1,* 115-125.

Lovinger, D. M., White, G., & Weight, F. F. (1989). Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science, 243,* 1721-1724.

Macdonald, J. F., Bartlett, M. C., Mody, I., Pahapill, P., Reynolds, J. N., Salter, M. W. et al. (1991). Actions of ketamine, phencyclidine and MK-801 on NMDA receptor currents in cultured mouse hippocampal neurones. *J Physiol, 432,* 483-508.

Mandel, P., Mack, G., & Kempf, E. (1979). Molecular basis of some models of aggressive behavior. In M.Sandler (Ed.), *Psychopharmacology of Aggression* (pp. 95-110). New York: Raven Press.

Mason, B. J. & Heyser, C. J. (2010). The neurobiology, clinical efficacy and safety of acamprosate in the treatment of alcohol dependence. *Expert.Opin.Drug Saf, 9,* 177-188.

Matthews, D. B., Overstreet, D. H., Rezvani, A. H., Devaud, L. L., & Morrow, A. L. (2001). Effects of sweetened ethanol solutions on ethanol self-administration and blood ethanol levels. *Pharmacol Biochem Behav, 68,* 13-21.

Mayer, S., Harris, B. R., Gibson, D. A., Blanchard, J. A., Prendergast, M. A., Holley, R. C. et al. (2002). Acamprosate, MK-801, and ifenprodil inhibit neurotoxicity and calcium entry induced by ethanol withdrawal in organotypic slice cultures from neonatal rat hippocampus. *Alcohol Clin Exp.Res., 26,* 1468-1478.

Mealing, G. A., Lanthorn, T. H., Murray, C. L., Small, D. L., & Morley, P. (1999). Differences in degree of trapping of low-affinity uncompetitive N-methyl-D-aspartic acid receptor antagonists with similar kinetics of block. *J Pharmacol Exp.Ther., 288,* 204-210.

Mehta, A. K. & Ticku, M. K. (1988). Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves gamma-aminobutyric acidA-gated chloride channels. *Journal of Pharmacology and Experimental Therapeutics, 246,* 558-564.

Miczek, K. A. (1987). The psychopharmacology of aggression. In L.L.Iversen, S. D. Iversen, & S. H. Snyder (Eds.), *Handbook of Psychopharmacology, Volume 19: New Directions in Behavioral Pharmacology* (pp. 183-328). New York: Plenum.

Miczek, K. A., Barros, H. M., Sakoda, L., & Weerts, E. M. (1998). Alcohol and heightened aggression in individual mice. *Alcohol Clin Exp Res, 22,* 1698-1705.

Miczek, K. A., Faccidomo, S. P., Fish, E. W., & DeBold, J. F. (2007). Neurochemistry and Molecular Neurobiology of Aggressive Behavior. In J.Blaustein (Ed.), *Behavioral Neurochemistry, Neuroendocrinology and Molecular Neurobiology* (3rd ed., pp. 285-336). New York: Springer.

Miczek, K. A. & Fish, E. W. (2005). Dopamine, Glutamate and Aggression. In W.J.Schmidt & M. E. A. Reith (Eds.), *Dopamine and Glutamate in Psychiatric Disorders* (pp. 237-266). Totowa, New Jersey: Humana Press.

Miczek, K. A., Fish, E. W., DeBold, J. F., & de Almeida, R. M. M. (2002). Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and □-aminobutyric acid systems. *Psychopharmacology, 163,* 434-458.

Miczek, K. A. & Haney, M. (1994). Psychomotor stimulant effects of d-amphetamine, MDMA and PCP: Aggressive and schedule-controlled behavior in mice. *Psychopharmacology, 115,* 358-365.

Miczek, K. A., Hussain, S., & Faccidomo, S. (1998). Alcohol-heightened aggression in mice: attenuation by 5-HT1A receptor agonists. *Psychopharmacology, 139,* 160-168.

Miczek, K. A. & O'Donnell, J. M. (1978a). Intruder-evoked aggression in isolated and nonisolated mice: effects of psychomotor stimulants and L-dopa. *Psychopharmacology (Berl), 57,* 47-55.

Miczek, K. A. & O'Donnell, J. M. (1978b). Intruder-evoked aggression in isolated and nonisolated mice: Effects of psychomotor stimulants and l-dopa. *Psychopharmacology, 57,* 47-55.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H. et al. (1992). Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science, 256,* 1217-1221.

Mulvihill, L. E., Skilling, T. A., & Vogel-Sprott, M. (1997). Alcohol and the ability to inhibit behavior in men and women. *J Stud.Alcohol, 58,* 600-605.

Nakahiro, M., Arakawa, O., & Narahashi, T. (1991). Modulation of gamma-aminobutyric acid receptor-channel complex by alcohols. *J Pharmacol Exp.Ther., 259,* 235-240.

New, A. S., Buchsbaum, M. S., Hazlett, E. A., Goodman, M., Koenigsberg, H. W., Lo, J. et al. (2004). Fluoxetine increases relative metabolic rate in prefrontal cortex in impulsive aggression. *Psychopharmacology, 176,* 451-458.

Newman, E.L., Chu, A., Bahamon, B., Takahashi, A., DeBold, J.F., & Miczek, K.A. (2012). NMDA receptor antagonism: Escalation of aggressive behavior in alcohol-drinking mice. *Psychopharmacology, in progress*.

Parsons, C. G., Gruner, R., Rozental, J., Millar, J., & Lodge, D. (1993). Patch clamp studies on the kinetics and selectivity of N-methyl-D-aspartate receptor antagonism by memantine (1-amino-3,5-dimethyladamantan). *Neuropharmacology, 32,* 1337-1350.

Parsons, C. G., Panchenko, V. A., Pinchenko, V. O., Tsyndrenko, A. Y., & Krishtal, O. A. (1996). Comparative patch-clamp studies with freshly dissociated rat hippocampal and striatal neurons on the NMDA receptor antagonistic effects of amantadine and memantine. *Eur J Neurosci, 8,* 446-454.

Parsons, C. G., Quack, G., Bresink, I., Baran, L., Przegalinski, E., Kostowski, W. et al. (1995). Comparison of the potency, kinetics and voltage-dependency of a series of uncompetitive NMDA receptor antagonists in vitro with anticonvulsive and motor impairment activity in vivo. *Neuropharmacology, 34,* 1239-1258.

Peterson, J. B., Rothfleisch, J., Zelazo, P. D., & Pihl, R. O. (1990). Acute alcohol intoxication and cognitive functioning. *J Stud.Alcohol, 51,* 114-122.

Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y., & Patra, J. (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet, 373,* 2223-2233.

Rewerski, W., Kostowski, W., Piechocki, T., & Rylski, M. (1971). The effects of some hallucinogens on aggressiveness of mice and rats. I. *Pharmacology, 5,* 314-320.

Rhodes, J. S., Best, K., Belknap, J. K., Finn, D. A., & Crabbe, J. C. (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiol Behav, 84,* 53-63.

Rhodes, J. S., Ford, M. M., Yu, C. H., Brown, L. L., Finn, D. A., Garland, T., Jr. et al. (2007). Mouse inbred strain differences in ethanol drinking to intoxication. *Genes Brain Behav, 6,* 1-18.

Ridderinkhof, K. R., de, V. Y., Bramlage, A., Spaan, M., Elton, M., Snel, J. et al. (2002). Alcohol consumption impairs detection of performance errors in mediofrontal cortex. *Science, 298,* 2209-2211.

Rogers, J., Wiener, S. G., & Bloom, F. E. (1979). Long-term ethanol administration methods for rats: advantages of inhalation over intubation or liquid diets. *Behav Neural Biol, 27,* 466-486.

Rossetti, Z. L., Carboni, S., & Fadda, F. (1999). Glutamate-induced increase of extracellular glutamate through N-methyl-D-aspartate receptors in ethanol withdrawal. *Neuroscience, 93,* 1135-1140.

Samson, H.H. (1986). Initiation of ethanol reinforcement using a sucrose-substitution procedure in food-sated and water-sated rats. *Alcohol Clin Exp Res, 10,* 436-442.

Schweizer, T. A., Vogel-Sprott, M., Dixon, M. J., & Jolicoeur, P. (2005). The stage-specific effect of alcohol on human information processing. *Psychopharmacology (Berl), 178,* 52-57.

Spanagel, R. (2000). Recent animal models of alcoholism. *Alcohol Res.Health, 24,* 124-131.

Spanagel, R. (2009). Alcoholism: a systems approach from molecular physiology to addictive behavior. *Physiol Rev, 89,* 649-705.

Spanagel, R. & Kiefer, F. (2008). Drugs for relapse prevention of alcoholism: ten years of progress. *Trends Pharmacol Sci., 29,* 109-115.

Stahl, S. M. (1998). Mechanism of action of serotonin selective reuptake inhibitors: Serotonin receptors and pathways mediate therapeutic effects and side effects. *Journal of Affective Disorders, 51,* 215-235.

Stepanyan, T. D., Farook, J. M., Kowalski, A., Kaplan, E., Barron, S., & Littleton, J. M. (2008). Alcohol withdrawal-induced hippocampal neurotoxicity in vitro and seizures in vivo are both reduced by memantine. *Alcohol Clin Exp.Res., 32,* 2128-2135.

Sukhotina, I. A. & Bespalov, A. Y. (2000). Effects of the NMDA receptor channel blockers memantine and MRZ 2/579 on morphine withdrawal-facilitated aggression in mice. *Psychopharmacology, 149,* 345-350.

Takadera, T., Suzuki, R., & Mohri, T. (1990). Protection by ethanol of cortical neurons from N-methyl-D-aspartate-induced neurotoxicity is associated with blocking calcium influx. *Brain Res., 537,* 109-114.

Takahashi, A., Quadros, I. M., de Almeida, R. M., & Miczek, K. A. (2011). Brain serotonin receptors and transporters: initiation vs. termination of escalated aggression. *Psychopharmacology (Berl), 213,* 183-212.

Tolliver, G. A., Sadeghi, K. G., & Samson, H. H. (1988). Ethanol preference following the sucrose-fading initiation procedure. *Alcohol, 5,* 9-13.

Tsai, G. & Coyle, J. T. (1998). The role of glutamatergic neurotransmission in the pathophysiology of alcoholism. *Annu.Rev Med., 49,* 173-184.

Tyler, C. B. & Miczek, K. A. (1982). Effects of phencyclidine on aggressive behavior in mice. *Pharmacol Biochem Behav, 17,* 503-510.

Van Erp, A. M. M. & Miczek, K. A. (2000). Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. *Journal of Neuroscience, 20,* 9320-9325.

Watt, A. J., Sjostrom, P. J., Hausser, M., Nelson, S. B., & Turrigiano, G. G. (2004). A proportional but slower NMDA potentiation follows AMPA potentiation in LTP. *Nat.Neurosci, 7,* 518-524.

Wilmot, C. A., Vander Wende, C., & Spoerlein, M. T. (1987). The effects of phencyclidine on fighting in differentially housed mice. *Pharmacol Biochem Behav, 28,* 341-346.

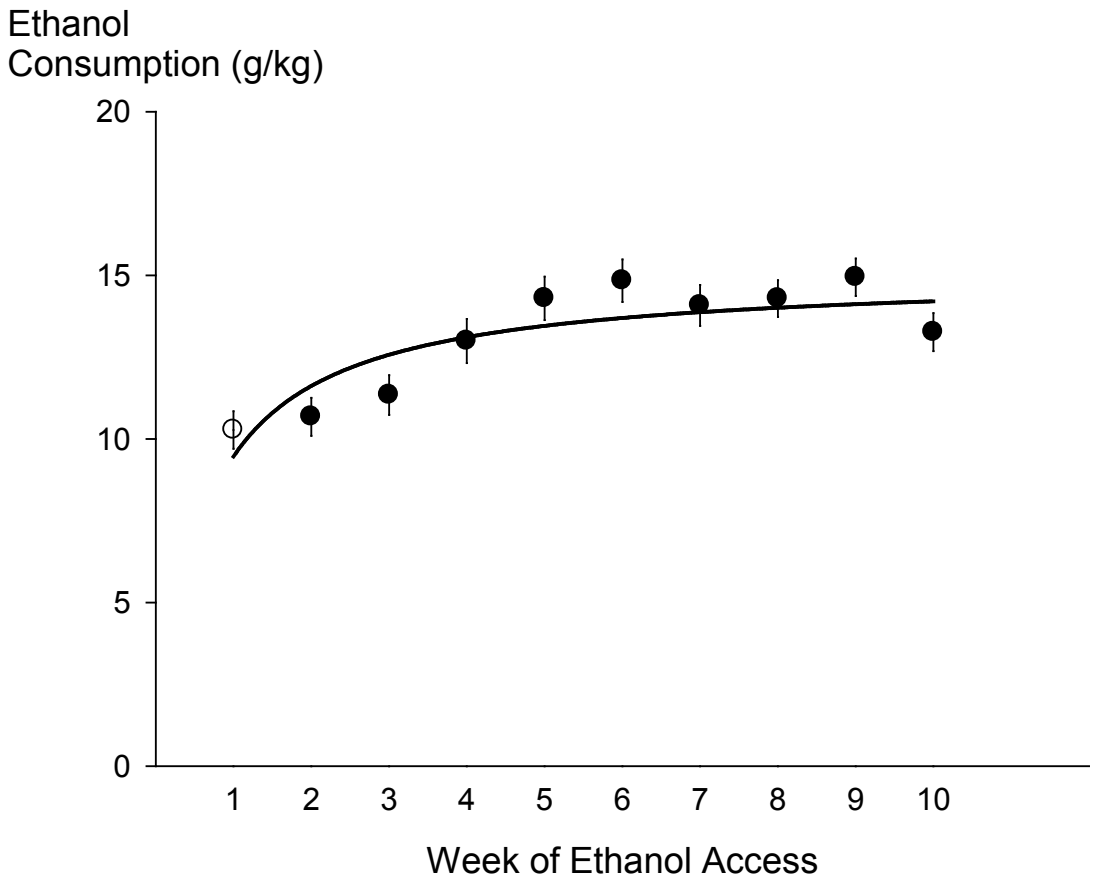
Wise, R. A. (1973). Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia, 29,* 203-210.

World Health Organization. (2006). Facts on Interpersonal Violence and Alcohol. Geneva, Switzerland.

World Health Organization. (2011). Global Status Report on Alcohol and Health. Geneva, Switzerland.

Figure 3. **Average weekly g/kg ethanol consumption for each week of intermittent access.** **A)** The average weekly g/kg ethanol consumption for all subjects (n=71), regardless of consumption group, each week of the intermittent access procedure. **B)** The average weekly g/kg ethanol consumption for each consumption group (High, On/Off, and Low) for each week of the intermittent access procedure. Error bars denote standard error of the mean. Asterisks denote significance ($p<.05$) compared to the average g/kg ethanol consumption of the first week of intermittent access within each group.

A)



B)

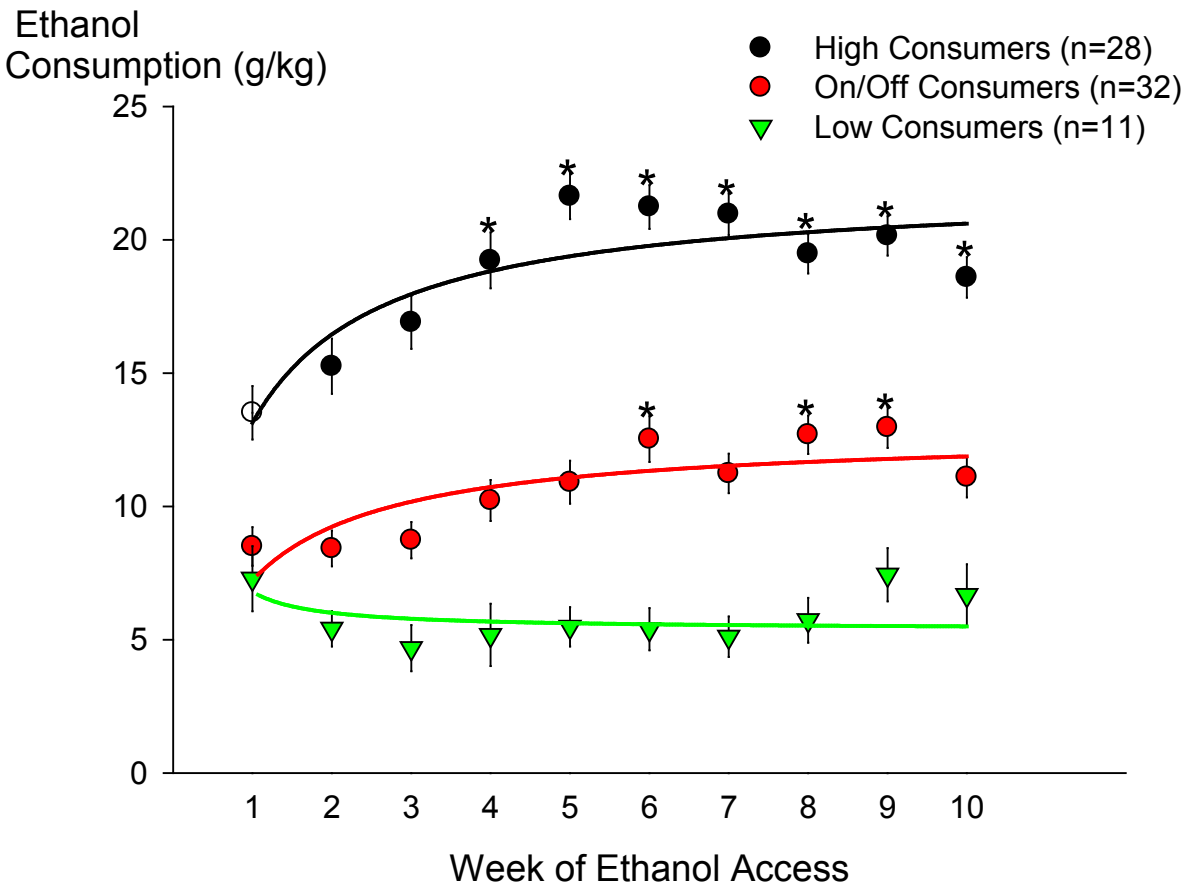
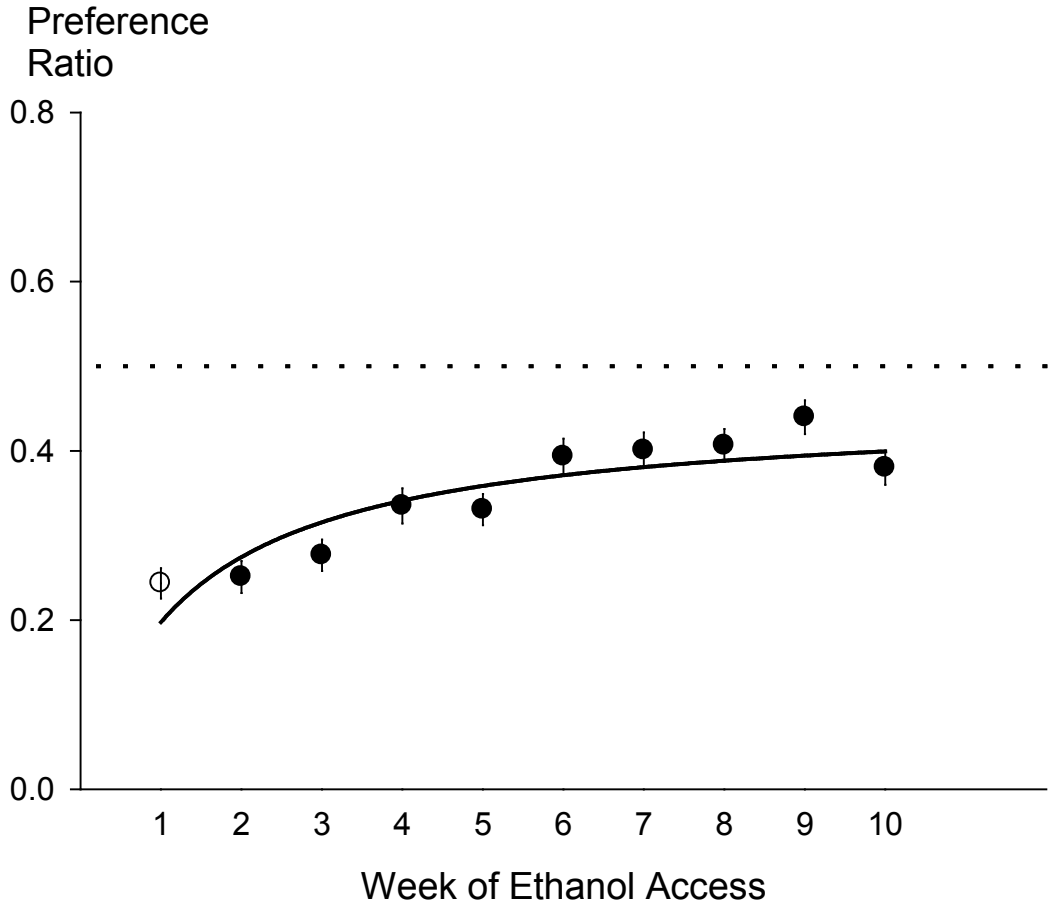

