The role of the NMDA receptor in mediating aggression during withdrawal from ethanol in outbred male mice.

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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. 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 and aggression during withdrawal periods was assessed after 1-2 and 4-5 weeks of exposure to ethanol. After 16 days of exposure to ethanol, mice drank ethanol in a large range from 25-300 grams/kilogram (g/kg) of body weight. Handling-induced convulsions (HIC) scores were assessed at the end of the 4-5 week testing session to determine withdrawal severity. Neither ethanol-drinking mice nor water-drinking mice reached median HIC scores above 0. During the 1-2 and 4-5 week aggression assessments, memantine was injected (0, 3, 5, 10 mg/kg, i.p.) 8 hours into withdrawal. Memantine did not appear to significantly increase aggression in ethanol-drinking mice but increased aggression in the water-drinking mice at varying doses. For many aggressive behaviors ethanol-drinking and water-drinking mice has significantly different baseline behaviors. These findings suggest that withdrawal appeared to have a depressive affect on aggression in ethanol-drinking mice particularly after 4-5 weeks exposure to ethanol perhaps due to a decrease in extracellular glutamate. Future studies should use microdialysis to determine glutamate levels in the brain in both the withdrawal period as well as during drinking in order to determine if withdrawal or exposure to ethanol is having lasting effects on aggression. It would also be a good idea to use an NMDA receptor agonist to further probe aggressive

behavior and see if aggression can be heightened in ethanol-drinking mice after 1-2 and 4-5 week exposure to ethanol.

Alcohol is one of the most commonly used drugs in the world. According to the National Institute on Drug Abuse (NIAAA), a survey conducted in the United States reported about half of Americans aged 12 or older had used alcohol at least once (NIAAA, 2011). Withdrawal from alcohol, characterized by symptoms such as nausea, tremors, anxiety, and sensory disturbances, has been an area of interest because of the positive as well as negative reinforcing effects it can have on drinking behavior (Stockwell, 1994; Valdez & Koob, 2004; Gilpin & Koob, 2008). Withdrawal has also been shown to have both acute and long-term effects on neurochemistry and glutamate in particular (Gilpin & Koob, 2008; Melendez, Hicks, Cagle et al., 2005). The aim of the current study is to further explore the role of glutamate mediated withdrawal from alcohol using a rodent model.

According to the World Health Organization (WHO) about 45% of the global population 15 years of age or older has never consumed alcohol (WHO, 2011). Despite a seemingly significant proportion of abstainers, alcohol use is still one of the leading causes of health issues world wide. In the United States alone, 18 million people have been diagnosed with an alcohol use disorder. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines alcohol use disorders as either abusing alcohol or being dependent on alcohol (alcoholism) (2007). Alcohol abuse, according to the Center for Disease Control and Prevention (CDC), is drinking that has negative effects on health, personal relationships or ability to work (2012). Dependence is characterized based on symptoms including craving, loss of control, physical dependence and tolerance (NIAAA 2007). With an average of 2.5 million deaths per year related to alcohol it is clear that more needs to be done to understand what leads to alcoholism in order to prevent it (WHO, 2011).

Modeling Alcoholic Drinking

Many different models of drinking have been created and used in order to better understand specific features of the behavior. An experiment conducted in the 1920s provided rats 25-30 days old with only alcohol and food pellets for several months (Richter, 1926). The animals were given 8, 10 or 16% solutions of ethanol by volume and consumed about 2 grams or 5-8 grams per kilogram of body weight (g/kg). This method failed to induce high concentrations of alcohol in the blood and animals showed no signs of dependence even after months of exposure. Although the rats that had access to ethanol ate less food than rats that only drank water, the animals weighed roughly the same amount. This suggested that rats could have been using ethanol as an additional source of calories.

Another method used to encourage animals to drink is based on a behavior produced by restricting the diet of the animal. In 1961, Falk discovered that rats maintained at 70-80% of their normal body weight increased their water intake during testing sessions by more than three times their normal intake (Falk, 1961). This increase in water consumption specifically occurred after the rats pressed a lever to receive a food pellet. Schedule induced polydipsia has also been used to increase intake of ethanol. One experiment specifically studied the caloric motivations behind drinking alcohol in rats (Holman & Myers, 1968; Meisch & Thompson, 1972). Although rats consumed more ethanol when maintained at a certain percentage below their normal weight, they still did not drink enough to display signs of dependence.

Forced exposure to alcohol using chambers filled with ethanol vapor has achieved much higher blood ethanol concentrations (Rogers, Wiener & Bloom, 1979; Becker & Lopez, 2004). Using this method, animals are exposed to a controlled amount of ethanol vapor for a specific amount of time which often leads to the animal voluntarily drinking more after vapor exposure (Griffin et al., 2009). A draw back to this method, however, is that animals do not voluntarily

drink ethanol, which lowers its translational value when comparing it to how humans consume alcohol.

Using sucrose to increase voluntary ethanol consumption helps to counteract the aversive taste of pure ethanol solution. In a study conducted by Tolliver, Sadeghi, and Samson, rats were trained to lever press for a sucrose reward. As test sessions progressed, ethanol was mixed into the sucrose solutions in increasing concentrations. Rats that previously showed a low preference for ethanol (preference below 25%) showed a significant increase in preference for 10% ethanol after the sucrose fading procedure. Average preference never went above 50% using this method. Although sucrose fading increases preference, the caloric content and change in taste are aspects that make this method less translatable.

Training an animal or using a food reward is not always necessary in order to escalate drinking. When the period of time the animal has access to ethanol is restricted, escalated drinking can be established particularly in C57BL/6J (B6) mice, a strain bred for high preference for ethanol (Rhodes et al., 2005). With this model, referred to as 'drinking in the dark', Rhodes demonstrated that when B6 mice are given access to 20% ethanol solution two or four hours into the dark portion of an alternating light/dark cycle (12hrs a day with lights on, 12hrs a day with lights off), the mice drink enough to reach blood ethanol concentrations (BEC) of around 1mg/ml.

Method	*Author(s)*	*Ethanol Concentration(s) Presented*	*Results*
Forced Drinking	Richter, 1926; Lieber and DeCarli, 1982	8, 10, 16%	Animals consumed 5-8g/kg; 100-150 mg/dl
Schedule-Induced Polydipsia	Holman & Myers, 1968; Meisch & Thompson 1972	3-14, 16, 18, 20%; 2, 4, 8, 16, 32%	Less ethanol was consumed the higher the concentration rose

Forced Inhalation of Ethanol Vapor	Rogers, Weiner & Bloom, 1979; Becker & Lopez, 2004; Griffin et al., 2009	22-25 mg/l of air; 15-20 mg/l of air; 15-20 mg/l	Animals reached BAC of 100+mg/dl; chronic exposure led to escalated drinking later on
Sucrose Fading	Tolliver et al., 1988	10%, 20%	EtOH paired with sucrose increased preference of initially low preference rats
Drinking in the Dark	Rhodes, Best, Belknap, Finn & Crabbe, 2005	20%	BEC >1.0mg/ml

Intermittent Access to Ethanol

Creating an animal model that incorporates all aspects of drinking behavior demonstrated by humans has proven difficult. The models summarized above all cover different aspects of the behavior and result in varying degrees of intoxication. When deciding what model of drinking to use, it is important to incorporate certain criteria. According to Lester and Freed alcohol should be voluntarily orally ingested and the animal should drink enough to produce signs of intoxication for long periods of time and eventually show signs of withdrawal (1973). Selectively breeding rodents for high preferences for ethanol will not necessarily result in these animals drinking enough to become intoxicated therefore paradigms have to be developed to induce escalated drinking.

One model that incorporates voluntary drinking and results in escalated drinking is the intermittent access procedure. Several experiments have used intermittent or every other day access and have produced varying levels of success. In one study, male Wistar rats were split into five groups that each had different combinations of either intermittent or continuous access, free or forced choice, and the use of acclimation prior to access to 20% ethanol. It was found

that schedule of exposure to ethanol was the most significant factor in escalating drinking and that intermittent access produced the most significant increase in ethanol with the longest lasting effects on intake. It also showed that acclimation was unnecessary to get rats to drink 20% ethanol solutions (Wise, 1973).

Intermittent access has also been found to increase preference for ethanol in some Long-Evans rats to levels of rats that were selectively bred for their preference to ethanol over water (Simms et al., 2008). Long-Evans rats have even been trained to press a lever in order to receive 20% ethanol as a reward without the use of sucrose fading and consumed an average of 1.5 g/kg of ethanol in a 30-minute period (Simms et al., 2010). A study done with B6 mice demonstrated that intermittent access to 15% ethanol solution escalates drinking to high and stable levels (Melendez, 2011).

The intermittent access paradigm adapted by Hwa and colleagues (2011) took Wise's model a step further and B6 mice were given intermittent access to 20% ethanol on Mondays, Wednesdays, and Fridays. Male mice consumed on average 20 g/kg/24hr shown below in Figure 1 and after 16 weeks of access showed significant signs of seizure activity during withdrawal from drinking. This paradigm has a relatively high level of external validity due to its voluntary nature and oral administration and is able to induce escalated drinking behavior that leads to signs of dependence. It is therefore the model of drinking that has been chosen for the current study.

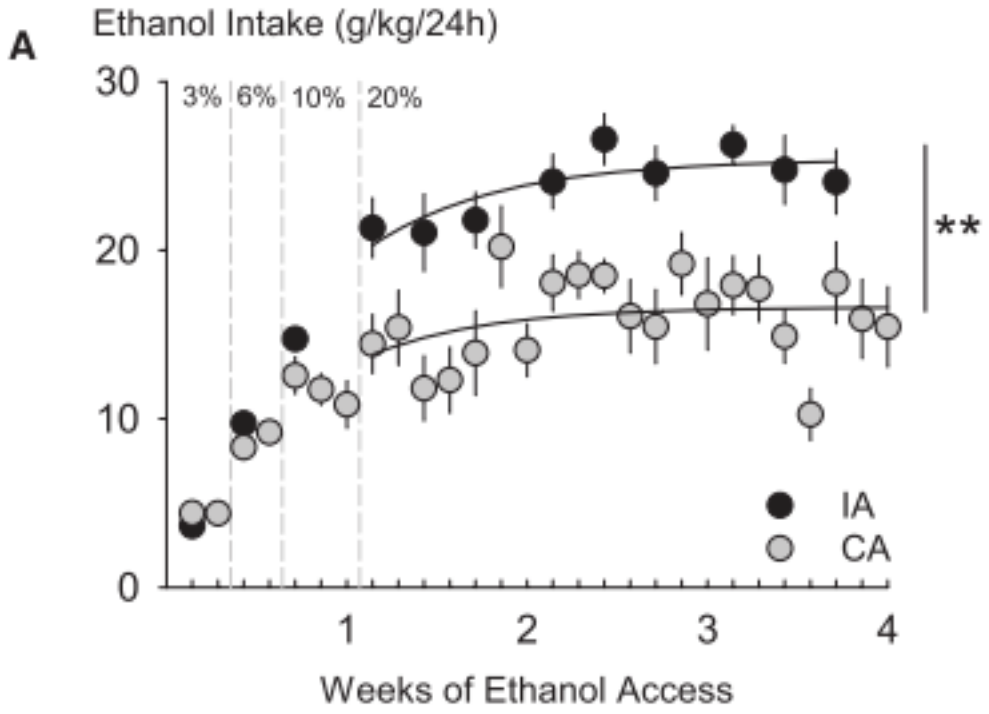

Figure 1 (Hwa et al., 2011) Intake of ethanol in g/kg of body weight of mice given intermittent access (IA) and continuous access (CA) to solution. IA mice drank significantly more 20% ethanol solution than CA mice.

Glutamate and the NMDA Receptor

Glutamate is one of the most abundant excitatory neurotransmitters in the central nervous system (Lovinger, 2008; Spanagel, 2009). Glutamaturgic neurotransmission has been studied in the past in conjunction with conditions such as anxiety, depression and schizophrenia (Conn & Pin, 1997; Skolnick et al., 2009; Coyle, 2006). Glutamate and its relationship to alcohol have also been of interest in the research world. Many studies have been conducted in order to better understand alcohol and its effects on glutamate.

