THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOBEHAVIORAL HEALTH

COLTON RUGGERY
SPRING 2019

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Pre-Medicine
with honors in Biobehavioral Health

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ABSTRACT

A major issue in the United States today is the prevalence of alcohol addiction and binge alcohol consumption. Binge drinking can lead to alcohol dependence, and while there are approved drugs to help treat alcoholics, these drugs are mostly ineffective or have unwanted side effects. However, a new drug with anti-addictive properties, 18-Methoxycoronaridine (18-MC), has potential to one day be used in clinical settings as a therapeutic tool. 18-MC is an α3β4 nicotinic acetylcholine receptor (nAChR) antagonist that has been shown to reduce alcohol self-administration, and other abused drugs. However, we know of no research that has examined the effect of 18-MC on binge alcohol consumption and other alcohol-related behaviors. The current study investigated 18-MC's efficacy in reducing alcohol consumption in C57BL/6J male and female mice. It also determined the effect that 18-MC has on basal locomotor activity in addition to alcohol's sedative-hypnotic properties and alcohol metabolism. Finally, the study determined whether or not 18-MC can be considered a specific treatment for alcohol by testing its effect on saccharin consumption. The results determined that 18-MC reduced ethanol consumption in male and female mice while having no effect on saccharin consumption. There were no effects observed on the ethanol-induced sedation or metabolism. Locomotor sedating effects were observed for high doses of 18-MC in male and female mice, but these effects were brief. The results of this study provide evidence of 18-MC's efficacy in reducing alcohol consumption in mice by antagonizing α3β4 nAChRs while having little effect on other alcohol-related behaviors. Further research on the underlying mechanisms behind 18-MC's actions should be conducted in order to determine its potential as a clinical anti-addiction drug.

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PREFACE

Data presented in this thesis are accepted for publication in the journal *Alcohol.*: Miller, C. N., Ruggery, C., & Kamens, H. M. (2019). The α3β4 nicotinic acetylcholine receptor antagonist 18-Methoxycoronaridine decreases binge-like ethanol consumption in adult C57BL/6J mice. *Alcohol*. https://doi.org/10.1016/J.ALCOHOL.2018.11.006

ACKNOWLEDGEMENTS

I would like to thank Dr. Helen Kamens and Carley Miller for giving me the opportunity to explore my interest in undergraduate research and for their role in critiquing my honors thesis. I will be forever grateful for their help, support, guidance and teaching. Thank you to Schreyer Honors College for their support of my education and allowing me to be part of a world-class honors program. I also extend sincere thanks to the entirety of the Kamens Lab for their guidance and support throughout the completion of my work. A special thanks to Dr. David Vandenbergh for his role as the faculty reader of my honors thesis. Finally, thank you to the Rodney A. Erickson Discovery Grant Program, as this project was supported by an Erickson Discovery Grant for the summer of 2018.

Chapter 1

Background Information

Binge drinking is defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as a pattern of alcohol intake that elevates an individual's blood alcohol concentration (BAC) to at least 0.08 g/dL (Stahre et al., 2014). Moreover, binge drinking is responsible for over half of the 88,000 alcohol-associated deaths that occur annually in the United States. This equates to one in ten deaths among adults aged 20 to 64 years old (Stahre et al., 2014). While binge drinking on its own is a major health problem, it does not exist in isolation. Approximately 10.5% of binge drinkers also meet criteria for a diagnosis of alcohol dependence (Esser et al., 2014). Within the last twenty years, the American Psychiatric Association has devised a new term, alcohol use disorder, which combines the criteria for both alcohol abuse and alcohol dependence. This disorder, according to NIAAA, is defined as a chronic relapsing disease in which an individual has an impaired ability to stop or control alcohol use despite adverse social, occupational, and health consequences ("Alcohol Facts and Statistics", 2018). Binge drinking, when it becomes a habit, can lead to heavy drinking which greatly increases the risk for alcohol use disorder (Cservenka & Brumback, 2017). Many individuals who are afflicted by alcohol dependence or alcohol use disorder seek medication to treat their illness. Unfortunately, the three FDA approved medications are either not very effective or have adverse side effects (Boothby & Doering, 2005; Brewer, 1992; Petrakis et al., 2007). Therefore, research is needed to identify new drugs that could treat this deadly disease.

Alcohol use and abuse is generally comorbid with the use of other drugs, the most prominent being nicotine. Studies have shown that around 80% of people who are addicted to alcohol also smoke cigarettes. Nicotine causes its effects by binding to nicotinic acetylcholine receptors (nAChRs). Further research suggests that alcohol may also target these same receptors both directly and indirectly to cause effects (Li et al., 2007). This research is supported by genetic evidence that shows similar genetic factors influencing both alcohol and nicotine use. Several human and animal studies have investigated the various genes encoding the subunits of different nAChRs. Mutations or knockouts of these genes lead to behavioral and neurological changes associated with addiction and reward. Because nicotine binds to nAChRs and alcohol also targets these receptors, it is clear that there is a common neural basis between the addiction of these drugs (Schlaepfer et al., 2008).

Effects of Alcohol

Alcohol is a depressant drug that acts in the central nervous system. Alcohol's effects are produced through a number of different ligand-gated ion channels. Some of these channels include glycine receptors (GlyRs), 5-HT_3 receptors, GABA receptors, glutamate receptors and as mentioned above, nicotinic acetylcholine receptors (nAChR). Alcohol interacts with these channels through both excitatory and inhibitory actions in the brain depending on the receptor. Research has provided evidence that there is a relationship between some of these receptors and the mesolimbic dopamine system which is responsible for the rewarding effects of drugs of abuse, including alcohol. For example, research suggests that a major connection exists between nAChRs and the mesolimbic dopamine system (Söderpalm et al., 2009). Alcohol's involvement

at nAChRs are shown to have both inhibitory and excitatory effects on the pathway (Kamens et al., 2010; Larsson & Engel, 2004; Pierce & Kumaresan, 2006). Increasing levels of dopamine as a result of alcohol and other addictive drugs can cause reinforcement which could eventually lead to dependence. The increased dopamine levels promote a "high" or rewarding action that plays into eventual dependence on the drug (Nutt et al., 2015). The α4β2, α4α5β2, α6β2β3, and α4α6β2β3 nicotinic acetylcholine receptors are found on dopamine terminals and influence dopamine release (Pierce & Kumaresan, 2006).

Location of nAChRs and α3β4 nAChR

Taking a deeper look, nAChRs are neurotransmitter receptors that are made up of 5 subunits including α, β, γ, δ and ε. Depending on the composition of the subunits, the receptors can have different actions (Hurst et al., 2013). Muscle nAChRs are formed from a combination of the five subunits in a 2:1:1:1 ratio with α1, β1, δ, and γ or ε, but neuronal nAChRs are formed from homopentamers or heteropentamers of α and β subunits. The main human neuronal α subunits are α2-α7 and β subunits are β2-β4 subunits (Albuquerque et al., 2009). These subunits combine into different functional receptors. Each subunit can have a unique function, but they are sometimes hard to differentiate due to subunits overlapping both genetically and functionally. Neuronal nAChRs are located throughout the central nervous system but many are located in the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), and in the habenulo-interpeduncular (Hb-IPN) pathway. The nAChRs located in the Hb-IPN are especially associated with drug reinforcement (Zoli et al., 2018). These brain regions are abundant in α3 and β4 subunits that dominate cholinergic activity (Quick et al., 1999; Grady et al., 2009). With

large quantities of α3 and β4 subunits and the association with drug reinforcement, studies have shown the α3β4 nAChRs in the Hb-IPN pathway indirectly modulate the mesolimbic dopamine pathway making them key mediators in drug addiction (Glick et al., 2008; Arias et al., 2010).

The α3β4 nAChR is composed of the α3 and β4 subunits that are encoded by the α3 and β4 genes which are located in a gene cluster with α5. This cluster is located on chromosome 15 in humans and chromosome 9 in mice. Several studies have investigated this gene cluster in regard to the addictive action of drugs, including alcohol. The gene cluster has been associated with alcohol preference in both alcohol preferring C57BL/6J mice and non-preferring DBA/2J mice (Symons et al., 2010), providing evidence for the genetic association with preference. These researchers investigated different nAChR subunit gene expressions thought to be associated with alcohol preference. They studied an F_2 population of alcohol preferring and alcohol non-preferring crossed mice and looked at a two-bottle choice, alcohol preference paradigm. They observed that there were differences in expression of the *CHRNA5* and *CHRNB4* genes between the alcohol preferring and non-preferring mice suggesting an association with alcohol preference (Symons et al., 2010). Transgenic studies have also investigated the overexpression of these genes. Researchers have found that transgenic mice overexpressing the α3, α5, and β4 subunits drank less ethanol in a two-bottle choice paradigm, but no other significant effect on alcohol-related behaviors were observed (Gallego et al., 2012). This, again, helps to confirm the role that the *CHRNA3-CHRNA5-CHRNB4* gene cluster plays in alcohol preference. Further, the *CHRNA3* gene has been shown to influence ethanol-induced locomotor activity. Using heterozygous α3 subunit knockout mice, researchers found that the mice lacking the α3 subunit exhibited greater locomotor depression compared to wild-type mice when acutely treated with ethanol (Kamens et al., 2009). This suggests that the *CHRNA3* gene, along with the

rest of the cluster, could have a potential role in mediating alcohol related behaviors and addictive traits. Taking all of this together, there is a potential for therapeutic treatment of addictive effects by using drugs that target nAChRs but finding safe and effective drugs has been challenging (Hurst et al., 2013).

Common nAChR Drug Targets

Aside from the three FDA-approved medications for alcohol misuse referenced previously, a number of other drugs have been shown to effectively decrease alcohol consumption, including some that target nAChRs. Three drugs include mecamylamine, varenicline (Chantix®), and N,N-decane-1,10-diyl-bis-3-picolinium diiodide (bPiDI). Mecamylamine is a nicotinic acetylcholine receptor antagonist with a wide range of action. It influences all nAChR subtypes and is not specific in its action (Nickell et al., 2013). Mecamylamine has been shown to decrease alcohol consumption and preference in a rodent model (Farook et al., 2009). Similar effects have been reported in clinical trials, but due to mecamylamine's wide range of effects and locations of action, its efficacy as a therapeutic treatment is still unclear (Blomqvist et al., 2002).

Varenicline is an α4β2 partial agonist that is approved by the FDA as a smoking cessation drug (Jordan & Xi, 2018). Further, there is evidence that varenicline decreases alcohol consumption in both humans and in animal models (Froehlich et al., 2017; Kamens et al., 2010; Kamens et al., 2018). There are at least two reasons why varenicline may decrease alcohol consumption. It could either decrease the rewarding effects of alcohol or enhance the negative effects of this drug. In adult mice, data suggests that the latter is true because varenicline

enhances negative effects of alcohol (ataxic and sedative effects), without influencing alcohol reward (Gubner et al., 2014; Kamens et al., 2010; Randall et al., 2015). Research has shown that varenicline reduces alcohol consumption and cravings in both smoking and non-smoking clinical populations, highlighting its potential efficacy for alcohol use disorder treatment (Litten et al., 2013; McKee et al., 2009).

Much like varenicline, bPiDI has specific action at nAChRs, but it works as an α6β2 antagonist. Past research has provided evidence that bPiDI decreases alcohol consumption in a rodent model, but it also reduces locomotor activity. Further, there is evidence that it is not specific for alcohol because it can lead to decreased saccharin consumption (Kamens et al., 2017; Srisontiyakul et al., 2016). While varenicline, bPiDI, and mecamylamine are possible options for reducing alcohol consumption, they are not the best choices for therapeutic use. This is due to adverse effects or non-specificity. It is clear that nAChRs are viable targets to reduce alcohol consumption, but more research is needed to find safe and effective treatment options.

Drugs that Target α3β4 Receptors

As previously mentioned, a key nAChR that influences the dopamine pathway is the α3β4 nAChRs. They are abundantly located in the Hb-IPN and have interactions that are associated with alcohol-related behaviors and addiction (Grady et al., 2009; Kamens et al., 2009; Quick et al., 1999). With these behaviors and interactions in mind, there are a number of drugs that target the receptor. AT-1001, mecamylamine, α-conotoxins, and dextromethorphan are a few popular modulators of the α3β4 receptor. AT-1001 is a partial agonist of α3β4 nAChRs that promotes antagonistic effects through receptor desensitization (Tuan et al., 2015).

Mecamylamine, as mentioned earlier, is a non-specific nAChR antagonist that targets several different subtypes including α3β4, α3β2, α7, and α4β2. While mecamylamine has shown some specificity at α3β4 nAChRs, it exhibits similar specificity at other receptors such as α3β2, α4β4, and α2β4. In addition to this, kinetic research provides evidence of a complex interactions with α3β4 nAChRs. Specifically, it suggests multiple binding sites or a longer-lasting inactive receptor state (Papke et al., 2001). α-conotoxins are peptides extracted from cone snails (Wu et al., 2018). The α-conotoxin AuIB is selective for α3β4 and inhibits nicotine stimulated norepinephrine release in the brain (Luo et al., 1998). However, conotoxins cause poisonous and unwanted effects that deem them unsafe for clinical use. In particular, α-conotoxins can cause muscle paralysis in humans which could cause major health problems or death (Bokor & Anderson, 2012). Dextromethorphan and its metabolite dextrorphan are derivatives of codeine and morphine that have been utilized as cough medicine. They are similar in structure and function to other opiates but possess a low affinity for opiate receptors which makes them much less addictive. In addition to their effects on opiate receptors, these derivatives have been implicated as noncompetitive, antagonists at α3β4 nAChRs. However, much like with mecamylamine, these drugs only have partial selectivity at α3β4 receptors with nonspecific actions at other locations (Damaj et al., 2005; Hernandez et al., 2000).

Even with all of the drugs that have potential actions at α3β4 nAChRs, all of the drugs mentioned have shortcomings. The main problems are that the drugs are either unsafe and have adverse side effects, such as with α-conotoxin AuIB, or they are not specific, such as with mecamylamine. For these reasons, they cannot be effective treatment options designed to target α3β4 nAChRs.