Ethanol
Consumption (g/kg)
High Consumers (n=28)
On/Off Consumers (n=32)
Low Consumers (n=11)
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
Week of Ethanol Access

Figure 4. **Average weekly preference ratios for ethanol over water for each week of intermittent access. A)** The average weekly ethanol preference ratio for all subjects (n=71), regardless of consumption group, each week of the intermittent access procedure. **B)** The average weekly ethanol preference ratio for each consumption group (High, On/Off, and Low) for each week of the intermittent access procedure. Error bars denote standard error of the mean. The dashed line marks the .5 preference ratio. Asterisks denote significance ($p<.05$) compared to the average ethanol preference ratio of the first week of intermittent access within each group.

A)



B)

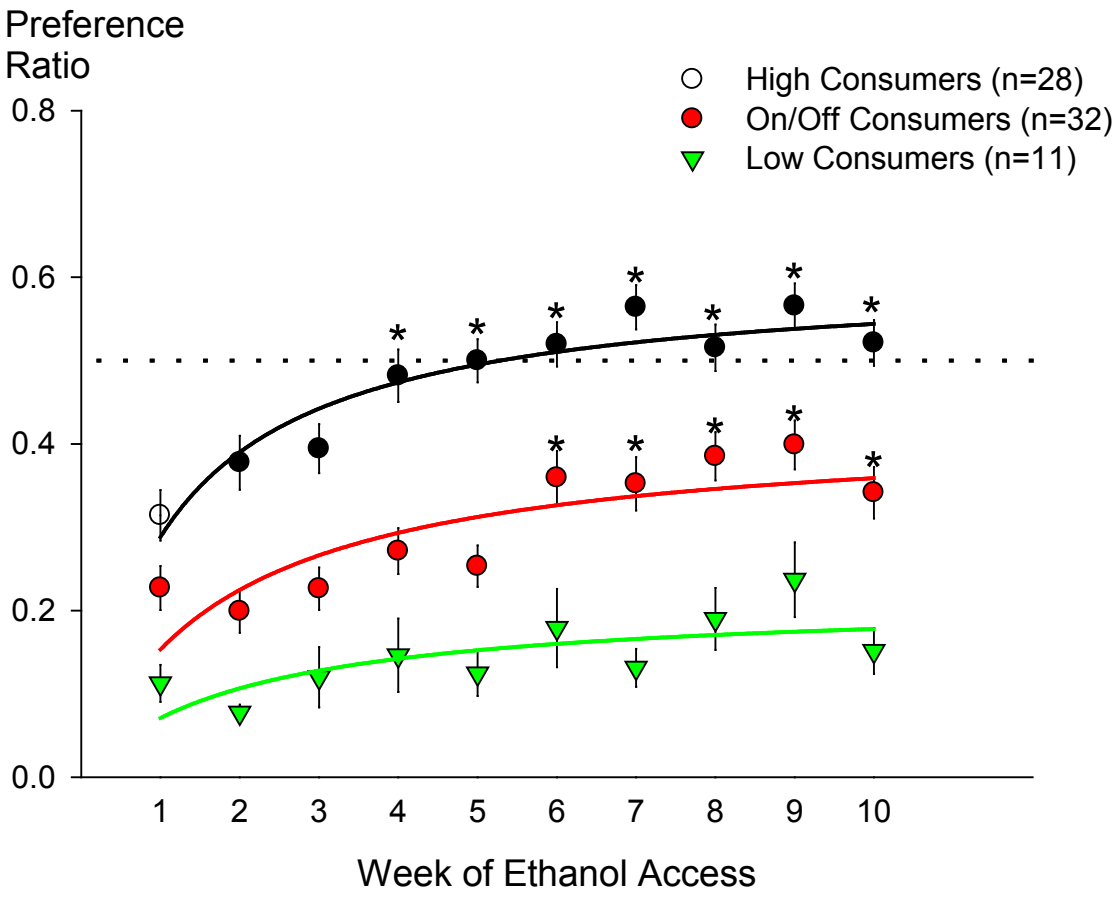
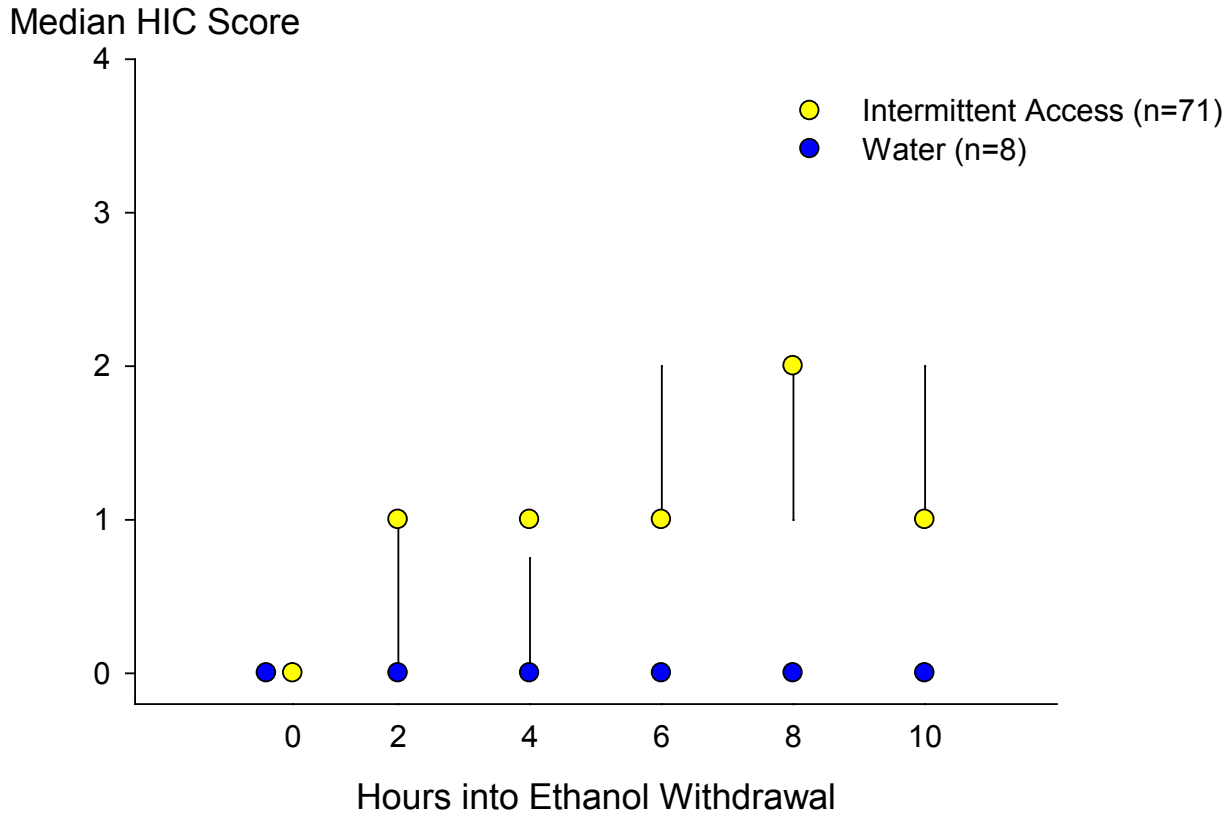
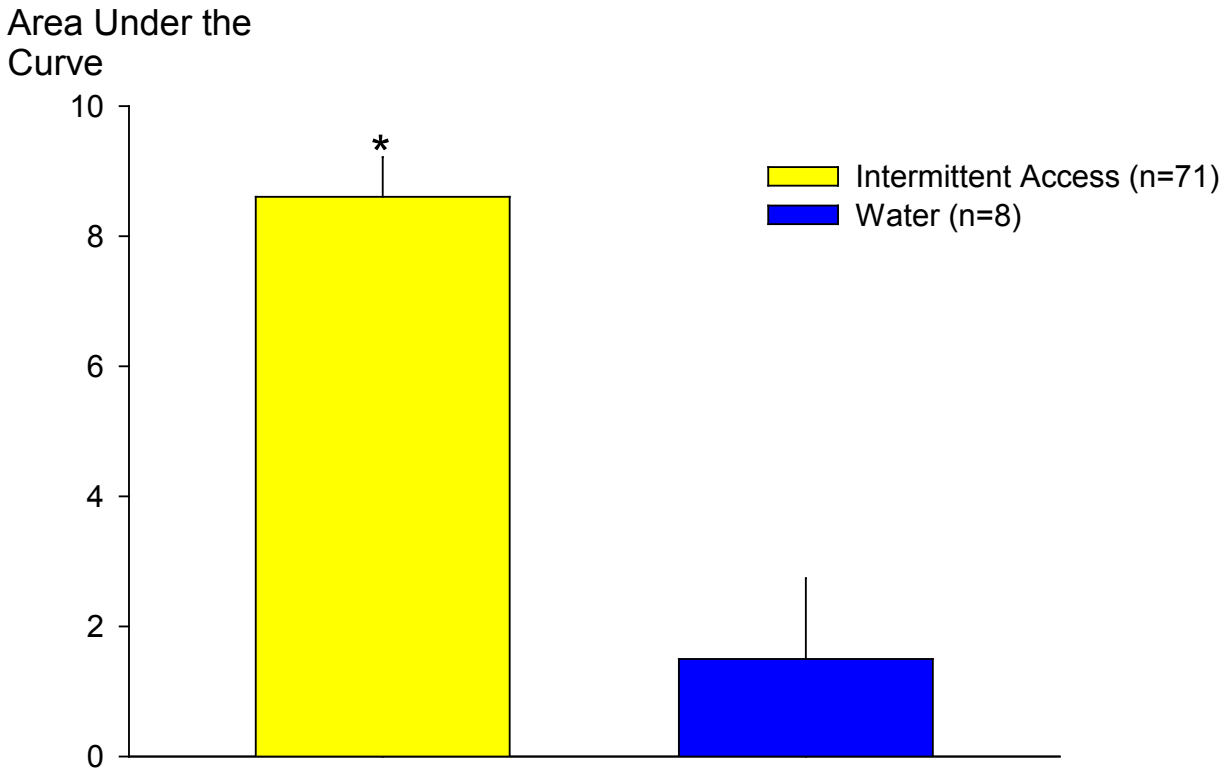

Preference
Ratio
High Consumers (n=28)
On/Off Consumers (n=32)
Low Consumers (n=11)
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7
8
9
10
Week of Ethanol Access

Figure 5. Severity of Handling-Induced Convulsion (HIC) scores for mice in ethanol withdrawal and water control mice. A) The median HIC score for each assessment (from 0-10 hours into ethanol withdrawal, assessed every 2 hours) in water control and IAA mice. Error bars denote interquartile ranges. **B)** The area under the curve for ethanol-withdrawn and water control mice. **C)** The area under the curve for IAA mice in each ethanol consumption group. Error bars denote standard error of the mean. Asterisks denote significance ($p<.05$) compared to water control.

A)



B)


Area Under the Curve
10
8
6
4
2
0
*
Intermittent Access (n=71)
Water (n=8)

C)

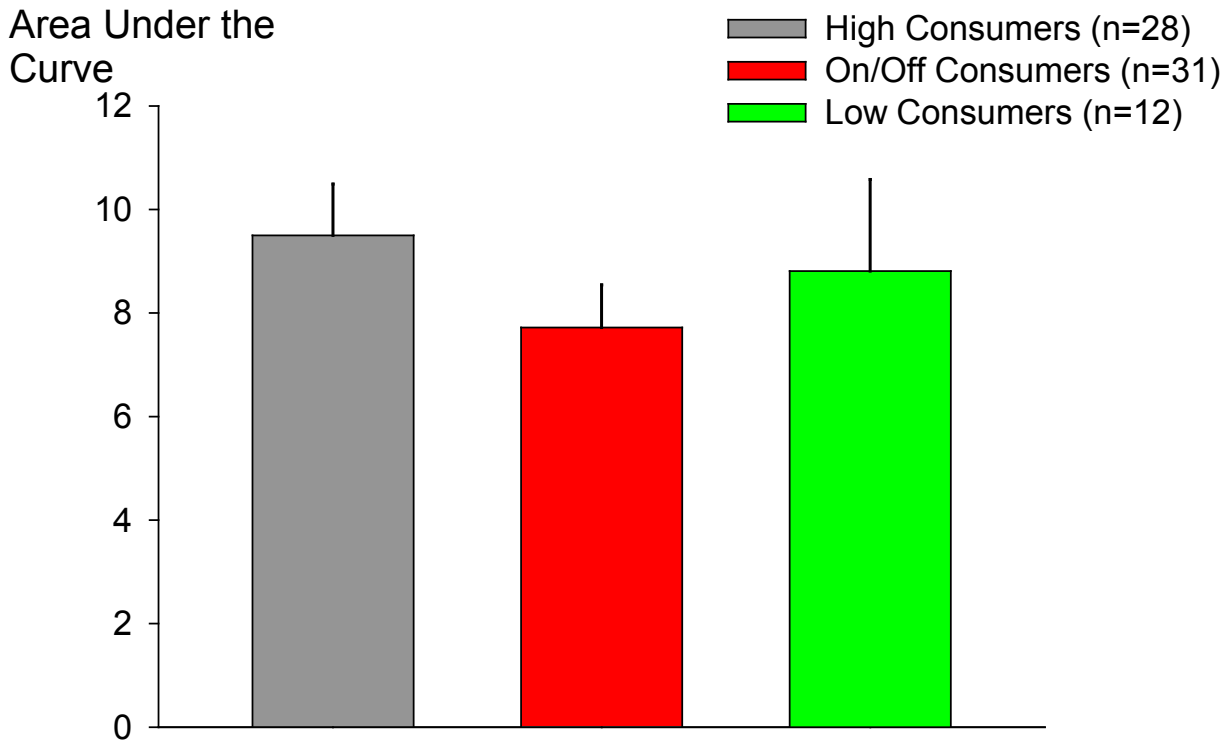

Area Under the Curve
High Consumers (n=28)
On/Off Consumers (n=31)
Low Consumers (n=12)
12
10
8
6
4
2
0

Figure 6. Correlation between 1-hour g/kg ethanol intake and blood ethanol concentration (mg/dl). The blood ethanol concentration for each of the 24 animals blood was collected from plotted against that animal's g/kg ethanol intake in the one hour prior to blood collection. There was a significant positive correlation between blood ethanol concentration and ethanol intake ($p<.05$).

