Effect of Adolescent Alcohol Use on Encoding of Decision-Related Variables in Prefrontal Cortex

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DEDICATION

For Jeff, my brother and my muse.

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LIST OF ABBREVIATIONS

AIE	Adolescent intermittent ethanol
AP	Anterior-posterior
ANOVA	Analysis of variance
AUD	Alcohol use disorder
BLA	Basolateral amygdala
BEL	Blood ethanol levels
cm	Centimeter
CDC	Center for disease control
EtOH	Ethanol
dlPFC	Dorsal lateral prefrontal cortex
FR-1	Fixed ratio of 1
g	Gram
GABA	Gamma-Aminobutyric acid
h	Hours
ICSS	Intracranial self-stimulation
IL	Infralimbic
IP	Intraperitoneal
ITI	Inter-trial interval
kg	Kilogram
LTP	Long term potentiation
mg	Milligram
ml	Milliliter
ML	Medial-lateral
mPFC	Medial prefrontal cortex
NAc	Nucleus accumbens core
NP	Negative press
NR	Negative reward
OFC	Orbitofrontal cortex
PD	Postnatal day
PFC	Prefrontal cortex
PL	Prelimbic
PNN	Peri-neuronal nets
PP	Positive press
PR	Positive reward
RM- ANOVA	Repeated measures analysis of variance
s	Seconds
sp	Spikes
TTL	Transistor-transistor logic
TMS	Trans-magnetic cranial stimulation
VR-1	Variable ratio of 1
VR-3	Variable ratio of 3
vHipp	Ventral Hippocampus
VSTR	Ventral Striatum
μm	Micrometers

SUMMARY

Prefrontal Cortex is important for executive control and decision-making behavior. Specifically, two sub-regions of Prefrontal Cortex, medial Prefrontal Cortex and Orbitofrontal Cortex are implicated in guiding optimal behavior and updating the economic value of rewards that result from choice behaviors. Extensive anatomical reorganization that is essential for the maturation of these regions is present until early adulthood. Thus, refinement of these areas and the complex decision-making they are involved in can be hindered if exposure to neurotoxins, such as alcohol, coincides with their critical developmental window. This is problematic because onset of alcohol experimentation and abuse is most prevalent during the teenage years. This dissertation sought to examine in greater depth how exposing Prefrontal Cortex to alcohol during its development would alter long-term function and subsequent decision-making behavior in adulthood. Alcohol was administered to male and female adolescent rats using a voluntary, intermittent, exposure paradigm. This was followed by a probabilistic risk task in adulthood to assess how variable amounts of alcohol affect long-term decision-making behavior. Neural activity was recorded concurrently in Orbitofrontal Cortex and medial Prefrontal Cortex as animals performed the task. Adolescent alcohol use was associated with a decrease in risk preference in adulthood and an inability to modify choice-behavior as the task contingencies changed. The inability to adjust their responses accordingly was only present when the delivery of a reward was not certain, suggesting alcohol-induced behavioral deficits are only evident when there is some degree of uncertainty in the outcome. Increased adolescent alcohol use was also correlated with the inability to discriminate reward size thorough neural encoding in OFC, providing insight as to why animals could no longer distinguish the changing parameters of the task. Interestingly, alcohol affected these neurons differently in males and females, although in both scenarios there was an overlap in the firing rates used to distinguish different reward sizes. Neurons in mPFC that responded at the time of the decision were

also affected by prior alcohol use and exhibited a distinct switch in the signaling of the lever that was acted on. Collectively the results provide novel insight into Prefrontal Cortex's capacity to encode decision-related elements and under what circumstances those variables are most necessary to guide advantageous behavior. Furthermore, they support current developmental and alcohol literature by emphasizing the importance of continued brain development in adolescence and expanding on the extended repercussions of adolescent alcohol use.

I. CHAPTER 1: Alcohol-induced alterations to adolescent prefrontal cortex

A. Background

Adolescence is a period of extensive physiological, psychological and social development (Bava & Tapert, 2010; L. P. L. Spear, 2000). The onset of puberty shepherds in an influx of sex-hormones that are imperative for secondary sex characteristics and the shaping of neural circuits (Vigil et al., 2016). This immature cortical state is idiomatic to adolescents wherein some neural processes are hindered or exacerbated compared to adults. These physiological changes are accompanied by the onset of many psychological disorders that are disproportionately present between sexes (Raznahan et al., 2014). Females are overwhelmingly more likely to develop mood, anxiety, and eating disorders, whereas males are more frequently plagued by behavioral and substance abuse disorders, both of which contribute to an increased incidence of accidental death. Furthermore, adolescents value their relationships with peers more than adults, choosing to spend a third of their time with their age group (Primus & Kellogg, 1989; L. P. L. Spear, 2000). This developmental hallmark is starkly different from children who heavily rely on, and actively crave, their parents' approval and affection.

The imbalance in neuronal activity and shift in social inclination can catalyze impulsive, sensation seeking, and destructive behaviors such as reckless driving, illicit drug use, and binge drinking (Albert & Steinberg, 2011; Steinberg, 2010; Steinberg et al., 2008; Van Hoorn, Crone, & Van Leijenhorst, 2017). Exposure to pernicious environmental factors during this period have prolonged consequences that can be seen in adulthood because of the fragile, malleable, state of the brain at this time.

1. *Neurostructural and functional changes that occur in adolescence*

In humans, the incipiency of adolescence is around 12 years of age; however, the brain doesn't resemble its adult state until around 20 years of age (Bava & Tapert, 2010; L. P. L. Spear, 2000). A similar progression occurs in rats but is condensed into a period beginning around postnatal day (PD) 27, and continuing until adulthood around PD 55 (L. P. L. Spear, 2000). Extreme cortical reorganization occurs during adolescence as the brain approaches its adult form. Grey matter volumes peak at the beginning of adolescence, then steadily decline beginning in the striatum and sensorimotor cortices, and continuing laterally until reaching dorsal lateral prefrontal cortex (dlPFC) (Giedd et al., 1999; Gogtay et al., 2004; Sowell et al., 2004; Sowell, Trauner, Gamst, & Jernigan, 2002). Peak cortical thickness occurs one to two years earlier in females than males; this coincides with the beginning of puberty, indicating the difference in developmental trajectories is likely a result of hormonal influence (Giedd et al., 1999; Peper et al., 2008; Perrin et al., 2009; Shaw et al., 2008). Sub-regions of prefrontal cortex (PFC) develop at differential rates in males and females as well (Giedd et al., 1999; Gogtay et al., 2004), giving increased weight to the influence of steroidal hormones on brain development.

The mechanisms underlying decreasing grey matter volumes include selective synaptic pruning, diminution in glial cells, and ebbing of intra-cortical myelin (Shaw et al., 2008). Throughout adolescence there is increased myelination and organization of white matter tracts yielding greater white matter density and volume (Barnea-Goraly et al., 2005; Bonekamp et al., 2007; Mukherjee et al., 2001; Schmithorst, Wilke, Dardzinski, & Holland, 2002). Changes in neurotransmission occur during this period in the form of increased GABAergic and dopaminergic synapses to PFC and a refinement of excitatory, glutamatergic connections (Bava

& Tapert, 2010; F. T. Crews, Vetreno, Broadwater, & Robinson, 2016). Specific alterations in gene transcription during adolescence can permanently change developing synaptic connections.

Neural development during adolescence is integral for strengthening synaptic connections that are responsible for complex cognition and behavior. Ongoing brain development is particularly important for the maturation and fine-tuning of structures involved in the reward system including Basolateral Amygdala (BLA), Ventral Striatum (VSTR), Ventral Hippocampus (vHipp), and PFC. These structures project multidirectional axons that communicate the various aspects required to guide adaptive, goal-directed behaviors. BLA contains subpopulations of neurons that differentially fire in anticipation of rewarding or aversive outcomes (Schoenbaum, Chiba, & Gallagher, 1998), this is essential for integrating reward value with emotional processing (Wassum & Izquierdo, 2015). Simultaneously, VSTR aides in stimulus-response learning via reward prediction error, affecting reward interpretation and motivation (O'Doherty et al., 2004; Schultz, Apicella, Scarnati, & Ljungberg, 1992). Memories of spatial-reward context are harbored in vHipp, directing future appetitive motivation (Riaz, Schumacher, Sivagurunathan, Van Der Meer, & Ito, 2017). Finally, PFC, which is reviewed further under *Overview of role of Prefrontal Cortex in decision-making,* is paramount for behavioral flexibility and proper evaluation of risk-reward situations.

2. *Behavioral phenotypes of adolescence*

Sensation seeking and heightened risk taking behaviors exhibited in adolescents are due in part to social influence, but are largely attributed to limited PFC growth (L. P. L. Spear, 2000). Males appear to be increasingly sensation seeking compared to females because rewards are more salient to them (Becker, Perry, & Westenbroek, 2012; Hammerslag & Gulley, 2015). While these behaviors are crucial for cultivating the skills and knowledge needed for

psychological maturity and social independence, they alternatively can lead to engagement in illegal activities and experimentation with substance use.

Risk taking in adolescents is likely caused by an unbalanced corticolimbic system in which the fully-developed striatum is more active than PFC, resulting in more bottom-up reward processing and less top-down inhibitory control (Ernst, 2014; Hammerslag & Gulley, 2015; Steinberg, 2010; Steinberg et al., 2008). Adolescence is the only period in which this functional imbalance occurs because in children both structures are underdeveloped and in adults both structures are in their mature, final state. Furthermore, adolescent substance use seems to be perpetuated by the development of sensation-seeking behavior before inhibitory control as well as greater dopamine release in VSTR in response to rewarding stimuli (Aarts et al., 2010; Koepp et al., 1998; Steinberg et al., 2008). Unfortunately, initial experimentation with illicit substances overwhelmingly coincides with this time of developmental vulnerability. Due to the dynamic, synaptic remodeling that occurs in adolescents' PFC, this region is especially susceptible to atypical development due to neurotoxin exposure, such as drugs and alcohol.

3. *Overview of role of Prefrontal Cortex in decision-making*

Prefrontal Cortex (PFC) is one of the last brain areas to mature and is necessary for executive functions such as planning, problem solving, reward assessment, inhibitory control, and motivated behaviors. Excitatory projections from PFC influence Nucleus Accumbens core (NAc) in a top down manner, effectively directing the motor commands that carry out a decision. The pruning of glutamatergic synapses as well as the integration of GABAergic interneurons in PFC is critical for the refinement of the corticolimbic system and generating outputs for optimal decision-making. Patients with PFC damage show impaired decision-making in the Iowa Card Sorting task, defective social behavior, and impaired moral reasoning (Anderson, Bechara,

Damasio, Tranel, & Damasio, 1999). Furthermore, PFC hypoactivity is associated with increased risky decisions (Orsini, Moorman, Young, Setlow, & Floresco, 2015). One aspect of optimal decision-making can be actively refraining from a behavior, also known as inhibitory control. Frontal cortex remodeling has profound effects on inhibitory control such that as the frontal cortex matures there is decreased connectivity and activation but increased efficiency, yielding enhanced inhibitory control (Blakemore & Choudhury, 2006).

PFC is thought to play a critical role in decision-making by coalescing a number of inputs. In particular, it is essential for integrating risk and reward as well as individual motivated drives. During goal directed behavior, PFC has separate sensory, motor, and outcome signals that are encoded by subtypes of interneurons and different layers of excitatory pyramidal cells (Pinto & Dan, 2015). It is important to emphasize that a decision encompasses motivational, cognitive, and emotional sub-processes and one of these may influence the decision outcome more than the others. The subjective weight of these factors varies between individuals and manifests as individual differences and preferences. There are different sub-regions of PFC that are defined anatomically as well as functionally. Sub-regions of PFC have different developmental trajectories as greater quantities of inhibitory synapses are incorporated, these synaptic changes likely align with critical periods of development for complex cognitive skills (Caballero & Tseng, 2016; Shaw et al., 2008). Orbitofrontal Cortex (OFC) and medial Prefrontal Cortex (mPFC) are of particular interest because of their proposed roles in behavioral flexibility, inhibitory control, and decision-making under uncertainty.

4. *Orbitofrontal Cortex*

OFC is critical for outcome guided behaviors, behavioral flexibility, and decisions in which the outcome is uncertain (Murray, O'Doherty, & Schoenbaum, 2007).

OFC is typically not implicated in the original acquisition of learned associations but instead appears to continually encode expected reward value and update changes to it by changing neural firing rates in response to reward-relevant stimuli. Interestingly, the encryption of OFC firing rates can reflect changes in reward features that are value independent, such that individual preference is reflected in overall firing rate. A study using extracellular recordings in non-human primates corroborated that neurons in OFC can not only discriminate between a preferred juice flavor and a non-preferred juice flavor, but they linearly discounted the preferred juice as the ratio of available juice quantities changed (Padoa-Schioppa & Assad, 2006). Similar studies in rats have demonstrated changes in OFC firing rate correspond to changes in quantity of the reward (i.e. drops of juice) and sensory features (i.e. flavor of juice) as well (Stalnaker et al., 2014). This substantiates that OFC neurons can consistently identify and signal the economic value of rewards based on multiple pertinent variables and independent of visuospatial factors.

Performance during reversal learning and reinforcer devaluation tasks are also OFC dependent. Individuals with OFC damage have difficulty adapting their behavior in reversal tasks when the previously rewarded cue no longer predicts the reward (Boulougouris, Dalley, & Robbins, 2007). However, it is unclear if this behavioral inflexibility is due to the inability of OFC to predict the expected outcome or the incapacity to signal the frequency of the rewarded event (Murray et al., 2007). Moreover, OFC lesions in rats impaired their ability to properly devalue a reward when it was subsequently paired with LiCl, underscoring the importance of OFC in updating and modifying the value of reinforcers (Gallagher, McMahan, & Schoenbaum, 1999; Hamilton & Brigman, 2015).

Accurate updating of anticipated consequences is crucial for determining future behavior, especially when the outcome is uncertain. OFC is necessary for the revision of mental representations of changing reward magnitudes and frequencies in probabilistic discounting tasks (Orsini et al., 2015). In humans, OFC activity positively correlates with increased ambiguity in the odds of winning a card gambling task (M. Hsu, Bhatt, Adolphs, Tranel, & Camerer, 2005). The subjective value of uncertain rewards has also been shown to correlate with OFC activity in rats performing a probabilistic discounting task, such that rats that showed an increased preference for risky rewards had increased firing during the reward period compared to rats that had a decreased preference for risky rewards (Roitman & Roitman, 2010). Furthermore, inactivation of OFC in rats has been shown to strengthen the impact of large, risky rewards on subsequent decisions leading to an overall greater predilection for risky choices (Orsini et al., 2015; St Onge & Floresco, 2010). This supports the theory that OFC damage can exacerbate poor decision-making because of the inability to accurately appraise rewards when there is some degree of uncertainty in the outcome.

5. *Medial Prefrontal Cortex*

The prelimbic (PL) and infralimbic (IL) regions of mPFC play various, and seemingly complementary roles in the initiation of cue-predicting rewarded behaviors. Studies suppressing PL activity in rats by way of GABA agonist infusions, resulted in the loss of behavioral response to a cue that signaled sucrose delivery (Ishikawa, Ambroggi, Nicola, & Fields, 2008). The silencing of PL also led to a dampening of down-stream NAc cue-evoked firing, indicating that PL is necessary for responses to reward-predictive cues. In contrast, increased neuronal activity in IL due to an injection of a glutamate agonist, aided in the suppression of cocaine seeking in cocaine exposed rats (Peters, LaLumiere, & Kalivas, 2008). Moreover, populations of neurons

in IL respond specifically to anticipated aversive stimuli, providing insight as to why mPFC would promote inhibition of behavior (David R. Euston, Gruber, & McNaughton, 2012; Orsini et al., 2015). Thus, it appears the two regions play opposing roles to generate a delicate balance between PL initiating reward-seeking responses and IL actively inhibiting behavioral responses.

Aside from cue-directive action, both PL and IL contain populations of neurons that respond at the time of reward, implicating mPFC in a more intricate role in decision-making. Inactivation of PL during a probabilistic risk task that was designed to shift the risky lever from advantageous to disadvantageous within a session, led to an increase in risky choices and an inability to accurately adjust behavior as the probability of reward delivery changed (St Onge & Floresco, 2010). However, the opposite effect was seen when the order of probabilities was reversed, alluding that PL may guide and promote optimal choice behavior by tracking previous patterns of outcomes. mPFC also guides behavior by refining value representations. Physiological recordings in IL have suggested it may have a similar role to OFC in encoding reward value during appetitive instrumental behavior (Burgos-Robles, Bravo-Rivera, & Quirk, 2013). There is growing evidence that the roles of mPFC's sub-regions in choice behaviors is context sensitive, emphasizing the complexity of mPFC circuitry (D. R. Euston & McNaughton, 2006). Furthermore, mPFC likely represents some information through its dichotomies and more complex properties of decisions are relayed to downstream connections such as BLA and NAc.

There is evidence that mPFC is also involved set-shifting, which can contribute to optimal decision-making under changing circumstances. Inactivation of mPFC impairs set-shifting and produces increased regressive errors (Hamilton & Brigman, 2015; Ragozzino,

2007). This result suggests damage to mPFC can limit one's ability to alter behaviors in visuo-spatial dimensions as the guiding rules change.

In conclusion, mPFC appears to sum information about context and motivational state to drive or inhibit decision execution.

6. *Adolescent alcohol use and its immediate effects*

The period of time imperative to PFC maturation coincides with the onset of experimentation with alcohol. Alcohol is the most used intoxicating substance amongst adolescents and 11% of alcohol in the US is consumed by people under the age of 20. The CDC cites 26% of 8th graders and 68% of 12th graders have tried alcohol (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2015). This high quantity of underage alcohol use results in an annual death toll of 4,300 minors (US Department of Justice, 2002) and is largely attributed to binge drinking, which comprises 90% of liquor consumption amongst teens.

Binge drinking is a pattern of excessive alcohol consumption over a short period of time, causing blood ethanol levels to rise to 0.08% or higher. In adult males consuming 5 or more drinks over the course of 2 hours is considered a binge. Body size and the amount of alcohol consumed to be considered a binge are directly correlated, thus 4 or more drinks over a 2-hour period is considered a binge for adult women, and the intake of even fewer drinks qualifies as a binge for most teens. College students, who are classified as being in late adolescence, are particularly vulnerable to binge drinking behavior. This is especially alarming because 44% of college students report binge drinking twice a month and 19% binge drink more than 3 times a week (Wechsler, Dowdall, Davenport, & Castillo, 1995).

The adolescent brain is particularly sensitive to the damaging effects of alcohol because of its ongoing development. Neuroimaging studies have revealed decreased white matter and

PFC volume in adolescent alcohol users as compared to healthy age-matched controls. More strikingly, there is a negative correlation between number of drinks per drinking episode and prefrontal grey matter volume (Bava & Tapert, 2010; Bellis et al., 2005; Feldstein Ewing, Sakhardande, & Blakemore, 2014; McQueeny et al., 2009; Vetreno, Yaxley, Paniagua, & Crews, 2015). Ethanol induced glutamate-excitotoxicity is related to axonal loss and increased cortical neurodegeneration. In combination with alcohol inhibited neurogenesis via decreased cell proliferation in the ventral hippocampus, results in the loss of the corticolimbic system's integrity (Fulton T Crews et al., 2004; Fulton T Crews, Mdzinarishvili, Kim, He, & Nixon, 2006). Additionally, alcohol exposure during visual cortex development permanently disrupts the evolvement of ocular dominance columns, further demonstrating how alcohol can disrupt cortical pathways during critical periods (Medina, Krahe, Coppola, & Ramoa, 2003). Adolescent intermittent ethanol (AIE) use has been documented to decrease dopamine markers in the infralimbic and prelimbic cortices (Boutros, Semenova, Liu, Crews, & Markou, 2015) and change mesolimbic dopamine signaling during a decision making task (N. A. Nasrallah et al., 2011).

The detrimental, behavioral manifestations that are a result of adolescent alcohol use are apparent when examining attention, information recall, object recognition, and inhibitory control. Adolescent rats who voluntarily consumed high quantities of alcohol also had increased risk preference for large-risky rewards over small-certain ones when compared to controls (Clark et al., 2012; McMurray, Amodeo, & Roitman, 2014). Adolescent males are more likely to engage in sensation seeking behaviors such as drinking but females are more vulnerable to exhibiting telescoping, a phenomenon in which there is a rapid progression from first use to daily use (Becker et al., 2012; Cotto et al., 2010). Furthermore, individuals who start drinking at the age

of 14 are more likely to have an alcohol use disorder in adulthood (Substance Abuse and Mental Health Services Administration, 2014), and if they do become abstinent, they are still marked by the prolonged effects of their teenage binge-drinking.

7. *Long-term consequences of alcohol use: Effects on executive control in adulthood*

Heavy alcohol use in adolescence is associated with a wide range of enduring anatomical and behavioral consequences that are still evident in adulthood. Individuals who try alcohol before age 14 are more likely to exhibit antisocial behavior (Foster, Hicks, Iacono, & McGue, 2014) and endure increased sensitivity to alcohol-driven memory impairment in adulthood (L. P. Spear & Swartzwelder, 2014). AIE causes a decrease in hippocampal volume (Bava & Tapert, 2010) and increased hippocampal excitability, such that long term potentiation (LTP) induction in the region more closely resembles an adolescent than an adult (L. P. Spear & Swartzwelder, 2014). Adult rats who were exposed to AIE present with increased anxiety which is potentially related to alcohol-induced epigenetic modifications in the amygdala (Pandey, Sakharkar, Tang, & Zhang, 2014). Additionally, adolescent alcohol use permanently alters mesolimbic dopamine signaling (N. A. Nasrallah et al., 2011; Pascual, Boix, Felipo, & Guerri, 2009), inhibiting an individual's ability to predict reward delivery and assess risks. AIE results in altered PFC volume in humans (Bava & Tapert, 2010) and rodents (Coleman, Liu, Oguz, Styner, & Crews, 2014). Altered adult OFC volume following AIE seems to be driven by altered expression of extracellular matrix proteins, and it's deformity is associated with deficits in OFC-dependent reversal learning (Coleman et al., 2014). This suggests adolescent alcohol use is preventing the proper refinement of synaptic connections that are essential for proper function by disrupting peri-neuronal net formation (PNN).

Due to alcohol induced atypical development of OFC, the electrophysiological properties of this area are also disrupted. Adolescent alcohol exposure causes hypoactivity of OFC in a dose dependent manner in response to rewards (McMurray, Amodeo, & Roitman, 2015). Rats who chronically consumed high levels of alcohol in adolescence had indiscriminate OFC activity in response to rewards and omissions in a probabilistic discounting task in adulthood. Likewise, AIE exposure causes blunted cell activation in OFC and PL mPFC when exposed to acute doses of alcohol again in adulthood (Liu & Crews, 2015).

These anatomical and functional changes present as behavioral deficits in spatial learning (Lindsay M. Squeglia, Schweinsburg, Pulido, & Tapert, 2011), reversal learning (Coleman et al., 2014), inhibitory control (Winward, Hanson, Bekman, Tapert, & Brown, 2011), memory retrieval (Brown, Tapert, Granholm, & Delis, 2000), and reward discounting (Clark et al., 2012; McMurray et al., 2015). In fact, there is a correlation between hangover symptoms experienced as a teen and visual-spatial task performance 3 years later (L. M. Squeglia, Jacobus, & Tapert, 2009). Interestingly, chronic exposure to low amounts of alcohol throughout adolescence showed decreased preference for large/risky rewards in adulthood male rats (McMurray et al., 2015), indicating that the effect of alcohol is not uniform across doses and can yield different behavioral consequences. Finally, it's been suggested that adolescent substance abuse may affect inhibitory control in adulthood more in males than females (Foster et al., 2014; Hicks, Iacono, & McGue, 2010).

B. Conclusion

It has been established that PFC is critical for directing decisions, especially ones in which the outcome is uncertain or contains an aversive consequence. Neural activity in regions of PFC, such as mPFC and OFC, have been shown to be necessary for advantageous decision-

making, including reward evaluation and cue sensitivity (Orsini et al., 2015). Due to adolescents' plastic and developing PFC, they are more susceptible to anatomical and functional disruption of this area due to neurotoxin exposure, resulting in permanent damage. However, it is not understood how a persistent, insult to PFC during development can differentially affect males and females, as well as different sub-regions of PFC.

Alcohol is the most widely used intoxicating substance among youths and initial experimentation with alcohol often transpires with maturation of PFC. Adolescents frequently engage in risky behaviors, such as binge drinking, that result in immediate and lasting consequences. Immediate impacts range from reduced verbal and spatial memory (Brown et al., 2000) to impaired decision-making (McMurray et al., 2014). Previous studies have demonstrated long-term repercussions of adolescent drinking on male rats' risk preference and OFC function (Boutros et al., 2015; Clark et al., 2012; McMurray et al., 2015; N. a Nasrallah, Yang, & Bernstein, 2009) however, whether these results are similar in females has yet to be determined. Furthermore, there is a gap in the literature as to how this effect translates to another sub region of PFC, mPFC, that is theorized to play a different role in decision-making. Additionally, adolescence typically marks the onset of addiction and other psychiatric disorders, providing further incentive to evaluate the long-term effects of adolescent alcohol use on neural activity.

The purpose of this thesis was to test how different sub regions of PFC encode decision-related variables and how decisions that are dependent on PFC activity are altered by variable alcohol consumption in males and females. The following two aims of this dissertation were conducted concurrently and followed the timeline illustrated in Figure 1.1.

1. *Aim1: To discern the repercussions of adolescent alcohol use in males and females on decision-making involving uncertainty in reward outcome in adulthood.*

Previous alcohol research in rodents has often used compulsory routes of administration via vapor inhalation (Badanich, Becker, & Woodward, 2011; Boutros et al., 2015; Coleman et al., 2014) or intraperitoneal (IP) injections (Pandey et al., 2014). While these mechanisms allow for the administration of more regulated doses, they minimize the prospect of evaluating the effects of individual differences in alcohol intake. Additionally, these alcohol paradigms are stressful for the animal, creating a confounding factor. Two ways to assess voluntary alcohol consumption are a two-bottle choice (Laviola, Macrì, Morley-Fletcher, & Adriani, 2003; Pandey et al., 2014; Pascual et al., 2009) or administering alcohol through another medium, such as gelatin (McMurray et al., 2014, 2015; N. A. Nasrallah et al., 2011; Peris et al., 2006). The latter method uses sweetener to elicit alcohol ingestion that can mimic adolescent drinking patterns without requiring restriction of food and fluid and reducing the stressful component of administration. Since the technique relies on voluntary intake, variable alcohol doses between subjects is inevitable. Past studies utilizing voluntary administration have collapsed animals into one group regardless of the amount of alcohol ingested or have arbitrarily divided them between high and low alcohol consumers. This type of analysis disregards individual differences and subtleties in drinking behavior that could help to explain later patterns of behavior or neural activity. Furthermore, the individual differences in consumption can be leveraged by correlating variability in alcohol dosing with variability in behavior.