18-Methoxycoronaridine

Within the last twenty years a new drug has emerged, 18-Methoxycoronaridine (18-MC), that has the potential to effectively reduce the addictive properties of alcohol and other drugs. Ibogaine is derived from a West African plant and disrupts addictive behaviors. Ibogaine has been shown to treat opioid, stimulant, alcohol, and nicotine use, but it has harsh side effects making it unsafe for clinical use and nonspecific actions in the brain (Glick et al., 2000). Historically, it has been used in healing ceremonies and religious initiations throughout West Africa, but in the United States it is rated by the DEA as a schedule 1 drug because of its low therapeutic value and abuse liability (Mash, 2018; Glick et al., 2000; Noller et al., 2018). 18-MC is a derivative of ibogaine which antagonizes α3β4 nicotinic acetylcholine receptors (nAChRs) with greater specificity. Prior research in rats has shown that 18-MC decreases morphine and cocaine intravenous self-administration and oral self-administration of alcohol and nicotine (Glick et al., 2000). These results are similar to those seen with ibogaine, but 18-MC has fewer side effects resulting in a greater therapeutic index.

As mentioned, 18-MC's primary action occurs at the α3β4 nAChR but 18-MC also has partial action at other locations. Similar to ibogaine, 18-MC has a low affinity for the kappa, mu, and delta opioid receptors (Antonio et al., 2013; Glick & Maisonneuve, 2006; Glick et al., 2000). Additionally, 18-MC also has a low affinity for 5-HT_3 serotonin receptors and significantly lower affinities for sigma 2, NMDA, sodium channels, and 5-HT serotonin transporter compared to ibogaine (Glick et al., 2000). This suggests that 18-MC's anti-addictive properties are centered around the α3β4 nAChR and not dependent upon the other possible targets. This makes 18-MC a more viable therapeutic possibility with less aversive side effects due to the lower affinities at these other sites, especially the 5-HT_3 serotonin receptor and 5-HT serotonin

transporter which have been linked to ibogaine's hallucinogenic properties (Glick & Maisonneuve, 2006; Glick et al., 2000; Wei et al., 1998).

Research with methamphetamine and nicotine has provided evidence that 18-MC acts in the MH-IPn pathway. As stated previously, these brain regions are abundant in α3 and β4 nAChR subunits. Local injections of 18-MC in the MHb, IPn, and the basolateral amygdala decreased methamphetamine self-administration, without decreasing sucrose self-administration, in rats (Glick et al., 2008). Similarly, local injections of 18-MC in the medial habenula, basolateral amygdala, and dorsolateral tegmentum caused decreased nicotine self-administration in the same rodent model (Glick et al., 2011). Based on these findings, it is possible that 18-MC would work in similar ways to reduce alcohol self-administration.

While 18-MC has been shown to decrease drug self-administration in rats, there is no data on the role of 18-MC in binge-like alcohol consumption or other alcohol-related behaviors. This is important because in addition to nAChRs influencing alcohol consumption, these receptors are also involved in alcohol's locomotor and sedative-hypnotic properties (Kamens et al., 2010; Sharma et al., 2014; Wu et al., 2014). This experiment seeks to determine the role of α3β4 nicotinic receptors in these behaviors. We hypothesized that 18-MC would reduce binge-like alcohol intake while also affecting alcohol-related behaviors. Testing the effects that 18-MC has on alcohol-related behaviors including sedative effects and metabolism will lead to a better understanding of how 18-MC may alter alcohol consumption and its ability to treat alcohol addiction. These results may provide additional data needed for clinical acceptance of 18-MC as a therapy for alcohol addiction.

Chapter 2

Materials and Methods

Animals

Male and female C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME) were used in all experiments. All mice were housed 2-4 per cage in standard cages except for the drinking in the dark procedure where mice were singly housed. Water and rodent chow (Lab Rodent Diet 5001, PMI Nutrition International, Inc., Brentwood, MO) were readily available. All mice were 6 weeks of age when arriving. All studies were approved by the Institutional Animal Care and Use Committee (IACUC).

Drugs

All drugs were prepared fresh on the day of testing. Two-hundred proof ethanol was diluted in saline to a 20% v/v solution for injections or in tap water for drinking solutions. 18-MC (18-Methoxycoronaridine hydrochloride) was purchased from Obiter Research, LLC (Champaign, IL) and was diluted in saline for intraperitoneal (i.p.) injections. 18-MC was injected at a volume of 10 ml/kg at doses of 0, 10, 20, 30, or 40 mg/kg. 18-MC doses were chosen based on prior experiments (Glick et al., 2000; Rezvani et al., 1997; Rezvani et al., 2016). The 40 mg/kg 18-MC dose was only used in the locomotor activity experiment and was not included in alcohol experiments because of sedative effects (see results below).

Locomotor Activity

Locomotor activity was monitored in 104 C57BL/6J mice (52 males and 52 females) across two experiments. Activity was measured in four, VersaMax testing chambers and analyzed via Accuscan software system. The activity boxes are made of clear plastic and are 16”x16”x12”. The mice were tested in a three-day paradigm. Groups were assigned prior to Day 1. On days 1 and 2, the mice were given a saline injection before being placed in the Accuscan testing chambers. Locomotor activity was recorded for 60 minutes in 10 minute bins. Day 1 and day 2 allowed the mice to habituate to the testing chambers and receiving injections. On day 3, the mice received an injection of saline or 18-MC (20, 30, or 40 mg/kg) before being placed in the VersaMax test chambers. Locomotor activity was recorded for 60 minutes in six, ten-minute epochs. The first experiment tested the 20 mg/kg and 40 mg/kg doses with saline while the second experiment tested the 30 mg/kg dose with saline.

Drinking in the Dark

The effect that 18-MC has on ethanol consumption was tested in a two-day drinking in the dark (DID) procedure (Kamens et al., 2017; Rhodes et al., 2005). Adult male and female mice (N=24 total) were tested in four, two-day DID sessions. Mice were housed on a reverse light-dark cycle – lights on at 10 PM, lights off at 10 AM. On the first day, the weight of the animals was recorded one hour before the dark cycle. The animal’s water was removed 3 hours into the dark cycle and replaced with a single bottle containing 20% ethanol. Initial ethanol fluid levels were recorded and a final fluid level was recorded after a 2 hour ethanol exposure time. At the end of the 2 hours, the ethanol tube was removed and water bottles were placed back

on the cages. On day 2 the protocol was repeated, but mice were given an acute injection of saline or 18-MC (10, 20, or 30 mg/kg) 30 minutes prior to the drinking session. The day 2 drinking session lasted 4 hours with 20% ethanol fluid level readings recorded at initial exposure, 30 minutes, 1 hour, 2 hours, and 4 hours. The primary dependent variable was ethanol consumption (g/kg). In order to be certain that the effects of 18-MC were specific for ethanol consumption, the same protocol was repeated with 0.033% saccharin (Kamens et al., 2017). The primary dependent variable was saccharin consumption (mg/kg).

LORR

The loss of righting reflex (LORR) procedure was used to test the effects of 18-MC on ethanol sedation (Crabbe et al., 2006; Kamens et al., 2010). Adult male and female naïve mice (N=44 total) were tested at 6-8 weeks old. Mice were moved to the procedure room on the testing day and left undisturbed for 30-45 minutes. After 30-45 minutes of acclimation, the mice received an acute injection of saline or 18-MC (10, 20, or 30 mg/kg) as pretreatment. The mice were immediately placed in individual holding cages after the pretreatment and left undisturbed for 30 minutes. After 30 minutes, the mice received an acute injection of ethanol (4 g/kg). After the ethanol treatment, the mice were monitored for visual impairment or sedation. At the time of impaired movement, the mice were placed on their backs in a plastic, V-shaped trough. The initial loss of righting reflex time from the treatment injection, or latency to LORR, was recorded. Loss of righting reflex was defined as the time that it took for the mice to lose the capability to turn over and stand upright on their paws. Mice were then left undisturbed until they could right themselves. A full righting event was accomplished when the mouse could turn

and have all four paws touching the trough. After one righting event, the mouse would again be placed on its back in the plastic trough. The final righting reflex time was recorded when the animal could fully right itself two times in the span of 1 minute. Once the mice successfully righted themselves, they were returned to their home cage. The dependent variables being monitored were latency to LORR and duration of LORR. Latency to LORR was described as the time it took between the ethanol injection and the initial loss of righting reflex. Duration of LORR was described as the time it took between the initial loss of righting reflex and when the animal could fully right itself twice in one minute.

Metabolism

A standard ethanol metabolism protocol was followed in order to determine the effects that 18-MC had on ethanol metabolism (Kamens et al., 2010). Adult male and female mice (N=30 total) were tested for ethanol metabolism via tail blood samples. The mice were moved to the testing room and allowed to acclimate for 30-45 minutes. Three groups were pre-determined to receive a pre-treatment of saline or 18-MC (20 or 30 mg/kg) before being placed in a holding cage for 30 minutes. After 30 minutes, each group received an injection of ethanol (4 g/kg) and the animals were returned to the holding cages. Tail blood was collected at varying time points (30, 60, 120, and 180 minutes) following the ethanol injection. Blood was collected in capillary tubes and put on ice. Once all blood was collected, the blood ethanol concentration (BEC) was measured using an Analox System.

Statistical Analysis

The results were analyzed using SPSS statistical software. The dependent variables included locomotor activity, alcohol consumption, saccharin consumption, latency to LORR, duration of LORR, and blood ethanol content (BEC). Locomotor activity, alcohol consumption, saccharin consumption, and blood ethanol content data were analyzed using repeated measures analysis of variance (ANOVA). Latency to LORR and duration of LORR data was analyzed using factorial ANOVA analyses. The independent variables included sex, 18-MC dose, and time. $\alpha<0.05$ was significant and significant variables were compared via a Tukey's post hoc analysis.

Chapter 3

Results

Locomotor Activity

The high (40 mg/kg) dose of 18-MC significantly reduced locomotor activity in male and female mice (Fig. 1 & 2). Locomotor activity was monitored over two separate experiments. Between the experiments, there were no significant differences between the saline treatment groups so the experiments were combined for analysis. When the combined data set was analyzed, there was a significant main effect of sex ($F=22.657$, $p<0.001$) so males and females were analyzed separately. In males, there was a significant time X dose interaction ($F=1.974$, $p<0.05$) thus each time point was analyzed separately. At the 10-minute time point, there were no significant effects of dose. At the 20-minute time point, the 30 mg/kg and 40 mg/kg 18-MC doses led to significantly reduced locomotor activity compared to saline treatment and 20 mg/kg 18-MC dose ($F=9.196$, $p<0.001$, Post Hoc p-values < 0.05). At the 30-minute time point, the 30 mg/kg and 40 mg/kg 18-MC doses led to significantly reduced locomotor activity compared to saline treatment ($F=9.308$, $p<0.001$, Post Hoc p-values < 0.05). At the 40-minute time point, the 30 mg/kg and 40 mg/kg 18-MC doses led to significantly reduced locomotor activity compared to saline treatment. The 40 mg/kg 18-MC dose also led to significantly reduced locomotor activity compared to the 20 mg/kg 18-MC dose ($F=8.914$, $p<0.001$, Post Hoc p-values < 0.05). At the 50-minute time point, the 30 mg/kg and 40 mg/kg 18-MC doses reduced locomotor activity compared to saline treatment ($F=5.188$, $p<0.01$, Post Hoc p-values < 0.05). At

the 60-minute time point, there were no significant effects of 18-MC treatment on locomotor activity. In female mice, there was a significant main effect of time and dose observed. The 40 mg/kg 18-MC dose significantly decreased locomotor activity compared to saline treatment ($F=3.535$, $p<0.05$, Post Hoc p-value < 0.05). When the main effect of time was analyzed, the 10-minute time point was significantly different from all other time points where locomotor activity decreased after the first 10 minutes ($F=37.002$, $p<0.001$, Post Hoc p-values < 0.05).

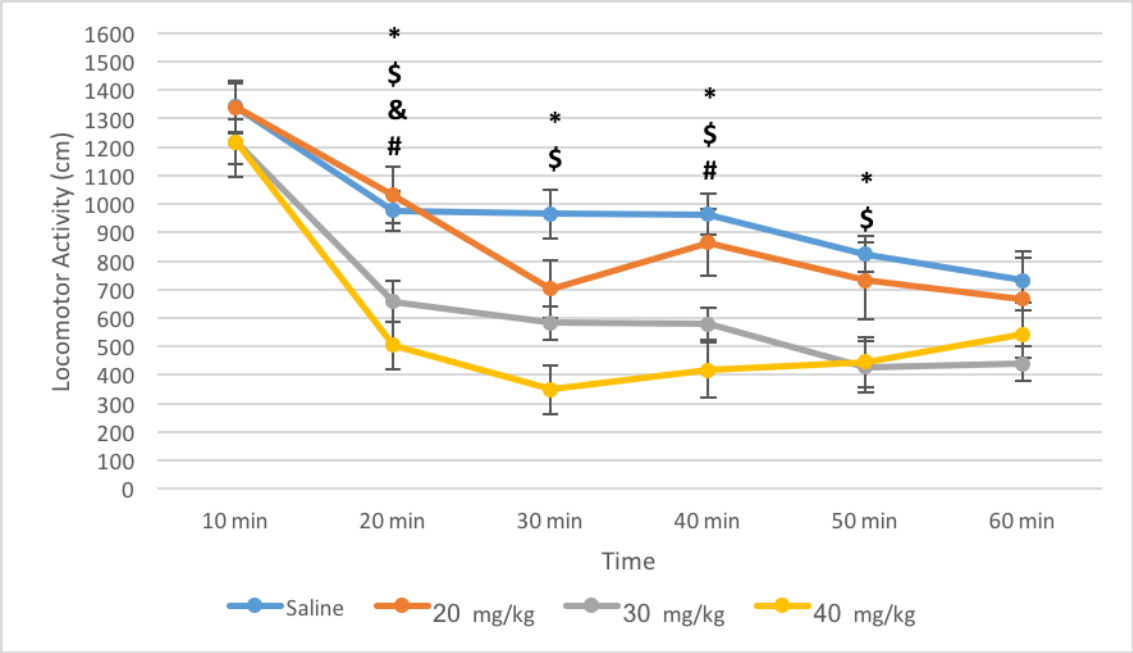

Figure 1. 18-MC significantly reduced locomotor activity in C57BL/6J male mice during a one-hour time period. There was a significant time X dose interaction on locomotor activity. Data represents mean ± SEM locomotor activity in male mice. N = 10 – 11/dose. * = saline significantly different from 30 mg/kg 18-MC. $ = saline significantly different from 40 mg/kg 18-MC. & = 20 mg/kg 18-MC significantly different from 30 mg/kg 18-MC. # = 20 mg/kg 18-MC significantly different from 40 mg/kg 18-MC.

