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Predicting Alcohol Consumption in Adolescent
Rhesus Macaques (*Macaca mulatta*)

Andrea N. Sorenson

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

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ABSTRACT

Predicting Alcohol Consumption in Adolescent Rhesus Macaques (*Macaca mulatta*)

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Master of Science

Numerous studies show that a low level of response to the intoxicating effects of alcohol is considered a risk factor for future alcoholism. However, assessing this sensitivity usually requires administering a controlled dose of alcohol, which has a number of inherent problems. Early observations in our lab suggest that the response to anesthetics that show cross tolerance with alcohol, like ketamine, are blunted in nonhuman primates at risk for high alcohol intake, and may be a viable measure of future alcohol consumption. This study was designed to test potential predictors of future alcohol consumption using the change in ketamine across repeated exposures (i.e., tolerance). In addition, potential mediating factors of alcohol consumption, including early temperament and behavior, were assessed.

Subjects were 16 three-year-old, alcohol naïve rhesus macaque males raised by their biological mothers. *Ketamine Exposure*-Each subject was exposed to three 10.0 mg/kg intramuscular doses of ketamine. The time from injection to recovery from anesthetic was recorded for each dose, to be used as a measure of subject's sensitivity and developed tolerance. *Alcohol Intake Assessment*-Two weeks after the final ketamine dose, subjects were allowed ad libitum access to a palatable 8.4% alcohol solution for two-hours a day, five days a week, for six weeks. During the Two-Choice phase of testing, subjects were simultaneously given ad libitum access to the 8.4% alcohol solution and to a sweetened solution for two-hours a day, five days a week, for four weeks. Solution consumption was recorded daily and averaged across the weeks for each phase of alcohol testing. *Temperament and Behavior*-As infants, all subjects participated in a bio-behavioral assessment (BBA), when they were between 90 and 120 days of age. Data collected during the BBA on subjects' temperament (Vigilance, Gentleness, Confidence, and Nervousness) and Behavior (Activity and Emotionality) were used in analyses.

Results showed a relationship between the tolerance developed between ketamine doses and average alcohol consumption during the Alcohol-Only phase ($r = 0.61$, $R^2 = 0.372$, $F(1,14) = 8.300$, $p = 0.012$). Average alcohol consumption during the Alcohol-Only phase was also related to ratings of Confidence ($r = 0.499$, $R^2 = 0.249$, $F(1,14) = 4.647$, $p = 0.049$), Activity (Day 1: $r = 0.503$, $R^2 = 0.253$, $F(1,14) = 4.732$, $p = 0.047$; Day 2: $r = 0.455$, $R^2 = 0.207$, $F(1,14) = 3.652$, $p = 0.077$), and Emotionality ($r = 0.466$, $R^2 = 0.217$, $F(1,14) = 3.885$, $p = 0.069$).

The results of this study suggest that change in ketamine recovery time and early life temperament and behaviors may be measures of future risk for alcohol abuse disorders. This data is limited by the small sample size and future study is necessary to further tease out the relationships between these variables and alcohol consumption.

Keywords: alcohol, ketamine, temperament, rhesus macaque, inherent sensitivity, tolerance

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Predicting Alcohol Consumption in

Adolescent Rhesus Macaques

(Macaca mulatta)

The rate of alcohol abuse and dependency in American adults increased by 7.41% between 1992 and 2002, leading to approximately 17.6 million adults in the United States that either abuse or are dependent on alcohol (i.e. alcoholism), and this rate continues to increase (National Institutes on Alcohol Abuse and Alcoholism [hereafter NIAAA], 2004). Not only are more adults abusing and showing dependence on alcohol than in the past; but, alcohol consumption and abuse is increasing in adolescents as well. A 2008 study by Johnston, O'Malley, Bachman, and Schulenberg showed an alarming number of underaged 8th, 10th, and 12th graders had used alcohol (39%, 62%, and 72% respectively). Many of the adolescents who reported having consumed alcohol also report participating in heavy drinking (more than five consecutive drinks at a time). Early use of alcohol is thought to be an important risk factor for alcoholism, with as much as 47% of adolescents who used alcohol at an early age became alcohol dependent by the age of 21 (NIAAA, 2006). Finding ways to more accurately identify individuals who have a propensity for alcoholism is a major focus and initiative of the NIAAA. Because alcohol use disorders are modulated by CNS mechanisms, and because administering alcohol to children is not possible, researchers have increasingly used animal models, including nonhuman primates.

Why Use Nonhuman Primates

Nonhuman primates, particularly rhesus macaques, are ideally suited as models for human behavior and physiology, particularly for the study of alcoholism. Rhesus macaques, like humans, live in complex social groups, forming specific and long term social bonds and

relationships within their groups (Gerald & Higley, 2002; Weinstein & Capitanio, 2012). Like humans, they also demonstrate individual temperaments and personalities that are relatively stable from an early age (Capitanio, Mason, Mendoza, Del Rosso, & Roberts, 2006; Gerald & Higley, 2002), and their physiological responses, especially during periods of stress, parallel humans in many ways (Higley & Bennett, 1999; Sorenson, Sullivan, Mendoza, Capitanio, & Higley, 2013). In addition, nonhuman primates are phylogenetically similar to humans, sharing a large percentage of their DNA, often allowing genetic studies of the same systems seen in humans. These similarities between nonhuman primates and humans more readily allow researchers to generalize their results from an animal model to humans than is possible when using nonhuman subjects who are less closely related and are behaviorally less similar.

Until recently, modeling alcoholism using nonhuman primates was thought to be difficult if not impossible because it was thought that nonhuman primates would not consume alcohol in amounts that would lead to abuse or dependency (Meisch, Hanninfield, & Thompson, 1975; Crowley, Weisbard, & Hydingier-MacDonald, 1983). More recently, however, researchers have shown that like humans, about 15-20 percent of rhesus macaques will drink to intoxication on a more or less daily basis if given the opportunity (Barr, Schwandt, Newman, & Higley, 2004; Higley & Linnoila, 1997).

An advantage to using a nonhuman primate model of alcohol abuse is that developmental patterns of drinking are known in detail and their development can be closely controlled to model underlying variables of human alcohol abuse acquisition. The current study, among others from our laboratory, models the typical acquisition pattern seen in teens as they first begin to use alcohol. Among humans there is a progression from palatable, sweetened, low ethanol concentration drinks to drinks with higher concentrations of alcohol (Lanier, Hayes, & Duffy,

2005). As with humans, when an alcohol solution is palatable, rhesus macaques, and other nonhuman primate species, drink initially at low rates, but over time, some subjects drink alcohol to excess, showing about the same percentage of abuse as humans (Ervin, Palmour, Young, Guzman-Flores, & Juarez, 1990; Higley, Hasert, Suomi, & Linnoila, 1991).

Limitations on Alcoholism Research Designed to Investigate Risk

Studies show that one of the major risk factors for alcohol abuse and alcoholism is inherent sensitivity or changes in sensitivity to alcohol, with relative insensitivity and large changes in sensitivity after initial exposure being predictive of future high alcohol intake and alcohol abuse (Barr et al., 2004; Schuckit, 1994). One primary limitation in performing research on adolescent alcohol use and abuse is that the majority of human alcoholism research that focuses on risk factors in children and adolescents must be retrospective, since it is not ethical to expose underage subjects to alcohol. In addition, most often, when individuals tested are of legal drinking age, they typically are not alcohol naïve, but instead, have a prior, uncontrolled (and often unknown), history of alcohol consumption which confounds interpretations. Nonhuman primate models enable researchers to have greater control over a subject's history of alcohol consumption. Researchers also have greater control over the developmental environments of nonhuman primates than they do in human subjects. Social settings, living conditions, and even with laboratory dwelling monkeys consume is controlled and maintained by the researchers. The control that comes with nonhuman primate studies also extends to a comprehensive medical history. Nonhuman primate research allows researchers to control for a wide variety of external factors that could constrain or influence the data on sensitivity to alcohol that is collected.

One problem with studies of alcohol sensitivity is the narrow safety range of alcohol consumption. Low doses of alcohol often produces little variation among subjects and

administration of larger doses of alcohol, at levels that produce significant intoxication, whether in humans or nonhuman primates, produces intoxication in some but can lead to unconsciousness in others and high alcohol intake may increase the risk to the health of a subject. These risks include accidents, vomiting, alcohol poisoning, and possibly death depending on the dose and rate of infusion or consumption. Moreover, exposing adolescents to alcohol when they are alcohol naïve is widely considered unethical and as noted above, suggest that it may increase the risk for alcohol abuse and alcoholism. Because of these risk factors, it is difficult to study the effects of high doses of alcohol on young human subjects.