When glutamate is released from the presynaptic terminal it binds to both ionotropic and metabotropic postsynaptic receptors. There are three types of ionotropic receptors that are classified by agonists that have a high affinity to each receptor. The *N*-methyl-D-aspartate

receptor (NMDAR) in particular is an ionotropic, ligand-gated glutamate receptor composed of NR1, NR2 (A-D) and NR3 subunits (Spanagel, 2009). A typical NMDAR is composed of a combination of four of these three different subunits (Johnson & Kotermanski, 2006). The NR2 subunit in particular contains the binding site for glutamate. Because the NMDA receptor is more potently inhibited by alcohol out of all the ionotropic receptors it will therefore be the focus of this study (Thoma et al., 2011; Gass & Olive, 2008; Johnson & Kotermanski, 2006).

Normally, extracellular levels of glutamate in the brain are very low with concentrations of glutamate within cells much higher (Danbolt, 2001). High concentrations of glutamate in the synaptic cleft can be highly toxic because excessive activation of NMDARs is harmful to the brain and can lead to neuronal cell death (Danbolt, 2001; Fadda & Rossetti, 1998). Glutamate uptake by surrounding glial cells keeps extracellular concentrations of glutamate low by quickly removing glutamate from the extracellular space when it is released (Danbolt, 2001).

Glutamate and Alcohol

Although the exact site of action is largely unknown, alcohol has been shown to have varying effects on the NMDA receptor and glutamate depending on history of ethanol exposure and whether or not alcohol is currently in the system. A study conducted by Lovinger, White and Weight demonstrated that when ethanol is acutely administered it inhibits the NMDA receptor very potently at concentrations of ethanol that produce intoxication (1989). This inhibition appears to reduce ion currents produced by NMDA stimulation (Lovinger et al., 1989; Krystal et al., 1998). Different areas of the brain such as the hippocampus, cerebellum, nucleus accumbens, amygdala and ventral tegmental area all had similar reduced NMDA function as a result of acute ethanol exposure (Clapp et al., 2008).

After repeated exposure to ethanol, however, increases in basal extracellular glutamate levels have been found (Melendez et al., 2005). In a study done by Melendez and colleagues rats were given intoxicating doses of ethanol for seven consecutive days. After a 24 hour period of abstinence, rats were again given ethanol and microdialysis was used to determine glutamate levels in the nucleus accumbens. Glutamate in the extracellular region had increased despite a normal expression of GLAST and GLT1 that are responsible for glutamate uptake. This study seemed to point to an inhibition of glutamate uptake in the rats that had repeated exposure to ethanol (2005).

After chronic exposure to alcohol an increase in the number of NMDA receptors as well as a sensitization of these receptors has been found (Tsai & Coyle, 1998; Gass & Olive, 2008). A study conducted by Iorio and colleagues determined that this increase in NMDA receptor function is related to an increase in the number of receptors as opposed to a change in the physical composition of the receptor itself (1992). Despite this increase in receptor expression chronic ethanol exposure has been seen to lower extracellular glutamate levels and raise glutamine levels in humans with alcohol use disorder as well as humans recovering from chronic alcohol use (Thoma et al., 2011). From these studies it appears that actual neuroadaptation takes place in response to ethanol's depressive effects on the brain.

The NMDA Receptor and Withdrawal from Alcohol

Withdrawal symptoms from alcohol are the consequence of the cessation or decrease of regular intake of large quantities of alcohol (Saitz, 1998). Initial symptoms of withdrawal include tremors, anxiety, insomnia, restlessness, and nausea. More serious symptoms seen in 10% of alcohol dependent individuals include a low-grade fever, rapid breathing, tremors,

excessive sweating as well as seizures (Trevisan et al., 1998). Glutamate hyperexcitability may be a cause of these withdrawal symptoms (Tsai & Coyle, 1998).

An experiment conducted by Dahchour and De Witte studied how chronic exposure to ethanol effects glutamate over time. After mice were subjected to 4 weeks of chronic ethanol exposure glutamate was analyzed in the hippocampal region of their brain. Microdialysis was first performed after the initial four weeks of exposure and significantly elevated levels of glutamate were found 8 hours into withdrawal. After another week of chronic ethanol exposure glutamate was again elevated but not as significantly as the first time. A third round of microdialysis was performed after yet another week of chronic ethanol exposure and this time glutamate levels were significantly lower than mice that had no ethanol exposure (1999).

Withdrawal from ethanol is thought to initally result in a hyperexcitability of the NMDA receptor with prolonged withdrawal eventually reducing its expression and functionality (Gass & Olive, 2008). This could be a result of increased levels of glutamate seen in cortical regions of the brain (Lovinger, 2008; Rossetti et al., 1999). The increased number of receptors could also explain the reason for increased sensitivity of NMDARs as well as the seizures seen in severely withdrawan indviduals (Grant et al., 1990).

Multiple episodes of withdrawal have been shown to lead to an increase in the occurrence and a severity of seizures (Becker, Diaz-Granados, & Weathersby, 1997). In one study, the more periods of withdrawal mice experienced, the more severe their seizures would be. The peak of this seizure activity occurred around eight hours into withdrawal for all mice including the mice that had only experienced one period of withdrawal (Becker et al., 1997). In another experiment, mice that received constant exposure to ethanol showed little signs of seizure activity suggesting that experiencing periods of withdrawal has some significant effect on subsequent seizures

(Becker, Diaz-Granados, & Hale, 1997). NMDA antagonists have been shown to decrease the severity and occurrence of seizures due to withdrawal (Grant et al., 1990).

Memantine

Memantine is a drug currently used to help treat Alzheimer's disease and has been used with some success to reduce withdrawal symptoms of patients that have already stopped drinking (Gass & Olive, 2008). It is an uncompetitive NMDA receptor antagonist. Uncompetitive antagonists still allow for some normal neurotransmission while blocking excessive activation of channels (Chen & Lipton, 1997). This characteristic makes them useful for decreasing neurotoxicity that results from ethanol withdrawal (Stepanyan et al., 2008). Memantine is also an open-channel blocker which means it binds preferentially to NMDA receptors that are already activated and open and has little effect on receptors that are closed (Chen & Lipton, 1997; Chen et al., 1992). Because of this agonists, like glutamate, are required in order for memantine to bind to receptors. The more glutamate available to bind, the better memantine can bind.

There are several other drugs that have the same actions as memantine but are not used as widely in treatment because of their negative side effects. What differentiates memantine as a therapeutic drug aside from its ability to allow neurotransmission is thought to be partly due to the way in which memantine binds with NMDA receptors. When memantine binds to open channels, it allows NMDA agonists to unbind from the receptor which causes the receptor to close and trap memantine inside (Chen et al., 1992). An experiment studying the way memantine binds to receptors discovered that when agonists are quickly removed from the receptor therefore trapping memantine within the receptor, some memantine can actually unbind from receptors. This differs from the actions of phencyclidine and MK-108 which are both NMDA antagonists (Blanpied et al., 1997).

Another characteristic of memantine is that it is partly voltage-dependent. At resting potentials NMDA receptors are blocked by magnesium. When a depolarization occurs, glycine and glutamine bind to NMDA receptors and magnesium is then released. This allows for the binding of memantine. NMDA receptors are also highly permeable to calcium and when channels are depolarized, there is a large influx of calcium into cells (Johnson & Kotermanski, 2006). Increased calcium levels and NMDA toxicity have been linked (Ahern, Lustig & Greenberg, 1994).

Aggression

Aggression and its relationship with neurotransmitters such as serotonin and GABA have been studied rather extensively but glutamate and its role in aggression is not as well understood and has had very different results depending on the drug used and the brain site targeted. The hypothalamus is one brain region that has been implicated in mediating aggression (Haller, 2012). In one study, glutamate was injected directly into the hypothalamus of cats. The result was heightened aggression that was comparable to direct electrical stimulation of the hypothalamus (Brody, DeFeudis & DeFeudis, 1969). In another experiment the NMDA antagonists PCP, dizolcipine and memantine were administered via intraperitoneal injection and although there was a trend towards increased aggression, the change in aggression was not significant (Belozertseva & Bespalov, 1999).

Aggression and its relationship to alcohol has been an area of interest because of alcohol's ability to increase aggression in both animals and humans (Miczek et al., 1992; Giancola et al., 2009). In one study, alcohol increased aggression in both men and women but had a more profound effect on men (Giancola et al., 2009). In studies done with CFW mice alcohol had differing effects on aggression. Alcohol was found to both increase aggression and

decrease aggression. Heightened aggression seemed unrelated to the actual amount of alcohol in the blood stream and appeared to be a matter of individual differences (Miczek et al., 1998).

Previous Research on the NMDAR, Withdrawal and Aggression

A recent study examined the relationship between alcohol, the NMDA receptor and aggression. Male Swiss Webster mice were trained to self-administer ethanol and once this behavior was reliably established aggression testing began. After mice ingested 1g/kg of ethanol memantine was administered prior to aggression testing via intraperitoneal injection at the doses 0mg/kg (vehicle – distilled water), 1mg/kg, 3mg/kg, 10mg/kg and 17mg/kg. It was found that the 1mg/kg and 3mg/kg doses of memantine increased aggression in mice that had ingested ethanol.

Previous research has been conducted on aggression related to alcohol but few studies have studied aggression during withdrawal from alcohol. A study was conducted that explored the relationship between the NMDA receptor and aggression during withdrawal from ethanol after 8-10 weeks of intermittent ethanol exposure. Outbred Carworth Farm Webster male mice were given access to 20% ethanol solution on the schedule previously outlined by Hwa and colleagues (2011). Aggression was assessed 8 hours into withdrawal from ethanol and memantine was administered via intraperitoneal injection 20 minutes prior to aggression testing. The doses administered were vehicle (distilled water), 3mg/kg, 5mg/kg, 10mg/kg and 30mg/kg. It was found that the 5mg/kg dose of memantine significantly increased aggression in mice that had consumed alcohol compared to mice that drank only water (Nathanson et al., *unpublished*). A similar experiment using C57BL/6J mice instead of CFWs also observed aggression during withdrawal from ethanol at 8-10 weeks after intermittent access to alcohol. Conversely, memantine decreased aggression in mice that had consumed ethanol (Dodman et al.,

unpublished).

Objective

The purpose of the current study was to expand upon previous work that examined the role of the NMDA receptor and aggressive behavior of mice during withdrawal after 8-10 weeks of exposure to ethanol via the intermittent access paradigm. Specifically, the current study examined aggression during withdrawal after 1-2 weeks exposure to ethanol and 4-5 weeks exposure in conjuncture with several doses of memantine. Previous work has shown that low doses of memantine heighten aggression after several weeks of exposure to ethanol and that by 11 weeks, mice begin to show signs of seizure activity due to withdrawal. We would therefore expect to find either a trend towards a significant increase in aggression as time passed or a more specific time point at which aggression heightens. This is the first experiment conducted that compares aggression during withdrawal over time.

Methods

Subjects

Adult, male Swiss Carworth Farm Webster (CFW) mice were received from Charles River Laboratories International Inc. (Wilmington, MA), weighing 21-23 g upon arrival. Forty-three mice were individually housed in polycarbonate cages (28 x 17 x 12 cm) with stainless steel wire mesh lids and pine shaving bedding. They were given at least two weeks to habituate to vivarium conditions that consisted of a 12-hour reversed light/dark cycle (lights off at 7 AM) with constant temperature (21 ± 2º Celsius) and humidity (25%). Thirty-one mice were assigned the experimental role of resident and were given intermittent access to alcohol. Twelve mice were also assigned the role of resident but were allocated to the control group and were given only water for the entirety of the experiment. Intruder mice that were used to instigate

aggression were housed in groups of 8 to11 in 46 x 24 x 16 cm polycarbonate cages with stainless steel wire mesh lids and corncob shavings used as bedding. Intruders were younger and smaller than resident mice. Standard rodent chow (LabDiet 5001 Rodent Diet, PMI Nutrition International, Brentwood, MO) and tap water were provided to the animals at all times under all conditions. All experimental procedures were approved by the Tufts University Institutional Animal Care and Use Committee and were in accordance with the NIH Guide for Care and Use of Laboratory Animals.