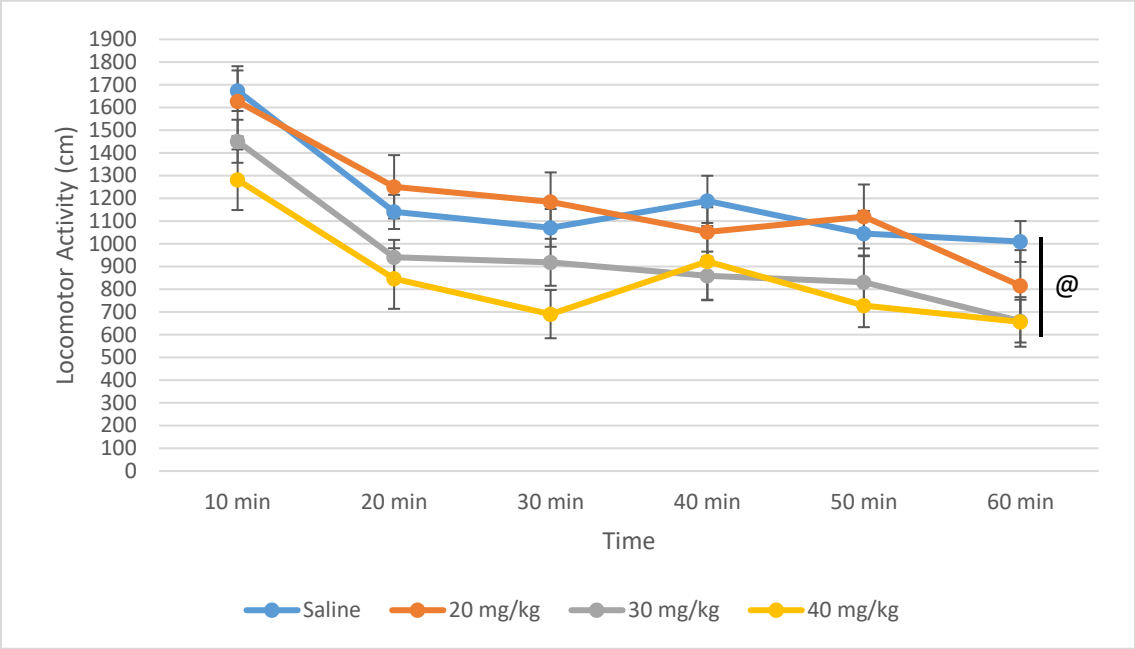

Figure 2. 18-MC affected locomotor activity in C57BL/6J female mice such that there was a main effect of dose during the one-hour time period. 40 mg/kg 18-MC decreased locomotor activity compared to saline treatment. Additionally, locomotor activity decreased after the first 10-minute time point. Data represents mean ± SEM locomotor activity in female mice. N = 10 – 11/dose. @ = significant main effect of 40 mg/kg 18-MC.

Drinking in the Dark

Overall, 18-MC treatment significantly reduced ethanol consumption in both male and female C57BL/6J mice while having no effect on saccharin consumption (Fig. 3 & 4). In the ethanol experiment, there was a significant dose X time interaction ($F=2.421$, $p<0.05$). Due to this interaction, each time point was analyzed separately. At the 30-minute time point, there were no significant effects. At the 60-minute time point, the 30 mg/kg 18-MC dose reduced ethanol consumption compared to saline treatment (main effect of dose; $F=4.563$, $p<0.01$, Post Hoc p-value < 0.05). At the 120-minute time point, the 30 mg/kg 18-MC dose significantly reduced ethanol consumption compared to saline treatment and the 10 mg/kg 18-MC dose in both male and female mice (main effect of dose; $F=5.991$, $p<0.01$, Post Hoc p-values < 0.05). At the 240-minute time point, the 30 mg/kg 18-MC dose significantly reduced ethanol consumption

compared to saline treatment in both male and female mice (main effect of dose; $F=4.458$, $p<0.01$, Post Hoc p-value < 0.05). Additionally, at the 240 time point, there was a significant main effect of sex ($F=10.073$, $p<0.01$) where females drank significantly more ethanol than males (8.7 ± 0.5, 6.4 ± 0.2 g/kg; mean ± SEM). However, this effect did not interact with 18-MC dose. In the saccharin experiment, there was a significant effect of time such that consumption increased over time ($F=36.291$, $p<0.001$, Post Hoc p-values < 0.05).

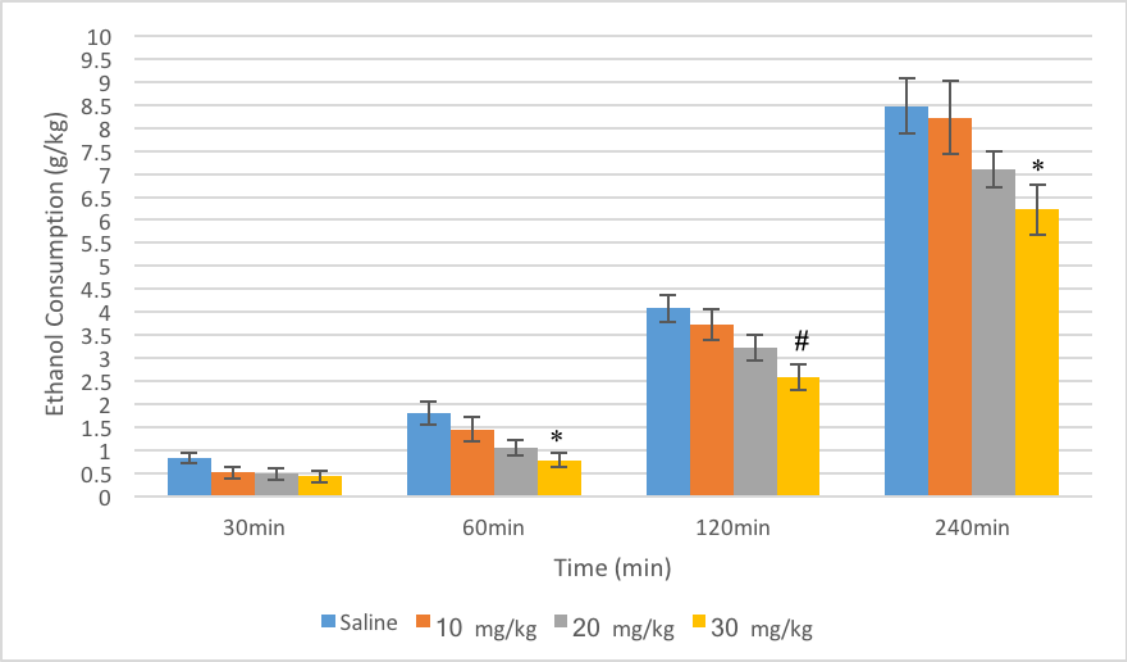

Figure 3. 18-MC significantly reduced dinking in the dark ethanol consumption in both male and female C57BL/6J mice. There was a significant main effect of dose and significant dose X time interaction. Data represent mean ± SEM ethanol consumption in male and female mice. N = 12/sex. * = significantly different from saline. # = significantly different from 10 mg/kg 18-MC.

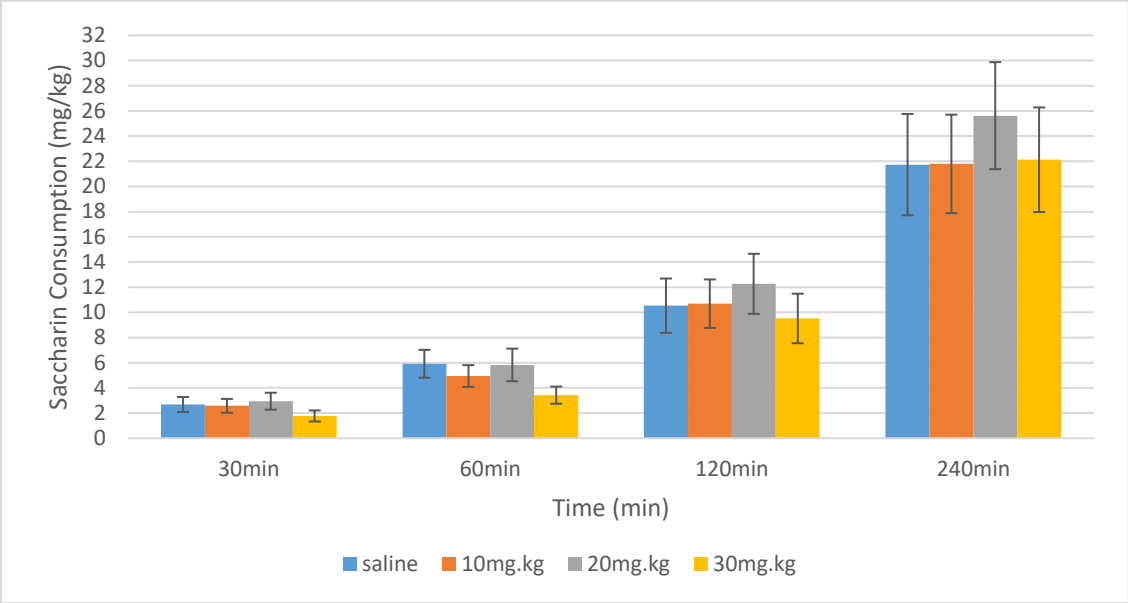

Figure 4. 18-MC had no effect on saccharin consumption in male or female C57BL/6J mice. There was a significant main effect of time, such that mice consumed more saccharin over the 2-hour experiment. Data represent mean ± SEM saccharin consumption in male and female mice. N = 12/sex.

LORR

18-MC treatment had no effect on ethanol sedation. Statistical analyses revealed no significant main effects or interactions on the latency to LORR or duration of LORR in male and female C57BL/6J mice (Fig. 5 & 6).

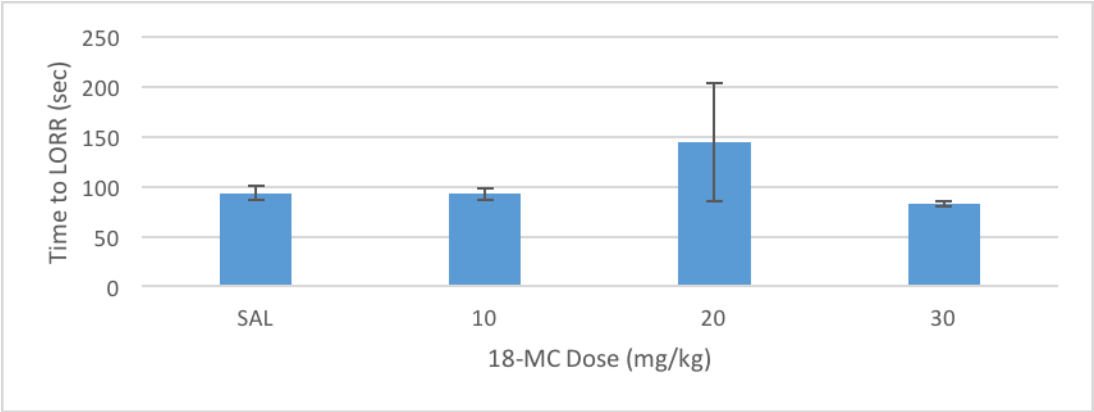

Figure 5. 18-MC did not affect the time to loss of righting reflex in male or female C57BL/6J mice. There was no significant main effect of 18-MC treatment on time to LORR in male or female mice. Data represent mean ± SEM time to LORR in seconds in male and female mice. N = 5 – 6/dose/sex.

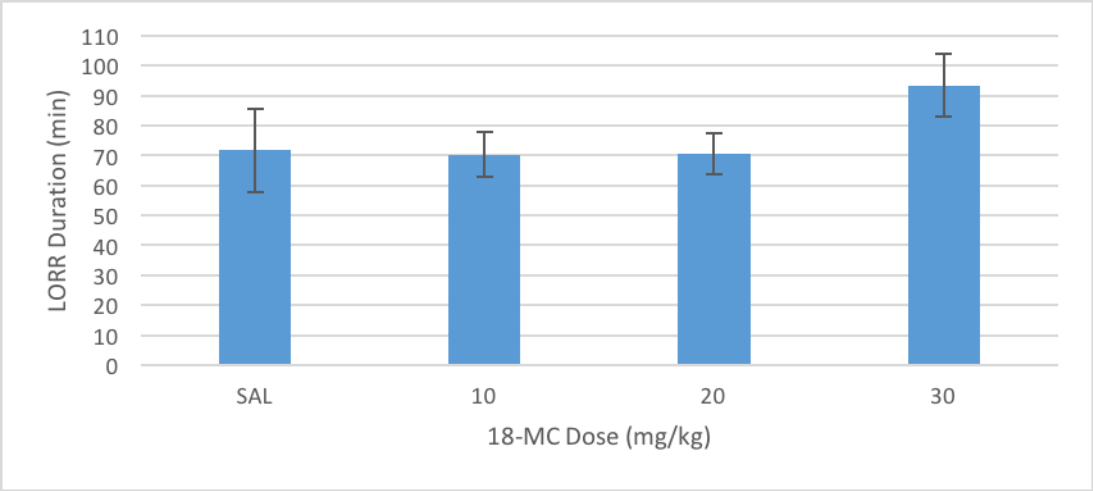

Figure 6. 18-MC did not affect loss of righting reflex duration in male or female C57BL/6J mice. There were no significant effects of 18-MC treatment on LORR duration in male or female mice. Data represent mean ± SEM duration in minutes of LORR in male and female mice. N = 5 – 6/dose/sex.