In monkeys, there are two methods of alcohol administration, oral and intravenous. If consumed orally, the alcohol must pass through the stomach before entering into the blood stream, and prior food consumption as well as other factors can affect the rate of absorption. Intravenous injections of alcohol bypass the stomach, directly entering the blood stream. This is a quicker method of delivery that provides a more accurate blood alcohol concentration than oral ingestion; however, because intravenous doses are quickly transported in a much less diluted fashion than orally consumed alcohol, it quickly crosses the blood brain barrier leading to high brain alcohol concentrations that may suppress breathing and other vital brain centers. Because it does not have to pass through the gut, it is rapidly infused throughout the brain and body, making it more dangerous than oral consumption, particularly as the dosage of alcohol increases. Because of these limitations, finding predictive measures of alcoholism that does not endanger the subjects has marked utility.

Alcoholism

Types of alcoholism. Research on alcoholism has shown that there are two primary types of alcoholism and that these two groups are separated by differences in behavior and

biological characteristics. Until Cloninger's 1987 research, most research on alcoholism focused on alcoholics as a unified group, with similar underlying behavioral and biological causes. Cloninger showed that there were at least two subgroups of alcoholism (1987). One subgroup, discussed by Cloninger, is Type I alcoholism. This type of alcoholism is characterized by being high in perfectionism, introversion and stress induced alcohol intake to mitigate anxiety (Cloninger, 1987; Rice, & Van Arsdale, 2010). The other subgroup, discussed by Cloninger, is Type II alcoholism. This type of alcoholism is characterized by impaired impulse control, violence and aggression, particularly following alcohol intake (Cloninger, 1987), and low CNS serotonin functioning (SOURCE). Those alcoholics that fall into this category are initially motivated to consume alcohol for its euphoric effects. Because they also show high levels of impulsivity, this initial motivation is then followed by episodes of binge-like, excessive alcohol intake. Type 2 alcoholics also show high tolerance and studies suggest that the characteristic low CNS serotonin seen in Type 2 alcoholics is in part a mediating factor of the relative insensitivity to alcohol (Heinz et al., 1998; Heinz, Jones et al., 2003).

Alcoholism in nonhuman primates. Cloninger's Type II classification of alcohol abuse disorders is further supported by research using a nonhuman primate model of alcoholism. Studies show that nonhuman primates who consume alcohol also exhibit behavioral and biological traits similar to those seen in humans with Type II alcoholism (Higley & Linnoila, 1997). As in humans, low or impaired CNS serotonin functioning, impulsive behavior and high levels of aggression form the bases of Type II alcoholism (Higley & Linnoila, 1997). In rhesus macaques Type II alcoholism is expressed through risk taking, antisocial behavior such as social alienation, difficulties in relationships, higher rates of aggression, and a lack of impulse control (Cloninger, 1987; Higley, Suomi, and Linnoila, 1996 a and b). Centrally, rhesus macaques that

demonstrate Type II-like high alcohol intake also show impaired CNS serotonin, as measured by low CSF 5-HIAA concentrations (Higley et al., 1996b). Soubrié's now classic review of behavioral psychopathology in animals and humans with impaired CNS serotonin functioning show difficulties in withholding impulses, and excessive and inappropriate aggression, particularly following provocation (Soubrié, 1986). Impaired CNS serotonin, as measured by low levels of CSF 5-HIAA may explain, at least in part, why impulsivity may modulate Type II alcoholism. In studies using rhesus macaques, subjects who made impulsive long, dangerous leaps at heights of more than ten meters and entered baited traps repeatedly (i.e. exhibited impulsive and dangerous behavior), engaged in impulsive aggression and violence, showed low levels of CSF 5-HIAA (Mehlman et al, 1994; Westergaard et al., 2003). This suggests that impulsive behavior is directly related to levels of CSF 5-HIAA and may be why impulsivity plays such a huge role in Type II alcoholism.

Sensitivity to Alcohol and Ketamine and High Alcohol Intake

In Schuckit's original 1985 study of sensitivity to the intoxicating effects of alcohol, he and his colleagues found that there are stable individual differences in nonalcoholic male college students for alcohol, with males who had a close alcoholic relative showing less sensitivity to the intoxicating effects of alcohol. In Schuckit's study, subjects were given weight controlled specific doses of alcohol and then their ability to maintain balance without swaying was measured. The less a subject swayed while standing on one foot the more insensitive he was considered to be to the intoxicating effects of alcohol. Schuckit consistently found that those subjects who had a family history of alcoholism swayed less than those subjects who did not have a family history of alcoholism. Those with a family history of alcoholism also reported fewer feelings of intoxication (Schuckit, 1985). In a follow up study ten years later, he and his

colleagues found that the subjects who as college students exhibited relative insensitivity to the effects of alcohol were more likely to have developed alcoholism or other alcohol abuse disorders than those men who showed high sensitivity to the intoxicating effects of alcohol and this relationship was independent of and similar in effect size to that of family history for alcoholism (Schuckit, 1994). This indicates that initial sensitivity to alcohol may be a predictor of risk for alcohol abuse disorders. Studies suggest that this relative insensitivity to alcohol is related to serotonin deficits. Subjects with relative insensitivity to alcohol were more likely to be classified as being homozygous for the short allele of the serotonin transporter genotype (Barr, et al., 2003). Given the limitations of using under age subjects for these types of studies, using a nonhuman primate model may enable further research and understanding of the relationship between inherent and acquired sensitivity, the intoxicating effects of alcohol and the acquisition of alcohol abuse and dependency.

Alcohol is glutamate receptor antagonist acting on the N-methyl-D-aspartate (NMDA) (Anis, Berry, Burton, & Lodger, 1983; Orser, Pennefather, & MacDonald, 1997). Many studies show that the effect of alcohol on NMDA receptors is related to the development of alcohol dependency (Nagy, J., 2008). In order to test this hypothesis, human subjects with and without a family history of alcoholism, and with no personal history of alcohol dependency, were injected with low doses of ketamine. Ketamine is an anesthetic that also affects the NMDA glutamate receptor. One study showed that individuals with a family history of alcoholism took longer to experience the effects of ketamine and recovered faster than those who did not have a family history of alcohol consumption (Petrakis, 2004). Still other studies in nonhuman primates show that low central serotonin functioning is related to decreased sensitivity to ketamine, with subjects possessing low CSF 5-HIAA waking up quicker from ketamine and other forms of

anesthesia (Heinz, et al., 1998; and Heinz, Schafer, Higley, Krystal, & Goldman, 2003), suggesting that Type II alcoholics with low or impaired central serotonin may also show relative insensitivity to the intoxicating and rewarding effects of alcohol. This suggests that those with a family history of alcoholism are more likely to be insensitive to the effects of ketamine. Rapid tolerance to ketamine has been shown to develop in macaque species after repeated exposures (Pouget, Wattiez, Rivaud-Pechoux, & Gaymard, 2010). This is similar to the tolerance that develops on the NMDA glutamate receptor after repeated exposure to alcohol. Still other studies show that there is a cross-tolerance effect between alcohol and ketamine (Krystal et al., 2003). Such cross-tolerance may allow ketamine sensitivity to be used as a predictive measure of alcoholism.

A nonhuman primate model is ideal for studying this relationship between alcohol and ketamine. Ketamine can be easily administered and within a few weeks its potential to predict high alcohol intake and abuse can be measured. Ketamine is a short-lived anesthetic that is injected intramuscularly, most commonly used in animal care and research, and because of its wide dose safety range and short recovery time, it is often used in children. Moreover, ketamine can be given in large doses, without the risk for breathing suppression seen with alcohol. Ketamine is a regulated substance, thus even in nonhuman primates all previous exposures to ketamine are recorded for each subject. The nature of the nonhuman primate model allows researchers control for subject's previous exposure to ketamine and alcohol.

Hypotheses

In this study the relationship between the change in ketamine tolerance and subsequent alcohol intake was assessed in adolescent rhesus macaques. Adolescent nonhuman primate subjects were used because they are at an age comparable to when most humans first use alcohol.

It is hypothesized that adolescent macaque subjects' change in tolerance to ketamine, as measured by the change from an initial dose to subsequent doses, will be predictive of alcohol intake. Additionally, it is hypothesized that early life temperament and behavioral ratings will have an influential relationship with alcohol consumption in adolescence.

