The effects of voluntary adolescent alcohol consumption on alcohol palatability

by

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Abstract

The relationship between age, alcohol intake, and the hedonic value of alcohol is key to understanding the motivation to consume alcohol. It is uncertain whether alcohol drinking during adolescence changes alcohol's hedonic value as measured by taste reactivity during adulthood. The current study compared voluntary ethanol (20% v/v) consumption among adolescent and adult Long-Evans rats in an intermittent access 2-bottle choice (IAE) paradigm and analyzed the effects of IAE on taste reactivity in adulthood compared to alcohol-naïve controls (CTRL). Blood ethanol was determined after a 28-min access period. For taste reactivity, orally infused fluids included water, ethanol (5, 20, & 40% v/v), and sucrose (0.01, 0.1, 1 M). IAE results indicate that adolescents drank more alcohol during IAE but had a lower rate of change in alcohol consumption across time compared to adults due to initially high adolescent drinking. During taste reactivity testing for ethanol, IAE rats had greater hedonic responding, less aversive responding, and a more positive relationship between hedonic responses and ethanol concentration than CTRL rats. Hedonic responses had positive while aversive responses had negative relationships with ethanol concentration and Total Ethanol Consumed during IAE. Adolescent+IAE rats displayed less hedonic and more aversive responses to ethanol than Adult+IAE rats. The adolescent group displayed less hedonic responding to sucrose than the adult group, but adolescent hedonic responding increased more steeply across sucrose concentrations. Hedonic responding for sucrose was unrelated to ethanol consumption. While many rats did not drink excessively, these results suggest alcohol consumption influences the future hedonic and aversive value of alcohol in a way that makes alcohol more palatable with greater prior consumption. However, it appears that those drinking alcohol as adolescents may be

more resistant to this palatability shift than those first drinking as adults, suggesting different mechanisms of vulnerability to consumption escalation for adolescents and adults.

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Dedication

In honor of my late aunt Theresa Simmons who was taken from us much too soon.

Chapter 1 - **Introduction**

Adolescents between the ages of 12 and 20 tend to consume approximately 90% of their alcohol in binge drinking sessions. While much of alcohol-related disease deaths occur among adults, the groundwork for the development of alcohol problems is often laid during childhood and adolescence. In humans, individuals who are exposed to alcohol in adolescence are more likely to develop dependence than those exposed in adulthood (Bonomo, Bowes, Coffey, Carlin, & Patton, 2004). In animal models, a growing body of research shows that adolescent alcohol binging may change incentive motivation for alcohol later in life and does change the neurobiological mechanisms of motivation and reward (Alaux-Cantin et al., 2013; but see also: Gilpin, Karanikas, & Richardson, 2012; Granholm, Rowley, Ellgren, Segerström, & Nylander, 2015; Shnitko, Spear, & Robinson, 2016). However, little is known about how the hedonic value ("liking") component of motivation is affected by adolescent binge drinking and what persisting changes in hedonic value this binging may cause. In light of the changes in motivation, reward, and their underlying mechanisms, a better understanding of changes in the hedonic components of motivation are of significant interest to public health and to existing hypotheses surrounding addiction.

The goals of this study are to 1) uncover differences in the palatability of ethanol ("liking" and aversive) following intermittent access to ethanol in both adolescents and adults and 2) to identify individual difference patterns of consumption that predict "liking" and "disliking" of varying concentrations of ethanol to clarify the relationship between previous alcohol consumption and hedonic and aversive taste responding.

Adolescent Binge Drinking

Binge drinking is defined by the National Institute of Alcohol Abuse and Alcoholism (NIAAA) as an alcohol drinking pattern that brings blood ethanol concentration (BEC) to 80mg/dL or above (Crabbe, Harris, & Koob, 2011). In adults, this pattern equates to consuming 5 or greater drinks for men and 4 or greater drinks for women in about 2 hours. In adolescents, 3% of 8th graders, 10% of 10th graders, and 16% of 12th graders reported binge drinking within the last 2 weeks and close to double the previous percentages reported being drunk in the past 12 months (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2017). In humans, adolescents who use alcohol are significantly more likely to develop dependence than individuals who are exposed in adulthood (Bonomo et al., 2004; Grant & Dawson, 1998) with earlier exposure associated with greater risk (DeWit, Adlaf, Offord, & Ogborne, 2000; Hingson, Heeren, & Winter, 2006). As a critical vulnerable period in the development of substance use disorders (SUDs)(Crews, He, & Hodge, 2007), further experimental inquiry into alcohol, the most widely used drug among adolescents (Johnston et al., 2017), is imperative to forming a more complete picture of addiction and addiction theory. However, legal, ethical, and moral problems exist with experimental manipulation among adult humans and to a greater extent among adolescents or children.

Animal Models of Adolescence

Given the similarity between humans and other mammals in reward-motivated behavior and pleasure (Berridge, 2000), animal models are widely used to advance our understanding of both the behavioral and neurobiological underpinnings of these behaviors. Animal models can enable understanding of a portion of humans' adolescent development and drug-related behavior, thereby aiding in advancing our knowledge of both.

Adolescence is a period of significant change as the body and nervous system mature. In humans this period tends to be between the ages of 12 to 25 years of age (Crews et al., 2007) and in rats it tends to be between postnatal day (PND) 28 to 42 (Spear, 2000). In rats, however, determination of what period constitutes adolescence depends on the metric of comparison to humans. Of particular interest is the period during which there is an increase in behaviors that correlate with engaging in drug-seeking/taking behavior such as increased risk taking and novelty and sensation-seeking (Andrucci, Archer, Pancoast, & Gordon, 1989; Feldstein & Miller, 2006; Wingo, Nesil, Choi, & Li, 2016) which extend beyond puberty and into late adolescence/early adulthood in both humans (Gardner & Steinberg, 2005) and in rats (out to around about PND 63) (Spear, 2000).

Adolescent Neural Development

Several neurodevelopmental changes correlate with the above behaviors during this period of heightened risk. Neural changes during adolescent development are geared towards improved specialization and increased efficiency. These improvements are accomplished through myelination and reduction in gray matter associated with the elimination of unneeded synapses (synaptic pruning) which occurs from birth into late adolescence/early adulthood (for a review see: Crews et al., 2007). Axon and synapse overproduction occurs early in development in many regions followed by a high rate of pruning extending into late adolescence/early adulthood (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Andersen & Teicher, 2004; Crews et al., 2007; Giedd et al., 1999; Semple, Blomgren, Gimlin, Ferriero, & Noble-Haeusslein, 2013). Within the prefrontal cortex (PFC), a protracted period of pruning and increased myelination starts around the onset of adolescence, peaking in late adolescence/early adulthood making the

PFC one of the last areas to undergo this process (Crews, Vetreno, Broadwater, & Robinson, 2016; Fuster, 2002; Petanjek et al., 2011; Romer, 2010; Semple et al., 2013).

Adolescent Behavioral Development

Earlier in adolescent development, mesocorticostriatal reward circuitry begins to mature in the ventral striatum which is thought to stimulate dopamine systems leading to increased risk taking and impulsive behaviors (Casey, Getz, & Galvan, 2008; Chambers, Taylor, & Potenza, 2003; Galvan et al., 2006; Romer, 2010). However, later in adolescence the neuroplastic changes within the PFC parallel the shift toward less risky and impulsive behavior (Chambers et al., 2003). The earlier development of mesocorticostriatal reward circuitry (e.g. nucleus accumbens), the delay in PFC development, and the progression of behaviors from more to less impulsive and risky across development has led to the suggestion that the extended process of pruning/myelination of the PFC is essential for increased capacity for behavioral regulation by this region (Crews et al., 2016).

Other research has revealed increased sensitivity to appetitive stimuli and decreased sensitivity to aversive stimuli among adolescents. One study examined taste reactivity to sucrose among adolescent rats compared to adult rats. In a series of experiments by Wilmouth and Spear (2009) adolescent and adult rats were given 2-3 different concentrations of sucrose, and hedonic and aversive responses were measured using taste reactivity. They found a greater shift in hedonic responses over sucrose concentrations indicative of higher sensitivity to the rewarding tastant and lower reaction to quinine's aversive taste in adolescents compared to adults. However, interpreting the results was complicated by the finding that adults displayed greater hedonic responses and fewer aversive responses to water than adolescents. In adult rats, research employing taste reactivity to solutions of sucrose mixed with quinine shows that the mixture has

a similar taste to alcohol and is similarly avoided when a conditioned taste aversion to alcohol is developed (Kiefer, Bice, Orr, & Dopp, 1990). Combined, the evidence suggests that taste reactions to sucrose and quinine as appetitive/aversive tastants may translate well to alcohol.

Binge Alcohol and the Adolescent Brain

Binge/binge-like ethanol exposure during adolescence has been linked to multiple, lasting neurophysiological changes in myelination, dopaminergic mechanisms, glutamatergic mechanisms, and epigenetic mechanisms. These changes directly affect the function of mesocorticostriatal reward structures like the PFC and nucleus accumbens (NAc). In the past 10 years, several key studies have emerged on the neural substrates of alcohol binging in adolescents and the lasting effects in adults who binged as adolescents.

In one study, the administration of alcohol (3 g/kg i.p.) to rats in a binge-like pattern (2 days injected then 2 days with no injections) for 2 weeks starting at PND 25 increased markers of neuroinflammation and cellular death in the neocortex, hippocampus, and cerebellum compared to saline treated controls when measured 24 hours after the last administration. In the same study, rats injected with ethanol as above without receiving anti-inflammatory treatment had significant deficits in discrimination learning (Y-maze & object recognition) and motor learning (rotarod & beam walking) as adolescents. The same behavioral deficits were found except in the rotarod task in adults who were binge-like exposed as adolescents (PND 60) (Pascual, Blanco, Cauli, Miñarro, & Guerri, 2007).

In another study by the same group using the same method of binge-like ethanol exposure as above, they found increased voluntary ethanol consumption and preference in adulthood after binge-like exposure during adolescence. The group also found increased basal dopamine (DA) levels in the Nucleus Accumbens (NAc) shell among binge-like exposed adolescents compared

to binge-like exposed adults; however, the DA response to ethanol challenge (3 g/kg i.p.) was similar between the groups. Further, binge-like exposed adolescent rats also showed reductions in DA receptor D2 in the PFC hippocampus, striatum, and NAc compared to controls and binge-like exposed adults. They also showed lower levels of NR2B-NMDA phosphorylation in the PFC, hippocampus, and NAc as well as changes in histone 3 and 4 acetylation in the PFC, NAc, and striatum compared to age-matched controls. Neither of the glutamatergic nor the epigenetic effects found among binge-like exposed adolescents were found between binge-like exposed adults (Pascual, Boix, Felipo, & Guerri, 2009).

In a study focusing on myelin, rats in early adolescence were trained to respond for a glucose/saccharin solution (supersac) in an operant box and switched them to supersac with 8-10% alcohol (binge alcohol self-administration). The daily overnight sessions were binge sessions in the sense that there were 30-minute access periods during which operant responses would earn the supersac-alcohol solution with 60- to 90-minute time out periods between the 6 access periods. The study revealed that adolescents who engaged in binge alcohol self-administration from PND 28 to 42 had damaged and reduced the density of myelin on medial PFC axons projecting to the corpus callosum (Vargas, Bengston, Gilpin, Whitcomb, & Richardson, 2014).

Another study using a binge-like model gave intermittent ethanol (5 g/kg i.g.) in a similar pattern to that of previous researchers (2 days gavage then 2 days off) (Pascual et al., 2007; Pascual et al., 2009) starting at PND 25. There was an abstinence period from PND 55-79, and an ethanol challenge on PND 80 followed by perfusions and brain harvest. Multiple markers of neuronal activation (c-Fos, ERG1, & pERK1/2) were measured and animals who received binge-

like ethanol had decreased activation in response to ethanol challenge in the PFC and amygdala and increased activation in the NAc core and shell (Liu & Crews, 2015).

Combined, the above indicate multiple models of binge-like alcohol during adolescence result in the disruption of normal PFC and NAc function along with damage to myelin. If myelination and/or synaptic pruning mature the PFC and therefore PFC-related behaviors, and alcohol binging during development disrupts or interrupts this process, one would expect behaviors typical of adolescents to persist (Crews et al., 2016).

Adolescent Alcohol and Behavior

Adolescent Intermittent Ethanol (AIE) is a general term referring to any procedure that is used to cause blood ethanol concentrations (BEC) in excess of 100mg/dl intermittently during adolescence (Neurobiology of Adolescent Drinking in Adulthood Consortium) and is stricter than the NIAAA definition of binge drinking (80mg/dl) (Crabbe et al., 2011). While several studies have observed the maintenance of adolescent behavioral phenotypes into adulthood after AIE such as increased reactivity/sensitivity to some rewarding effects of alcohol (Alaux-Cantin et al., 2013), others have reported decreased reactivity to ethanol's aversive taste (Anderson, Varlinskaya, & Spear, 2010; for a review see: Spear & Swartzwelder, 2014). Similar to humans, elevated alcohol consumption during adolescence occurs in rats (Daoura, Haaker, & Nylander, 2011; Doremus, Brunell, Rajendran, & Spear, 2005) and, although more mixed, studies have reported adolescent rats exposed to intermittent ethanol also have increased consumption in adulthood (Spear, 2014; Amodeo, Kneiber, Wills, & Ehlers, 2017; Doremus et al., 2005; Pascual et al., 2009; but see also: Gilpin et al., 2012; Labots et al., 2018). However, among the mixed results, there is significant variation in administration route (including differences in voluntary or experimenter administration methods) and assessment of adult ethanol intake (e.g., operant self-

administration, location and duration of access, intermittent schedule, etc.). Additional research has observed that adult animals that had undergone AIE had higher levels of disinhibition, impulsive-like behavior, and impairments in the overshadowing of contextual fear by predictive stimuli when compared to Chronic Intermittent Ethanol (CIE) exposed adults (Spear & Swartzwelder, 2014). Together these findings indicate that several motivation and reward-related behaviors are altered by AIE.

Intermittent Access to Ethanol 2 Bottle Choice

Many models of AIE and binge drinking exist, and among these models is the Intermittent Access to Ethanol 2 Bottle Choice (IAE) procedure (Wise, 1973; Simms et al., 2008). Briefly, the IAE procedure offers a choice between 2 bottles in the home cage during the night cycle. One bottle contains an ethanol solution (20% v/v) and the other contains water. Access is limited to 24-hour periods, 3 days a week (e.g. M, W, F) with ethanol solution and water (ETOH days) and the intervening days with 2 bottles of water.

Intermittent Access to Ethanol in Adults. Among models of voluntary binge-like alcohol consumption in rodents, the IAE procedure is widely used for a number of reasons (for reviews see: Carnicella, Ron, & Barak, 2014; Fritz & Boehm, 2016). IAE results in many rats drinking ethanol. This simple procedure has produced final ethanol intakes averaging around 5.5 g/kg/24 hours in Long-Evans rats (Simms et al., 2008; Carnicella, Amamoto, & Ron, 2009; Carnicella, Kharazia, Jeanblanc, Janak, & Ron, 2008; Carnicella, He, Yowell, Glick, & Ron, 2010; Barak, Carnicella, Yowell, & Ron, 2011; Barak, Ahmadiantehrani, Kharazia, & Ron, 2011; Ahmadiantehrani, Barak, & Ron, 2014; Hwa, DeBold, & Miczek, 2013; Meyer, Long, Fanselow, & Spigelman, 2013; Li et al., 2012; For a review see: Carnicella et al., 2014). As a result of this consumption, the procedure can produce pharmacologically relevant BECs which

correlate with consumption after about 4 weeks and meets the qualifications of an AIE procedure for some animals. But, due to the procedure's voluntary nature, not all animals' BECs reach the higher 100mg/dl threshold of AIE (Carnicella et al., 2009; Hwa et al., 2013; Li et al., 2012; Simms et al., 2008). Additionally, any mean BEC calculation will be an underestimate of the true peak BEC in a voluntary access procedure because the mean will contain data from animals that did not consume during the selected time sample (Spoelder, Tsutsui, Lesscher, Vanderschuren, & Clark, 2015). Importantly, after 3-4 weeks of intermittent access, approximately 40% of Long-Evans rats reach BECs observed in selectively bred alcohol preferring strains (Bell, Rodd, Lumeng, Murphy, & McBride, 2006a; Carnicella et al., 2014; Simms et al., 2008).

IAE is widely used as a model of binge drinking because the data collected to date suggest it is a valid model. It has good face validity as a model of binge drinking. The ethanol is consumed not only intermittently but voluntarily in this paradigm, as opposed to forced choice or vapor chamber procedures which lack face validity for binge drinking because they remove the element of choice that contributes to individual variability in consumption, and lack comparable patterns of drinking to that of human alcoholics (Carnicella et al., 2014; Fritz & Boehm, 2016; Koob, 2003; Koob & Volkow, 2010). The paradigm has good predictive validity when considering the attenuation of intake during the procedure by medications used to treat alcoholism such as acamprosate and naltrexone (Li et al., 2010; Sabino, Kwak, Rice, & Cottone, 2013; Simms et al., 2008). Finally, it has good construct validity given the neural changes found after the procedure and the strong relationship between BEC and alcohol consumption (Carnicella et al., 2009; Li et al., 2012; Simms et al., 2008).

Intermittent Access to Ethanol in Adolescents. IAE has been carried out in adolescent animals in the absence of sucrose fading procedures. The fading procedures are a potential

confound (Augier et al., 2014; Simms et al., 2008; Simms, Bito-Onon, Chatterjee, & Bartlett, 2010) because sweeteners serve as potent rewards and, as such, share similar paths of neural activation with drugs of abuse (Avena, Rada, & Hoebel, 2008; Shariff et al., 2017; Westwater, Fletcher, & Ziauddeen, 2016). Given the delay to the primary reinforcing effects of ingested alcohol (Bice & Kiefer, 1990), the taste, smell, and oral sensation of ethanol is paired with the immediately reinforcing sucrose and thus could act as a conditioned reinforcer in the absence of sucrose (McCusker & Bell, 1988) and confound future taste reactivity. Additionally, once the sucrose or saccharin is removed completely, levels of ethanol consumption tend to decrease indicating that the sweet incentive may be driving consumption (Carrillo et al., 2008; Koob & Weiss, 1990).

We have already used IAE with adolescents in our lab and achieved average 6-week final consumption levels above 4.5 g/kg/24 hours (Wukitsch, Reinhardt, Kiefer, & Cain, 2019). We also found a general pattern of increasing consumption from the first week which is somewhat inconsistent with other studies that used IAE with adolescents and somewhat more consistent with the findings of IAE among adults. There are a few studies using IAE methods nearly identical to the current proposal with adolescent rats that found very high ethanol consumption (usually > approximately 6g/kg/24hr) in the first week of IAE among Long-Evans rats starting drinking between PND 23-27 (DiLeo, Wright, Mangone, & McDannald, 2015; DiLeo, Wright, & McDannald, 2016; Fisher, Bright, Gallo, Pajser, & Pickens, 2017) and in adolescent CD rats (an outbred strain of Sprague-Dawley rats) that started at PND 28 (Schramm-Sapyta et al., 2014). High and low drinking rats which usually comprised the top and bottom $1/3^{rd}$ or $1/4^{th}$ of drinkers tended to drop during the first week and diverge after 1 to 2 weeks. Judging from the graphs in the literature, high drinkers appeared to have an overall increase in consumption (g/kg/24hr)

from week 1 onward whereas low drinkers seemed to have a distinct decrease in consumption from week 1 onward in studies which separated these groups (DiLeo et al., 2015; DiLeo et al., 2016; Schramm-Sapyta et al., 2014). In those studies that did not separate these groups, the results are more mixed (Fisher et al., 2017; Wukitsch et al., 2019) demonstrating the importance of individual differences in ethanol consumption among adolescents. In studies among adults using the same paradigm, initially low ethanol consumption occurs early on and steadily increases to over 4 g/kg/24hr by around 3-4 weeks with stable drinking around the same time (Carnicella et al., 2014) – an effect observed in our previous work (Wukitsch et al., 2019), but with greater variability characteristic of adolescents. Considering the previous success our lab has had and our previous data patterns, I hypothesize the current study would show a similar pattern without large peak drinking initially. However, given the variability in the literature, the first day spike in drinking and subsequent decline across week 1 may be present among our adolescent group and alternative analytical strategies should be considered.