Metabolism

Overall, 18-MC treatment had no effect on ethanol metabolism in male or female C57BL/6J mice (Fig. 7 & 8). Statistical analyses revealed a significant time X sex interaction ($F=5.675$, $p<0.001$), so male and female mice were analyzed separately. Males showed a significant time X dose interaction ($F=2.673$, $p<0.05$) so each dose was analyzed separately. There were no significant group differences for the males at any one time point but there were varying blood ethanol concentrations based on 18-MC dose. Saline treated mice showed significantly higher blood ethanol content at the 30-minute and 60-minute time points compared to the 180-minute time point (main effect of time; $F=9.946$, $p<0.01$, Post Hoc p-values < 0.05). The 20 mg/kg 18-MC dose mice also showed significantly higher blood ethanol content at the 30-minute and 60-minute time points compared to the 180-minute time point (main effect of time; $F=8.059$, $p<0.01$, Post Hoc p-values < 0.05). The 30 mg/kg 18-MC dose mice showed

significantly higher blood ethanol content at the 60-minute time point compared to the 180-minute time point (main effect of time; $F=6.274$, $p<0.01$, Post Hoc p-value < 0.05). Female mice showed a significant effect of time where blood ethanol content levels decreased over time ($F=111.044$, $p<0.001$, Post Hoc p-values < 0.05).

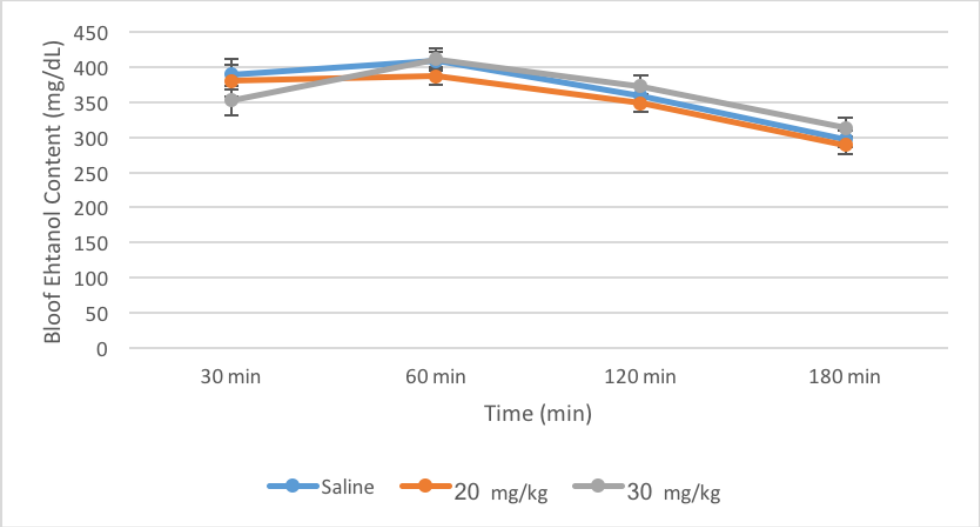

Figure 7. 18-MC had no effect on blood ethanol content in C57BL/6J male mice over a 180-minute time period. There was a significant time X dose interaction but no significant group differences at any time point. Data represents mean ± SEM blood ethanol content (mg/dL) in male C57BL/6J mice. N = 5/dose.

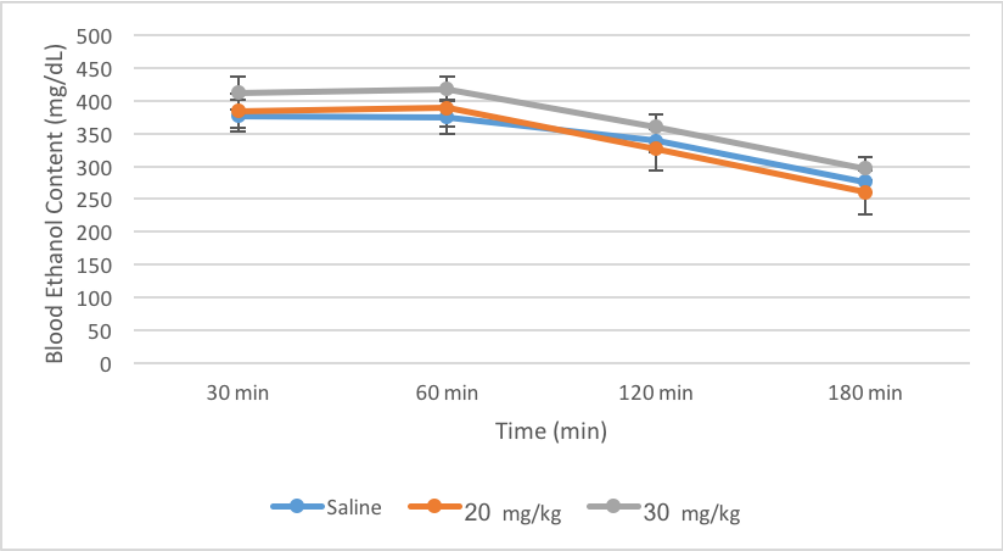

Figure 8. 18-MC had no effect on blood ethanol content in C57BL/6J female mice over a 180-minute time period. There was a significant effect of time where blood ethanol content decreased over the course of the experiment. Data represents mean ± SEM blood ethanol content (mg/dL) in female C57BL/6J mice. N = 5/dose.

Chapter 4

Discussion

Our results showed that 18-MC was effective at decreasing alcohol consumption while having little effect on other alcohol-related behaviors. 18-MC treatment reduced ethanol consumption in the drinking in the dark paradigm in both male and female C57BL/6J mice without affecting saccharin consumption, which highlights 18-MC's specificity for alcohol. At the same time, there were no effects on alcohol's sedative effects and 18-MC did not alter alcohol metabolism. 18-MC did reduce locomotor activity at high doses, but the effects were not long lasting and did not occur at the same time that alcohol consumption effects occurred. These results support the hypothesis that 18-MC can effectively reduce alcohol consumption while having little effect on other alcohol-related behaviors.

As mentioned above, there was an effect on locomotor activity where 18-MC decreased the activity in male and female mice. In males, this decrease subsided after 50 minutes and the effects on consumption occurred after one hour, which surpasses the 50-minute time point where locomotion was diminished. In females, as well as in the males, the 40 mg/kg dose of 18-MC significantly decreased locomotor activity. Due to the strong sedating effects of the 40 mg/kg 18-MC dose, it was not used in the ethanol experiments.

Drinking and Alcohol-Related Behaviors

In this study, 18-MC decreased ethanol consumption while having no effect on saccharin consumption in C57BL/6J mice. As highlighted, the 30 mg/kg dose of 18-MC significantly reduced binge-like alcohol consumption. The reduction occurred after the one-hour mark and continued into the two-hour mark. This is significant because it provides evidence that 18-MC does, in fact, decrease alcohol consumption which is consistent with prior research (Glick et al., 2000). It is also important that 18-MC did not affect saccharin consumption because this suggests that 18-MC is selective for alcohol. This result is similar to results found in a previous alcohol consumption experiment in rats. In this prior study, researchers administered 18-MC to rats and tested alcohol consumption and preference in a two bottle choice paradigm where the rats could choose between alcohol and water. The researchers found that 18-MC significantly decreased alcohol consumption and only the highest dose of 18-MC (40 mg/kg) decreased food intake. Both the 5 mg/kg and 20 mg/kg doses of 18-MC significantly decreased alcohol consumption without affecting food intake (Rezvani et al., 1997). This is similar to the results of the present study where 40 mg/kg 18-MC had locomotor sedating effects but the other doses that influence alcohol consumption (20 mg/kg and 30 mg/kg) did not have sedating effects that would influence alcohol consumption. This supports the idea that 18-MC, at appropriate doses, is specific for alcohol with no other strong influences or major side effects. These results back the notion that 18-MC has the potential to be an effective treatment for those with alcohol use disorder but requires further research.

Regarding the other alcohol-related behaviors, 18-MC had no effect on the metabolic or sedative-hypnotic properties of alcohol as demonstrated in the metabolism and LORR

experiments respectively. After evaluating the results, there is evidence to support 18-MC's efficacy at reducing alcohol consumption without affecting these other alcohol-related behaviors.

Brain Regions

Prior research has shown that 18-MC works by antagonizing α3β4 nicotinic acetylcholine receptors to influence the mesolimbic dopamine pathway. In particular, the α3β4 nAChR modulates this pathway indirectly from other brain regions (Arias et al., 2010; Glick et al., 2008). These brain regions mainly include the medial habenula (MHb) and interpeduncular nucleus (IPn) where α3 and β4 subunits are highly expressed (Quick et al., 1999; Grady et al., 2009). These two regions together make up the medial habenula-interpeduncular (MHb-IPn) pathway (Quick et al., 1999; Grady et al., 2009). Research with methamphetamine and nicotine has provided evidence that 18-MC acts on this pathway. Local injections of 18-MC in the MHb, basolateral amygdala, IPn, and dorsolateral tegmentum decreased nicotine self-administration and methamphetamine self-administration, without decreasing sucrose self-administration (Glick et al., 2011; Glick et al., 2008). Based on these results, in conjunction with our findings, we hypothesize that 18-MC would work in similar brain regions, and on similar receptors, to reduce alcohol consumption, but further research is necessary to address this question.

18-MC has actions at other receptors, including the kappa, mu, and delta opioid receptors along with the 5-HT_3 serotonin receptors, sigma 2, NMDA, sodium channels, and 5-HT serotonin transporter, but our data is consistent with prior research demonstrating that α3β4 nicotinic acetylcholine receptors are involved in ethanol-mediated behaviors. Previous research tested the effects of the α3β4 nAChR partial agonists, CP-601932 and PF-4575180, on alcohol

preference and consumption in ethanol seeking rats (Chatterjee et al., 2011). Both drugs are selective for α3β4 nAChRs and decreased ethanol consumption and self-administration in male rats. Further, these drug treatments had no effects on sucrose consumption, much like the effects with saccharin in the present study (Chatterjee et al., 2011). The similarities in findings with CP-601932, PF-4575180, and 18-MC suggest that α3β4 receptors are important in alcohol consumption. While CP-601932 and PF-4575180 are partial agonists and 18-MC is an antagonist, each drug has a high affinity for α3β4 and caused the same reduction in consumption. This occurs because partial agonists can cause similar effects to antagonists. When a partial agonist binds to a receptor and displaces a full agonist, it causes reduced activity leading to antagonistic behavior (Ariëns, 1983). Thus, α3β4 nAChRs are implicated in alcohol consumption and the dense expression of α3β4 receptors in the MHb-IPn pathway may be the site of action.

Conclusion

In conclusion, this research helps provide evidence of the action and efficacy of 18-MC as a potential therapeutic drug to combat alcohol addiction. These results show that 18-MC was able to effectively reduce ethanol consumption in C57BL/6J mice with actions specific for alcohol. 18-MC did not have any major effect on other alcohol-related behaviors such as the sedative-hypnotic, metabolic, or basal locomotor activity. Future research should aim to discover a better understanding of the underlying mechanisms behind 18-MC that cause decreased alcohol self-administration. In doing this, 18-MC testing can progress further in the next steps to becoming a potential clinical tool in combating alcohol addiction.

Appendix

SPSS Statistical Analysis

Saline Experiments 1&2

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

time	Dependent Variable
1	D3_LOCO5
2	D3_LOCO10
3	D3_LOCO15
4	D3_LOCO20
5	D3_LOCO25
6	D3_LOCO30
7	D3_LOCO35
8	D3_LOCO40
9	D3_LOCO45
10	D3_LOCO50
11	D3_LOCO55
12	D3_LOCO60

Between-Subjects Factors

		N
EXP	1	20
	2	19

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	7043423.959	11	640311.269	17.708	.000
	Greenhouse-Geisser	7043423.959	7.716	912847.526	17.708	.000
	Huynh-Feldt	7043423.959	10.208	690020.298	17.708	.000
	Lower-bound	7043423.959	1.000	7043423.959	17.708	.000
time * EXP	Sphericity Assumed	510694.215	11	46426.747	1.284	.231
	Greenhouse-Geisser	510694.215	7.716	66187.405	1.284	.253
	Huynh-Feldt	510694.215	10.208	50030.976	1.284	.236
	Lower-bound	510694.215	1.000	510694.215	1.284	.264
Error(time)	Sphericity Assumed	14716566.845	407	36158.641		
	Greenhouse-Geisser	14716566.845	285.488	51548.875		
	Huynh-Feldt	14716566.845	377.680	38965.730		
	Lower-bound	14716566.845	37.000	397745.050		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	136072750.668	1	136072750.668	530.145	.000
EXP	540603.488	1	540603.488	2.106	.155
Error	9496828.324	37	256671.036		

Locomotor Activity

General Linear Model

Within-Subjects Factors

Measure: LOCO

Time	Dependent Variable
1	D3_LOCO10
2	D3_LOCO20
3	D3_LOCO30
4	D3_LOCO40
5	D3_LOCO50
6	D3_LOCO60

Between-Subjects Factors

		N
Dose	0	39
	20	22
	30	21
	40	22
Sex	F	52
	M	52

Tests of Within-Subjects Effects

Measure: LOCO

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	30729207.535	5	6145841.507	86.442	.000
	Greenhouse-Geisser	30729207.535	4.255	7221082.165	86.442	.000
	Huynh-Feldt	30729207.535	4.802	6399058.853	86.442	.000
	Lower-bound	30729207.535	1.000	30729207.535	86.442	.000
Time * Dose	Sphericity Assumed	1401689.688	15	93445.979	1.314	.189
	Greenhouse-Geisser	1401689.688	12.766	109794.744	1.314	.202
	Huynh-Feldt	1401689.688	14.406	97296.085	1.314	.192
	Lower-bound	1401689.688	3.000	467229.896	1.314	.274
Time * Sex	Sphericity Assumed	344202.699	5	68840.540	.968	.437
	Greenhouse-Geisser	344202.699	4.255	80884.480	.968	.428
	Huynh-Feldt	344202.699	4.802	71676.867	.968	.435
	Lower-bound	344202.699	1.000	344202.699	.968	.328
Time * Dose * Sex	Sphericity Assumed	1434344.334	15	95622.956	1.345	.171
	Greenhouse-Geisser	1434344.334	12.766	112352.591	1.345	.185
	Huynh-Feldt	1434344.334	14.406	99562.756	1.345	.175
	Lower-bound	1434344.334	3.000	478114.778	1.345	.264
Error(Time)	Sphericity Assumed	34127079.535	480	71098.082		
	Greenhouse-Geisser	34127079.535	408.527	83536.989		
	Huynh-Feldt	34127079.535	461.006	74027.424		
	Lower-bound	34127079.535	96.000	355490.412		