Methods

Subjects

Subjects were 16 male rhesus macaques raised with their biological mothers in one of 8 0.2 hectare (30.5 m x 61 m) field cages at the California National Primate Research Center (CNPRC) in Davis California. Subjects were approximately three years old (see Table 1). Each field cage contained a social group of 100-150 rhesus macaques, living in a social condition that closely parallels the natural condition. For the purposes of this study, subjects were removed from their field cages and moved into individual cages (60 x 65 x 79 cm, Lab Products Inc., Maywood NJ), within the indoor testing room, two weeks prior to the beginning of testing and were housed in the same location for the duration of the study. During the entirety of testing, subjects were given ad libitum access to water, along with dietary appropriate standard monkey chow, thrice a week fruits and vegetables, and foods that encourage foraging (i.e., enrichment). Other forms of enrichment (toys, puzzles, etc.) were also provided each day. All produce, enrichment, and evening portion of standard monkey chow was provided after testing on applicable days. On the completion of this study subjects were returned to the general research pool of the CNPRC.

BBA

All subjects for this study were selected from a cohort of 3-year-old males born in 2007, tested in a bio-behavioral assessment (BBA [see Capitanio, Mendoza, & Cole, 2011]). The BBA

is a 25-hour long test, during which time rhesus macaque infants between 90 and 120 days of age are separated from their mother and their social group and placed in an individual cage in an indoor testing room with up to 8 other similarly aged subjects, who were also housed alone. All infants are placed in holding cages, 60 x 65 x 79 cm (Lab Products Inc., Maywood, NJ) and had no visual or physical contact with the other subjects. During the BBA, subjects were tested using a number of behavioral paradigms and observations are performed periodically throughout testing. All testing was performed individually and any interactions observed are between the subject and the observer. At the end of the 25-hour testing period, infants were returned to their mothers and their social group. All behavior temperament data used in this study were collected during the BBA.

Temperament. Temperament measures taken during the BBA were used to assess the relationship between early infant temperament and alcohol intake. One trained observer, with 10 years' experience collecting BBA behavioral data and an established reliability rating of greater than 85% agreement, collected all of the behavioral data for each subject. At the end of testing, the observer completed a rating of 16 traits for each animal. This rating uses a 7-point Likert scale for each individual temperament trait. Using factor analysis, four factors were previously identified (each named for the trait with the highest loading) and are as follows: Vigilance (vigilant, not depressed, not tense, and not timid); Gentle (calm, flexible, gentle, and curious); Confidence (bold, active, confident, curious, and playful); and Nervous (fearful, nervous, timid, not calm, and not confident). The standardized temperament factor scores were used in statistical analyses (See Golub, Hogrefe, Widaman, and Capitanio, 2009; Weinstein and Capitanio, 2008 for further details).

Holding cage observations. Objective behavior data was also collected. In order to assess the effects of separation and relocation on infant behavior during the BBA, behavioral observations were completed at the beginning (Day 1) and the end (Day 2) of the 25-hour testing period. The data collected on Day 1 is considered representative of the subjects' immediate response to the separation and relocation, whereas the data collected on Day 2 is representative of the infant's adaptation to the situation. Using the ObserverTM software, a trained observer watched and recorded infant behavior for a 5-minute period each day. This data included activity states (sit, stand, locomote, etc.), as well as event states (vocalizations, self-directed behaviors, lipsmacking, etc.). Using factor analysis, a two-factor solution was found to fit the data and the categories were labeled "Activity" and "Emotionality". The Activity variable indicates the proportion of time the subject spent active and exploring the environment, as well as whether they ate food, drank water, and/or crouched during the observation. The Emotionality variable is representative of the amount of time the subject spent cooing and barking (measures of anxiety), and whether they scratched, displayed threats, and/or lipsmacked (measures of irritation/aggression). Scores for Activity and Emotionality were z-scored and the data from Day 1 and Day 2 are used in the analyses. (see Golub et al., 2009 for further methodological details)

Adolescent Ketamine Exposure

Two to three weeks after being moved into the indoor testing room for this study, the adolescent subjects were anesthetized using ketamine in order to collect both blood and CSF samples. Prior to testing all subjects had been exposed to ketamine (Mean = 10). All subjects were exposed to ketamine twice yearly for standard health checks, additional exposures for injury or illness. Preliminary analyses showed no relationship between previous exposures to

ketamine or the time since subjects' last ketamine exposure and the amount of time to subjects took to recover from ketamine.

Experimental dosing of ketamine. Following acclimation to the testing environment, subjects were again anesthetized for cerebrospinal fluid (CSF) removal. Ketamine dosage was based on weight (10 mg/kg body weight; Table 1) and was injected intramuscularly, four subjects received additional 30 mg doses of ketamine to maintain anesthesia for CSF removal. Preliminary analyses showed that these subjects showed no differences from the other subjects on any of the variables studied. All subjects were anesthetized within five minutes of researchers entering the testing room, and the specific time of injection was recorded for each subject.

Measuring ketamine recovery time. After the CSF sample collection was completed, subjects were placed back into their individual cages and monitored as they recovered from anesthesia. Using a standardized scoring system, every five minutes a subject's "wakefulness" was assessed on a scale of 0-3. A score of 0 was given to those subjects who were lying down and unmoving. A score of 1 was given to subjects who were lying down but had begun moving. A score of 2 was given if subjects were attempting to sit up, move their limbs with some voluntary control; but were unable to sit up without falling over and/or hold their head above their shoulders. A score of 3 was given when they were able to sit up on their own, did not fall over, and could hold their head above the shoulders. Once subjects received a rating of 3, they were considered fully awake from anesthesia and the time since the original injection was recorded in seconds. The time between initial injection and receiving a rating of 3 is considered to be the amount of time (in seconds) it took the subjects to recover from the dose of ketamine (hereafter called Ketamine Recovery Time [KRT]). This time to recover was used as a measure

of a subject's sensitivity to alcohol. Coders were blind to the animals' genotype, previous behavior, and response to ketamine.

Between 3 and 10 days following this baseline sample collection participants took part in behavioral testing, after which they were anesthetized for a second time. Ketamine dosage was 10 mg/kg, and identical methods were used to measure KRT2 as those used for KRT1. A third dose of ketamine was administered between 4 and 7 days later and identical behavioral coding was performed to measure recovery time. The average time to receive a rating of 3 for the three doses of ketamine are as follows: KRT 1 = 4147.44 sec, KRT 2 = 2933.63 sec, KRT 3 = 2989.44 sec. No subject required a second dose of ketamine during the second or third KRT measurement. Preliminary analysis showed no relationship between ketamine dosage and KRTs.

Ketamine tolerance. Change in tolerance to ketamine was measured by subtracting the KRTs for episodes 2 and 3 from KRT 1, and KRT 3 was subtracted from KRT 2. This produced three measures of change in time to recover, in seconds, between ketamine doses (hereafter called change in Ketamine Recovery Time [change in KRT]). The reduction in time taken to recover from ketamine was considered representative of the degree at which subjects develop a tolerance to ketamine from dose to dose.

Training and Experimental Alcohol-Only Phase

Two weeks after subjects were given their third dose of ketamine, the training and Two-phase alcohol exposure paradigm began. Using an established protocol (Higley et al., 1996 a and b), subjects were introduced to the automated alcohol dispenser used to measure alcohol intake. This was done by allowing subjects to consume an aspartame-sweetened vehicle for three consecutive days from the drinking apparatus. On each of those three days, subjects were given access to 200 mls of the sweet vehicle for 60 minutes to allow them to become familiar with the

apparatus and drinking station. All subjects consumed the sweetened solution. After this three-day introduction, ethanol was added to the aspartame solution in sufficient quantities to produce an 8.4% alcohol aspartame solution and subjects were allowed *ad libitum* access during testing times. Prior to this study all subjects were alcohol naïve.

The alcohol/aspartame mixture was dispensed via home-cage drinking spouts. The amount of solution consumed was measured via a computerized system that automatically weighed and recorded the amount of the alcohol and aspartame solution consumed. Subjects were given access to the alcohol/aspartame mixture for two-hours at a time, five days a week, for six consecutive weeks. Testing was performed at the same time each day, during the early afternoon. A red light was turned on above the alcohol drinking spout on each apparatus, indicating to subjects that the alcohol solution was available for consumption. Subjects were observed during the full two-hour period to assure: they had continued access to the alcohol/aspartame solution, the safety of the animals, and that the equipment was functioning properly. After three weeks of alcohol testing four subjects were removed from testing because they did not consume sufficient alcohol to meet the pre-established criteria used in other studies (Higley et al., 1996a), an amount necessary to experience the pharmacological effects of alcohol. These subjects were removed from the remainder of testing, resulting in a sample size of 12 subjects for some portions of analyses.