A prominent aspect of decision-making that relies on PFC function is the processing of rewards obtained under conditions of uncertainty. A way to model decision-making when the outcome is unknown is by using a probabilistic risk task in which the delivery of a reward on any

given trial can be estimated but is unknown until the trial is complete. By changing the probability of reward delivery, we can examine how individual's behavior changes in relation to the changing parameters of the task. Additionally, the behavior during the probabilistic risk task can be contrasted to behavior in magnitude discrimination and extinction, in which the outcome is known. This comparison will expose if the effects of alcohol are uniform across all decisions, or if it is the probabilistic aspect the task that alcohol is disrupting.

2. *Aim 2: To compare how alcohol alters the neural encoding of subcomponents of decision making in Orbitofrontal Cortex and medial Prefrontal Cortex.*

Adolescence is a critical period for the proper development of prefrontal cortex (PFC). Sub-regions of PFC, specifically OFC and mPFC, both appear to be crucial areas in directing choices and signaling information pertinent to uncertain outcomes. However, it is unclear if these regions encode overlapping aspects of decision-making or are solely responsible for communicating different facets of a choice. Furthermore, these regions do not have identical cellular compositions and trajectories of maturation, suggesting alcohol will not induce parallel effects on them.

Recording from single cells in awake and behaving animals in real time enables us to relate neural activity to ongoing behavior on a millisecond time scale. This technique allows us to visualize how specific events are represented as patterns of action potentials and how neural activity changes from event to event. By comparing aspects of the task to OFC and mPFC activity, we can determine how specific characteristics such as action initiation and reward value are encoded. Neural activity can also be related to sex and estrous cycle stage to determine if sex hormones alter neural excitability. Finally, this approach can identify how alcohol disrupts

patterns and magnitudes of neural activity, and whether this disturbance correlates with behavioral performance on the probabilistic risk task, which is thought to be PFC dependent.

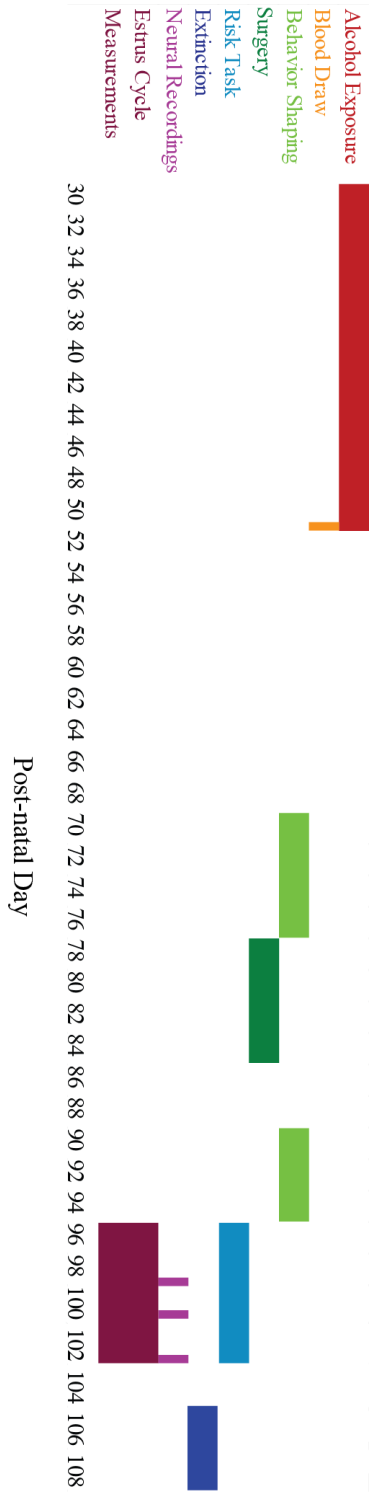


Figure 1.1 A predefined schedule of events was organized to assess the effect of adolescent alcohol use on decision-behavior and neural activity. Rats had intermittent access to alcoholic or

control gelatin from PD 30-51, tail blood was drawn on PD51 to assess BEL. On PD 70 rats were trained to lever press for sugar pellets until they reached criteria. Upon completion, around PD 75, rats had microelectrodes surgically implanted in OFC and mPFC. They were allowed at least one week to recover from surgery. Around PD 90 rats were reintroduced to the task in the form of magnitude discrimination for a maximum of 5 days. Immediately upon completion of magnitude discrimination, rats were tested on the probabilistic risk task and subsequent extinction sessions. Estrous cycle stage measurements were taken daily during the risk task.

II. CHAPTER 2: Adolescent alcohol use leads to long lasting deficits in decision-making

A. Rationale

Adolescents frequently engage in risky behaviors, such as binge drinking, that result in immediate and lasting consequences. Due to adolescents' plastic and developing PFC, they are more susceptible to permanent anatomical and functional disruption of this area due to neurotoxin exposure. Previous studies have demonstrated long-term repercussions of adolescent drinking on male rats' risk preference (Boutros et al., 2015; Clark et al., 2012; McMurray et al., 2015; N. a Nasrallah et al., 2009) however, whether these results are similar in females has yet to be determined. I sought to examine the spectrum and severity of behavioral deficits during decision-making involving uncertainty in the outcome that arise from variable adolescent alcohol use.

During adolescence (PD30-51) rats had limited, (2h) intermittent access (2 days on, 2 days off, 2 days on, 1 day off) to a sweetened gelatin containing 10% alcohol or no alcohol. The non-alcoholic gelatin was calorie matched to the alcohol-containing gelatin using additional polycose. After prolonged abstinence, rats were trained and tested in adulthood (PD70) on a probabilistic risk task in which they had the choice between small-certain rewards or large-risky rewards. The rats' sensitivity to the changing frequency of the large reward's delivery and their preference for the larger, riskier reward was measured by exposing them to an array of reward contingencies.

Animals were predicted to exhibit variable alcohol consumption patterns consisting of some engaging in binge-like alcohol intake and others consuming low amounts of alcohol. There was no anticipated effect of alcohol on weight. It was hypothesized that alcohol consumption in adolescents would positively correlate with preference for the risky lever in

adulthood regardless of the session parameters. This bias would be measured as a decrease in lose-shift choices, an increase in win-stay choices, and an overall increase in risky lever selection across probability sessions. This hypothesis was based off of the results of similar studies in male rats (Boutros et al., 2015; Clark et al., 2012; McMurray et al., 2015; N. A. Nasrallah et al., 2011). Additionally, females were expected to be more risk averse compared to males, this would be seen as a decrease in the proportion of risky lever presses across all probabilities and increased lose-shift behavior compared to males. Estrous stage was not presumed to have an effect on proclivity for the risky lever. All animals, regardless of experimental group and sex, were predicted to highly favor the risky lever during magnitude discrimination and shy away from it during extinction to demonstrate that the behavioral shortcomings are only apparent when there is some degree of uncertainty in the outcome.

B. Materials and methods

1. Animals

Male and female Long-Evans rats were acquired from Charles River Laboratory (Wilmington, MA) on PD 22. Rats were housed in groups of 3 during adolescence (2 AIE, 1 control) in polycarbonate cages (45x24x20 cm) and were provided with ad libitum chow (LabDiet 5012 Richmond, IN) and water. The colony was maintained on a 12:12 light/dark cycle. Animals were treated under the approval of the Animal Care Committee of the University of Illinois at Chicago and in accordance with the National Institutes of Health's guidelines.

2. Alcohol administration

Rats were given access to alcohol in the form of a "gelatin shot" that was comprised of 2.5% Knox gelatin (Kraft Foods, Northfield, IL), 10% Polycose (SolCarb, Pawcatuck, CT), and 10% EtOH (190 proof) by weight. Control animals were given non-alcoholic gelatin that

contained additional polycose to match the caloric content of alcohol in the experimental gelatin. Rats had access to gelatin using a 'drinking in the dark' paradigm (Bell et al., 2011; McMurray et al., 2015) that allowed them to consume the gelatin at the beginning of the dark cycle. Rats were given intermittent access to the gelatin throughout adolescence (2 days on, 2 days off, 2 days on, 1 day off) starting with 24 hours of access on PD 30 and PD 31, 6 hours on PD 34, 3 hours on PD 35, and 2 hours on all subsequent access days until PD 51 (Figure 2.1). Before the gelatin became available for consumption, rats were weighed and transferred from their home cage to a larger polycarbonate cage (52x28x21 cm) that was equipped with a mesh divider to ensure that rats could only access to their own gelatin while still maintaining social contact with cage mates (Figure 2.2). Rats had ongoing access to chow and water while housed in the larger apparatus. Jars of prepared gelatin were weighed before and after the access periods to determine the amount consumed.

3. *Blood ethanol levels*

Blood was collected via tail nick on the last day of AIE (PD 51) and emulsified with heparin. Blood plasma was extracted from each sample and blood ethanol levels (BEL) were measured at a later time using an AM1 alcohol analyzer (Analox).

4. *Behavioral training and probabilistic risk task*

Behavior training and testing were conducted in operant chambers (35 x 32 x 33 cm) equipped with a central pellet dispenser, two levers on either side of the pellet dispenser, one cue light above each lever, a house light in the back of the chamber, and an overhead fan (Med Associates St Albans, VT). An infrared beam was positioned at the opening of the pellet dispenser to mark head entries into the area. Rats were food restricted to 85% of free-feeding bodyweight during behavior shaping and the probabilistic risk task.

On PD 70 rats began magazine training which consisted of the dispensing of sugar pellets (45mg; BioServ, Frenchtown, NJ) at variable intervals (20, 30, 60, or 90 s) for 45 minutes. Magazine training continued daily until rats ate 90% of the dispensed sugar pellets for 2 consecutive days. Rats were trained to lever press for sugar pellets using a fixed ratio of 1 (FR-1) schedule containing a forced choice block (12 trials per lever) and a free choice block (88 trials). The FR-1 schedule continued until rats completed 50% of trials for 2 days. Side bias during FR-1 training was determined to be present if a rat pressed one lever for more than 70% of completed trails. If a side bias was observed, the favored lever became the small, certain lever during magnitude discrimination and the risk task. This ensured that any initial spatial preference was superseded by the preference for the large reward. If a bias was not observed, then the small, certain lever was assigned at random. Magnitude discrimination consisted of a block of forced-choice trials (12 trials) and a block of free-choice trials (88 trials), wherein one lever delivered a large payout (three 45mg sucrose pellets) when it was pressed (varied ratio of 1: VR-1) and the other lever delivered a small payout (one 45mg sucrose pellet) when it was pressed (fixed ratio of 1: FR-1). Rats performed magnitude discrimination until they were selecting the VR-1 lever on 70% of completed free-choice trials for 2 consecutive days or for a maximum of 5 consecutive days. Rats then performed two days of an "anchor session" in which the VR-1 lever's ratio of delivery changed to 3 (VR-3). This transformed it the large, risky lever and when pressed it resulted in the delivery of 3 sugar pellets 33% of the time (VR-3); the intention of this was to expose rats to the uncertainty aspect of the task without contaminating the data with carry-over effects caused by the risky lever bias evoked during magnitude discrimination.

During the risk task, the lever associated with the FR-1 schedule during magnitude

discrimination was designated the small, certain payoff lever (one 45mg sucrose pellet, 100% of the time) and the VR-1 lever became associated with a potentially large, risky reward (zero or three 45mg sucrose pellets, 16%, 33%, or 66% of the time). Each session began with a block of 12 forced trials per lever followed by 76 free choice trials. The rats were tested on each probability for 2 consecutive days but only data from the second day was analyzed because it was further removed from the effects of the previous contingency. The large, risky lever probabilities were ordered pseudo- randomly to ensure the 33% session did not immediately follow the two days of "anchor sessions" (Figure 2.3B). Extinction sessions (large, risky lever payoff was 0%) were conducted following completion of the task.

Sessions began with the illumination of the house light. Trials were initiated by the animal making a head entry into the central dispenser, which subsequently generated the illumination of the cue light(s) for 2 seconds; this indicated which lever(s) were about to extend. After the lever(s) extended and the animal pressed one lever, the lever(s) retracted, the cue lights were extinguished, and a reward (or omission) was delivered 2 seconds after the lever press. Ten seconds after the reward (or omission) was dispensed, the house light was extinguished for a variable ITI (11, 13, 15, or 17s) and re-illuminated; animals were not able to initiate a new trial until the house light was re-illuminated (Figure 2.3A). Risk preference was calculated as the percentage of large, risky lever presses during the free-choice block.

5. *Lavage procedures*

The estrous cycle of female rats was monitored daily during the risk task. Samples were collected following completion of the behavior task by gently flushing the vaginal area using a disposable pipet filled with sterile water. Residual water containing cells was then examined on a glass slide under a microscope. Identification of estrous phase was based on the estimation of

relative concentrations of leukocytes, nucleated epithelial cells, and cornified epithelial cells (Pompili, Tomaz, Arnone, Tavares, & Gasbarri, 2010).

6. *Data analysis*

A hierarchical linear regression was used to examine weight as a factor of gelatin condition, sex, and PD.

Consumption between animals was normalized by dividing the amount of gelatin consumed on any day by the animal's weight that day (g/kg). Average alcohol and gelatin consumed was calculated as the average of all 2-hour standard access periods. A two-way ANOVA was used to examine the effect of sex and alcohol condition on average gelatin intake. A linear regression was used to test if gelatin consumed across standard access days could be explained by PD.

BEL was plotted against the amount of alcohol consumed on PD51; a regression line was generated based on this relationship. Five samples were removed from the analysis because of contamination that was apparent upon visual inspection and affirmed with a BEL level that was less than 10 mg/dl. The cause of the contamination is unclear, it is possible that the heparin and blood did not mix properly before the plasma was extracted.

Behavior during sessions in which the outcome was certain (magnitude discrimination and extinction) were analyzed with a hierarchical linear regression using the predictors of probability of risky lever payoff, average alcohol consumed, and sex. Data used for this analysis included behavior from the last day of magnitude discrimination and extinction.

A two-way RM-ANOVA was used to analyze the effect of sex and probability of risky lever delivery on percent of risky lever presses in control animals. A hierarchical linear regression was used to further examine if there was an underlying correlation between sugar

consumed in adolescence and risk preference in adulthood. A hierarchical linear regression was used to evaluate preference for the risky lever in animals that consumed alcohol in adolescence as predicted by probability of risky lever payoff, sex, and average alcohol consumed.

Further categorization of behavior employed quantification of win-stay decisions, lose-shift decisions, trials completed, and total pellets delivered. Separate linear regressions were used to examine trials completed and total pellets delivered over the session as a factor of sex, probability of risky lever payoff, and alcohol. A similar model was used to evaluate win-stay and lose-shift decisions; however, session half was incorporated as an independent variable. Win-stay decisions were defined as risky lever presses that immediately followed a risky lever press that had resulted in the delivery of 3 sugar pellets. Lose-shift decisions were identified as certain lever presses that immediately proceeded a risky lever press that resulted in the omission of sugar pellets.

A one-way RM-ANOVA was used to examine the effect of estrous cycle on risk preference.

R (version 3.4.3, R Core Team, 2017) and the R-packages *plyr* (Wickham, 2011)*, ggplot2* (Wickham, 2009)*, afex* (Singmann, Bolker, Westfall, & Aust, 2018)*, lmerTest* (Kuznetsova, Brockhoff, & Christensen, 2017)*, emmeans* (Lenth, 2018)*,* and *car* (Fox & Weisberg, 2011) were used for analyses.

C. Results

1. Weight

Access to alcohol did not affect the typical weight gain expected across adolescence in male or female animals (Figure 2.4). Model 1 tested the effect of PD, which showed a predicted, positive effect on weight, ($b = 0.01$, 95% CI [0.01, 0.01], $t(479) = 50.39$, $p < 0.001$) and

explained 84% of the variance, (R^2 = 0.84, F (1, 479) = 2539, p < 0.001). See Table 2.1 for full model results. Sex was incorporated into Model 2 to test the hypothesis that it moderates the effect of PD on weight. There was an effect of sex, indicating females weighed less than males (b = -0.08, 95% CI [-0.10, -0.07], t (477) = -12.80, p < 0.001), and an interaction between PD and sex denoting that males had accelerated weight gain compared to females, (b = 0.002, 95% CI [0.002, 0.003], t (477) = 17.19, p < 0.001). Overall this improved the model fit (R^2 = 0.95, F (3, 477) = 2868, p < 0.001). Finally, gelatin condition was added to the Model 3 to ensure that the alcohol condition had no effect on weight; this was confirmed by the results (b = -0.0002, 95% CI [-0.002, 0.002], t (476) = -0.21, p = 0.84). The interaction between sex and PD was quantified by examining the simple slopes of sex at -1 and +1 SD of PD. When rats were younger, male rats weighed more (b = -0.07, 95% CI [-0.08, -0.05], t (477) = -11.92, p < 0.001), but as they aged the effect of sex became stronger, and the weight discrepancy became larger (b = -0.1, 95% CI [-0.12, -0.09], t (477) = -13.43, p < 0.001).

2. *Variable alcohol consumption*

There was a significant effect of gelatin condition on average amount of gelatin consumed (F (1,33) = 110.19, p < 0.001) and a trend toward a main effect of sex (F (1,33) = 3.76, p =0.06), but there was no interaction between sex and gelatin condition on average gelatin consumed (F (1, 33) = 0.15, p = 0.71; Figure 2.5). Least-squared means post-hoc comparisons revealed the control group (M = 43.44, SE = 2.07) on average, consumed significantly more than the alcohol group (M = 15.20, SE = 1.71). Males on average consumed 1.83 g of alcohol per kg body weight each day (M = 1.83, SD = 0.96) and females consumed 1.21 g of alcohol per kg body weight (M = 1.21, SD = 0.74).

PD did not have a significant effect on gelatin consumed across the standard, 2 hour access days (b = 0.26, 95% CI [-0.15, 0.68], t (331) = 1.27, p = 0.20; Figure 2.6 E, F) and explained less than 1% of the variance (R^2 = 0.005, F (1, 331) = 1.61, p = 0.20).

3. *Blood ethanol levels*

The best fit linear regression had a slope of 18.93, such that for every 1g/kg alcohol, blood ethanol increased by 18.93mg/dl.

4. *Long term effects on risk preference*

During magnitude discrimination and extinction phases of testing (100% and 0% chance of large, risky reward delivery, respectively), rats reliably chose the lever with the larger reward option, regardless of prior alcohol consumption (Figure 2.7). Model 1 examined the percentage of large, risky lever choices during magnitude discrimination and extinction as a factor of probability of risky lever payoff, sex, and average alcohol consumed in adolescence. This model resulted in a significant, positive effect of probability (b = 0.76, 95% CI [0.72, 0.80], t (70) = 33.90, p < 0.001) such that magnitude discrimination elicited more risky lever presses than extinction. There was no effect of sex (b = -0.03, 95% CI [-0.05, -0.01], t (70) = -1.19, p = .24) or average alcohol (b = 0.02, 95% CI [0.01, 0.03], t (70) = 1.92, p = 0.06) on risk preference. Model 1 accounted for 94% of the variability in the dependent variable, (R^2 = 0.94, F (3, 70) = 384.5, p < 0.001). Model 2 examined if there was an interaction between any of the predictors in Model 1, this strengthened the effect of probability (b = 0.91, 95% CI [0.81, 1.07], t (66) = 9.73, p <0.001) and there was a significant moderation of sex on probability (b = -0.13, 95% CI [-0.18, -0.07], t (66) = -2.14, p = 0.04). Although Model 2 did improve the R^2 fit by 0.01 it was not a significantly better fit than Model 1 (F (1,66) = 2.48, p = 0.052), see Table 2.2.

During performance of the probabilistic risk task (16%, 33%, and 66% chance of large, risky reward), control animals showed a significant main effect of probability of risky lever payout on selection of the risky lever indicating that they pressed the lever more as it's probability of payout increased ($F(2,28) = 15.33$, $p < 0.001$). However, there was no effect of sex on lever preference ($F(1,13) = 0.18$, $p = 0.68$). Post-hoc tests using least-squared means revealed no difference between 16% ($M = 0.39$, $SE = 0.15$) and 33% ($M = 0.51$, $SE = 0.16$) or between 33% and 66% ($M = 0.74$, $SE = 0.16$), but there was a significant difference between 16% and 66%. A linear regression, Model 1 in Table 2.3, confirmed probability of risky lever payout was a strong predictor of preference for the risky lever ($b = 0.71$, 95% CI [0.55, 0.87], $t(42) = 4.35$, $p < 0.001$), but sex was not ($b = 0.04$, 95% CI [-0.01, 0.09], $t(42) = 0.58$, $p = 0.57$). Overall Model 1 accounted for 31% of the variability in the dependent variable ($R^2 = 0.31$, $F(2, 42) = 9.61$, $p < 0.001$). Average gelatin consumed in adolescence was incorporated into Model 2 but this did not have an effect on risk preference in adulthood ($b = 0.01$, 95% CI [0.00, 0.02], $t(41) = 1.25$, $p = 0.22$), nor did it increase the explained variance ($R^2 = 0.34$, $F(3, 41) = 7.007$, $p < 0.001$), ($F(1) = 1.56$, $p = 0.22$). A full comparison of models can be viewed in Table 2.3

The effects of probability of risky lever payoff and sex on preference for the risky lever in animals that had consumed alcohol in adolescence was examined in Model 1 (see Table 2.4). This model showed a significant effect of the probability of the risky lever ($b = 0.46$, 95% CI [0.32, 0.60], $t(63) = 3.18$, $p = 0.002$) such that as the probability of lever payoff increased so did their preference for the lever. There was also an effect of sex ($b = 0.12$, 95% CI [0.06, 0.18], $t(63) = 2.01$, $p = 0.005$) such that males selected the risky lever a greater portion of the time than females. Together these variables explained a significant portion of the variance in preference for the risky lever ($R^2 = 0.18$, $F(2, 63) = 7.08$, $p = 0.002$). Model 2 incorporated average

alcohol consumed to test the effect of adolescent alcohol use on percentage of risky lever choices. There was an effect of average alcohol consumed, but surprisingly it was in the opposite direction than originally hypothesized (b = -0.12, 95% CI [-0.15, -0.09], t (62) = -3.75, p <0.001), manifesting as increased alcohol use being associated with a decrease in preference for the risky lever. The effect of probability of payout (b = 0.46, 95% CI [0.43, 0.49], t (62) = 3.49, p < 0.001) and sex remained (b = 0.20, 95% CI [0.14, 0.26], t (62) = 3.40, p = 0.001). Model 2 increased explained variance to 33% (R^2 = 0.33, F (3, 62) = 10.4, p < 0.001) and ANOVA results show Model 2 was a significantly better fit than Model 1 (F (1) = 14.09, p < 0.001).

Win-stay decisions was evaluated by the factors sex, alcohol, probability of risky lever payoff, and session half which collectively explained 35% of variability (R^2 = 0.35, F (4, 177) = 23.78, p < 0.001). Sex was a significant, independent factor (b = -2.34, 95% CI [-2.97, -1.71], t (177) = -3.71, p = 0.003) indicating males engaged in more win-stay decisions than females. Additionally, there were significant effects of alcohol (b = -0.70, 95% CI [-1.00, -0.04], t (177) = -2.33, p = 0.02) and probability of risky lever payoff (b = 0.12, 95% CI [0.11, 0.13], t (177) = -4.34, p < 0.001), such that win-stay decisions decreased with increased adolescent alcohol use and increased as the probability of the risky lever increased. Finally, there was a positive effect of session half on number of win-stay decisions (b = 1.38, 95% CI [0.79, 1.97], t (177) = 2.33, p = 0.02), signifying that more of these decisions occurred in the second half of the session compared to the first. Risk preference as a function of consumption is shown for control animals and those who had access to alcohol in Figure 2.8.

The number of lose-shift decisions was negatively related to the probability of the risky lever payout (b = -0.03, 95% CI [-0.04, -0.03], t (177) = -4.38, p < 0.001) and session half (b = -

1.00, 95% CI [-1.32, -0.68], t (177) = -3.15, p = 0.002). These results indicate that as the probability of risky lever payoff increased, the number of lose-shift choices decreased, and more lose-shift decisions were made in the first half of the session than the second (Figure 2.9). However, sex (b = -0.45, 95% CI [-0.78, 0.11], t 177) = -1.34, p = .18) and adolescent alcohol use (b = 0.07, 95% CI [-0.09, 0.23], t (177) = 0.43, p = 0.66) did not significantly contribute to the variability in lose-shit decisions (R^2 = 0.15, F (4, 177) = 7.95, p < 0.001).

The number of trials completed was significantly predicted by sex (b = -9.93, 95% CI [-13.37, -6.49], t (88) = -2.89, p = 0.005) and probability of risky lever payoff (b = -0.16, 95% CI [-0.24, -0.08], t (88) = -2.08, p = 0.04), such that males completed more trials per session than females and less trials were completed as the probability of the risky lever increased (Figure 2.10A). Adolescent alcohol use did not significantly contribute (b = 0.16, 95% CI [-0.66, 0.97], t (88) = 0.19, p = 0.85) to the explained variance in the model (R^2 = 0.14, F (3, 88) = 4.71, p = 0.004).

Similarly, total number of pellets delivered during a session was predicted by sex (b = -12, 95% CI [-17.06, -8.45], t (88) = -2.96, p = 0.004), indicating males received more sugar pellets than females (Figure 2.10B). Probability of risky lever payoff was also a significant, positive factor of total sugar pellets received (b = 0.92, 95% CI [0.82, 1.02], t (88) = 9.46, p < 0.001). Again, alcohol consumed in adolescence was not related to total sugar pellets delivered (b = -0.50, 95% CI [-1.53, 0.53], t (88) = -0.48, p = 0.63). Overall sex, probability of risky lever payout and alcohol explained 53% of the variance (R^2 = 0.53, F (3, 88) = 32.9, p < 0.001).

5. Estrous cycle

There was no effect of estrous cycle on preference for the risky lever ($F(3,53) = 0.55$, $p = 0.65$; Figure 2.11). Female rats exhibited a normal estrous cycle that lasted between 3-5 days.