Analytical Considerations. Studies among adolescents often separate high and moderate drinkers into groups based on their drinking amounts and analyze them as groups (DiLeo et al., 2015; 2016; Schramm-Sapyta et al., 2014), however there are several issues with this from an analytical perspective (e.g. Cohen & Cohen, 1983; Humphreys, 1978; for review see: Young, 2016). Thus, in the proposed study, the data-rich continuum of consumption will not be broken into categories to use an ANOVA. Instead mean alcohol consumption and rate of change in alcohol consumption across time will be parameter estimates in repeated measures analyses. When assessing any pattern of behavior acquisition, data retention from the first to last session is incredibly important. The first week of access among adolescents may represent something akin to a learning/acquisition period during which heightened consumption among adolescents may

be due to novelty or unfamiliarity with the intoxicating effects of ethanol which are subject to considerable delay when ingested – recall that novelty-seeking is increased among adolescents (Wingo et al., 2016). In addition, steep increases in weight during the first week are a potential driving factor in the decreasing g/kg trend during the same period as noted in the literature (DiLeo et al., 2015). However, the weight increase among Long-Evans rats continues to be similarly steep from 3 to approximately 9 weeks according to weight charts from Charles River Laboratories (the strain and source of rats in both Fisher et al. and DiLeo et al.'s studies), such that increasing weight alone may not explain the observed initially high levels of g/kg consumption in previous work (DiLeo et al., 2015; 2016; Fisher et al., 2017). Alternatively, the similarity between high and low drinkers in the early sessions of IAE appears to be a consistent trend across studies which separate the two groups (DiLeo et al., 2015; DiLeo et al., 2016; Schramm-Sapyta et al., 2014); indicating that a divergence in drinking patterns among adolescent individuals occurs after 1-2 weeks of IAE.

Deciding on the best data analytic strategy when considering ethanol consumption as a *predictor* of other outcome variables (such as taste reactions) can be difficult. Analyses comparing the water (CTRL) and intermittent access to ethanol (IAE) groups can yield valuable information, but the voluntary nature of consumption creates high variability among the IAE group rendering a mean comparison between the IAE and CTRL groups potentially inappropriate depending on the aims of the analysis (Carnicella et al., 2014). The previous comparison would credit animals who drank very little under the same category as animals who drank very much, resulting in high variability which may reduce power (Rusticus & Lovato, 2014). In addition, it would also be theoretically inappropriate because animals who drink very little will likely not reach levels of intoxication associated with deficits/damage and therefore should be more similar

in the outcome variable to the controls. Therefore, the current experiment will use two separate models. The first model will compare ethanol access groups directly (IAE/CTRL) and will yield an overall mean comparison of IAE versus CTRL group. The second model will treat the total amount of alcohol consumed as a continuous predictor variable within the IAE group. The second model treats alcohol consumed as a continuous variable conferring the ability to make claims about those that drank more or less without the need for median splits to create low/high categories. Instead, the model will treat the total consumption as a continuous spectrum.

Taste reactivity data will be analyzed with the two separate models above. In addition to those models, a 2-stage model with a similar structure to the second model mentioned above will be tested to determine whether variables representing the *patterns of consumption* for each individual during IAE can better model the data. The total alcohol consumed from IAE will be replaced with unbiased estimates of the mean alcohol consumed per day (IMAC) and the rate of change in consumption (IRoC) for each individual. IMAC and IRoC refer specifically to the predictors derived from *individual-level* data and should not be confused with group-level outcomes in terms of rate of change or mean alcohol consumption comparisons. These unbiased estimates, known as Best Linear Unbiased Predictors, are generated by the first stage of the model which uses a multilevel model to predict alcohol consumption across time (among other group variables like age group [adolescent vs adult]). Because the multilevel model adjusts for the amount of data (e.g. missing data points) and variability in the data (e.g. outliers or strange consumption patterns) by comparing it to the other rats, it can effectively "shrink" the effect of those data issues on the results of the analysis through weighting based on estimations of certainty (restricted maximum likelihood; REML) akin to some Bayesian approaches (Gelman & Hill, 2007; McLean, Sanders, & Stroup, 1991). Considering that fistula patency loss is a

common issue in taste reactivity, bottles may be dropped or broken during IAE before they have been weighed, and the expected variability in consumption, this analytical model will do well to mitigate at least some portion of these issues compared to traditional techniques. A traditional calculation of each rat's best fit mean and rate of change values would not show this shrinkage and therefore be ill-equipped to deal with common problems associated with the current proposal's methods which create such data biases.

IAE does come with some drawbacks. While approximately 50-80% of adult Long-Evans rats reach excessive intake, only 50% of those rats have sufficiently high BECs to meet the 80mg/dl threshold for binge drinking (Bell et al., 2006a; Carnicella et al., 2014; Simms et al., 2008) and approximately 20% of Long-Evans rats fail to reach 3.5-4 g/kg/24 hours, which usually does not result in binge-level BECs (Carnicella et al., 2014). When looking at individual differences in consumption, this can be both advantageous and disadvantageous due to the consumption variability involved. While keeping data from all individuals poses minor challenges for claims about binge consumption if there are not enough rats who reach or exceed the threshold, rats below the binge BEC threshold should be kept in order to generalize better to a broader population and characterize individual differences. In addition, as with most other drugs, alcohol's effects lie along a spectrum based on dose. The current analytical approaches afford ways of looking at the magnitude of change across the entire range of voluntary drinking, including levels which exceed binge threshold. If the research question is specific to rats that must reach or exceed binge threshold, any challenges posed can be resolved easily with subsequent analyses focusing on those rats that meet binge criteria.

Alcohol and Sweet Solutions

Ethanol is often described as having a bitter-sweet taste, for example, avoidance of a 5% ethanol solution generalizes to sweet solutions (sucrose, glucose, fructose, and saccharin) with a bitter tastant (quinine HCl) added (Kiefer & Lawrence, 1988). In addition, taste reactivity to a sucrose-quinine HCl mixture was like that of an alcohol solution (Kiefer et al., 1990). However, it is ethanol's similarities with sweet tastants that may uncover interesting insights into how ethanol alters the neural substrates that underlie naturally reinforcing ingested stimuli (e.g. Thiele, Marsh, Ste Marie, Bernstein, & Palmiter, 1998). Sweet tastant consumption is consistently highly correlated with alcohol consumption in both rats (e.g. Dess, Badia-Elder, Thiele, Kiefer, & Blizard, 1998; Kampov-Polevoy, Kasheffskaya, & Sinclair, 1990) and humans (Kampov-Polevoy, Garbutt, & Janowsky, 1997; Kampov-Polevoy, Garbutt, & Khalitov, 2003; Kampov-Polevoy et al., 2014) suggesting overlap in mechanisms of ingestion of the two tastants.

However, unlike sucrose, alcohol has both hedonic and aversive aspects as a tastant stimulus (Kiefer, 1995) as well as post-ingestion effects (e.g. nausea) as many humans can readily attest. When compared in an associative learning task, voluntary adolescent alcohol exposure differentially affects consumption of alcohol- and sucrose-paired tastant stimuli (DiLeo et al., 2015). High amounts of voluntary alcohol consumption during adolescence resulted in higher levels of consumption of the tastant that was previously paired with alcohol compared to: A) a high adolescent alcohol consuming group that did not receive a pairing of alcohol with the tastant, B) water controls who never received alcohol prior to the pairings, and C) rats that voluntarily consumed low amounts of alcohol during adolescence – a pattern of effects not seen with the tastant that was previously paired with sucrose. Further, among water control and low drinking rats pairing a tastant with alcohol *reduced* consumption of the tastant compared to the

group that never received such a pairing indicating avoidance/aversion. The tastant that was previously paired with sucrose was consumed more regardless of voluntary adolescent alcohol consumption. There were no differences in consumption of the tastant except that those rats that received the sucrose-tastant pairings consumed much more than those that did not receive the pairings (DiLeo et al., 2015). It is important to note that incentive motivation was measured in that study in terms of consumption and does not dissociate incentive salience and hedonic value. If there is a reduction in aversion and/or an increase in liking of alcohol among high-drinking adolescent rats that parallels the differences seen in consumption between sucrose and alcohol-associated stimuli, one would expect sucrose and alcohol to have different outcomes after voluntary adolescent alcohol consumption in terms of hedonic value for those who consumed high vs low and no (water control) amounts of alcohol.

When observing the underlying neural substrates of the relationship between alcohol and sweet tastants, however, the evidence suggests high overlap between alcohol and sweet tastants in the gustatory system. For example, alcohol activates at least one pathway sensitive to sucrose in the gustatory system (Lemon, Brasser, & Smith, 2004). Further, sweet-sensitive and selective neurons of the gustatory system (nucleus of the solitary tract) respond to ethanol much more among strains of rats selectively bred for their alcohol preference (P rats) indicating that differences in the representation of ethanol in the gustatory system may be an important contributor to enhanced motivation to consume alcohol (Lemon, Wilson, & Brasser, 2011). Thus, if alcohol and sucrose are similarly represented in the taste system, one might expect similar hedonic value for those who consumed high vs low (IAE) and no (CTRL) amounts of alcohol during adolescence.

Incentive Motivation

Incentive motivation theory breaks down motivation into two categories: incentive salience ("wanting") and hedonic value ("liking"). "Wanting" is the willingness to attend to, approach, and/or respond for some stimulus (e.g., food, drugs, sex, and their associated stimuli) and is usually associated with mesocorticostriatal dopamine (Robinson & Berridge, 1993). "Liking" is the subjective pleasure experienced from interacting with some stimulus (e.g., how good or bad something feels to an individual) and is associated with a small region of the medial shell of the nucleus accumbens opioid system (Berridge, 2000; Peciña, Smith, & Berridge, 2006).

"Liking" and "wanting" were not considered to be very separable. In the 1990s, mesocorticostriatal dopamine was thought to be intimately involved in pleasure, and wanting followed naturally from that pleasurable, hedonic value of the stimulus in question (Berridge & Robinson, 2016). Though some evidence for separate "wanting" and "liking" systems existed at the time, the surprising results of two experiments initiated a change in the reward-motivation status quo and the separation of incentive motivation's two components. In the first study, Berridge, Venier, and Robinson (1989) expected rats who had depleted dopamine via intranigral 6-OHDA injections would have taste reactions to a sweet solution that indicate reduced "liking" or that there would be an overall reduction in reactions due to reduced sensory motor arousal. On the contrary, dopamine depleted rats' orofacial taste reactions to sweet substances were not significantly altered, but the lack of food reward-seeking and consuming indicated a distinct loss of motivation. That experiment was followed by another that electrically stimulated the lateral hypothalamus (medial forebrain bundle) to raise dopamine levels in the mesocorticostriatal system and found that "liking" was unchanged and "wanting" increased drastically (Berridge &

Valenstein, 1991). These findings led to the proposal that "liking" and "wanting" were behaviorally and neurally separable in some ways, with "wanting," but not "liking," mediated by mesolimbic dopamine (Berridge, 2007; Robinson & Berridge, 1993) which has significant implications for addiction and substance abuse.

Incentive-sensitization. The previous work by Robinson, Berridge, and colleagues and growing evidence for their position eventually led to their proposal that sensitization of "wanting" by drugs of abuse results in a behavioral profile consistent with that seen in addiction among humans. As "wanting" escalates to a level consistent with the pathology of addiction, users tend to want a drug more and may like the drug less, paralleling a shift seen in instrumental learning from goal-directed behavior (e.g., to enjoy the high) to compulsive use. The Incentive-Sensitization Theory of Addiction posits that the sensitization of the neural substrates of "wanting" explains the transition to a pathological level of "wanting" synonymous with craving. Sensitization of "wanting" systems occurs through incremental changes caused by repeated exposure to drugs of abuse. Further, this sensitization is long-lasting, which explains why craving and relapse (reinstatement of drug-taking) can remain after extended abstinence periods (Robinson & Berridge, 1993; Robinson & Berridge, 2003).

The Role of "Liking". In adolescents, factors such as maturing brain structures and differences in alcohol reward and aversion sensitivity all very likely contribute to vulnerability to developing alcohol use disorders and alcoholism. The causal mechanics of this vulnerability, however, are still being determined and indeed, many more factors than those addressed by the current proposal may contribute to this enhanced adolescent vulnerability. The focus of the proposed study is on adolescent vulnerability to alcohol and the lasting changes in behavior produced by adolescent binging. If heightened "liking" (or decreased disliking/aversion) for

alcohol in adulthood is related to the magnitude of alcohol consumption during adolescence to a greater extent than adults and age-matched controls, then it could be considered evidence of a dose-dependent change in hedonic mechanisms that are vulnerable to ETOH during adolescence.

Importantly, "liking" is largely independent of the incentive-sensitization process, and the "liking" responses to a given drug are often difficult to measure behaviorally without confounding factors related to "wanting" (Berridge, 2000). Most widely used behavioral paradigms (e.g. drinking ETOH in an operant paradigm) rarely separate the two components. Taste reactivity, a common measure of "liking" (Berridge, 2000) requires that the test substance be tasted, which many drugs of abuse are not. Alcohol, however, offers a unique opportunity to measure "liking" of a drug behaviorally because it is ingested and must be experienced by the gustatory system before it can have a pharmacological and behavioral effect. In addition, compared to other drugs of abuse, oral ingestion is the most common means by which alcohol is administered by both humans and animals in the population, thus lending face validity to a taste reactivity test using alcohol. Taste reactivity's method of oral fluid delivery then removes confounding factors related to "wanting."

Taste Reactivity

Taste reactivity entails the scoring of orofacial behavioral reactions to fluids infused directly into the mouth via a surgically implanted intraoral fistula, thereby removing interpretation problems related to approach/avoidance behaviors in other paradigms. The behavioral reactions are recorded and scored as aversive (e.g. gapes, forelimb flails, headshakes, passive drips, and fluid expulsions) and hedonic (e.g. tongue protrusions, lateral tongue protrusions) per standards set forth by Grill and Norgren (1978) and updated as the paradigm evolved with alcohol, e.g. no longer counting mouth movements as a "liking" response (Kiefer,

1995). The more of a given aversive or hedonic behavior present during a given trial, the greater the aversion or "liking", respectively, is assigned to the infused substance. Together hedonic and aversive responses make up "palatability." While many instrumental operant paradigms can measure concurrent hedonic value and incentive salience or incentive salience alone (e.g., outcome devaluation, cue-induced reinstatement of drug-seeking, and extinction responding), only taste reactivity has, so far, been lauded as perhaps the only method which can assess purely hedonic "liking" responding without the confounding approach behaviors which also measure "wanting" (Berridge, 2000). While, ultrasonic vocalizations are also used to measure affective state (Burgdorf, Panksepp, & Moskal, 2011), delivery of rewards into the mouth may interfere with the production of such vocalizations and may reflect direct reactions to stimuli aside from the tastant tested. Perhaps for the previously listed reasons, there is little to no research directly comparing taste reactivity and ultrasonic vocalizations in reaction to the same stimulus (Pelloux, Meffre, Giorla, & Baunez, 2014).

Alcohol and Taste Reactivity

Previous research that has looked at taste reactivity in adult outbred and selectively bred rat strains highlights the importance of palatability in alcohol consumption. In the following studies, taste reactivity to a range of ethanol doses was performed pre- and post-ethanol exposure. In Holtzman-derived rats (an outbred strain similar to Sprague-Dawley rats), pre-ethanol exposure taste reactivity to 3, 6, 9 and 12% ethanol doses showed a dose dependent increase in aversive responding as ethanol dose increased, but no differences in "liking" responding. Subsequent ethanol exposure consisted of 8 days of continuous access to 2 bottles (1 water, 1 increasing incrementally from 3-12% ethanol (v/v) every 48 hours). Pre-exposure taste reactions did not significantly correlate with grams of ethanol solution consumed at any alcohol

dose. Post-exposure taste reactivity indicated decreases in aversive responding to ethanol from pre- to post-exposure with post-exposure showing low aversion across all concentrations and again, no significant differences in "liking" (Kiefer & Dopp, 1989). The authors suggested that outbred rats habituate to the aversive qualities of ethanol after a relatively brief exposure period. They go on to explain that the lack of pre-exposure reactivity correlating with consumption was due to a lack of experience with "postingestional factors," such as ethanol's intoxicating effects, at the time of the initial taste reactivity test.

Intuitively, it makes sense that taste reactions of ethanol naïve rats should be predictive of later consumption, assuming taste plays a role in the likelihood of ingesting something. Those individuals that like something more should ingest more of it and, if that is true, the knowledge could therefore be used for early alcohol addiction vulnerability detection. Kiefer and Dopp's (1989) study correlated taste reactivity responses with grams of alcohol solution consumed and not the actual g/kg of pure alcohol consumption. Since there was no adjustment for weight, one might assume it is still possible that initial pre-exposure reactivity is related to later ethanol consumption dose (g/kg) and not necessarily the amount of solution consumed in outbred rats. However, no such correlations were found across several studies (including those presented below), with several metrics (e.g. g, g/kg ethanol, mL of solution consumed) from the same group (Kiefer, 1995).

The following studies indicate that the initially assumed direction of causality between palatability and ethanol consumption is likely reversed. Selectively bred alcohol-preferring (P) and high alcohol drinking (HAD) rats showed no differences in pre-ethanol exposure taste reactivity to ethanol across a range of concentrations compared to their non-preferring (NP) or low alcohol drinking (LAD) counterparts (Bice & Kiefer, 1990; Kiefer, Badia-Elder, & Bice,

1995). After 3 weeks of continuous access to 2 bottles (1 water, one 10% ethanol), however, lower amounts of aversive and higher amounts of "liking" responses specific to ethanol were present in P and HAD rats compared to pre-exposure taste reactions *and* to their respective NP and LAD strains who did not differ from pre- to post-ethanol exposure (Bice & Kiefer, 1990; Kiefer, Badia-Elder, & Bice, 1995). Additionally, unpublished data mentioned in Bice and Kiefer (1990) suggest outbred rats (Sprague-Dawley) show similar patterns of decreases and increases in aversive and hedonic responding respectively after 4 weeks of continuous ethanol access under the same conditions as P and HAD rats; however, these differences were not as strong as those found among selectively bred lines. These findings led Bice and Kiefer (1990) to posit that the taste of alcohol is associated with its positively reinforcing post-ingestion effects.

In another study by the same group (Kiefer & Badia-Elder, 1997), identical methods were used in another strain of rat selectively bred for sensitivity to the sedative effects of alcohol injections (alcohol neurosensitivity). Selection criteria for breeding was for those who slept longest or shortest after the injection and thus were dubbed high alcohol sensitive (HAS) and low alcohol sensitive (LAS) rats, respectively. There were no differences in taste reactivity between HAS, LAS, and control animals, though all showed a palatability shift after ethanol experience and along the alcohol concentration gradient, indicating that alcohol neurosensitivity may not be directly related to taste reactions (Kiefer & Badia-Elder, 1997). However, due to their lower sensitivity to alcohol, LAS rats form weaker alcohol-induced conditioned taste aversions (CTA), but not lithium chloride-induced CTA (Kulkosky, Carr, Flores, LaHeist, & Hopkins, 1995). The weaker alcohol-induced CTA of LAS rats parallels the CTA findings among adolescent rats which required both a higher dose of ethanol to induce aversion and more pairings of the sucrose CS with ethanol compared to adults (Anderson et al., 2010).

Additional support for both the developmental and alcohol exposure-related variability in alcohol palatability comes from fetal alcohol research. Studies on the taste reactivity of weanlings exposed to alcohol show a pattern of palatability similar to the studies in adult rats selectively-bred for alcohol preference and consumption. Prenatal alcohol during the last 4 days of gestation increased postnatal (PND 14) ethanol consumption and "liking" responses and decreased aversive responses to a 6% (v/v) ethanol solution compared to controls (Arias & Chotro, 2005).