Two-Choice Phase: Alcohol and Aspartame

Immediately following the sixth week of alcohol consumption, subjects were given access to both the sweetened solution from the Alcohol-Only phase of testing as well as the sweetened 8.4% alcohol solution. This portion of testing was completed in order to determine subject's preference and whether subjects' alcohol consumption from the first portion of testing

was due to the sweetness of the alcohol solution, or if they would choose to continue consuming the sweetened alcohol solution, even though a sweet nonalcoholic beverage was also available.

For this portion of testing, all procedures were identical to the Alcohol-Only phase, except subjects were given *ad libitum* access to both solutions, and a green light was turned on above a second adjacent drinking spout to indicate to subjects that the sweetened solution was available for consumption. Subjects were again observed during the full two-hour testing period. All analyses of alcohol consumption were performed using mg/kg, a typical measure of alcohol consumption. All procedures for this study were conducted according to the Guidelines for Use and Care of Laboratory Animals of the National Research Council and approved, prior to implementation, by the CNPRC.

Data Analysis

Prior to analyzing the data, a correlation was performed to assess interindividual stability between alcohol consumption during the Alcohol-Only phase of testing, weeks 1-6. Results showed that weeks 2-6 were significantly positively correlated at the $p < 0.01$ level. Thus we used the overall alcohol intake mean across weeks to perform our analyses. Because the first week is somewhat a learning phase and because correlations between the first week of alcohol consumption and later weeks were not as consistent as the correlation between subsequent weeks, the average of alcohol consumption during the Alcohol-Only phase of this study does not include the first week of testing.

Similar correlations were performed on the consumption data for both alcohol and aspartame solutions consumed during the Two-Choice phase of the study. As in the Alcohol-Only phase, the four weeks of aspartame consumption were positively correlated with one another at the $p < 0.015$ level or less. The four weeks of alcohol consumption data also highly

correlated with one another at the $p < 0.01$ level or less. Averages for both Alcohol and Aspartame consumption during the Two-Choice phase of testing were created using all four weeks of data. The averages created of solution consumption during the Alcohol-Only phase and the Two-Choice phase of the study are representative of the subject's typical drinking patterns during the course of testing.

Preliminary analyses, using Linear Regression and univariate Analysis of Variance (ANOVA), were also run to determine the effect (if any) of potential confounding variables on alcohol consumption and KRTs. These potential confounding variables include age of subjects at time of testing (days), the number of previous exposures the subject had had with ketamine, and the number of days between doses of ketamine. Additional preliminary analyses, again using Linear Regression, were run to determine the relationship(s) between average alcohol consumption during the Alcohol-Only phase and average alcohol and aspartame consumption (respectively) during the Two-Choice phase of testing.

In order to assess the relationship between average alcohol consumption (dependent variable [DV]) and sensitivity to ketamine, Linear Regressions were run for each change in KRT with change in KRT as the independent variable (IV). Linear Regression was also used to examine the relationship between average alcohol consumption (DV) and change in tolerance to ketamine, using the changes in KRT from the first dose of ketamine to the second and third doses (IV). In addition to assessing the previously mentioned relationship, Linear Regression was used to determine the predictive relationship of early life temperament (IV) and behavior (Activity and Emotionality) (IV) to average alcohol consumption (DV).

Supplementary analyses were run using univariate ANOVA to determine if there were significant differences between those four subjects who did not consume alcohol and were

removed from testing and those 12 subjects who meet the requirements for alcohol consumption. Specifically, univariate ANOVAs were run with KRT, change in KRT, early life temperament, and behavior (Activity and Emotionality) as the independent variables.

Results

Preliminary Analysis

The age of subjects was not related to average alcohol consumption ($p > 0.20$), KRTs ($p > 0.23$) nor the number of previous exposures to ketamine and average alcohol consumption ($p > 0.17$), KRTs ($p = 0.39$). Neither age nor previous exposures to ketamine were related to change in KRTs ($p > 0.45$). KRTs were not related to the number of days between ketamine testing ($p > 0.91$), although the number of days between ketamine doses 1 and 2 approached traditional levels of significance ($p > 0.09$). Therefore, age, prior ketamine exposure, and time between ketamine doses were not used in subsequent analyses.

Alcohol and Aspartame

Average alcohol consumption was not significantly related to consumption of the aspartame vehicle ($p > 0.33$). As has been shown in other studies (Higley, Hasert, Suomi, & Linnoila, 1991; Higley, Suomi, & Linnoila, 1996a), the average alcohol consumption during the Alcohol-Only phase of testing was significantly related to the average amount of alcohol consumed during the Two-Choice phase of testing ($r = 0.611$, $t(10) = 2.440$, $p = 0.035$; see Figure 1), with average alcohol consumption during the Alcohol-Only phase of testing accounting for a significant proportion of variance in average alcohol consumption during the Two-Choice phase of testing ($R^2 = 0.373$, $F(1,10) = 5.952$, $p = 0.035$).

Actual Ketamine Recovery Time

There was no significant relationship between average alcohol consumption during the Alcohol-Only phase of testing and the actual KRTs 1 ($p = 0.252$) and 3 ($p = 0.346$). However, a significant relationship was found for average alcohol consumption and KRT 2 ($r = -0.504$, $t(14) = -2.185$, $p = 0.046$).

Change in Ketamine Recovery Time

While there was not a significant relationship with absolute time to recover from ketamine, the change in KRT from dose 1 to dose 3 showed a significant relationship with average alcohol consumption ($r = 0.61$, $t(14) = 2.881$, $p = 0.012$; see Figure 2), and the change in KRT from dose 1 to dose 2 showed a nearly significant relationship with average alcohol consumption ($r = 0.487$, $t(14) = 2.085$, $p = 0.056$; see Figure 3). Not surprisingly given the minimal change in KRT between dose 2 and dose 3, no significant relationship was found between average alcohol consumption and the change in KRT from dose 2 to dose 3 ($p = 0.554$). Both the change in KRT between doses 1 and 3 and between doses 1 and 2 accounted for between 37 and 24% of the variance in average alcohol consumption (Change in KRT from 1 to 3: $R^2 = 0.372$, $F(1,14) = 8.300$, $p = 0.012$; Change in KRT from dose 1 to 2: $R^2 = 0.237$, $F(1,14) = 4.347$, $p = 0.056$).

Furthermore, the univariate ANOVA used to compare those subjects who met the pre-established alcohol intake criteria to be included in the study with those who did not meet the criteria showed that those subjects who were removed from the study showed that the non-drinkers had significantly lower change scores between ketamine doses 1 and 2 when compared to those who consumed sufficient alcohol to meet criteria ($p = 0.032$, $F(1,14) = 5.690$), with a similar trend being seen with the change between ketamine doses 1 and 3 ($p = 0.062$, $F(1,14) =$

4.117). No other significant relationships were found between those who consumed alcohol and those who did not consume alcohol.

BBA

Activity and emotionality. A significant relationship was found between Day 1 Activity and average alcohol consumption during the first 6 weeks of testing ($r = 0.503$, $t(14) = 2.175$, $p = 0.047$, see Figure 4) and a nearly significant relationship between Day 2 of Activity and average alcohol consumption ($r = 0.455$, $t(14) = 1.911$, $p = 0.077$, see Figure 5). Time spent being Active on both Day 1 and Day 2, accounting for between 20 and 25% of the variance in average alcohol consumption (Day 1: $R^2 = 0.253$, $F(1,14) = 4.732$, $p = 0.047$; Day 2: $R^2 = 0.207$, $F(1,14) = 3.652$, $p = 0.077$).

A trending relationship was found between average alcohol consumption and Day 1 Emotionality ($r = -0.466$, $t(14) = -1.971$, $p = 0.069$, see Figure 6). This trending relationship with alcohol intake accounting for about 22% of the variance in average alcohol consumption ($R^2 = 0.217$, $F(1,14) = 3.885$, $p = 0.069$). No significant relationship was found between Day 2 Emotionality and average alcohol consumption ($p = 0.242$).

Temperament. Confidence was related to average alcohol consumption ($r = 0.499$, $t(14) = 2.156$, $p = 0.049$, see Figure 7). Confidence was found to account for a significant proportion of variance in alcohol consumption ($R^2 = 0.249$, $F(1, 14) = 4.647$, $p = 0.049$). Vigilance, Gentleness, and Nervousness did not significantly correlate with average alcohol consumption (Vigilance, $p = 0.411$; Gentleness, $p = 0.235$, and Nervousness, $p = 0.998$).