Tests of Within-Subjects Contrasts

Measure: LOCO

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	21359727.781	1	21359727.781	180.488	.000
	Quadratic	5001175.491	1	5001175.491	82.053	.000
	Cubic	3841142.338	1	3841142.338	66.101	.000
	Order 4	420577.810	1	420577.810	7.319	.008
	Order 5	106584.115	1	106584.115	1.758	.188

Time * Dose	Linear	333099.375	3	111033.125	.938	.425
	Quadratic	583204.040	3	194401.347	3.189	.027
	Cubic	61873.597	3	20624.532	.355	.786
	Order 4	319944.548	3	106648.183	1.856	.142
	Order 5	103568.129	3	34522.710	.569	.636
Time * Sex	Linear	2.579	1	2.579	.000	.996
	Quadratic	254944.265	1	254944.265	4.183	.044
	Cubic	53079.154	1	53079.154	.913	.342
	Order 4	4951.206	1	4951.206	.086	.770
	Order 5	31225.495	1	31225.495	.515	.475
Time * Dose * Sex	Linear	26409.013	3	8803.004	.074	.974
	Quadratic	879923.157	3	293307.719	4.812	.004
	Cubic	27087.466	3	9029.155	.155	.926
	Order 4	28507.533	3	9502.511	.165	.919
	Order 5	472417.165	3	157472.388	2.597	.057
Error(Time)	Linear	11361053.598	96	118344.308		
	Quadratic	5851249.094	96	60950.511		
	Cubic	5578551.345	96	58109.910		
	Order 4	5516247.095	96	57460.907		
	Order 5	5819978.403	96	60624.775		

Tests of Between-Subjects Effects

Measure: LOCO

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	479438174.137	1	479438174.137	1026.460	.000
Dose	14703010.147	3	4901003.382	10.493	.000
Sex	10582825.944	1	10582825.944	22.657	.000
Dose * Sex	154195.234	3	51398.411	.110	.954
Error	44839590.709	96	467079.070		

General Linear Model

Within-Subjects Factors

Measure: LOCO

Time	Dependent Variable
1	D3_LOCO10
2	D3_LOCO20
3	D3_LOCO30
4	D3_LOCO40
5	D3_LOCO50
6	D3_LOCO60

Sex = F

Between-Subjects Factors[a]

		N
Dose	0	20
	20	11
	30	10
	40	11

a. Sex = F

Tests of Within-Subjects Effects[a]

Measure: LOCO

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	14604778.686	5	2920955.737	37.002	.000
	Greenhouse-Geisser	14604778.686	3.993	3657985.965	37.002	.000
	Huynh-Feldt	14604778.686	4.672	3125853.490	37.002	.000
	Lower-bound	14604778.686	1.000	14604778.686	37.002	.000
Time * Dose	Sphericity Assumed	956022.671	15	63734.845	.807	.669
	Greenhouse-Geisser	956022.671	11.978	79816.741	.807	.642
	Huynh-Feldt	956022.671	14.017	68205.685	.807	.661

	Lower-bound	956022.671	3.000	318674.224	.807	.496
Error(Time)	Sphericity Assumed	18945538.146	240	78939.742		
	Greenhouse-Geisser	18945538.146	191.644	98858.215		
	Huynh-Feldt	18945538.146	224.268	84477.168		
	Lower-bound	18945538.146	48.000	394698.711		

a. Sex = F

Tests of Between-Subjects Effects[a]

Measure: LOCO

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	313163136.395	1	313163136.395	516.190	.000
Dose	6433027.069	3	2144342.356	3.535	.022
Error	29120734.940	48	606681.978		

a. Sex = F

Post Hoc Tests
Dose

Multiple Comparisons[a]

Measure: LOCO

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0	20	12.91	119.364	1.000	-304.76	330.58
	30	244.89	123.155	.207	-82.87	572.65
	40	333.64*	119.364	.036	15.96	651.31
20	0	-12.91	119.364	1.000	-330.58	304.76
	30	231.98	138.937	.351	-137.78	601.75
	40	320.73	135.589	.098	-40.12	681.58
30	0	-244.89	123.155	.207	-572.65	82.87
	20	-231.98	138.937	.351	-601.75	137.78
	40	88.75	138.937	.919	-281.02	458.51
40	0	-333.64*	119.364	.036	-651.31	-15.96
	20	-320.73	135.589	.098	-681.58	40.12
	30	-88.75	138.937	.919	-458.51	281.02

Based on observed means.

The error term is Mean Square(Error) = 101113.663.[a]

*. The mean difference is significant at the .05 level.

a. Sex = F

Univariate Analysis of Variance

Between-Subjects Factors

		N
Time	10	52
	20	52
	30	52
	40	52
	50	52
	60	52

Tests of Between-Subjects Effects

Dependent Variable: LOCO

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15615975.683[a]	5	3123195.137	17.234	.000
Intercept	355778574.490	1	355778574.490	1963.170	.000
Time	15615975.683	5	3123195.137	17.234	.000
Error	55455322.827	306	181226.545		
Total	426849873.000	312			
Corrected Total	71071298.510	311			

a. R Squared = .220 (Adjusted R Squared = .207)

Post Hoc Tests
Time

Multiple Comparisons

Dependent Variable: LOCO

Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
10	20	474.96*	83.488	.000	235.53	714.39
	30	553.31*	83.488	.000	313.87	792.74

	40	498.13*	83.488	.000	258.70	737.57
	50	585.54*	83.488	.000	346.11	824.97
	60	711.46*	83.488	.000	472.03	950.89
20	10	-474.96*	83.488	.000	-714.39	-235.53
	30	78.35	83.488	.936	-161.09	317.78
	40	23.17	83.488	1.000	-216.26	262.61
	50	110.58	83.488	.771	-128.86	350.01
	60	236.50	83.488	.055	-2.93	475.93
30	10	-553.31*	83.488	.000	-792.74	-313.87
	20	-78.35	83.488	.936	-317.78	161.09
	40	-55.17	83.488	.986	-294.61	184.26
	50	32.23	83.488	.999	-207.20	271.66
	60	158.15	83.488	.408	-81.28	397.59
40	10	-498.13*	83.488	.000	-737.57	-258.70
	20	-23.17	83.488	1.000	-262.61	216.26
	30	55.17	83.488	.986	-184.26	294.61
	50	87.40	83.488	.902	-152.03	326.84
	60	213.33	83.488	.112	-26.11	452.76
50	10	-585.54*	83.488	.000	-824.97	-346.11
	20	-110.58	83.488	.771	-350.01	128.86
	30	-32.23	83.488	.999	-271.66	207.20
	40	-87.40	83.488	.902	-326.84	152.03
	60	125.92	83.488	.659	-113.51	365.36
60	10	-711.46*	83.488	.000	-950.89	-472.03
	20	-236.50	83.488	.055	-475.93	2.93
	30	-158.15	83.488	.408	-397.59	81.28
	40	-213.33	83.488	.112	-452.76	26.11
	50	-125.92	83.488	.659	-365.36	113.51

Based on observed means.

The error term is Mean Square(Error) = 181226.545.

*. The mean difference is significant at the 0.05 level.

Sex = M

Between-Subjects Factors[a]

		N
Dose	0	19
	20	11
	30	11
	40	11

a. Sex = M

Tests of Within-Subjects Effects[a]

Measure: LOCO

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	16487133.028	5	3297426.606	52.128	.000
	Greenhouse-Geisser	16487133.028	3.781	4360484.037	52.128	.000
	Huynh-Feldt	16487133.028	4.401	3746108.215	52.128	.000
	Lower-bound	16487133.028	1.000	16487133.028	52.128	.000
Time * Dose	Sphericity Assumed	1872838.072	15	124855.871	1.974	.018
	Greenhouse-Geisser	1872838.072	11.343	165108.158	1.974	.032
	Huynh-Feldt	1872838.072	13.203	141845.039	1.974	.024
	Lower-bound	1872838.072	3.000	624279.357	1.974	.130
Error(Time)	Sphericity Assumed	15181541.389	240	63256.422		
	Greenhouse-Geisser	15181541.389	181.490	83649.662		
	Huynh-Feldt	15181541.389	211.255	71863.739		
	Lower-bound	15181541.389	48.000	316282.112		

a. Sex = M

Tests of Between-Subjects Effects[a]

Measure: LOCO

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	175504833.405	1	175504833.405	535.932	.000
Dose	8420776.270	3	2806925.423	8.571	.000
Error	15718855.769	48	327476.162		

a. Sex = M

Post Hoc Tests
Dose

Multiple Comparisons[a]

Measure: LOCO

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0	20	78.13	88.512	.814	-157.43	313.69
	30	317.04*	88.512	.004	81.48	552.60
	40	388.39*	88.512	.000	152.83	623.95
20	0	-78.13	88.512	.814	-313.69	157.43
	30	238.91	99.617	.091	-26.21	504.03
	40	310.26*	99.617	.016	45.14	575.38
30	0	-317.04*	88.512	.004	-552.60	-81.48
	20	-238.91	99.617	.091	-504.03	26.21
	40	71.35	99.617	.890	-193.77	336.47
40	0	-388.39*	88.512	.000	-623.95	-152.83
	20	-310.26*	99.617	.016	-575.38	-45.14
	30	-71.35	99.617	.890	-336.47	193.77

Based on observed means.

The error term is Mean Square(Error) = 54579.360.[a]

*. The mean difference is significant at the .05 level.

a. Sex = M

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	0	19
	20	11
	30	11
	40	11

Tests of Between-Subjects Effects

Dependent Variable: D3_LOCO10

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	192525.011[a]	3	64175.004	.518	.672
Intercept	80397946.849	1	80397946.849	649.308	.000
Dose	192525.011	3	64175.004	.518	.672
Error	5943402.220	48	123820.880		
Total	92431930.000	52			
Corrected Total	6135927.231	51			

a. R Squared = .031 (Adjusted R Squared = -.029)

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	0	19
	20	11
	30	11
	40	11

Tests of Between-Subjects Effects

Dependent Variable: D3_LOCO20

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2347087.112[a]	3	782362.371	9.196	.000
Intercept	30865267.756	1	30865267.756	362.805	.000
Dose	2347087.112	3	782362.371	9.196	.000
Error	4083555.196	48	85074.067		
Total	41447942.000	52			
Corrected Total	6430642.308	51			

a. R Squared = .365 (Adjusted R Squared = .325)

Post Hoc Tests
Dose

Multiple Comparisons

Dependent Variable: D3_LOCO20

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0	20	-54.32	110.506	.961	-348.41	239.78
	30	319.05*	110.506	.029	24.95	613.15
	40	473.05*	110.506	.001	178.95	767.15
20	0	54.32	110.506	.961	-239.78	348.41
	30	373.36*	124.370	.021	42.37	704.36
	40	527.36*	124.370	.001	196.37	858.36
30	0	-319.05*	110.506	.029	-613.15	-24.95
	20	-373.36*	124.370	.021	-704.36	-42.37
	40	154.00	124.370	.606	-177.00	485.00
40	0	-473.05*	110.506	.001	-767.15	-178.95
	20	-527.36*	124.370	.001	-858.36	-196.37
	30	-154.00	124.370	.606	-485.00	177.00

Based on observed means.

The error term is Mean Square(Error) = 85074.067.

*. The mean difference is significant at the 0.05 level.

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	0	19
	20	11
	30	11
	40	11

Tests of Between-Subjects Effects

Dependent Variable: D3_LOCO30

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2855583.333[a]	3	951861.111	9.308	.000
Intercept	20723793.007	1	20723793.007	202.657	.000
Dose	2855583.333	3	951861.111	9.308	.000
Error	4908493.340	48	102260.278		
Total	33083331.000	52			
Corrected Total	7764076.673	51			

a. R Squared = .368 (Adjusted R Squared = .328)

Post Hoc Tests
Dose

Multiple Comparisons

Dependent Variable: D3_LOCO30

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0	20	263.21	121.155	.146	-59.23	585.65
	30	383.39*	121.155	.014	60.95	705.83
	40	617.57*	121.155	.000	295.14	940.01
20	0	-263.21	121.155	.146	-585.65	59.23
	30	120.18	136.355	.814	-242.71	483.07
	40	354.36	136.355	.058	-8.53	717.26
30	0	-383.39*	121.155	.014	-705.83	-60.95
	20	-120.18	136.355	.814	-483.07	242.71
	40	234.18	136.355	.326	-128.71	597.07
40	0	-617.57*	121.155	.000	-940.01	-295.14
	20	-354.36	136.355	.058	-717.26	8.53
	30	-234.18	136.355	.326	-597.07	128.71

Based on observed means.

The error term is Mean Square(Error) = 102260.278.

*. The mean difference is significant at the 0.05 level.

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	0	19
	20	11
	30	11
	40	11

Tests of Between-Subjects Effects

Dependent Variable: D3_LOCO40

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2558700.923[a]	3	852900.308	8.914	.000
Intercept	24505592.570	1	24505592.570	256.128	.000
Dose	2558700.923	3	852900.308	8.914	.000
Error	4592497.904	48	95677.040		
Total	36055725.000	52			
Corrected Total	7151198.827	51			

a. R Squared = .358 (Adjusted R Squared = .318)

Post Hoc Tests
Dose

Multiple Comparisons

Dependent Variable: D3_LOCO40

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0	20	98.31	117.190	.836	-213.58	410.19
	30	385.49*	117.190	.010	73.60	697.37
	40	546.85*	117.190	.000	234.96	858.74
20	0	-98.31	117.190	.836	-410.19	213.58
	30	287.18	131.893	.144	-63.84	638.20
	40	448.55*	131.893	.007	97.53	799.56
30	0	-385.49*	117.190	.010	-697.37	-73.60

	20	-287.18	131.893	.144	-638.20	63.84
	40	161.36	131.893	.615	-189.65	512.38
40	0	-546.85*	117.190	.000	-858.74	-234.96
	20	-448.55*	131.893	.007	-799.56	-97.53
	30	-161.36	131.893	.615	-512.38	189.65

Based on observed means.

The error term is Mean Square(Error) = 95677.040.

*. The mean difference is significant at the 0.05 level.