Initial Aggression Assessment

After animals were habituated to vivarium conditions, the forty-three singly housed mice classified as residents were assessed for aggressive behavior using the resident-intruder procedure detailed by Miczek and O'Donnell (Miczek & O'Donnell, 1978). A group housed intruder mouse was first placed in a perforated container and placed in the home cage of one of the residents for a maximum of five minutes. This was done to socially instigate and agitate the resident mouse (Fish, Faccidomo, & Miczek, 1999). After five minutes the container was removed and a new intruder was promptly placed directly into the home cage of the resident. Aggressive behavior by the resident toward the intruder was then observed. After this five-minute period, if no attack bite was delivered, the intruder was removed from the cage and the screening session ended. Otherwise, the session terminated five minutes after the first attack bite. The latency to the first attack bite and the number of attack bites were recorded during each session. Some animals that failed to attack within the five-minute period during the first five days of screening were then given up to 10 minutes to initiate the first attack bite. Three residents that failed to attack intruders during the screening sessions were not included in the

experiment. Intruders were paired with the same resident for each session unless intruders displayed aggressive behavior in which case they were replaced and were not used again.

Ethanol Intake Procedures

Three to four days prior to the presentation of alcohol, resident animals were provided with two 50 mL tubes (Nalgene) with no. 5 rubber stoppers (Fisher Scientific, Agawam, MA) containing stainless steel ball-bearing sipper (Ancare Corp., Bellmore, NY) filled with tap water in order to acclimate the animals to drinking from the sipper tubes. 20% w/v ethanol solutions were then prepared using tap water and 95% ethyl alcohol (Pharmaco-AAPER, Brookfield, CT). Drinking tubes were held in the wire mesh cage lid and given to mice 3 hours into their dark cycle (10 AM). Bottles were weighed to the nearest hundredth of a gram 24 hours after presentation in order to determine grams of fluid consumed. A drip cage was also set up without an animal in order to ascertain loss of fluid due to accidental spillage or evaporation. The drip data was averaged weekly and subtracted from the animals' intake. Animals were weighed every other day in order to calculate the gram per kilogram intake of ethanol.

Intermittent Access to Alcohol

Residents had intermittent access to alcohol (IAA) using the methods detailed by Hwa and colleagues (2011). Mice were given a daily choice of two bottles to drink from. On Mondays, Wednesdays and Fridays one bottled contained a 20% w/v ethanol solution and the other contained tap water. On Tuesdays, Thursdays, Saturdays and Sundays, the ethanol was replaced with tap water. Ethanol was presented for 24 hours and would then be removed, weighed and replaced by water. Ethanol bottles were rinsed thoroughly to ensure no ethanol remained. In order to avoid side preferences, the position of the ethanol bottle on the cage lid

was alternated on days that ethanol was presented. The control group was presented with water in both bottles every day.

Aggression During Withdrawal

After four or five days of exposure to ethanol, the aggressive behavior of 31 IAA and 12 control mice was assessed during the withdrawal period from alcohol in conjunction with memantine. Animals were tested at the peak of withdrawal as indicated by Hwa and colleagues (2011) eight hours after ethanol was removed from the cage. The aggression testing protocol during withdrawal was similar to the one used to screen the mice initially for aggression however no social instigation occurred before the intruder was placed directly into the home cage of the resident. The latency period to the first attack bite and the number of subsequent attack bites was measured and each session was recorded using a JVC Everio GZ-MG670 digital camera in order to later observe the sessions using Observer XT 9.0 software (Noldus; Wageningen, The Netherlands) for additional aggressive and non-aggressive behaviors of the resident. Aggressive behaviors analyzed included sideways threat, tail rattling, attack bites, and pursuit of the intruder. Non-aggressive behaviors included self-grooming, rearing, walking, and non-aggressive contact with the intruder mainly consisting of anonasal contact.

Pharmacological Manipulations

Four days prior to testing mice during withdrawal from ethanol, the 43 resident mice were given intraperitoneal (IP) injections of distilled water (dH_2O) to habituate them to the procedure. Memantine was purchased from Sigma-Aldrich (St. Louis. MO) and was mixed with dH_2O, known as the vehicle, to create 1.0mg/mL, 0.5mg/mL and 0.3mg/mL solutions. Each mouse was weighed and injected with either 0.1mL of drug per 10 grams of body weight or the equivalent volume of vehicle. Dosages were given in a random order with 10.0mg/kg being the

last dose given to each mouse. Injections were given 20 minutes prior to assessing their aggression during withdrawal.

Handling-Induced Convulsions

After seven to eight weeks of exposure to alcohol severity of withdrawal was assessed. This was done using the methods detailed by Goldstein (1972) in which physical signs of withdrawal are measured by assessing seizure activity. Thirty-one IAA and twelve water control mice were lifted by the tail and handling-induced convulsions (HIC) were graded on a scale from 0 to 4 (0 = no withdrawal signs; 1 = tonic convulsions induced by gently turning the mouse 180º; 2 = tonic convulsions when lifted without turning or tonic-clonic convulsions when turned 180º; 3 = tonic-clonic convulsions without turning the mouse; 4 = violent tonic-clonic convulsions when lifted that often continue when mouse is placed back in cage). Scores correlate with the severity of withdrawal. HIC scores were measured every two hours over a ten-hour period starting at 0 hours after ethanol was removed from the cage and replaced with water. This procedure was done twice with a day of ethanol access in between for IAA mice. The first round of HIC scores, no drug was administered. The second time HIC scores were measured, memantine was administered at 8 hours into withdrawal at the 5mg/kg dose to determine if memantine had an effect on withdrawal.

Statistical Analysis

Statistical analyses were performed with SigmaStat 11.0 (Systat Software, San Jose, CA). Multiple two-way, repeated measures ANOVAs were conducted to determine the effect of memantine on both aggressive and non-aggressive behaviors between the ethanol drinking and water drinking groups after 1-2 weeks exposure to ethanol. Post-hoc Bonferroni t-testing was

used if any main effects or interactions were found to be significant ($p<.05$). The same analysis was also conducted examining behaviors after 4-5 weeks exposure to ethanol.

In order to compare aggressive and non-aggressive behaviors within the ethanol-drinking group of mice, cumulative consumption was calculated for each mouse by totaling the amount of ethanol consumed over 16 days of exposure. Mice that consumed less than 100g/kg ethanol over four weeks were placed in the low consumption group (n=11) and mice that consumed more than 200g/kg ethanol were placed in the high consumption group (n=14). These groups were then compared using multiple two-way, repeated measures ANOVAs to determine the effect of memantine on aggressive and non-aggressive behaviors. Comparisons were done to examine 1-2 weeks ethanol exposure and 4-5 weeks exposure separately. Post-hoc Bonferroni t-testing was used if any main effects or interactions were found to be significant ($p<.05$).

In order to determine if time had an effect on aggressive behavior multiple one-way, repeated measures ANOVAs were conducted to analyze the behavior of all mice included in the experiment at the vehicle dose of memantine comparing 1-2 weeks exposure to ethanol and 4-5 weeks. Post-hoc Bonferroni t-testing was used if any main effects or interactions were found to be significant ($p<.05$). Multiple two-way, repeated measures ANOVAs were conducted to compare aggressive behavior at 1-2 weeks exposure and 4-5 weeks exposure between the ethanol drinking group of mice and the water drinking group of mice at the vehicle dose of memantine. Post-hoc Bonferroni t-testing was used if any main effects or interactions were found to be significant ($p<.05$).

Results

Ethanol Intake

Total ethanol intake of each of the individual 31 IAA mice was measured after 16 days of exposure to ethanol. Intake of each mouse was graphed and high and low intake groups were determined (See Figure 2A). Mean ethanol consumption was 245.92 ± 8.26 g/kg for the high intake group of mice and 71.40 ± 7.34 g/kg for the low intake group.

Behavior of EtOH v. Water-Drinking Mice – 1-2 Weeks

The aggressive and non-aggressive behavior of 31 ethanol-drinking and 12 water-drinking mice was analyzed in conjunction with different doses of memantine. Multiple two-way, repeated measures ANOVAs revealed that after 1-2 weeks exposure to ethanol or water a significant interaction was found between dose and drinking condition for the duration [$F(3,121)=6.658$, $p<.001$] and frequency [$F(3,121)=7.994$, $p<.001$; Fig. 3] of the resident mouse pursuing the intruder. The water-drinking group of resident mice pursued the intruder for longer and more times than the ethanol-drinking group of mice. Post-hoc Bonferroni t-tests indicated that the ethanol-drinking group had a significantly lower baseline pursuit frequency [$t=3.223$, $p<.05$] and duration [$t=3.150$, $p<.05$] at the vehicle dose than the water group and memantine at the 3mg/kg dose significantly increased pursuit frequency [$t=4.889$, $p<.05$] and duration [$t=4.724$, $p<.05$] of the water-controls compared to the ethanol-drinking mice. At the 5mg/kg [$t=2.528$, $p<.05$] and 10mg/kg [$t= 2.953$, $p<.05$] doses of memantine water-controls pursued intruders significantly less than at the vehicle dose.

A significant main effect of drinking type was found on the duration [$F(1,121)=5.327$, $p<.05$] and frequency [$F(1,121)=4.582$, $p<.05$] the resident tail rattled. Water control mice displayed these behaviors more than IAA mice. Post-hoc t-tests indicated that the control mice tail rattled significantly longer than the ethanol-drinking mice specifically at 3mg/kg [$t=2.729$, $p<.05$] and 5mg/kg [$t=2.171$, $p<.05$] of memantine. There was also a significant difference in the

number of tail rattles displayed between the water-drinking and ethanol-drinking mice at the 3mg/kg [t=2.729, p<.05] and 5mg/kg [t=2.171, p<.05] doses of memantine.

A significant interaction between drinking group and dose of memantine was found on the duration of the non-aggressive behaviors rearing [F(3,121)=6.588, p<.001; Fig. 4a] and walking [F(3,121)=3.101, p<.05; Fig. 4b]. Water control mice performed both behaviors longer than ethanol-drinking mice. The t-test revealed that memantine significantly increased rearing at the 5mg/kg [t=3.433, p<.05] and 10mg/kg [t=4.596, p<.05] dose but no specific dose significantly affected walking duration compared to vehicle. The 5mg/kg [t=3.087, p<.05] and 10mg/kg [t=4.502, p<.05] doses of memantine also significantly increased rearing duration within the water-drinking group of mice from baseline levels.

A significant main effect of drinking type was found on resident-intruder contact duration [F(1,121)=5.827, p<.05; Fig. 4c]. Ethanol-drinking resident mice spent more time in contact with the intruder mice than control mice. The post-hoc analysis revealed that the ethanol-drinking and water-drinking groups had significantly different baselines for the contact duration behavior at the vehicle dose [t=2.427, p<.05] of memantine. There was also a significant difference between groups at the 3mg/kg [t=2.291, p<.05] dose of memantine.

Sideways threat duration and frequency, attack bite duration and frequency, self-grooming duration and frequency, rearing frequency, walking frequency, and contact frequency behaviors were all analyzed as well but no significant effect of memantine or drining group was found.

Behavior of EtOH v. Control Mice – 4-5 Weeks

Multiple two-way, repeated measures ANOVAs were conducted to analyze aggressive and non-aggressive behavior of the same group of resident mice after 4-5 weeks exposure to

ethanol or water. Significant main effects of drinking group [$F(1,116)=10.671$, $p<.01$] as well as dose of memantine [$F(3,116)=4.171$, $p<.01$] were found for sideways threat duration. Drinking group [$F(1,116)=8.294$, $p<.01$] and dose of memantine [$F(3,116)=4.282$, $p<.01$] were also found to have main effects on bite frequency. Control mice displayed the sideways threat behavior and bit intruders more frequently than IAA mice. Further analysis revealed the vehicle [$t=3.541$, $p<.05$], 3mg/kg [$t=2.715$, $p<.05$], and 5mg/kg [$t=2.026$, $p<.05$] doses of memantine had a significant effect on sideways threat duration (See Figure 5a). Within the IAA group of mice memantine significantly increased sideways threat duration at the 3 g/kg [$t=3.100$, $p<.05$] and 5 g/kg [$t=2.973$, $p<.05$] doses of memantine.