D. Discussion

The lack of an effect of alcohol on weight, indicates that alcohol did not alter general body development and growth. This result infers that animals that ingested smaller quantities of gelatin consumed more chow to compensate for the lack of gelatin derived calories. Male rats weighed more and had accelerated weight gain over the AIE period compared to females (Figure 2.4); this is consistent with typical adolescent development patterns (L. Spear, 2000). Furthermore, while the beta coefficient for post-natal day in all of the models was fairly small, it is rational and reaffirms animals gained roughly 0.007 kg per day.

Control animals consumed approximately twice as much gelatin compared to alcohol animals (Figure 2.5A). This is likely due to (1) the enhanced palatability of the control gelatin which was comprised of a greater polycose content, (2) a decrease in the felicitousness of the experimental gelatin due to the bitter taste of alcohol, and (3) the intoxicating effects of alcohol may have created a ceiling effect, limiting consumption. Based on prior work examining voluntary alcohol consumption (Bell et al., 2006; Maldonado, Finkbeiner, Alipour, & Kirstein, 2008; Vetter, Doremus-Fitzwater, & Spear, 2007) it is likely the former two justifications, as previous studies have successfully elicited alcohol consumption superior to what was observed in this study. However, similar alcoholic gelatin models (McMurray et al., 2014; Peris et al., 2006) have observed alcohol intake comparable to what was documented in this study, reaffirming the method. There was a trend toward males consuming more gelatin than females, but this result wasn't significant. Human data mirrors these findings such that there is no difference in the number of binge drinking

episodes between adolescent boys and girls [i](Hamburg & Sharfstein, 2009; Schulte, Ramo, & Brown, 2009). Although daily consumption was normalized to weight to account for the discrepancy in size between sexes, this trend may indicate divergent patterns in escalation over time.

Control and alcohol groups exhibited a range of consumption that indicates discrete, individual differences were responsible for gelatin preference. This parallels the variable amount of alcohol (Schulte et al., 2009) and sugar sweetener beverages (Carwile et al., 2015; Imamura et al., 2015) consumed by human adolescents, and provides a valuable, translatable paradigm that represents a range of human behavior as opposed to exclusively modeling the extreme consumption and abuse that is exhibited by a subset of individuals. Binge drinking is characterized as consuming roughly 1g of alcohol per 1kg body weight over a 2-hour period (Leeman et al., 2010), indicating that ¾ of the males and ½ of the females in the experiment were achieving binge-like alcohol consumption (Figure 2.5B).

All rats ingested more gelatin during the extended access periods compared to the standard 2-hour sessions (Figure 2.6), suggesting intake was limited during smaller windows of time either by satiation or intoxication. There was no universal effect of PD on consumption during the standard access periods (Figure 2.6E, F), indicating that intake was stable within animals and there was no ubiquitous increase or decrease in consumption across AIE exposure.

Consumption of 1 g/kg alcohol was expected to evoke a BEL of 80 mg/dl. However, the levels seen were lower than expected. The 'drinking in the dark' paradigm has been previously documented to evoke BEL that are of a lower magnitude than would be expected for the amount of alcohol consumed (N. A. Nasrallah et al., 2011; Peris et al., 2006). Possible explanations for this phenomenon include: different foraging patterns exhibited by rodents which were not recorded

during this experiment, the length of time between alcohol administration and blood draw was not ideal for measuring maximal BEL, and increased alcohol clearance relative to adults (Doremus, Brunell, Rajendran, & Spear, 2005). Furthermore, adolescent rodents do not exhibit the same behavioral signs of intoxication that are seen in adults, especially not at BEL below 100 mg/dl. Thus, measuring behavior indicative of intoxication after AIE would have been moot.

Male and female controls selected the risky lever proportionally per session to the probability of risky lever payoff, this was reflected as a significant effect of probability (Figure 2.8A). This exemplifies that under control conditions, uncertainty in reward delivery results in a moderation of risk preference in response to the respective probability. However, the variability in the control group's behavior during the 33% session should be noted. It is cogent that this session would have the most variability because the two levers have the same objective value during this session, thus the subjective value is likely to be more inconstant. There was a trend toward control males modulating their behavior more than females in response to changing probability sessions, but again this was not significant. This suggests that males are either more sensitive to a changing environment, or females are more risk averse.

There is some evidence that increased sugar intake in childhood can alter memory and cause long term cognitive impairments (Noble, Hsu, & Kanoski, 2017). However, this study did not find any relationship between the amount of gelatin derived sugar consumed in adolescence and behavior on the probabilistic risk task in adulthood (Figure 2.8B). Increased sugar in adolescence appeared to have divergent effects on preference for the risky lever depending on the probability of payoff but this was not significant (Figure 6.5A). These results confirm that any effect seen in the experimental group is a result of their ingestion of alcohol and not confounding property of the gelatin medium.

Choices made during magnitude discrimination and extinction illustrate that certainty in the outcome promotes increased, advantageous behavior in rats. The probability of risky lever payout accounted for the most variability in these decisions, as indicated by its large beta coefficient in both models. The significant moderation of sex on probability in Model 2 seemed to be primarily driven by males during magnitude discrimination, but there was still no effect of alcohol on preference for the risky lever. Contrary to the hypothesis, there was a trend toward male controls exhibiting a decreased preference for the large/risky lever during magnitude discrimination compared to alcohol animals (Figure 2.7). However, control males still selected the large/risky lever the majority of the time during magnitude discrimination. This suggests they understood it was the advantageous option during that session but were continuing to sample from the small/certain lever because of side bias or innate, species-specific scavenging behavior.

Parametric modulation of decision-making in response to the changing probability sessions during the risk task was observed in alcohol animals and was reflected in the significant effect of probability seen in Table 2.4. This moderation of behavior in response to the likelihood of risky lever payoff was also observed during magnitude discrimination and extinction, both of which did not involve uncertainty in reward delivery. Interestingly, the effect of probability in relation to risky lever selection was stronger in sessions in which the outcome was concrete. Additionally, while there was an effect of alcohol on selection of the risky lever during the risk task, this effect was not significant during magnitude discrimination and extinction, suggesting uncertainty in outcome is needed to expose the long-term behavioral deficits generated by adolescent alcohol use.

Previous alcohol use in adolescence had a negative, linear relationship with risky lever preference in both sexes (Figure 2.8C). The effect of alcohol was most prominent in the 66% session, as the risky lever was more greatly favored by low-alcohol consuming animals in this

session than in the 16% and 33% (Figure 6.5B). Additionally, the incorporation of average alcohol use into the model increased the significance of the variables sex and probability, indicating that some of the prior variability within these terms was actually attributable to alcohol use. Although sex was not a predictor of risk task performance in control animals, it was present in alcohol animals and was consistent with the hypothesis that females are more risk averse than males. Furthermore, this phenotype appears to be amplified with alcohol as the slope of the regression line is steeper in females than males

There was no effect of estrous cycle stage on decision making in the probabilistic risk task (Figure 2.11), and alcohol did not cause deregulation or changes to the estrous cycle in females. Due to the relatively small sample size, the variable use of alcohol, and the multiple stages of the estrous cycle, it is possible that if an effect of estrous cycle did exist it would not be detected because of the small sample size and reduced power of the analysis.

An increase in win-stay decisions was associated with an increase in the probability of risky lever payoff, further emphasizing rats' parametric modulation of behavior in response to the different probability sessions. Win-stay decisions were also more likely to occur in the second half of a session than the first half, demonstrating that animals continued to make behavior adjustments throughout the task (Figure 2.9A). Alcohol use in adolescence was negatively related to number of win-stay decisions, foreshadowing that increased alcohol use in adolescence may result in abnormal decision-making because of reward encoding corruption. However, since alcohol use was associated with a decrease in preference for the risky lever in both males and females, there was presumably less opportunity to enact win-stay behavior.

Lose-shift behavior was negatively related to probability of risky lever payoff. This is potentially because there was a higher frequency of risky-lose events in sessions with a lower

probability of payoff that prompted the correction of behavior to the certain lever. Dissimilar to win-stay decisions, lose-shift behavior occurred more frequently in the first half of the session than the second half. This puts forth the idea that development of aversion to reward omissions early in the session may have eradicated risky lever sampling in the latter half of the session (Figure 2.9B). Alcohol was not a predictive factor of lose-shift decisions, but alcohol animals also had less opportunity to enact this behavior because they were not selecting the risky lever as often.

Males completed more trials than females but there was no effect of alcohol on either sex, indicating alcohol did not affect task engagement or motivation (Figure 2.10A). Less trials were completed as the probability of risky lever payout increased, this is presumably because rats received pellets more frequently during these sessions. Males also received more pellets over a session than females, this can be explained by their greater number of completed trials. Finally, the total number of pellets delivered during a session was positively related to the probability of risky lever payoff and was not affected by adolescent alcohol use (Figure 2.10B). This indicates that while alcohol animals show differences in the proportion of risky-lever presses compared to controls, they are somehow compensating to make up the deficit in pellets received. One possible explanation is that alcohol animals are completing more trials than controls, but that this effect is graded, and too small to be detected statistically with an ANOVA.

Sex, alcohol, and probability were predicted to have an effect on decision-making behavior under uncertainty, but the directionality and magnitude of these variables' impact was not congruent with the original theorem. Although there is a lack of literature describing the long-term effects of adolescent alcohol use on decision making in a probabilistic discounting task in female rats, there are ample studies documenting this phenomenon in male rats. The behavioral phenotypes observed in males in this study were discordant with results that have been replicated numerously (Boutros et

al., 2015; Clark et al., 2012; McMurray et al., 2015; N. A. Nasrallah et al., 2011). Though the results were unexpected, there are a few rational explanations that don't contradict or dismiss the conclusions of past studies, and instead offer alternative explanations that encompass all observed behavioral phenotypes. First, the task was a novel iteration of the probabilistic discounting task, designed so animals were not over-trained during acquisition or the task, and the sequence of probabilities was pseudorandomized. These are minor yet significant changes from previous studies which have trained rats on magnitude discrimination for up to 6 days (Clark et al., 2012; St Onge & Floresco, 2010) and the task for up to 24 days (St Onge & Floresco, 2010), until behavior was consistent. Furthermore, these experiments have utilized increasing or decreasing patterns of probabilistic discounting within a single session, despite evidence that that presentation order of probabilities can influence risky lever preference (Orsini et al., 2015). Thus, it is plausible that the unpredictable order of sessions combined with the lack of an ingrained, habit resulted in a behavior strategy that has not been seen before. Another experimental difference is this study used a sugar pellet ratio (1 v 3) that has been found to be more accurately discriminated compared to the ratio used in prior studies (2 v 4) (McMurray, Conway, & Roitman, 2017). Lastly, one can speculate that the amount of alcohol used in prior reports, the mechanism of alcohol delivery, and the long-term social isolation of rats could be confounding experimental factors. In conclusion, adolescent alcohol use does not universally cause increased risk preference in adulthood but does result in the inability to modulate behavior in response to changing contingencies. Tangential to the aforementioned rationalization, if alcohol-induced hypoactivity of the prefrontal cortex results in the inability to encode and update reward value, then previous patterns or cues could be playing a disproportionate role in decision making behavior to compensate.

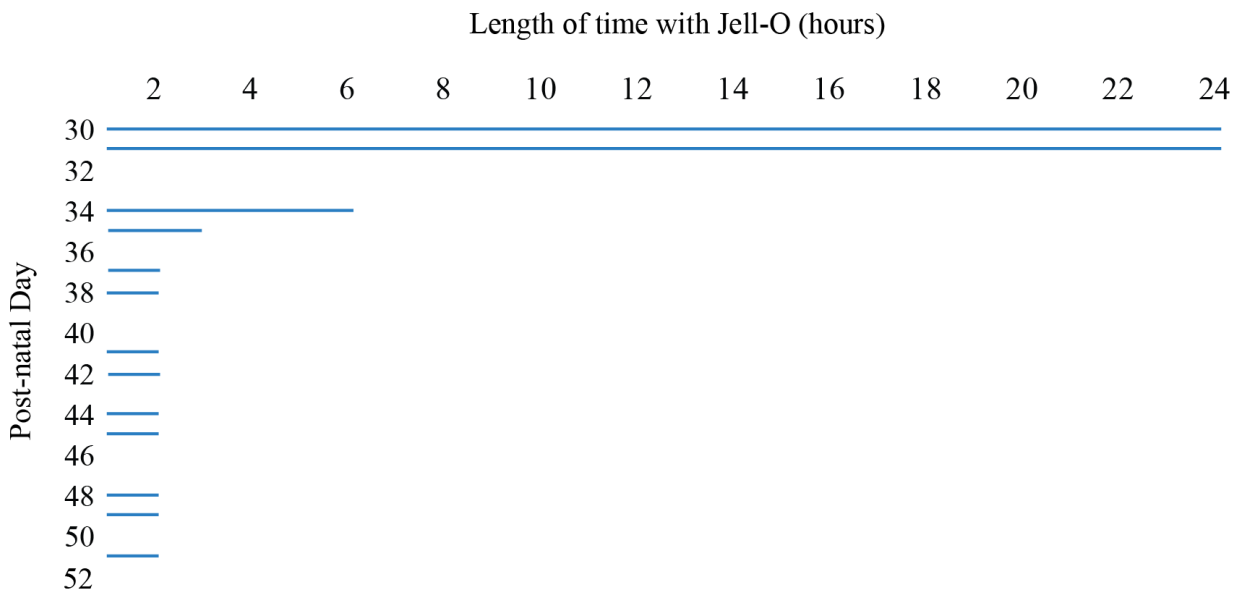


Figure 2.1 Schedule of AIE and gelatin administration. AIE and control gelatin was administered using a 2 days on, 1 day off, 2 days on-1 day off schedule. Blue lines indicate the length of time (hrs) rats had access to the gelatin on any given day during the AIE period.

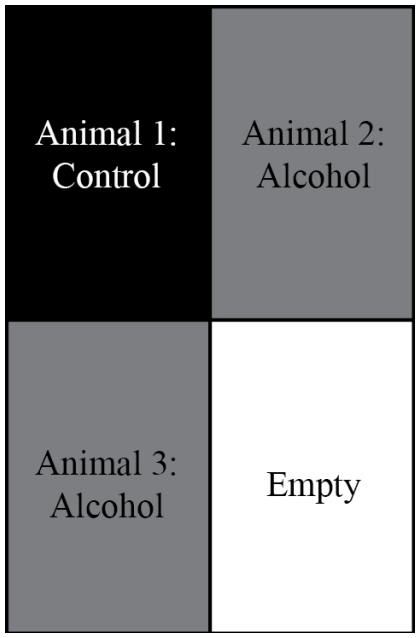
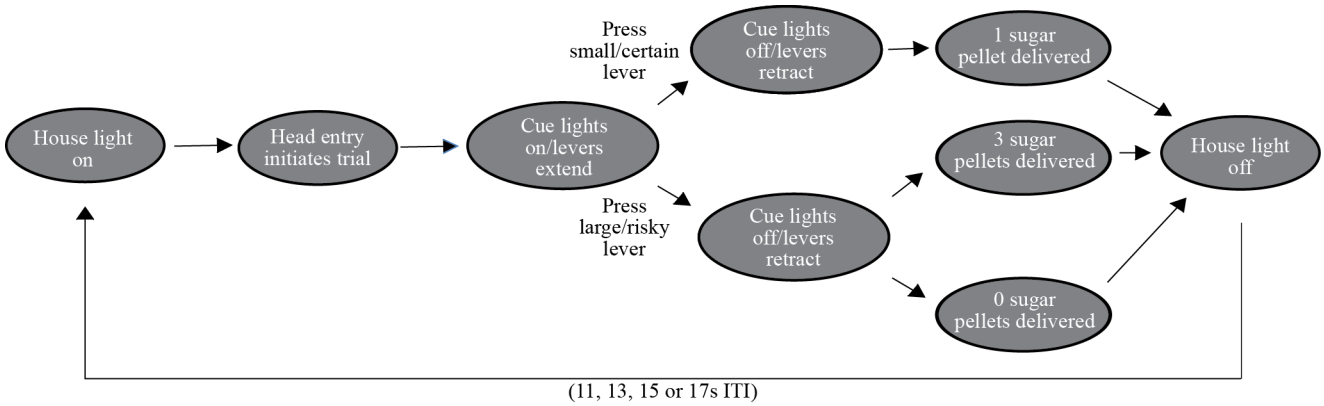


Figure 2.2 Schematic of AIE housing structure. Rats were housed during AIE such that both alcohol animals shared one wall with the control animal and one wall with an empty quadrant.

A.



B.

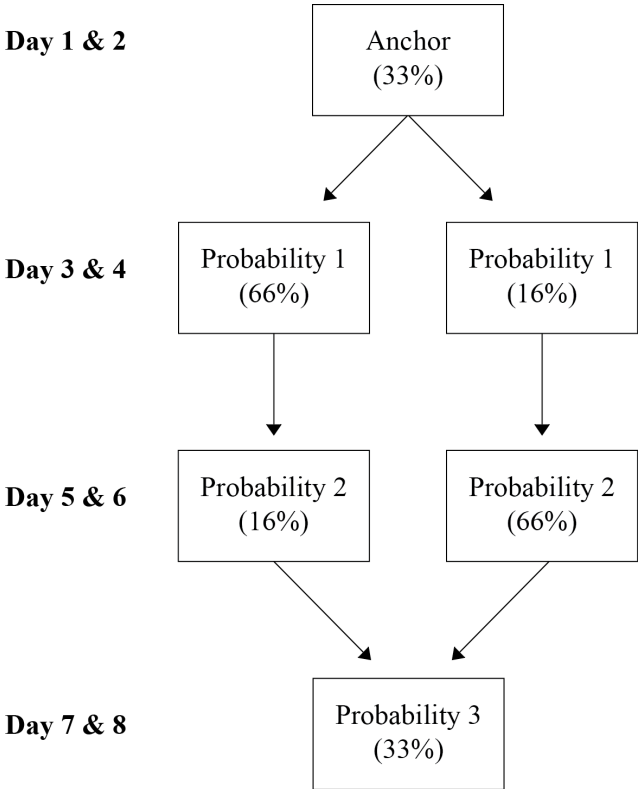


Figure 2.3 Order of behavioral events within a trial and order of session probabilities during the risk task. The sequence of behavioral events within a trial was static but the order of session probabilities was pseudo-random (A) The sequence of events during individual trials of magnitude discrimination, the probabilistic risk task, and extinction. (B) The order of probability sessions throughout the task was pseudo-randomized across animals.

Table 2.1

Summary of hierarchical regression analysis for variables predicting weight.

	Model 1	Model 2	Model 3
Intercept	-0.133*** (0.0058)	-0.086*** (0.0050)	-0.085*** (0.0050)
PD	0.007*** (0.0001)	0.006*** (0.0001)	0.006*** (0.0001)
Sex		-0.087*** (0.0068)	-0.087***(0.007)
PD x Sex		0.003*** (0.0002)	0.003 *** (0.0002)
Condition			-0.0002 (0.001)
R^2	0.841	0.948	0.948
F	2539	2868	2147
df_1	1	3	4
df_2	479	477	476
p	< 0.001	< 0.001	< 0.001

Note: * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

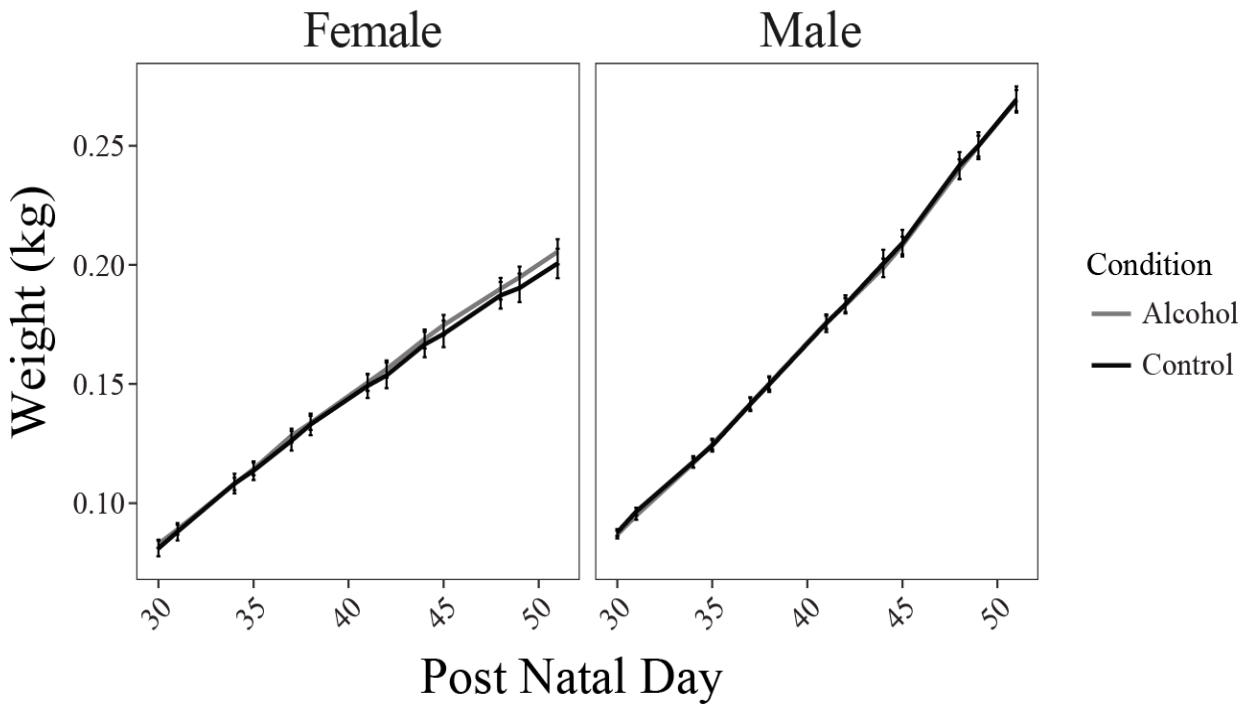
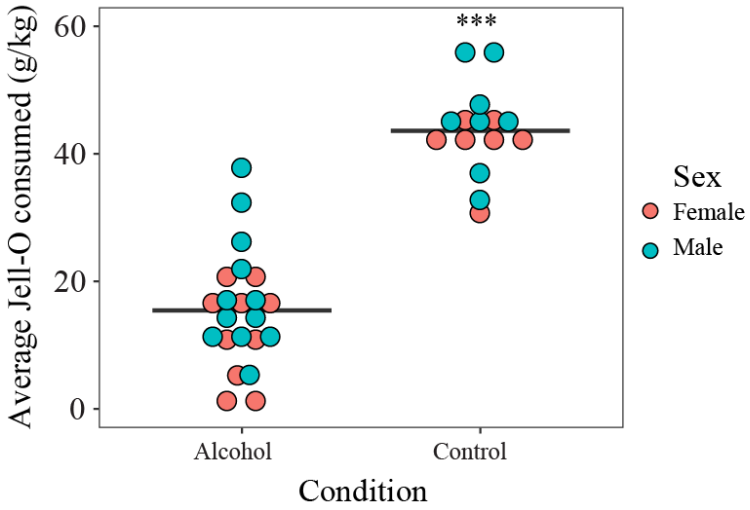


Figure 2.4 Weight of alcohol and control animals across all AIE days. Group is denoted by line color and error bars indicate SEM of each group by day.

A.



B.

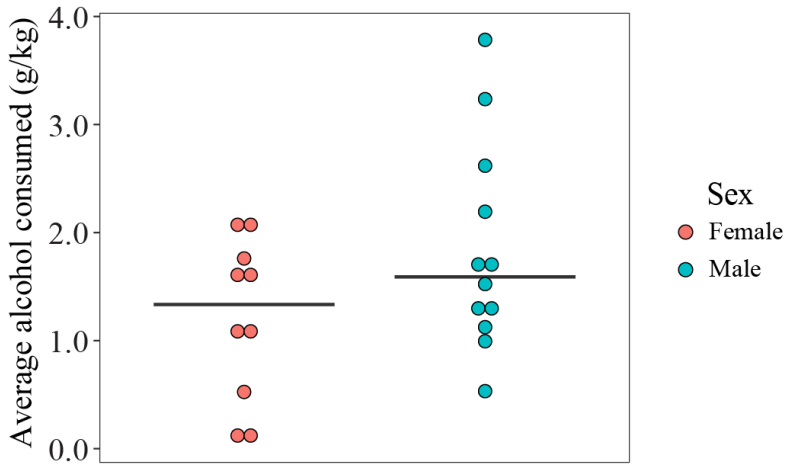


Figure 2.5 Average gelatin and alcohol consumption. (A) The average amount of gelatin consumed by individual rats across all 2-hour access days. Controls consumed significantly more gelatin than alcohol animals. (B) The average amount of alcohol consumed by individual rats in the alcohol group. Horizontal lines indicate the mean of each group (A) or sex (B).