Together these studies indicate that vulnerable sub-populations may be especially sensitive to ethanol-experience-induced palatability shifts and a blunted ability to form CTAs, all of which are conducive to repeated ethanol consumption. Considering the ethanol-related vulnerability of adolescents, similar palatability changes to what Kiefer and colleagues observed among P and HAD rats may occur in an adolescent alcohol exposed population. However, no research to date has looked at palatability differences that may be induced by adolescent alcohol access. Additionally, the relationship between ethanol consumption and palatability is likely influenced by more complex processes, such as ethanol-induced alterations to hedonic mechanisms and/or alcohol-specific learning which likely depend on individual vulnerabilities such as genetics, age of drinking onset etc...

The previous studies indicate prior alcohol drinking experience lowers aversive responses to and increases "liking" responses to alcohol. While no individual pre-ethanol exposure taste reaction was correlated with the total amount of alcohol consumed, patterns of voluntary consumption over time have not been used to predict palatability post-ethanol exposure. Together with evidence for alcohol-related problems in adolescent prefrontal cortical development and the maintenance of adolescent behavioral phenotypes into adulthood,

adolescence seems to be a logical timeframe for patterns of drinking to more drastically affect neural substrates of palatability compared to adulthood. If exposure to alcohol via IAE during adolescence is more strongly associated with a pattern of palatability conducive to increased consumption compared to adults and controls, then alcohol has induced either changes to or maintenance of adolescent typical reward sensitivity and/or blunted aversion response. In either case, the previous would be clear evidence for lasting changes to mechanisms of hedonic valuation by adolescent alcohol binging. In addition, if differences in palatability are predicted by individual differences in alcohol consumption – either total consumption or consumption pattern – then that would constitute evidence for a relationship between vulnerability to alcohol problems and hedonic mechanisms.

Chapter 2 - **Hypotheses**

Hypothesis 1 – Intermittent Access to Ethanol

I predict there will be higher consumption (g/kg) of ethanol among adolescent rats compared to adult rats. The nature of the alcohol consumption pattern among adults will be characterized by an increasing trend in alcohol consumption across time. Adolescents, however, may either increase alcohol consumption across time, as observed in our own lab and some of the literature, or decrease alcohol consumption across time as observed in other literature. I predict that alcohol consumption on day 1 will likely be high if the group's rate of change is negative or near zero and low if the rate of change is positive. In either case, I predict a significant interaction between age groups across time.

Finding that adolescents drink more alcohol than adults during IAE is consistent with previous literature that has explored IAE between similar age ranges (e.g. Schramm-Sapyta et al., 2014). If supported, this effect would lend support to existing literature surrounding adolescent binge-drinking. If unsupported, this finding would indicate that adolescents may not drink more alcohol on average compared to adults as found in other literature (Bell et al., 2006b; Pickens et al., in press). This may be due to a downward trend in drinking when averaged together (not separating low from high drinkers; Fisher et al., 2017). However, in that study the adolescents were not directly compared to adults. In studies that did separate high and low adolescent drinkers, it is difficult to tell whether the drinking trend would be flatter or still more negatively sloped given the omission of the data of the "middle" drinkers (DiLeo et al., 2015; DiLeo et al., 2016). While beyond the scope of this study, it is also possible that the timing of alcohol availability (when the bottles go onto the cages relative to the onset of the dark cycle) is an important factor related to consumption pattern as it appears to be with adults (Carnicella et

al., 2014), however, this has not yet been directly tested among adolescents, and the literature for adolescent IAE is currently sparse.

Adolescents and adults should differ in their drinking pattern with adolescents showing greater variability than adults (Schramm-Sapyta et al., 2014). The nature of the rate of change in ethanol consumption between the two age groups, however, has not yet been explored and will help to characterize the discrepancies found among adolescents in the literature and aid in developing new modeling strategies. As previously stated, the adolescent rate of change in consumption may increase over time or it may decrease slightly. I predict that there will either be a steeper increase or, conversely, a steeper decrease in adolescent alcohol consumption over time compared to adults. In either case, and regardless of the significance of the interaction between age group and time, the analytical strategy alone will help to better mathematically quantify aspects related to binge drinking patterns among both adolescents and adults which may serve as priors for later models.

Hypothesis 2 – Taste Reactivity: Alcohol

I hypothesize that compared to the adult IAE group and all water controls, the adolescent IAE group will have: 1) the greatest amount of "liking" responses and 2) the lowest amount of aversive responses. However, these effects will be qualified by an interaction: 3) I predict an ethanol concentration by alcohol consumption by age interaction such that as concentration (5-40% ethanol) and total ethanol consumption during IAE (total g/kg consumed) increase, the adolescent IAE and adult IAE groups will diverge in both "liking" and aversive responses. In this 3-way interaction, adolescents that had higher total ethanol consumption will have higher "liking" and lower aversive taste responses compared to adults with higher total ethanol. The difference between the age groups at different levels of consumption will be moderated by

ethanol concentration, such that, at higher ethanol concentrations, the predicted differences between the IAE age groups will increase in magnitude. Adult and adolescent water controls will show similar "liking" and aversive responding. For analyses using IMAC and IRoC instead of alcohol consumption during IAE, I have the same hypotheses, however, IMAC and IRoC will take the place of total ethanol consumption as predictors in the model. Further, I hypothesize that the models with IMAC and IRoC will have better fit in terms of their Akaike and Bayesian Information Criterion (AIC and BIC) than the model with total ethanol consumption as a predictor.

If supported, this pattern of results would suggest that binge drinking during adolescence produces lasting changes to the hedonic value of alcohol that are conducive to further consumption. Further, these hedonic value changes would depend on individual differences in alcohol consumption during adolescence with greater changes at higher levels of consumption. Such a finding would constitute preliminary evidence for hedonic value shift as factor in alcohol use disorders. If not supported, this indicates that age of consumption, alcohol drinking pattern, or both are not predictive of alcohol taste reactivity and would indicate that the hedonic value is not vulnerable to shift. This would suggest that some other mechanism mediates increased incentive motivation such as the proposed aberrated incentive salience mechanisms in the incentive motivation theory of addiction (Berridge & Robinson, 2016).

Hypothesis 3.1 – Taste Reactivity: Sucrose

I hypothesize that compared to the adult IAE group and all water controls, the adolescent IAE group will have: 1) the greatest amount of "liking" responses and 2) the lowest amount of aversive responses. However I anticipate a floor effect for aversive responses and additionally predict that these main effects of age will be qualified by an interaction: 3) Similar to alcohol, I

predict a sucrose concentration by alcohol consumption by age interaction such that as concentration (0.01-1M sucrose) and total ethanol consumption during IAE (total g/kg consumed) increase, the adolescent IAE and adult IAE groups will diverge in both "liking" and aversive responses (if there is not a floor effect). Specifically, adolescents that had higher total ethanol consumption will have higher "liking" and lower aversive taste responses to sucrose compared to adults with higher total ethanol consumption. This difference between the age groups will increase as concentration and total ethanol consumption increase. Adult and adolescent water controls will show similar "liking" and aversive responding. For analyses using IMAC and IRoC instead of total ethanol consumption during IAE, I have the same hypotheses. However, if one of the IMAC and IRoC models has better AIC/BIC, IMAC and IRoC will take the place of total ethanol consumption as predictors in the model.

If supported, adolescent binging changes hedonic value that generalizes to sucrose and perhaps other reinforcers. Together with a supported H2, this may indicate the presence of a positive feedback loop between adolescent alcohol consumption and hedonic value shift in adolescent individuals. If this adolescent-alcohol-fueled shift in hedonic value has generalized to a sucrose reinforcer, it may generalize to others. Notably, a hedonic shift that generalizes to other reinforcers proposed here was not seen in selectively bred, adult P and HAD rats (Bice & Kiefer, 1990; Kiefer, Badia-Elder, & Bice, 1995). At the very least it would indicate an interrelation between hedonic value and adolescent alcohol consumption, suggesting that hedonic value may be playing a larger role in the development of addiction or addiction vulnerability than previously expected (Berridge & Robinson, 2016). Thus, hedonic value warrants further exploration in the field of adolescent alcohol abuse. In addition, this effect facilitated by alcohol would seem to work in a way which increases vulnerability to addiction to other reinforcers and

could explain a portion of behavioral differences in adulthood resulting from adolescent alcohol observed in previous literature (for review see: Spear & Swartzwelder, 2014).

Hypothesis 3.2 – Taste Reactivity: Sucrose

Alternatively, prior ethanol experience may only change hedonic value for alcohol given that heavy adolescent alcohol consumption alters alcohol and sucrose-paired stimulus consumption differently (DiLeo et al., 2015). Thus, I hypothesize no differences predicted by total ethanol consumption or between the age and control groups. Additionally, I hypothesize a main effect of sucrose concentration (0.01-1M sucrose), with higher amounts of "liking" responses and lower amounts of aversive responses as sucrose concentration increases.

If H3.2 is supported along with H2, adolescent binging's effect on hedonic value is unique to alcohol and does not generalize to another reinforcer like sucrose indicating that alcohol likely gains access to hedonic value through experience-related mechanisms which also warrant further exploration.

Together, the current proposal attempts to characterize the impact of IAE at different ages on hedonic value to determine if alcohol drinking pattern and age of alcohol exposure are related to changes in hedonic value of alcohol in adulthood. In addition, this work will explore different analytical methods for comparing and making predictions from group and individual-level adult and adolescent voluntary intermittent alcohol intake patterns. This will advance our understanding of the impact of individual differences in alcohol consumption on hedonic "liking" and aversive "disliking" responses.

Chapter 3 - **General Method**

Animals

Sixty-four male, Long-Evans rats arrived in our lab at PND 21. They were singly housed in plastic shoebox cages (20 × 43 × 20 cm) with wire tops and bedding for the duration of the experiment. To ensure handling was similar between age groups, the adult rats were handled during adolescence 4 days per week. All rats were housed under a reversed 12 hour dark:light schedule at a temperature of 21 ±1°C and humidity between 30 and 50% along with ad libitum food and water access for the entire experiment. A dim red lamp was left on during the dark cycle. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (2011). The protocol was approved by the Institutional Animal Care and Use Committee of Kansas State University.

Intermittent Access to Ethanol 2-Bottle Choice

Intermittent access to a 20% ethanol solution (v/v) in a 2-bottle choice paradigm (IAE) occurred in the home cage consistent with methodology found in Simms et al. (2008). IAE began on PND 27 for adolescents and PND 69 for adults at the onset of the dark cycle, and alcohol placement alternated on the left or right of the cage for each exposure period. Alcohol-exposed rats (IAE rats, n = 22 adolescent, 22 adult) had concurrent access to water and a 20% alcohol (v/v) solution in tap water every other day, 3 days a week for 16 sessions while water controls (CTRL rats, n = 10 adolescent, 10 adult) always had 2 bottles of water. On intervening days (T, R, Sat, & Sun), both bottles contained only water. Bottles were weighed daily (excluding Sundays' water consumption) after 24 hours and consumption was recorded for both bottles. Rats were weighed daily during bottle changes. The experimental timeline can be found in Figure 1.

Blood Ethanol Concentration Determination

The day before blood collection, all rats had the lateral aspect of the hock region of one hind leg shaved to access the saphenous vein. Twenty-eight minutes after ethanol bottles were placed on the cage during the last ethanol access period of IAE, each rat (including water controls) was restrained with a towel, Vaseline was applied to the leg over the saphenous vein and a 22-gauge needle was used to puncture the vein. 36 μL of blood were collected at 30 min from when the bottles went on for each animal and placed into a heparinized aliquot for BEC analysis using the Analox AM1 Alcohol Analyzer (Analox, Stoubridge, UK).

Taste Reactivity

Surgery. After 1-5 rest days from the final IAE session, rats were anesthetized with a combination of ketamine (80 mg/kg; 1 mg/ml, i.p.) and diazepam (5 mg/kg; 1 mg/ml, i.p.) and underwent aseptic surgical implantation of intraoral fistulae. First the scalp was shaved and prepared aseptically by scrubbing with chlorhexidine followed by 70% isopropyl alcohol to rinse, and the region was allowed to air dry. The buccal region near the first maxillary molar was swabbed with a dilute chlorhexidine solution (1:10) and then rinsed with sterile saline. The fistula, made of 60-gauge polyethylene tubing, was inserted with a needle lateral to the first maxillary molar. After insertion, the needle was directed subcutaneously along the side of the rat's face to the top of the scalp, where it exited through an incision. Head mounts (Plastics One) were friction-fitted to the PE tubing and secured with screws and dental acrylic to the skull. After surgery, all rats were allowed to heal for approximately 1 week before taste reactivity testing began. Fistulas were flushed daily with sterile water to maintain patency. One rat from the Adult+IAE group died in surgery prior to taste reactivity testing.

Testing. Taste reactivity testing sessions began with 3 habituation sessions during which no fluids were infused through the fistula. The custom designed chamber was shaped like a trapezoidal prism stood on end with mirrors on the three shortest sides facing the interior of the chamber (Figure 2). Plexiglas panes form the front wall and chamber floor. An additional mirror is angled to visualize through the floor from the front to view the rat's ventral side. The top of the chamber was open and a weighted arm was mounted at the top to prevent the tangling or twisting of the tubing while infusing solutions. After habituation, the fistulas on the rats' heads were attached to tubing attached to a syringe driven by an infusion pump (KD Scientific). Rats received an intraoral infusion of distilled water at a rate of 1 mL/min for 1 minute to be used as a baseline. Following the water session, rats received a single, daily, 1-minute infusion of ethanol solutions (5%, 20%, & 40%; v/v) and sucrose solutions (0.01, 0.1 & 1M). The order was determined pseudo-randomly via partial Latin Square. Once the order of solution testing was determined for one group, members of each group were yoked to the same order as members of the first group to control for order effects. On the last day of testing, rats received a final infusion of water to check for any shifts in reactivity that may have been caused by the testing itself. For rats that did not complete all sessions (e.g. due to loss of fistula viability), we were able to maintain the data already collected from the rat due to the analytical technique we are using. All test sessions were recorded in high definition (Cannon Vixia HF R800; 1080p, 60 fps) and stored for later behavioral scoring per methods set forth in Grill and Norgren (1978) and refined by Kiefer (1995).

Each video was scored frame-by-frame by a trained rater blind to the experimental conditions for a duration of 1 min from the first appearance of one of the following behaviors: mouth movements, tongue protrusions, lateral tongue protrusions, paw licking, gapes, head

shakes, forelimb flails, fluid expulsions, and passive drips. Tongue protrusions, lateral tongue protrusions, and paw licking (that was not followed by forelimb flails or headshakes) were considered hedonic responses, while gapes, chin rubs, head shakes, forelimb flails, passive drips and fluid expulsions were considered aversive responses per the recommendations of Kiefer (1995). Scoring consisted of noting each instance of a behavior and time logging it at the first frame in which the behavior occurred. Behaviors were tallied into totals for each behavior for each animal for each concentration. The totals of the behaviors were then combined into their respective hedonic or aversive response totals to be analyzed. Multiple, well-trained scorers with established reliability, scored all the hedonic and aversive responses. A subset of 10 randomly selected videos were scored and correlated between all raters to assess interrater reliability. The overall average interrater reliability correlation coefficient was .93 with aversive response average reliability at .91, while Hedonic response average reliability was .96.

Taste Reactivity Data

The second stage models with IMAC and IRoC estimates from the complete drinking data and data excluding day 1 were compared to each other and to a model with Total Ethanol Consumed as a predictor based on their AIC and BIC when predicting aversive and hedonic taste reactions to ethanol. Table 1 shows the results of the Poisson mixed effects models. The AICs were relatively similar between the three models; however, BICs highlight the best predictive models were those with Total Ethanol Consumed (g/kg) during IAE as a predictor. Therefore, all subsequent analyses of taste reactions were performed using only the models with Total Ethanol Consumed (g/kg) as a predictor.

Total hedonic taste reactions were the sum of tongue protrusions, lateral tongue protrusions, and paw licks. Total aversive taste reactions were the sum of gapes, chin rubs, head

shakes, forelimb flails, passive drips, and fluid expulsions. Separate Poisson mixed effects models predicting total hedonic and total aversive taste reactions were performed for each substance (ethanol or sucrose) with the fixed effects of solution concentration (Concentration; ethanol: 5 to 40%, sucrose: 0.01-1M) or Trial (for water; trials 1 and 2), Age (adolescent/adult), and Condition (IAE/CTRL). Additional Poisson mixed effects models predicting total hedonic and total aversive taste reactions were also performed on the data from IAE rats exclusively. These additional models were also performed for each substance (ethanol, sucrose, and water) with the fixed effects of solution concentration (Concentration; ethanol: 0 to 40%, sucrose: 0.01-1M) or Trial (for water; trials 1 and 2), Age (adolescent/adult), and Total Ethanol Consumed (g/kg) during IAE (sum of the g/kg/24hr consumption for all drinking days). Models predicting both total hedonic and total aversive taste reactions to ethanol and sucrose taste reactions also included the random effects of the intercept and the interaction of the intercept and concentration while water analyses included only the random effect of the intercept. These random effects credit within subjects changes for each individual across reactivity sessions and create repeated measures analyses. Analyses for aversive behaviors in response to sucrose and water were analyzed; however, aversive responses to sucrose and water are historically near zero and a floor effect is likely to occur.

For those unfamiliar with these statistical approaches, Poisson models yield regression weights (*b*) that can be interpreted similar to the "rate of change" or the "slope" of a plotted line. The line in a Poisson regression – and its multi-level counterpart used in the current study – is analyzed as a line in a logarithmic space. The predicted variable is never transformed. Instead, the whole regression equation is resolved within a logarithmic space. When the *b* for any given predictor is in this logarithmic space and is back-transformed out of the logarithmic space and

into a standard linear slope, the slope will change depending on the values of continuous predictors the *b* is based on. This change in the rate of change – i.e. change in the slope of the line – results in the logarithmic curve seen in many of the graphs presented here. In the case of Poisson regression, we are dealing with counts or incidents of a given behavior as our predicted variable, so *b* is an *incidence rate ratio*. For example, the amount of hedonic responses predicted by a Poisson model for rats in the IAE condition, at a specific substance concentration, and with all other variables at their mean values can be determined using Equation (1) below, where $\hat{Y}$ is the predicted number of hedonic responses, *x* is the actual (Concentration) or effect coded (Condition) value of the predictor variable and Intercept is the grand mean.

$$\hat{Y} = e^{\text{Intercept} + \boldsymbol{b}_{Condition}\boldsymbol{x}_{Condition} + \boldsymbol{b}_{Concentration}\boldsymbol{x}_{Concentration} + \boldsymbol{b}_{Interaction}\boldsymbol{x}_{Condition}\boldsymbol{x}_{Concentration}} \quad (1)$$

Two sets of analyses were performed to answer two different and important theoretical questions. The first set of analyses included Condition as a fixed (main) effect in the analyses with all animals included to compare between IAE and CTRL rats at a macro-level, without accounting for IAE rats' drinking pattern. The second set of analyses includes only the IAE rats and examines Total Ethanol Consumed during IAE as a fixed (main) effect to account for any differences related to individual IAE rats' drinking patterns across IAE.

Chapter 4 - **Statistical Tests**

For IAE and BEC all statistical tests were performed using JMP® 12.1 (SAS Institute Inc., Cary, NC, USA). All Poisson mixed effects models were performed using the GLMER function from the lme4 package version 1.1-21 (Bates, Mächler, Bolker, & Walker, 2015) in R (R Core Team, 2019). Some Poisson generalized linear mixed effects models required an

increase in the number of iterations to reach convergence, the code for this is provided in Appendix A.

Intermittent Access to Ethanol 2 Bottle Choice Data

A linear multilevel model predicting alcohol consumed was performed on the ETOH groups with the full factorial fixed effects of Day (as a continuous predictor) and Age (adolescent/adult). In addition, the random effects of the intercept and the Day slope were specified to credit within subjects changes for each individual across time, thus creating a repeated measures analysis and providing IMAC (intercept) and IRoC (slope) estimates for individual rats. Previous literature shows that consumption is high and decreases initially, then increases after the first week in higher-drinking adolescents, but remains flat among lower-drinking adolescents (DiLeo et al., 2015; DiLeo et al., 2016; Fisher et al., 2017). Thus, higher drinking on day 1 creates a nonlinearity which may obfuscate the overall pattern of drinking across time in a linear model. The alternative strategy which addresses adolescents' similar drinking on day 1 was performed as well by using an identical linear multilevel model predicting alcohol consumed but excluding drinking data from day 1 to see if parameter estimates are affected.