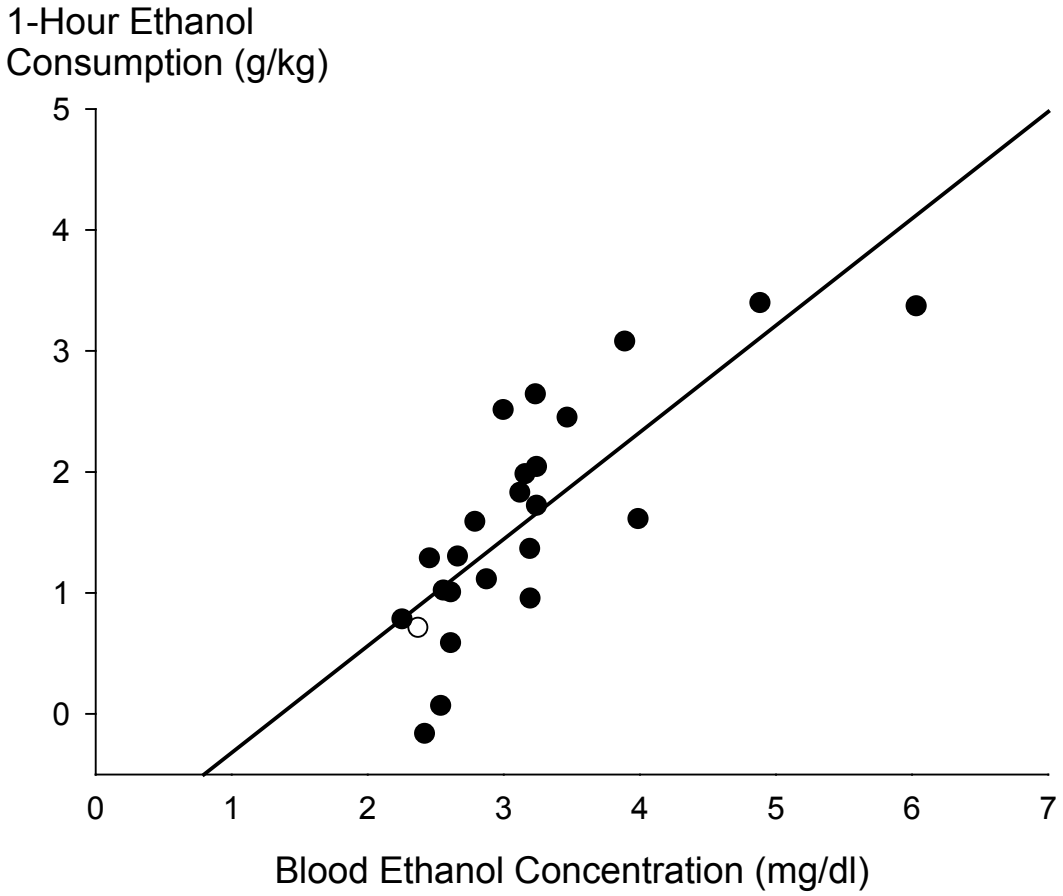
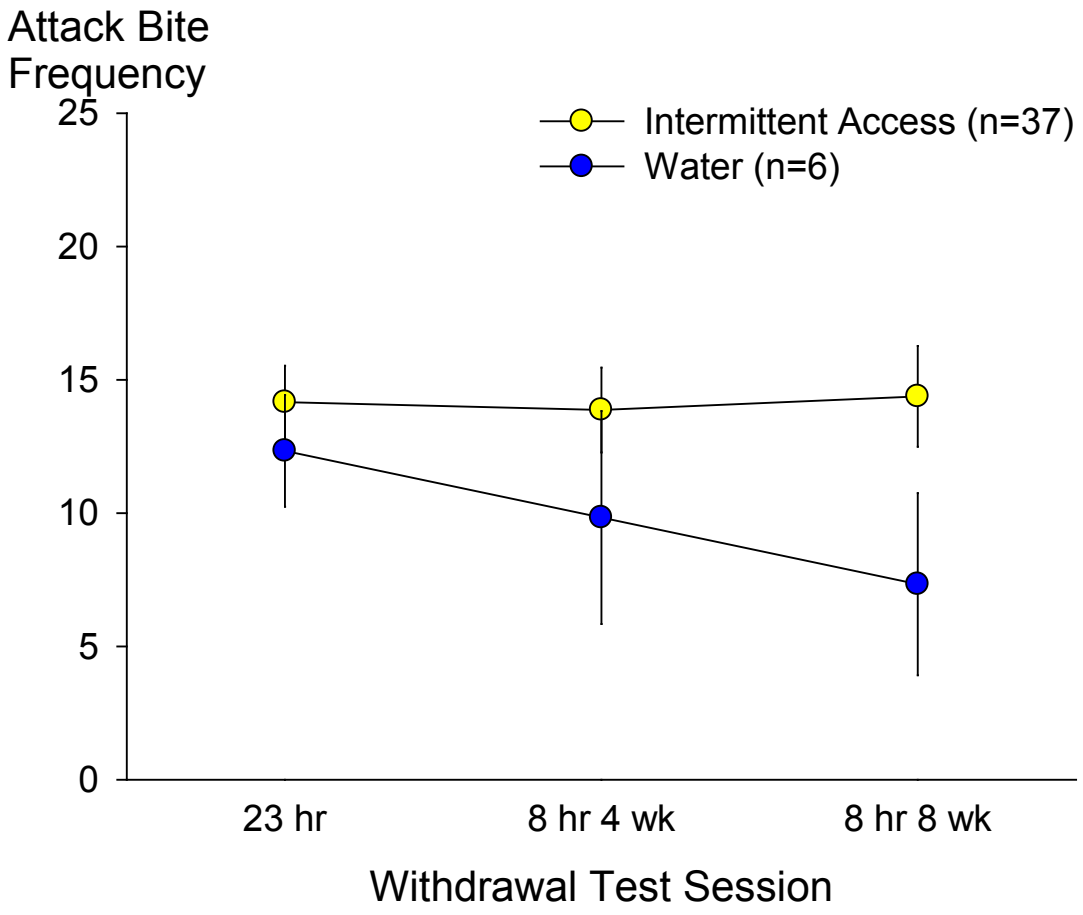


Figure 7. **The effect of ethanol withdrawal on aggressive behavior in IAA and water control mice at 23 and 8 hours into ethanol withdrawal. A)** The effect of ethanol withdrawal on **A)** attack bite frequency and **B)** sideways threat frequency in mice with intermittent access to ethanol and water control mic. Error bars denote standard error of the mean. Asterisks denote significance within the IAA or water control groups compared to vehicle ($p<.05$) and pound signs denote significance compared to water control ($p<.05$).

A)



B)

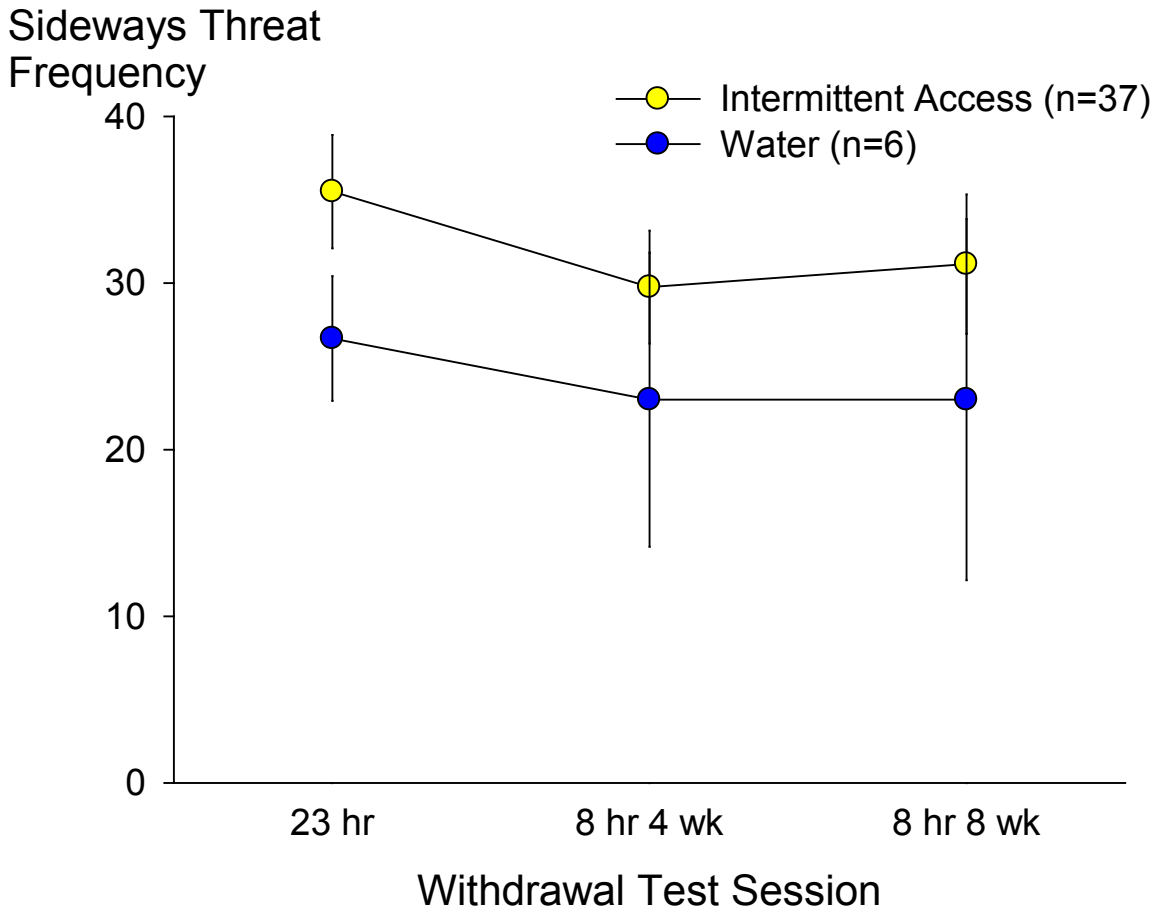
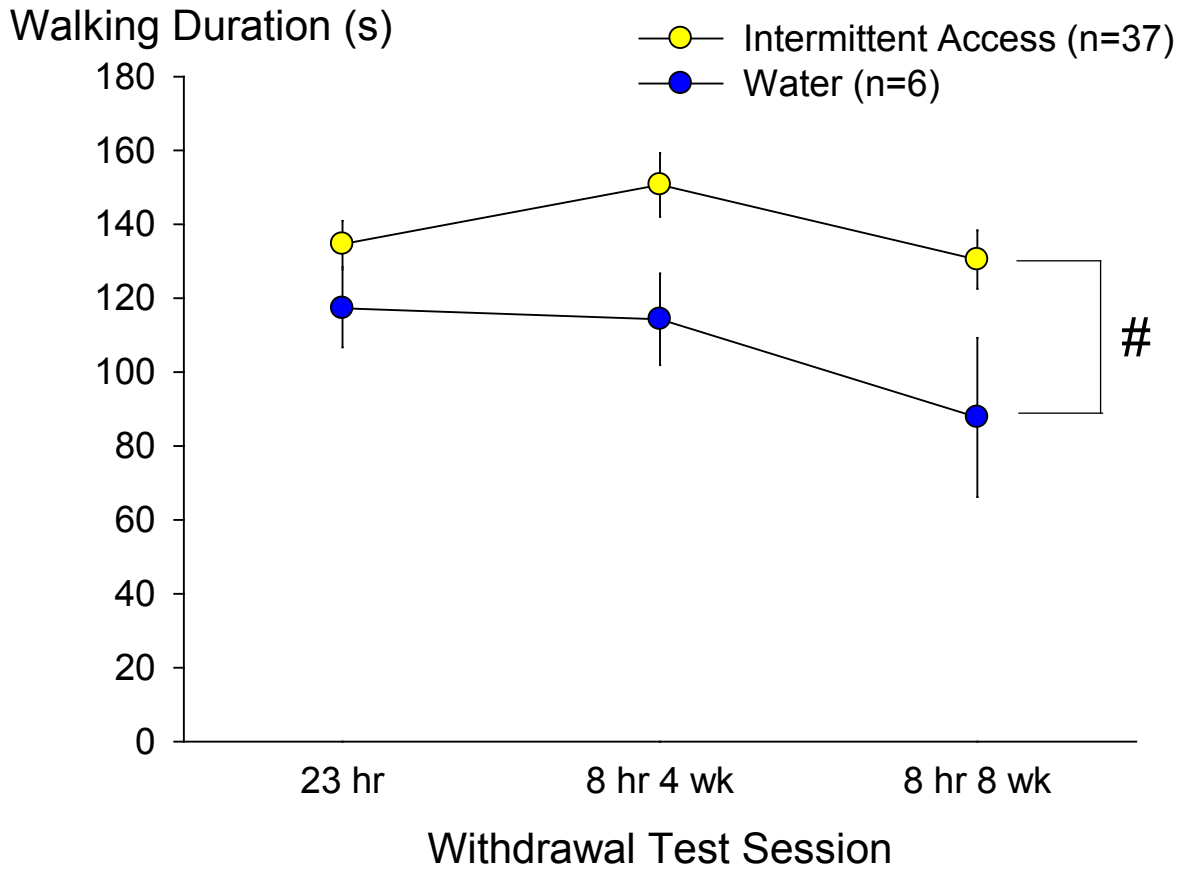
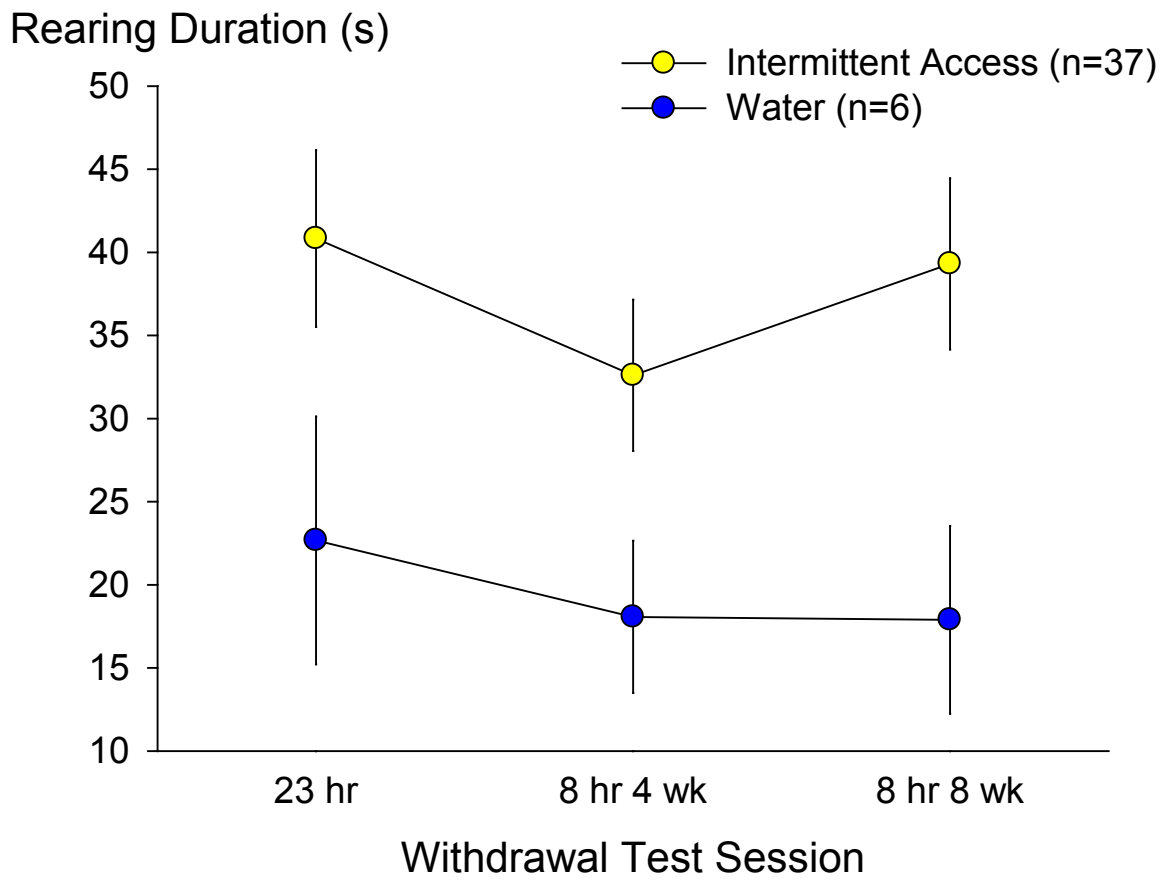

Sideways Threat Frequency
Intermittent Access (n=37)
Water (n=6)
40
30
20
10
0
23 hr
8 hr 4 wk
8 hr 8 wk
Withdrawal Test Session

Figure 8. The effect of ethanol withdrawal on non-aggressive behaviors in IAA and water control mice at 23 and 8 hours into ethanol withdrawal. The effect of ethanol withdrawal on seconds of **A)** walking duration, **B)** rearing duration, and **C)** self-grooming duration during an aggressive encounter in mice that had intermittent access to ethanol and water control mice. Error bars denote standard error of the mean. Asterisks denote significance compared to vehicle ($p<.05$) within the IAA or water control group and pound signs denote significance compared to water control ($p<.05$).