A significant difference in baseline values for bite frequency was found between the ethanol-drinking and water-drinking mice at the vehicle [$t=2.904$, $p<.05$] dose of memantine. The 3mg/kg [$t=2.994$, $p<.05$], and 5mg/kg [$t=2.293$, $p<.05$] were both found to decrease the number of attack bites of ethanol-drinking mice significantly (See Figure 5b).

Significant main effects were also found of drinking condition [$F(1,116)=8.468$, $p<.01$] and dose of memantine [$F(3,116)=3.319$, $p<.05$] on tail rattle duration. Water control mice tail rattled longer and more frequently than ethanol-drinking mice. The post-hoc test revealed that there was a significant difference in baseline tail rattling duration [$t=3.142$, $p<.05$] and tail rattling frequency [$t=2.805$, $p<.05$] behaviors at the vehicle dose of memantine, respectively. The 3mg/kg dose showed a significant trend toward increasing duration [$t=3.028$, $p<.05$; Fig. 5c] and frequency [$t=2.284$, $p<.05$] of tail rattles for the water-drinking group a significant increase in tail rattle frequency [$t=2.597$, $p<.05$] in the ethanol-drinking group of mice.

Analysis of rearing revealed significant main effects of both drinking condition [$F(1,116)=13.664$, $p<.001$] and dose of memantine [$F(3,116)=2.820$, $p<.05$] on rearing duration.

Water control mice reared longer on average than IAA mice. The post-hoc analysis revealed a significant effect of memantine on rearing duration at the 3mg/kg [t=3.719, p<.05], 5mg/kg [t=3.018, p<.05], and 10mg/kg [t=3.048, p<.05] doses. There was a significant main effect of dose on walking duration [F(3,116)=4.661, p<.05]. On average, water control mice walked for a longer time during the confrontation than ethanol-drinking mice. Further analysis did not reveal significant differences of memantine between doses on duration. A significant main effect of drinking condition was found on contact duration. Ethanol-drinking mice spent more time in contact with intruder mice than water controls. Further analysis revealed a significant difference in baseline behavior of walking duration at the vehicle dose of mememantine [t=3.555, p<.05]. At the 3mg/kg [t=3.314, p<.05], 5mg/kg [t=2.088, p<.05], and 10mg/kg [t=2.876, p<.05] doses of memantine this significant increase in contact duration in ethanol-drinking mice remained stable.

Pursuit duration and frequency, sidways threat frequency, attack bite duration, rearing frequency, walking frequency, self-grooming duration and frequency and contact frequency were all also analyzed but no significant effects of dose of memantine or drinking-group was found.

Behavior of High v. Low EtOH Intake Mice – 1-2 Weeks

Multiple two-way, repeated measures ANOVAs were conducted to determine the effect of total ethanol intake and dose of memantine on both aggressive and non-aggressive behaviors after 1-2 weeks exposure to ethanol. A significant main effect of total ethanol intake was found on duration of the self-groom behavior [F(1,67)=6.605, p<.05; Fig. 7]. Mice in the high ethanol intake group of mice groomed longer on average than mice in the low intake group. The t-test revealed that ethanol drinking mice had a significantly lower baseline for the self-groom

behavior than water-drinking mice after being injected with the vehicle dose of memantine [t=3.177, p<.05].

Behavior of High v. Low EtOH Intake Mice – 4-5 Weeks

Two-way, repeated measures ANOVAs were conducted to determine the effect of total ethanol intake and dose of memantine on both aggressive and non-aggressive behaviors after 4-5 weeks exposure to ethanol. A significant main effect of dose was found on pursuit frequency [F(3,65)=2.896, p<.05] and sideways threat frequency [F(3,65)=3.443, p<.05; Fig. 8]. A t-test revealed that the 5mg/kg dose of memantine significantly increased the frequency the low intake group of mice pursued intruders [t=2.461, p<.05]. The t-test also revealed that sideways threat frequency was significantly increased in the low intake group of mice at the 5 mg/kg dose of memantine [t=3.050, p<.05].

For sideways threat duration [F(3,65)=3.312, p<.05], attack bite frequency [F(3,65)=2.690, p<.05] and duration [F(3,65)=2.869, p<.05], and tail rattle frequency [F(3,65)=3.003, p<.05], there was a significant main effect of dose overall but post-hoc analysis did not reveal any significant effects of the differing doses of memantine on any of these aggressive behaviors. There was an overall significant main effect of dose [F(3,65)=3.946, p<.05] on contact frequency but pot-hoc analysis did not reveal any specific dose as being different from vehicle.

Behavior of All Mice Over Time – 1-2 v. 4-5 Weeks

One-way, repeated measures ANOVAs were conducted to determine if there was a change in behavior of mice due to the time of testing. Comparing all mice at the vehicle dose of memantine, a significant main effect of time was found for tail rattle duration [F(1,37)=5.037, p<.05]. Tail rattle duration increased over time from 1-2 weeks ethanol exposure to 4-5 weeks

exposure. Post-hoc analysis revealed a significant increase in tail rattle duration [t=2.194, p<.05].

Behavior of EtOH v. Control Mice – 1-2 v. 4-5 Weeks

Multiple two-way, repeated measures ANOVAs were performed to determine if drinking condition or time course had an effect on aggressive behaviors. A significant main effect of drinking condition was found on pursuit duration [F(1,36)=7.469, p<.01]. Water control mice pursued intruders for a longer time than ethanol-drinking mice. The t-test was conducted and revealed that duration was significantly higher for water control mice after 1-2 weeks exposure to ethanol than after 4-5 weeks. Pursuit frequency was similarly higher for water control mice than ethanol-drinking mice. A significant main effect of drinking condition [F(1,36)=8.762, p<.01] was found with post-hoc analysis resulting in a significant effect of time on pursuit frequency. Water control mice pursued intruders significantly more frequently after 1-2 weeks than 4-5 weeks [t=3.196, p<.05].

A significant main effect of drinking condition was also found on sideways threat duration [F(1,36)=9.643, p<.01] and frequency [F(1,36)=6.921, p<.05] and attack bite duration [F(1,36)=65.374, p<.05] and frequency [F(1,36)=9.161, p<.01; Fig. 9]. In each case, water control mice performed the behavior longer and more frequently than IAA mice. T-tests revealed that all behaviors of water-drinking mice were significantly increased over time (Sideways Threat Duration [t=3.241, p<.05], Sideways Threat Frequency [t=2.637, p<.05], Bite Duration [t=2.420, p<.05], Bite Frequency [t=3.114, p<.05]).

A significant interaction was found between drinking condition and time on duration [F(1,36)=4.391, p<.05] and frequency [F(1,36)=5.961, p<.05] of tail rattles displayed by the mice. Water control mice tail rattled longer and more frequently than IAA mice. Post-hoc

analysis revealed the difference in tail rattle duration between ethanol-drinking mice and water-drinking mice during the 1-2 week [t=2.045, p<.05] testing period and 4-5 week [t=4.003, p<.05] testing period was significantly different. There was also a significant increase found between testing times within the water-drinking group of mice for duration of tail rattling [t=3.002, p<.05]. T-tests also revealed that time significantly increased the number of times water control mice tail rattled compared to IAA mice [t=3.372, p<.05]. Water-drinking mice significantly tail rattled more at 4-5 weeks drinking than when they were tested at 1-2 weeks [t=3.292, p<.05]. There was a significant difference found within the water-drinking group of mice. Water-drinking mice tail rattled significantly more times during the 4-5 week testing period than at 1-2 weeks [t=3.292, p<.05].

HIC Scores

HIC scores were recorded for the 31 IAA and 12 control mice after about 7-8 weeks of exposure to ethanol or water. Median HIC scores for all groups was a score of zero. Because of this lack of variability no further statistical analysis was performed.

Discussion

The intermittent access paradigm used by Hwa and colleagues (2011) to escalate voluntary drinking of ethanol in C57BL/6J mice was used in the current study to attempt to escalate drinking in outbred CFW mice. As expected, this resulted in varying success. Ethanol intake of the 31 ethanol-drinking mice ranged from around 25g/kg to 300g/kg over 16 days of exposure to ethanol. This broad range of individual differences in the amount of ethanol ingested is not surprising due to the higher variability of the behaviors of outbred mice. Similar results were found in a study conducted by Nathanson and collegues (*unpublished*) in which mice were given intermittent access to ethanol for 31 days (See Figure 2B). Preference for

ethanol versus water never stably reached above 50% even after 5 weeks of intermittent access to alcohol. Similar results were found in the study by Nathanson and colleagues. Ethanol-drinking mice did not reach preference levels above 50% even after 10 weeks of intermittent access (*unpublished*).

Handling-induced convulsions (HIC) scores also provided some insight into the effects of intermittent access on withdrawal in CFW mice. HIC scores measured after about seven weeks exposure to ethanol were not significantly different than the scores produced by water-drinking mice. In the study done by Nathanson and colleagues, however, CFW mice began to show signs of seizure activity after about 11 weeks IAA (*unpublished*). A similar study with C57BL/6J mice found that after about 11 weeks IAA HIC scores were heightened in ethanol-drinking mice versus water controls (*unpublished*). These seizures are thought to be a result of the hyperexcitability of the central nervous system which accompanies withdrawal (Becker, 2008; Dachour & DeWitte, 2003). Seizures due to withdrawal from alcohol are on the more severe end of the spectrum of withdrawal symptoms, however, and it is possible that an individual is experiencing withdrawal without showing signs of seizure activity. It is therefore possible that the CFW mice in the current experiment could have experienced other symptoms of withdrawal that are less severe than seizures and harder to detect.

Aggressive and non-aggressive behavior was also assessed in relation to withdrawal from ethanol and interestingly water-drinking mice appeared to be more aggressive than ethanol-drinking mice. After 1-2 weeks of exposure to ethanol, the 31 IAA mice pursued intruders and tail rattled less than water-control mice but at stable levels over all doses of memantine. Memantine appeared to have no effect on aggression in the IAA mice. For tail rattling behavior ethanol-drinking mice and water-drinking mice had more similar baselines of behavior. At

3mg/kg and 5mg/kg memantine appeared to increase tail-rattling behavior in water-drinking mice. However, because these doses did not increase tail-rattling behavior significantly from the vehicle dose, this suggests that perhaps ethanol or withdrawal had depressive effects on tail rattling in the ethanol-drinking group.

Neither withdrawal nor memantine appeared to have lasting effects on locomotor activity as walking duration was very similar between the ethanol-drinking and water-drinking groups of mice. Memantine increased rearing behavior, however, in water-drinking mice at the 5mg/kg and 10mg/kg doses compared to ethanol-drinking mice and the vehicle dose. This suggests that memantine increased rearing and that withdrawal depressed this increase in ethanol-drinking mice. Time spent in contact with the intruder was the only behavior that ethanol-drinking mice did significantly more than water-drinking mice. After 1-2 weeks of drinking time, it seems water-control mice spent less time investigating intruders and more time displaying signs of aggression than ethanol-drinking mice. Interestingly, the actual number of attack bites between groups was not significantly different. Thus it appears that after 1-2 weeks of ethanol exposure there are not many differences between the two groups. This could be because the permanent effects of chronic ethanol exposure are only beginning to take place at this stage.

After 4-5 weeks of exposure to ethanol a significant difference in baseline appears for several aggressive behaviors. This suggests that ethanol or withdrawal, not memantine is affecting the aggressive behavior of IAA mice. It appears that ethanol exposure or withdrawal from intermittent ethanol access is suppressing aggressive behavior. In order to better determine if the exposure time is what is suppressing aggressive behavior or if withdrawal from ethanol is what is affecting this difference in behavior between groups, aggression should be tested when ethanol is still in the body of the mice. This can then be compared to animals during withdrawal.