Blood Ethanol Concentrations

Blood Ethanol Concentration (BEC; mg/dL) was analyzed with a Student's T-test comparing adolescent and adult BECs. In addition, a correlation between BEC and g/kg of ethanol consumed on the day of blood sampling was performed.

Chapter 5 - **Results**

Intermittent Access to Ethanol

The details for all fixed effects for all models predicting intermittent access to ethanol can be found in Table 2. To assess whether differences in alcohol consumption (g/kg) exist between adolescent and adult rats, a linear mixed effects model was performed on the days when ethanol was available. The fixed (main) effect of Day was not significant. The fixed (main) effect of Age was significant, $F(1,42) = 6.16$, $p = .017$ with adolescents (*mean* = 3.16 g/kg) drinking more alcohol than adults (*mean* = 1.97 g/kg), but the interaction of Age and Day qualifies this effect. The interaction of Age and Day was significant, $F(1,42) = 8.10$, $p = .007$ and the rate of change in ethanol drinking for adolescents ($b = -0.05$) was lower than and in the opposite direction of adults ($b = 0.03$; Figure 3A). While adolescents' alcohol consumption declined by 0.05 g/kg per day on average, adults' alcohol consumption increased by an average of 0.03 g/kg per day suggesting differences in ethanol consumption patterns between adolescents and adults over the drinking period.

To examine the effect of initially high drinking among adolescents, an identical linear mixed effects model was performed on alcohol consumed (g/kg) with day 1 excluded from the analysis. All fixed (main) effects and interactions were non-significant ($p > .05$), suggesting ethanol drinking remained similar between adults and adolescents across time after day 1 (Figure 3B). The lack of significant main effects in the model without data from day 1 implies that the differences between adolescent and adult alcohol consumption patterns are driven, in large part, by initially high drinking among adolescents on the first day of ethanol access.

Blood Ethanol Concentration

To examine the effect of Age on BEC (mg/dL) a Student's T-test was performed and was not significant ($t(42) = -0.52$, $p > .05$; Figure 4A). In addition, a significant correlation between BEC and ethanol consumed (g/kg), $r(42) = .68$, $p < .001$, indicates that the rats were consuming the ethanol from the bottles (Figure 4B). Together, these results suggest that the measure of ethanol intake was valid, and that Age group did not influence ethanol metabolism.

Taste Reactivity

Alcohol Hedonic. Means and SEMs are displayed in Table 3 and the details for all fixed effects can be found in Table 4. Analysis of total hedonic taste reactions to alcohol ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 175$) revealed a fixed (main) effect of Concentration that neared significance, $Z = 1.83$, $p = .067$, $b = 0.0079$, and was qualified by an interaction. The fixed (main) effect of Age was not significant ($p > .05$). The significant main effect of Condition ($Z = 2.97$, $p = .003$) showed that IAE rats (*mean* = 99.2) had more hedonic responding to ethanol compared to CTRL rats (*mean* = 57.5) overall and this was qualified by a significant interaction of Concentration and Condition ($Z = 2.02$, $p = .044$, $b = -0.0087$) such that, for every 1% increase in ethanol concentration, hedonic responding increases by an incidence rate ratio (b) of 0.0167 among IAE rats (i.e. in this case, with all other variables at their mean values, Equation [1] above applies), but decreases by an incidence rate ratio of 0.0008 among CTRL rats. No other interactions were significant ($p < .05$; Figure 5A).

Further analysis of hedonic taste reactions to alcohol among IAE rats with the full factorial fixed effect of Total Ethanol Consumed during IAE (all drinking days included) in the model instead of Condition ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 117$) shows a significant fixed (main) effect of Concentration ($Z = 3.00$, $p = .003$, $b = 0.0164$), such that for every 1% increase in

ethanol concentration, hedonic responding increases by an incidence rate ratio of 0.016. Interestingly, among IAE rats there was a significant main effect of Age when Total Ethanol Consumed was included in the model, $Z = 2.39$, $p = .017$, where Adolescent+IAE rats (*mean* = 77.2) displayed less hedonic responses to ethanol than Adult+IAE rats (*mean* = 122.9). The fixed (main) effect of Total Ethanol Consumed was significant ($Z = 2.38$, $p = .017$, $b = 0.009$), such that for every 1 g/kg increase in Total Ethanol Consumed, hedonic responding increased by an incidence rate ratio of 0.009. No interactions were significant ($p > .05$; Figure 5B). Together, these results indicate that prior ethanol experience increases "liking" of higher concentrations of ethanol; rats with adolescent ethanol experience have lower "liking" of ethanol than rats with ethanol experience as adults; and the amount of ethanol consumed during ethanol experiences has a positive relationship with the "liking" of ethanol.

Alcohol Aversive. Means and SEMs are displayed in Table 3 and the details for all fixed effects can be found in Table 4. Analysis of total aversive taste reactions to ethanol ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 175$) revealed a significant fixed (main) effect of Concentration $Z = 5.43$, $p < .001$, $b = 0.025$, such that, for every 1% increase in ethanol concentration, aversive responding increases by an incidence rate ratio of 0.025. The fixed (main) effect of Age was not significant ($p > .05$). The fixed (main) effect of Condition was significant $Z = 5.19$, $p < .001$, with IAE rats (*mean* = 7.41) emitting less aversive responses to ethanol than CTRL rats (*mean* = 24.51) overall. No interactions were significant ($p > .05$; Figure 6A).

Further analysis of aversive taste reactions to ethanol among IAE rats with the full factorial fixed effect of Total Ethanol Consumed during IAE in the model instead of Condition ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 117$) shows a significant fixed (main) effect of Concentration ($Z = 3.27$, $p = .001$, $b = 0.025$) similar to the previous analysis. Again, among IAE rats there was a

significant fixed (main) effect of Age when Total Ethanol Consumed was included in the model, $Z = 3.03$, $p = .002$, where Adolescent+IAE rats (*mean* = 10.51) displayed more aversive responses to ethanol than Adult+IAE rats (*mean* = 4.39). The fixed (main) effect of Total Ethanol Consumed was significant ($Z = 4.66$, $p < .001$, $b = -0.026$), such that for every 1 g/kg increase in Total Ethanol Consumed, aversive responding decreased by an incidence rate ratio of 0.026. No interactions were significant ($p > .05$; Figure 6B). Together, these results indicate that prior ethanol experience decreases "disliking" of ethanol; rats with adolescent ethanol experience have greater "disliking" of ethanol than rats with ethanol experience as adults; and the amount of ethanol consumed during ethanol experiences is inversely related to the "disliking" of ethanol.

Sucrose Hedonic. Means and SEMs are displayed in Table 5 and the details for all fixed effects can be found in Table 6. Analysis of total hedonic taste reactions to sucrose ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 172$) revealed a significant fixed (main) effect of Concentration, $Z = 6.57$, $p < .001$, $b = 0.53$, such that for every 1M increase in sucrose concentration, hedonic responding increases by an incidence rate ratio of 0.53. The fixed (main) effect of Age was also significant ($Z = 2.64$, $p = .008$) with less hedonic responding to sucrose from adolescents (*mean* = 126) compared to adults (*mean* = 164). Qualifying the previous effects, there was a significant interaction of Concentration and Age ($Z = 2.28$, $p = .023$, $b = 0.18$) with adolescent ($b = 0.72$) hedonic responding increasing with sucrose concentration more steeply than adults ($b = 0.35$). No other interactions were significant ($p < .05$; Figure 7A).

Further analysis of hedonic taste reactions to sucrose among IAE rats with the full factorial fixed effect of Total Ethanol Consumed during IAE in the model instead of Condition ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 114$) shows a similar fixed (main) effect of Concentration ($Z = 5.25$, $p < .001$, $b = 0.48$), such that for every 1M increase in sucrose concentration, hedonic responding

increases by an incidence rate ratio of 0.48. Interestingly, among IAE rats the main effect of Age was only nearing significance ($Z = 1.90$, $p = .058$). The fixed (main) effect of Total Ethanol Consumed was not significant ($p > .05$). A significant interaction qualified the fixed (main) effects of Age and Concentration, $Z = 2.63$, $p = .009$, $b = 0.24$, such that Adolescent+IAE rats ($b = 0.72$) had a steeper increase in the relationship between hedonic responding and sucrose concentration compared to Adult+IAE rats ($b = 0.24$). All other interactions were non-significant ($p > .05$; Figure 7B). These results indicate that prior ethanol experience does not increase the "liking" of sucrose. However, rats with alcohol experience during adolescence displayed a stronger positive relationship between "liking" and sucrose concentration suggesting Adolescent+IAE rats are more sensitive to changes in the hedonic value of sucrose than rats with ethanol experience as adults.

Sucrose Aversive. Means and SEMs are displayed in Table 5 and the details for all fixed effects can be found in Table 6. Analysis of total aversive taste reactions to sucrose ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 172$) revealed no significant fixed (main) effects or interactions (ps $> .05$) with the fixed (main) effect of Concentration nearing significance ($Z = -1.83$, $p = .067$, $b = -0.716$; Figure 8A).

Further analysis of aversive taste reactions to sucrose among IAE rats with the full factorial fixed effect of Total Ethanol Consumed during IAE in the model instead of Condition ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 114$) showed no significant fixed (main) effects. There was, however, a significant interaction of Concentration and Total Ethanol Consumed ($Z = 2.65$, $p = .008$, $b = 0.038$) such that, for every 1 g/kg increase in Total Ethanol Consumed, the rate of decline in aversive responding as Concentration increases is made more and more positive by an incidence rate ratio of 0.038. There was also a significant interaction of Age and Total Ethanol Consumed,

$Z = 2.34$, $p = .019$, $b = -0.021$, where Adolescent+IAE rats ($b = -0.026$) displayed decreasing aversive responses to sucrose compared to increasing aversive responses displayed by Adult+IAE rats ($b = 0.016$) as Total Ethanol Consumed increased. No other interactions were significant (ps > .05; Figure 8B). Combined, these results show that greater levels of ethanol consumption may increase the "disliking" of higher sucrose concentrations. In addition, the age at which ethanol experience occurs interacts with the amount of ethanol consumed to influence the "disliking" of sucrose. However, given that the number of total aversive responses to sucrose was very low overall, it is likely that there is a floor effect and these results should be interpreted with caution.

Water Hedonic. Means and SEMs are displayed in Table 7 and the details for all fixed effects can be found in Table 8. Analysis of total hedonic taste reactions ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 113$) yielded no significant fixed (main) effects of Age or Condition, but did reveal a significant fixed (main) effect of Trial, ($Z = 9.49$, $p < .001$, $b = -0.09$) that was qualified by the significant interaction of Trial and Condition, $Z = 7.71$, $p < .001$, $b = -0.07$. Planned contrasts indicated that during the first water trial, IAE and CTRL rats had similar amounts of hedonic responding to water ($p > .05$). However, during the second water trial, there was significantly greater hedonic responding from IAE rats compared to CTRLs, $Z = 2.45$, $p = .014$ – an effect that was driven largely by a decline in hedonic responding among CTRL rats from the first to last water trial, $Z = 10.29$, $p < .001$, while IAE rats' hedonic responding did not change ($p > .05$). No other interactions were significant ($p < .05$; Figure 9A).

Further analysis of hedonic taste reactions to water among IAE rats with the full factorial fixed effect of Total Ethanol Consumed during IAE in the model instead of Condition ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 74$) shows the fixed (main) effects of Age and Total Ethanol Consumed were not

significant (*p*s > .05). Similar to the previous analysis, there was a significant fixed (main) effect of Trial ($Z = 2.63$, $p = .008$, $b = -0.03$), with a decreasing trend in hedonic responding from trial 1 to trial 2 that was qualified by an interaction. The interactions between Trial and Age along with Age and Total Ethanol Consumed were not significant (*p*s > .05). There was a significant interaction of Trial and Total Ethanol Consumed ($Z = 5.55$, $p < .001$, $b = 0.002$), but this interaction was further qualified by the significant three-way interaction of Trial, Age, and Total Ethanol Consumed, $Z = 2.77$, $p = .006$, $b = 0.001$. Planned contrasts indicated that from water trial 1 to trial 2, Adolescent+IAE rats' relationship between hedonic responding and Total Ethanol Consumed changed significantly from a negative ($b = -0.001$) to a positive rate of change ($b = 0.005$), $Z = 5.41$, $p < .001$. The same relationship in Adult+IAE rats also significantly increased from trial 1 ($b = 0.003$) to trial 2 ($b = 0.006$), $Z = 2.17$, $p = .030$. However, Adolescent+IAE and Adult+IAE rats' rates of change did not significantly differ from each other on either trial (*p*s > .05; Figure 9B). Overall, these results indicate that prior ethanol experience and age do not affect the initial "liking" of water. However, prior alcohol experience does affect the "liking" of water on the final trial, which occurred at the end of taste reactivity testing, in a manner related positively to the amount of ethanol consumed during IAE with a stronger increase in this relationship from the first to last water trial among adolescents.

Water Aversive. Means and SEMs are displayed in Table 7 and the details for all fixed effects can be found in Table 8. Analysis of total aversive taste reactions to water ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 113$) revealed a significant fixed (main) effect of Trial ($Z = 9.44$, $p < .001$, $b = -0.41$) with an increasing trend in aversive responding from trial 1 to trial 2 that was qualified by interactions. The fixed (main) effect of Age was not significant ($p > .05$). The fixed (main) effect of Condition was significant ($Z = 2.47$, $p = .014$) with IAE rats (*mean* = 2.60) displaying less

aversive responding than CTRL rats (*mean* = 5.62), but this effect was also qualified by interactions. The interaction of Age and Condition was not significant ($p > .05$). There were significant interactions between Trial and Age ($Z = 5.63$, $p < .001$, $b = 0.24$) as well as Trial and Condition ($Z = 4.86$, $p < .001$, $b = -0.21$) both of which were qualified by the significant three-way interaction of Substance, Age, and Condition, $Z = -4.04$, $p < .001$, $b = -0.17$. Planned contrasts indicated that all groups significantly increased aversive responding from water trial 1 to trial 2 (Zs = -6.86 – -6.64, all ps < .001) except Adolescent+IAE rats which significantly decreased ($Z = 3.32$, $p < .001$). On the initial water trial, Adolescent+IAE rats (*mean* = 3.83) displayed significantly greater aversive responses compared to Adult+IAE rats (*mean* = 1.19), $Z = 3.03$, $p = .003$. On the final water trial, Adolescent+CTRL rats (*mean* = 12.07) displayed significantly greater aversive responses compared to Adolescent+IAE rats (*mean* = 2.48), $Z = 3.58$, $p < .001$; Figure 10A).

Further analysis of aversive taste reactions to water among IAE rats with the full factorial fixed effect of Total Ethanol Consumed during IAE in the model instead of Condition ($df_{\text{Parameter}} = 11$, $df_{\text{Residual}} = 74$) showed no significant fixed (main) effects ($p > .05$). The interaction of Age and Total Ethanol Consumed was not significant ($p > .05$). There was a significant interaction of Trial and Age ($Z = 3.99$, $p < .001$) as well as Trial and Total Ethanol Consumed ($Z = 3.35$, $p < .001$, $b = 0.010$), but these were qualified by the significant interaction of Trial, Age, and Total Ethanol Consumed, $Z = 5.74$, $p < .001$, $b = -0.017$. Planned contrasts for Adolescent+IAE rats between water trial 1 ($b = -0.028$) and trial 2 ($b = -0.014$) indicated a significantly less steep decline in aversive responding during trial 2 as Total Ethanol Consumed increased, $Z = -2.26$, $p = .024$. Adult+IAE rats' rate of change in aversive responding as Total Ethanol Consumed changed significantly, $Z = 5.35$, $p < .001$, from an increasing trend on trial 1 ($b = 0.022$) to a

decreasing trend on trial 2 (b = -0.033). Additionally, there was a significant difference in the rate of change in aversive responses as Total Ethanol Consumed increased on water trial 1 between Adolescent+IAE and Adult+IAE rats, Z = -2.80, p = .005, with Adolescent+IAE rats showing a decreasing trend and Adult+IAE rats showing an increasing trend (Figure 10B). Combined, these results show that alcohol experience during adolescence alters aversive responding to water and that there is a relationship between lower aversive responding to water and higher alcohol consumption which is present on the first water trial among adolescents. However, as with sucrose, the number of total aversive responses to water were very low overall, thus it is likely that there is a floor effect and these results should also be interpreted with caution.

Chapter 6 - **Discussion**

The overarching goals of this study were to determine whether adolescent alcohol drinking affected the palatability of alcohol and to determine the relationship between individual drinking pattern during IAE and the "liking" and "disliking" of alcohol after alcohol experience. Overall, this study supports a connection between individual voluntary drinking pattern, age of alcohol initiation, and the hedonic value of alcohol with greater drinking associated with higher "liking" and lower levels of "disliking." However, drinking pattern and age relate to the "liking" and "disliking" of alcohol independent from one another.

As hypothesized (H1), adolescents drank more overall during IAE than adults. As was further hypothesized, adults had a steady upward rate of change in drinking across time, while adolescents had a downward rate of change in drinking during IAE. However, the descending rate of change in drinking and higher overall alcohol drinking by adolescents were due to

initially high adolescent consumption with age group differences disappearing when day 1 was not included in the analysis.

The general hypothesis (H2) that adolescent IAE alters the palatability of alcohol across a range of alcohol concentrations in a manner positively related to alcohol consumption during IAE was partially supported with IAE rats showing greater hedonic and fewer aversive responses to alcohol compared to CTRL rats. Both Adolescent+IAE and Adult+IAE rats displayed increased "liking" and decreased "disliking" as the amount of alcohol they consumed during IAE increased, supporting the hypothesis. However, contrary to my hypotheses, Adolescent+IAE rats showed surprisingly *less* overall "liking" and greater overall "disliking" of alcohol compared to Adult+IAE rats. In addition, Adolescent+IAE rats were hypothesized to display higher magnitude rates of change across total ethanol consumed during IAE moderated by alcohol concentration. This three-way interaction was hypothesized to occur such that Adolescent+IAE rats would diverge from Adult+IAE rats to have steeper and steeper relationships between "liking" and "disliking" responses and total ethanol consumed during IAE as alcohol concentration tested during taste reactivity increased; however, these interactions were not present.

The hypothesis (H3.2) predicting that age and prior alcohol experience would not affect the hedonic value of sucrose was partially met. As predicted, "liking" increased as sucrose concentration increased indicating that taste reactivity was sensitive to changes in hedonic value. In further support of H3.2, total ethanol consumption during IAE was not predictive of sucrose "liking." In support of the alternative hypothesis (H3.1), the adolescent group had a more positive rate of change in "liking" across sucrose concentrations. However, neither hypothesis predicted the adolescent group would have less hedonic responses to sucrose overall.

While analyses of aversive responding to sucrose yielded several significant effects, most animals exhibited few if any aversive responses to sucrose. With responses very low, the effects found are difficult to interpret due to the low rates of responding resulting in a floor effect and will not be further discussed.

As hypothesized, no differences between age and condition were found on the initial water trial and there was no relationship between Total Ethanol Consumed during IAE and initial water "liking." The current study's taste reactivity testing occurred when all rats were young adults or adults. Thus, the lack of differences between conditions and age groups supports the hypothesis that differences in taste reactions between adolescents and adults on initial water trials may be limited to testing during adolescence and not during young adulthood (Wilmouth & Spear, 2009). While Total Ethanol Consumed appears unrelated to initial hedonic responding to water, the reduction in hedonic responding from trial 1 to trial 2 among CTRL rats and lack of change among IAE rats begins to elucidate a pattern. I conjecture that this lack of change among IAE rats combined with the strengthening of a positive relationship between "liking" and total ethanol consumed during IAE on the final water trial implies that associative learning between the context and substances encountered during non-water taste reactivity trials may be involved. However, the current study was not designed to test conditioning or contextual learning.