Discussion

Our hypotheses were largely supported. Change in KRTs from the first exposure to the second and third was related to average alcohol consumption. Our results suggest that rapid

tolerance to ketamine is predictive of high alcohol intake, with subjects who demonstrate the largest decrease in ketamine recovery (i.e., acquisition of tolerance) consuming the most alcohol. Or put another way, subjects who show minimal or an increase in sensitivity to ketamine, as demonstrated by an increase in KRT between doses, consumed the least amount of alcohol. This suggests a cross-tolerance between ketamine and alcohol, possibly as a result of common actions on the NMDA receptor. This is consistent with other studies in mice and rats (Fidecka & Langwiriski, 1989; Khanna, Wu, & Kalant, 1991; Khanna, Kalant, Shah, & Chau, 1992; Khanna, Shah, Weiner, Wu, & Kalant, 1993) and with nonhuman primates using a drug discrimination paradigm (Ford, Davis, McCracken, & Grant, 2005).

Given that ketamine and alcohol show cross-tolerance, our findings suggest that the change in ketamine recovery time between doses may provide measure of an individual's susceptibility to high alcohol intake and possibly alcohol abuse disorders. This is further supported by the relationship found between those who consumed alcohol and those who were removed from testing because they did not consume alcohol. Those subjects who were removed from testing because they did not consume enough alcohol to qualify for the study showed lower change scores in KRT than those who consumed enough alcohol to qualify for the study. Interestingly, unlike Schuckit and others (Schuckit, 1985; Schuckit, 1994) finding that the response to a single dose was predictive of alcohol intake and abuse, the relationship between the actual time to recover from a dose of ketamine was at best weak (only the change from dose 1 to dose 3); on the other hand, we found a much stronger relationship for the change between and early dose and subsequent doses. This finding is consistent with an earlier study from our laboratory, showing that the change in levels of intoxication between an initial IV dose of alcohol and a second IV dose of alcohol were predictive of subsequent oral alcohol intake, and

while the change in tolerance was predictive of future alcohol intake, the actual level of intoxication was not statistically significant. Our subject population was relatively small, and it may be that we were underpowered to detect a relationship between the absolute time to wake up and alcohol intake. What is clear is that the change in tolerance is much more strongly related to future alcohol consumption than the actual time to recover.

The relationship between temperament and alcohol consumption (i.e., temperamental measures of high Confidence, and temperamental holding cage behaviors [high Activity-seen as a measure of coping- and low Emotionality]) and high alcohol intake, suggest that early life temperament is related to alcohol consumption later in life. Our analyses show that high Activity (significant for Day 1 and trending for Day 2), low emotionality (trending Day 1), and high confidence ratings is related to higher alcohol intake. This data suggests that the measures of Confidence, Activity, and Emotionality obtained during the BBA, as infants, may provide additional measures for alcohol abuse disorder risk factors.

Reflecting on the definitions for these behavioral categories, those subjects who consumed the most alcohol were also the most likely to be bold and curious, to eat and drink in a new environment, and were the least likely to exhibit anxiety. Previous research shows that temperament measures early in life, such as Confidence, are related to future impulsivity (Capitanio, 1999), one of the hallmark traits of Type II alcoholism and abuse. In behavioral and temperament assessments, rates of impulsivity are often determined by short latency to approach novel stimuli, being less restrained and taking risks in new situations (Parker & Bagby, 1997). Directly related to impulsivity is novelty seeking, which has been characterized as locomotor response in a new environment and includes eating and drinking new foods (i.e. Activity), and is often shown to be related to ADHD (Bird & Schenk, 2013). The Confidence and Activity

behaviors assessed during the BBA seem to be measuring rates of impulsivity and novelty seeking. Research has shown impulsivity and novelty seeking are both related to alcohol consumption, with those high in impulsivity and novelty seeking consuming more alcohol and at higher risk for long-term alcohol abuse (Cloninger, 1987; Hayton, Mahony, & Olmstead, 2012; Cloninger & Sigvardsson, 2006).

BBA Emotionality is a measure of subject's irritation, aggression, and anxiety over the novel situation they have been placed in. Type I alcoholism, previously defined as being characterized by being high in perfectionism, introversion, anxiety and stress (Cloninger, 1987; Rice, & Van Arsdale, 2010), and would be reflected in a high Emotionality rating. Distress vocalizations, freezing (i.e., a lack of activity) and self-directed behaviors have been found to be indicators of stress and anxiety in nonhuman primates (Hrdina, von Kulmiz, & Stretch, 1979; Baker & Aureli, 1997), but in this study, subjects who consumed the most alcohol also had the lowest emotionality ratings. This suggests that the alcohol consumption patterns seen in this study are not reflective of Type I alcoholism.

Initial analysis of alcohol consumption during the alcohol-only phase and the Two-Choice phase of the study showed significant relationships between average alcohol consumption during both parts of testing, with no relationship between alcohol intake and aspartame solution consumption. As has been shown in other studies (Vivian, Higley, Linnoila, & Woods, 1999), our findings suggest that consumption of alcohol is not driven by a preference for sweetened beverages, but instead for the pharmacological effects of alcohol. They also suggest that alcohol drinking patterns established when only alcohol is available are predictive of alcohol consumption under other conditions, such as when other reinforcers are available. Our data also showed that individual patterns of alcohol consumption stabilized after the first few

weeks when only alcohol was available. This, along with the correlation between average alcohol consumption during the Alcohol-Only phase and average alcohol consumption during the Two-Choice phase, suggests that the alcohol consumption pattern seen throughout testing is potentially representative of long-term alcohol consumption.

The predictive relationships of change in KRT, Confidence, Activity, and Emotionality to average alcohol consumption may be interrelated. In Meyer and Phillips study on the effect ethanol has on locomotion in mice, they found that ketamine and ethanol produced additive effects in a dose-response curve (2003). The subjects in this study showed cross tolerance between alcohol and ketamine doses, suggesting that they are both acting through a common mechanism, possibly the NMDA receptor. This cross-tolerance related to a subject's levels of Activity after dosing. As mentioned previously, high activity is also related to impulsivity and novelty seeking. While no direct relationship was seen between the change in KRT and early life temperament and behavior in this study, it is possible that with a larger sample size that the relationship between these measures of alcohol consumption would emerge.

One limitation of this study is that the results are based on a relatively small sample size, and should be considered a pilot study. All results and implications of this data warrant further scrutiny and testing with a larger sample size. Thus using change in KRT and early life temperament and behavior as measures of future risk for alcoholism is premature, although promising.

An additional limitation is the short period of time alcohol consumption data was collected. The predictive measures found may be predictive of initial alcohol consumption, but it is possible that they are not predictive of long-term alcohol consumption. However, previous research by (Higley et al., 1996a) indicate that differences in alcohol intake are stable after the

first few weeks of alcohol consumption, although as a group alcohol intake increases with continued exposure. This suggests that the rates of alcohol consumption seen in this study may be reflective of subject's long-term use.

Alcoholism is a prevalent disorder in society and nonhuman primate research is currently being used to study the underlying etiology of alcoholism. One of the difficulties with studying of inherent tolerance to alcohol as a predictive measure of future risk is the narrow window of alcohol safety. Patients (human and nonhuman alike) must be continually monitored for breathing and other health problems after modest doses of alcohol. The benefit of finding alternative measures, like change in ketamine recovery time over repeated doses of ketamine, temperament, behavior and serotonin genotype, as predictive measures of long-term alcohol consumption patterns is that they are relatively safe. Ketamine can be administered in doses that produce unconsciousness, as was done in this study, but these doses seldom resulting in medical difficulties or interventions (*typically the higher the dose, the longer the time to recover, and one must administer an extraordinary dose of ketamine to put a subject at risk*). As noted earlier, it can be administered intramuscularly avoiding variations in absorption and it has a rapid onset (90-120 seconds for unconsciousness). It will be interesting to see if a lower dose that does not produce unconsciousness can be used in humans, where behaviors and ratings for the degree of its effect can be measured. Being able to use any or all of these measures to predict future alcohol consumption has potential application in clinical settings. This research also has the potential to aid alcohol researchers in subject selection (i.e., Pre-determine which subjects are likely to consume alcohol).