Similar to the results seen after 1-2 weeks of drinking time water-drinking mice reared significantly more than IAA mice and memantine seemed to increase this behavior in the water-drinking mice compared to the ethanol-drinking mice. At the 3mg/kg dose in particular memantine increased rearing duration in water control mice significantly more than the vehicle dose. Walking duration between the two groups was not significantly different but a significant difference in baseline was found for contact with intruders between the two groups. Once again ethanol-drinking mice spent significantly more time non-aggressively contacting intruders than water-control mice.

A previous experiment using CFW mice also studied aggressive and non-aggressive behaviors during withdrawal after 8-10 weeks of exposure to ethanol. In that study, conducted by Nathanson and colleagues (*unpublished*), a preliminary look at aggression after 4-5 weeks of drinking was done without the use of memantine. Mice that were exposed to ethanol had no significant differences in aggressive behavior than mice that only drank water. It was suggested that perhaps this lack of difference could have been due to the smaller number of mice used in the control group and the high variability in their aggressive behavior within the group versus the ethanol group.

The ethanol-group in Nathanson's study was also found to have higher locomotor activity than the water-control group and it was believed to be due to withdrawal and accompanying glutamate hyperexcitability (*unpublished*). The difference in baseline aggression between groups found in the current study seems to suggest that a cause other than memantine treatment was affecting aggressive behavior. It is possible that the mice in the current experiment were not experiencing the same hyperexcited state that the mice in Nathanson's study did (*unpublished*). It is also possible that a batch effect occurred and that the small number of water-drinking mice

could have been a factor in this experiment as well. Adding another batch of water-drinking animals in the future could be useful.

Nathanson and colleagues (*unpublished*) found that after 8-10 weeks exposure to ethanol aggression during withdrawal was heightened in the ethanol-drinking mice compared to water-drinking mice. Memantine also only had an effect on aggression in the mice that consumed high levels of ethanol compared to animals that consumed low levels. This was significant at the 5mg/kg dose of memantine. Memantine was also found to increase aggression in the water-drinking animals but only at the 3mg/kg dose of memantine. Increased locomotor activity in the ethanol group as well as HIC scores higher than the water control group indicated that CFW mice were experiencing glutamate hyperexcitability due to withdrawal.

A trend seems to appear by 4-5 weeks of exposure in which as mice ingest progressively more ethanol, memantine has less of an effect in heightening aggression. In terms of non-aggressive behavior after 4-5 weeks of ethanol exposure, both high and low intake groups seemed to display similar behaviors. Comparing behavior of ethanol-drinking and water-drinking mice over the two test sessions also shows a slight increase in aggressive behavior of water control mice from 1-2 weeks to 4-5 weeks water drinking. The difference for most aggressive behavior is not significant within the water-drinking group but is significantly higher than the ethanol-drinking group by the second testing session. This also seems to support the idea that withdrawal from ethanol seems to have some depressive effect on aggression.

The difference in aggressive behavior found in CFW mice after 4-5 weeks of drinking ethanol and 8-10 weeks drinking ethanol suggests that, either progressively or at a specific time, aggression of the ethanol withdrawn mice heightens. CFW mice showed a lack of seizure activity after only 4-5 weeks of drinking. When examining this lack of seizure activity in

conjunction with ethanol-drinking mice displaying less aggressive behavior than water-drinking mice, a hyperexcited state was probably not reached. Because memantine requires glutamate present in order to bind to and open NMDA channels, perhaps lower levels of extracellular glutamate prevented memantine from binding and therefore prevented any heightened aggression due to memantine (Fadda & Rossetti, 1998). The time period from 4 weeks to 10 weeks ethanol exposure should be studied in greater depth in order to determine when exactly aggression heightens.

One drawback to this study was that the same mice were used for testing aggression at both 1-2 and 4-5 weeks exposure to ethanol. Because of this, the results obtained at the 4-5 week mark could have been affected by prior experience with memantine at the 1-2 week mark. In the future, different mice should be used for just testing aggression after 4-5 weeks exposure to ethanol. In order to better understand the role of the NMDA receptor in mediating aggression during withdrawal, receptor expression should be assessed at the three different time points and glutamate levels should also be looked at to determine how these two things could be affecting aggression.

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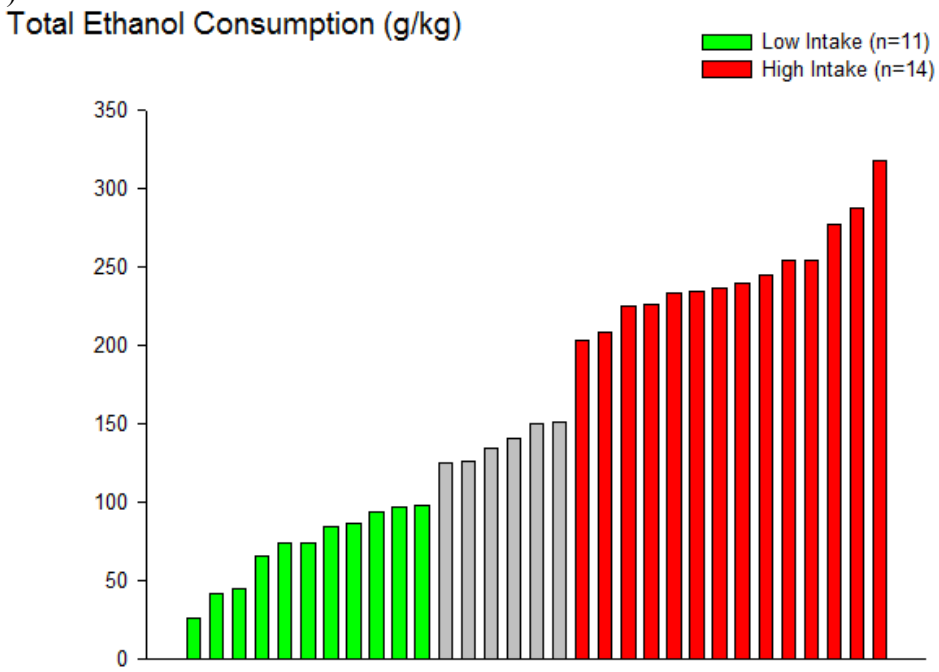
Figure 2. Total ethanol consumption of individual IAA mice. A) Individual intake of IAA mice after 16 days access to ethanol. Mice were grouped into high intake and low intake groups based on their individual total consumption. B) Individual intake of IAA mice after 31 days access to ethanol.

A)



B)

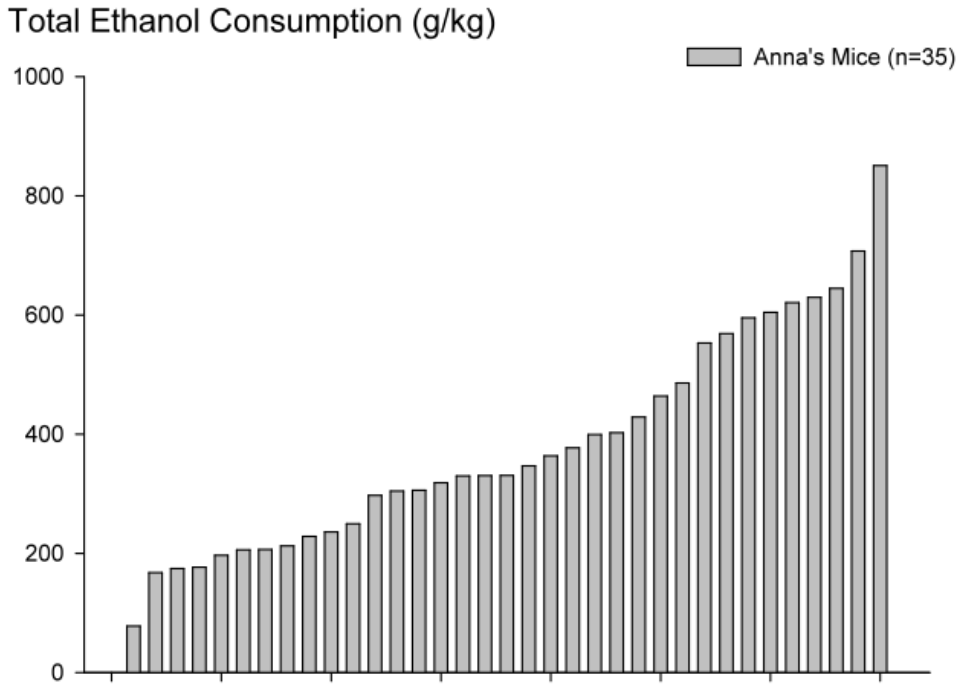


Figure 3. The effect of memantine treatment on aggressive behavior during withdrawal from ethanol in IAA and water control mice after 1-2 weeks of drinking. The effect of memantine treatment on pursuit duration in mice with intermittent access to ethanol and water control mice 8 hours into the ethanol withdrawal period. Pound signs indicate significance between groups ($p<.05$).