While analyses of aversive responding to water yielded several significant effects, most animals exhibited few, if any, aversive responses to water. With responses very low, the effects are difficult to interpret due to a floor effect and will not be further discussed.

Intermittent Access to Ethanol

Interestingly, the overall average final alcohol consumption during the last few sessions was considerably lower than what has been observed in previous research with Long-Evans rats

(Carnicella et al., 2014), including within our own lab (Wukitsch et al., 2019). Typical averages for Long-Evans rats average approximately 5.5g/kg/24hr after 4-5 weeks with about 40% reaching BECs usually observed among rats bred for high alcohol consumption or preference (Carnicella et al., 2014). However, the BECs after the final 28-minute consumption test corroborate that many rats in the current study did not drink very excessively with only 4 of 44 rats above the binge-drinking threshold of 80mg/dL. Although higher BECs were not achieved, the overall change in alcohol consumption over time in the current study appears to be consistent with previous research where adults increased consumption (e.g. Schramm-Sapyta et al., 2014; Simms et al., 2008) and adolescents decreased consumption over time (DiLeo et al., 2015; DiLeo et al., 2016; Fisher et al., 2017; Schramm-Sapyta et al., 2014; but see: Doremus et al., 2005; Wukitsch et al., 2019).

However, claims about rate of change must be extrapolated from looking at the graphs of previous research because rates of change in alcohol consumption across time were not directly analyzed previously for adolescents and adults. In the current study, rate of change was analyzed and compared between the two age groups. When comparing the analysis of the complete alcohol drinking data with the data excluding day 1 drinking, the rate of decline in drinking across time became much flatter for adolescents without day 1 and adult's rate of change showed little change. Although adolescents displayed higher average consumption compared to adults, as in some other literature (Daoura et al., 2011; Doremus et al., 2005; García-Burgos, González, Manrique, & Gallo, 2009; see also: Schramm-Sapyta et al., 2014), the literature is mixed and other studies found that groups with adult-onset alcohol access consumed and preferred alcohol more than rats with adolescent-onset access (Siegmund, et al., 2005; Bell et al., 2006b; Pickens et al., in press). In light of these mixed results, the finding that without day 1 drinking there were

no longer mean differences between adult and adolescent rats in the present report is interesting. However, comparison to previous reports is difficult as day 1 drinking was not excluded. Voluntary alcohol intake is known to be altered between rat strains and environmental/social conditions (Carnicella et al., 2014). There are studies that do not show a pattern of very high initial adolescent drinking and some differences compared to the current study in rat strain (Bell et al., 2006b: P rats; Daoura et al., 2011: Wistar; Doremus et al., 2005: Sprague-Dawley; Schramm-Sapyta et al., 2014: CD) or environment (Wukitsch et al., 2019) were present but not in all previous research (Pajser, Breen, Fisher, & Pickens, 2018; Pickens et al., in press). Although the magnitude of the initial adolescent binge varies, some studies using methods consistent with the current study have found high initial drinking during IAE among adolescent rats (DiLeo et al., 2015; DiLeo et al., 2016; Fisher et al., 2017); however, others have not (Pajser, et al., 2018; Pickens et al., in press).

The current study suggests that differences in alcohol drinking patterns (mean and rate of change) between adolescent and adult Long-Evans rats may be driven primarily by the initially high drinking of adolescents when initially high levels of consumption are present in adolescents. While other aspects of drinking pattern differences between age groups remain to be further explored, the analytical approach to the IAE data presented here affords new ways to compare drinking patterns across time. The current analytical approach offers rate of change in addition to mean differences to more completely represent, compare, and elucidate patterns and magnitudes of change in drinking data. Rate of change in alcohol drinking during adolescence is heritable in humans (Edwards et al., 2017) and may be helpful as a phenotypical metric for selective breeding in future research to look at the escalation of drinking and exploring the behavioral epigenetics of alcohol use disorders and alcoholism in animal models.

Taste Reactivity

Analyses. When analyzing taste reactivity, two separate analyses were used to answer different theoretical questions concerning hedonic and aversive taste reactions for each substance. In one analysis CTRL rats are compared to IAE rats to see what differences may exist as a result of IAE, regardless of how much any individual rat drank during IAE. In another analysis, data from only the IAE rats was analyzed but used Total Ethanol Consumed during IAE to account for differences in taste reactions based on individuals' levels of consumption. The former can answer questions concerning the hedonic value of a substance after alcohol experience compared to no alcohol experience. The latter can answer questions about how the magnitude of alcohol experience is related to hedonic value among rats that had access to alcohol. While reanalyzing a subset of the same data may inflate family-wise error rates, the addition of a variable (Total Ethanol Consumed) that assesses a theoretically relevant source of variability among the IAE rats was also important. In addition, it was not possible to test the hypotheses concerning the magnitude of alcohol experience without the latter analysis.

Ethanol. Previous research in adult outbred rats (Kiefer & Badia-Elder, 1997; Kiefer, Bice, & Badia-Elder, 1994) as well as strains selectively bred for alcohol-related traits like preference (Bice & Kiefer, 1990), drinking (Kiefer et al., 1995), and sedative sensitivity (Kiefer & Badia-Elder, 1997), shows alcohol-naïve rats have relatively flat or slightly increased hedonic responses to increasing ethanol concentrations. Further, the same research shows that prior voluntary alcohol experience results in a positive relationship between hedonic responses and alcohol concentrations accompanied by an increase in hedonic responses overall compared to naïve rats. In accord with previous research, the current study found flat/very slightly decreasing

hedonic responding from alcohol-naïve CTRL rats along with the upward-shift and increasing trend in hedonic responding from alcohol-experienced IAE rats.

Alcohol-naïve rats have shown a relatively flat trend in aversive responses in the past with slight increases or very slight decreases in response to increasing alcohol concentrations, though there is some variability (c.f. Kiefer et al., 1994). In addition, compared to alcohol-naïve rats, alcohol-experienced rats have previously had a generally flatter or decreasing trend in aversive responding to increasing ethanol concentrations and less aversive responses overall, especially among strains selectively bred for high alcohol preference and drinking (Bice & Kiefer, 1990; Kiefer et al., 1995; Kiefer & Badia-Elder, 1997; Kiefer et al., 1994). In line with these previous findings, the current study found a steeper increase in aversive responding with increasing alcohol concentrations among alcohol-naïve CTRL rats compared to alcohol-experienced IAE rats which showed only a slight increasing trend and less overall aversive responding.

Previous research performed taste reactivity testing both before and after voluntary access to alcohol and did not find a relationship between adult, alcohol-naïve rat's hedonic or aversive responding and subsequent ethanol consumption (Bice, Kiefer, & Elder, 1992; Kiefer & Dopp, 1989). However, the current report examined the relationship between consumption and taste reactivity which occurred *after* alcohol access. The amount of alcohol consumed during IAE shows distinct positive and negative relationships with hedonic and aversive responses to alcohol, respectively. Since alcohol-naïve taste reactivity was previously not correlated with voluntary alcohol consumption (Bice et al., 1992; Kiefer & Dopp, 1989) and the current study shows that the magnitude of voluntary alcohol consumption is related to alcohol-experienced IAE rats' taste reactivity, it appears the hedonic and aversive value of alcohol are moderated by

the amount of experience one has with alcohol. In other words, while naïve hedonic and aversive responses do not predict alcohol consumption, alcohol consumption appears to predict post-experience hedonic and aversive responses to alcohol. Thus, "liking" and "disliking" of alcohol are more greatly influenced by greater amounts of alcohol consumption; however, the mechanisms of this influence are not known.

Habituation to the aversive aspects of alcohol's taste may explain decreased "disliking" among alcohol-experienced rats as has been previously proposed (Kiefer & Dopp, 1989). There is some evidence that suggests reduced "disliking" results from higher levels of alcohol consumption per day that result from intermittent access models. For example, in a study on conditioned place preference (CPP) for alcohol, rats with intermittent access appear to have approximately equal or slightly *less* total ethanol consumed during home cage access overall compared to the continuous access rats, but intermittent access rats drank more per day. During CPP testing only rats that had home cage access to alcohol on a 48-hour intermittent basis did not develop a conditioned place aversion (CPA) towards the alcohol-paired chamber compared with groups that had continuous alcohol access or water access alone (Williams, Nickel, & Bielak, 2018). Further, after place preference testing, all groups consumed similar amounts of alcohol during oral operant self-administration (Williams et al., 2018). Taken together, these findings and those of the current report support that higher voluntarily alcohol consumption per drinking session results in lower levels of aversion. The magnitude of reduction in "disliking" of alcohol appears to be related to the level of intoxication reached during drinking sessions, which was driven by the intermittent schedule. However, whether decreased "disliking" is due to post-consumption pharmacological effects which act alongside or instead of habituation remains a

question for future research in addition to the contribution of conditioned aversion, or lack thereof, in this process.

The increased "liking" of alcohol among alcohol-experienced rats supports the hypothesis that "liking" of alcohol's taste may be altered by alcohol's post-consumption pharmacological effects (Bice & Kiefer, 1990). This hypothesis and the current results align with the findings of another study which allowed adolescent rats to have intermittent access to alcohol and then tested their appetitive learning in adulthood by pairing flavors with an alcohol or sucrose solution (DiLeo et al., 2015). The study showed that all rats consumed more of a sucrose-paired flavor solution than rats without any flavor pairing. Naïve controls and lower-drinking rats showed aversion towards flavors previously-paired with alcohol. Heavy-drinking rats, however, consumed much more of the flavor solutions paired previously with alcohol compared to heavy drinking rats with no flavor pairings, naïve controls, and lower-drinking rats (DiLeo et al., 2015). These results lead to the conclusion that alcohol alters aspects of appetitive learning through heavy alcohol consumption (DiLeo et al., 2015). While most rats in the current study did not drink as heavily as those in previous studies, the impacts of alcohol likely fall along a dose-response gradient. Thus, the current study extends the previous study's conclusion, implying that the changes to appetitive learning that occur from higher levels of drinking may be linked to changes in the "liking" of alcohol that occur with alcohol experience.

Due to the lower-than-expected amounts of drinking in this experiment, it is difficult to assess how these results advance the incentive sensitization theory. The Incentive-sensitization Theory of Addiction is specifically concerned with addiction and addiction-like states characterized by compulsive drug-seeking and drug-taking (Berridge & Robinson, 2016). Under this theory, hedonic value does not increase as craving and drug-taking reach pathological levels.

However, the current study found a positive relationship between hedonic value and prior alcohol intake. Given the low BECs and generally lower-than-usual consumption of the rats in the current study, an addiction-like state is unlikely to have been achieved for most rats and the current study was not intended to assess craving-levels or markers of withdrawal. Thus, a reduction in "liking" hypothesized under Incentive-sensitization Theory would not be expected to occur in this case. However, the alcohol experience dependent shift in alcohol's palatability without the induction of an addiction-like state suggests that enhanced "liking" and reduced "disliking" may be part of a feedback loop that increases motivation to drink through mechanisms separate from incentive salience. These hedonic and aversive changes, as the current study shows, are worth further exploration as driving forces behind alcohol drinking prior to the development of addiction and are potential sources of vulnerability to repeated alcohol exposure that may lead to addiction.

It appears that repeated lower doses of alcohol are relevant for understanding the early stages of alcohol-taking and alcohol's effects on behavior. BECs below the threshold for binging obtained in the current study have been shown to have behavioral effects in previous literature (e.g. Pickens et al., in press; Fisher et al., 2017; van Erp & Miczek, 1997; Waller, Murphy, McBride, Lumeng, & Li, 1986) and the current findings align with other findings relating level of alcohol consumption and alcohol-associated learning (DiLeo et al., 2015). Thus, the current report supports the hypothesis that there is a link between "liking" and "disliking" alcohol's taste and prior experience. Previous researchers have suggested the focus of alcohol research should be on the pharmacological effects of alcohol alone, suggesting a dissociation between sensory or other properties and alcohol's intoxicating effects (e.g. Lester & Freed, 1973). However, the results herein provide additional evidence for the hypothesis that an association, rather than a

dissociation, between alcohol's sensory and post-consumption effects exists (Brasser, Castro, & Feretic, 2015) and is intertwined with alcohol's hedonic value.

Other variables, such as age of alcohol initiation, also influence the "liking" and "disliking" of alcohol. One of the goals of the current study was to determine whether lasting changes to the hedonic value of alcohol result from alcohol consumption during adolescence. This goal has significant public health implications that may inform identification of vulnerable individuals, preventative interventions, and public policy. There is a mixed literature in rodents concerning whether alcohol binging during adolescence predicts adult alcohol use. Some studies have shown that adolescent alcohol increases intake in adulthood (e.g. Alaux-Cantin et al., 2013; Amodeo et al., 2017). However, another study shows no difference between ages of drinking onset and drinking behavior in adulthood (Labots et al., 2018). In the current study, rats with adolescent access to alcohol show less "liking" and more "disliking" of alcohol compared to rats which had access during adulthood. However, adolescents consumed more alcohol than adults on average when including the initial high drinking and similar amounts when not including initial drinking, suggesting that adults may be more vulnerable to changes in alcohol palatability induced by prior alcohol consumption than adolescents. This finding aligns with the finding that higher levels of prior alcohol consumption resulted in greater loss of control over drinking among adults, a result not found among adolescent-onset drinkers (Labots et al., 2018). This vulnerability may be a contributing factor behind the consistent, steady increase in consumption that is often seen during IAE in adult rats (Carnicella et al., 2014) and may act as a catalyst for escalation of intake.

Conversely, the adolescent+IAE group's reduced "liking" and increased "disliking" may indicate a weaker association between alcohol's taste and the post-consumption effects of

alcohol. Evidence from ethanol-induced CTA supports this as adolescents need higher doses of ethanol and more pairings of ethanol with sucrose to develop a CTA (Anderson et al., 2010). The case may be that more alcohol is consumed because adolescents tend to be inaccurate when matching intake with their desired satiation point. This explanation might also explain why adolescents' alcohol consumption is considerably more variable than adults' (Schramm-Sapyta et al., 2014). However, the opposite may also be true. Since the adolescent+IAE group had very high initial alcohol consumption, the resulting high intoxication may have resulted in aversive outcomes which subsequently led to both decreased "liking" and consumption – i.e. a conditioned taste aversion. Future studies that explore limiting initial adolescent drinking may help resolve these competing hypotheses.

Notably, taste reactivity testing occurred in the current study when members of the adolescent and adult group were all adults. The adolescent group was not age-matched with the adult group for taste reactivity testing to avoid a prolonged abstinence period which previous research has shown can alter taste reactivity after just one month (Kiefer et al., 1995; Kiefer et al., 1994). While differences in age at the testing point exist in the current study, all rats in the present report were tested in adulthood; thus, one would not expect differences in taste reactivity as the majority of taste reactivity experiments have been conducted in adult rats (e.g. Berridge & Schulkin, 1989; Bice et al., 1992; Delamater, LoLordo, & Berridge, 1986; Kiefer et al., 1994).

Sucrose. Including sucrose as a tastant was done primarily to see whether any differences in palatability of alcohol were unique to alcohol itself or affected another rewarding substance especially among the adolescent+IAE group. The amount of "liking" and "disliking" responses to sucrose were similar to those observed in previous research (e.g. Flynn & Grill, 1988; Kiefer et al., 1995). The differences in "liking" related to prior alcohol experience observed for alcohol

are not present for sucrose and are likely to be unique to alcohol. While alcohol experience and total alcohol consumed did not influence the "liking" of sucrose, somewhat surprisingly, age group did. In the past, adolescents have been shown to consume large amounts of sucrose compared to adults (e.g. Franklin, Wearne, Homewood, & Cornish, 2017; Rodríguez-Ortega, Alcaraz-Iborra, de la Fuente, & Cubero, 2019). Importantly, in the current experiment, both cohorts were adults at the time of taste reactivity testing. Despite the fact that both groups were adults at testing, the group that received IAE or water during adolescence had less hedonic responding to sucrose compared to the group that received IAE or water during adulthood. In addition, the group that received IAE or water during adolescence had a stronger positive relationship between hedonic responding and sucrose concentration. The steeper increase in hedonic responding across sucrose concentrations indicates a higher sensitivity to changes in hedonic value among the group that received IAE or water during adolescence. The steeper increase in hedonic responding across sucrose concentrations among the group that received IAE or water during adolescence is consistent with the findings of previous research that performed taste reactivity *during* adolescence and compared hedonic responding to adults (Wilmouth & Spear, 2009). However, this is the first study comparing the effects of adolescent versus adult alcohol exposure and taste reactivity in adults.

Water. Water trials were the first and last trials of taste reactivity. The lack of differences between groups on the initial water trial indicates that all the groups "liked" water, a relatively neutral and familiar tastant, similarly. Thus, IAE and age do not affect the initial "liking" of water and all groups' reactivity was similar at the start of taste reactivity testing.

On the final trial, however, IAE rats' "liking" did not decline compared to the initial trial, whereas CTRL rats' "liking" did decline. Both Adolescent+IAE and Adult+IAE rats showed a

positive shift (i.e. become more positively inclined) in the relationship between hedonic responses and total ethanol consumed from the first to the final trial. Adolescent+IAE rats saw a larger shift in this relationship compared to Adult+IAE rats, but the two groups did not differ from each other on either trial. Together, the results imply a few conclusions. First, that prior experience with alcohol affects final water trial "liking" in a manner positively related to the magnitude of prior alcohol intake. Second that rats which had greater intake of alcohol during adolescence are more vulnerable to the shift in water's hedonic value that occurs from the beginning to the end of taste reactivity. And finally, that the cause of differences between rats with and without alcohol experience on the final trial may be the result of divergent associative learning occurring over the course of taste reactivity testing.

While the current study was not designed to test associative learning, from the first to last trial the reduced "liking" among naive controls and the strengthening of the positive relationship between "liking" and total alcohol consumed among IAE rats aligns with that hypothesis. The amount of hedonic and aversive responding for water are comparable to prior literature (e.g. Kiefer & Badia-Elder, 1997); however, there were no studies in my search which tested or compared initial and final water taste reactivity. Thus, future studies are necessary to explore hypotheses surrounding appetitive learning and hedonic value.

The present research has explored analytical approaches for modelling and comparing drinking data across time and using voluntary consumption to predict taste reactivity. Stand-alone, these analytical approaches will help facilitate the understanding and interpretation of drinking behavior in general. However, the current findings also add to a small but growing body of research (e.g. Pickens et al., in press; Fisher et al., 2017; van Erp & Miczek, 1997; Waller et al., 1986) demonstrating the importance and impact of moderate levels of alcohol intake and

challenge a focus on specific cut-offs for intoxication levels (e.g. 80 or 100 mg/dL) or consumption amounts when exploring the long-term effects of alcohol. In addition, these findings support a link between alcohol's hedonic, sensory, and post-consumption effects which may be important to the early parts of the process by which alcoholism develops. This is the first study exploring the effects of adolescent alcohol on the hedonic and aversive value of alcohol in adulthood. In sum, consumption of alcohol influences the future hedonic and aversive value of alcohol in a way that makes alcohol more palatable with greater prior consumption. This alcohol-experience-related change in palatability may lead to a cycle of increasing intake that has the potential to drive higher intake which may eventually result in an addiction-like state in vulnerable individuals. However, it appears that those drinking alcohol as adolescents may be more resistant to this palatability shift than those first drinking as adults, suggesting different mechanisms of vulnerability to consumption escalation for adolescents and adults. More research is needed to explore the behavioral and neurological mechanisms of these effects to better understand how drinking influences hedonic and aversive value and how hedonic and aversive value then impact drinking behavior.