A)



B)


Rearing Duration (s)
Intermittent Access (n=37)
Water (n=6)
50
45
40
35
30
25
20
15
10
23 hr
8 hr 4 wk
8 hr 8 wk
Withdrawal Test Session

C)

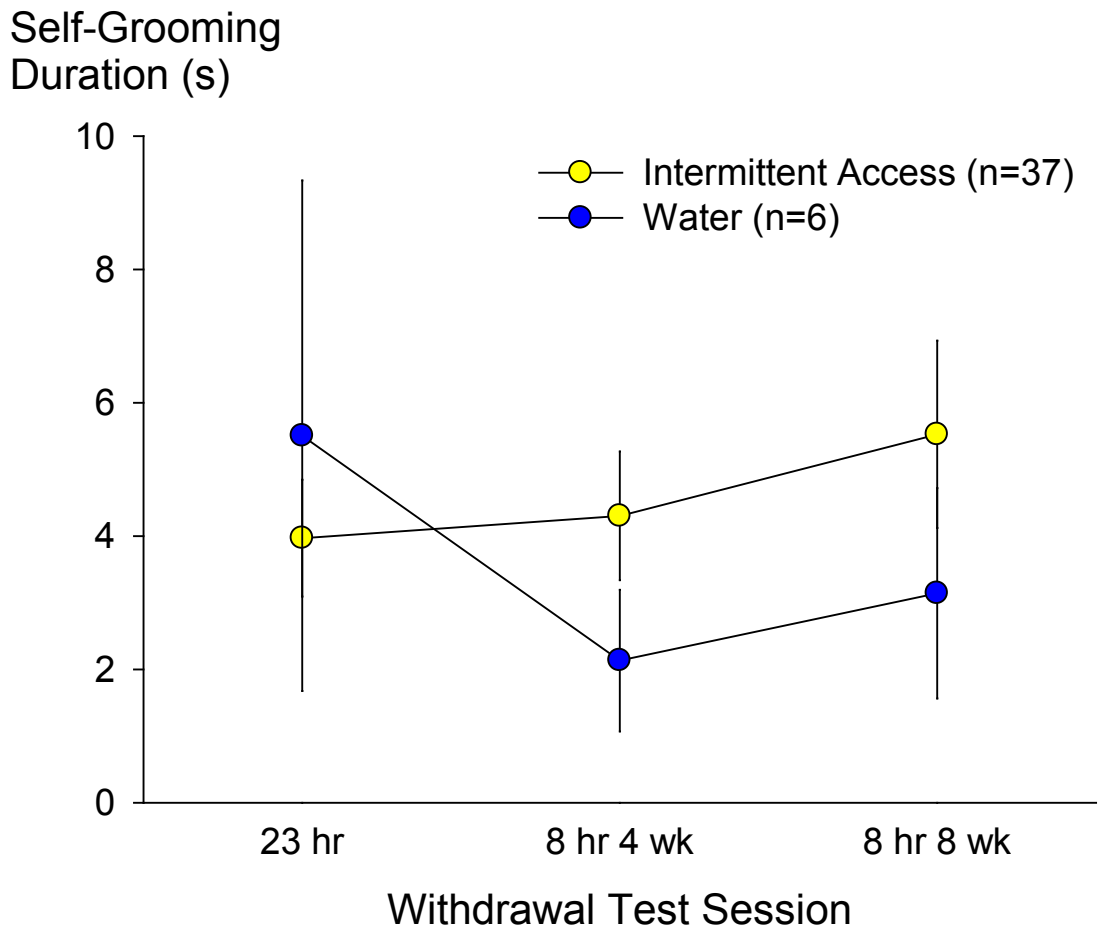
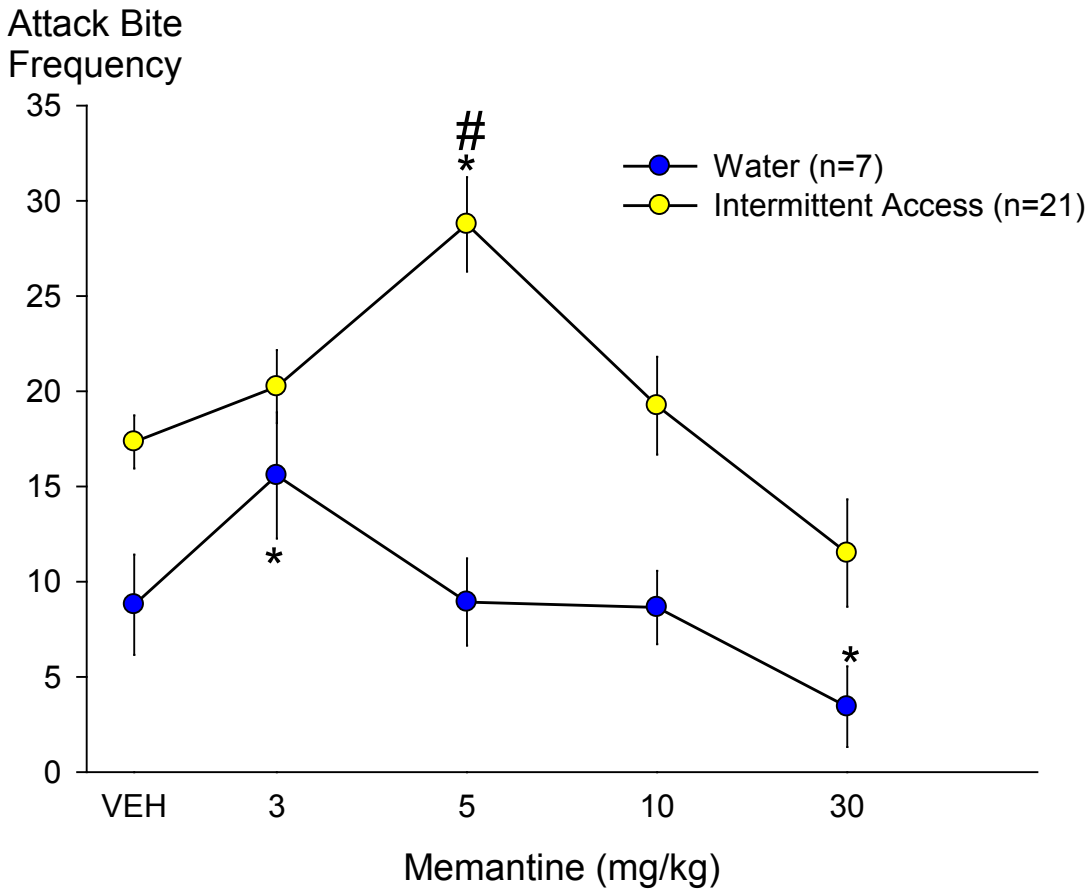

Self-Grooming
Duration (s)
10
8
6
4
2
0
Intermittent Access (n=37)
Water (n=6)
23 hr
8 hr 4 wk
8 hr 8 wk
Withdrawal Test Session

Figure 9. **The effect of memantine treatment on aggressive behavior in IAA and water control mice at 8 hours into ethanol withdrawal. A)** The effect of memantine treatment on **A)** attack bite frequency and **B)** sideways threat frequency in mice with intermittent access to ethanol and water control mice at 8 hours into the ethanol withdrawal period. Error bars denote standard error of the mean. Asterisks denote significance within the IAA or water control groups compared to vehicle ($p<.05$) and pound signs denote significance compared to water control ($p<.05$).

A)



B)

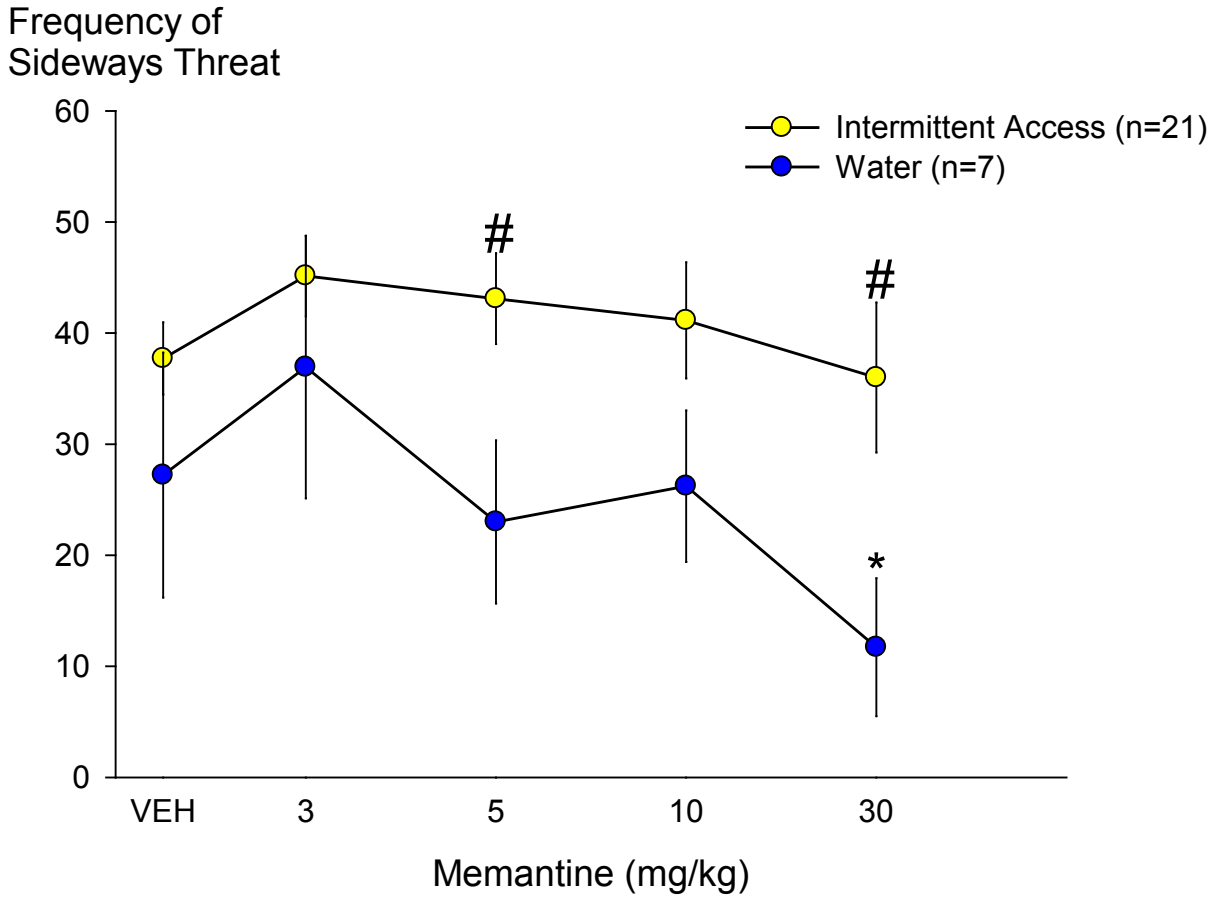
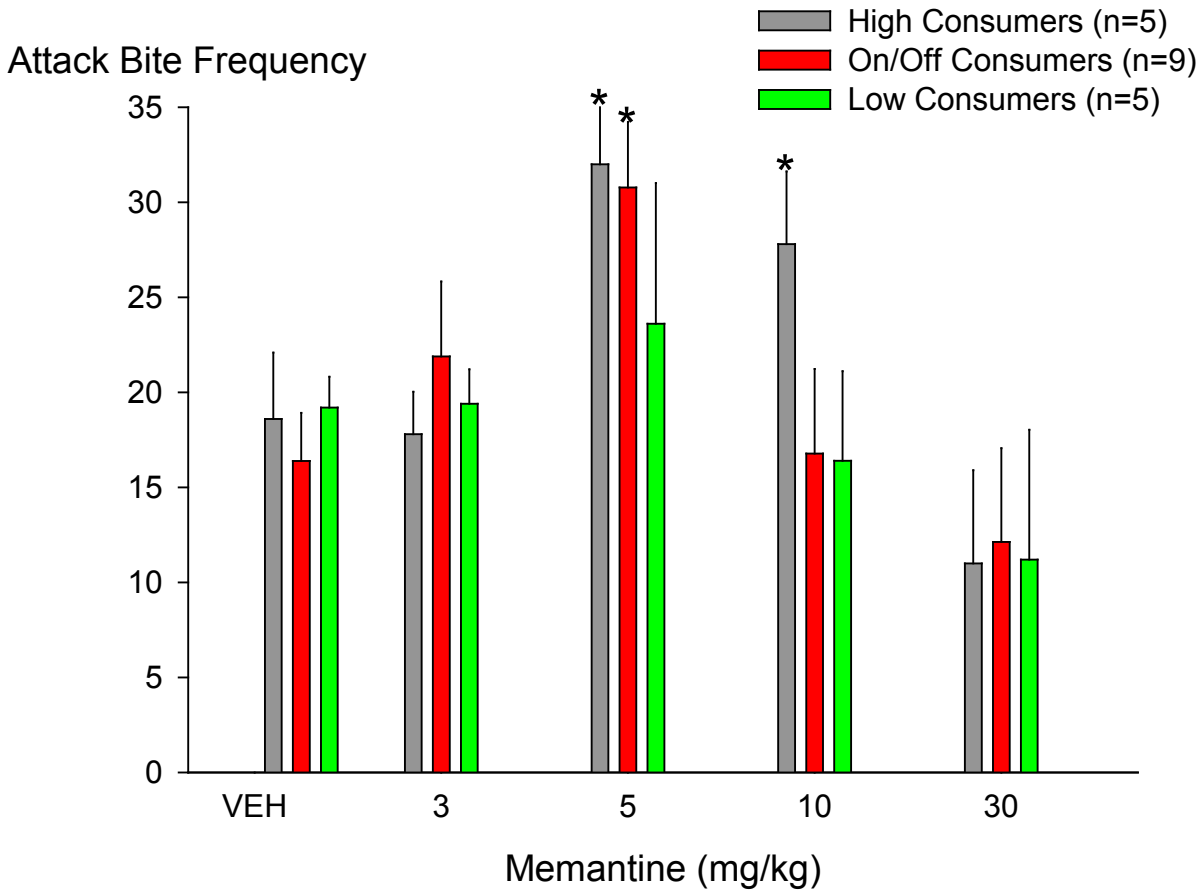

Frequency of
Sideways Threat
Intermittent Access (n=21)
Water (n=7)
60
50
40
30
20
10
0
#
#
*
VEH
3
5
10
30
Memantine (mg/kg)

Figure 10. **The effect of memantine treatment on aggressive behavior in High, On/Off, and Low ethanol consumption groups at 8 hours into ethanol withdrawal. A)** The effect of varying doses of memantine on **A)** attack bite frequency and **B)** sideways threat frequency in mice that drank High, On/Off, or Low levels of ethanol at 8 hours into the ethanol withdrawal period. Error bars denote standard error of the mean. Asterisks denote significance within the IAA or water control groups compared to vehicle ($p<.05$) and pound signs denote significance compared to the Low group ($p<.05$).

A)



B)

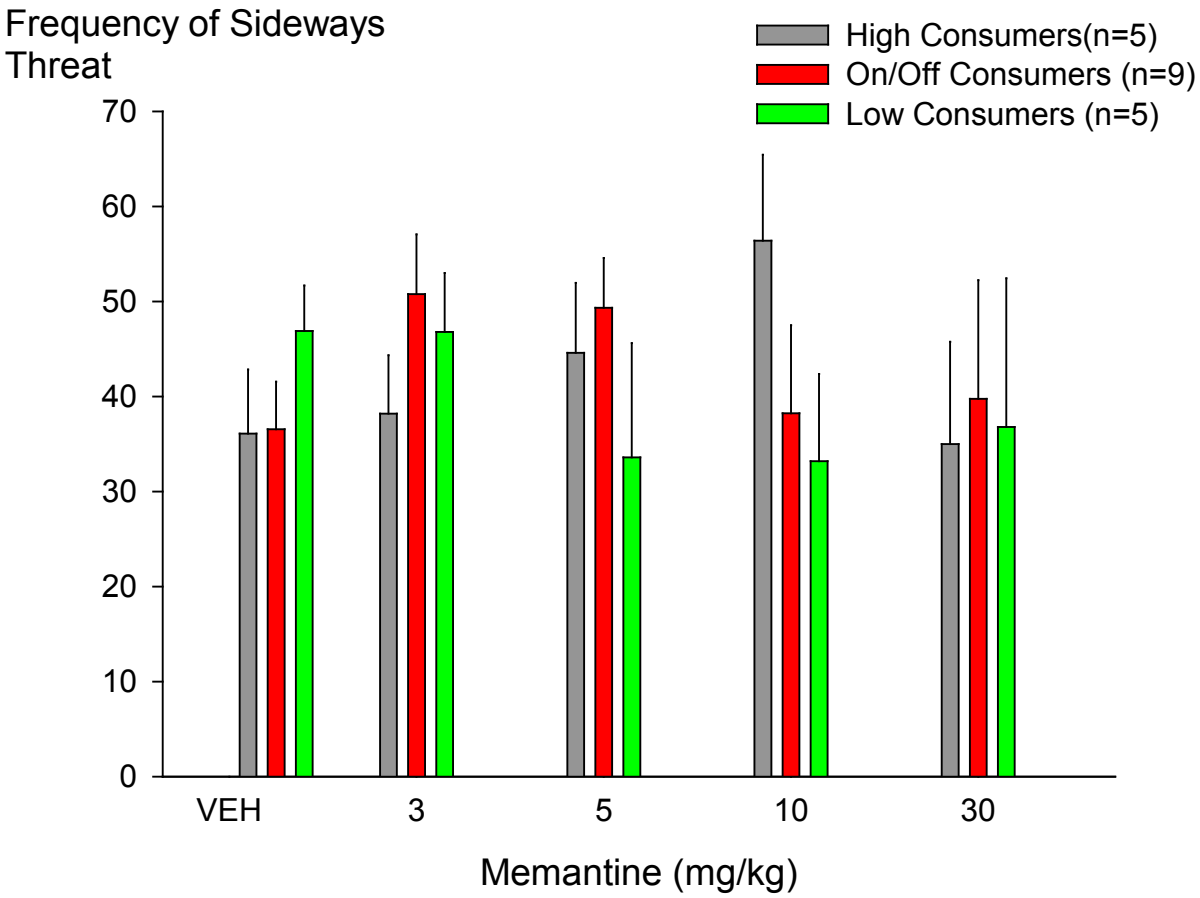
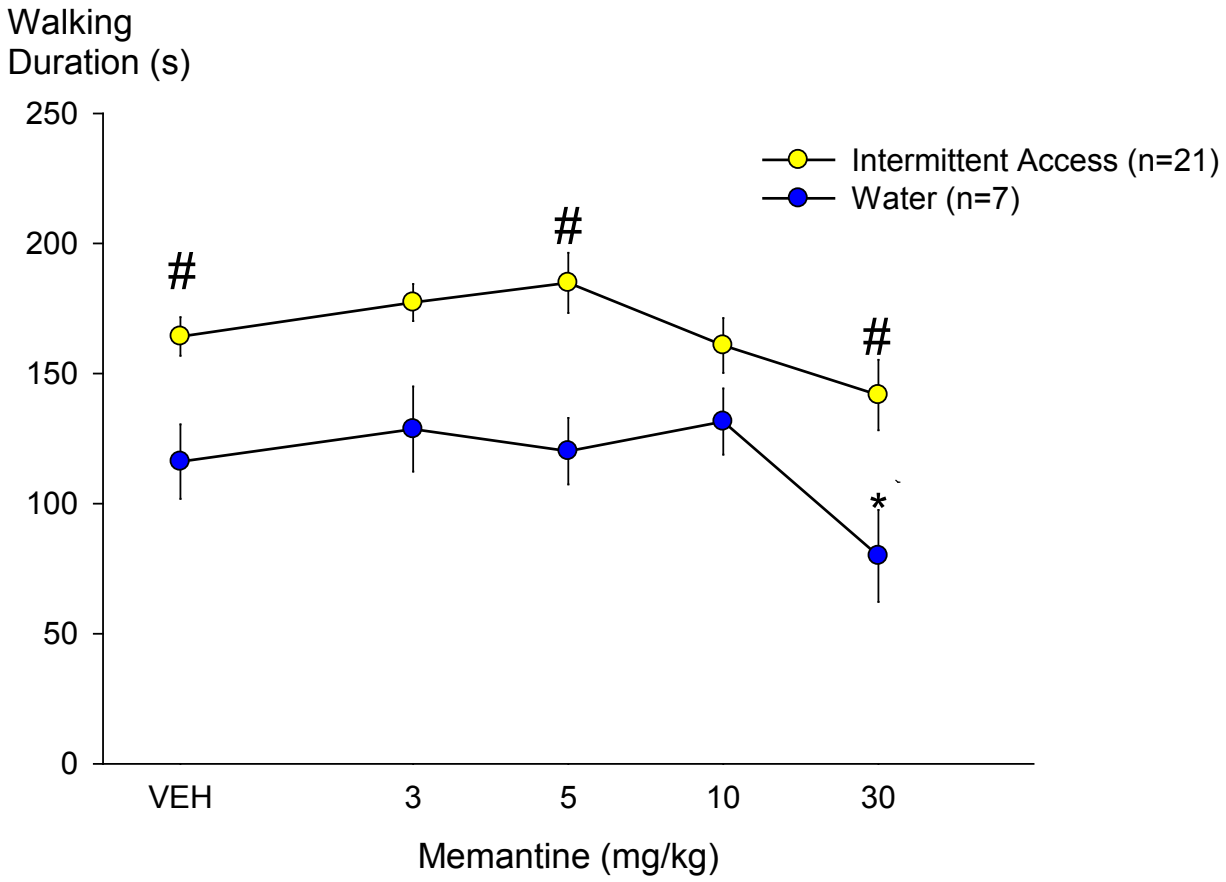
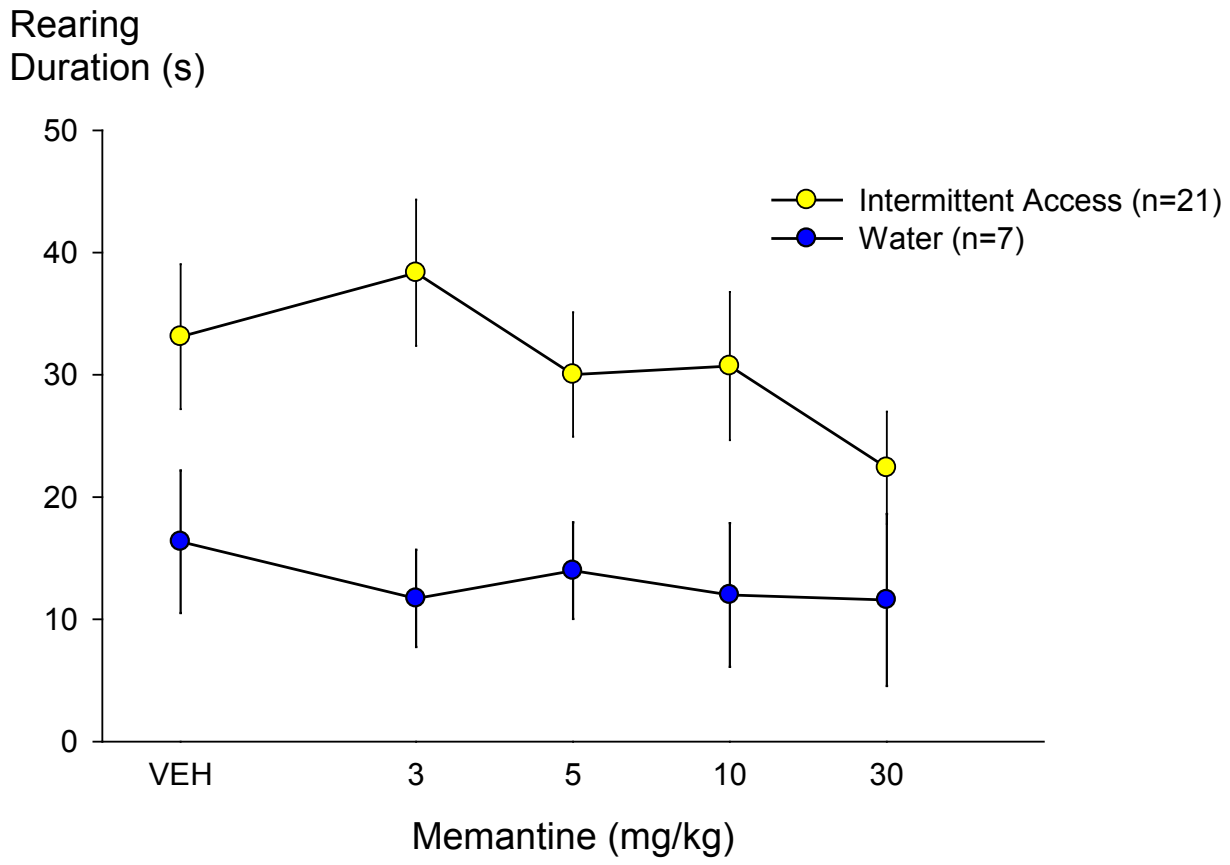