*** $p < 0.001$

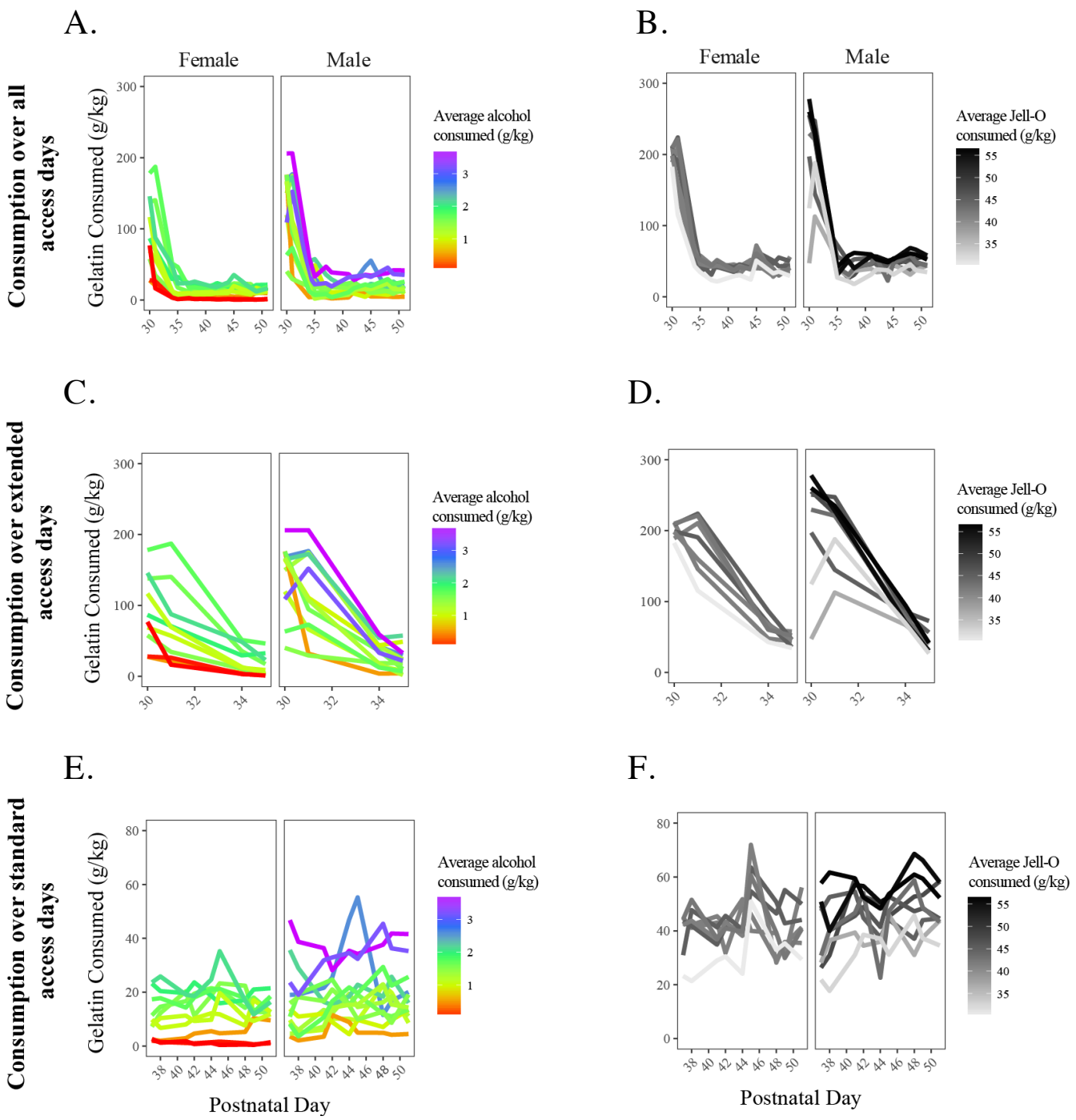


Figure 2.6 Patterns of individual gelatin consumption across AIE days. Lines indicate the behavior of individual rats and are colored based on the average alcohol consumed if they were in the alcohol group (A, C, E) or the average gelatin consumed if they were in the control group

(B, D, F). Panel rows are based on patterns of gelatin consumption over all of adolescence (A, B), solely extended access days (C, D), or exclusively 2-hour standard access days (E, F).

Table 2.2

Summary of linear regression analysis for variables predicting large/risky lever presses during magnitude discrimination and extinction.

	Model 1	Model 2
Intercept	0.058 (0.0382)	0.022 (0.066)
Probability	0.759 (0.022) ***	0.913 (0.094) ***
Average EtOH	0.021 (0.011)	-0.003 (0.059)
Sex	-0.027 (0.023)	0.010 (0.041)
Probability*Average EtOH		-0.056 (0.083)
Probability*Sex		-0.126 (0.059) *
Average EtOH*Sex		0.001 (0.033)
Probability*Average EtOH*Sex		0.060 (0.047)
R^2	0.943	0.950
F	384.5	180.2
df_1	3	7
df_2	70	66
p	< 0.001	< 0.001

Note: * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

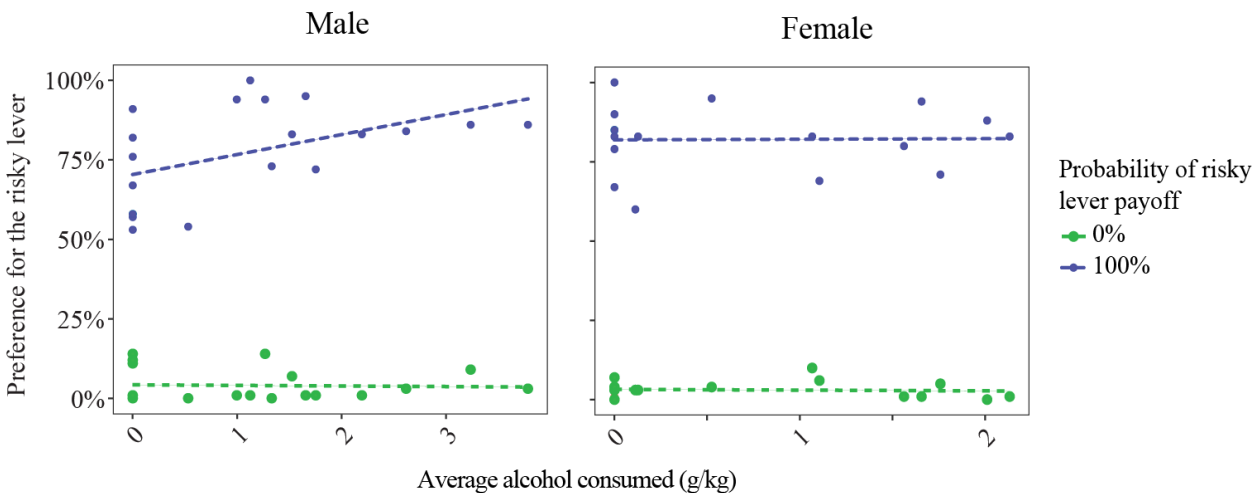


Figure 2.7 Preference for the risky lever during magnitude discrimination and extinction. Dots denote individual rat's percentage of large/risky lever presses based on the average amount of alcohol they consumed in adolescence. Lines represent the simple slopes of each probability session across average alcohol consumption. Dots and lines are colored based on the probability of the risky lever payoff.

Table 2.3

Summary of hierarchical regression analysis for variables predicting large/risky lever presses in control rats.

	Model 1	Model 2
Intercept	-0.001 (0.125)	-0.245 (0.231)
Probability	0.705*** (0.162)	0.705*** (0.0.161)
Sex	0.039 (0.068)	0.011 (0.071)
Average gelatin		0.007 (0.005)
R^2	0.314	0.225
F	9.605	7.007
df_1	2	3
df_2	42	41
p	<0.001	<0.001

Note: * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

Table 2.4

Summary of hierarchical regression analysis for variables predicting large/risky lever presses in alcohol rats.

	Model 1	Model 2
Intercept	-0.006 (0.113)	0.066 (0.104)
Probability	0.461** (0.145)	0.461*** (0.001)
Sex	0.122* (0.060)	0.200** (0.059)
Average EtOH		-0.124*** (0.033)
R^2	0.184	0.335
F	7.081	10.40
df_1	2	3
df_2	63	62
p	0.002	<0.001

Note: * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

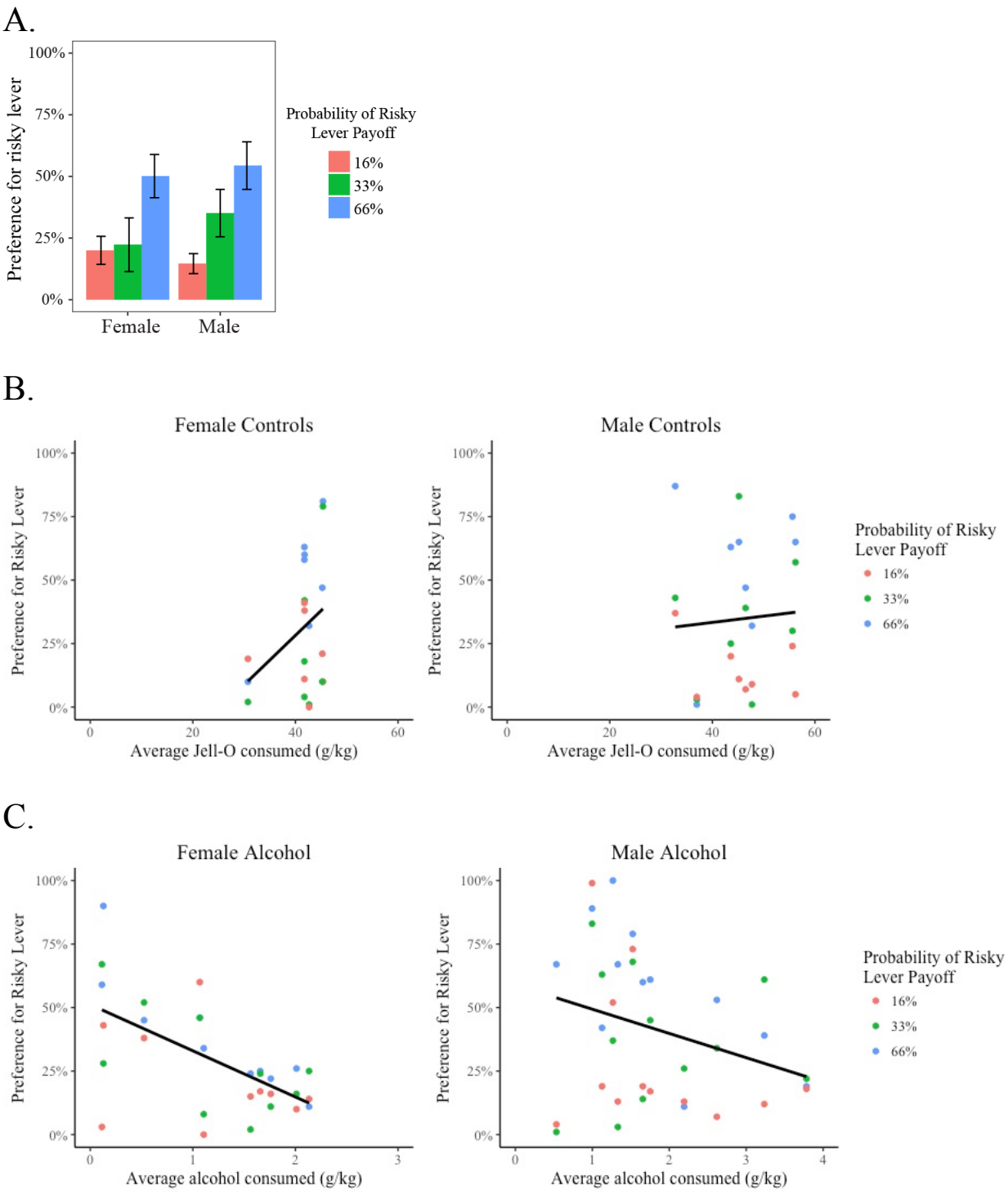


Figure 2.8 Effect of adolescent alcohol use on probabilistic risk task performance. (A) Average risky lever preference of control animals colored by session. Error bars indicate SEM. (B) The percentage of risky lever presses by control animals across the range of gelatin consumed in

adolescence. (C) The percentage of risky lever presses by alcohol animals across the range of alcohol consumed in adolescence. Dots indicate the behavior of individuals and dot color is representative of the session probability and the black lines denote the linear regression fit of gelatin (B) or alcohol (C) as a predictor of preference for the risky lever for each sex.

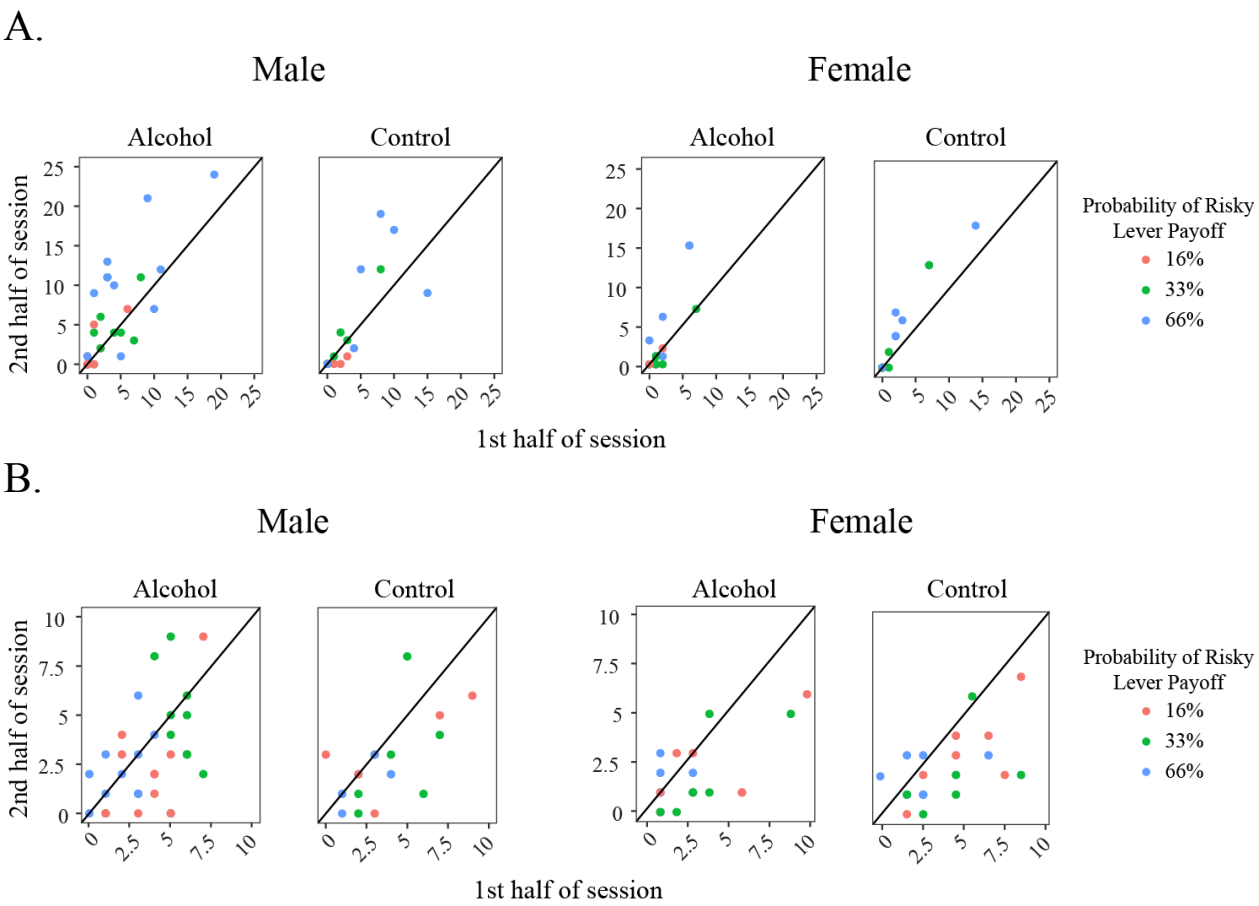
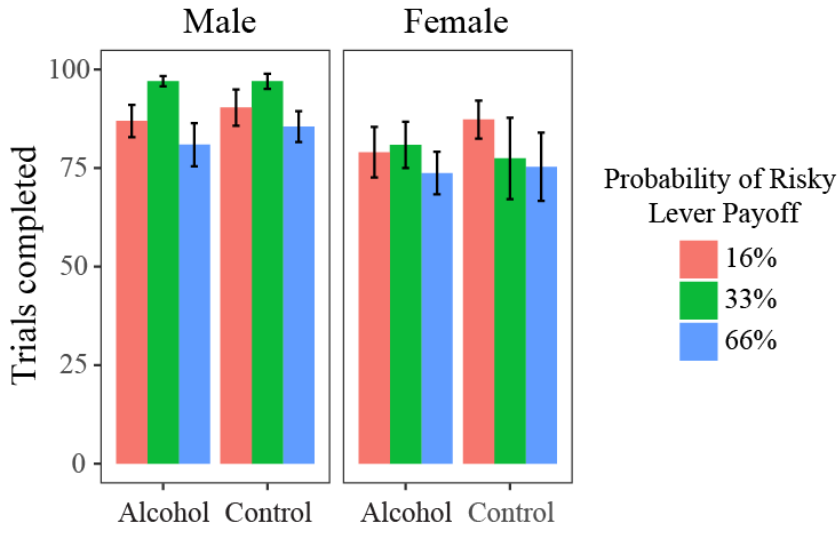


Figure 2.9 Win-stay and lose-shift decisions indicate ongoing adjustments in decision-making behavior. (A) A comparison of the number of win-stay decisions in the first half of the session vs the 2nd half in males and females across groups. (B) A comparison of the number of lose-shift decisions in the first half of the session vs the 2nd half as a factor of sex and group. Dots represent the behavior of individuals and are colored based on the probability of the risky lever payoff of the session. Black lines assist in the visualization of which session half the majority of events occurred.

A.



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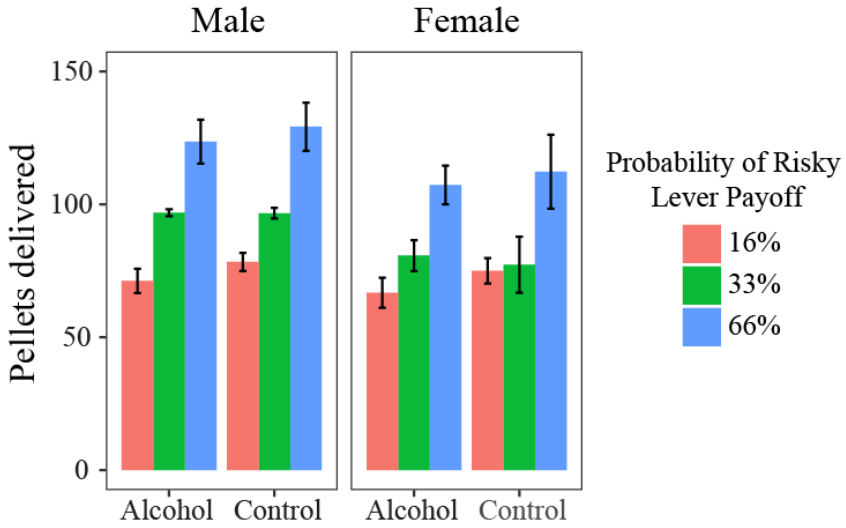


Figure 2.10 The number of trials completed and total pellets delivered are indicative of task engagement. (A) Number of trials completed as a factor of sex and group. (B) Total pellets received over a session as a factor of sex and group. Bar color indicates the probability of risky lever reward delivery, error bars denote SEM.

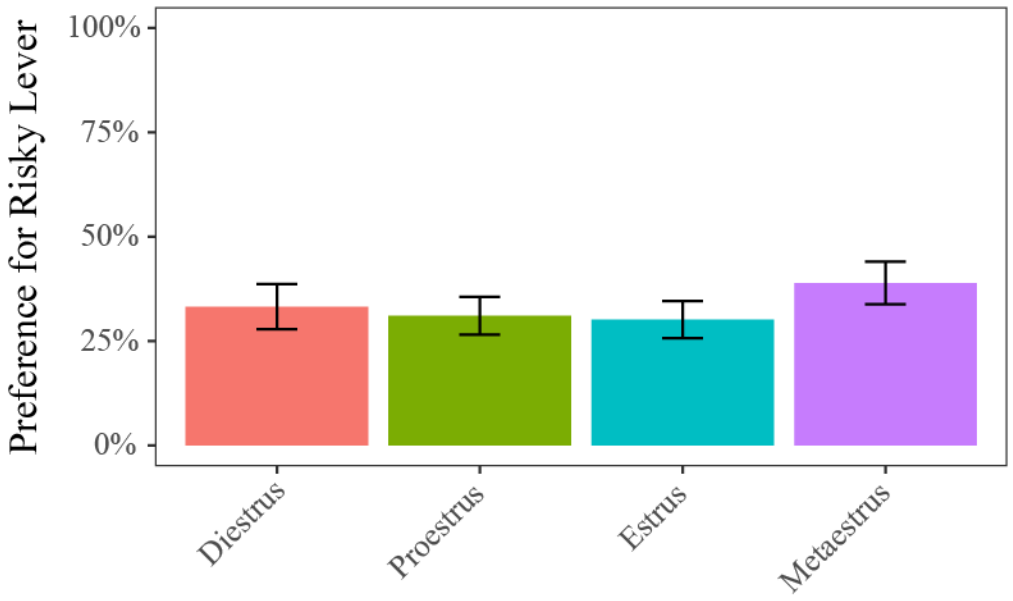


Figure 2.11 Effect of estrous cycle on probabilistic risk task performance. Estrous cycle stage was not a predictor of female rats' performance on the probabilistic risk task. Error bars indicate SEM.

III. CHAPTER 3: Neural encoding of decision-related factors is permanently altered by adolescent alcohol use

A. Rationale

Neural activity in sub-regions of PFC, such as mPFC and OFC, has been shown to be necessary for conventional patterns of decision-making, including preference for large-risky rewards over small-certain ones and vice versa (Orsini et al., 2015). Pruning and strengthening of synaptic connections continues in these cortical regions until early adulthood, imparting insight into the mechanisms underlying maladaptive behaviors exhibited by teens. Adolescence also coincides with the onset of addiction and other psychiatric disorders that involve aberrant decision-making, providing further incentive to evaluate the long-term effects of adolescent binge drinking on neural activity in these critical regions. The purpose of the following study was to probe how decision-dependent variables are relayed within OFC and mPFC, as well as how binge drinking in males and females alters said encoding.

Extracellular, single cell, electrophysiological recordings were used to record action potentials in OFC and mPFC and align them to events that occurred during the probabilistic risk task. Thus, based on the timing and patterns of activity, it could be established what specific role these regions were playing in the decision. Additionally, by measuring discrepancies in neural activity in rats who had consumed alcohol in adolescence compared to those who had not, it could be ascertained how alcohol was changing the neural basis of behavior.

It was hypothesized that OFC and mPFC would both contain neurons that altered their firing rates at the time of the lever press or at the time of reward delivery. However, reward encoding neurons in OFC and neurons active at the time of lever press in mPFC were of greater interest due to the regions proposed roles in behavior. Neurons in OFC and mPFC that

responded during reward delivery were expected to exhibit alcohol-dependent hypoactivity regardless of sex. It was predicted that this blunted activity would result in the inability to discriminate different reward sizes through firing rate. Previous research has shown stunted OFC activity in high alcohol consuming male rats (McMurray et al., 2015) and a similar result was anticipated in females. Alcohol was hypothesized to have a positive effect on the firing rate in press-responsive neurons at the time of lever press. This was thought to align with the predicted risky behavior in alcohol animals. Finally, females were predicted to have to have greater changes in firing rates regardless of prior alcohol use.

B. Materials and methods

1. Animals

Animals used during the behavioral task were simultaneously used for electrophysiological experiments. The colony was maintained on a 12:12 reversed light/dark cycle. Animals were treated under the approval of the Animal Care Committee of the University of Illinois at Chicago and in accordance with the National Institutes of Health's guidelines.

2. Surgical procedures

Once animals completed behavior shaping (~PD 77), they were anesthetized with ketamine-xylazine (0.1, 0.05 ml/kg, IP) and stainless-steel Teflon insulated electrode microwire arrays (MicroProbes, Gaithersburg, MD) were implanted bilaterally. The electrode arrays were organized into two rows of four microwires (50 μm diameter; tip separation .25mm) and were guided stereotaxically into OFC (AP +3.2, ML -3.0 relative to bregma, and -4.0 mm relative to the brain's surface) and mPFC (AP +3.3, ML +1.2 relative to bregma, and -3.5 mm relative to the brain's surface at a 10° angle). Surgical steel screws and dental cement were used to secure the implanted electrodes; ground wires from each array were secured around one of the distal

surgical screws. Animals were given at least one week of recovery time after surgery before continuing onto magnitude discrimination.

3. Electrophysiology recordings

Electrophysiological recordings began when the rats were roughly PD 100. Electrode arrays were connected to a recording cable that was attached to a motorized commutator (Plexon, Dallas, TX) allowing rats to move freely in the operant chamber. Electrical signals detected at the electrode tip were amplified and transduced via the OmniPlex system (Plexon, Dallas, TX). Events such as lever presentation, lever press, sugar pellet delivery, and nose port entry were time stamped onto the neural activity data using transistor- transistor logic (TTL). Individual waveform statistics were performed during the recording session to identify waveforms belonging to individual neurons (PlexControl). All recorded waveforms were evaluated again and refined after recordings had ended (Offline Sorter). The data was exported to Matlab and R for additional analysis.

4. Histology

When animals completed behavioral testing and extinction, the final electrode position was marked by passing electrical current down each electrode. The rats were euthanized, perfused, and the brains were removed and allowed to post-fix in a Potassium Ferricyanide, formalin mixture for 24 hours before being moved to a 20% sucrose solution. Brains were sliced at 40μm to assess electrode placement. Neurons were only included in the analyses if the wire they were recorded on had a verified placement in one of the regions of interest.

5. Data analysis

The mean firing rate and standard deviation of the firing rate were calculated for each neuron over an entire session. The average time-course of the response for each neuron was

aligned to the time of lever press on each trial and the average firing rate during baseline (8-5 s before trial initiation), the time of the lever press (2s before lever press to 1s after lever press), and the reward period (2-8 s after lever press) were calculated for each neuron. Neurons were excluded if they exhibited a baseline firing rate less than .3 sp/s or greater than 20 sp/s. Student's t-tests were performed on each neuron to identify which ones, on average, exhibited a significant change in firing rate at the time of lever press or reward compared to baseline. Neurons were grouped based on event and direction of response and could be sorted into multiple groups. Firing rates were further sorted based on the lever that was pressed and the number of sugar pellets that resulted from the lever press. Average firing rates were z-transformed using the neuron's baseline mean and standard deviation firing rate. Hierarchical linear regressions were performed on each response group to test the relationship between z-transformed firing rate, sex, alcohol consumed in adolescence, and event (lever pressed or number of sugar pellets delivered). Analyses were repeated using more conservative degrees of freedom in a mixed model regression analysis, but the results were almost identical. Tables containing the more conservative results can be found in the appendix. Analyses were done separately in mPFC and OFC based on a priori hypotheses. At least one model for each neuron population tested the interaction between sex and alcohol; if the moderation was significant the sexes were analyzed separately to prevent the complicated interpretation of a three-way interaction. Neurons with z-scores above 10 were verified as outliers and excluded.

Independent one-way ANOVA's examined the effect of estrous cycle stage on baseline firing rate. Neurons were grouped based on their anatomical location and if on average they had a significant increase in firing rate at the time of lever press or reward, or a significant decrease at the time of lever press or reward.

R (version 3.4.3, R Core Team, 2017) and the R-packages *plyr* (Wickham, 2011), *ggplot2* (Wickham, 2009), *afex* (Singmann et al., 2018), *lmerTest* (Kuznetsova et al., 2017), *emmeans* (Lenth, 2018), and *car* (Fox & Weisberg, 2011) were used for analyses.