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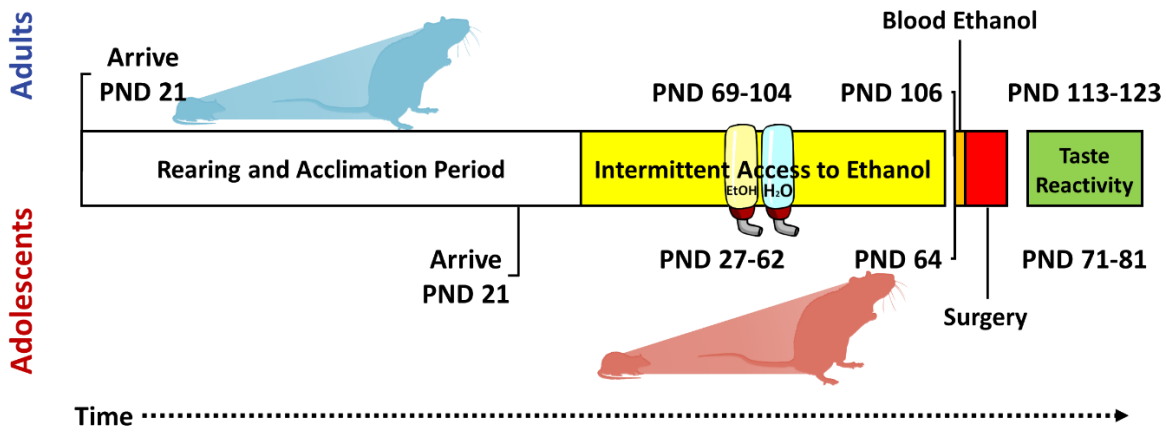

Figure 1. Experimental timeline. Postnatal days (PND) are listed for adult (top) and adolescent (bottom) rats for each stage of the experiment.

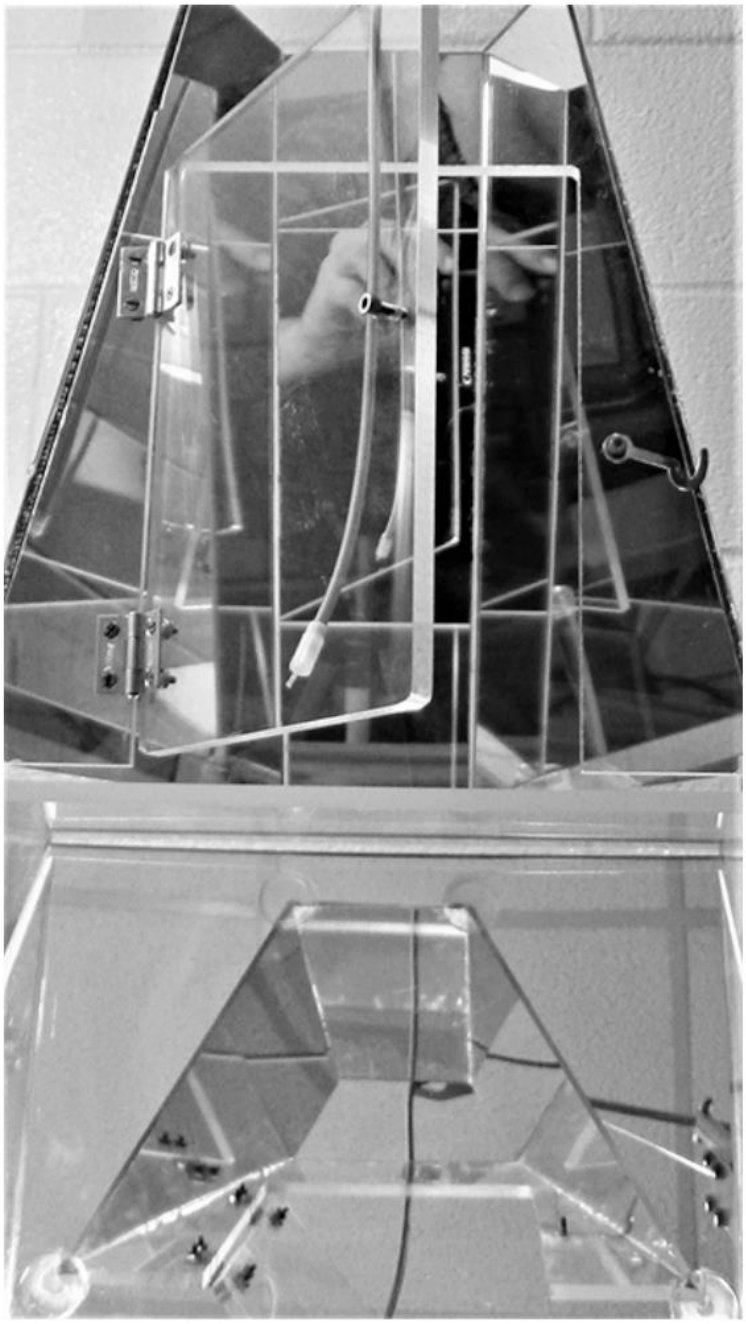

Figure 2. The taste reactivity chamber I designed with the lower panel showing the swivel (Harvard Apparatus) and leash (Plastics One) that are anchored to the side of the chamber. The swivel allows the tubing to spin freely, preventing twists/kinks in the line. Together, these design features allow for minimally inhibited movement, prevent tubing disconnection, damage, and obstruction all while maximizing the useable field of view for video scoring. The design modifications improved taste reactivity workflow and scoring accuracy.

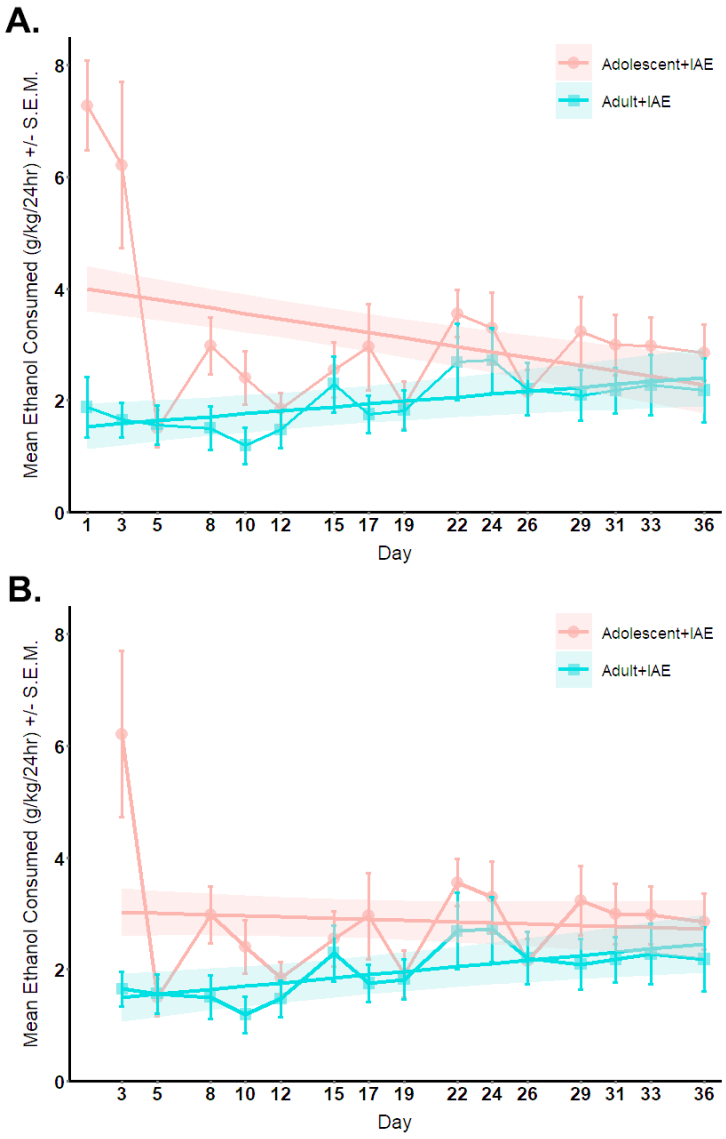

Figure 3. Mean ethanol consumed (g/kg/24hr) during Intermittent Access to Ethanol (IAE) for adolescent and adult rats in the IAE conditions. The days shown on the x-axis are the alcohol exposures during the IAE period. The trend line represents the predicted values for Adolescent+IAE (red line) and Adult+IAE (blue line) rats. Error ribbons represent ±1 S.E.M. A: When including the first day of drinking in the analysis, Adolescent+IAE rats had higher overall consumption than Adult+IAE rats, but Adolescent+IAE rats had a declining rate of change in consumption compared to Adult+IAE rats' inclining rate of change. B: When excluding the first day, drinking was similar between the two groups.

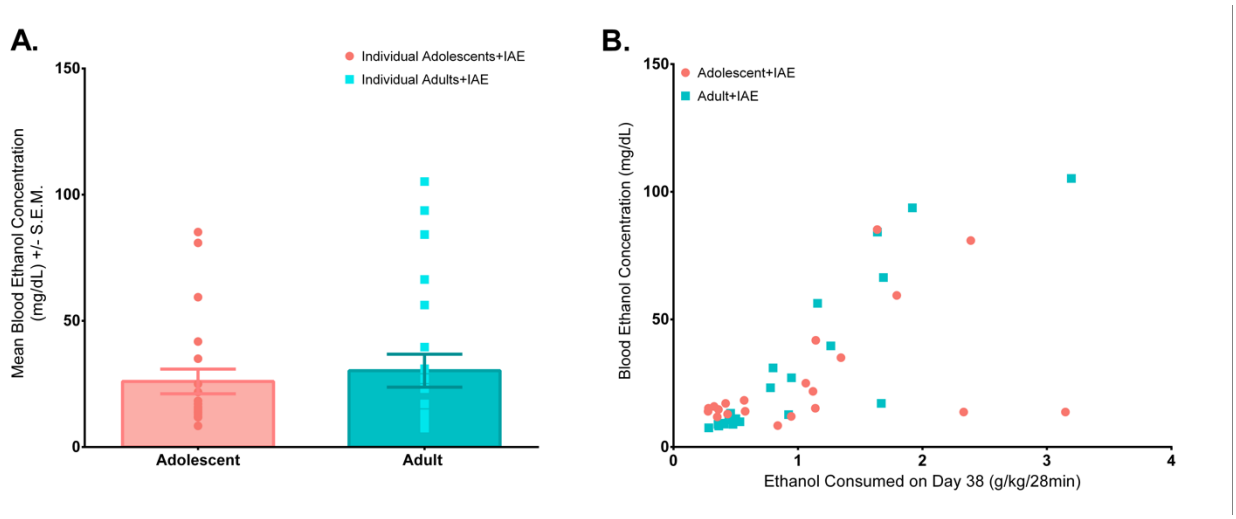

Figure 4. A: Mean and individual blood ethanol concentrations (BECs) and B: individual BECs plotted against grams ethanol consumed (g/kg/28min) during the brief ethanol access on the 38th day of IAE. There was no difference between BECs of rats in the adult and adolescent groups. Ethanol consumption and BEC were correlated.

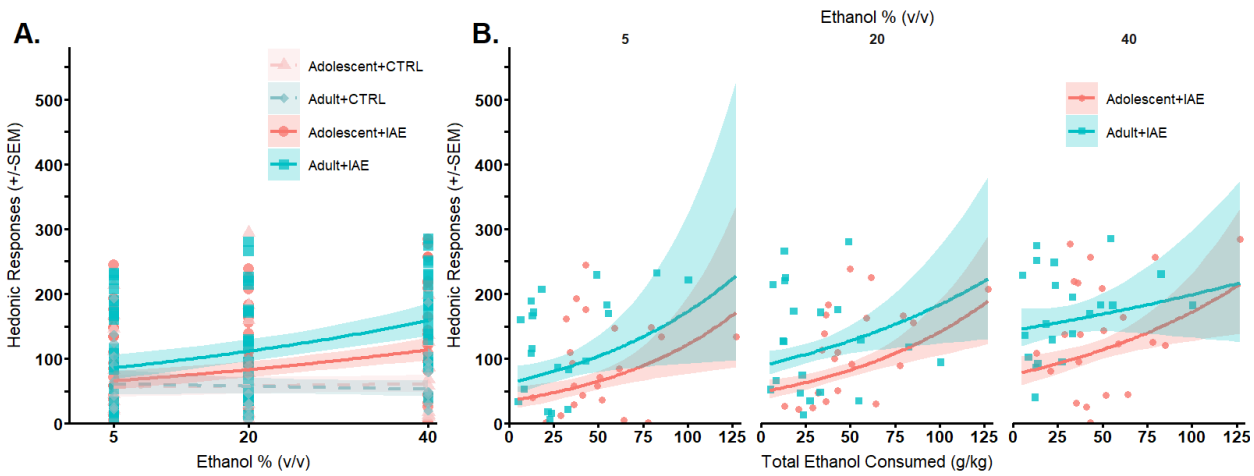

Figure 5. Individual and predicted hedonic responses to ethanol during taste reactivity trials. Error ribbons represent ±1 S.E.M. Adolescent (red) and Adult (blue) rats received either intermittent access to ethanol (IAE; solid line) or only water (CTRL; broken line) prior to taste reactivity testing. A: Analysis of all conditions' hedonic responses to ethanol revealed, IAE rats had greater hedonic responding and a more positive relationship between hedonic responses and ethanol concentration than CTRL rats. B: When examining the impact of ethanol consumption on hedonic responding to ethanol among only the IAE group, the upper x-axis breaks the figure into three panels for each ethanol concentration tested (5, 20, & 40%) to show any change in the relationship between Total Alcohol Consumed (lower x-axes) and hedonic responding during taste reactivity across concentration. There were positive relationships between hedonic responses and ethanol concentration as well as Total Ethanol Consumed during IAE. Additionally, Adolescent+IAE rats displayed less hedonic responses to ethanol than Adult+IAE rats.

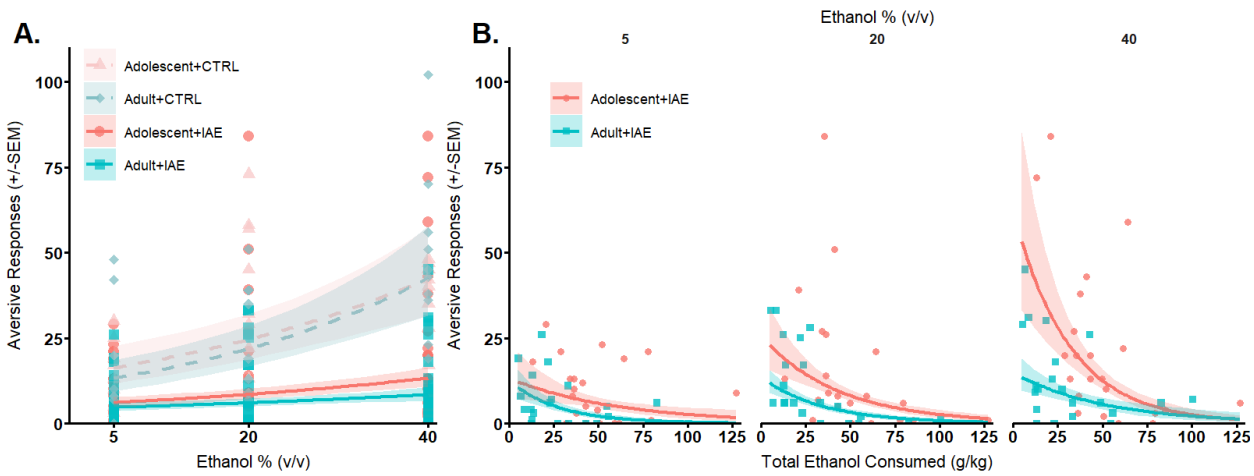

Figure 6. Individual and predicted aversive responses to ethanol. Error ribbons represent ±1 S.E.M. Adolescent (red) and Adult (blue) rats received either intermittent access to ethanol (IAE; solid line) or only water (CTRL; broken line) prior to taste reactivity testing. A: Analysis of all conditions' aversive responses to ethanol, there was a positive relationship between aversive responding and ethanol concentration. Additionally, IAE rats displayed less aversive responses to ethanol than CTRL rats. B: When examining the impact of ethanol consumption on aversive responding to ethanol among only the IAE group, the upper x-axis breaks the figure into three panels for each ethanol concentration tested (5, 20, & 40%) to show any change in the relationship between Total Alcohol Consumed (lower x-axes) and aversive responding during taste reactivity across concentration. There were negative relationships between aversive responses and ethanol concentration as well as the Total Ethanol Consumed during IAE. In addition, Adolescent+IAE rats displayed more aversive responses to ethanol than Adult+IAE rats.

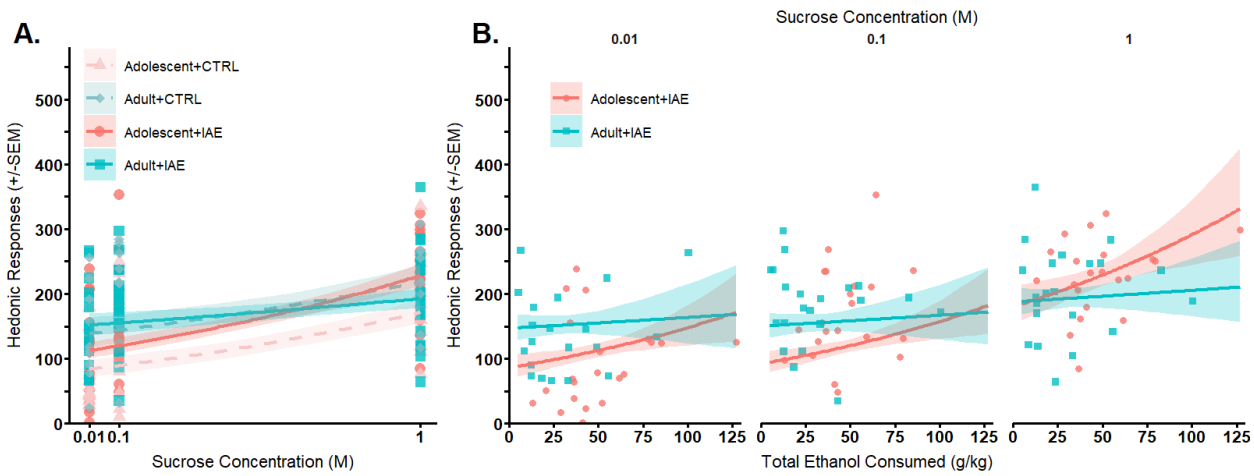

Figure 7. Individual and predicted hedonic responses to sucrose. Error ribbons represent ±1 S.E.M. Adolescent (red) and Adult (blue) rats received either intermittent access to ethanol (IAE; solid line) or only water (CTRL; broken line) prior to taste reactivity testing. A: Analysis of all conditions' hedonic responses to sucrose, there was a positive relationship between hedonic responding and sucrose concentration. Additionally, the Adolescent group displayed less hedonic responses to sucrose than the Adult group. However, the Adolescent group had a stronger positive relationship between hedonic responding and concentration than the Adult group. B: When examining the impact of ethanol consumption on hedonic responding to sucrose among only the IAE group, the upper x-axis breaks the figure into three panels for each sucrose concentration tested (0.01, 0.1, 1.0 M) to show any change in the relationship between Total Alcohol Consumed (lower x-axes) and hedonic responding during taste reactivity across concentration. There was a positive relationship between hedonic responses and sucrose concentration with the Adolescent+IAE rats displaying a stronger positive relationship between hedonic responding and sucrose concentration than Adult+IAE rats.