Frequency of Sideways Threat
High Consumers(n=5)
On/Off Consumers (n=9)
Low Consumers (n=5)
70
60
50
40
30
20
10
0
VEH
3
5
10
30
Memantine (mg/kg)

Figure 11. The effect of memantine treatment on non-aggressive behaviors in IAA and water control mice at eight hours into ethanol withdrawal. The effect of varying doses of memantine on seconds of **A)** walking duration, **B)** rearing duration, and **C)** self-grooming duration during an aggressive encounter in mice that had intermittent access to ethanol and water control mice at eight hours into the ethanol withdrawal period. Error bars denote standard error of the mean. Asterisks denote significance compared to vehicle ($p<.05$) within the IAA or water control group and pound signs denote significance compared to water control ($p<.05$).

A)



B)


Rearing
Duration (s)
50
40
30
20
10
0
Intermittent Access (n=21)
Water (n=7)
VEH
3
5
10
30
Memantine (mg/kg)

C)

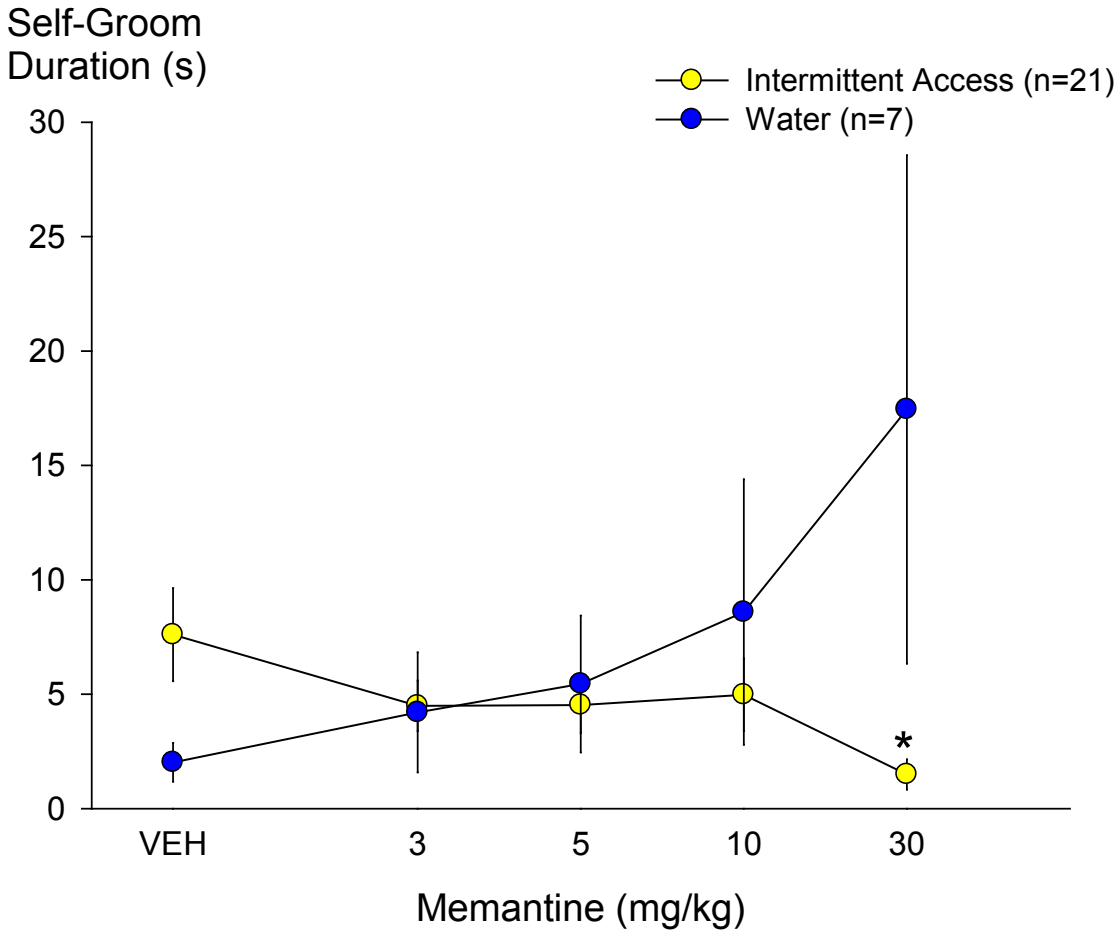
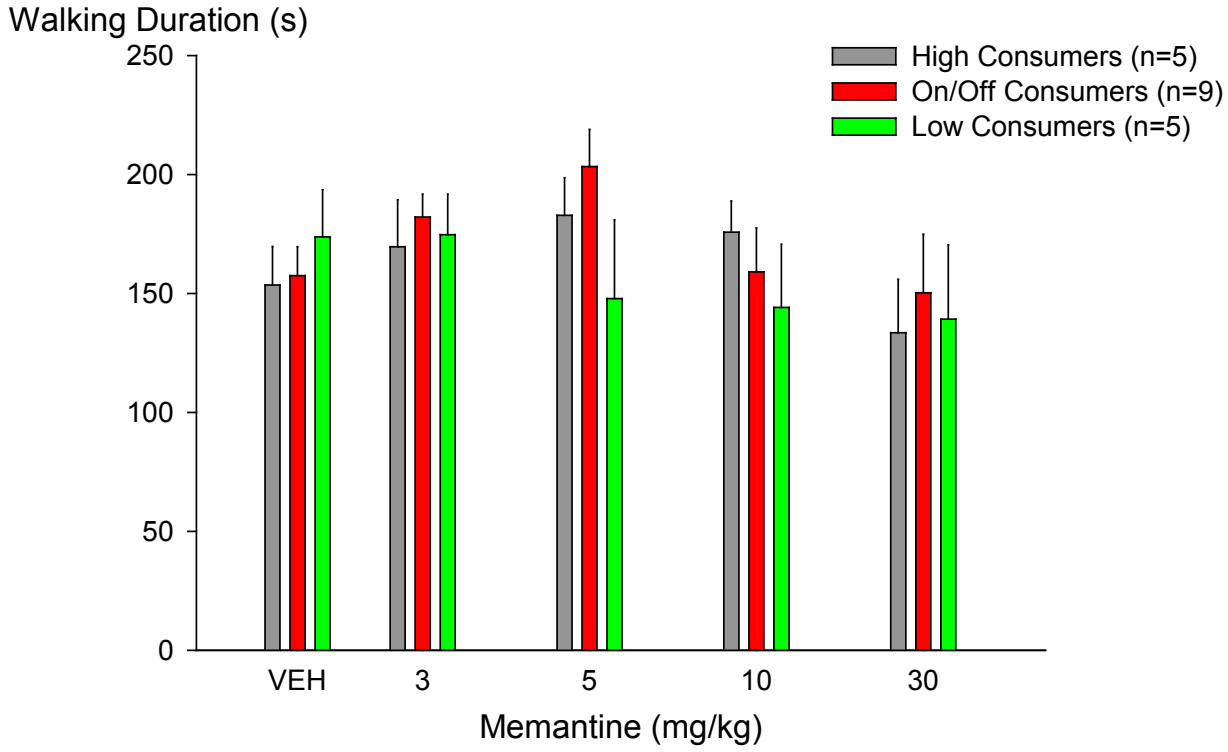
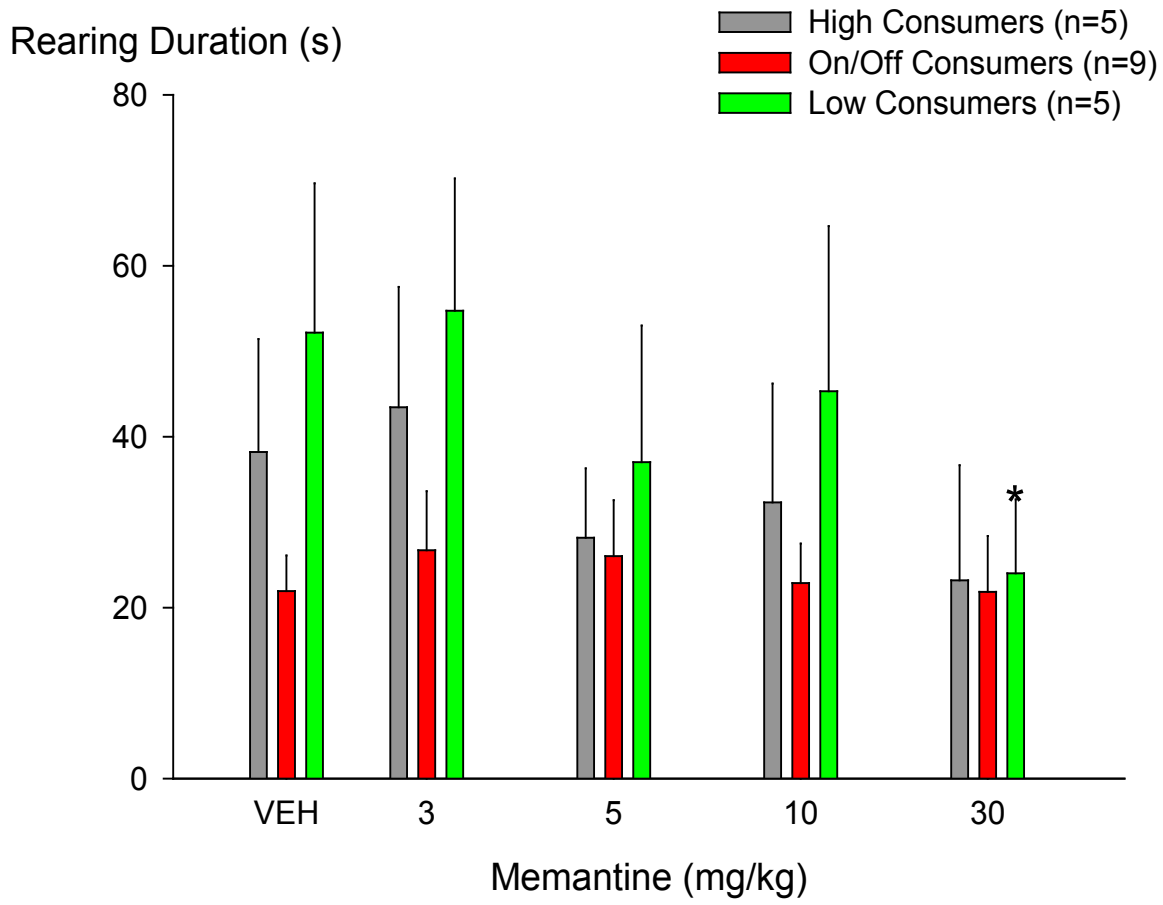

Self-Groom
Duration (s)
Intermittent Access (n=21)
Water (n=7)
30
25
20
15
10
5
0
VEH
3
5
10
30
*
Memantine (mg/kg)

Figure 12. The effect of memantine treatment on non-aggressive behaviors in High, On/Off, and Low ethanol consumption groups at 8 hours into ethanol withdrawal. The effect of varying doses of memantine on seconds of **A)** walking duration, **B)** rearing duration, and **C)** self-grooming duration during an aggressive encounter in mice that drank High, On/Off, or Low levels of ethanol at eight hours into the ethanol withdrawal period. Error bars denote standard error of the mean. Asterisks denote significance compared to vehicle ($p<.05$) within the IAA or water control group and pound signs denote significance compared to the Low group ($p<.05$).