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1300171

Table 1*Demographics for subjects' age and weight*

Subjects	Average Weight (kg)	Age (days)
1	5.61	1163
2	4.72	1185
3	5.2	1118
4	6.56	1166
5	4.81	1176
6	6.17	1083
7	5.69	1142
8	6.05	1179
9	5.41	1133
10	5.33	1187
11	5.01	1138
12	5.11	1148
13	4.87	1148
14	5.38	1123
15	5.21	1127
16	4.97	1141
Average	5.38	1147.31

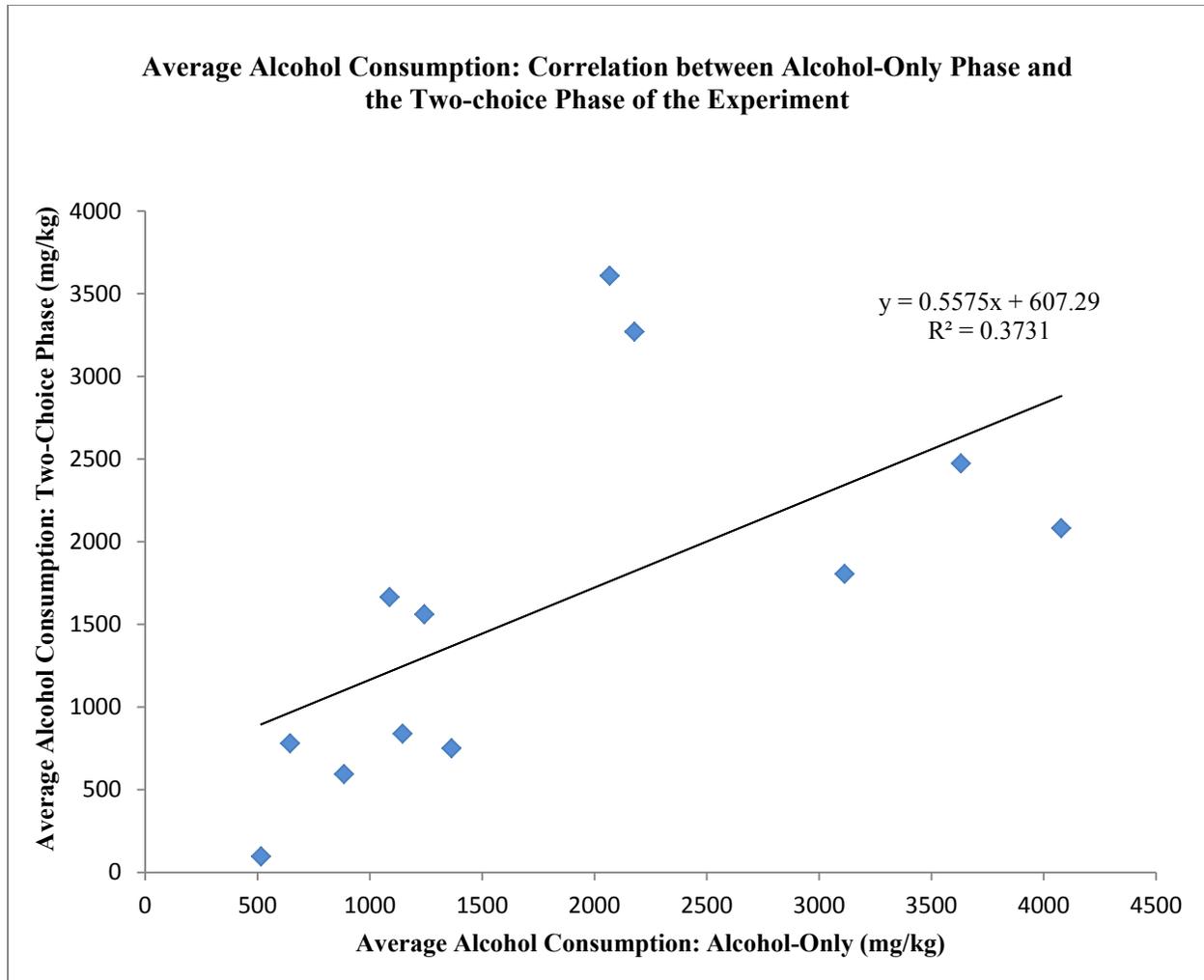


Figure 1

Scatter plot of the relationship between average alcohol consumption during the Alcohol-Only phase of testing and average alcohol consumption during the Two-Choice phase of testing ($n = 12$, $p = 0.035$). The Y-axis reflects alcohol intake during the Two-Choice phase of the experiment. The X-axis shows alcohol intake during the Alcohol-Only phase of the experiments. Alcohol is reported in mg/kg.

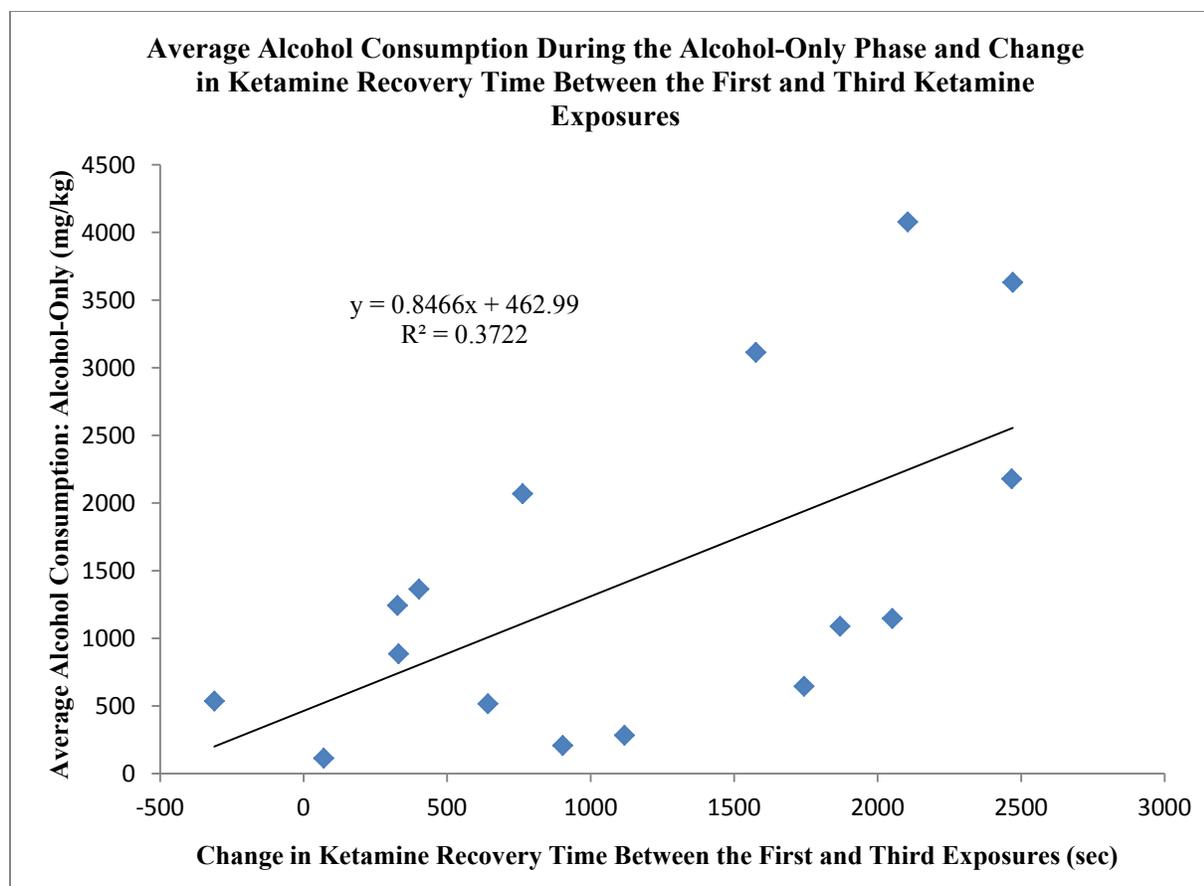


Figure 2

Scatter plot of the relationship between the Change in Ketamine Recovery Times between the first and third ketamine exposures, and average alcohol consumption during the Alcohol-Only phase of testing ($n = 16$, $p = 0.012$). The Y-Axis shows the average alcohol consumption during the Alcohol-Only phase of testing and the X-Axis shows the Change in Ketamine Recovery Time between the first and third doses. Alcohol consumption is reported in mg/kg and change in ketamine recovery time is in seconds.