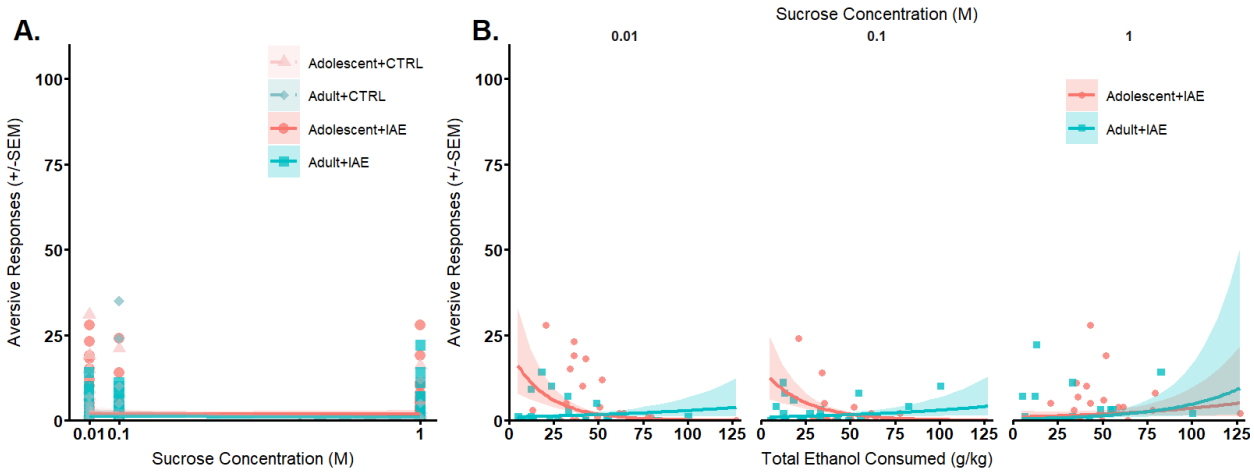

Figure 8. Individual and predicted aversive responses to sucrose. Error ribbons represent ±1 S.E.M. Adolescent (red) and Adult (blue) rats received either intermittent access to ethanol (IAE; solid line) or only water (CTRL; broken line) prior to taste reactivity testing. A: Analysis of all conditions' aversive responses to sucrose very low aversive responding and high potential for a floor effect. B: When examining the impact of ethanol consumption on aversive responding to sucrose among only the IAE group, the upper x-axis breaks the figure into three panels for each sucrose concentration tested (0.01, 0.1, 1.0 M) to show any change in the relationship between Total Alcohol Consumed (lower x-axes) and aversive responding during taste reactivity across concentration. However, the potential for a floor effect remains.

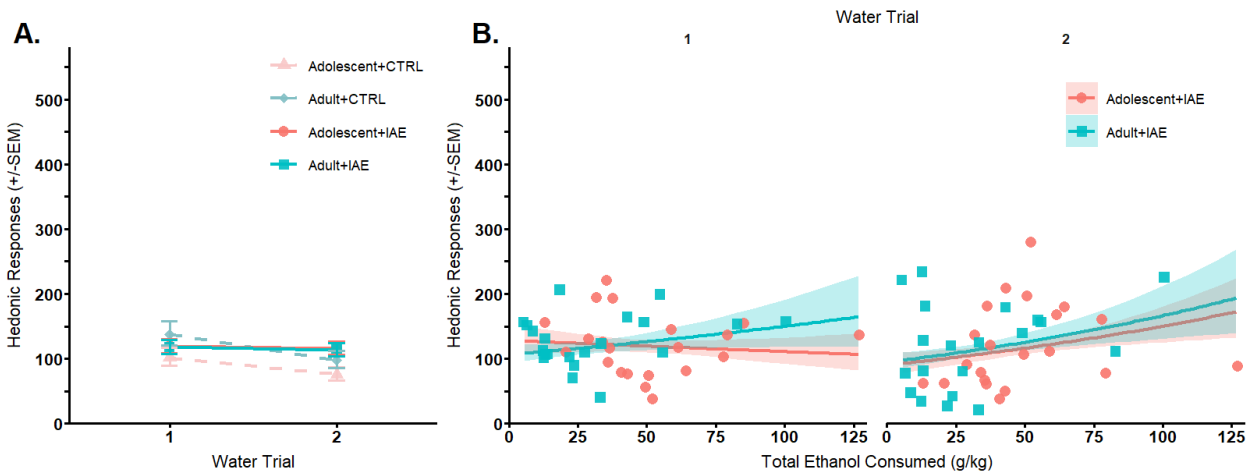

Figure 9. Individual and predicted hedonic responses to water. Error ribbons represent ±1 S.E.M. Adolescent (red) and Adult (blue) rats received either intermittent access to ethanol (IAE; solid line) or only water (CTRL; broken line) prior to taste reactivity testing. A: Analysis of all conditions' hedonic responses to water revealed that all groups responded similarly to water on the initial trial. However, on the second trial IAE rats displayed greater hedonic responding to water than CTRL rats driven by the decline in hedonic responding for CTRL rats from trial 1 to trial 2. B: When examining the impact of ethanol consumption on hedonic responding to water among only the IAE group, the upper x-axis breaks the figure into two panels, one for each water trial, to show any change in the relationship between Total Alcohol Consumed (lower x-axes) and hedonic responding during taste reactivity across the first and final trial. Adolescent+IAE and Adult+IAE's relationships between hedonic responding and total ethanol consumed increased differently with Adolescent+IAE increasing more than Adult+IAE from trial 1 to trial 2.

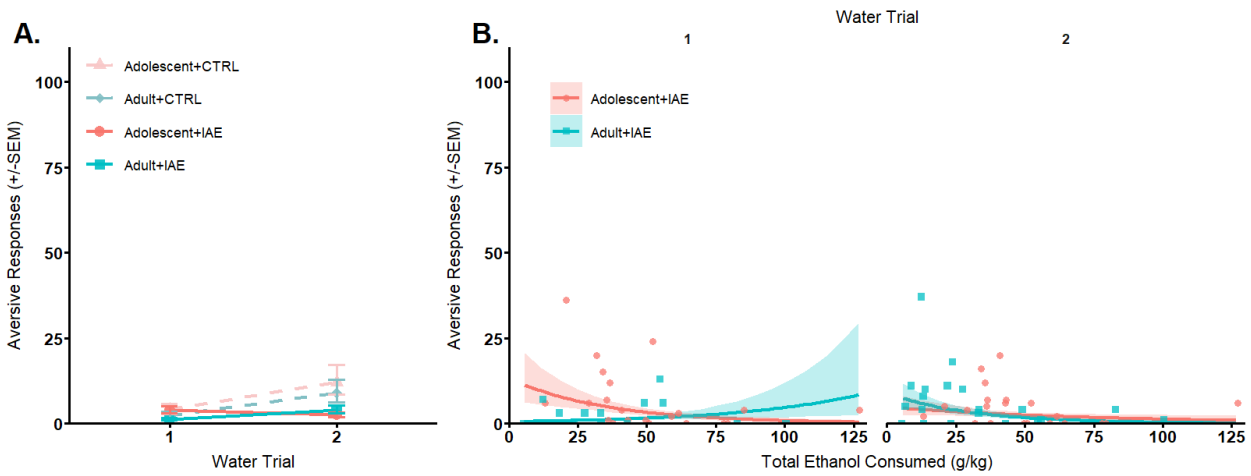

Figure 10. Individual and predicted aversive responses to water. Error ribbons represent ±1 S.E.M. Adolescent (red) and Adult (blue) rats received either intermittent access to ethanol (IAE; solid line) or only water (CTRL; broken line) prior to taste reactivity testing. A: Analysis of all conditions' aversive responses to water revealed very low aversive responding and high potential for a floor effect. B: When examining the impact of ethanol consumption on aversive responding to water among only the IAE group, the upper x-axis breaks the figure into two panels, one for each water trial, to show any change in the relationship between Total Alcohol Consumed (lower x-axes) and aversive responding during taste reactivity across the first and final trial. However, the potential for a floor effect remains.

Table 1.

Comparison of Poisson Multi-level Models Predicting Taste Reactivity Responses to Alcohol

Table 1

Comparison of Poisson Multi-level Models Predicting Total Hedonic and Aversive Responses to Alcohol

Model	$df_{Parameter}$	*AIC*	*BIC*
Predicting Total Hedonic Responses			
Total Ethanol Consumed (g/kg) as a Predictor	11	2420	2452
IMAC & IRoC (Days 1-36) as Predictors	15	2423	2466
IMAC & IRoC (Days 3-36) as Predictors	15	2425	2468
Predicting Total Aversive Responses			
Total Ethanol Consumed (g/kg) as a Predictor	11	1130	1161
IMAC & IRoC (Days 1-36) as Predictors	15	1133	1175
IMAC & IRoC (Days 3-36) as Predictors	15	1132	1175

Table 2.
Intermittent Access to Ethanol Results

Table 2

Intermittent Access to Ethanol Results

Model and Fixed Effect	*df*	*F*	*p*	*b*
LMM predicting g/kg Ethanol Consumption (Day 1-36)				
Age (Adolescent/Adult)	1, 42	6.16	*.017	
Day (1-36)	1, 42	< 1	>.050	-0.01
Age*Day	1, 42	8.10	*.007	
LMM predicting g/kg Ethanol Consumption (Day 3-36)				
Age (Adolescent/Adult)	1, 42	3.35	.074	
Day (3-36)	1, 42	< 1	>.050	0.01
Age*Day	1, 42	1.89	.177	

Note. LMM = Linear Multilevel Model. * denotes $p < .05$.

Table 3.
Total Hedonic and Aversive Taste Reactivity Responses to Alcohol

Table 3

Total Hedonic and Aversive Taste Reactivity Responses to Alcohol

	Ethanol Concentration (v/v)					
	5%		20%		40%	
Group	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
Hedonic responses						
Adolescent+CTRL	84.70	22.61	96.10	29.41	80.00	19.18
Adolescent+IAE	90.00	14.77	113.48	15.35	141.00	18.25
Adult+CTRL	89.22	18.93	45.89	11.38	74.80	15.52
Adult+IAE	121.95	16.84	128.00	17.65	171.95	14.71
Aversive responses						
Adolescent+CTRL	12.20	3.32	36.80	6.72	39.20	3.13
Adolescent+IAE	9.50	1.93	15.62	4.53	21.82	5.03
Adult+CTRL	18.11	5.52	24.56	5.51	48.30	7.61
Adult+IAE	6.38	1.60	11.00	2.51	12.67	2.72

Table 4.
Taste Reactivity to Alcohol Results

Table 4

Taste Reactivity to Alcohol Results

Model and Fixed Effects	*df*	*Z*	*p*	*b*
GLMM predicting hedonic taste reactions	11, 175			
Concentration (Ethanol: 5-40%)		1.83	.067	0.784
Age (Adolescent/Adult)		-0.76	.448	-0.069
Condition (IAE/CTRL)		-2.97	*.003	-0.272
Concentration*Age		0.27	.787	0.117
Concentration*Condition		-2.02	*.044	-0.873
Age*Condition		0.88	.381	0.080
Concentration*Age*Condition		0.48	.630	0.208
GLMM predicting hedonic taste reactions for IAE rats	11, 117			
Concentration (Ethanol: 5-40%)		3.00	*.003	1.638
Age (Adolescent/Adult)		-2.39	*.017	-0.232
Total Ethanol Consumed (during IAE)		2.38	*.017	0.009
Concentration*Age		0.13	.900	0.069
Concentration*Total Ethanol Consumed		-0.78	.433	-0.016
Age*Total Ethanol Consumed		0.49	.621	0.002
Concentration*Age*Total Ethanol Consumed		0.18	.855	0.004
GLMM predicting aversive taste reactions	11, 175			
Concentration (Ethanol: 5-40%)		5.43	*< .001	0.025
Age (Adolescent/Adult)		1.00	.318	0.114
Condition (IAE/CTRL)		5.19	*< .001	0.598
Concentration*Age		-0.02	.983	-0.009
Concentration*Condition		1.19	.235	0.542
Age*Condition		-0.54	.586	-0.062
Concentration*Age*Condition		-0.64	.525	-0.287
GLMM predicting aversive taste reactions for IAE rats	11, 117			
Concentration (Ethanol: 5-40%)		3.27	*.001	2.454
Age (Adolescent/Adult)		3.03	*.002	0.436
Total Ethanol Consumed (during IAE)		-4.66	*< .001	-0.026
Concentration*Age		0.18	.859	0.130
Concentration*Total Ethanol Consumed		0.01	.993	0.000
Age*Total Ethanol Consumed		0.38	.705	0.002
Concentration*Age*Total Ethanol Consumed		-1.59	.112	-0.045

Note. GLMM = Generalized Linear Multilevel Model. IAE = Intermittent Access to Ethanol group. CTRL = Water control group. All models were Poisson GLMMs and fixed effects were contrast coded (i.e. sum-to-zero) or mean-centered. * denotes $p < .05$.

Table 5.
Total Hedonic and Aversive Taste Reactivity Responses to Sucrose

Table 5

Total Hedonic and Aversive Taste Reactivity Responses to Sucrose

	Sucrose Concentration (Molarity)					
	0.01		0.10		1.00	
Group	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
Hedonic responses						
Adolescent+CTRL	71.70	16.13	139.30	28.58	183.70	25.26
Adolescent+IAE	94.05	14.60	168.05	16.06	227.38	12.93
Adult+CTRL	136.20	22.37	200.78	27.79	220.89	20.76
Adult+IAE	140.75	14.17	179.81	13.16	202.90	15.40
Aversive responses						
Adolescent+CTRL	8.40	3.29	3.40	2.12	2.40	1.54
Adolescent+IAE	6.90	1.91	2.71	1.27	5.48	1.53
Adult+CTRL	2.20	0.79	8.22	4.28	2.00	1.36
Adult+IAE	2.60	0.93	2.81	0.80	3.52	1.26

Table 6.
Taste Reactivity to Sucrose Results

Table 6

Taste Reactivity to Sucrose Results

Model and Fixed Effects	*df*	*Z*	*p*	*b*
GLMM predicting hedonic taste reactions	11, 172			
Concentration (Sucrose: 0.01-1 M)		6.57	*< 0.001	0.534
Age (Adolescent/Adult)		-2.64	*0.008	-0.132
Condition (IAE/CTRL)		-1.52	0.129	-0.076
Concentration*Age		2.28	*0.023	0.185
Concentration*Condition		0.69	0.489	0.056
Age*Condition		-1.39	0.166	-0.070
Concentration*Age*Condition		-0.63	0.528	-0.051
GLMM predicting hedonic taste reactions for IAE rats	11, 114			
Concentration (Sucrose: 0.01-1 M)		5.25	*< 0.001	0.481
Age (Adolescent/Adult)		-1.90	0.058	-0.093
Total Ethanol Consumed (during IAE)		1.69	0.092	0.003
Concentration*Age		2.63	*0.009	0.241
Concentration*Total Ethanol Consumed		-0.12	0.907	0.000
Age*Total Ethanol Consumed		1.13	0.257	0.002
Concentration*Age*Total Ethanol Consumed		-0.07	0.946	0.000
GLMM predicting aversive taste reactions	11, 172			
Concentration (Sucrose: 0.01-1 M)		-1.83	0.067	-0.716
Age (Adolescent/Adult)		0.89	0.372	0.184
Condition (IAE/CTRL)		-0.26	0.797	-0.053
Concentration*Age		0.12	0.906	0.039
Concentration*Condition		-1.81	0.071	-0.592
Age*Condition		-0.54	0.587	-0.112
Concentration*Age*Condition		-0.21	0.834	-0.069
GLMM predicting aversive taste reactions for IAE rats	11, 114			
Concentration (Sucrose: 0.01-1 M)		-1.14	0.253	-0.482
Age (Adolescent/Adult)		1.33	0.184	0.291
Total Ethanol Consumed (during IAE)		-0.54	0.588	-0.005
Concentration*Age		-0.36	0.718	-0.124
Concentration*Total Ethanol Consumed		2.65	*0.008	0.038
Age*Total Ethanol Consumed		-2.34	*0.019	-0.021
Concentration*Age*Total Ethanol Consumed		1.69	0.091	0.024

Note. GLMM = Generalized Linear Multilevel Model. IAE = Intermittent Access to Ethanol. CTRL = Water control. All models were Poisson GLMMs and fixed effects were contrast coded (i.e. sum-to-zero) or mean-centered. * denotes $p < .05$.

Table 7.
Total Hedonic and Aversive Taste Reactivity Responses to Water

Table 7

Total Hedonic and Aversive Taste Reactivity Responses to Water

	Trial 1		Trial 2	
Group	*M*	*SEM*	*M*	*SEM*
Hedonic responses				
Adolescent+CTRL	116.90	18.83	86.90	20.02
Adolescent+IAE	120.52	10.23	119.95	13.92
Adult+CTRL	144.56	16.31	105.40	24.07
Adult+IAE	127.76	8.75	119.35	15.13
Aversive responses				
Adolescent+CTRL	4.90	1.62	14.60	4.39
Adolescent+IAE	6.95	2.08	4.67	1.22
Adult+CTRL	3.22	1.62	12.90	3.44
Adult+IAE	1.95	0.75	6.55	1.95

Table 8.
Taste Reactivity to Water Results

Table 8

Taste Reactivity to Water Results

Model and Fixed Effects	*df*	*Z*	*p*	*b*
GLMM predicting hedonic taste reactions	11, 113			
Time (Trial 1/Trial 2)		9.49	*< .001	-0.088
Age (Adolescent/Adult)		-1.14	0.255	-0.066
Condition (IAE/CTRL)		-1.24	0.213	-0.072
Concentration*Age		-0.75	0.455	-0.007
Concentration*Condition		7.71	*< .001	-0.072
Age*Condition		-1.27	0.203	-0.073
Concentration*Age*Condition		-0.47	0.642	-0.004
GLMM predicting hedonic taste reactions for IAE rats	11, 74			
Time (Trial 1/Trial 2)		2.63	*0.008	-0.028
Age (Adolescent/Adult)		-0.34	0.732	-0.021
Total Ethanol Consumed (during IAE)		1.39	0.164	0.003
Concentration*Age		1.39	0.164	-0.015
Concentration*Total Ethanol Consumed		-5.55	*< .001	0.002
Age*Total Ethanol Consumed		-0.59	0.555	-0.001
Concentration*Age*Total Ethanol Consumed		-2.77	*0.006	0.001
GLMM predicting aversive taste reactions	11, 113			
Time (Trial 1/Trial 2)		-9.44	*< .001	-0.405
Age (Adolescent/Adult)		1.25	0.212	0.195
Condition (IAE/CTRL)		2.47	*0.014	0.386
Concentration*Age		5.63	*< .001	0.242
Concentration*Condition		-4.86	*< .001	-0.209
Age*Condition		0.15	0.880	0.024
Concentration*Age*Condition		-4.04	*< .001	-0.174
GLMM predicting aversive taste reactions for IAE rats	11, 74			
Time (Trial 1/Trial 2)		-0.69	0.492	-0.044
Age (Adolescent/Adult)		1.53	0.125	0.347
Total Ethanol Consumed (during IAE)		-1.54	0.123	-0.013
Concentration*Age		3.99	*< .001	0.258
Concentration*Total Ethanol Consumed		3.35	*0.001	0.010
Age*Total Ethanol Consumed		-0.88	0.377	-0.008
Concentration*Age*Total Ethanol Consumed		-5.74	*< .001	-0.017

Note. GLMM = Generalized Linear Multilevel Model. IAE = Intermittent Access to Ethanol. CTRL = Water control. All models were Poisson GLMMs and fixed effects were contrast coded (i.e. sum-to-zero) or mean-centered. * denotes $p < .05$.