A)



B)


Rearing Duration (s)
High Consumers (n=5)
On/Off Consumers (n=9)
Low Consumers (n=5)
80
60
40
20
0
VEH
3
5
10
30
*
Memantine (mg/kg)

C)

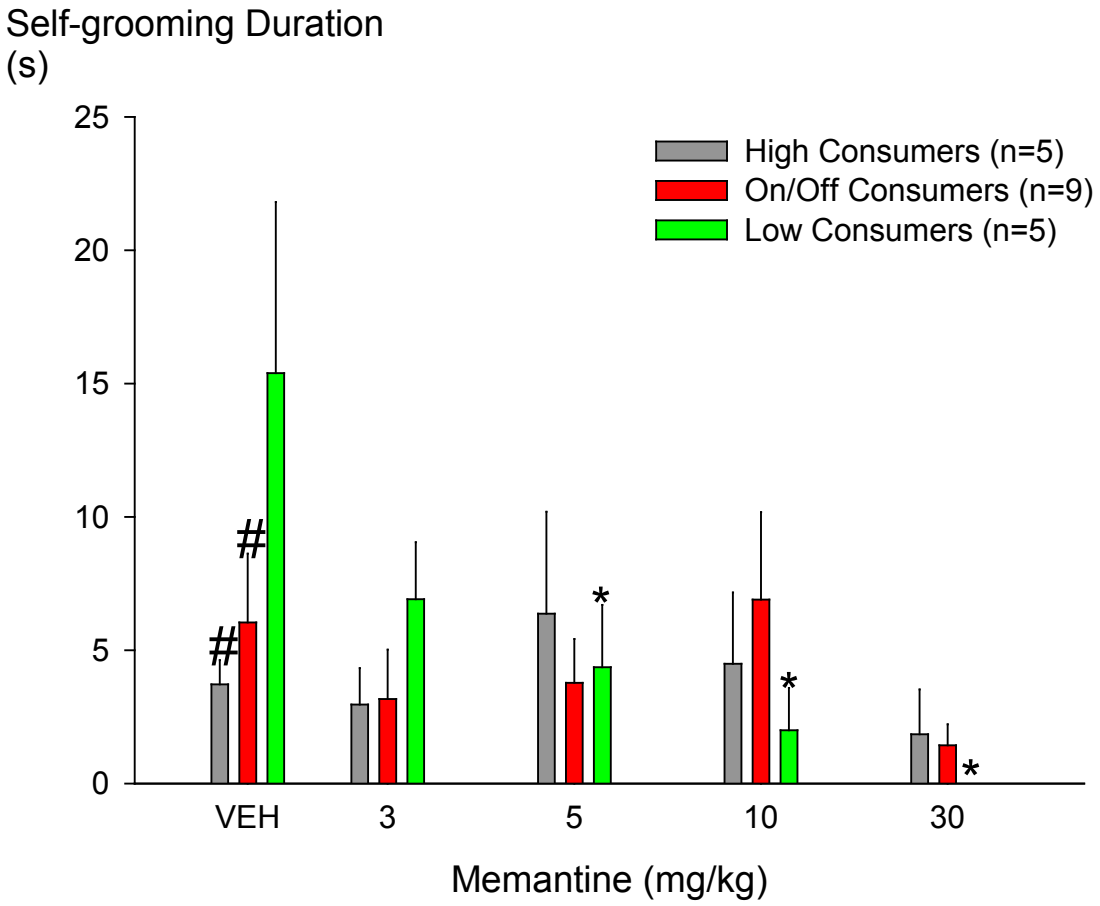
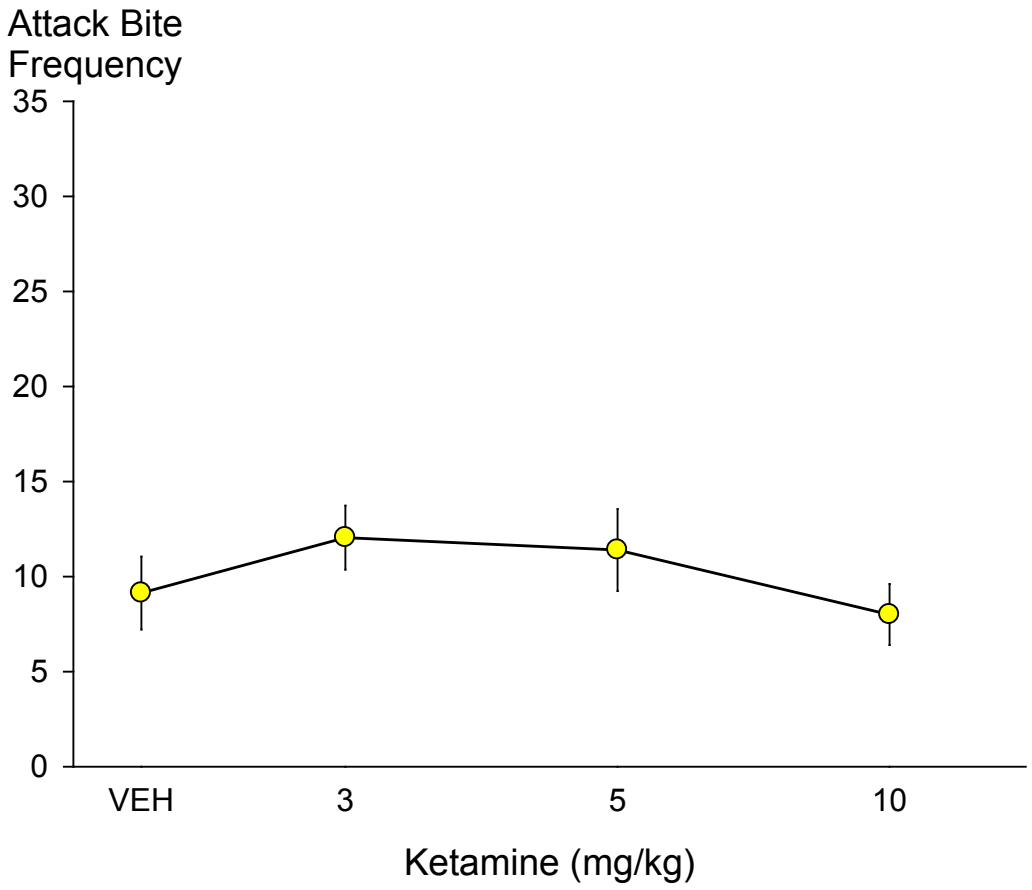

Self-grooming Duration
(s)
25
20
15
10
5
0
High Consumers (n=5)
On/Off Consumers (n=9)
Low Consumers (n=5)
#
#
*
*
*
VEH
3
5
10
30
Memantine (mg/kg)

Figure 13. **The effect of ketamine treatment on aggressive behavior in IAA mice at eight hours into ethanol withdrawal. A)** The effect of varying doses of ketamine on **A)** attack bite frequency and **B)** sideways threat frequency in mice with intermittent access to ethanol (n=23) at 8 hours into the ethanol withdrawal period. Error bars denote standard error of the mean.

A)



B)

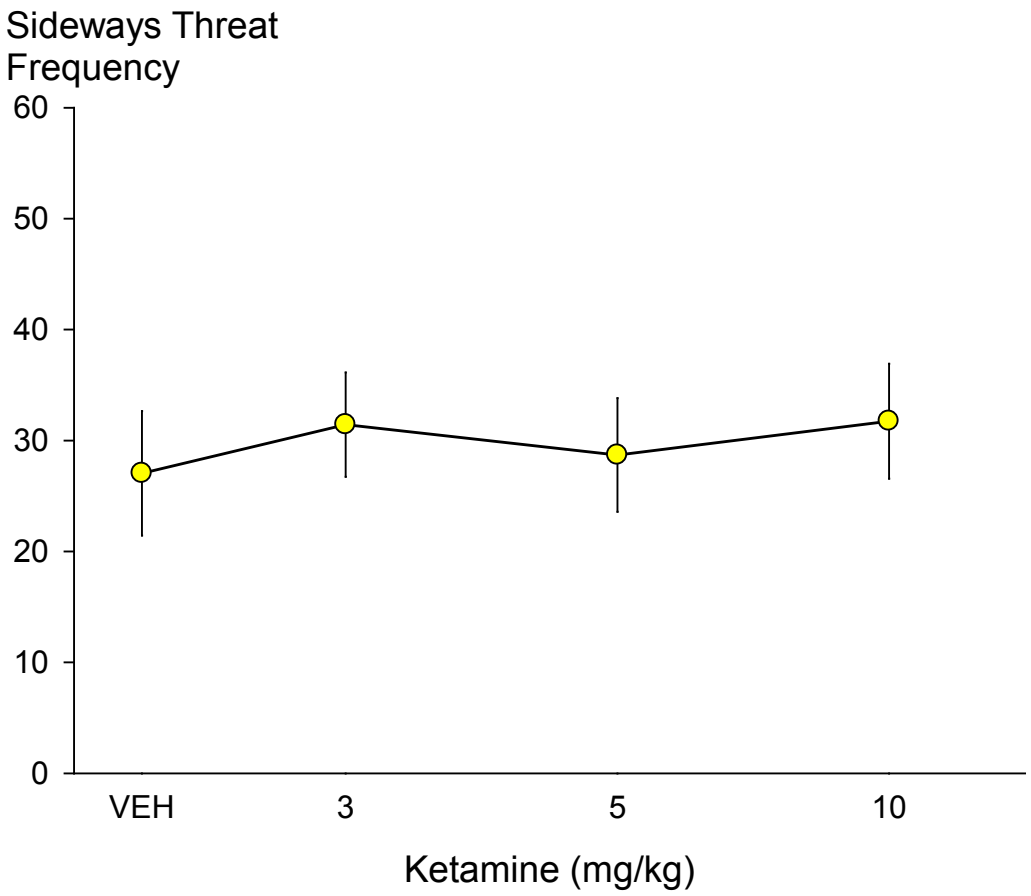
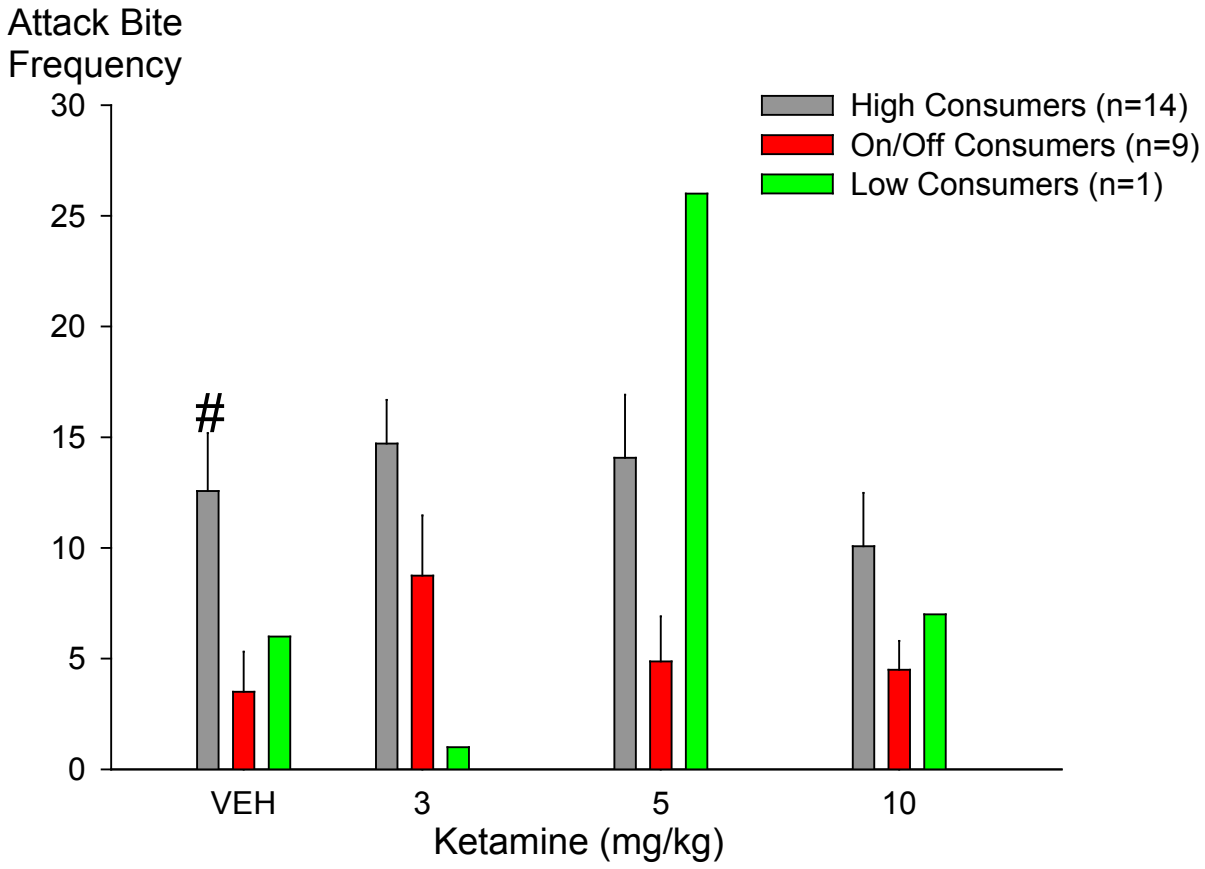

Sideways Threat Frequency
60
50
40
30
20
10
0
VEH
3
5
10
Ketamine (mg/kg)

Figure 14. **The effect of ketamine treatment on aggressive behavior in High, On/Off, and Low ethanol consumption groups at eight hours into ethanol withdrawal. A)** The effect of varying doses of ketamine on **A)** attack bite frequency and **B)** sideways threat frequency in mice that drank High, On/Off, and Low levels of ethanol at 8 hours into the ethanol withdrawal period. Error bars denote standard error of the mean. Pound signs denote significance compared to the On/Off group ($p<.05$).

A)



B)

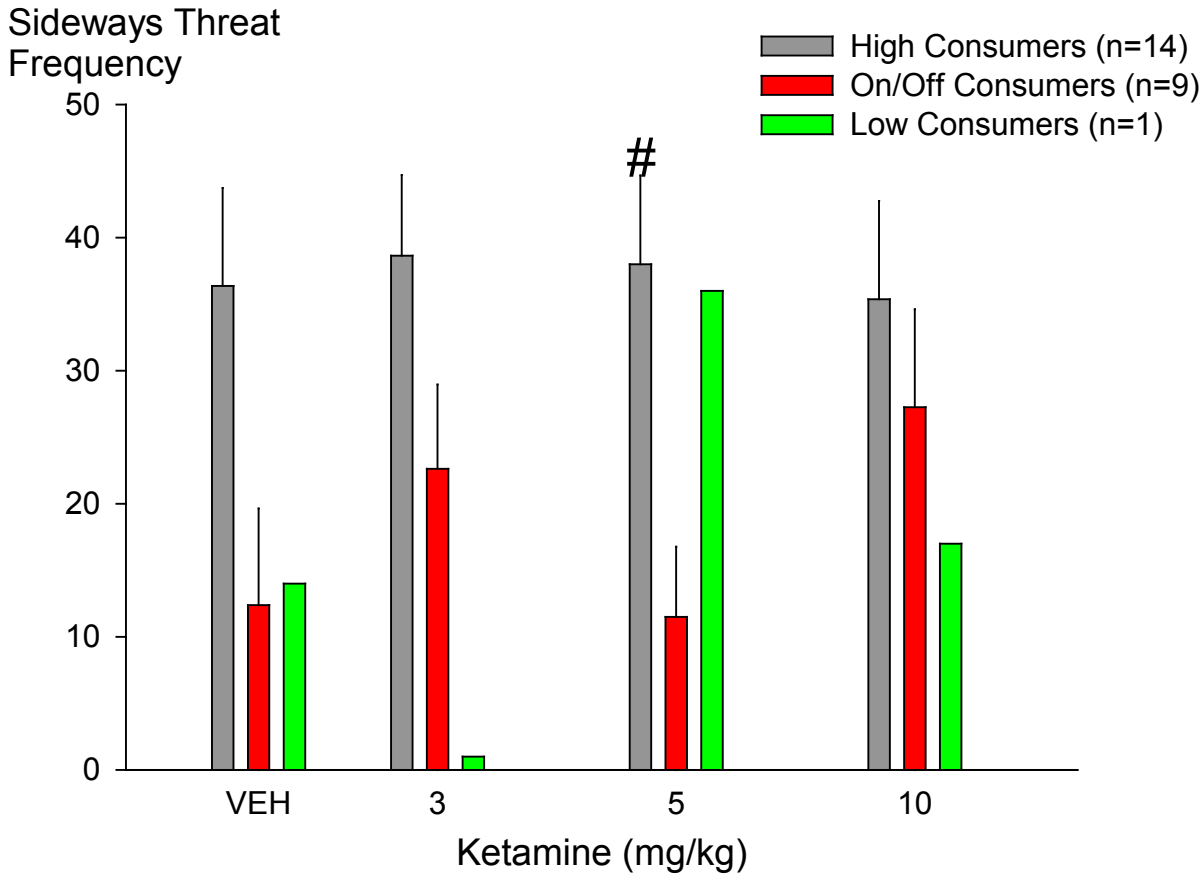
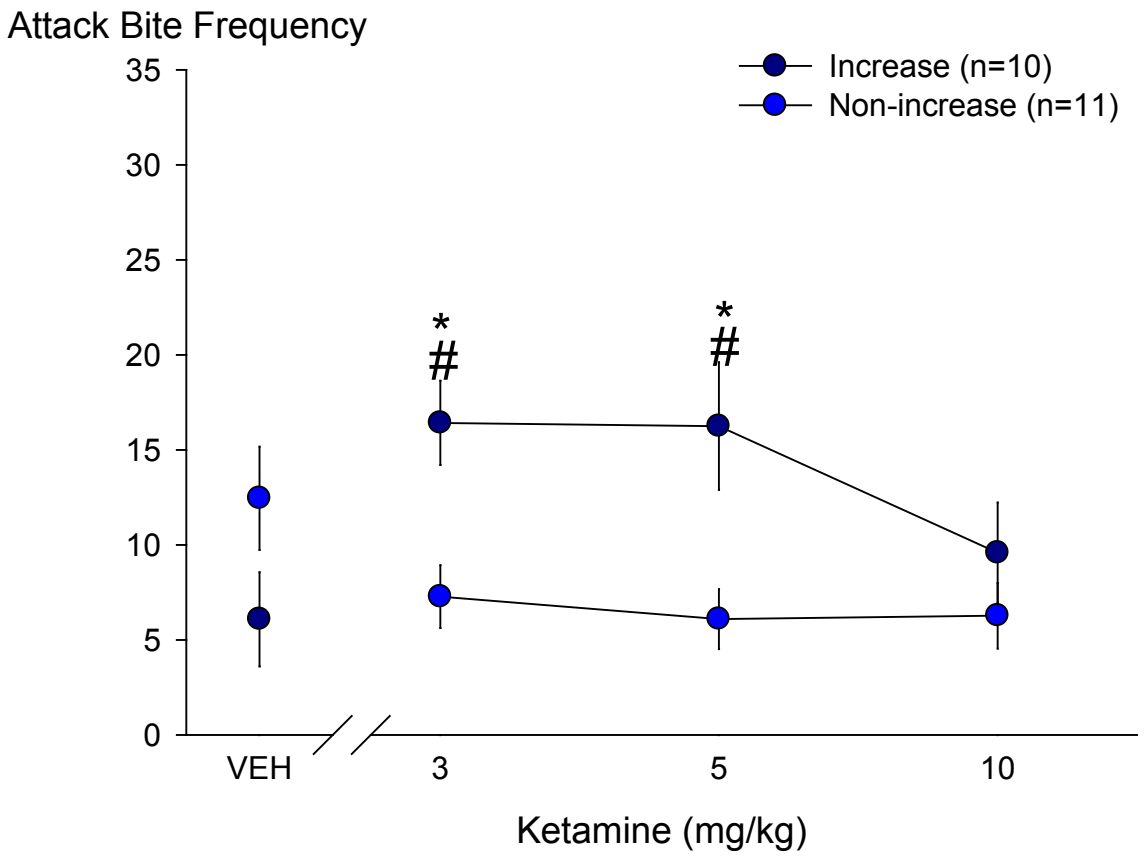

Sideways Threat Frequency
High Consumers (n=14)
On/Off Consumers (n=9)
Low Consumers (n=1)
50
40
30
20
10
0
#
VEH
3
5
10
Ketamine (mg/kg)

Figure 15. **The effect of ketamine treatment on aggressive behavior in the Increase and Non-increase groups at eight hours into ethanol withdrawal. A)** The effect of varying doses of ketamine on **A)** attack bite frequency and **B)** sideways threat frequency in mice that experienced an increase in attack bites (Increase group) or no change in attack bites (Non-increase group) after ketamine treatment at 8 hours into the ethanol withdrawal period. Error bars denote standard error of the mean. Asterisks denote significance within the Increase or Non-increase groups compared to vehicle, while pound signs denote significance between groups ($p<.05$).