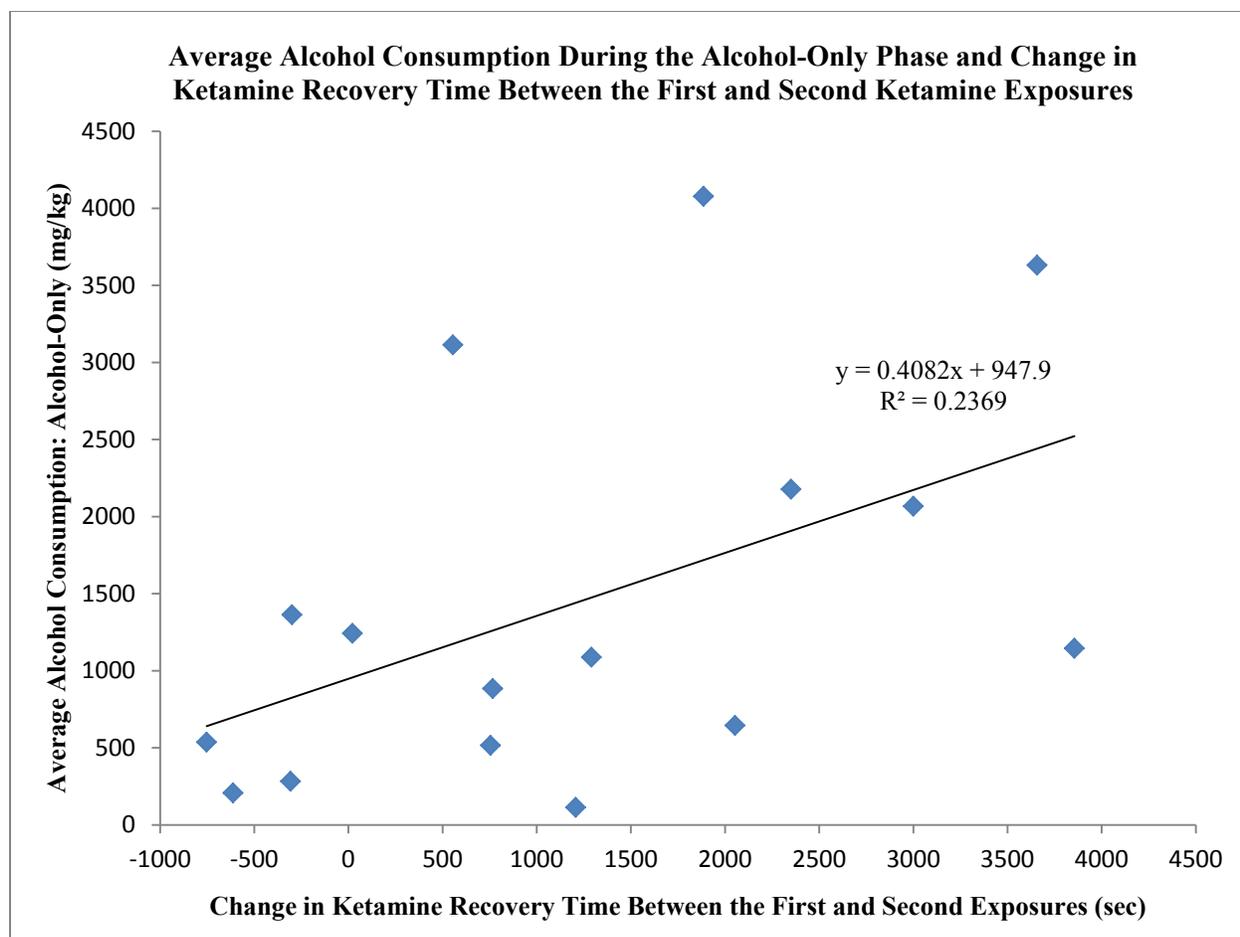


Figure 3

Scatter plot of the relationship between the Change in Ketamine Recovery Times between the first and second ketamine exposures, and average alcohol consumption during the Alcohol-Only phase of testing ($n = 16, p = 0.056$). The Y-Axis shows the average alcohol consumption during the Alcohol-Only phase of testing and the X-Axis shows the Change in Ketamine Recovery Time between the first and second doses. Alcohol consumption is reported in mg/kg and change in ketamine recovery time is in seconds.

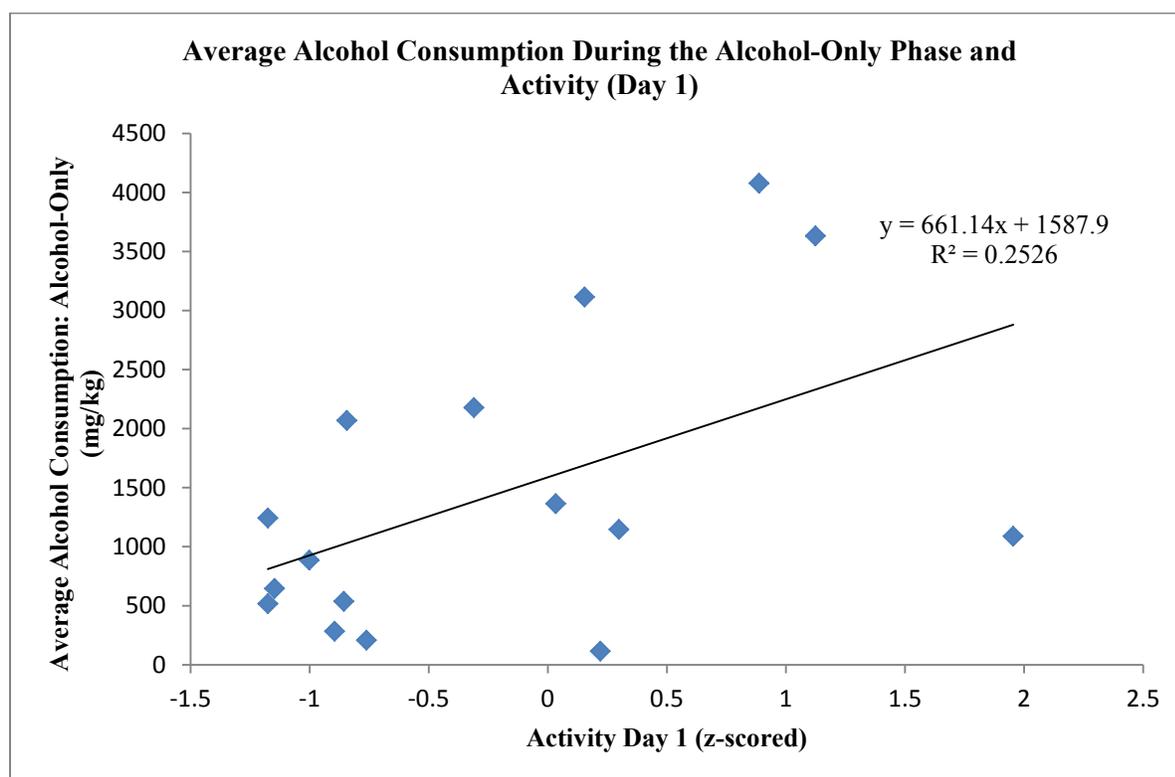


Figure 4

Scatter plot of the relationship between Activity during the first day of BBA testing and average alcohol consumption during the Alcohol-Only phase of testing ($n = 16$, $p = 0.047$). The Y-Axis shows the average alcohol consumption during the Alcohol-Only phase of testing and the X-Axis shows Activity during the first day of BBA testing. Alcohol consumption is reported in mg/kg and Activity is z-scored.