C. Results

1. Demographics and time course of neural data

In total, 1,186 neurons were recorded; 60 were excluded due to electrode misplacement (15) or poor isolation with too few actions potentials identified (45). Electrode placements can be seen in Figure 3.1. Of the remaining 1126 neurons, 493 of these neurons were recorded in mPFC and 633 were recorded from OFC. 578 of the neurons came from males and 548 came from females. Control animals contributed 482 neurons to the total, while 644 neurons were in alcohol animals.

Neurons were grouped into 4 categories based on their average patterns of activity: increased activity at lever press (PP), decreased activity at lever press (NP), increased activity at reward delivery (PR) or decrease in activity at reward delivery (NR). mPFC contained 108 PP neurons, 126 NP neurons, 115 PR neurons, and 163 NR neurons. OFC contained 139 PP neurons, 104 NP neurons, 173 PR neurons, and 152 NR neurons (Table 3.1). mPFC and OFC contained different distributions of neurons that was confirmed by a chi-squared analysis (χ^2 = 15.20, p = 0.002).

Environmental stimuli that occurred during the baseline period, such as the ignition of the house light, did not have an effect on the average baseline firing rate of neurons (2s before house light, M = 2.45, SD = 0.07 sp/s; 2s after house light, M = 2.54, SD = 0.07 sp/s; t (1125) = -0.87, p = 0.38) (Figure 3.2). Time course of neural activity in mPFC and OFC for each group (PP, NP, PR, NR) aligned to the time of lever press can be seen separately for controls and alcohol-

consuming animals in Figure 3.3, Figure 3.4, and Figure 3.5 For OFC, PR neurons are shown separately by sex, as discussed below.

2. Neural activity in mPFC

Model PPm1 examined the neural activity in mPFC's PP subpopulation of neurons, at the time of lever press as a function of sex, alcohol, and lever pressed. This model resulted in a significant effect of sex (b = 0.39, 95% CI [0.22, 0.55], t (212) = 2.38, p = 0.02), indicating females had greater changes in firing rate from baseline than males. There was a trend towards the risky lever eliciting a greater change in firing rate than the certain lever, but it was not significant (b = 0.28, 95% CI [0.13, 0.42], t (212) = 1.90, p = 0.06). There was no effect of alcohol on change in firing rate during the time of lever press (b = -0.09, 95% CI [-0.19, 0.01], t (212) = -0.93, p = 0.35). Model PPm1 accounted for 6% of the variability in the firing rate at the time of lever press, (R^2 = 0.06, F (3, 212) = 4.65, p = 0.004). Model PPm2 tested the interaction between sex and alcohol and confirmed that the effect of alcohol was similar between sexes (b = -0.18, 95% CI [-0.38, 0.02], t (211) = -0.91, p = 0.36), directing the continuation of the analysis without the interaction term. Model PPm3 tested if there was an interaction between lever pressed and alcohol, because it was hypothesized that neurons would show increased activity to the risky lever with increased adolescent alcohol use. There was a trend towards a significant moderation that contradicted our hypothesis (b = -0.32, 95% CI [-0.49, -0.25], t (211) = -1.87, p = 0.06), such that increased alcohol use was correlated with a decrease in firing during risky lever presses and an increase in firing during certain lever presses. The addition of this interaction term also increased the beta coefficient and significance of the lever press term (b = 0.48 95% CI [0.30, 0.66], t (211) = 2.65, p = 0.009). Model PPm3 increased the explained variance by 2% (R^2 = 0.08, F (4, 211) = 4.40, p = 0.002), and although it was not a significant

improvement from Model PPm1 ($F (1, 211) = 3.90$, $p = 0.06$), it trended toward being a better fit (refer to Table 3.2 for model comparisons), suggesting Model PPm3 is the best fit model for mPFC's PP subpopulation's neural activity. The average responses of mPFC PP neurons at the time of lever press are shown as a function of alcohol consumption in Figure 3.6.

Model NPm1 sought to model the neural activity in mPFC's NP subpopulation of neurons, at the time of lever press, as a function of sex, alcohol, and lever pressed. This did not reveal a main effect of sex ($b = 0.02$, 95% CI [-0.04, 0.08], $t (248) = 0.31$, $p = 0.76$) or alcohol ($b = 0.04$, 95% CI [0.01, 0.07, $t (248) = 1.64$, $p = 0.10$) on change in neural activity. There was also a trend towards the risky lever generating a greater increase in firing compared to the certain lever ($b = 0.09$, 95% CI [0.04, 0.14], $t (248) = 1.86$, $p = 0.06$). Overall, Model NPm1only explained 3% of the variance and was not a significant predictor of change in firing rate at lever press ($R^2 = 0.03$, $F (3, 248) = 2.18$, $p = 0.09$). Similar to Model PPm1, Model NPm2 confirmed that there was no interaction between sex and adolescent alcohol use ($b = 0.08$, 95% CI [0.01, 0.15], $t (247) = 1.17$, $p = 0.24$). Finally, Model NPm3 tested if alcohol moderated the effect of lever. The beta coefficients for the other variables remained constant, but Model NPm3 did not yield a significant interaction ($b = -0.03$, 95% CI [-0.07, 0.01], $t (248) = -0.59$, $p = 0.55$) and the correlation coefficient did not increase ($R^2 = 0.03$, $F (4, 247) = 1.72$, $p = 0.15$). Refer to Table 3.3 for mPFC NP model comparisons. The average responses of mPFC NP neurons at the time of lever press are shown as a function of alcohol consumption in Figure 3.7.

mPFC's activity in the PR subpopulation during the reward period was first examined using Model PRm1as a factor of sex, adolescent alcohol use, and reward size. The number of sugar pellets delivered had a positive relationship with firing rate such that 1 sugar pellet increased normalized firing at the time of reward by 0.37 ($b = 0.37$, 95% CI [0.29, 0.45], $t (340)$

= 4.29, $p < 0.001$) and 3 sugar pellets increased the firing rate by 0.50 (b = 0.50, 95% CI [0.41, 0.59], t (340) = 5.81, $p < 0.001$) compared to 0 pellets. However, there was not a significant difference in firing rate between 1 and 3 sugar pellets (b = 0.13, 95% CI [0.04, 0.22], t (340) = 1.53, p = 0.13). There was a trend toward a significant, negative relationship between alcohol and change in firing rate (b = -0.08, 95% CI [-0.13, -0.03], t (340) = -1.66, p = 0.10). In total, Model PRm1 explained 10% of the variance in firing rate ($R^2 = 0.10$, F (4, 340) = 1.72, $p < 0.001$), full results can be seen in Table 3.4. The interaction between sex and alcohol was examined in Model PRm2, but it was not significant (b = 0.06, 95% CI [-0.03, 0.15], t (339) = 0.626, p = 0.53) and the overall model was not significantly different than Model PRm1 (F (1, 339) =0.39, p = 0.53). Lastly, Model PRm3 examined if alcohol moderated the effect of reward size on firing rate during the reward period. There was no difference between alcohol's effect on reward when comparing 0 and 1 pellets (b = 0.11, 95% CI [0.01, 0.21], t (338) = 1.11, p = 0.27) or 0 and 3 pellets (b = -0.76, 95% CI [-0.18, 0.02], t (338) = -7.61, p = 0.45), but the comparison between 1 pellet and 3 pellets verged on significance (b = -0.19, 95% CI [-0.29, -0.09], t (338) = -1.875, p = 0.06). However, Model PRm3 only explained 1% more variance in the dependent variable ($R^2 = 0.11$, F (6, 338) = 7.14, $p < 0.001$) than Model PR1 and was not statistically different (F (2,338) = 1.78, p = 0.17). The average responses of mPFC PR neurons during the reward period are shown as a function of alcohol consumption in Figure 3.8.

Model NRm1 examined the relationship between mPFC's NR population's firing rate during the reward period, sex, alcohol, and number of sugar pellets delivered, results from the model can be seen in Table 3.5. Similar to Model PRm1, the number of sugar pellets delivered correlated with firing rate, but in the NR population this relationship was negative such that 1 sugar pellet decreased normalized firing at the time of reward by 0.31 compared to 0 pellets (b =

-0.31, 95% CI [-0.36, -0.26], t (484) = -5.76, p < 0.001) and 3 sugar pellets decreased the firing rate by 0.46 compared to 0 pellets (b = -0.46, 95% CI [-0.51, -0.41], t (484) = -8.48, p < 0.001) and .15 compared to 1 pellet (b = -0.15, 95% CI [-0.20, -0.10], t (484) = -2.72, p = 0.007). There was no detectable relationship between firing rate during the reward period and sex (b = 0.04, 95% CI [-0.01, 0.09], t (484) = 0.70, p = 0.49) or alcohol (b = 0.02, 95% CI [0.00, 0.04], t (484) = 0.68, p = 0.50). In total, Model NRm1 explained 14% of the variance in firing rate (R^2 = 0.14, F (4, 484) = 1.72, p < 0.001). Model NRm2 integrated the interaction between alcohol and sex into the model but it was not significant (b = -0.04, 95% CI [-0.12, 0.02], t (483) = -0.70, p = 0.49) nor was it a better fit than Model NRm1 (F (1, 483) = 0.48, p = 0.49). Model NPm3 examined the interaction between alcohol and number of sugar pellets. This resulted in an increased magnitude of the beta coefficients for the effect of reward size and a significant difference between alcohol's effect on the firing rate to 0 vs 3 sugar pellets (b = 0.14, 95% CI [0.09, 0.19], t (482) = 2.84, p = 0.005). However, there was no difference between alcohol's effect on 0 vs 1 pellet (b = 0.06, 95% CI [0.02, 0.12], t (482) = 1.34, p = 0.18) or 1 vs 3 pellets (b = 0.07, 95% CI [0.02, 0.12], t (482) = 1.50, p = 0.13). Model NRm3 explained 15% of the variance in firing rate during the reward period (R^2 = 0.15, F (6, 482) = 14.09, p < 0.001) and was significantly better at explaining the variance than Model NRm1(F (2, 482) = 4.04, p = 0.02). The average responses of mPFC NR neurons during the reward period are shown as a function of alcohol consumption in Figure 3.9.

Estrous cycle stage did not have an effect on baseline firing rate of mPFC PP and PR neurons (F (3, 100) = 0.94, p =0.42), nor did it effect mPFC NP and NR neurons' baseline firing rate (F (3, 46) = 0.26, p = 0.85).

3. Neural activity in OFC

The PP population of neurons in OFC were first examined using Model PPo1. This model looked at the relationship between firing rate at the time of lever press, sex, adolescent alcohol use, and lever pressed. Model PPo1 explained 2% of the variance in firing rate at the time of lever press (R^2 = 0.02, F (3, 274) = 1.39, p = 0.25) and neither sex (b = 0.11, 95% CI [-0.01, 0.23], t (274) = 0.79, p = 0.43), alcohol (b = -0.06, 95% CI [-0.12, 0.00], t (274) = -1.03, p = 0.30), or lever pressed (b = 0.14, 95% CI [0.02, 0.26], t (274) = 1.14, p = 0.26) had a main effect on firing rate. Model PPo2 tested the interaction between sex and adolescent alcohol use but the new term was not significant (b = 0.06, 95% CI [-0.10, 0.22], t (273) = 0.37, p = 0.71). The final model for OFC's PP population, Model PPo3, probed if there was an interaction between alcohol and lever pressed on firing rate. However, this relationship did not appear to exist (b = 0.003, 95% CI [-0.11, 0.11], t (272) = 0.03, p = 0.98) and did not increase the model fit (R^2 = 0.02, F (4, 273) = 1.04, p = 0.39). Model comparisons can be found in Table 3.6, and the average responses of OFC PP neurons at the time of lever press are shown as a function of alcohol consumption in Figure 3.10.

Model NPo1 aimed to look at if the change in firing rate during lever press in OFC's NP neurons could be explained by sex, alcohol, and lever pressed. Model NPo1 showed males had an increased firing rate during lever press than females (b = -0.12, 95% CI [-0.17, -0.07], t (204) = -2.10, p = 0.04), the risky lever caused an increase in firing compared to the certain lever (b = 0.14, 95% CI [0.09, 0.21], t (204) = 2.87, p = 0.005), and there was a trend toward increased alcohol causing a decrease in firing at the time of lever press (b = -0.04, 95% CI [-0.06, -0.02], t (204) = -1.94, p = 0.05). Overall the model explained 6% of the variance in firing rate at the time of lever press (R^2 = 0.06, F (3, 204) = 4.69, p = 0.003). Model details can be viewed in

Table 3.7. The following model tested for an interaction between sex and alcohol, but this relationship was non-existent (b = -0.03, 95% CI [-0.08, 0.02], t (203) = -0.65, p = 0.52) and the model was not a better fit than Model NPo1 (F (1,203) = 0.42, p = 0.52). Model NPo3 tested if alcohol moderated the effect of lever pressed, resulting in a trend toward a significant, negative relationship (b = -0.07, 95% CI [-0.11, -0.03], t (203) = -1.69, p = 0.09). This suggests increased adolescent alcohol use decreases firing rate at the time of lever press more when the risky lever is pressed than the certain. However, this model obliterated the emerging main effect of alcohol (b = -0.009, 95% CI [-0.03, 0.03], t (203) = -0.31, p = 0.76) and was not significantly different than Model NPo1(F (1, 203) = 2.84, p = 0.09). The average responses of mPFC NP neurons at the time of lever press are shown as a function of alcohol consumption in Figure 3.11.

The relationship between the activity of OFC's PR population during reward delivery, sex, adolescent alcohol use, and number of sugar pellets delivered was modeled in Model PRo1. This model indicated that females had an increased firing rate compared to males (b = 0.26, 95% CI [0.19, 0.33], t (514) = 3.87, p < 0.001) and there was a positive correlation between number of sugar pellets delivered and firing rate such that 1 sugar pellet increased firing by 0.27 compared to 0 pellets (b = 0.27, 95% CI [0.19, 0.33], t (514) = 3.87, p < 0.001), 3 sugar pellets increased firing by 0.61 compared to 0 pellets (b = 0.61, 95% CI [0.53, 0.69], t (514) = 7.80, p < 0.001), and 3 sugar pellets increased firing by 0.33 compared to 1 pellet (b = 0.33, 95% CI [0.25, 0.41], t (514) = 4.29, p < 0.001). Although, Model PRo1 did not reveal a main effect of alcohol (b = -0.02, 95% CI [-0.05, 0.01], t (514) = -0.66, p = 0.51), it did explain 14% of the variability in firing rate (R^2 = 0.14, F (4, 514) = 20.08, p < 0.001). Model PRo2 revealed the interaction between sex and alcohol was significant (b = 0.18, 95% CI [0.11, 0.25], t (513) = 2.46, p = 0.01) and was almost entirely responsible for the previously seen effect of sex (b = 0.10, 95% CI [0.00,

0.20], t (513) = 2.46, p = 0.01). This result indicated that as females had increased volumes of alcohol in adolescence, they had increased neural activity during the reward period in adulthood compared to males of the same condition. Additionally, Model PRo2 explained more of the variance in the firing rate than Model PRo1 (F (1, 513) = 6.06, p = 0.01); a comparison of the two models can be found in Table 3.8, with average neural responses shown in Figure 3.12. This result prompted the analysis of the sexes to be done separately for OFC's PR population in order to avoid possible three-way interactions. Models of separate male and female OFC PR activity can be seen in Table 3.9, with average neural responses for each sex shown in Figure 3.13. Model PRo.M1 re-evaluated the relationship between firing rate of OFC PR neurons in males, alcohol, and reward size. While the positive correlation between reward size and firing rate persisted (0 v 1: b = 0.30, 95% CI [0.20, 0.40], t (278) = 3.08, p = 0.002; 0 v 3: b = 0.56, 95% CI [0.46, 0.66], t (278) = 5.79, p < 0.001; 1 v 3: b = 0.26, 95% CI [0.16, 0.36], t (278) = 2.71, p = 0.01), a trend toward a negative effect of alcohol emerged as well (b = -0.05, 95% CI [-0.08, -0.02], t (278) = -1.74, p = 0.08). Overall Model PRo.M1 was a significant predictor of firing rate during the reward period in male OFC PR neurons (R^2 = 0.12, F (3, 278) = 12.20, p < 0.001).

The interaction term between alcohol and reward size was incorporated into Model PRo.M2. This revealed that alcohol had a negative effect on firing rate when 3 pellets were delivered compared to 0 (b = -0.15, 95% CI [-0.22, -0.08], t (276) = -2.22, p = 0.03), and a trend toward a negative effect on firing rate when 1 pellet was delivered compared to 0 (b = -0.12, 95% CI [-0.19, -0.05], t (276) = -1.77, p = 0.08). Alcohol did not significantly moderate the effect of reward size on firing rate when 1 and 3 pellets were contrasted (b = -0.03, 95% CI [-0.10, 0.04], t (276) = -0.45, p = 0.66), indicating alcohol had a similar effect on the encoding of these rewards. Additionally, while Model PRo.M2 trended toward being a significantly better model than

Model PRoM.1, it fell short in a statistical comparison (F (2, 276) = 2.75, p = 0.07). Analyses were repeated for female OFC PR neurons in Model PRo.F1 and Model PRo.F2. In Model PRo.F1, when there was no interaction term, there was a trend toward a significant, positive effect of alcohol on firing rate (b = 0.13, 95% CI [0.06, 0.20], t (233) = 1.82, p = 0.07), and a positive contrast between 0 and 1 pellets' effect on firing rate (b = 0.24, 95% CI [0.12, 0.36], t (233) = 1.90, p = 0.05). 3 sugar pellets also resulted in a greater increase in firing rate when compared to 0 (b = 0.66, 95% CI [0.54, 0.78], t (233) = 5.30, p < 0.001) or 1 pellet (b = 0.42, 95% CI [0.30, 0.54], t (233) = 3.37, p < 0.001). Cumulatively, Model PRo.F1 explained 12% of the variance in firing rate of the OFC PR population (R^2 = 0.12, F (3, 233) = 10.69, p < 0.001). The incorporation of the interaction term into Model PRo.F2 did not result in any additional effects and was not a better model fit than Model PRo.F1 (F (2, 231) = 0.77, p = 0.47).

Model NRo1 examined sex, alcohol, and reward size as predictors of OFC's NR population's firing rate during the reward period. This revealed females had a decreased firing rate during the reward period compared to males (b = -0.09, 95% CI [-0.13, -0.05], t (451) = -2.24, p = 0.03) and there was a negative relationship between reward size and firing rate (0 v 1: b = -0.38, 95% CI [-0.43, -0.33], t (451) = -7.89, p < 0.001; 0 v 3: b = -0.61, 95% CI [-0.66, -0.56], t (451) = -12.63, p < 0.001; 1 v 3: b = -0.23, 95% CI [-0.28, -0.18], t (451) = -4.74, p < 0.001). These factors explained 27% of the variability in firing rate (R^2 = 0.27, F (4, 451) = 41.97, p < 0.001). Model NRo2 tested if alcohol moderated the effect of sex on firing rate but the interaction term of not significant (b = 0.02, 95% CI [-0.03, 0.07], t (450) = 0.44, p = 0.66) and did not pull any of the explained variability from the main effect of sex (b = -0.11, 95% CI [-0.16, -0.06], t (451) = -2.06, p = 0.04). Finally, Model NRo3 probed if alcohol moderated the effect of reward size. This did not result in any additional significant terms or an increase in

explained variance compared to Model NRo1 ($F(2, 449) = 1.32$, $p = 0.27$). Model comparisons for OFC's NR population can be found in Table 3.10. The responses of OFC NR neurons during the period of reward processing are shown in Figure 3.14.

Estrous cycle stage did not have an effect on baseline firing rate of OFC PP and PR neurons ($F(3, 26) = 0.18$, $p = 0.90$), nor did it effect OFC NP and NR neurons' baseline firing rate ($F(3, 46) = 0.26$, $p = 0.85$).

D. Discussion

In addition to being engaged at the time of lever press, the PP population of mPFC appears to also be capable of discriminating choice-related cues through the frequency of action potentials. In this paradigm, the risky lever was often encoded by greater augmentation of number of action potentials from baseline compared to the certain lever. Furthermore, the effect of actionable lever on firing rate became more pronounced when a portion of the variability in the term was removed by assimilating the interaction between adolescent alcohol use and lever pressed. The moderation had concealed the magnitude of selected lever's impact because increased alcohol consumption in adolescence was tied to a decrease in firing to the risky lever but not the certain (Figure 3.6). This change in neural activity is of interest because it pertains to the behavior seen during the probabilistic risk task. Akin to the behavior results, wherein increased alcohol was associated with an abatement in preference for the risky lever in both sexes, the neural activity in mPFC's PP population mimics this finding in the decrement of action potentials associated with the risky lever. Moreover, this espial infers that the favored lever is signaled by greater changes in activity from baseline in this population of neurons. The mPFC PP neurons in females had a superior change in magnitude of firing rate at the time of

press compared to males, however there was no interplay between sex and alcohol suggesting mPFC's PP neuron population is affected uniformly by alcohol regardless of sex.

Peculiarly, mPFC's NP population did not embody the inverse properties of PP neurons by encoding lever partiality through reductions in firing rate frequency compared to baseline. Instead an emerging trend indicated that the risky lever was associated with an inferior decrease in activity at the time of lever press compared to the certain lever. Albeit there was no significant interaction between previous alcohol use and lever interaction, visualization of the simple slopes indicates that increased alcohol use resulted in the overlap of encoding patterns for lever selected (Figure 3.7). This permits speculation that while mPFC's PP and NP neuron populations are active at the time of lever press, they are encoding different properties of the decision-behavior.

Upon superficial examination of PR neurons in mPFC, it's clear that this population of neurons is able to discriminate rewarded responses from unrewarded responses by positive changes in firing rate. However, further dissection of the interplay between reward size and alcohol unveils an alternate theory. Although not statistically significant, increased adolescent alcohol use results in a decrease in the rate-encoding of 3 sugar pellets relative to alcohol's moderation of the encoding of 1 sugar pellet (Figure 3.8). The addition of the interaction removed the variance in neural activity caused by alcohol, leading to the contrast between non-zero reward sizes being elevated to significance as well. This result and the graphical support suggest mPFC's PR neurons can ordinarily differentiate between reward sizes on rewarded and non-rewarded trials, but the calamitous properties of alcohol cause a reduction in the firing rate in response to 3 pellets so that it is encoded at the same frequency as 1 or 0 pellets. Strikingly, alcohol did not inhibit the ability to discriminate 1 pellet from 0. This indicates that prior binge

drinking (more than 1 g/kg of alcohol on average) results in the loss of ability to discriminate reward size while retaining the capability to determine if a decision was rewarded or not. However, extreme alcohol use (4 g/kg) produces a valuation bias toward the certain lever over either risky lever outcome, evocative of behavioral consequences of alcohol use seen in the probabilistic risk task.

Liken to mPFC's PR neurons, the NR population within mPFC possessed similar, yet inverse properties such as the ability to discriminate rewarded and non-rewarded trial outcomes by graded, reductions in relative firing rate. Furthermore, the relation between the ramifications of previous alcohol use on the encoding of no reward and maximal reward shows increased alcohol is associated with reduced abatement of firing to 3 sugar pellets relative to 0. This finding indicates that increased alcohol results in the encoding rate of 3 sugar pellets resembling that of 0 or 1 (Figure 3.9), giving further weight to the thesis that the reward encoding neurons in mPFC can usually discriminate between rewarded and unrewarded decisions and reward size within rewarded trials, but adolescent alcohol use hinders the adult brain from being able to properly encrypt reward size. Moreover, the activity in mPFC's reward encoding neurons provides additional insight to the alcohol-induced, certain lever bias that was observed in the probabilistic risk task. Due to the loss of ability to discriminate between non-zero reward size, but the preservation of the capacity to discern rewarded from unrewarded choices, a bias toward the certain lever could develop even when the risky lever is objectively more advantageous because the unrewarded trials are not associated with the certain lever.

None of the predicted factors had a discernible effect on the activity of the PP population in OFC at the time of lever press (Figure 3.10), but the NP population did exhibit compelling patterns of activation. Females had a preponderant, negative change in firing rate at the time of

lever selection compared to males. This finding is reminiscent of activity seen in the mPFC's PP cells, in that females' neurons in both groups generate a greater change in firing rate in the direction of activity that characterizes that population of neurons compared to males. The emerging negative effect of alcohol was dissipated by the interaction between adolescent alcohol use and selected lever but said interaction did cause the main effect of lever to become even stronger. Furthermore, the interrelationship suggests the negative impact of alcohol on firing rate that was previously seen was actually due to its negative moderation on the encoding of the risky lever more so than the certain (Figure 3.11). This interaction is meaningful because it indicates that increased alcohol use correlates with a decrease in firing at the time of risky lever press, so that the normalized change in firing rate more closely resembles that of the certain lever.

PR neurons in OFC were able to discriminate and encode reward size through graded increases in firing from baseline. However, adolescent alcohol use causes sexually dimorphic, long-lasting changes in this population's neural activity (Figure 3.12). In males, prior alcohol use was inversely related to the encoding of reward size (Figure 3.13A); as a result, there was hypoactivity during the reward period of non-zero rewards. This finding is similar to what was reported in McMurray et al., 2015 and supports the hypothesis that adolescent alcohol use disrupts OFC's dynamic reward encoding that is essential for goal-directed behavior. This effect was most prominent for large rewards associated with risk, but a similar, negative effect was imposed on the encoding of small, certain rewards as well. In females, juvenile alcohol use resulted in an overall positive effect on the encoding of reward size, thus there was no differing interactions between alcohol and specific reward values. However, alcohol had the most substantial, positive effect on small certain rewards (Figure 3.13B). Consequentially, under

control conditions, 1 sugar pellet was valued similarly to 0, but at higher levels of alcohol this relationship resulted in the encoding of 1 sugar pellet more closely resembling the encoding of 3 pellets. Another point of interest is the contrast between the sexes is in the encoding of 0 sugar pellets. Females appeared to exhibit increased firing for reward omissions compared to males whose firing rate did not increase from baseline when there was a lack of reward. In summary, the sexually divergent data suggests that alcohol causes hypoactivity and the inability to encode economic value of rewards in males; however, adolescent alcohol use in females results in only fixed, definite rewards increasing in value. Both scenarios result in an overlap in the encoded value of larger and smaller rewards, yielding similar risk-averse phenotypes.