A)



B)

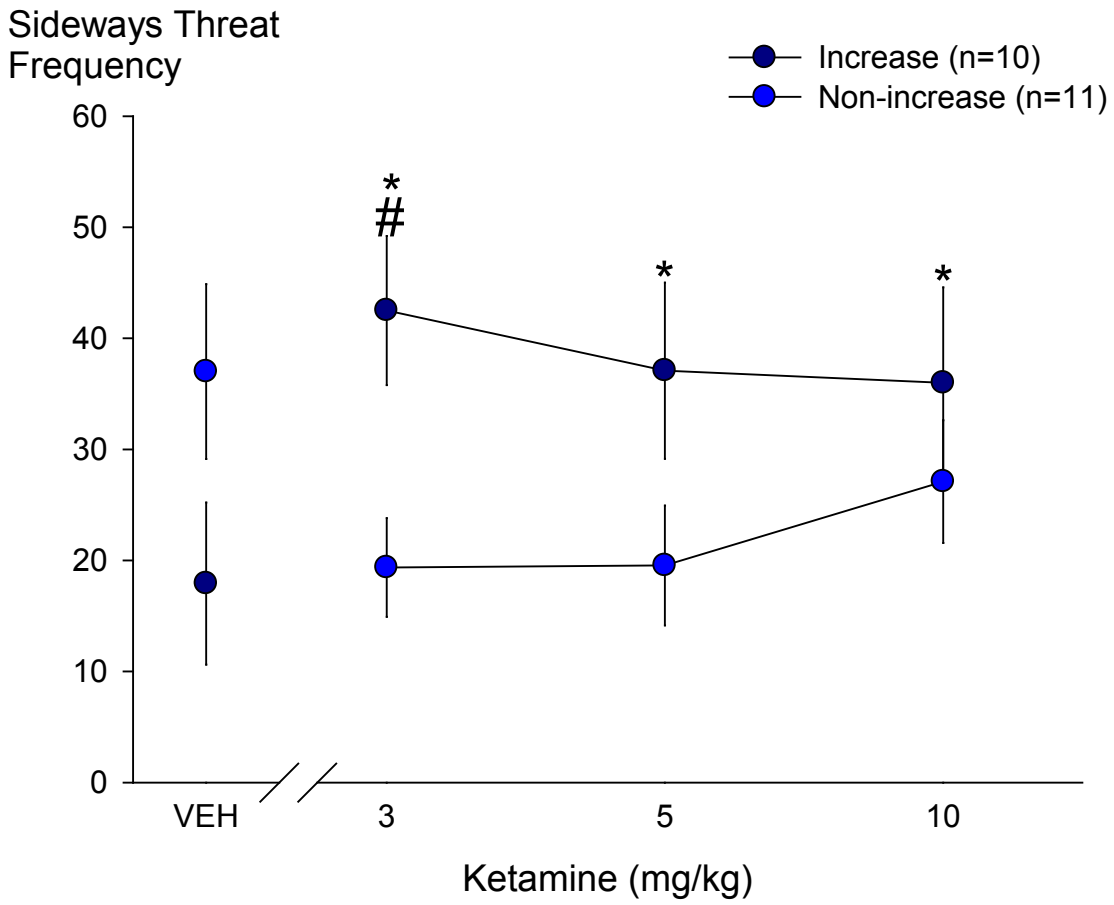
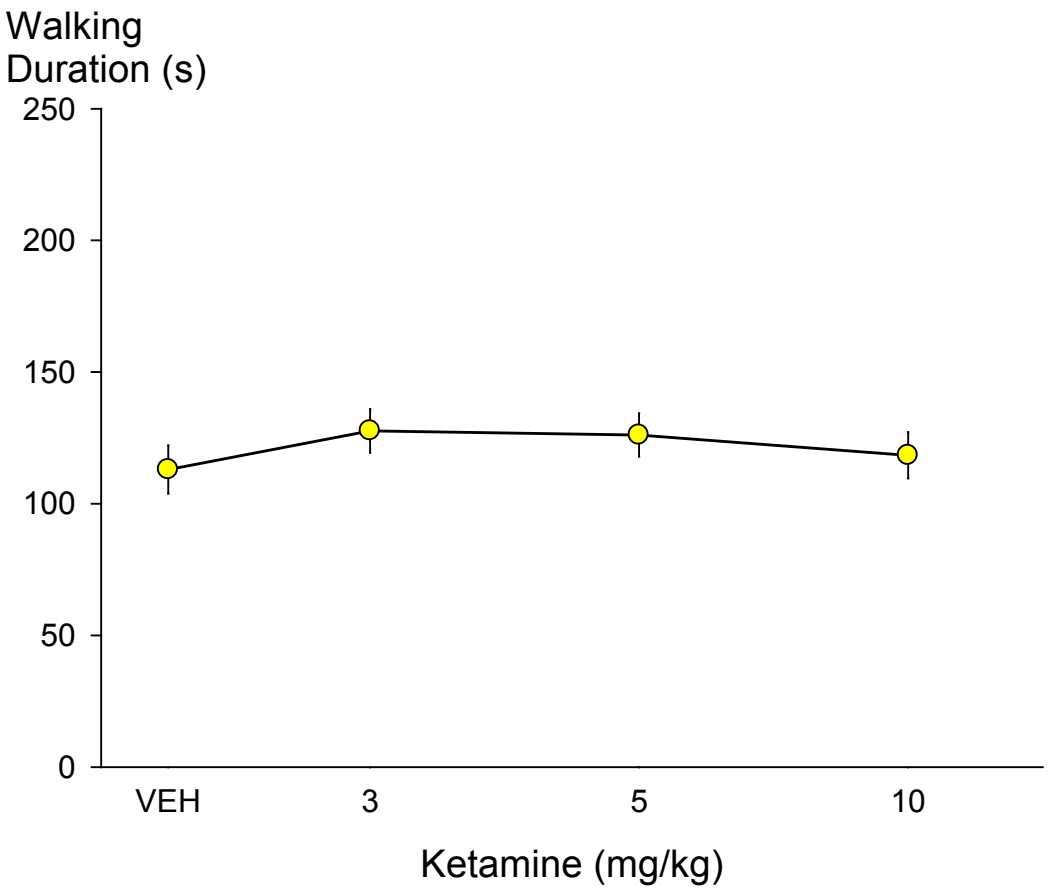
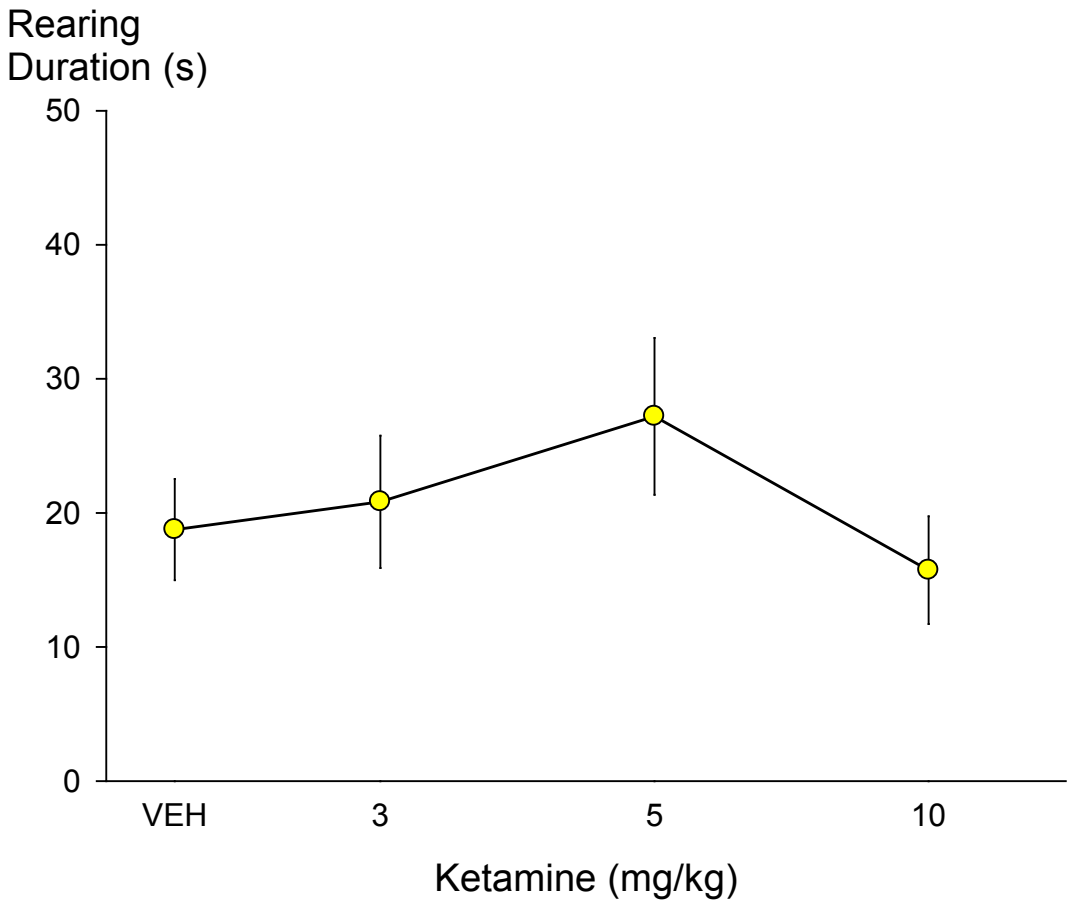

Sideways Threat Frequency
Increase (n=10)
Non-increase (n=11)
60
50
40
30
20
10
0
*
#
*
*
VEH
3
5
10
Ketamine (mg/kg)

Figure 16. The effect of ketamine treatment on non-aggressive behaviors in IAA mice at eight hours into ethanol withdrawal. The effect of varying doses of ketamine on seconds of **A)** walking duration, **B)** rearing duration, and **C)** self-grooming duration during an aggressive encounter in mice that had intermittent access to ethanol (n=23) at eight hours into the ethanol withdrawal period.

A)



B)



C)

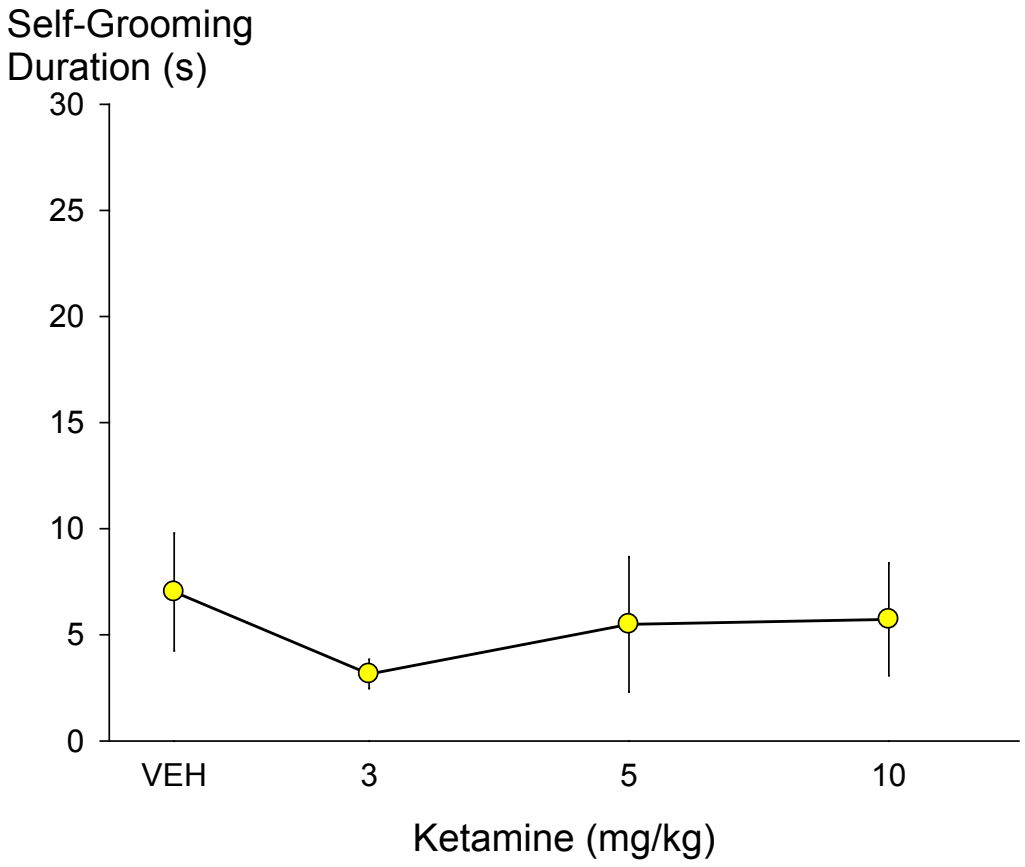
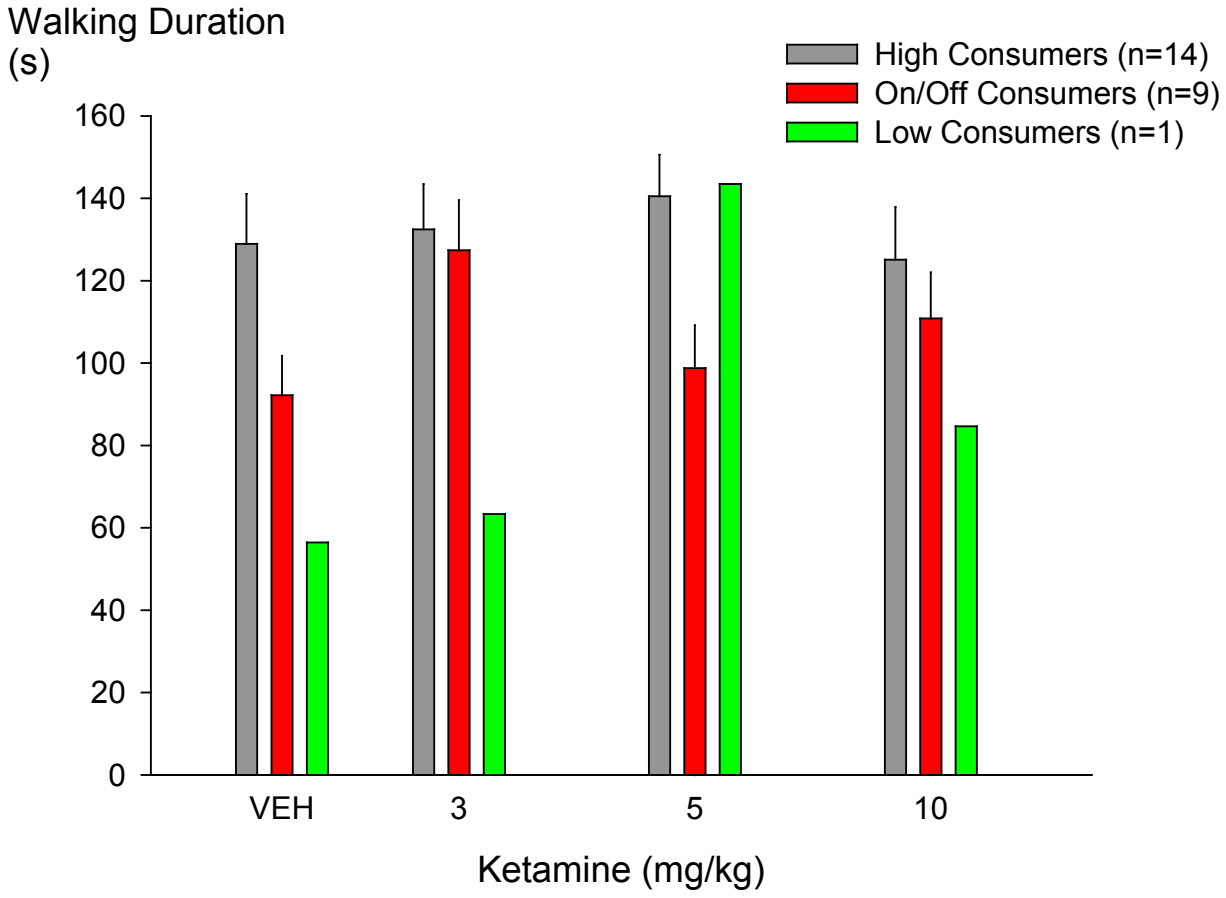
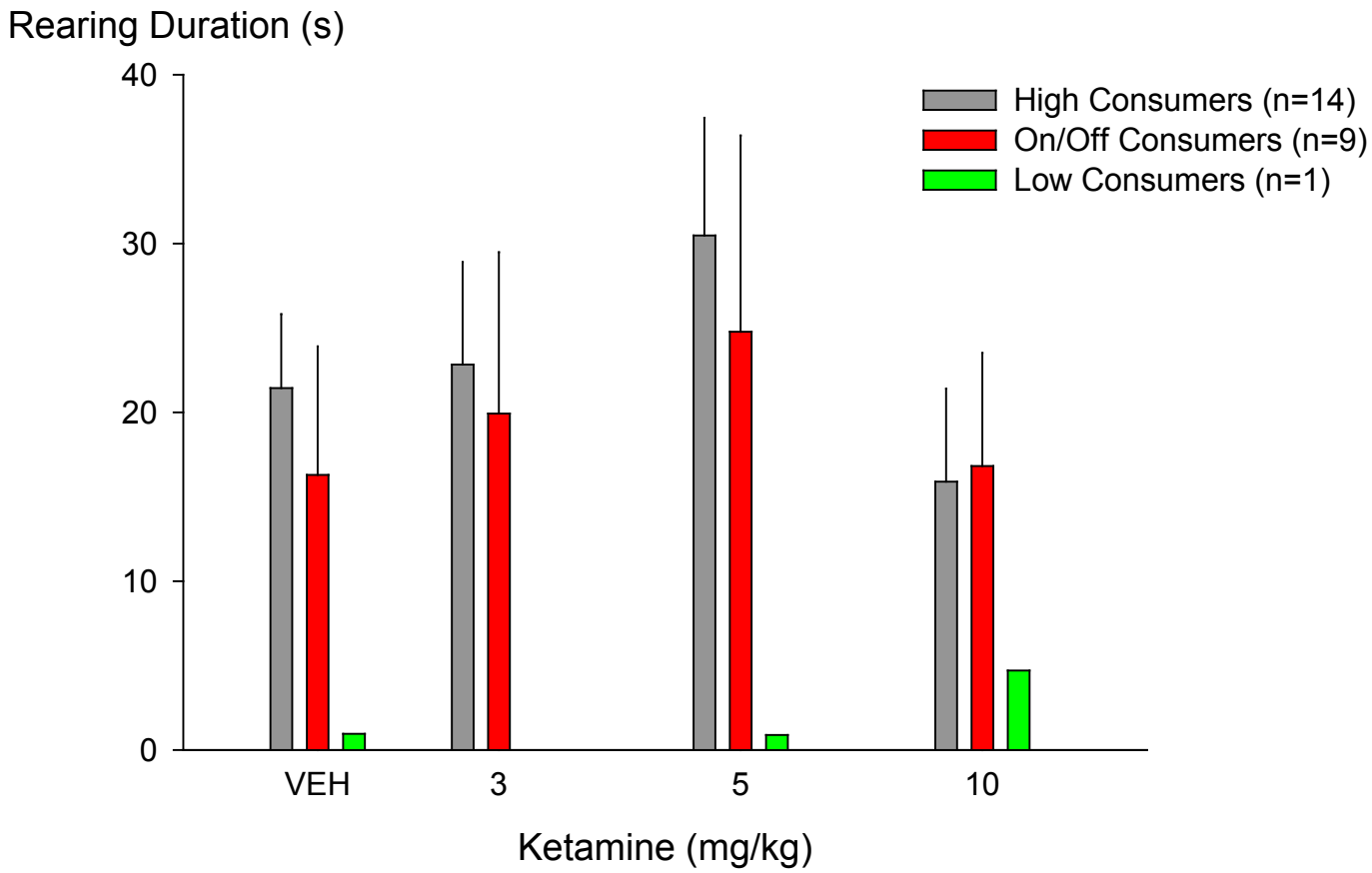

Self-Grooming
Duration (s)
30
25
20
15
10
5
0
VEH
3
5
10
Ketamine (mg/kg)

Figure 17. The effect of ketamine treatment on non-aggressive behaviors in High, On/Off, and Low ethanol consumption groups at eight hours into ethanol withdrawal. The effect of varying doses of ketamine on seconds of **A)** walking duration, **B)** rearing duration, and **C)** self-grooming duration during an aggressive encounter in mice that drank High, On/Off, or Low levels of ethanol at eight hours into the ethanol withdrawal period.