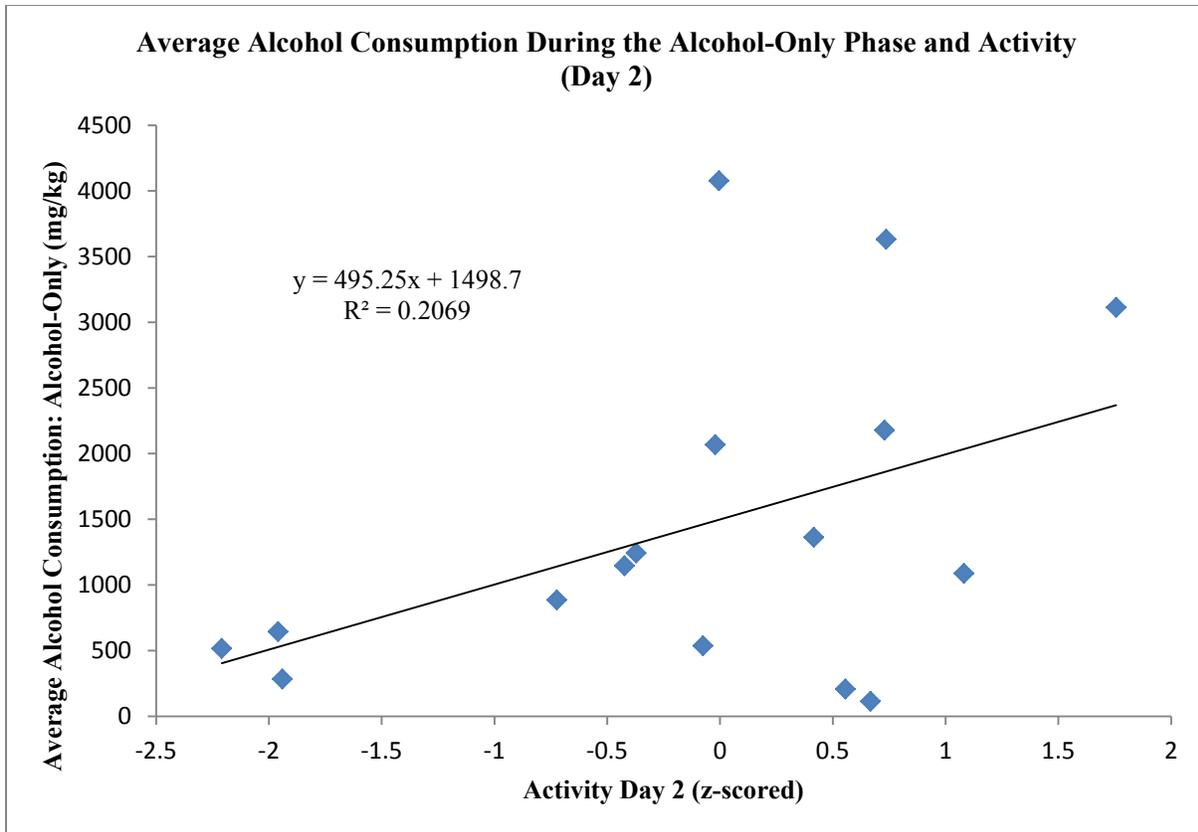


Figure 5

Scatter plot of the relationship between Activity during the second day of BBA testing and average alcohol consumption during the Alcohol-Only phase of testing ($n = 16$, $p = 0.077$). The Y-Axis shows the average alcohol consumption during the Alcohol-Only phase of testing and the X-Axis shows Activity during the second day of BBA testing. Alcohol consumption is reported in mg/kg and Activity is z-scored.

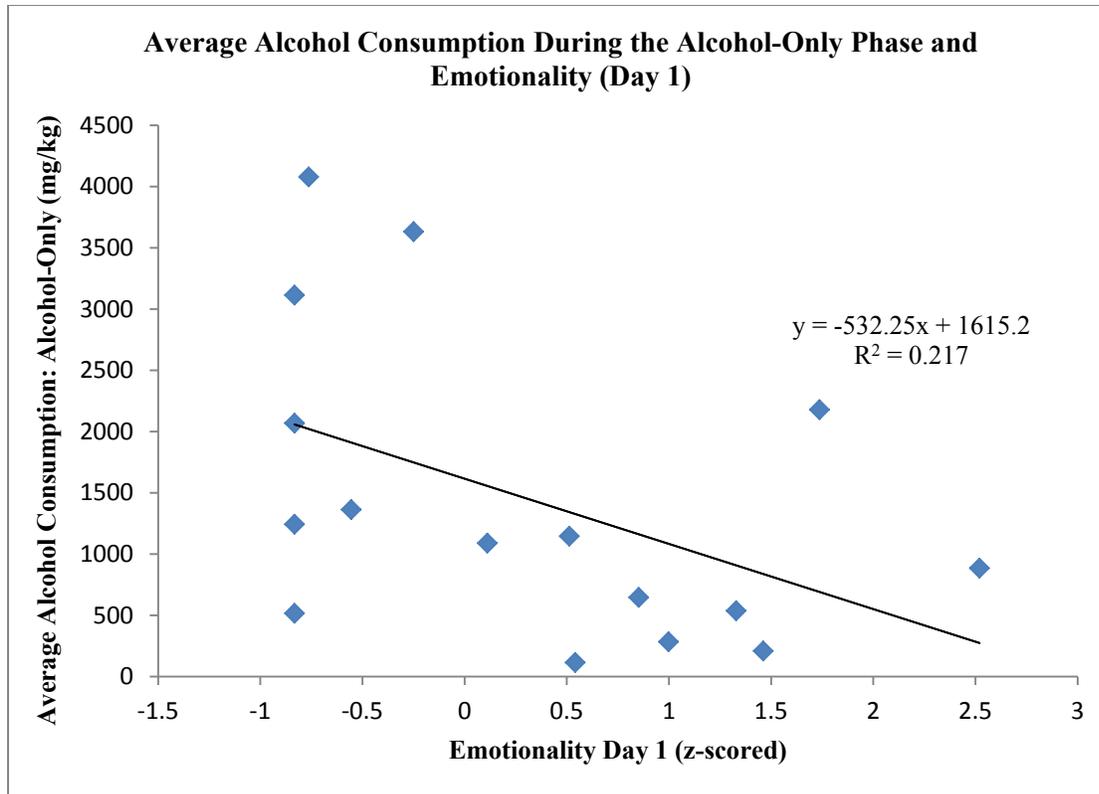


Figure 6

Scatter plot of the relationship between Emotionality during the first day of BBA testing and average alcohol consumption during the Alcohol-Only phase of testing ($n = 16$, $p = 0.069$). The Y-Axis shows the average alcohol consumption during the Alcohol-Only phase of testing and the X-Axis shows Emotionality during the first day of BBA testing. Alcohol consumption is reported in mg/kg and Emotionality is z-scored.

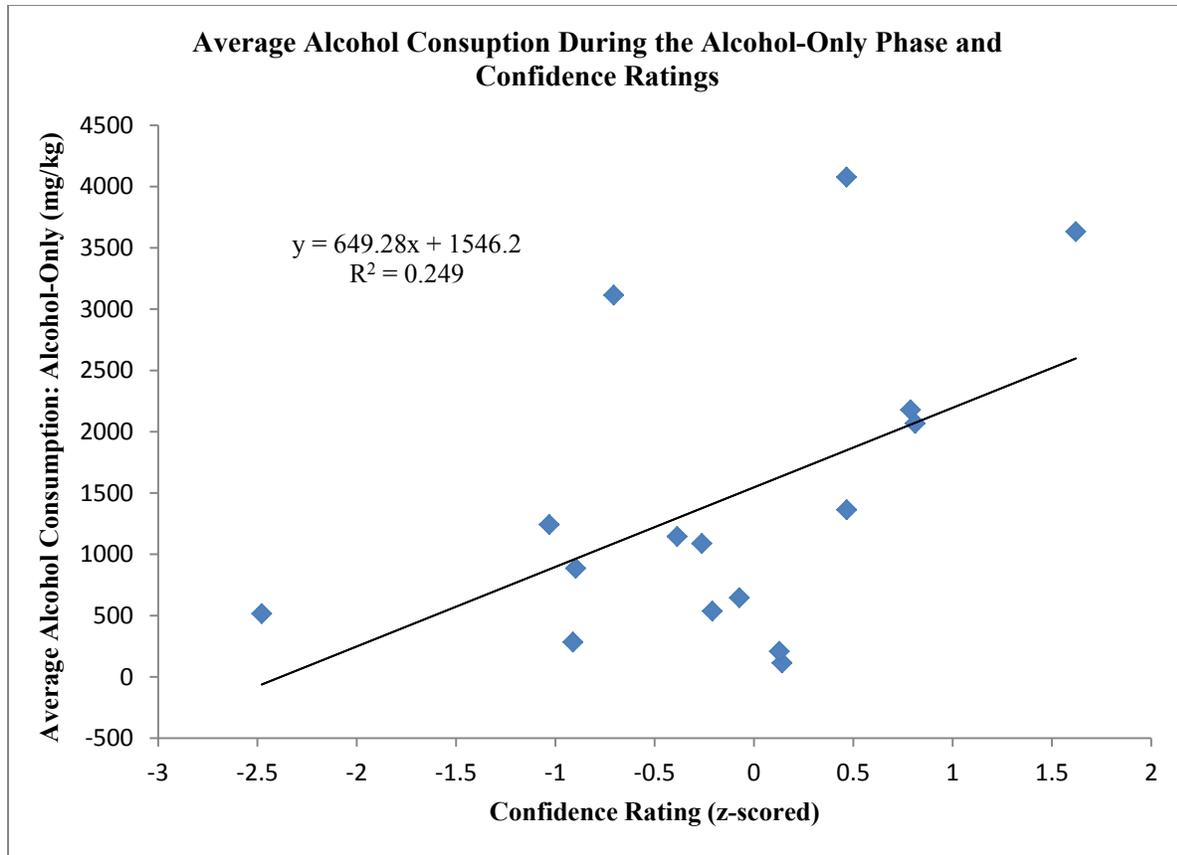


Figure 7

Scatter plot of the relationship between BBA Confidence Rating and average alcohol consumption during the Alcohol-Only phase of testing ($n = 16, p = 0.049$). The Y-Axis shows the average alcohol consumption during the Alcohol-Only phase of testing and the X-Axis shows the BBA Confidence rating. Alcohol consumption is reported in mg/kg and the Confidence rating is z-scored.