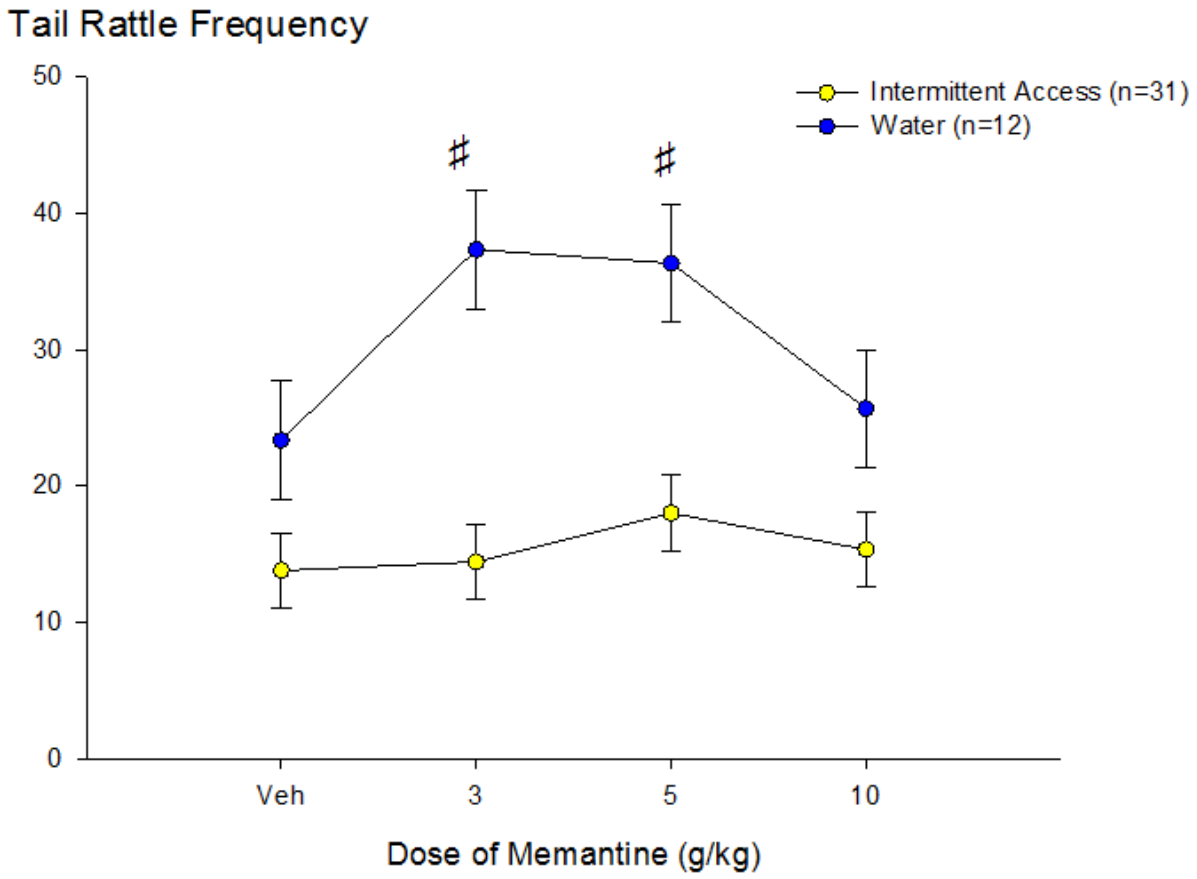
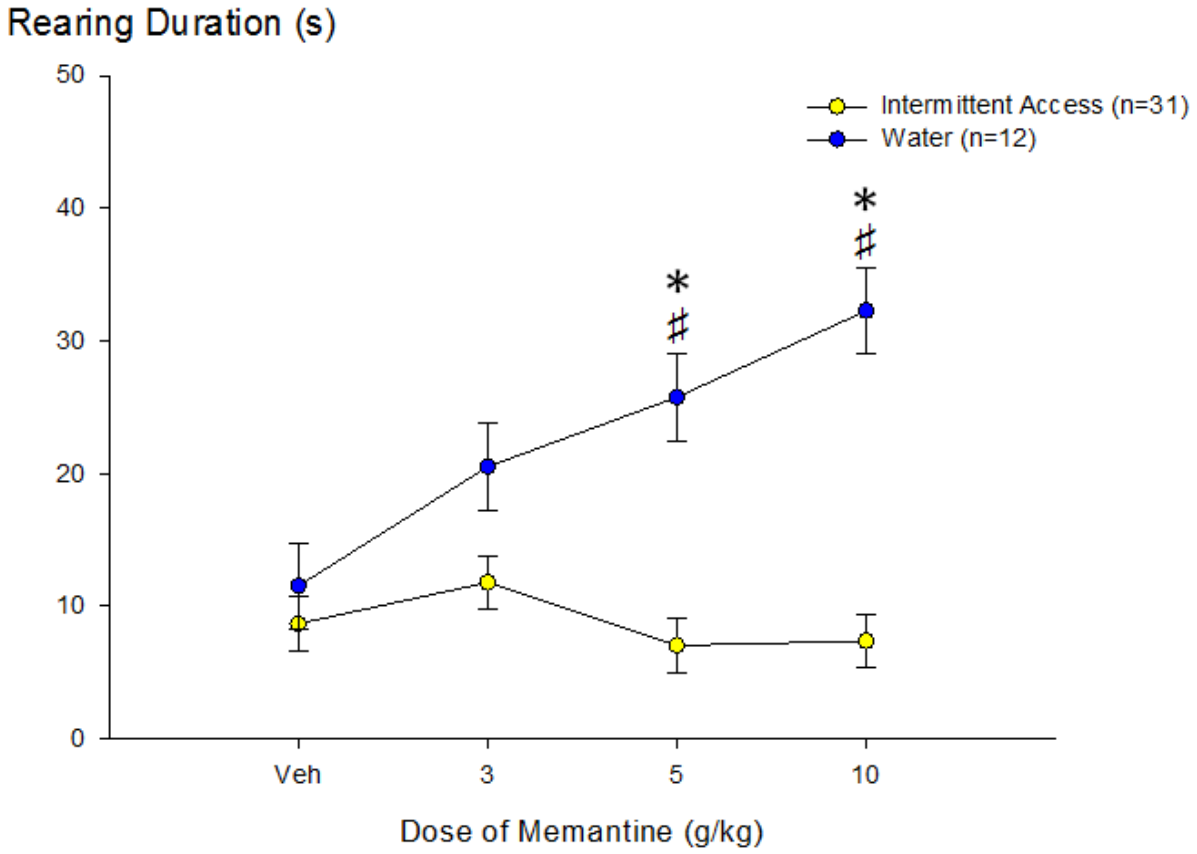


Figure 4. The effect of memantine treatment on non-aggressive behavior during withdrawal from ethanol in IAA and water control mice after 1-2 weeks of drinking. The effect of memantine treatment on A) rearing duration, B) walking duration and C) contact duration in mice with intermittent access to ethanol and water control mice 8 hours into the ethanol withdrawal period. Pound signs indicate significance between groups ($p<.05$) and asterisks indicate significance within groups compared to the vehicle dose ($p<.05$).

A)



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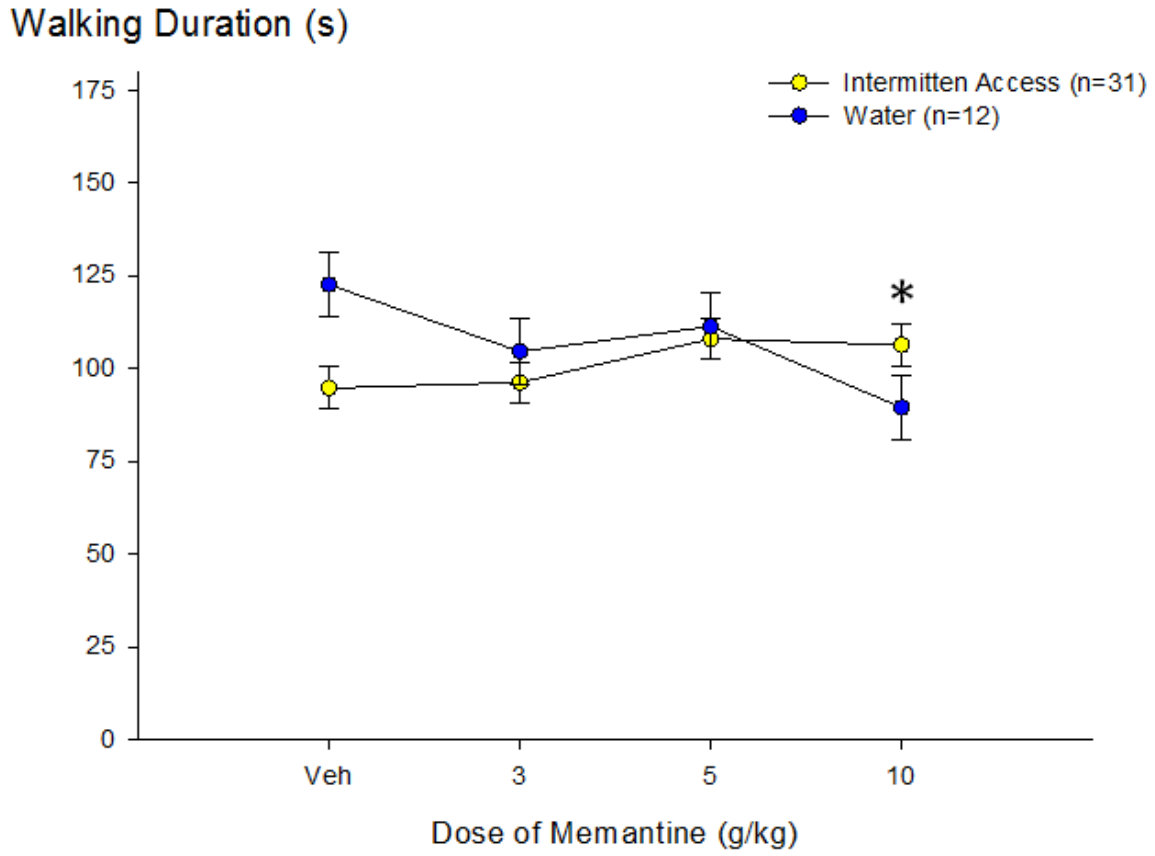

Walking Duration (s)
Intermitten Access (n=31)
Water (n=12)
175
150
125
100
75
50
25
0
*
Veh
3
5
10
Dose of Memantine (g/kg)

C)