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	0	19
	20	11
	30	11
	40	11

Tests of Between-Subjects Effects

Dependent Variable: D3_LOCO50

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1648313.085[a]	3	549437.695	5.188	.003
Intercept	18120592.505	1	18120592.505	171.091	.000
Dose	1648313.085	3	549437.695	5.188	.003
Error	5083775.608	48	105911.992		
Total	28059458.000	52			
Corrected Total	6732088.692	51			

a. R Squared = .245 (Adjusted R Squared = .198)

Post Hoc Tests
Dose

Multiple Comparisons

Dependent Variable: D3_LOCO50

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0	20	93.01	123.299	.874	-235.13	421.16
	30	396.11*	123.299	.012	67.96	724.25
	40	379.20*	123.299	.018	51.05	707.34
20	0	-93.01	123.299	.874	-421.16	235.13
	30	303.09	138.769	.142	-66.22	672.41
	40	286.18	138.769	.180	-83.13	655.50
30	0	-396.11*	123.299	.012	-724.25	-67.96
	20	-303.09	138.769	.142	-672.41	66.22
	40	-16.91	138.769	.999	-386.22	352.41
40	0	-379.20*	123.299	.018	-707.34	-51.05
	20	-286.18	138.769	.180	-655.50	83.13
	30	16.91	138.769	.999	-352.41	386.22

Based on observed means.

The error term is Mean Square(Error) = 105911.992.

*. The mean difference is significant at the 0.05 level.

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	0	19
	20	11
	30	11
	40	11

Tests of Between-Subjects Effects

Dependent Variable: D3_LOCO60

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	691404.879[a]	3	230468.293	1.759	.168
Intercept	17378773.746	1	17378773.746	132.648	.000
Dose	691404.879	3	230468.293	1.759	.168
Error	6288672.890	48	131014.019		
Total	26689620.000	52			
Corrected Total	6980077.769	51			

a. R Squared = .099 (Adjusted R Squared = .043)

LORR Duration

Univariate Analysis of Variance

Between-Subjects Factors

		N
18-MC	10	12
	20	11
	30	12
	Sal	10
Sex	F	23
	M	22

Tests of Between-Subjects Effects

Dependent Variable: LORR Duration (m)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9352.684[a]	7	1336.098	1.223	.315
Intercept	260688.743	1	260688.743	238.644	.000
@18MC	4529.613	3	1509.871	1.382	.263
Sex	3691.031	1	3691.031	3.379	.074
@18MC * Sex	1138.826	3	379.609	.348	.791
Error	40417.826	37	1092.374		
Total	314094.206	45			
Corrected Total	49770.510	44			

a. R Squared = .188 (Adjusted R Squared = .034)

Time to LORR

Univariate Analysis of Variance

Between-Subjects Factors

		N
18-MC	10	12
	20	11
	30	12
	Sal	10
Sex	F	23
	M	22

Tests of Between-Subjects Effects

Dependent Variable: Time to LORR (s)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1323.533[a]	7	189.076	.544	.795
Intercept	352689.070	1	352689.070	1015.646	.000
@18MC	903.723	3	301.241	.867	.467
Sex	271.678	1	271.678	.782	.382
@18MC * Sex	107.801	3	35.934	.103	.958
Error	12848.467	37	347.256		
Total	367952.000	45			
Corrected Total	14172.000	44			

a. R Squared = .093 (Adjusted R Squared = -.078)

EtOH and Saccharin DID

EtOH DID

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

Dose	Time	Dependent Variable
1	1	SAL_30Con
	2	SAL_60Con
	3	SAL_120Con
	4	SAL_240Con
2	1	D10_30Con
	2	D10_60Con
	3	D10_120Con
	4	D10_240Con
3	1	D20_30Con
	2	D20_60Con
	3	D20_120Con
	4	D20_240Con
4	1	D30_30Con
	2	D30_60Con
	3	D30_120Con
	4	D30_240Con

Between-Subjects Factors

		N
Sex	F	12
	M	12

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Dose	Sphericity Assumed	92.650	3	30.883	6.780	.000

	Greenhouse-Geisser	92.650	2.194	42.223	6.780	.002
	Huynh-Feldt	92.650	2.558	36.219	6.780	.001
	Lower-bound	92.650	1.000	92.650	6.780	.016
Dose * Sex	Sphericity Assumed	3.847	3	1.282	.282	.839
	Greenhouse-Geisser	3.847	2.194	1.753	.282	.776
	Huynh-Feldt	3.847	2.558	1.504	.282	.807
	Lower-bound	3.847	1.000	3.847	.282	.601
Error(Dose)	Sphericity Assumed	300.631	66	4.555		
	Greenhouse-Geisser	300.631	48.275	6.228		
	Huynh-Feldt	300.631	56.276	5.342		
	Lower-bound	300.631	22.000	13.665		
Time	Sphericity Assumed	2824.044	3	941.348	308.966	.000
	Greenhouse-Geisser	2824.044	1.361	2074.560	308.966	.000
	Huynh-Feldt	2824.044	1.486	1900.345	308.966	.000
	Lower-bound	2824.044	1.000	2824.044	308.966	.000
Time * Sex	Sphericity Assumed	100.957	3	33.652	11.045	.000
	Greenhouse-Geisser	100.957	1.361	74.164	11.045	.001
	Huynh-Feldt	100.957	1.486	67.936	11.045	.001
	Lower-bound	100.957	1.000	100.957	11.045	.003
Error(Time)	Sphericity Assumed	201.087	66	3.047		
	Greenhouse-Geisser	201.087	29.948	6.715		
	Huynh-Feldt	201.087	32.694	6.151		
	Lower-bound	201.087	22.000	9.140		
Dose * Time	Sphericity Assumed	31.758	9	3.529	2.421	.012
	Greenhouse-Geisser	31.758	2.527	12.566	2.421	.085
	Huynh-Feldt	31.758	3.012	10.544	2.421	.073
	Lower-bound	31.758	1.000	31.758	2.421	.134
Dose * Time * Sex	Sphericity Assumed	6.648	9	.739	.507	.869
	Greenhouse-Geisser	6.648	2.527	2.631	.507	.648
	Huynh-Feldt	6.648	3.012	2.207	.507	.680
	Lower-bound	6.648	1.000	6.648	.507	.484
Error(Dose*Time)	Sphericity Assumed	288.530	198	1.457		
	Greenhouse-Geisser	288.530	55.598	5.190		
	Huynh-Feldt	288.530	66.265	4.354		
	Lower-bound	288.530	22.000	13.115		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	3904.612	1	3904.612	419.491	.000
Sex	30.023	1	30.023	3.226	.086
Error	204.776	22	9.308		

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

Dose	Dependent Variable
1	SAL_30Con
2	D10_30Con
3	D20_30Con
4	D30_30Con

Between-Subjects Factors

		N
Sex	F	12
	M	12

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Dose	Sphericity Assumed	2.380	3	.793	1.986	.125
	Greenhouse-Geisser	2.380	2.789	.853	1.986	.129
	Huynh-Feldt	2.380	3.000	.793	1.986	.125
	Lower-bound	2.380	1.000	2.380	1.986	.173
Dose * Sex	Sphericity Assumed	.949	3	.316	.793	.502

	Greenhouse-Geisser	.949	2.789	.340	.793	.495
	Huynh-Feldt	.949	3.000	.316	.793	.502
	Lower-bound	.949	1.000	.949	.793	.383
Error(Dose)	Sphericity Assumed	26.358	66	.399		
	Greenhouse-Geisser	26.358	61.360	.430		
	Huynh-Feldt	26.358	66.000	.399		
	Lower-bound	26.358	22.000	1.198		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	29.979	1	29.979	76.757	.000
Sex	.956	1	.956	2.447	.132
Error	8.592	22	.391		

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

Dose	Dependent Variable
1	SAL_60Con
2	D10_60Con
3	D20_60Con
4	D30_60Con

Between-Subjects Factors

		N
Sex	F	12
	M	12

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Dose	Sphericity Assumed	14.673	3	4.891	4.563	.006
	Greenhouse-Geisser	14.673	2.464	5.955	4.563	.010
	Huynh-Feldt	14.673	2.925	5.017	4.563	.006
	Lower-bound	14.673	1.000	14.673	4.563	.044
Dose * Sex	Sphericity Assumed	3.134	3	1.045	.975	.410
	Greenhouse-Geisser	3.134	2.464	1.272	.975	.398
	Huynh-Feldt	3.134	2.925	1.072	.975	.409
	Lower-bound	3.134	1.000	3.134	.975	.334
Error(Dose)	Sphericity Assumed	70.737	66	1.072		
	Greenhouse-Geisser	70.737	54.210	1.305		
	Huynh-Feldt	70.737	64.345	1.099		
	Lower-bound	70.737	22.000	3.215		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	154.855	1	154.855	110.731	.000
Sex	.347	1	.347	.248	.623
Error	30.766	22	1.398		

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

Dose	Dependent Variable
1	SAL_120Con
2	D10_120Con
3	D20_120Con
4	D30_120Con

Between-Subjects Factors

		N
Sex	F	12
	M	12

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Dose	Sphericity Assumed	29.876	3	9.959	5.991	.001
	Greenhouse-Geisser	29.876	2.440	12.243	5.991	.003
	Huynh-Feldt	29.876	2.892	10.331	5.991	.001
	Lower-bound	29.876	1.000	29.876	5.991	.023
Dose * Sex	Sphericity Assumed	.425	3	.142	.085	.968
	Greenhouse-Geisser	.425	2.440	.174	.085	.946
	Huynh-Feldt	.425	2.892	.147	.085	.965
	Lower-bound	.425	1.000	.425	.085	.773
Error(Dose)	Sphericity Assumed	109.709	66	1.662		
	Greenhouse-Geisser	109.709	53.685	2.044		
	Huynh-Feldt	109.709	63.622	1.724		
	Lower-bound	109.709	22.000	4.987		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1112.837	1	1112.837	283.287	.000
Sex	1.443	1	1.443	.367	.551
Error	86.423	22	3.928		

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

Dose	Dependent Variable
1	SAL_240Con
2	D10_240Con
3	D20_240Con
4	D30_240Con

Between-Subjects Factors

		N
Sex	F	12
	M	12

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Dose	Sphericity Assumed	77.479	3	25.826	4.458	.007
	Greenhouse-Geisser	77.479	1.788	43.329	4.458	.021
	Huynh-Feldt	77.479	2.024	38.274	4.458	.017
	Lower-bound	77.479	1.000	77.479	4.458	.046

Dose * Sex	Sphericity Assumed	5.986	3	1.995	.344	.793
	Greenhouse-Geisser	5.986	1.788	3.348	.344	.687
	Huynh-Feldt	5.986	2.024	2.957	.344	.713
	Lower-bound	5.986	1.000	5.986	.344	.563
Error(Dose)	Sphericity Assumed	382.357	66	5.793		
	Greenhouse-Geisser	382.357	39.339	9.719		
	Huynh-Feldt	382.357	44.536	8.585		
	Lower-bound	382.357	22.000	17.380		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5430.985	1	5430.985	426.597	.000
Sex	128.235	1	128.235	10.073	.004
Error	280.081	22	12.731		

Means

Case Processing Summary

	Cases					
	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
240Con * Sex	96	100.0%	0	0.0%	96	100.0%

Report

240Con

Sex	Mean	N	Std. Deviation	Std. Error of Mean
F	8.677245465610708	48	3.615105614892864	.521795549976831
M	6.365728066322461	48	1.673711023881222	.241579377545867
Total	7.521486765966586	96	3.033390794773874	.309594151568813

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	10	24
	20	24
	30	24
	SALINE	24

Tests of Between-Subjects Effects

Dependent Variable: 60Con

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14.673[a]	3	4.891	4.286	.007
Intercept	154.855	1	154.855	135.702	.000
Dose	14.673	3	4.891	4.286	.007
Error	104.985	92	1.141		
Total	274.512	96			
Corrected Total	119.657	95			

a. R Squared = .123 (Adjusted R Squared = .094)

Post Hoc Tests
Dose

Multiple Comparisons

Dependent Variable: 60Con

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
10	20	.400338827711252	.308374643196771	.566	-.406558901993237	1.207236557415742
	30	.669403448349400	.308374643196771	.139	-.137494281355089	1.476301178053889
	SALINE	-.359365852784178	.308374643196771	.650	-1.166263582488667	.447531876920311
20	10	-.400338827711252	.308374643196771	.566	-1.207236557415742	.406558901993237
	30	.269064620638147	.308374643196771	.819	-.537833109066342	1.075962350342637
	SALINE	-.759704680495430	.308374643196771	.073	-1.566602410199919	.047193049209059
30	10	-.669403448349400	.308374643196771	.139	-1.476301178053889	.137494281355089

	20	-.269064620638147	.308374643196771	.819	-1.075962350342637	.537833109066342
	SALINE	-1.028769301133578*	.308374643196771	.007	-1.835667030838067	-.221871571429089
SALINE	10	.359365852784178	.308374643196771	.650	-.447531876920311	1.166263582488667
	20	.759704680495430	.308374643196771	.073	-.047193049209059	1.566602410199919
	30	1.028769301133578*	.308374643196771	.007	.221871571429089	1.835667030838067

Based on observed means.

The error term is Mean Square(Error) = 1.141.

*. The mean difference is significant at the 0.05 level.

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	10	24
	20	24
	30	24
	SALINE	24

Tests of Between-Subjects Effects

Dependent Variable: 120Con

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29.876[a]	3	9.959	4.627	.005
Intercept	1112.837	1	1112.837	517.077	.000
Dose	29.876	3	9.959	4.627	.005
Error	198.000	92	2.152		
Total	1340.713	96			
Corrected Total	227.875	95			

a. R Squared = .131 (Adjusted R Squared = .103)

Post Hoc Tests
Dose

Multiple Comparisons

Dependent Variable: 120Con

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
10	20	.494038793014415	.423494451212122	.649	-.614083157879789	1.602160743908619
	30	1.132591929501100*	.423494451212122	.043	.024469978606896	2.240713880395305
	SALINE	-.352181732086715	.423494451212122	.839	-1.460303682980919	.755940218807489
20	10	-.494038793014415	.423494451212122	.649	-1.602160743908619	.614083157879789
	30	.638553136486685	.423494451212122	.437	-.469568814407519	1.746675087380889
	SALINE	-.846220525101130	.423494451212122	.196	-1.954342475995335	.261901425793074
30	10	-1.132591929501100*	.423494451212122	.043	-2.240713880395305	-.024469978606896
	20	-.638553136486685	.423494451212122	.437	-1.746675087380889	.469568814407519
	SALINE	-1.484773661587815*	.423494451212122	.004	-2.592895612482019	-.376651710693611
SALINE	10	.352181732086715	.423494451212122	.839	-.755940218807489	1.460303682980919
	20	.846220525101130	.423494451212122	.196	-.261901425793074	1.954342475995335
	30	1.484773661587815*	.423494451212122	.004	.376651710693611	2.592895612482019

Based on observed means.