A)



B)


Rearing Duration (s)
40
30
20
10
0
VEH
3
5
10
Ketamine (mg/kg)
High Consumers (n=14)
On/Off Consumers (n=9)
Low Consumers (n=1)

C)

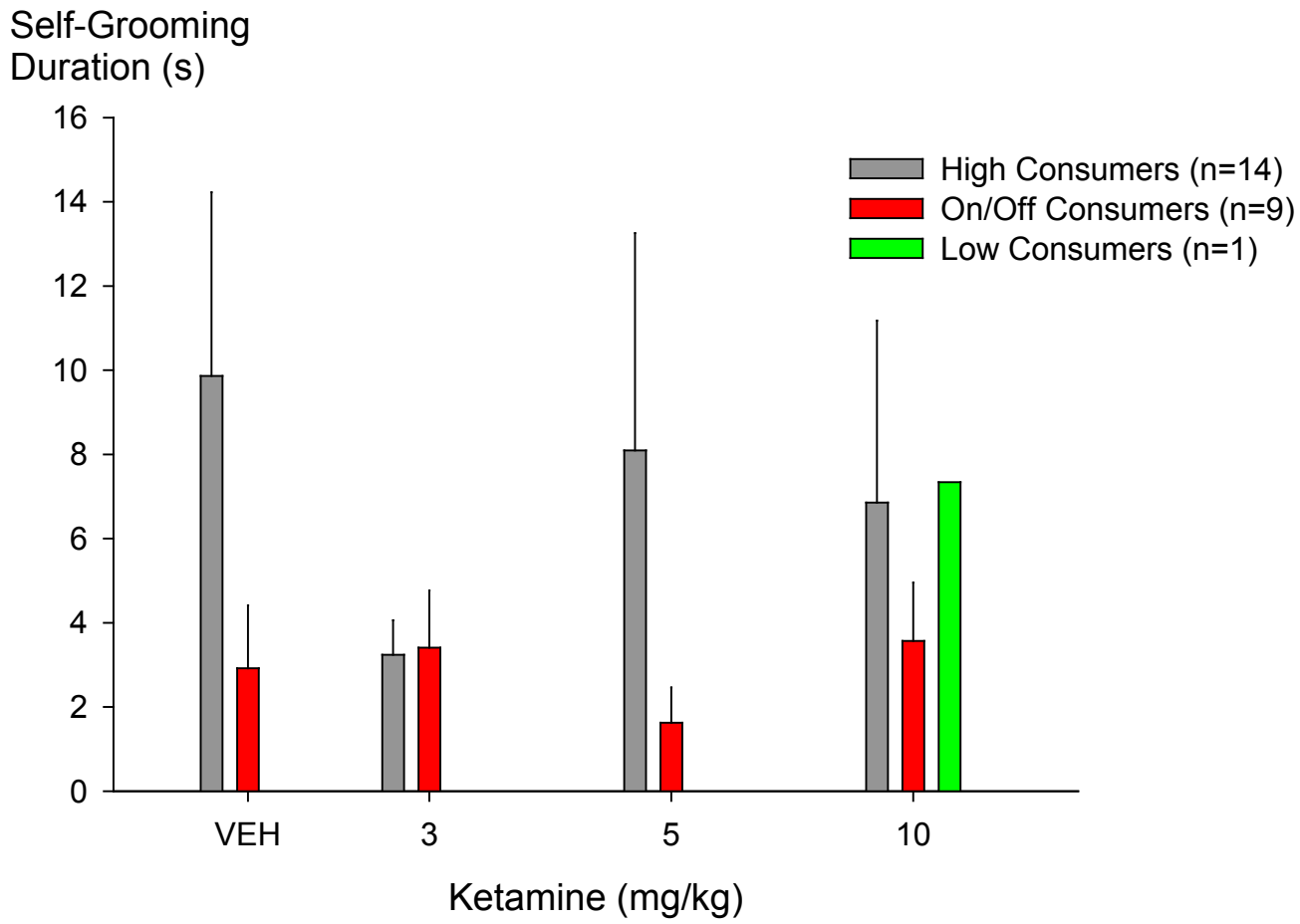
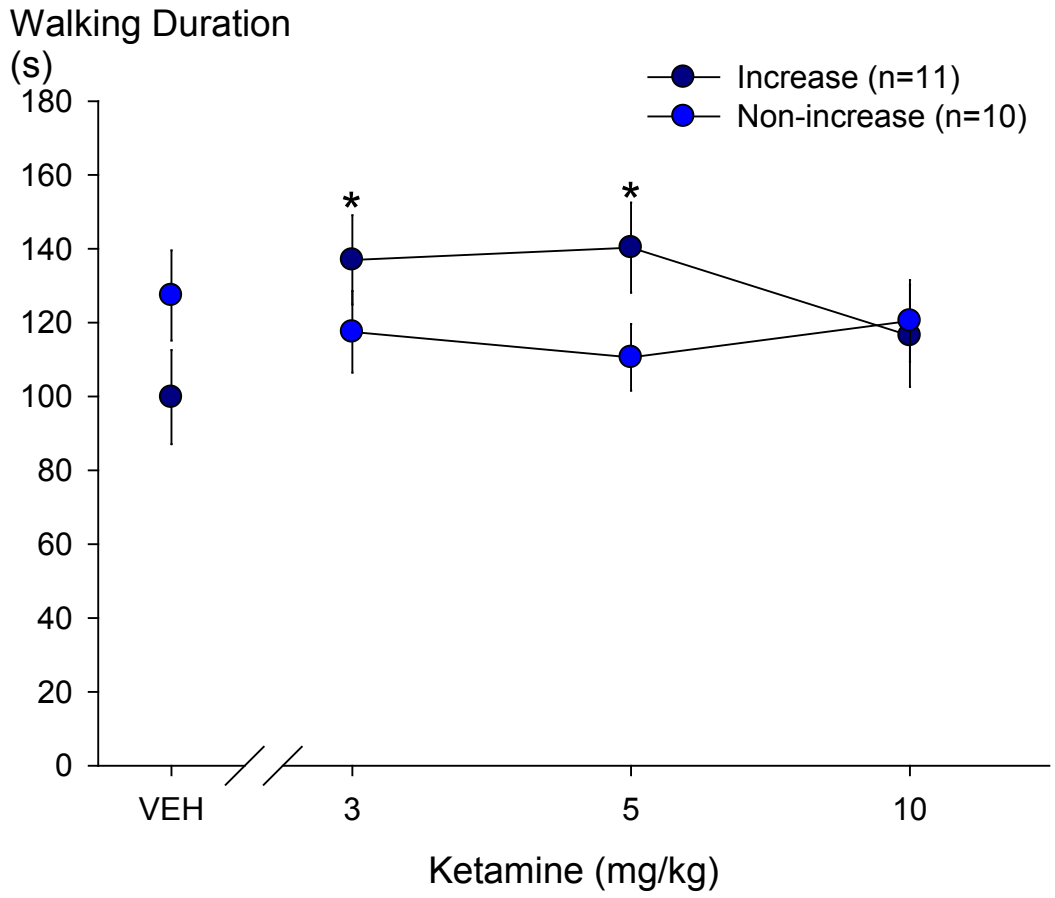
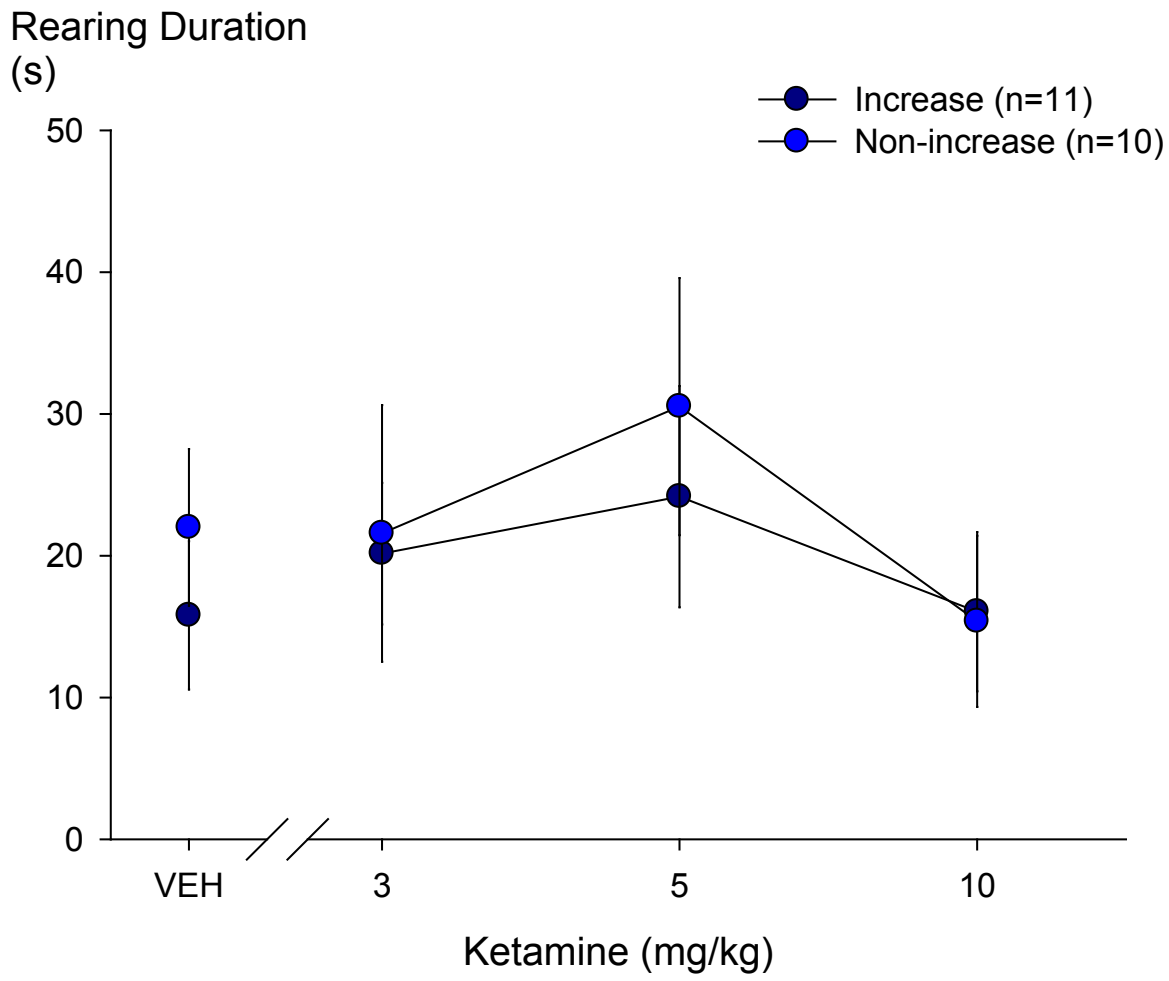

Self-Grooming
Duration (s)
16
14
12
10
8
6
4
2
0
VEH
3
5
10
Ketamine (mg/kg)
High Consumers (n=14)
On/Off Consumers (n=9)
Low Consumers (n=1)

Figure 18. The effect of ketamine treatment on non-aggressive behaviors in the Increase and Non-increase groups at eight hours into ethanol withdrawal. The effect of varying doses of ketamine on seconds of **A)** walking duration, **B)** rearing duration, and **C)** self-grooming duration during an aggressive encounter in mice that experienced an increase in attack bites (Increase group) or no change in attack bites (Non-increase group) after ketamine treatment at eight hours into the ethanol withdrawal period. Asterisks denote significance within the Increase or Non-increase group compared to vehicle (p<.05).

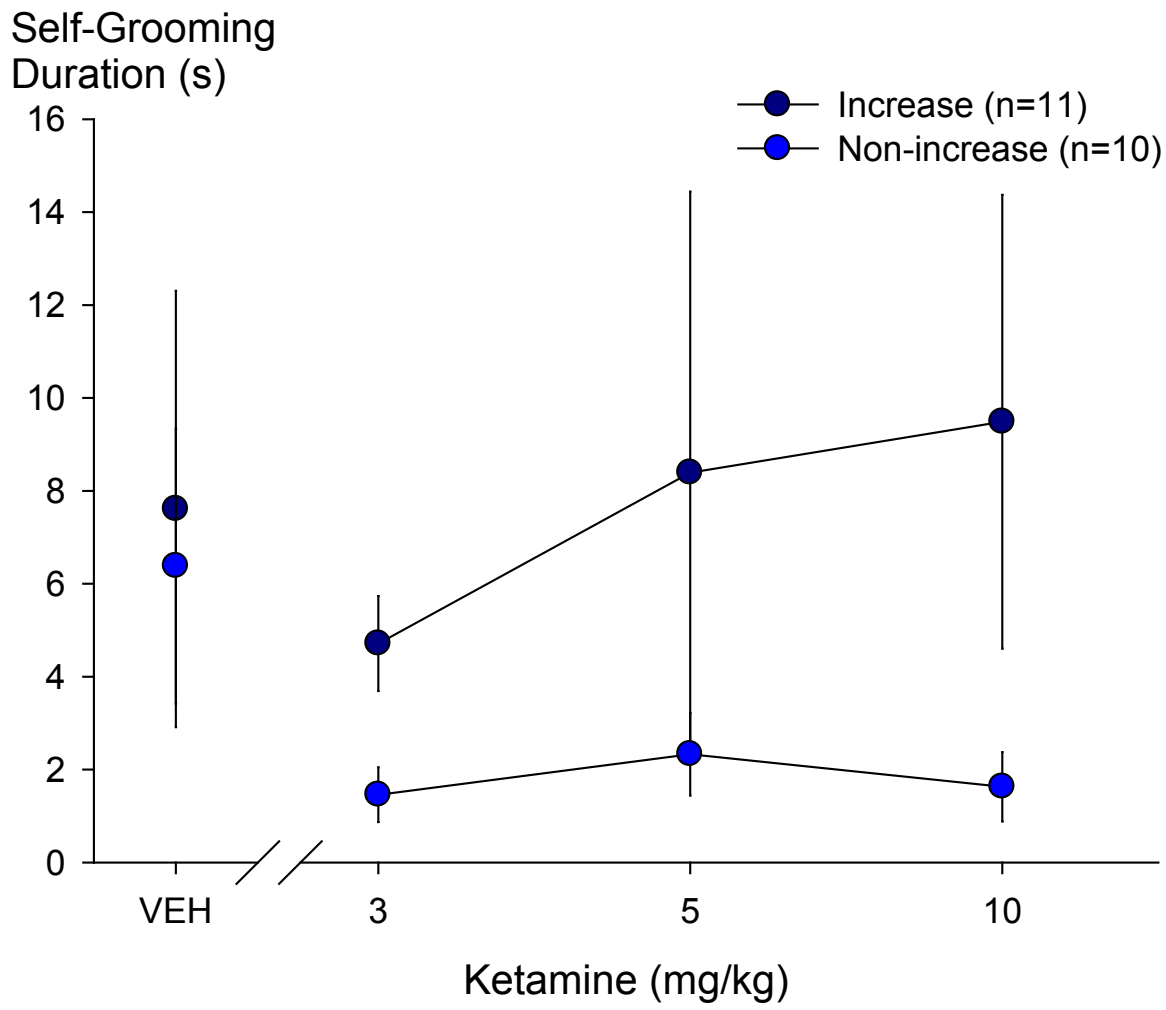
A)



B)


Rearing Duration
(s)
Increase (n=11)
Non-increase (n=10)
50
40
30
20
10
0
VEH
3
5
10
Ketamine (mg/kg)

C)


Self-Grooming
Duration (s)
Increase (n=11)
Non-increase (n=10)
16
14
12
10
8
6
4
2
0
VEH
3
5
10
Ketamine (mg/kg)