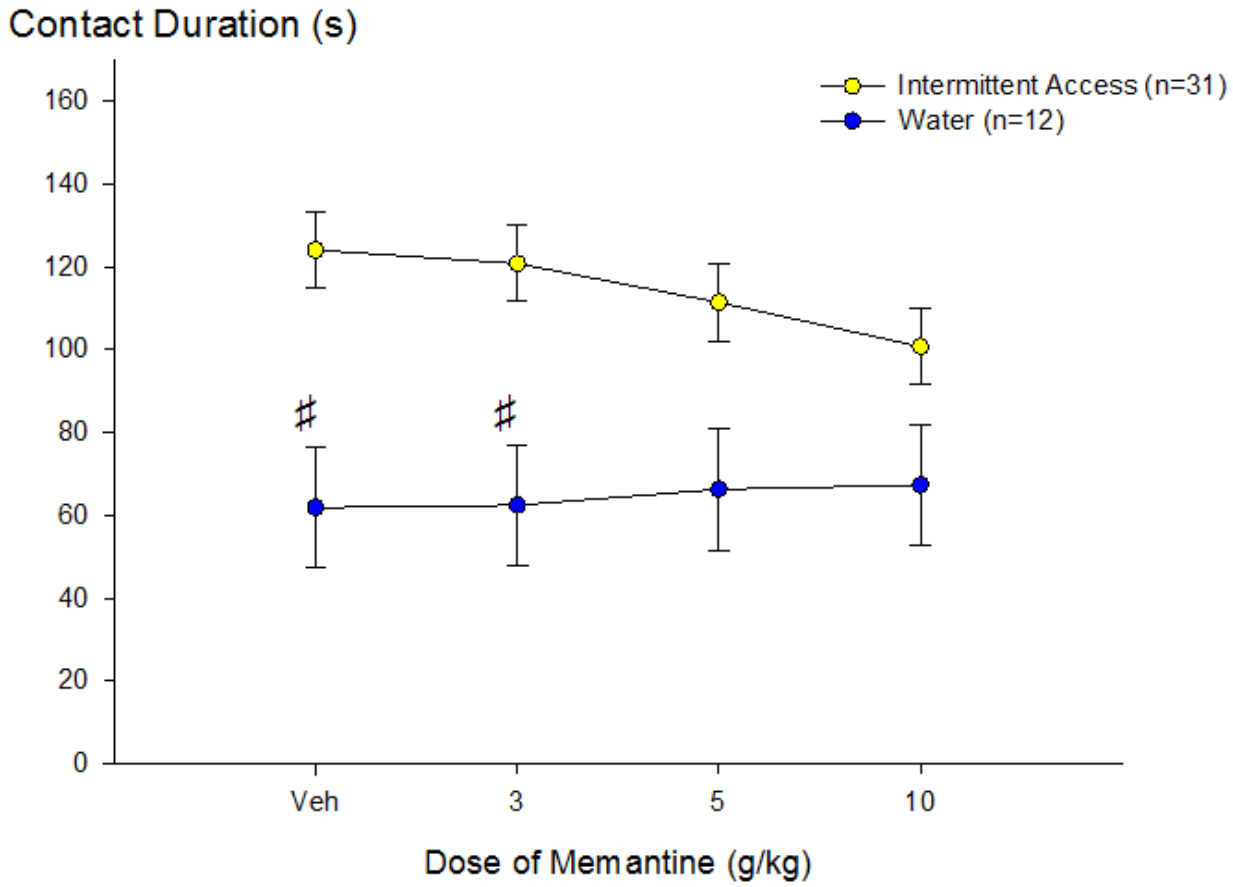
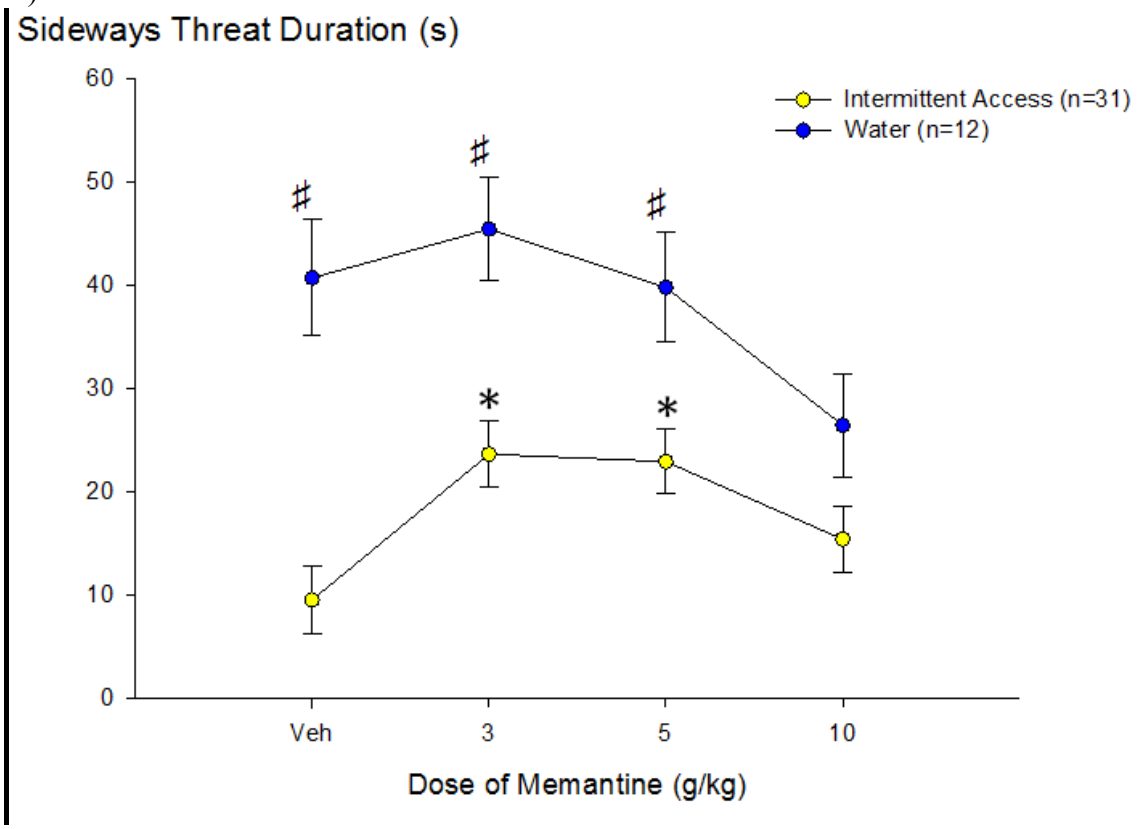
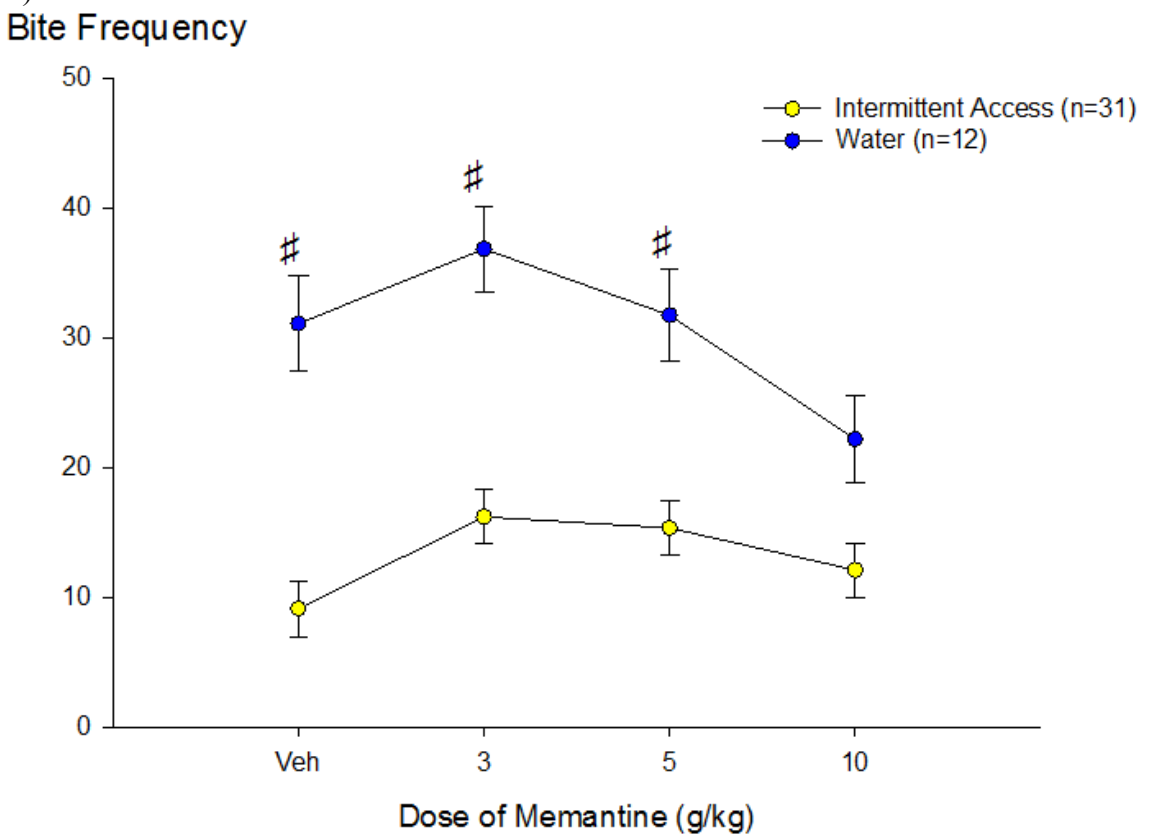

Contact Duration (s)
Intermittent Access (n=31)
Water (n=12)
160
140
120
100
80
60
40
20
0
#
#
Veh
3
5
10
Dose of Memantine (g/kg)

Figure 5. The effect of memantine treatment on aggressive behavior during withdrawal from ethanol in IAA and water control mice after 4-5 weeks of drinking. The effect of memantine treatment on A) sideways threat duration, B) bite frequency and C) tail rattle duration in mice with intermittent access to ethanol and water control mice 8 hours into the ethanol withdrawal period. Pound signs indicate significance between groups ($p<.05$) and asterisks indicate significance within groups compared to the vehicle dose ($p<.05$).

A)



B)


Bite Frequency
50
40
30
20
10
0
Intermittent Access (n=31)
Water (n=12)
#
#
#
Veh
3
5
10
Dose of Memantine (g/kg)

C)

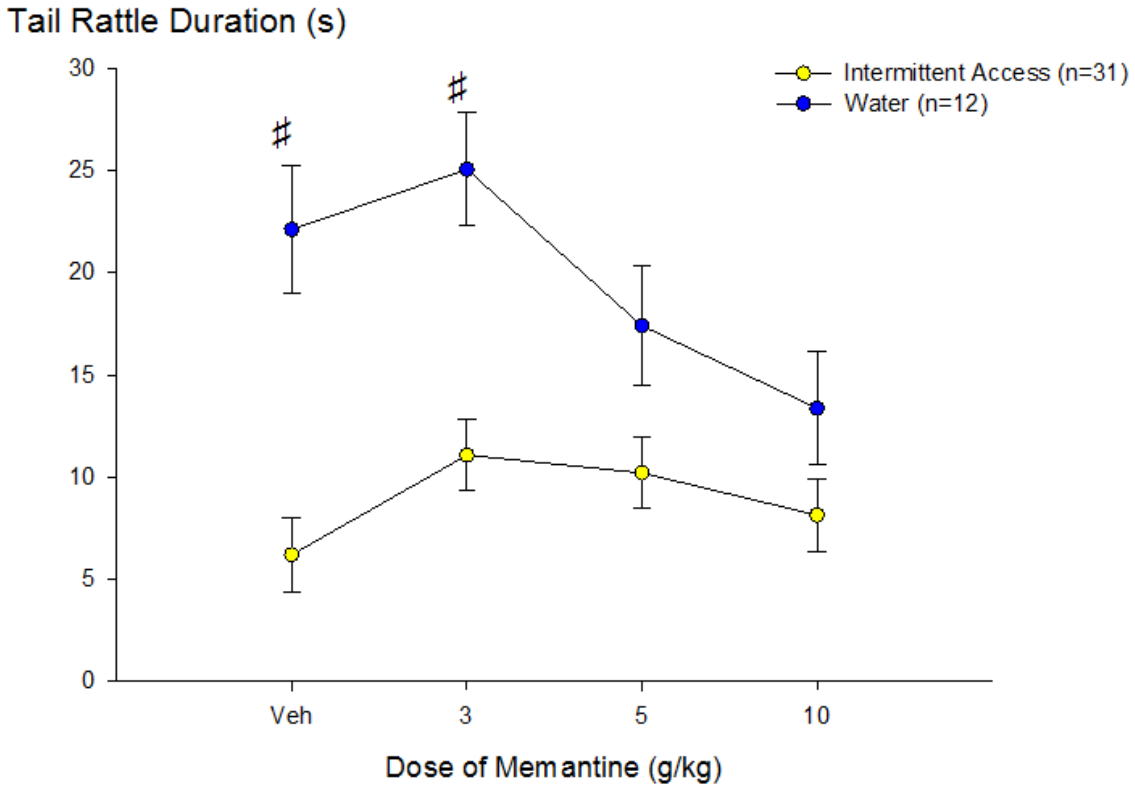
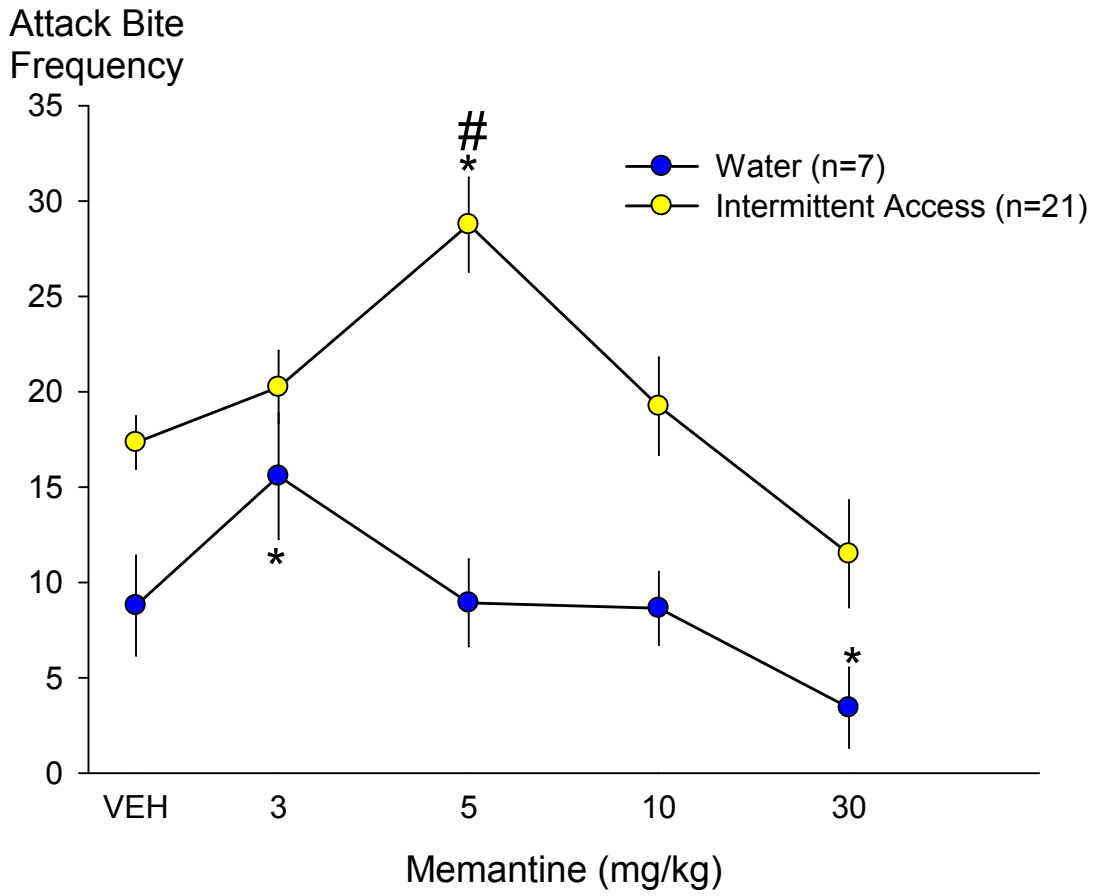

Tail Rattle Duration (s)
Intermittent Access (n=31)
Water (n=12)
#
#
30
25
20
15
10
5
0
Veh
3
5
10
Dose of Memantine (g/kg)

Figure 6. The effect of memantine treatment on aggressive behavior during withdrawal from ethanol in IAA mice and water control mice after 8-10 weeks of drinking. 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 ($p<.05$) (Nathanson, *unpublished*).