The error term is Mean Square(Error) = 2.152.

*. The mean difference is significant at the 0.05 level.

Univariate Analysis of Variance

Between-Subjects Factors

		N
Dose	10	24
	20	24
	30	24
	SALINE	24

Tests of Between-Subjects Effects

Dependent Variable: 240Con

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	77.479[a]	3	25.826	2.983	.035
Intercept	5430.985	1	5430.985	627.182	.000
Dose	77.479	3	25.826	2.983	.035
Error	796.659	92	8.659		
Total	6305.124	96			
Corrected Total	874.139	95			

a. R Squared = .089 (Adjusted R Squared = .059)

Post Hoc Tests
Dose

Multiple Comparisons

Dependent Variable: 240Con

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
10	20	1.081450137053688	.849477253035744	.582	-1.141304820372479	3.304205094479854
	30	2.002685776247582	.849477253035744	.093	-.220069181178584	4.225440733673748
	SALINE	-.246661286414026	.849477253035744	.991	-2.469416243840193	1.976093671012140
20	10	-1.081450137053688	.849477253035744	.582	-3.304205094479854	1.141304820372479
	30	.921235639193895	.849477253035744	.700	-1.301519318232272	3.143990596620061
	SALINE	-1.328111423467714	.849477253035744	.405	-3.550866380893881	.894643533958452
30	10	-2.002685776247582	.849477253035744	.093	-4.225440733673748	.220069181178584
	20	-.921235639193895	.849477253035744	.700	-3.143990596620061	1.301519318232272
	SALINE	-2.249347062661609*	.849477253035744	.046	-4.472102020087775	-.026592105235442
SALINE	10	.246661286414026	.849477253035744	.991	-1.976093671012140	2.469416243840193
	20	1.328111423467714	.849477253035744	.405	-.894643533958452	3.550866380893881
	30	2.249347062661609*	.849477253035744	.046	.026592105235442	4.472102020087775

Based on observed means.

The error term is Mean Square(Error) = 8.659.

*. The mean difference is significant at the 0.05 level.

Saccharin DID

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

Dose	Time	Dependent Variable
1	1	SAL_30Con
	2	SAL_60Con
	3	SAL_120Con
	4	SAL_240Con
2	1	D10_30Con
	2	D10_60Con
	3	D10_120Con
	4	D10_240Con
3	1	D20_30Con
	2	D20_60Con
	3	D20_120Con
	4	D20_240Con
4	1	D30_30Con
	2	D30_60Con
	3	D30_120Con
	4	D30_240Con

Between-Subjects Factors

		N
Sex	F	12
	M	12

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Dose	Sphericity Assumed	302.268	3	100.756	1.088	.360
	Greenhouse-Geisser	302.268	2.792	108.278	1.088	.358
	Huynh-Feldt	302.268	3.000	100.756	1.088	.360
	Lower-bound	302.268	1.000	302.268	1.088	.308
Dose * Sex	Sphericity Assumed	342.078	3	114.026	1.232	.305
	Greenhouse-Geisser	342.078	2.792	122.539	1.232	.305
	Huynh-Feldt	342.078	3.000	114.026	1.232	.305
	Lower-bound	342.078	1.000	342.078	1.232	.279
Error(Dose)	Sphericity Assumed	6110.503	66	92.583		
	Greenhouse-Geisser	6110.503	61.415	99.495		
	Huynh-Feldt	6110.503	66.000	92.583		
	Lower-bound	6110.503	22.000	277.750		
Time	Sphericity Assumed	23586.033	3	7862.011	36.291	.000
	Greenhouse-Geisser	23586.033	1.044	22591.766	36.291	.000
	Huynh-Feldt	23586.033	1.100	21437.519	36.291	.000
	Lower-bound	23586.033	1.000	23586.033	36.291	.000
Time * Sex	Sphericity Assumed	138.388	3	46.129	.213	.887
	Greenhouse-Geisser	138.388	1.044	132.554	.213	.659
	Huynh-Feldt	138.388	1.100	125.782	.213	.672
	Lower-bound	138.388	1.000	138.388	.213	.649
Error(Time)	Sphericity Assumed	14297.956	66	216.636		
	Greenhouse-Geisser	14297.956	22.968	622.510		
	Huynh-Feldt	14297.956	24.205	590.705		
	Lower-bound	14297.956	22.000	649.907		
Dose * Time	Sphericity Assumed	159.364	9	17.707	.653	.751
	Greenhouse-Geisser	159.364	3.501	45.521	.653	.607
	Huynh-Feldt	159.364	4.434	35.943	.653	.642
	Lower-bound	159.364	1.000	159.364	.653	.428
Dose * Time * Sex	Sphericity Assumed	435.971	9	48.441	1.786	.073
	Greenhouse-Geisser	435.971	3.501	124.531	1.786	.148
	Huynh-Feldt	435.971	4.434	98.328	1.786	.131
	Lower-bound	435.971	1.000	435.971	1.786	.195
Error(Dose*Time)	Sphericity Assumed	5370.064	198	27.122		

	Greenhouse-Geisser	5370.064	77.020	69.723		
	Huynh-Feldt	5370.064	97.544	55.053		
	Lower-bound	5370.064	22.000	244.094		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	40515.936	1	40515.936	38.634	.000
Sex	20.675	1	20.675	.020	.890
Error	23071.762	22	1048.716		

Univariate Analysis of Variance

Between-Subjects Factors

		N
Time	30	96
	60	96
	120	96
	240	96

Tests of Between-Subjects Effects

Dependent Variable: DID

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	23586.033[a]	3	7862.011	59.455	.000
Intercept	40515.936	1	40515.936	306.395	.000
Time	23586.033	3	7862.011	59.455	.000
Error	50249.029	380	132.234		
Total	114350.998	384			
Corrected Total	73835.062	383			

a. R Squared = .319 (Adjusted R Squared = .314)

Post Hoc Tests
Time

Multiple Comparisons

Dependent Variable: DID

Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
30	60	-2.535003143055060	1.659783407712687	.422	-6.817966060084116	1.747959773973996
	120	-8.258877660329938*	1.659783407712687	.000	-12.541840577358993	-3.975914743300883
	240	-20.326717776057410*	1.659783407712687	.000	-24.609680693086467	-16.043754859028354
60	30	2.535003143055060	1.659783407712687	.422	-1.747959773973996	6.817966060084116
	120	-5.723874517274878*	1.659783407712687	.003	-10.006837434303934	-1.440911600245823
	240	-17.791714633002353*	1.659783407712687	.000	-22.074677550031410	-13.508751715973297
120	30	8.258877660329938*	1.659783407712687	.000	3.975914743300883	12.541840577358993
	60	5.723874517274878*	1.659783407712687	.003	1.440911600245823	10.006837434303934
	240	-12.067840115727474*	1.659783407712687	.000	-16.350803032756530	-7.784877198698418
240	30	20.326717776057410*	1.659783407712687	.000	16.043754859028354	24.609680693086467
	60	17.791714633002353*	1.659783407712687	.000	13.508751715973297	22.074677550031410
	120	12.067840115727474*	1.659783407712687	.000	7.784877198698418	16.350803032756530

Based on observed means.

The error term is Mean Square(Error) = 132.234.

*. The mean difference is significant at the 0.05 level.

Metabolism

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

Time	Dependent Variable
1	@30_BEC_mgdladjust
2	@60_BEC_mgdladjust
3	@120_BEC_mg.dladjust
4	@180_BEC_mg.dladjust

Between-Subjects Factors

		N
SEX	F	15
	M	15
Dose	0	10
	20	10
	30	10

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	209961.911	3	69987.304	172.767	.000
	Greenhouse-Geisser	209961.911	1.623	129358.583	172.767	.000
	Huynh-Feldt	209961.911	2.087	100621.010	172.767	.000
	Lower-bound	209961.911	1.000	209961.911	172.767	.000

Time * SEX	Sphericity Assumed	6896.295	3	2298.765	5.675	.002
	Greenhouse-Geisser	6896.295	1.623	4248.842	5.675	.010
	Huynh-Feldt	6896.295	2.087	3304.943	5.675	.005
	Lower-bound	6896.295	1.000	6896.295	5.675	.025
Time * Dose	Sphericity Assumed	3502.102	6	583.684	1.441	.211
	Greenhouse-Geisser	3502.102	3.246	1078.831	1.441	.244
	Huynh-Feldt	3502.102	4.173	839.164	1.441	.233
	Lower-bound	3502.102	2.000	1751.051	1.441	.256
Time * SEX * Dose	Sphericity Assumed	4807.002	6	801.167	1.978	.080
	Greenhouse-Geisser	4807.002	3.246	1480.809	1.978	.129
	Huynh-Feldt	4807.002	4.173	1151.841	1.978	.110
	Lower-bound	4807.002	2.000	2403.501	1.978	.160
Error(Time)	Sphericity Assumed	29166.965	72	405.097		
	Greenhouse-Geisser	29166.965	38.954	748.746		
	Huynh-Feldt	29166.965	50.080	582.409		
	Lower-bound	29166.965	24.000	1215.290		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	15132499.304	1	15132499.304	2059.353	.000
SEX	1923.194	1	1923.194	.262	.614
Dose	9602.341	2	4801.170	.653	.529
SEX * Dose	4904.840	2	2452.420	.334	.720
Error	176356.321	24	7348.180		

General Linear Model

Within-Subjects Factors

Measure: MEASURE_1

Time	Dependent Variable
1	@30_BEC_mgdladjust
2	@60_BEC_mgdladjust
3	@120_BEC_mg.dladjust
4	@180_BEC_mg.dladjust

SEX = F

Between-Subjects Factors[a]

		N
Dose	0	5
	20	5
	30	5

a. SEX = F

Tests of Within-Subjects Effects[a]

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	132799.215	3	44266.405	111.044	.000
	Greenhouse-Geisser	132799.215	1.965	67597.360	111.044	.000
	Huynh-Feldt	132799.215	2.737	48517.449	111.044	.000
	Lower-bound	132799.215	1.000	132799.215	111.044	.000
Time * Dose	Sphericity Assumed	1707.518	6	284.586	.714	.641
	Greenhouse-Geisser	1707.518	3.929	434.580	.714	.588

	Huynh-Feldt	1707.518	5.474	311.916	.714	.629
	Lower-bound	1707.518	2.000	853.759	.714	.509
Error(Time)	Sphericity Assumed	14351.025	36	398.640		
	Greenhouse-Geisser	14351.025	23.575	608.746		
	Huynh-Feldt	14351.025	32.846	436.922		
	Lower-bound	14351.025	12.000	1195.919		

a. SEX = F

Tests of Between-Subjects Effects[a]

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	7396616.021	1	7396616.021	678.310	.000
Dose	12547.743	2	6273.871	.575	.577
Error	130853.715	12	10904.476		

a. SEX = F

Univariate Analysis of Variance

Between-Subjects Factors

		N
Time	30	15
	60	15
	120	15
	180	15

Tests of Between-Subjects Effects

Dependent Variable: BEC_mg/dl adjust

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	132799.215[a]	3	44266.405	15.546	.000
Intercept	7396616.021	1	7396616.021	2597.582	.000
Time	132799.215	3	44266.405	15.546	.000
Error	159460.001	56	2847.500		
Total	7688875.237	60			
Corrected Total	292259.216	59			

a. R Squared = .454 (Adjusted R Squared = .425)

Post Hoc Tests
Time

Multiple Comparisons

Dependent Variable: BEC_mg/dl adjust

Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
30	60	-2.745135493333351	19.485037038664807	.999	-54.339318373096820	48.849047386430120
	120	49.191566179999940	19.485037038664807	.067	-2.402616699763534	100.785749059763420
	180	113.086961599999940*	19.485037038664807	.000	61.492778720236466	164.681144479763420
60	30	2.745135493333351	19.485037038664807	.999	-48.849047386430120	54.339318373096820
	120	51.936701673333290*	19.485037038664807	.048	.342518793569816	103.530884553096770
	180	115.832097093333290*	19.485037038664807	.000	64.237914213569810	167.426279973096770
120	30	-49.191566179999940	19.485037038664807	.067	-100.785749059763420	2.402616699763534
	60	-51.936701673333290*	19.485037038664807	.048	-103.530884553096770	-.342518793569816
	180	63.895395420000000*	19.485037038664807	.009	12.301212540236527	115.489578299763480
180	30	-113.086961599999940*	19.485037038664807	.000	-164.681144479763420	-61.492778720236466
	60	-115.832097093333290*	19.485037038664807	.000	-167.426279973096770	-64.237914213569810
	120	-63.895395420000000*	19.485037038664807	.009	-115.489578299763480	-12.301212540236527

Based on observed means.

The error term is Mean Square(Error) = 2847.500.

*. The mean difference is significant at the 0.05 level.