Appendix A - R Code for Analysis of Taste Reactivity

```
library("MASS")
library("lattice")
library("boot")
library("car")
library("emmeans")
library("lme4")
library("zoo")
library("tidyr")
library("multcomp")
library("foreign")
library("msm")
library("ggplot2")
library("effects")
library("lmerTest")

#Read in data file with auto-headings and blanks/ N/As set to blank ("")
#Laptop
#myd <- read.csv("C:/Users/Thomas/Google Drive/Grad/Lab/Projects/Phase II
Projects - Analysis & Write-up/Masters/Results/Analyses/ABHV2018.csv",
na.strings="\"\"")
#Home
myd <- read.csv("C:/Users/Kiersten/Google Drive/Grad/Lab/Projects/Phase II
Projects - Analysis & Write-up/Masters/Results/Analyses/ABHV2018.csv",
na.strings="\"\"")
View(myd)


# ETHANOL #####

###Data Frame Preparation###

##Subset for Substance
mydetoh <- subset(myd, Substance == "Ethanol")
View(mydetoh)

##Rescaling
    #Rescale concentration to avoid issues with eigen values
    mydetoh$recoded.conc <- car::recode(mydetoh$Concentration, "5 =.05; 20
=.20; 40 =.40")

    ##Subset for Consumption Pattern Variables
    mydetoh.noCTRL <- subset(mydetoh, Condition != "CTRL")
    View(mydetoh.noCTRL)

##Centering
    #center concentration to avoid issues with variance inflation
    mydetoh$c.conc <- mydetoh$recoded.conc-mean(mydetoh$recoded.conc)
    #center TOTAL.ETOH.Swap.Consumed..g.kg. to avoid issues with variance
inflation
    mydetoh.noCTRL$c.totale <-
mydetoh.noCTRL$TOTAL.ETOH.Swap.Consumed..g.kg.-
mean(mydetoh.noCTRL$TOTAL.ETOH.Swap.Consumed..g.kg.)
```

```
    #center concentration for noCTRL subset to avoid issues with variance
inflation
    mydetoh.noCTRL$c.conc <- mydetoh.noCTRL$recoded.conc-
mean(mydetoh.noCTRL$recoded.conc)
    #center IMAC and IROC for noCTRL subset to avoid issues with variance
inflation
    mydetoh.noCTRL$c.MAC <- mydetoh.noCTRL$MAC-mean(mydetoh.noCTRL$MAC)
    mydetoh.noCTRL$c.ROC <- mydetoh.noCTRL$ROC-mean(mydetoh.noCTRL$ROC)
    mydetoh.noCTRL$c.MAC3 <- mydetoh.noCTRL$MAC3-mean(mydetoh.noCTRL$MAC3)
    mydetoh.noCTRL$c.ROC3 <- mydetoh.noCTRL$ROC3-mean(mydetoh.noCTRL$ROC3)

###Variable Coding Adjustment###

    #adjust Age to contrast coding
    contrasts(mydetoh$Age)=contr.sum(2)
    contrasts(mydetoh$Age)

    contrasts(mydetoh.noCTRL$Age)=contr.sum(2)
    contrasts(mydetoh.noCTRL$Age)

    #adjust Condition to contrast coding
    contrasts(mydetoh$Condition)=contr.sum(2)
    contrasts(mydetoh$Condition)

    contrasts(mydetoh.noCTRL$Condition)=contr.sum(2)
    contrasts(mydetoh.noCTRL$Condition)

## ETHANOL AVERSIVES ANALYSES#####

    ### Ethanol Aversives GLMER (with EtOH vs CTRL)####
    Eavers <-glmer(Total.Aversive ~ c.conc*Age*Condition
                     + (c.conc|RatID), data=mydetoh, family=poisson)
    summary(Eavers)

        #Post Hocs & Planned Contrasts

        #Checking to see if Adolescent vs Adult IAE rats were different
        Eavers.emm.c<- emmeans(Eavers,~ Condition)
        summary(Eavers.emm.c, type = "response")

    ### Ethanol Aversives GLMER (EtOH Group Only: Total EtOH Consumed) ######

    EaversTot <-glmer(Total.Aversive ~ c.conc*Age*c.totale
                   + (c.conc|RatID), data=mydetoh.noCTRL, family=poisson)

        # Model did not converge, used code below to extend # of iterations
and start from where the previous model left off.
        ss1 <- getME(EaversTot,c("theta","fixef"))
        EaversTot <-
update(EaversTot,start=ss1,control=glmerControl(optCtrl=list(maxfun=2e6)))
        summary(EaversTot)

        #Post Hocs & Planned Concrasts
        EaversTot.emm.a<- emmeans(EaversTot,~ Age)
        summary(EaversTot.emm.a, type = "response")
```

```
    ### Ethanol Aversives GLMER (EtOH Group Only: IMAC & IROC) ######

    EaversMR <-glmer(Total.Aversive ~ c.conc+Age+c.MAC+c.ROC
                     + c.conc:Age
                     + c.conc:c.MAC
                     + c.conc:c.ROC
                     + Age:c.MAC
                     + Age:c.ROC
                     + c.conc:Age:c.MAC
                     + c.conc:Age:c.ROC
                     + (c.conc|RatID), data=mydetoh.noCTRL, family=poisson)

        # Model did not converge, used code below to extend # of iterations
and start from where the previous model left off.
        ss3 <- getME(EaversMR,c("theta","fixef"))
        EaversMR <-
update(EaversMR,start=ss3,control=glmerControl(optCtrl=list(maxfun=2e6)))
        summary(EaversMR)

    # Check Variance Inflation & Compare AIC/BIC
    vif(EaversMR)
    AIC(EaversMR, EaversTot)
    BIC(EaversMR, EaversTot)

    ### Ethanol Aversives GLMER (EtOH Group Only: IMAC3 & IROC3) ######

    EaversMR3 <-glmer(Total.Aversive ~ c.conc+Age+c.MAC3+c.ROC3
                     + c.conc:Age
                     + c.conc:c.MAC3
                     + c.conc:c.ROC3
                     + Age:c.MAC3
                     + Age:c.ROC3
                     + c.conc:Age:c.MAC3
                     + c.conc:Age:c.ROC3
                     + (c.conc|RatID), data=mydetoh.noCTRL, family=poisson)

        # Model did not converge, used code below to extend # of iterations
and start from where the previous model left off.
        ss12 <- getME(EaversMR3,c("theta","fixef"))
        EaversMR3 <-
update(EaversMR3,start=ss12,control=glmerControl(optCtrl=list(maxfun=2e9)))
        summary(EaversMR3)

    # Check Variance Inflation & Compare AIC/BIC
    vif(EaversMR3)
    AIC(EaversMR3, EaversMR, EaversTot)
    BIC(EaversMR3, EaversMR, EaversTot)

## ETHANOL HEDONICS ######

    ### Ethanol Hedonics GLMER (with EtOH vs CTRL)####
    Ehed <-glmer(Total.Hedonic...MM.~c.conc*Age*Condition
                    + (c.conc|RatID), data=mydetoh, family=poisson) #Either
syntax works for Intercept & Slope inclusion
    summary(Ehed)

        #Post Hocs & Planned Contrasts
```

```
        #Use emmeans to get means for Conditions and summary toe back-
transform using 'type="response"'
        Ehed.emm.c <- emmeans(Ehed,~ Condition)
        summary(Ehed.emm.c, type = "response")

        #Getting bs for Condition. Remember that c.conc was rescaled so move
the decimal to the left 2 times.
        Ehed.emm.c_c <- emtrends(Ehed, ~c.conc*Condition, var="c.conc")
        Ehed.emm.c_c


    ### Ethanol Hedonics GLMER (EtOH Group Only: Total EtOH Consumed) ######

    EhedTot <-glmer(Total.Hedonic...MM. ~ c.conc*Age*c.totale
                    + (c.conc|RatID), data=mydetoh.noCTRL, family=poisson)
    summary(EhedTot)

        #Post Hocs and Planned Contrasts
        EhedTot.emm.a <- emmeans(EhedTot,~ Age)
        summary(EhedTot.emm.a, type = "response")


    ### Ethanol Hedonics GLMER (EtOH Group Only: IMAC & IROC) ######

    EhedMR <-glmer(Total.Hedonic...MM. ~ c.conc+Age+c.MAC+c.ROC
                   + c.conc:Age
                   + c.conc:c.MAC
                   + c.conc:c.ROC
                   + Age:c.MAC
                   + Age:c.ROC
                   + c.conc:Age:c.MAC
                   + c.conc:Age:c.ROC
                   + (c.conc|RatID), data=mydetoh.noCTRL, family=poisson)
    summary(EhedMR)

    # Check Variance Inflation & Compare AIC/BIC
    vif(EhedMR)
    AIC(EhedMR, EhedTot)
    BIC(EhedMR, EhedTot)


    ### Ethanol Hedonics GLMER (EtOH Group Only: IMAC3 & IROC3) ######

    EhedMR3 <-glmer(Total.Hedonic...MM. ~ c.conc+Age+c.MAC3+c.ROC3
                  + c.conc:Age
                  + c.conc:c.MAC3
                  + c.conc:c.ROC3
                  + Age:c.MAC3
                  + Age:c.ROC3
                  + c.conc:Age:c.MAC3
                  + c.conc:Age:c.ROC3
                  + (c.conc|RatID), data=mydetoh.noCTRL, family=poisson)
    summary(EhedMR3)

    # Check Variance Inflation & Compare AIC/BIC
    vif(EhedMR3)
```

```
    AIC(EhedMR3, EhedMR, EhedTot)
    BIC(EhedMR3, EhedMR, EhedTot)

    ##MR3 Models no longer appear after this point: Similar AICs, with BICs
favoring the less complex model.
    #Use less complex Total Ethanol variable for interpretation from here on
out.

# SUCROSE #####

###Data Frame Preparation###

    ##subset for Substance
    mydsuc <- subset(myd, Substance == "Sucrose")
    View(mydsuc)
    ##Subset for Consumption Pattern Variables
    mydsuc.noCTRL <- subset(mydsuc, Condition != "CTRL")
    View(mydsuc.noCTRL)

    ##Rescaling
    #convert concentration to molarity and center to avoid issues with
scaling & vif
    mydsuc$molarity <-recode(mydsuc$Concentration, ".34=.01; 3.4=.1; 34=1")
    mydsuc$c.molarity <-mydsuc$molarity-mean(mydsuc$molarity)
    #convert concentration to molarity and center to avoid issues with
scaling & vif
    mydsuc.noCTRL$molarity <-recode(mydsuc.noCTRL$Concentration, ".34=.01;
3.4=.1; 34=1")
    mydsuc.noCTRL$c.molarity <-mydsuc.noCTRL$molarity-
mean(mydsuc.noCTRL$molarity)

    ##Centering
    #center TOTAL.ETOH.Swap.Consumed..g.kg. to avoid issues with variance
inflation
    mydsuc.noCTRL$c.totale <- mydsuc.noCTRL$TOTAL.ETOH.Swap.Consumed..g.kg.-
mean(mydsuc.noCTRL$TOTAL.ETOH.Swap.Consumed..g.kg.)
    #center IMAC and IROC for noCTRL subset to avoid issues with variance
inflation
    mydsuc.noCTRL$c.MAC <- mydsuc.noCTRL$MAC-mean(mydsuc.noCTRL$MAC)
    mydsuc.noCTRL$c.ROC <- mydsuc.noCTRL$ROC-mean(mydsuc.noCTRL$ROC)

###Variable Coding Adjustment###

    #adjust Age and Condition to contrast coding for both datasets
    contrasts(mydsuc$Age)=contr.sum(2)
    contrasts(mydsuc$Age)

    contrasts(mydsuc$Condition)=contr.sum(2)
    contrasts(mydsuc$Condition)

    contrasts(mydsuc.noCTRL$Age)=contr.sum(2)
    contrasts(mydsuc.noCTRL$Age)

    contrasts(mydsuc.noCTRL$Condition)=contr.sum(2)
    contrasts(mydsuc.noCTRL$Condition)

## SUCROSE AVERSIVES #####
```

```
    ### Sucrose Aversives GLMER (with EtOH vs CTRL)####
    Savers <-glmer(Total.Aversive ~ c.molarity*Age*Condition
                   + (c.molarity|RatID), data=mydsuc, family=poisson)
        # Model did not converge, used code below to extend # of iterations
and start from where the previous model left off.
        ss4 <- getME(Savers,c("theta","fixef"))
        Savers <-
update(Savers,start=ss4,control=glmerControl(optCtrl=list(maxfun=2e6)))
        summary(Savers)

        #Post Hocs & Planned Contrasts
        Savers.emm.a <- emmeans(Savers, ~ Age)
        summary(Savers.emm.a, type="response")


    ### Sucrose Aversives GLMER (EtOH Group Only: Total EtOH Consumed) ######

    SaversTot <-glmer(Total.Aversive ~ c.molarity*Age*c.totale
                      + (c.molarity|RatID), data=mydsuc.noCTRL,
family=poisson)

        # Model did not converge, used code below to extend # of iterations
and start from where the previous model left off.
        ss5 <- getME(SaversTot,c("theta","fixef"))
        SaversTot <-
update(SaversTot,start=ss5,control=glmerControl(optCtrl=list(maxfun=2e6)))
        summary(SaversTot)

        ##Post Hocs & Planned Contrasts

        #Age*Total Ethanol Manual Calculation
        #Adolescent
        Savers.Ado.b <-fixef(SaversTot)[4]+fixef(SaversTot)[7]
        Savers.Ado.b
        #Adult
        Savers.Adu.b <-fixef(SaversTot)[4]-fixef(SaversTot)[7]
        Savers.Adu.b


## SUCROSE HEDONICS #####

    ### Sucrose Hedonics GLMER (with EtOH vs CTRL)####
    Shed <-glmer(Total.Hedonic...MM. ~ c.molarity*Age*Condition
                   + (c.molarity|RatID), data=mydsuc, family=poisson)
    summary(Shed)

        #Post Hocs & Planned Contrasts

        Shed.emm.a <- emmeans(Shed, ~ Age)
        summary(Shed.emm.a, type="response")

        Shed.emm.m_a <- emtrends(Shed, ~c.molarity*Age, var="c.molarity")
        Shed.emm.m_a


    ### Sucrose Hedonics GLMER (EtOH Group Only: Total EtOH Consumed) ######
```

```
    ShedTot <-glmer(Total.Hedonic...MM. ~ c.molarity*Age*c.totale
                    + (c.molarity|RatID), data=mydsuc.noCTRL,
family=poisson)
    summary(ShedTot)

        #Post Hocs & Planned Contrasts
        #Age*Molarity using emtrends
        ShedTot.emm.a_mol<- emtrends(ShedTot, ~ Age, var = "c.molarity")
        ShedTot.emm.a_mol


# WATER #####

###Data Frame Preparation###

    #Load new dataset made in excel to get rid of additional variables in
Substance column. subset(), and select(filter(),cols) will not work for this.
    #Laptop
    mydh2o <- read.csv("C:/Users/Thomas/Google Drive/Grad/Lab/Projects/Phase
II Projects - Analysis & Write-up/Masters/Results/Analyses/ABHV2018H2O.csv",
na.strings="\"\"")
    #Home computer
    #mydh2o <- read.csv("C:/Users/Kiersten/Google
Drive/Grad/Lab/Projects/Phase II Projects - Analysis & Write-
up/Masters/Results/Analyses/ABHV2018H2O.csv", na.strings="\"\"")
    View(mydh2o)

    #Adjust contrasts to sum-to-zero
    contrasts(mydh2o$Substance)=contr.sum(2)
    contrasts(mydh2o$Substance)

    contrasts(mydh2o$Age)=contr.sum(2)
    contrasts(mydh2o$Age)

    contrasts(mydh2o$Condition)=contr.sum(2)
    contrasts(mydh2o$Condition)

    ##Filter for Consumption Pattern Variables
    library("dplyr", lib.loc="~/R/win-library/3.6")
    mydh2o.noCTRL <- filter(mydh2o, Condition != "CTRL")
    View(mydh2o.noCTRL)
    detach("package:dplyr", unload=TRUE)

    ##Centering
    #center TOTAL.ETOH.Swap.Consumed..g.kg. to avoid issues with variance
inflation
    mydh2o.noCTRL$c.totale <- mydh2o.noCTRL$TOTAL.ETOH.Swap.Consumed..g.kg.-
mean(mydh2o.noCTRL$TOTAL.ETOH.Swap.Consumed..g.kg.)

###Variable Coding Adjustment###

    #Adjust contrasts to sum-to-zero
    contrasts(mydh2o.noCTRL$Substance)=contr.sum(2)
    contrasts(mydh2o.noCTRL$Substance)

    contrasts(mydh2o.noCTRL$Age)=contr.sum(2)
```

```
    contrasts(mydh2o.noCTRL$Age)


## WATER AVERSIVES #####

    ### Water Aversives GLMER (with EtOH vs CTRL)####
    Havers <-glmer(Total.Aversive ~ Substance*Age*Condition
                   + (1|RatID), data=mydh2o, family=poisson)
    summary(Havers)

        #Post Hocs & Planned Contrasts

            #Condition
            Havers.emm.c <- emmeans(Havers, ~ Condition)
            summary(Havers.emm.c, type = "response")
            #Substance*Age
            Havers.emm.s_a <- emmeans(Havers, ~ Substance*Age)
            #run Z-tests no adjustment for familywise error rate
            pairs(Havers.emm.s_a, adjust="None")
            Havers.emm.s_a
            #Visualizing the differences
            plot(Havers.emm.s_a)

            #Age*Condition
            Havers.emm.s_c <- emmeans(Havers, ~ Substance*Condition)
            #run Z-tests no adjustment for familywise error rate
            pairs(Havers.emm.s_c, adjust="None")
            Havers.emm.s_c
            #Visualizing the differences
            plot(Havers.emm.s_c)

            #Substance*Age*Condition
            Havers.emm.s_a_c <- emmeans(Havers, ~ Substance*Age*Condition)
            #run Z-tests no adjustment for familywise error rate
            pairs(Havers.emm.s_a_c, adjust="None")
            Havers.emm.s_a_c
            #Visualizing the differences
            plot(Havers.emm.s_a_c)


    ### Water Aversives GLMER (EtOH Group Only: Total EtOH Consumed) ######

        HaversTot <-glmer(Total.Aversive ~ Substance*Age*c.totale
                          + (1|RatID), data=mydh2o.noCTRL, family=poisson)

            # Model did not converge, used code below to extend # of
iterations and start from where the previous model left off.
            ss8 <- getME(HaversTot,c("theta","fixef"))
            HaversTot <-
update(HaversTot,start=ss8,control=glmerControl(optCtrl=list(maxfun=2e6)))
            summary(HaversTot)

        #Post Hocs & Planned Contrasts

            #Substance*Age
            HaversTot.emm.s_a <- emmeans(HaversTot, ~ Substance*Age)
            #run Z-tests no adjustment for familywise error rate
```

```
            pairs(HaversTot.emm.s_a, adjust="None")
            HaversTot.emm.s_a
            #Visualizing the differences
            plot(HaversTot.emm.s_a)

            #Substance*c.totale
            HaversTot.emt.s_c <- emtrends(HaversTot, ~ Substance,
var="c.totale")
            #run Z-tests no adjustment for familywise error rate
            pairs(HaversTot.emt.s_c, adjust="None")
            HaversTot.emt.s_c
            #Visualizing the differences
            plot(HaversTot.emt.s_c)

            #Substance*Age*c.totale
            HaversTot.emt.s_a_c <- emtrends(HaversTot, ~ Substance*Age, var =
"c.totale")
            #run Z-tests no adjustment for familywise error rate
            pairs(HaversTot.emt.s_a_c, adjust="None")
            #pairs looks alot like Substance*Age but it is different, it is
the comparisons of slopes
            HaversTot.emt.s_a_c
            #Visualizing the differences
            plot(HaversTot.emt.s_a_c)


## WATER HEDONICS #####

    ### Water Hedonics GLMER (with EtOH vs CTRL)####
    Hhed <-glmer(Total.Hedonic...MM. ~ Substance*Age*Condition
                    + (1|RatID), data=mydh2o, family=poisson)
    summary(Hhed)

        #Post Hocs & Planned Contrasts

            #Substance*Condition
            Hhed.emm.s_c <- emmeans(Hhed, ~ Substance*Condition)
            #run Z-tests no adjustment for familywise error rate
            pairs(Hhed.emm.s_c, adjust="None")
            Hhed.emm.s_c
            #Visualizing the differences
            plot(Hhed.emm.s_c)


    ### Water Hedonics GLMER (EtOH Group Only: Total EtOH Consumed) ######

    HhedTot <-glmer(Total.Hedonic...MM. ~ Substance*Age*c.totale
                        + (1|RatID), data=mydh2o.noCTRL, family=poisson)
    summary(HhedTot)

        #Post Hocs & Planned Contrasts
            #Substance*c.totale
            HhedTot.emt.s_c <- emtrends(HhedTot, ~ Substance, var="c.totale")
            #run Z-tests no adjustment for familywise error rate
            pairs(HhedTot.emt.s_c, adjust="None")
            HhedTot.emt.s_c
            #Visualizing the differences
```

```
            plot(HhedTot.emt.s_c)

            #Substance*Age*c.totale
            HhedTot.emt.s_a_c <- emtrends(HhedTot, ~ Substance*Age, var =
"c.totale")
            #run Z-tests no adjustment for familywise error rate
            pairs(HhedTot.emt.s_a_c, adjust="None")
            #pairs looks alot like Substance*Age but it is different, it is
the comparisons of slopes
            HhedTot.emt.s_a_c
            #Visualizing the differences
            plot(HhedTot.emt.s_a_c)
```