The NR population of neurons in OFC had decreased activity in females compared to males, further emphasizing that females tend to have greater changes in activity in line with the direction of communication for that population. Additionally, reward size was negatively correlated with firing rate but no main effect or interactions involving alcohol, suggesting this population is distinctly different from OFC's PR population and might be protected from the toxic repercussions of alcohol (Figure 3.14).

There was no detectable effect of estrous cycle stage on baseline firing rate despite evidence that estrous cycle stage can alter dopamine signaling and thus cortical excitability (Morisette & Di Paolo, 1993; Pompili et al., 2010; Vandegrift, You, Satta, Brodie, & Lasek, 2017). However, upon visual inspection of the data there did appear to be a trend in all OFC neurons such that rats in metaestrus had a decreased firing rate compared to rats in diestrus, who had a decreased firing rate in relation to rats in proestrus, and rats in estrus had the highest baseline firing rate. This observation suggests that fluctuating female hormones may have a

graded effect on baseline cortical firing rate, but the present study was too underpowered to detect such an effect.

The multitude of observed main effects and interactions modeled in the neural data speaks to the complexity of prefrontal cortex's role in volition behavior and the extent of toxic assault it can endure.

Table 3.1

Summary of neuron distributions in mPFC and OFC.

	mPFC	**OFC**
Increase in activity during press (PP)	108	139
Decrease in activity during press (NP)	126	104
Increase in activity reward delivery (PR)	115	173
Decrease in activity reward delivery (NR)	163	152

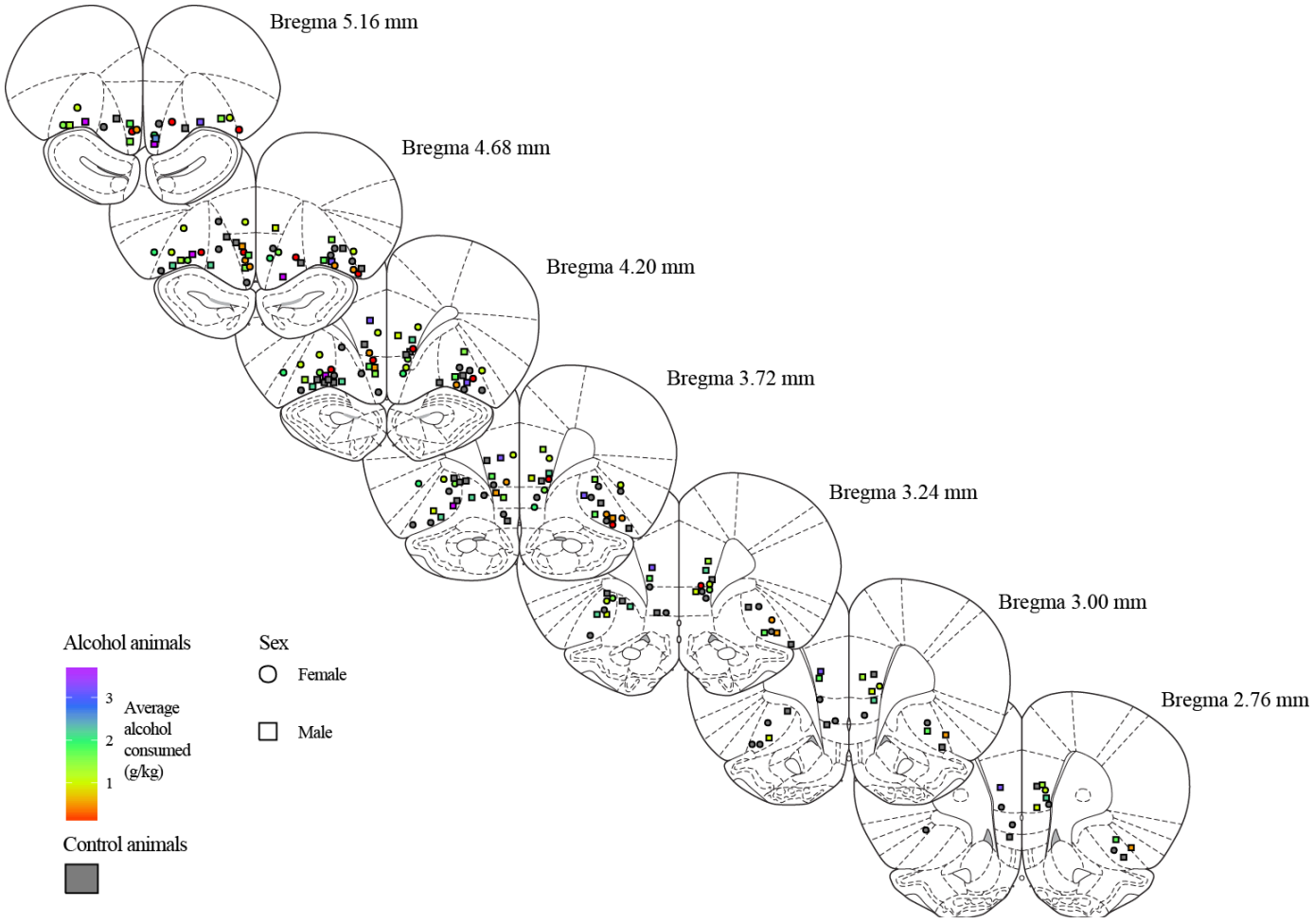


Figure 3.1 Histologically confirmed electrode placement in OFC and mPFC. The shape of label indicates what sex the wire was in and color indicates the average amount of alcohol consumed in adolescence.

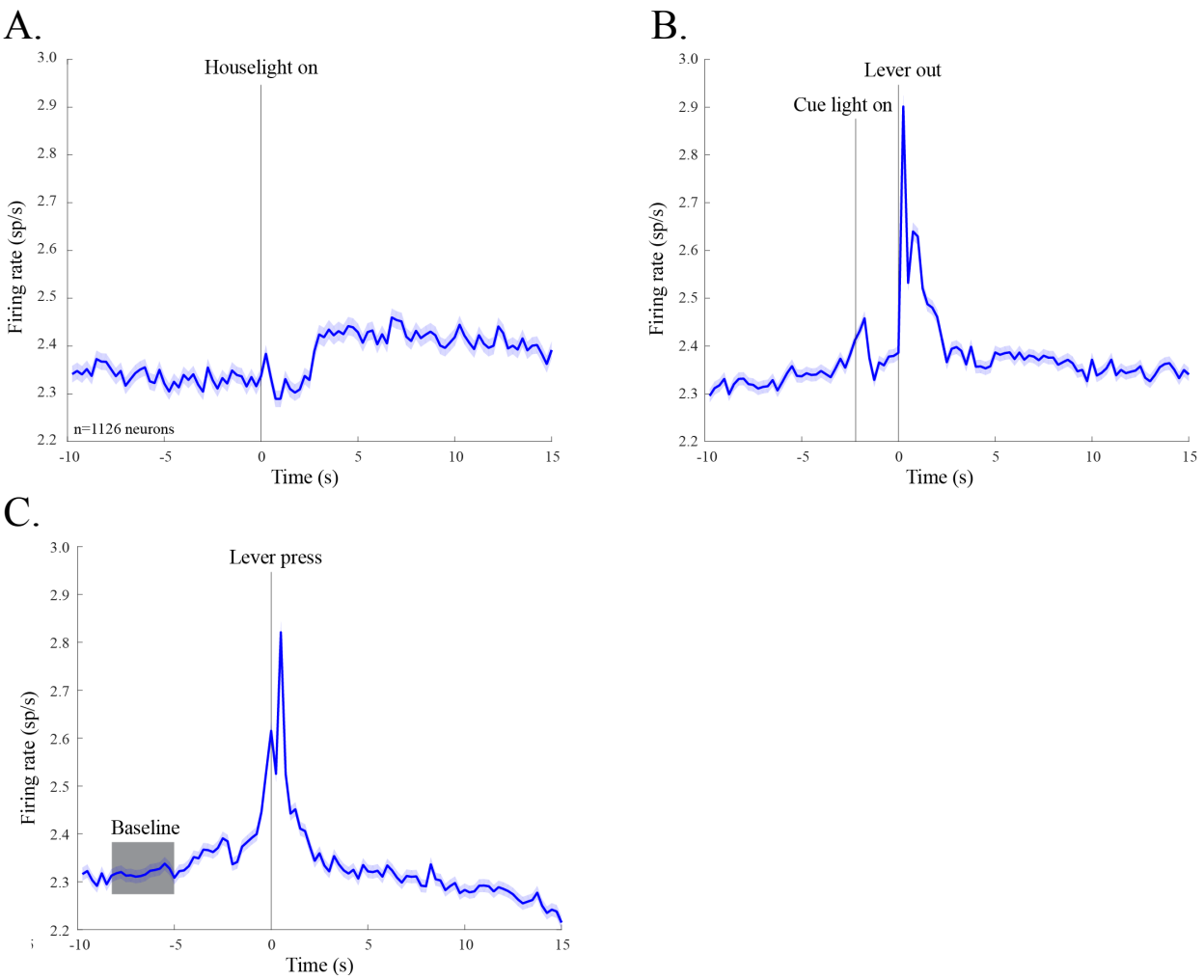


Figure 3.2 Average activity of all neurons aligned to external stimuli. (A) Neural activity aligned to the ignition of the house-light reveals no effect of the house-light on baseline activity. (B) Activity aligned to the extension of the levers shows a brief increase in activity at the time of trial initiation, denoted as the time the cue lights turned on. (C) Activity aligned to the time of lever press shows earlier environmental stimuli did not cause any fluctuations in baseline firing rate.

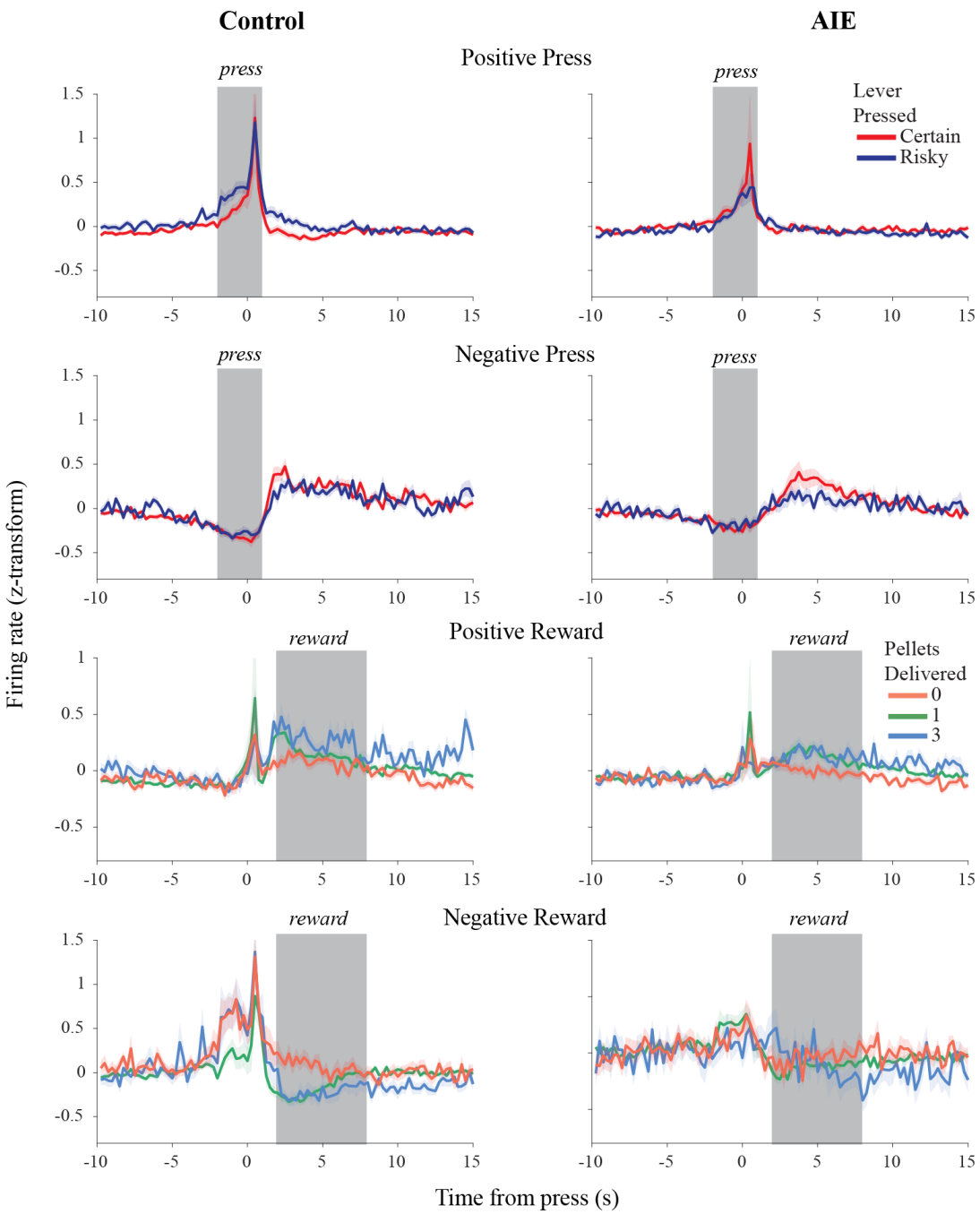


Figure 3.3 Time course of average neural activity in of four response types (PP, NP, PR, NR) in mPFC. Averages were calculated in 250ms bins aligned to time of lever press. Gray shaded regions indicate period of response that was compared to baseline. Neurons from control and AIE animals are presented separately. Color of line indicates the lever acted on or the outcome of the trial.

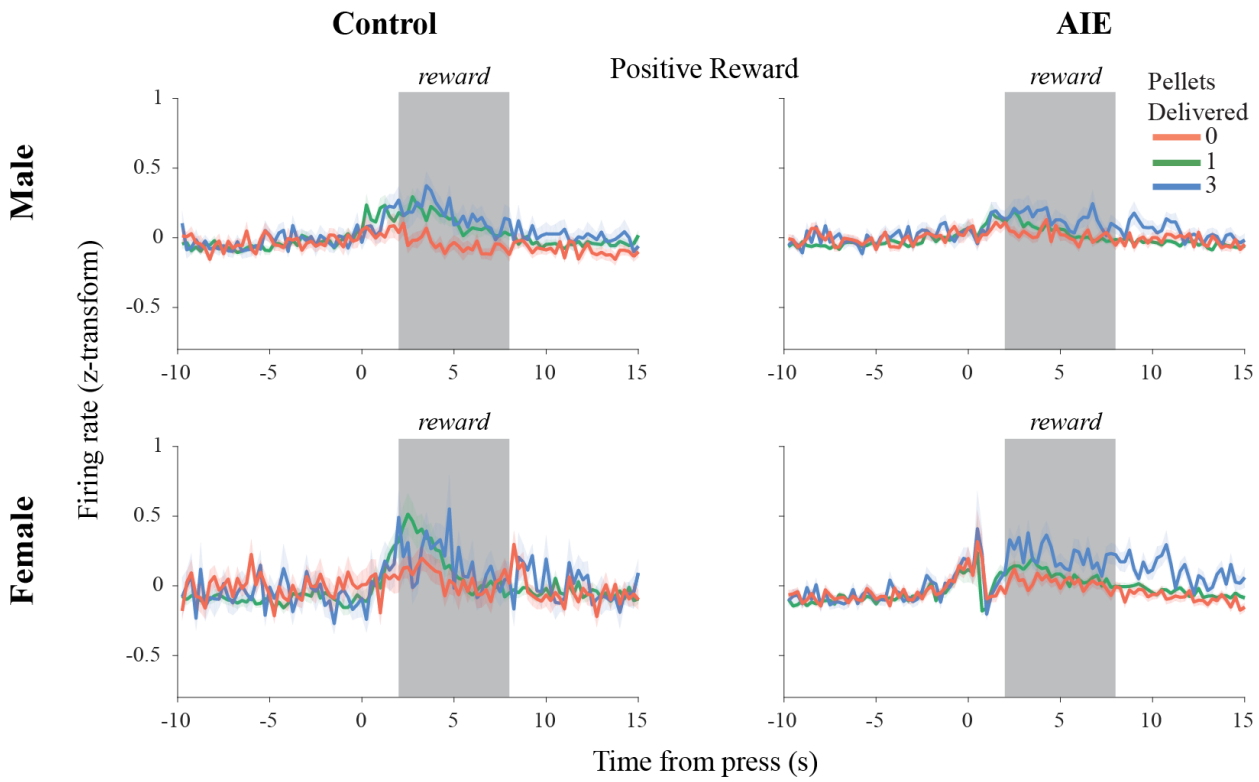


Figure 3.4 Time course of average neural activity of response type PP in OFC. Averages were calculated in 250ms bins aligned to time of lever press. Gray shaded regions indicate period of response that was compared to baseline. Neurons are presented separately based on sex and control and AIE condition. Color of line indicates the outcome of the trial.

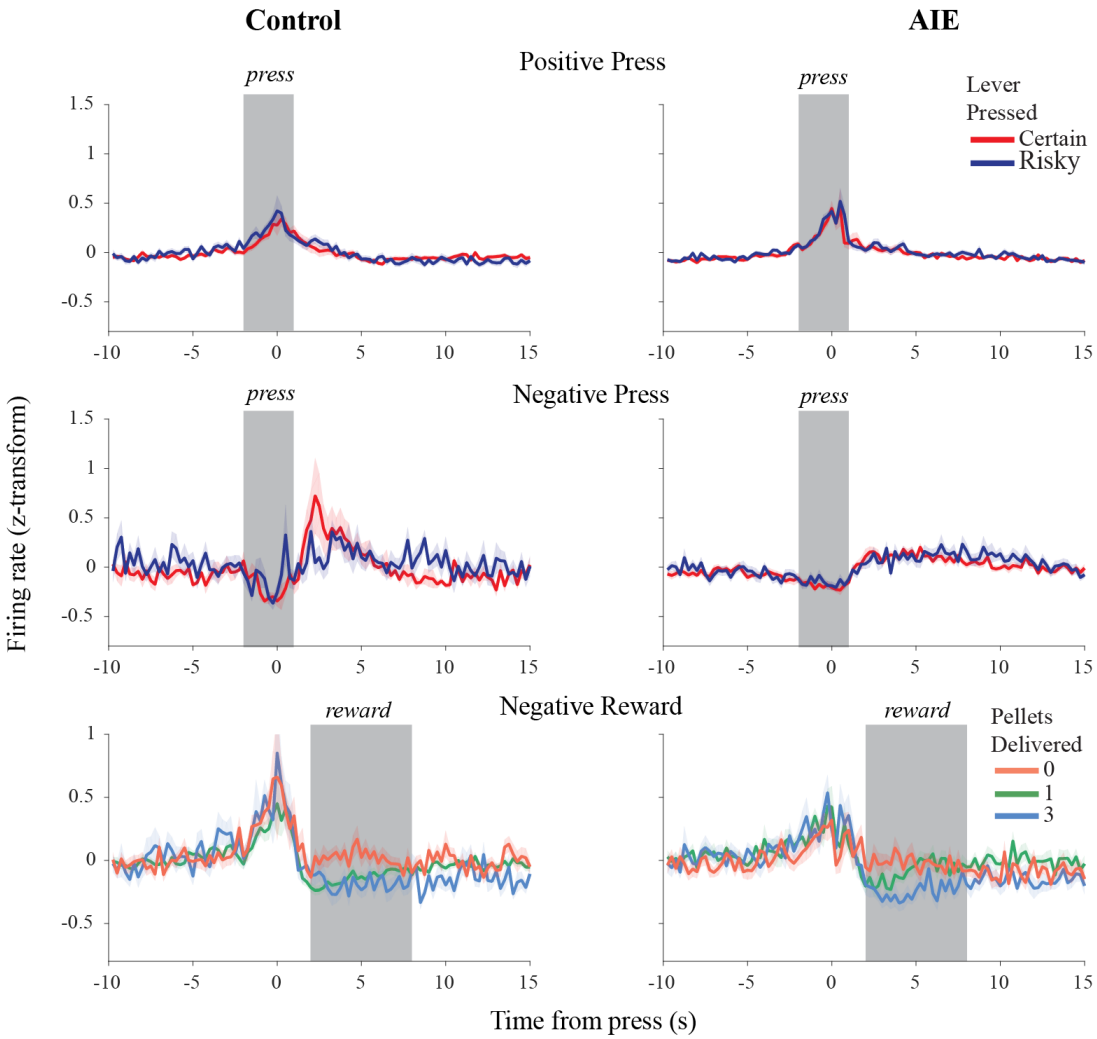


Figure 3.5 Time course of average neural activity in of three response types (PP, NP, NR) in OFC. Averages were calculated in 250ms bins aligned to time of lever press. Gray shaded regions indicate period of response that was compared to baseline. Neurons from control and AIE animals are presented separately. Color of line indicates the lever acted on or the outcome of the trial.

Table 3.2

Summary of hierarchical regression analysis for variables predicting change in firing rate during lever press in mPFC PP neurons.

	Model PPm1	Model PPm2	Model PPm3
Intercept	0.723*** (0.164)	0.648*** (0.183)	0.623*** (0.172)
Sex	0.386* (0.161)	0.49* (0.200)	0.386* (0.160)
Average EtOH	-0.090 (0.097)	-0.016 (0.126)	0.072 (0.130)
Lever Pressed	0.276^ (0.145)	0.276^ (0.145)	0.476** (0.180)
Average EtOH*Sex		-0.179 (0.196)	
Average EtOH*Lever Pressed			-0.325^ (0.174)
R^2	0.062	0.065	0.077
F	4.651	3.694	4.398
df_1	3	4	4
df_2	212	211	211
p	0.004	0.006	0.002

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

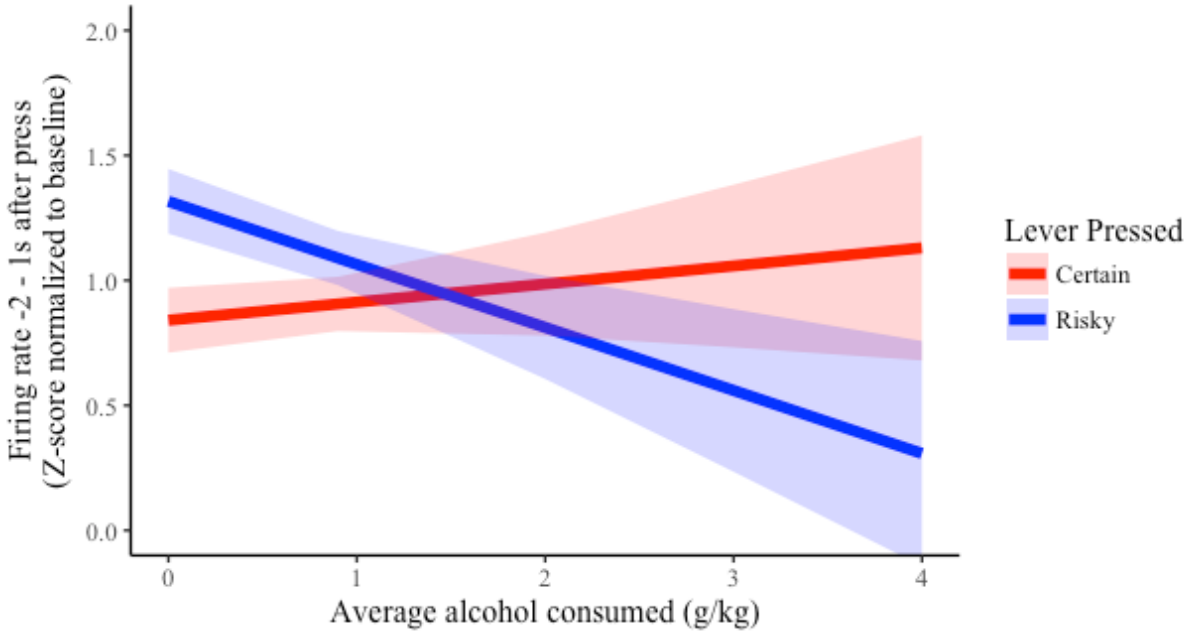


Figure 3.6 Simple slopes of acted on lever, moderated by alcohol consumed in adolescence, predicting firing rate at the time of lever press in mPFC's PP population.

Table 3.3

Summary of hierarchical regression analysis for variables predicting change in firing rate during lever press in mPFC NP neurons.

	Model NPm1	Model NPm2	Model NPm3
Intercept	-0.586*** (0.056)	-0.568*** (0.058)	-0.597*** (0.059)
Sex	0.018 (0.058)	-0.017 (0.066)	0.018 (0.058)
Average EtOH	0.042 (0.026)	0.030 (0.028)	0.055 (0.034)
Lever Pressed	0.092^ (0.050)	0.092^ (0.050)	0.115^ (0.063)
Average EtOH*Sex		0.084 (0.072)	
Average EtOH*Lever Pressed			-0.026 (0.043)
R^2	0.026	0.031	0.027
F	2.179	1.979	1.718
df_1	3	4	4
df_2	248	247	247
p	0.091	0.098	0.147

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

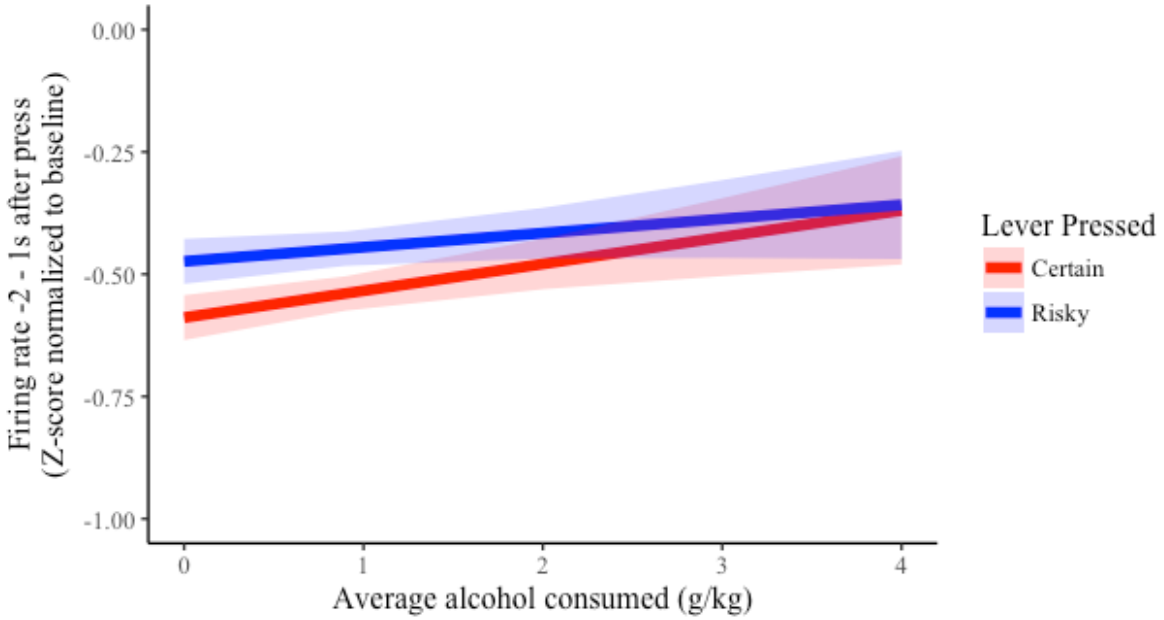


Figure 3.7 Simple slopes of acted on lever, moderated by alcohol consumed in adolescence, predicting firing rate at the time of lever press in mPFC's NP population.