SEX = M

Between-Subjects Factors[a]

		N
Dose	0	5
	20	5
	30	5

a. SEX = M

Tests of Within-Subjects Effects[a]

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	84058.990	3	28019.663	68.083	.000
	Greenhouse-Geisser	84058.990	1.250	67258.136	68.083	.000
	Huynh-Feldt	84058.990	1.558	53959.156	68.083	.000
	Lower-bound	84058.990	1.000	84058.990	68.083	.000
Time * Dose	Sphericity Assumed	6601.586	6	1100.264	2.673	.030
	Greenhouse-Geisser	6601.586	2.500	2641.064	2.673	.093
	Huynh-Feldt	6601.586	3.116	2118.845	2.673	.075
	Lower-bound	6601.586	2.000	3300.793	2.673	.110
Error(Time)	Sphericity Assumed	14815.940	36	411.554		
	Greenhouse-Geisser	14815.940	14.998	987.890		
	Huynh-Feldt	14815.940	18.694	792.554		
	Lower-bound	14815.940	12.000	1234.662		

a. SEX = M

Tests of Between-Subjects Effects[a]

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	7737806.477	1	7737806.477	2040.623	.000
Dose	1959.438	2	979.719	.258	.776
Error	45502.607	12	3791.884		

a. SEX = M

Univariate Analysis of Variance

Dose = 0

Between-Subjects Factors[a]

		N
Time	30	5
	60	5
	120	5
	180	5

a. Dose = 0

Tests of Between-Subjects Effects[a]

Dependent Variable: BEC_mg/dl adjust

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	35283.024[b]	3	11761.008	9.946	.001
Intercept	2645867.598	1	2645867.598	2237.517	.000
Time	35283.024	3	11761.008	9.946	.001
Error	18920.024	16	1182.502		
Total	2700070.645	20			
Corrected Total	54203.048	19			

a. Dose = 0

b. R Squared = .651 (Adjusted R Squared = .585)

Post Hoc Tests

Time

Multiple Comparisons[a]

Dependent Variable: BEC_mg/dl adjust

Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
30	60	-18.458669799999996	21.748577171364758	.831	-80.681780589976140	43.764440989976150
	120	30.669789799999990	21.748577171364758	.511	-31.553320989976157	92.892900589976140
	180	92.198689100000020*	21.748577171364758	.003	29.975578310023877	154.421799889976170

60	30	18.458669799999996	21.748577171364758	.831	-43.764440989976150	80.681780589976140
	120	49.128459599999985	21.748577171364758	.150	-13.094651189976162	111.351570389976130
	180	110.657358900000020*	21.748577171364758	.001	48.434248110023870	172.880469689976170
120	30	-30.669789799999990	21.748577171364758	.511	-92.892900589976140	31.553320989976157
	60	-49.128459599999985	21.748577171364758	.150	-111.351570389976130	13.094651189976162
	180	61.528899300000035	21.748577171364758	.053	-.694211489976112	123.752010089976180
180	30	-92.198689100000020*	21.748577171364758	.003	-154.421799889976170	-29.975578310023877
	60	-110.657358900000020*	21.748577171364758	.001	-172.880469689976170	-48.434248110023870
	120	-61.528899300000035	21.748577171364758	.053	-123.752010089976180	.694211489976112

Based on observed means.

The error term is Mean Square(Error) = 1182.502.

*. The mean difference is significant at the 0.05 level.

a. Dose = 0

Dose = 20

Between-Subjects Factors[a]

		N
Time	30	5
	60	5
	120	5
	180	5

a. Dose = 20

Tests of Between-Subjects Effects[a]

Dependent Variable: BEC_mg/dl adjust

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	30159.070[b]	3	10053.023	8.059	.002
Intercept	2464874.175	1	2464874.175	1975.844	.000
Time	30159.070	3	10053.023	8.059	.002
Error	19960.071	16	1247.504		
Total	2514993.317	20			
Corrected Total	50119.142	19			

a. Dose = 20

b. R Squared = .602 (Adjusted R Squared = .527)

Post Hoc Tests
Time

Multiple Comparisons[a]

Dependent Variable: BEC_mg/dl adjust

Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
30	60	-7.004828539999949	22.338347909516774	.989	-70.915285113176860	56.905628033176970
	120	31.711048080000012	22.338347909516774	.506	-32.199408493176904	95.621504653176930
	180	91.346750500000040*	22.338347909516774	.004	27.436293926823126	155.257207073176970
60	30	7.004828539999949	22.338347909516774	.989	-56.905628033176970	70.915285113176860
	120	38.715876619999960	22.338347909516774	.340	-25.194579953176955	102.626333193176880
	180	98.351579039999990*	22.338347909516774	.002	34.441122466823074	162.262035613176920
120	30	-31.711048080000012	22.338347909516774	.506	-95.621504653176930	32.199408493176904
	60	-38.715876619999960	22.338347909516774	.340	-102.626333193176880	25.194579953176955
	180	59.635702420000030	22.338347909516774	.072	-4.274754153176886	123.546158993176950
180	30	-91.346750500000040*	22.338347909516774	.004	-155.257207073176970	-27.436293926823126
	60	-98.351579039999990*	22.338347909516774	.002	-162.262035613176920	-34.441122466823074
	120	-59.635702420000030	22.338347909516774	.072	-123.546158993176950	4.274754153176886

Based on observed means.

The error term is Mean Square(Error) = 1247.504.

*. The mean difference is significant at the 0.05 level.

a. Dose = 20

Dose = 30

Between-Subjects Factors[a]

		N
Time	30	5
	60	5
	120	5
	180	5

a. Dose = 30

Tests of Between-Subjects Effects[a]

Dependent Variable: BEC_mg/dl adjust

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25218.482[b]	3	8406.161	6.274	.005
Intercept	2629024.143	1	2629024.143	1962.100	.000
Time	25218.482	3	8406.161	6.274	.005
Error	21438.451	16	1339.903		
Total	2675681.076	20			
Corrected Total	46656.933	19			

a. Dose = 30

b. R Squared = .541 (Adjusted R Squared = .454)

Post Hoc Tests
Time

Multiple Comparisons[a]

Dependent Variable: BEC_mg/dl adjust

Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
30	60	-59.067743319999980	23.150837332727040	.089	-125.302748253179960	7.167261613180003
	120	-20.351866699999960	23.150837332727040	.816	-86.586871633179940	45.883138233180020
	180	39.283835720000010	23.150837332727040	.357	-26.951169213179966	105.518840653179990
60	30	59.067743319999980	23.150837332727040	.089	-7.167261613180003	125.302748253179960
	120	38.715876620000020	23.150837332727040	.369	-27.519128313179962	104.950881553180000
	180	98.351579039999990*	23.150837332727040	.003	32.116574106820010	164.586583973179980
120	30	20.351866699999960	23.150837332727040	.816	-45.883138233180020	86.586871633179940
	60	-38.715876620000020	23.150837332727040	.369	-104.950881553180000	27.519128313179962
	180	59.635702419999970	23.150837332727040	.085	-6.599302513180007	125.870707353179950
180	30	-39.283835720000010	23.150837332727040	.357	-105.518840653179990	26.951169213179966
	60	-98.351579039999990*	23.150837332727040	.003	-164.586583973179980	-32.116574106820010
	120	-59.635702419999970	23.150837332727040	.085	-125.870707353179950	6.599302513180007

Based on observed means.

The error term is Mean Square(Error) = 1339.903.

*. The mean difference is significant at the 0.05 level.

a. Dose = 30

Univariate Analysis of Variance

Time = 30

Between-Subjects Factors[a]

		N
Dose	0	5
	20	5
	30	5

a. Time = 30

Tests of Between-Subjects Effects[a]

Dependent Variable: BEC_mg/dl adjust

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3741.388[b]	2	1870.694	.770	.484
Intercept	2099734.135	1	2099734.135	864.676	.000
Dose	3741.388	2	1870.694	.770	.484
Error	29140.177	12	2428.348		
Total	2132615.700	15			
Corrected Total	32881.565	14			

a. Time = 30

b. R Squared = .114 (Adjusted R Squared = -.034)

Post Hoc Tests
Dose

Multiple Comparisons[a]

Dependent Variable: BEC_mg/dl adjust

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0	20	9.749945539999999	31.166315646290577	.948	-73.397535363318610	92.897426443318610
	30	37.295960460000000	31.166315646290577	.477	-45.851520443318606	120.443441363318610

20	0	-9.749945539999999	31.166315646290577	.948	-92.897426443318610	73.397535363318610
	30	27.546014920000005	31.166315646290577	.660	-55.601465983318604	110.693495823318610
30	0	-37.295960460000000	31.166315646290577	.477	-120.443441363318610	45.851520443318606
	20	-27.546014920000005	31.166315646290577	.660	-110.693495823318610	55.601465983318604

Based on observed means.

The error term is Mean Square(Error) = 2428.348.

a. Time = 30

Time = 60

Between-Subjects Factors[a]

		N
Dose	0	5
	20	5
	30	5

a. Time = 60

Tests of Between-Subjects Effects[a]

Dependent Variable: BEC_mg/dl adjust

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1769.426[b]	2	884.713	.981	.403
Intercept	2427910.276	1	2427910.276	2691.036	.000
Dose	1769.426	2	884.713	.981	.403
Error	10826.657	12	902.221		
Total	2440506.359	15			
Corrected Total	12596.083	14			

a. Time = 60

b. R Squared = .140 (Adjusted R Squared = -.003)

Post Hoc Tests
Dose

Multiple Comparisons[a]

Dependent Variable: BEC_mg/dl adjust

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	20	21.203786800000046	18.997067215623332	.523	-29.477798455589850	71.885372055589940
	30	-3.313113059999978	18.997067215623332	.983	-53.994698315589870	47.368472195589916
20	0	-21.203786800000046	18.997067215623332	.523	-71.885372055589940	29.477798455589850
	30	-24.516899860000024	18.997067215623332	.427	-75.198485115589920	26.164685395589870
30	0	3.313113059999978	18.997067215623332	.983	-47.368472195589916	53.994698315589870
	20	24.516899860000024	18.997067215623332	.427	-26.164685395589870	75.198485115589920

Based on observed means.

The error term is Mean Square(Error) = 902.221.

a. Time = 60

Time = 120

Between-Subjects Factors[a]

		N
Dose	0	5
	20	5
	30	5

a. Time = 120

Tests of Between-Subjects Effects[a]

Dependent Variable: BEC_mg/dl adjust

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1509.872[b]	2	754.936	.890	.436
Intercept	1945430.137	1	1945430.137	2292.960	.000
Dose	1509.872	2	754.936	.890	.436

Error	10181.233	12	848.436		
Total	1957121.241	15			
Corrected Total	11691.105	14			

a. Time = 120

b. R Squared = .129 (Adjusted R Squared = -.016)

Post Hoc Tests
Dose

Multiple Comparisons[a]

Dependent Variable: BEC_mg/dl adjust

Tukey HSD

(I)	(J)				95% Confidence Interval	
Dose	Dose	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
0	20	10.791203820000021	18.422117809388350	.830	-38.356494808827560	59.938902448827605
	30	-13.725696039999946	18.422117809388350	.742	-62.873394668827530	35.422002588827640
20	0	-10.791203820000021	18.422117809388350	.830	-59.938902448827605	38.356494808827560
	30	-24.516899859999967	18.422117809388350	.406	-73.664598488827550	24.630798768827617
30	0	13.725696039999946	18.422117809388350	.742	-35.422002588827640	62.873394668827530
	20	24.516899859999967	18.422117809388350	.406	-24.630798768827617	73.664598488827550

Based on observed means.

The error term is Mean Square(Error) = 848.436.

a. Time = 120

Time = 180

Between-Subjects Factors[a]

		N
Dose	0	5
	20	5
	30	5

a. Time = 180

Tests of Between-Subjects Effects[a]

Dependent Variable: BEC_mg/dl adjust

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1540.338[b]	2	770.169	.909	.429
Intercept	1348790.920	1	1348790.920	1591.419	.000
Dose	1540.338	2	770.169	.909	.429
Error	10170.480	12	847.540		
Total	1360501.738	15			
Corrected Total	11710.818	14			

a. Time = 180

b. R Squared = .132 (Adjusted R Squared = -.013)

Post Hoc Tests
Dose

Multiple Comparisons[a]

Dependent Variable: BEC_mg/dl adjust

Tukey HSD

(I) Dose	(J) Dose	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0	20	8.898006940000016	18.412387198304860	.880	-40.223731745136305	58.019745625136340
	30	-15.618892920000008	18.412387198304860	.681	-64.740631605136330	33.502845765136314
20	0	-8.898006940000016	18.412387198304860	.880	-58.019745625136340	40.223731745136305
	30	-24.516899860000024	18.412387198304860	.406	-73.638638545136350	24.604838825136298
30	0	15.618892920000008	18.412387198304860	.681	-33.502845765136314	64.740631605136330
	20	24.516899860000024	18.412387198304860	.406	-24.604838825136298	73.638638545136350

Based on observed means.

The error term is Mean Square(Error) = 847.540.

a. Time = 180

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Academic Vita

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Education

Pennsylvania State University, University Park, PA

B.S. in Pre-Medicine with honors in Biobehavioral Health

Projected Graduation: May 2019

Thesis title, The Effects of the α3β4 Nicotinic Acetylcholine Receptor Antagonist, 18-Methoxycoronaridine (18-MC), on Alcohol-Related Behaviors

Research Experience & Fellowships

- **University Health Services Clinic Intern**, Penn State University
  Measure patients' vital signs and collect other pertinent, medical information
  January 2018 – December 2018
- **Behavioral Neurogenetics Lab**, Penn State University
  Conduct independent research on the effect of an experimental drug 18-Methoxycoronaridine on alcohol behaviors
  Lab Head: Dr. Helen Kamens August 2017 – Present
- **Translational Neuroimaging and Systems Neuroscience Lab**, Penn State University
  Researched effects of nicotine on activated brain regions and resting-state brain networks of rats
  Lab Head: Dr. Nanyin Zhang September 2016 – August 2017
- **Atlantis Project Fellowship**, Vigo, Spain
  Shadowed neurological and orthopedic surgeons at Hospital `Alvaro Cunqueiro, which serves over 600,000 residents. May, 2017

Shadowing experience

- Dr. Cesáreo Conde Alonso, M.D., Neurosurgery, Vigo, Spain, May, 2017
- Dr. Gregory Fulchiero, M.D., Dermatology, Altoona, PA, April, 2015
- Dr. George Cummings III, D.O., General Surgery, Altoona, PA, January, 2015

Honors and Awards

- Schreyer Honors College – One of only 300 students chosen out of over 3,900 applicants
- Dean's List - Penn State University, Fall 2015, Spring 2016, Spring 2017, Fall 2017, Spring 2018, Fall 2018
- Erickson Discovery Grant – Penn State University, Summer 2018

Community Service & Volunteer Activities

- Saint Mary Catholic Church - Volunteer for various activities including soup kitchen, food drives, and decorating church for holidays. Hollidaysburg, PA, 2013-Current
- UPMC Altoona Hospital, Patient Support Volunteer May, 2017 - August, 2017
- THON Fundraising, Penn State University October, 2016
- Autism Walk – Setup committee member. Altoona, PA, Summers, 2013 – 2015