A)



B)

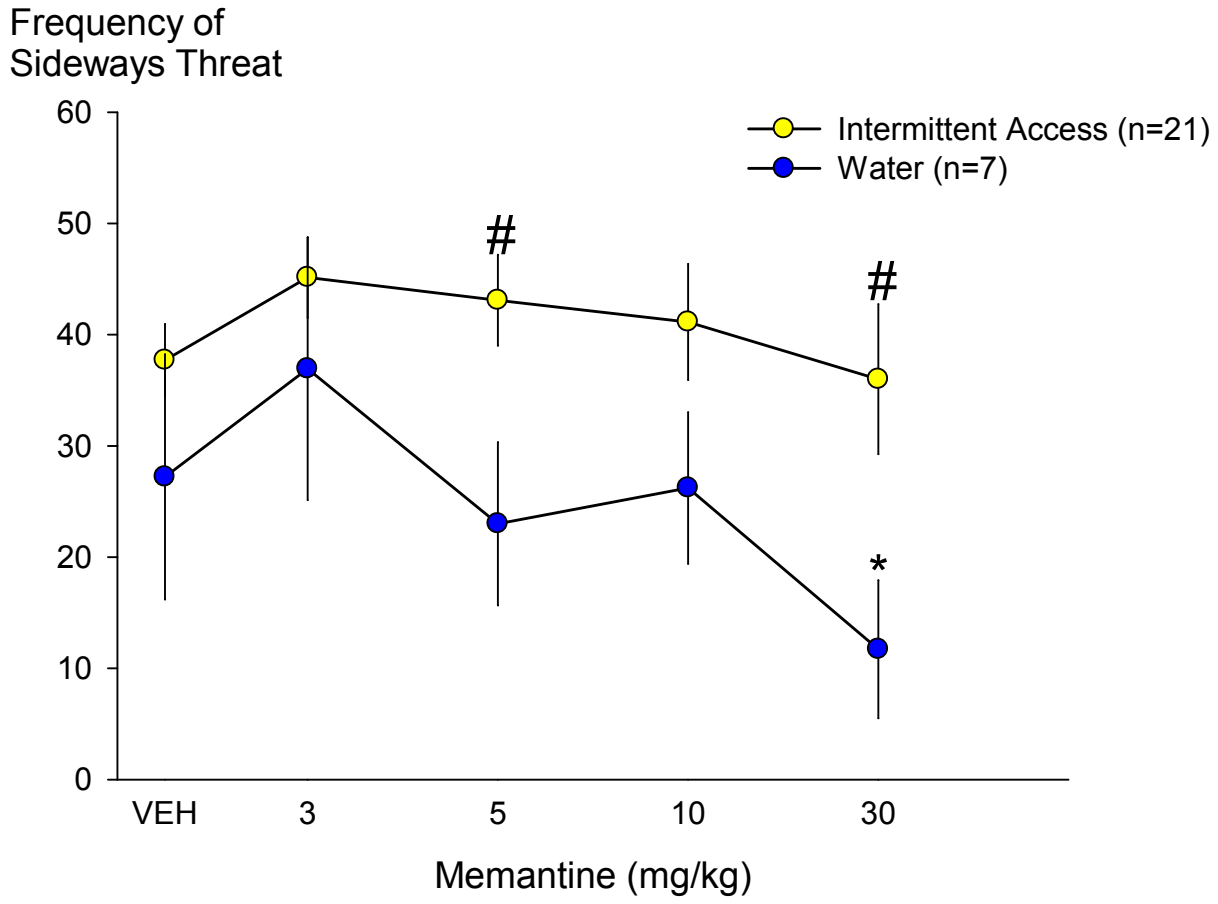

Frequency of
Sideways Threat
60
50
40
30
20
10
0
Intermittent Access (n=21)
Water (n=7)
#
#
*
VEH
3
5
10
30
Memantine (mg/kg)

Figure 7. The effect of memantine treatment on non-aggressive behavior during withdrawal from ethanol in high and low intake mice after 1-2 weeks of drinking. The effect of memantine treatment on self-grooming behavior in mice that consumed more than 200g/kg (high intake) or less than 100g/kg (low intake) ethanol total over 5 weeks 8 hours into the ethanol withdrawal period. Pound signs indicate significance between groups ($p<.05$).

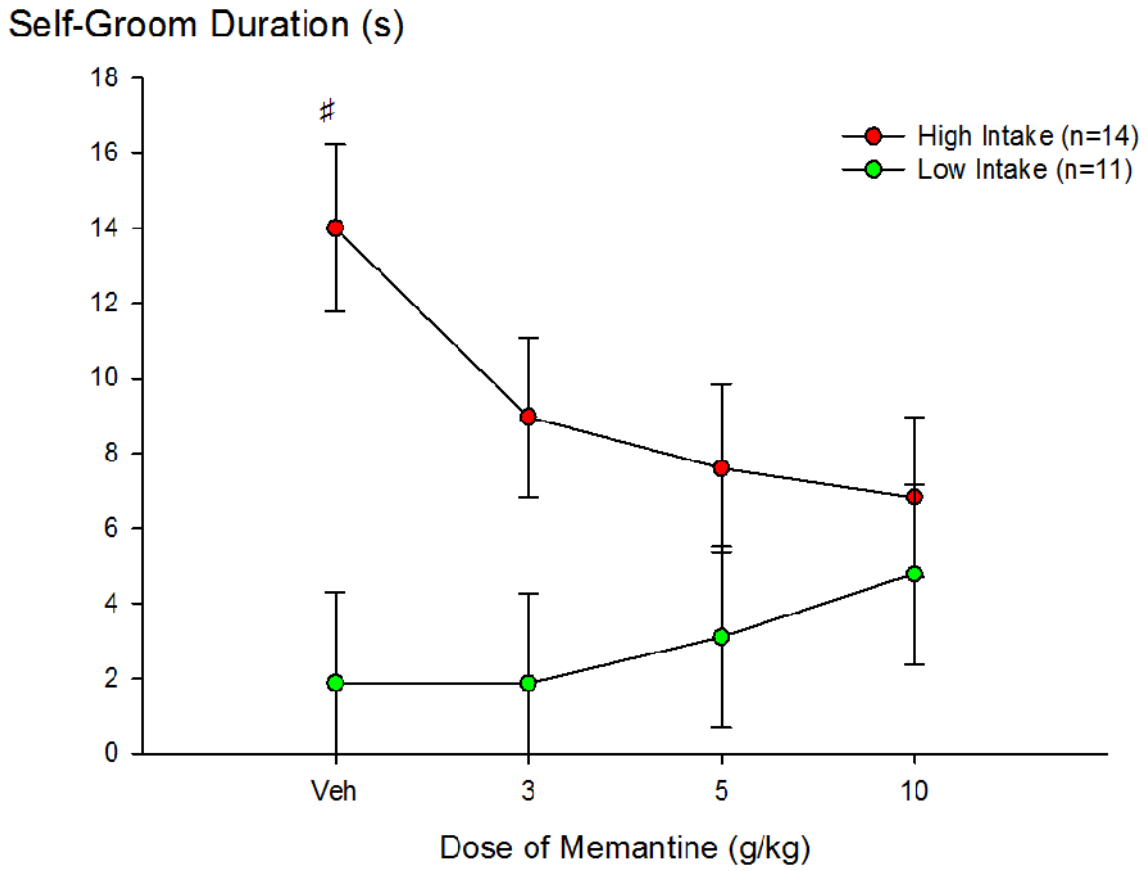


Figure 8. The effect of memantine treatment on aggressive behavior during withdrawal from ethanol in high and low intake mice after 4-5 weeks of drinking. The effect of memantine treatment on sideways threat duration in mice that consumed more than 200g/kg (high intake) or less than 100g/kg (low intake) ethanol total over 5 weeks 8 hours into the ethanol withdrawal period. Asterisks indicate significance within groups compared to the vehicle dose ($p<.05$).

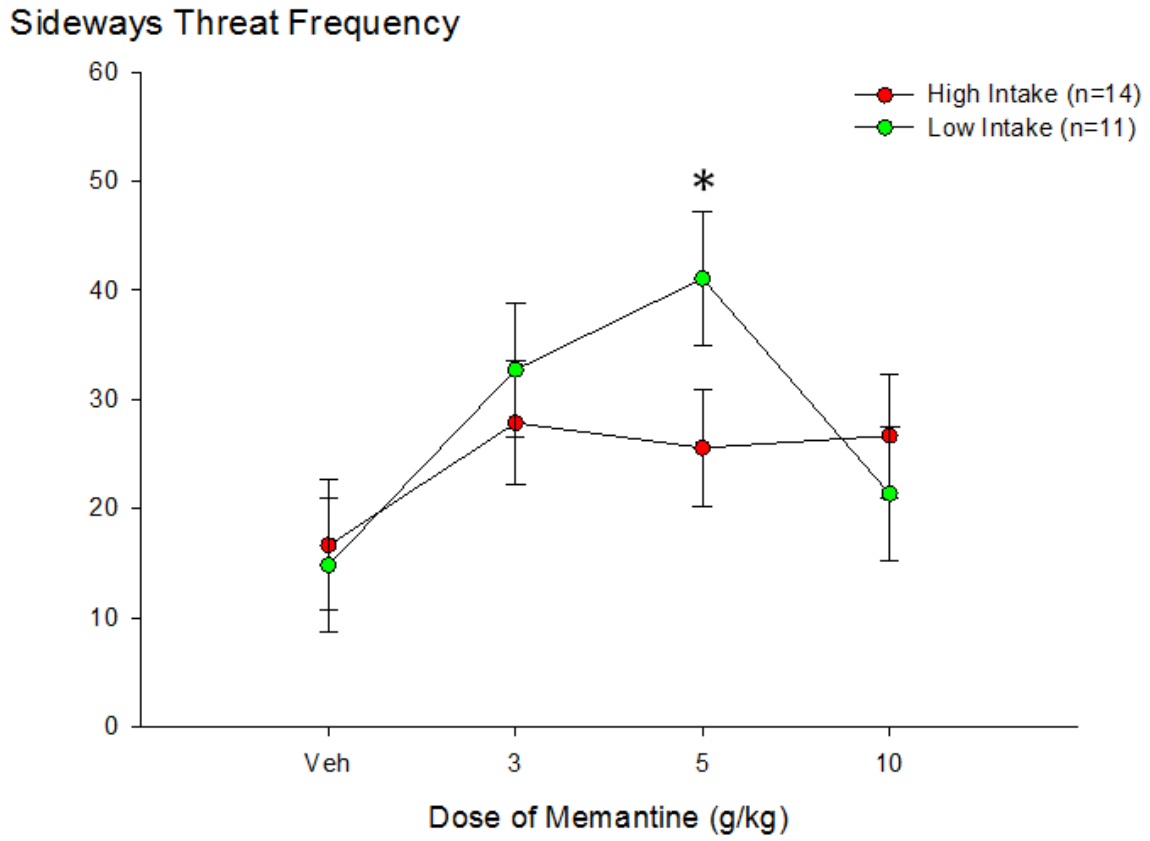


Figure 9. The effect of time course on aggressive behavior during withdrawal from ethanol in IAA and water control mice after 1-2 weeks of drinking. The effect of memantine treatment on bite frequency in mice with intermittent access to ethanol and water control mice 8 hours into the ethanol withdrawal period. Pound signs indicate significance between groups ($p<.05$).