Table 3.4

Summary of hierarchical regression analysis for variables predicting change in firing rate during the reward period in mPFC PR neurons.

	Model PRm1	Model PRm2	Model PRm3
Intercept	0.388*** (0.089)	0.417*** (0.100)	0.397*** (0.097)
Sex	-0.066 (0.078)	-0.107 (0.102)	-0.066 (0.078)
Average EtOH	-0.076 (0.046)	-0.101^ (0.061)	-0.088 (0.074)
Reward 0 v Reward 1	0.366*** (0.085)	0.366*** (0.085)	0.286* (0.111)
Reward 0 v Reward 3	0.496*** (0.085)	0.496*** (0.085)	0.550*** (0.111)
Reward 1 v Reward 3	0.130 (0.085)	0.130 (0.085)	0.264 * (0.111)
Average EtOH*Sex		0.058 (0.092)	
Average EtOH*Reward 0 v 1			0.111 (0.998)
Average EtOH*Reward 0 v 3			-0.076 (0.998)
Average EtOH*Reward 1 v 3			-0.187^ (0.100)
R^2	0.103	0.104	0.112
F	9.769	7.879	7.135
df_1	4	5	6
df_2	340	399	338
p	< 0.001	< 0.001	< 0.001

Note: ^ p < 0.1 * p < 0.05, ** p < 0.01; *** p < 0.001

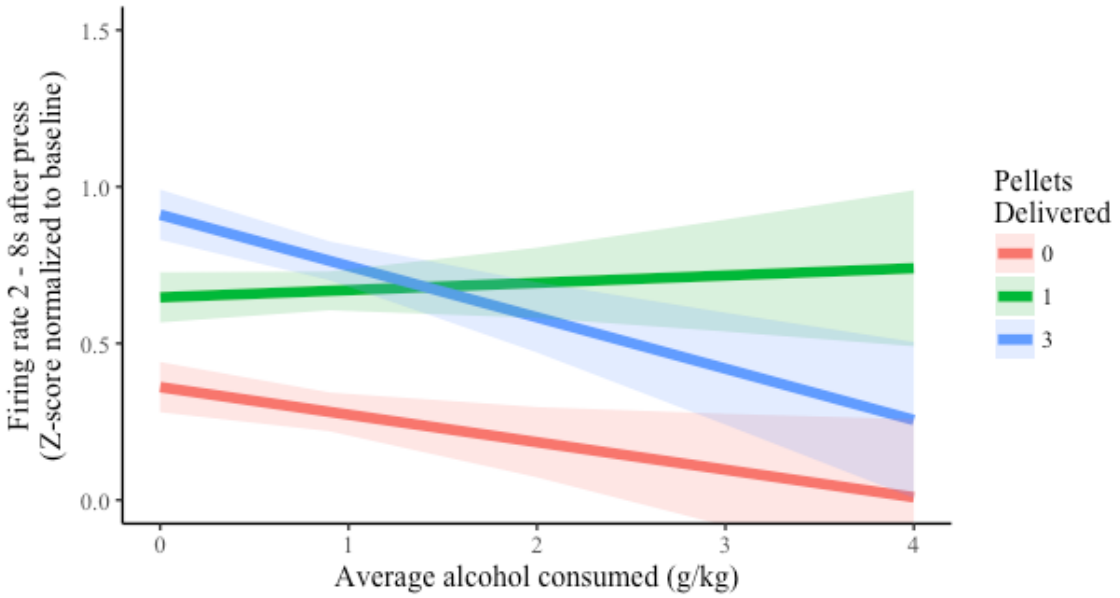


Figure 3.8 Simple slopes of reward size, moderated by alcohol consumed in adolescence, predicting firing rate during the reward period in mPFC's PR population.

Table 3.5

Summary of hierarchical regression analysis for variables predicting change in firing rate during the reward period in mPFC NR neurons.

	Model NRm1	Model NRm2	Model NRm3
Intercept	-0.130* (0.056)	-0.139* (0.058)	-0.083 (0.059)
Sex	0.036 (0.052)	0.053 (0.057)	0.036 (0.052)
Average EtOH	0.016 (0.024)	0.227 (0.023)	-0.052 (0.037)
Reward 0 v Reward 1	-0.310*** (0.054)	-0.310*** (0.054)	-0.355*** (0.063)
Reward 0 v Reward 3	-0.456*** (0.054)	-0.456*** (0.054)	-0.552*** (0.063)
Reward 1 v Reward 3	-0.146** (0.054)	-0.146** (0.054)	-0.197 ** (0.063)
Average EtOH*Sex		-0.047 (0.068)	
Average EtOH*Reward 0 v 1			0.065 (0.049)
Average EtOH*Reward 0 v 3			0.139** (0.049)
Average EtOH*Reward 1 v 3			0.073 (0.049)
R^2	0.135	0.136	0.149
F	18.88	15.19	14.09
df_1	4	5	6
df_2	484	483	482
p	< 0.001	< 0.001	< 0.001

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

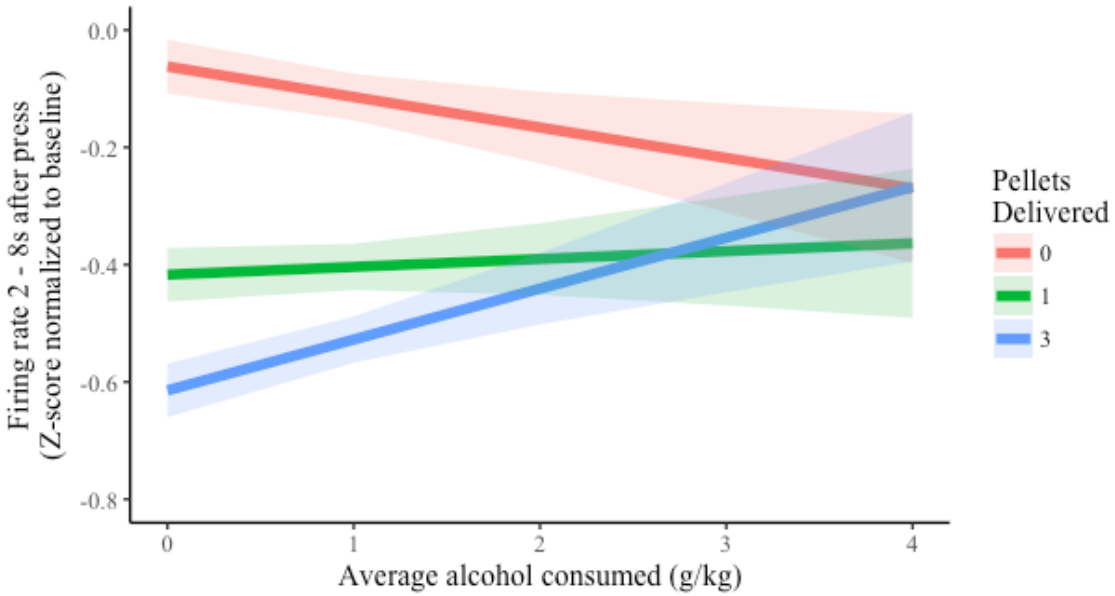


Figure 3.9 Simple slopes of reward size, moderated by alcohol consumed in adolescence, predicting firing rate during the reward period in mPFC's NR population.

Table 3.6

Summary of hierarchical regression analysis for variables predicting change in firing rate during lever press in OFC PP neurons.

	Model PPo1	Model PPo2	Model PPo3
Intercept	0.875*** (0.134)	0.890*** (0.140)	0.876*** (0.144)
Sex	0.107 (0.136)	0.071 (0.167)	0.107* (0.136)
Average EtOH	-0.065 (0.063)	-0.076 (0.070)	-0.066 (0.085)
Lever Pressed	0.140 (0.123)	0.140 (0.122)	0.137 (0.161)
Average EtOH*Sex		0.061 (0.162)	
Average EtOH*Lever Pressed			0.003 (0.114)
R^2	0.015	0.015	0.015
F	1.389	1.295	1.038
df_1	3	4	4
df_2	274	273	273
p	0.247	0.370	0.388

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

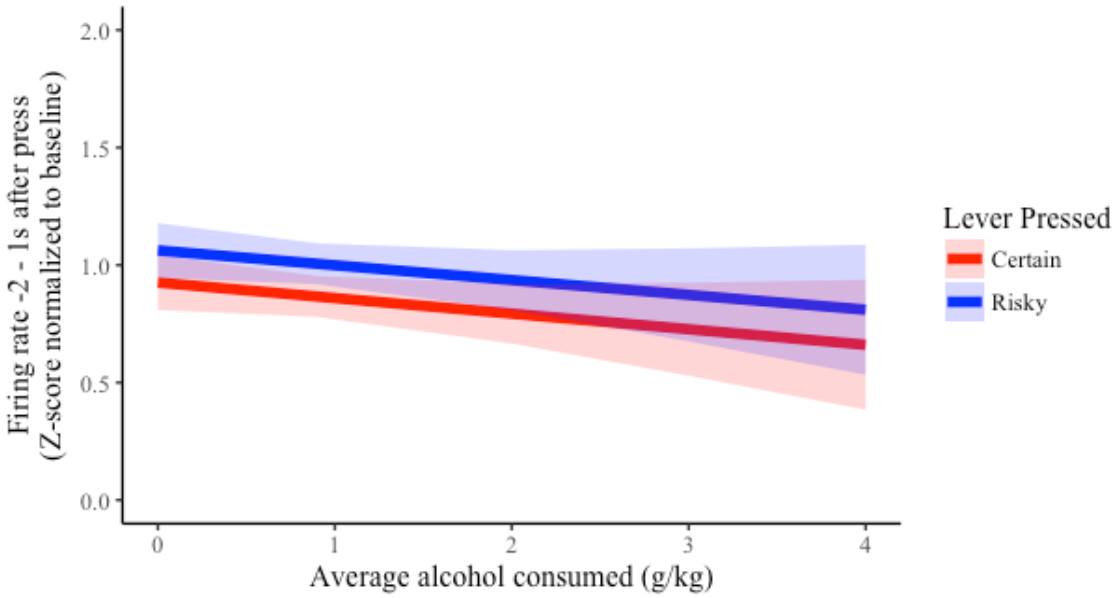


Figure 3.10 Simple slopes of activated lever, moderated by alcohol consumed in adolescence, predicting firing rate at the time of lever press in OFC's PP population.

Table 3.7

Summary of hierarchical regression analysis for variables predicting change in firing rate during lever press in OFC NP neurons.

	Model NPo1	Model NPo2	Model NPo3
Intercept	-0.412*** (0.62)	-0.428*** (0.067)	-0.455*** (0.067)
Sex	-0.115* (0.055)	-0.078 (0.079)	-0.115* (0.055)
Average EtOH	-0.044^ (0.022)	-0.035 (0.026)	-0.009 (0.030)
Lever Pressed	0.142** (0.049)	0.142** (0.049)	0.229** (0.071)
Average EtOH*Sex		-0.033 (0.051)	
Average EtOH*Lever Pressed			-0.069^ (0.041)
R^2	0.064	0.066	0.077
F	4.685	3.609	4.257
df_1	3	4	4
df_2	204	203	203
p	0.003	0.007	0.002

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

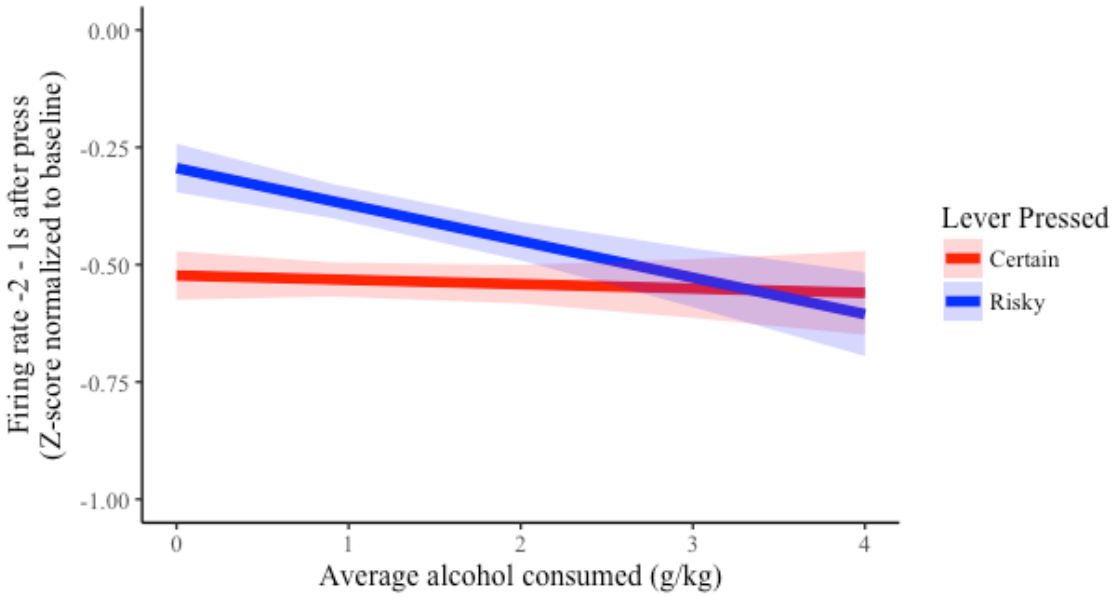


Figure 3.11 Simple slopes of activated lever, moderated by alcohol consumed in adolescence, predicting firing rate at the time of lever press in OFC's NP population.

Table 3.8

Summary of hierarchical regression analysis for variables predicting change in firing rate during the reward period in OFC PR neurons.

	Model PRo1	Model PRo2
Intercept	0.191* (0.076)	0.240** (0.079)
Sex	0.262*** (0.068)	0.097 (0.095)
Average EtOH	-0.018 (0.027)	-0.048 (0.030)
Reward 0 v Reward 1	0.272*** (0.078)	0.272*** (0.077)
Reward 0 v Reward 3	0.606*** (0.078)	0.606*** (0.077)
Reward 1 v Reward 3	0.334*** (0.078)	0.334*** (0.077)
Average EtOH*Sex		0.179* (0.073)
R^2	0.135	0.145
F	20.08	17.43
df_1	4	5
df_2	514	513
p	< 0.001	< 0.001

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

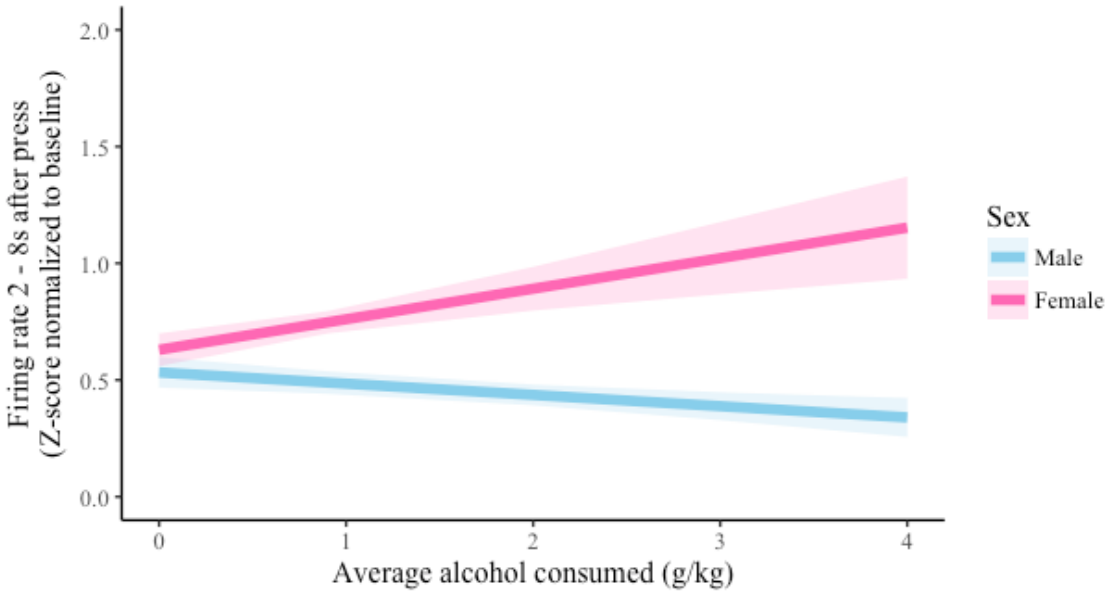


Figure 3.12 Simple slopes of sex, moderated by alcohol consumed in adolescence, predicting firing rate during the reward period in OC's PR population.

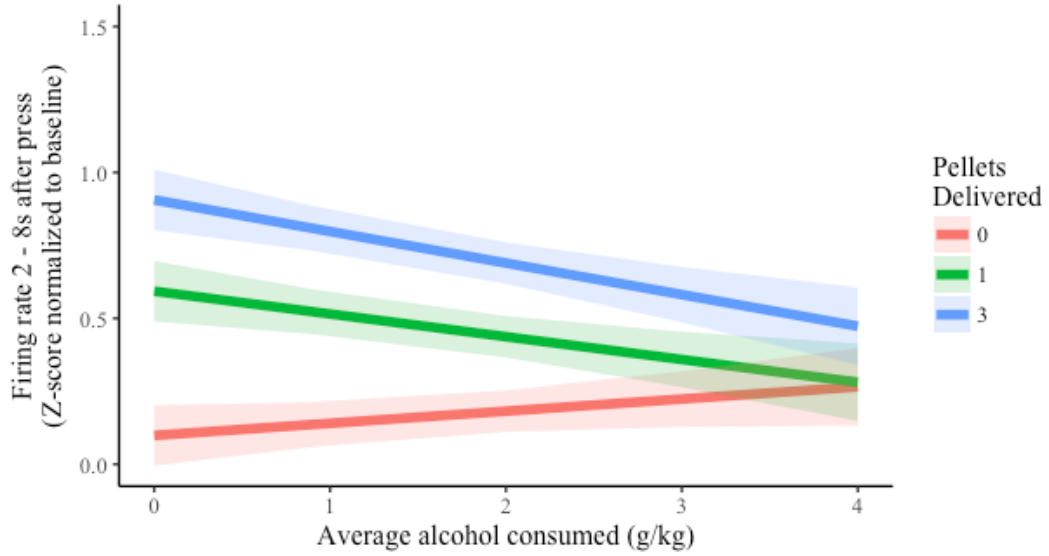
Table 3.9

Summary of hierarchical regression analysis for variables predicting change in firing rate during the reward period in OFC PR neurons.

	Males		*Females*	
	Model PRo.M1	Model PRo.M2	Model PRo.F1	Model PRo.F2
Intercept	0.244** (0.082)	0.099 (0.103)	0.331** (0.104)	0.393** (0.131)
Average EtOH	-0.048^ (0.028)	0.042 (0.048)	0.1131^ (0.072)	0.051 (0.124)
Reward 0 v Reward 1	0.300** (0.097)	0.494*** (0.146)	0.239* (0.124)	0.080 (0.185)
Reward 0 v Reward 3	0.564*** (0.097)	0.807*** (0.146)	0.656*** (0.124)	0.629*** (0.185)
Reward 1 v Reward 3	0.264*** (0.097)	0.313* (0.146)	0.417*** (0.124)	0.548** (0.185)
Average EtOH* Reward 0 v 1		-0.120^ (0.068)		0.203 (0.176)
Average EtOH* Reward 0 v 3		-0.150* (0.068)		0.035 (0.176)
Average EtOH* Reward 1 v 3		-0.030 (0.068)		-0.169 (0.176)
R^2	0.116	0.134	0.121	0.127
F	12.20	8.51	10.69	6.709
df_1	3	5	3	5
df_2	278	276	233	231
p	< 0.001	< 0.001	< 0.001	< 0.001

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

A.



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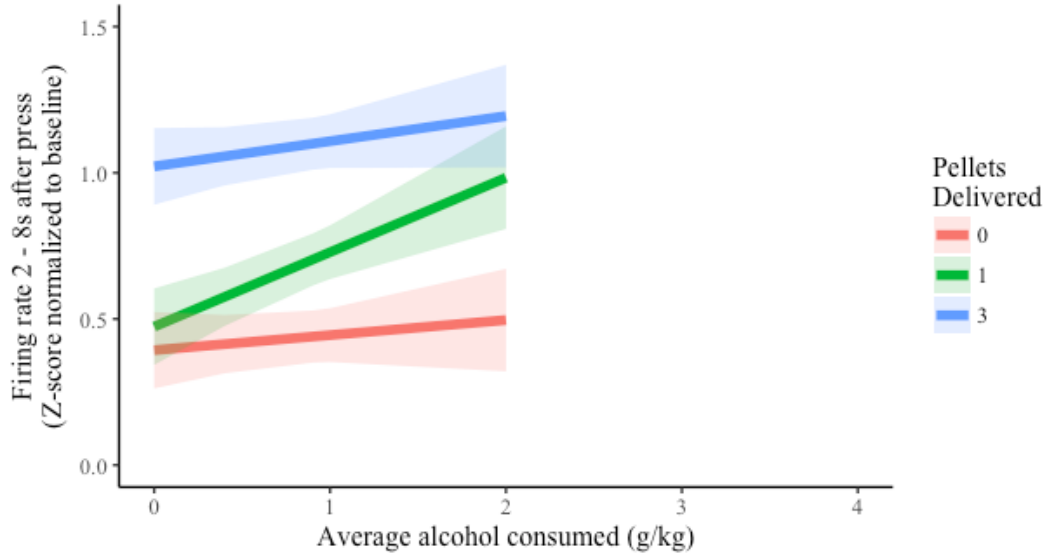

Figure 3.13 Simple slopes of reward size, moderated by alcohol consumed in adolescence, predicting firing rate during the reward period in OFC's PR population in (A) males and (B) females.

Table 3.10

Summary of hierarchical regression analysis for variables predicting change in firing rate during the reward period in OFC NR neurons.

	Model NRo1	Model NRo2	Model NRo3
Intercept	0.049 (0.047)	0.055 (0.049)	-0.084 (0.051)
Sex	-0.094* (0.042)	-0.107* (0.052)	-0.094* (0.042)
Average EtOH	-0.009 (0.020)	-0.015 (0.024)	-0.053 (0.034)
Reward 0 v Reward 1	-0.379*** (0.048)	-0.379*** (0.048)	-0.431*** (0.061)
Reward 0 v Reward 3	-0.607*** (0.048)	-0.607*** (0.048)	-0.660*** (0.061)
Reward 1 v Reward 3	-0.228** (0.048)	-0.228** (0.048)	-0.229 ** (0.061)
Average EtOH*Sex		-0.020 (0.045)	
Average EtOH*Reward 0 v 1			0.065 (0.047)
Average EtOH*Reward 0 v 3			0.065 (0.047)
Average EtOH*Reward 1 v 3			0.001 (0.047)
R^2	0.271	0.272	0.276
F	41.97	33.55	28.46
df_1	4	5	6
df_2	451	450	449
p	< 0.001	< 0.001	< 0.001

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

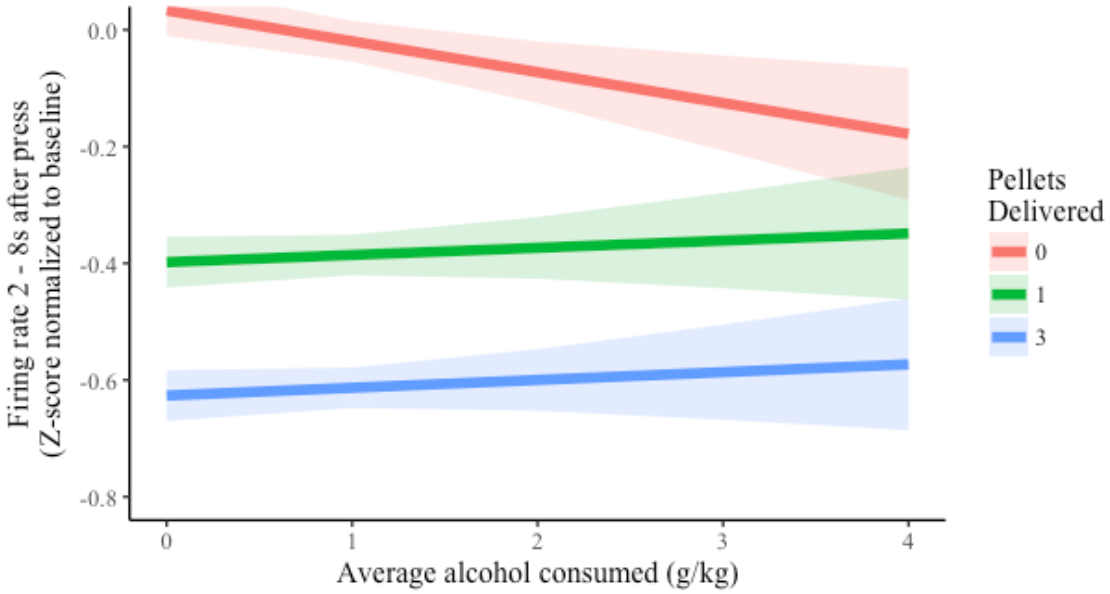

Figure 3.14 Simple slopes of reward size, moderated by alcohol consumed in adolescence, predicting firing rate during the reward period in OFC's NR population.

IV. CHAPTER 4: Concluding Discussion

A. Prospectus

Prefrontal cortex is essential for choice behaviors, especially when there is uncertainty in the outcome. Various sub-regions within PFC are active at different times during a decision and are thought to encode an assortment of facets needed for optimal behavior. In particular, mPFC has been implicated in guiding cue directed behaviors while OFC updates outcomes and reward values. Due to the caudal-dorsal pattern of brain maturation, PFC and its substructures experience active synaptic strengthening and pruning into early adulthood. The proper development of these structures is critical to their function but can be derailed by malignant insult, such as exposure to toxic substances. Unfortunately, this crucial period of cortical development typically coincides with onset of alcohol use. Furthermore, adolescents are more likely to engage in a repeated pattern of drinking that increases one's blood ethanol level to almost 1%, wreaking havoc on the developing brain. The purpose of this dissertation was to correlate the long-lasting effects of adolescent alcohol use on decision-making behavior and neural activity while concurrently examining the independent roles of mPFC and OFC in adult choice-behavior. Adolescent rats were given voluntary, intermittent access to alcohol in a gelatin medium over a period of time that is analogous with 13-20 years of age in humans. Once the rats reached adulthood, they performed a probabilistic risk task to assess how exposure to alcohol during development affected their extended decision-making behavior. Neural activity in OFC and mPFC was concurrently recorded as rats performed the behavioral task, enabling the correlation between environmental events and cellular activity to be examined. Alcohol use in adolescence results in a linear decrease in risk preference that indicated animals are no longer modulating their behavior in response to the changing parameters but are instead shifting their

preference towards a certain reward, even when it is disadvantageous. Additionally, increased alcohol use was associated with a diminished ability to differentially encode reward sizes in OFC and a shift in the neural activity of mPFC at the time of the choice. The results indicate that adolescent alcohol use alters the development of PFC sub-regions such that they are no longer able to properly function and thus can no longer promote advantageous choice-behavior when the outcome is uncertain.

B. Implications for frontal lobe development, choice behavior, and the effects of alcohol

Previous research stipulates that PFC is essential for advantageous decision-making when there is uncertainty in the outcome (Ming Hsu, Bhatt, Adolphs, Tranel, & Camerer, 2005; Orsini et al., 2015; St. Onge, Stopper, Zahm, & Floresco, 2012) and adolescent alcohol use compromises the function of PFC and subsequent choice behavior (Clark et al., 2012; McMurray et al., 2014; N. a Nasrallah et al., 2009). The work outlined in this thesis supports the neurofunctional findings in the published literature and elaborates on the uniqueness of substructures within PFC as it pertains to their roles in signaling various aspects of decisions. Additionally, their singularity is emphasized by alcohol's heterogeneous effects on neuronal sub-populations' utilities.

Female OFC neurons appeared to indicate the subjective value of the reward. This was seen as 1 sugar pellet being encoded similarly to 0 in controls who had a greater preference for the larger, riskier rewards, and 1 sugar pellet being encoded similarly to 3 in alcohol rats who had a greater preference for small, certain rewards. This finding is similar to other studies which have documented changes in OFC firing rates in response to the subjective value of risky rewards (Roitman & Roitman, 2010). Although, the change in encoding and lever preference

seen in the results were also correlated to alcohol use, so it cannot be definitively concluded that this change in neural activity was solely due to subjective preference. It is also likely that some neurons in OFC are simultaneously signaling the probability of reward delivery through changes in firing rate (Burke & Tobler, 2011). Unfortunately, we were unable to tease this effect from the data.

While OFC appears to be signaling subjective reward value, mPFC's activation during lever press and the reward period are likely working in concert to direct future behavior. Lesions to mice mPFC result in the mice being unable to adjust behavior as reward contingencies change (Laskowski et al., 2016), providing a possible explanation for the persistent behavior seen in alcohol animals as the task cycled through the different session probabilities.

mPFC and OFC contained populations of neurons that responded similarly to overlapping facets, suggesting theses subpopulations might project cumulative information to the same downstream targets. However, the differential impact of adolescent alcohol use on adult mPFC and OFC activity may provide further insight into PFC's extended development. Alcohol appeared to more severely affect the encoding of cues related to uncertainty than those that corresponded to known outcomes. This was illustrated by the negative correlation between alcohol and activity at the time of risky lever press in mPFC's PP population, alcohol-induced hypoactivity to large, risky rewards in mPFC's PR and NR populations, and a greater decrease in firing rate in OFC's NP population's response at the time of risky lever press. Interpreted together with the behavior from sessions that had no uncertainty in outcome (i.e. magnitude discrimination and extinction), suggests there are other structures or sub-populations of neurons that can generate advantageous behavior when the outcome is certain and are not as susceptible

to the deleterious effects of alcohol because their vulnerable period of maturation concluded before the time of alcohol use.

It was apparent that there was a divergence in male and female OFC PR neurons' ability to encode rewards and how alcohol differentially effects their integrity. Alcohol-induced disinhibition observed in female OFC PR neurons suggests that the inhibitory properties of these cells did not fully develop and perhaps were not completely integrated, whereas in males a graded decrease in activity indicates suppression of overall activity. Slightly different trajectories of frontal lobe maturation combined with variable alcohol consumption patterns could explain this disparate neural activity. Alcohol is thought to disrupt the dynamic reorganization of PFC leading to an imbalance in glutamatergic and GABAergic input. Mechanisms underlying this irregularity include alterations in GABAα expression and reduced GABAα tonic currents in mPFC (Centanni, Burnett, Trantham-Davidson, & Chandler, 2017), disruption of microglia-dependent synaptic pruning, premature extracellular protein infrastructure formation (Coleman et al., 2014; Costa et al., 2007), and derangement of neurotransmitter-guided synaptic stabilization (F. T. Crews et al., 2016). Interestingly, despite the sexually dimorphic OFC PR activity, the behavioral phenotypes were the same across sexes. This is to say that while OFC's encoding of rewards is critical for idyllic behavior, there must be other components driving the sexes toward similar decisions. VTA and NAc are also involved in reward-directed behaviors and project axons to PFC. However, these structures reach their adult form earlier than PFC, implicating them as the common factor. Furthermore, AIE has been shown to alter tonic dopamine signaling in response to risky rewards (N. A. Nasrallah et al., 2011), suggesting OFC's inability to assess reward size may lead to VTA dopamine neurons producing an inaccurate reward prediction error (Fiorillo, Tobler, & Schultz, 2003). This further

highlights the wide spread impact of alcohol and stresses the complexity of its long-term effects on the brain's reward circuitry.

Additionally, sex differences in the presence of extracellular dopamine (Riccardi et al., 2011) and expression of cortical dopamine receptors (Kaasinen, Någren, Hietala, Farde, & Rinne, 2001) could offer and explanation as to why females tended to exhibit greater changes in cortical activity from baseline compared to males. This also supports the growing literature surrounding sex differences in the dopamine system.

Alcohol-induced hypoactivity observed in reward encoding neurons was reminiscent of previous studies (McMurray et al., 2015), although the risk preference behavior associated with prior alcohol use contradicted what has been reported (Clark et al., 2012; McMurray et al., 2014; N. A. Nasrallah et al., 2011). However, a mutual feature between studies was the apparent lack of adaptive behavior between sessions, suggesting an integral source of information needed for the decision has been lost. A feasible rationale underlying the inconsistency in behavior is alcohol is acting on OFC value-coding neurons similarly by dampening the differences in firing rate that are critical for appraising and communicating rewards. The failure to discriminate reward size leads to an inability to communicate updated value information that is essential for optimal behavior under uncertainty. mPFC's decision-guiding properties likely play a role in determining which direction the behavior will skew toward after loss of reward valuation. Activity in mPFC at lever press showed a transition in dominant activity that was correlated with lever preference, indicating it is still guiding behavior at the time of the decision. Thus, the lack of ability to encode rewards can manifest as an array of behavioral deficits depending on other contextual factors like length of training, order of probabilities, structure of behavioral sessions, and number of behavioral sessions.

C. Future directions

As with the saying "as one door closes, two more open", the conclusion of this project brings forth the prospect of many future directions.

Additional studies can focus on modifying and further examining voluntary alcohol consumption behavior to confirm the translational value of this model. It would be of interest to examine if different patterns of alcohol consumption (i.e. one large binge upon initial access or small, periodic samplings across the access period) correlate with behavior on the risk task and alcohol challenges in adulthood. It is possible that one, large binge causes an accelerated rise in BEL compared to smaller, staggered patterns of alcohol intake. A large spike in BEL could overwhelm the brain with alcohol, resulting in more pronounced damage and leading to more severe long-term effects on behavior. Additionally, while there was no effect of control gelatin on behavior, some studies have reported altered behavioral phenotypes in adulthood following high levels of childhood sugar intake (Carwile et al., 2015; Imamura et al., 2015). It would be interesting to repeat the study with a compound that does not contain high quantities of a sweetened carbohydrate while also masking the bitter flavor of the alcohol. This would produce a true control and might elicit increased alcohol intake.

Modifications to the behavioral task could also be made to assess how alcohol alters the subjective value of other rewards. A similar probabilistic task design utilizing intracranial self-stimulation (ICSS) would provide an alternative to using sugar pellets as a reward. In an ICSS paradigm the current elicited upon press of the risky lever would be the most pleasurable to the rat but would only be delivered probabilistically, whereas a press on the certain lever would always deliver a current that was a third the strength of the optimal current. Along with the elimination of sugar as a reward, PFC's ability to evaluate non-physical rewards could also be

evaluated. One challenge of this proposed paradigm would be the possibility of the ICSS causing too much electrical noise that would disrupt the neural recording process. Another proposed variation of the task would give rats the choice between sugar pellets or timed access to alcoholic gelatin. This would allow further evaluation of how adolescent alcohol use changes the valuation of alcohol in adulthood.

An alternative direction for future experiments would be to concentrate on the mechanism of action underlying alcohol-induced disruption of PFC activity and reward encoding. It is known that extracellular matrices, also known as perineuronal nets (PNN) develop around GABAergic interneurons (Costa et al., 2007; Lasek, 2016) during adolescence and play a role in finalizing synaptic connections. However, the development of PNNs throughout adolescence and the trafficking of various involved proteins has not been well studied. There is evidence that AIE alters the expression of PNN-associated proteins in OFC (Coleman et al., 2014), prompting further investigation into the role of PNNs during development as well as alcohol's effect on them. This could provide insight as to how adolescent alcohol use alters neural activity in PFC. The effect of alcohol on the mesocortical system could also be examined in greater detail in an effort to discern how the areas within this circuit work together to estimate probability, assess rewards, and how alcohol causes these properties to go awry. It is well documented the PFC and VTA are capable of estimating the frequency of reward and signaling its value (Büchel et al., 2017; Ferenczi et al., 2016; Fiorillo et al., 2003; Ishikawa et al., 2008; Mayberg, 1990; Schoenbaum et al., 1998; Schultz et al., 1992; St Onge & Floresco, 2010). Additionally PFC, VTA, and NAc send reciprocal axons and these areas' functions can be disrupted with alcohol use (Boutros et al., 2015; Pascual et al., 2009; L. P. Spear, 2016; Trantham-Davidson et al., 2016). By deciphering how alcohol affects one brain region, and how

the disruption of that area alters downstream signaling, it's possible to develop a more harmonious picture of neuroeconomics and the brain's reward circuitry.

Finally, additional effort could be directed toward discerning more of the electrophysical properties of PFC neurons. This could include further examination of OFC and mPFC reward-encoding neurons during a probabilistic risk task to evaluate how firing rates change as a factor of the probability of reward delivery. Alternatively, activity in cue directive neurons in mPFC can be correlated with individuals' behavior in an effort to relate neural activity with lever preference.

D. Significance for the prevention and treatment of alcohol use disorders

People who start drinking before the age of 15 are four times more likely to develop alcohol use disorder (AUD) at some point in their life than those who wait until they are of legal age (Grant & Dawson, 1998). While social circumstances and demographics play a substantial role in the initiation of alcohol use and subsequent AUD development, the research presented in this dissertation begs the question - does alcohol-altered reward encoding promote the enhanced neuroeconomic value of alcohol and thus contribute to later AUD? The answer is likely more complicated than a binary yes or no, but introduces a more fascinating observation; why are some individuals more protected from the long-term effects of adolescent alcohol use and associated increased AUD incidence than others? The answer may lie in the genetics of African Americans and Asians, who are less likely to drink than their Native American and White counterparts (Hamburg & Sharfstein, 2009). Alternatively, a higher cognitive baseline could provide a protective quality or mask the resulting alcohol-deficits. The best way to prevent escalating alcohol use and extended cognitive deficiencies is preventative cognitive behavioral

therapy targeted toward children that fit the criteria for being at-risk for early alcohol use or AUD.

Alas, while preventative, proactive treatments are idyllic it is unlikely they will ever be globally utilized, contributing to the ongoing problem at hand. Thus, using other substances in conjunction with alcohol may reduce drinking or protect the neuroanatomy of PFC from alcohol's deleterious effects. If atypical formation of PNN are responsible for altered neural activity, a therapeutic designed to inhibit their formation or increase their plasticity could be taken simultaneously with alcohol to rescue proper synapse formation. However, a drug of this nature would undoubtedly have unintended side effects on the integrity of synapses and extracellular proteins in other brain regions, suggesting this would not be an ideal solution. Additionally, CB_1 receptor availability and binding has been implicated in stress induced alcohol seeking in mice (Racz et al., 2003) and alcohol dependence in humans (Hirvonen et al., 2013). This presents the endocannabinoid system as another potential therapeutic target for reducing alcohol seeking and consumption. LSD, although illegal and not readily available, has also shown promise in reducing long-term alcohol seeking in humans with AUD (Krebs & Johansen, 2012). Finally, while alcohol may inhibit athletic performance, exercise may reduce some of alcohol's harmful effects on the brain and liver (El-Sayed, Ali, & Ali, 2005).

Retroactive therapeutics may also be harnessed to alleviate some of the resulting consequences of adolescent alcohol use. RNA therapy targeted to promote transcription of genes related to axon growth and synapse formation could reinstate neural connections that were inhibited by alcohol use and remedy any functional deficits. Another solution might be to act on other structures, like VTA, to try to rescue the ambiguous reward signal and thus the behavioral phenotype (Schindler, Soden, Zweifel, & Clark, 2016). Non-pharmacological techniques such as

trans-magnetic cranial stimulation (TMS) could be utilized to reverse hypo-frontality exhibited by reward encoding neurons. If this therapy were successful in lessening the effects of adolescent alcohol use, it could have additional implications in the treatment of other psychiatric disorders that involve maladaptive decision-making and dysfunctional cortical activity.

The continued study of adolescent brain development and its hindrance due to substance use is crucial. This line of research provides insight into the mechanisms underlying typical PFC maturation and function and affords clarity as to how alterations to its integrity can drastically affect long-term advantageous behavior. The latter of which may also aide in the understanding and treatment of addiction and other psychiatric disorders that onset during adolescence.

V. Cited Literature

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VI. Appendix

Table 6.1

Summary of mixed linear regression analysis for variables predicting change in firing rate during lever press in mPFC PP neurons.

	Model PPm1	Model PPm2	Model PPm3
Fixed Effects			
Intercept	0.723*** (0.177)	0.648*** (0.199)	0.623*** (0.182)
Sex	0.386* (0.182)	0.494* (0.225)	0.386* (0.182)
Average EtOH	-0.090 (0.109)	-0.016 (0.142)	0.072 (0.130)
Lever Pressed	0.276* (0.120)	0.276* (0.120)	0.476** (0.146)
Average EtOH*Sex		-0.179 (0.221)	
Average EtOH*Lever Pressed			-0.325* (0.141)
Random Effect			
N group	108	108	108
Observations	216	216	216

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

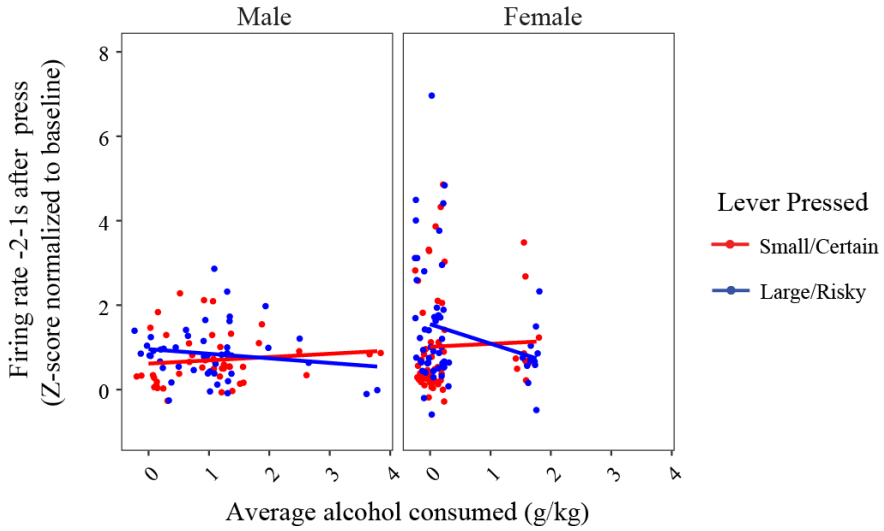
Table 6.2

Summary of mixed linear regression analysis for variables predicting change in firing rate during lever press in mPFC NP neurons.

	Model NPm1	Model NPm2	Model NPm3
Fixed Effects			
Intercept	-0.586*** (0.056)	-0.568*** (0.058)	-0.597*** (0.059)
Sex	0.018 (0.060)	-0.017 (0.067)	0.018 (0.060)
Average EtOH	0.042 (0.026)	0.030 (0.028)	0.055 (0.033)
Lever Pressed	0.092^ (0.047)	0.092^ (0.047)	0.115^ (0.060)
Average EtOH*Sex		0.084 (0.074)	
Average EtOH*Lever Pressed			-0.026 (0.041)
Random Effect			
N group	126	126	126
Observations	252	252	252

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

A.



B.

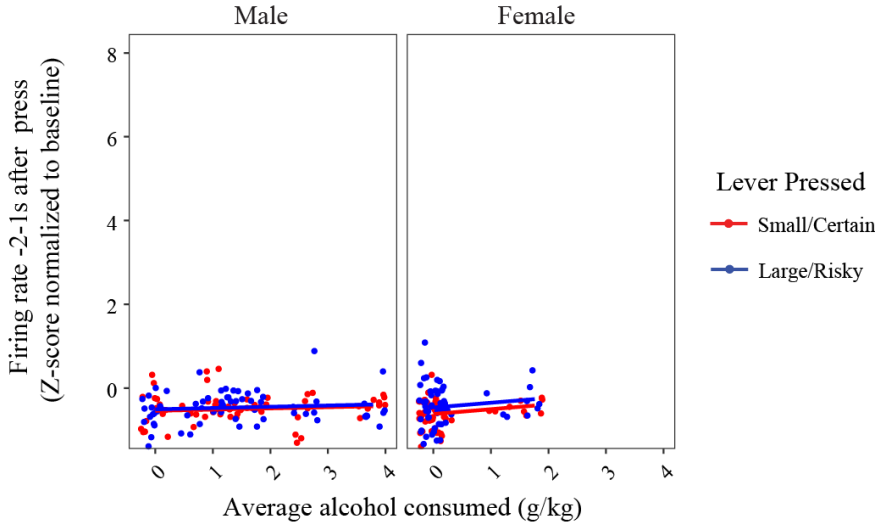


Figure 6.1 Patterns of neural activity in mPFC at the time of lever press as a factor of alcohol consumed in adolescence and lever pressed. (A) Change in normalized firing rate from baseline in neurons that on average exhibited an increase in firing rate at the time of lever press. (B) Change in normalized firing rate from baseline in neurons that on average exhibited a decrease in firing rate at the time of lever press.

Table 6.3

Summary of mixed linear regression analysis for variables predicting change in firing rate during the reward period in mPFC PR neurons.

	Model PRm1	Model PRm2	Model PRm3
Fixed Effects			
Intercept	0.388*** (0.099)	0.417*** (0.114)	0.397*** (0.105)
Sex	-0.066 (0.095)	-0.107 (0.124)	-0.066 (0.095)
Average EtOH	-0.076 (0.056)	-0.102 (0.074)	-0.088 (0.074)
Reward 0 v Reward 1	0.366*** (0.073)	0.366*** (0.073)	0.286** (0.095)
Reward 0 v Reward 3	0.496*** (0.073)	0.496*** (0.073)	0.550*** (0.095)
Reward 1 v Reward 3	0.130^ (0.073)	0.130^ (0.073)	0.264** (0.095)
Average EtOH*Sex		0.058 (0.112)	
Average EtOH*Reward 0 v 1			0.111 (0.085)
Average EtOH*Reward 0 v 3			-0.076 (0.085)
Average EtOH*Reward 1 v 3			-0.187* (0.085)
Random Effects			
N group	115	115	115
Observations	345	345	345

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

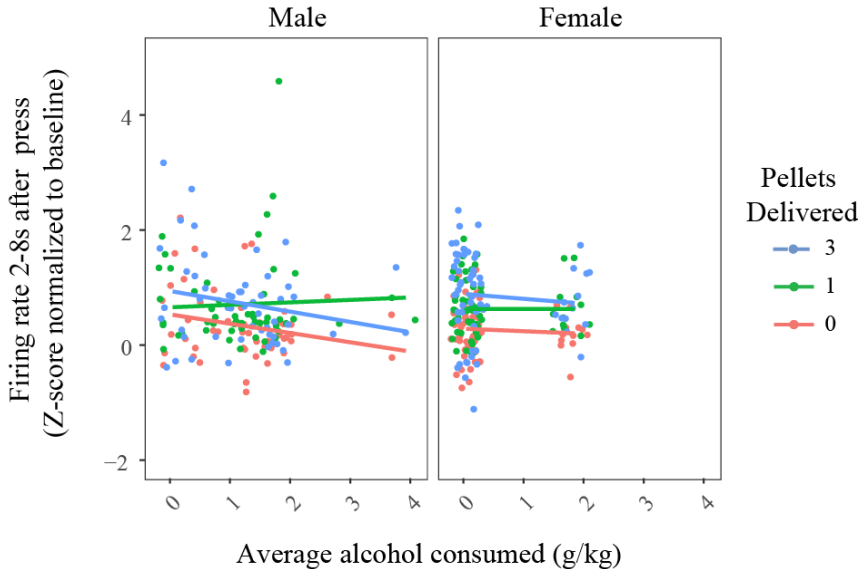
Table 6.4

Summary of mixed linear regression analysis for variables predicting change in firing rate during the reward period in mPFC NR neurons.

	Model NRm1	Model NRm2	Model NRm3
Fixed Effects			
Intercept	-0.130 * (0.060)	-0.139* (0.062)	-0.083 (0.063)
Sex	0.036 (0.060)	-0.053 (0.066)	0.036 (0.061)
Average EtOH	0.016 (0.027)	-0.023 (0.029)	-0.052 (0.037)
Reward 0 v Reward 1	-0.310*** (0.049)	-0.310*** (0.049)	-0.355** (0.057)
Reward 0 v Reward 3	-0.456*** (0.049)	-0.456*** (0.049)	-0.552*** (0.057)
Reward 1 v Reward 3	-0.146** (0.049)	-0.146**(0.049)	-0.197***(0.057)
Average EtOH*Sex		-0.047 (0.078)	
Average EtOH*Reward 0 v 1			0.065 (0.044)
Average EtOH*Reward 0 v 3			0.139** (0.044)
Average EtOH*Reward 1 v 3			0.073^ (0.044)
Random Effects			
N group	163	163	163
Observations	489	489	489

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

A.



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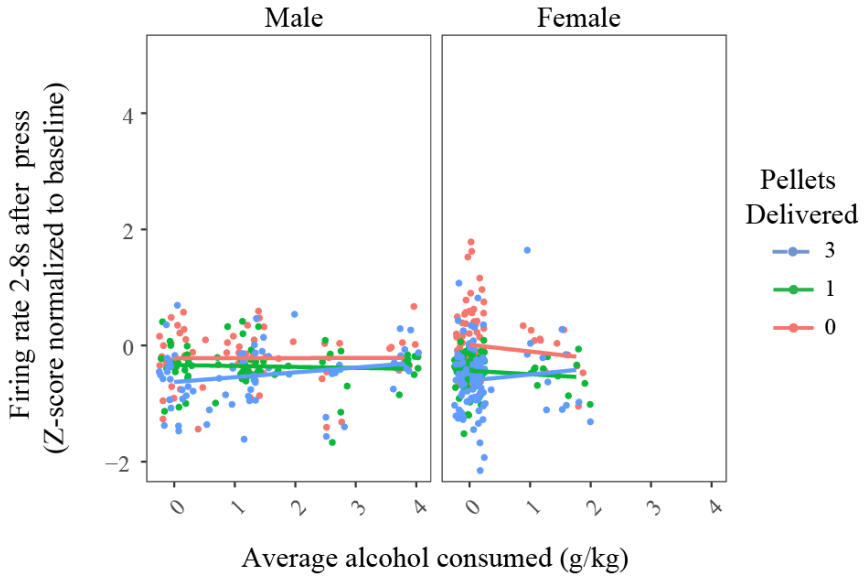


Figure 6.2 Patterns of neural activity in mPFC during the reward period as a factor of alcohol consumed in adolescence and lever pressed. (A) Change in normalized firing rate from baseline in neurons that on average exhibited an increase in firing rate during the reward period. (B) Change in normalized firing rate from baseline in neurons that on average exhibited a decrease in firing rate during the reward period.

Table 6.5

Summary of mixed linear regression analysis for variables predicting change in firing rate during lever press in OFC PP neurons.

	Model PPo1	Model PPo2	Model PPo3
Fixed Effects			
Intercept	0.875*** (0.159)	0.890*** (0.167)	0.876*** (0.161)
Sex	0.107 (0.178)	0.071 (0.217)	0.106 (0.178)
Average EtOH	-0.065 (0.082)	-0.076 (0.091)	-0.066 (0.087)
Lever Pressed	0.140* (0.063)	0.140* (0.063)	0.137^ (0.082)
Average EtOH*Sex		0.060 (0.211)	
Average EtOH*Lever Pressed			0.003 (0.058)
Random Effect			
N group	139	139	139
Observations	278	278	278

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

Table 6.6

Summary of mixed linear regression analysis for variables predicting change in firing rate during lever press in OFC NP neurons.

	Model NPo1	Model NPo2	Model NPo3
Fixed Effects			
Intercept	-0.412*** (0.063)	-0.428*** (0.068)	-0.455*** (0.067)
Sex	-0.115* (0.056)	-0.078 (0.081)	-0.115* (0.056)
Average EtOH	-0.044^ (0.023)	-0.035 (0.027)	-0.009 (0.030)
Lever Pressed	0.142** (0.047)	0.142** (0.047)	0.229** (0.067)
Average EtOH*Sex		-0.033 (0.052)	
Average EtOH*Lever Pressed			-0.069^ (0.038)
Random Effect			
N group	104	104	104
Observations	208	208	208

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

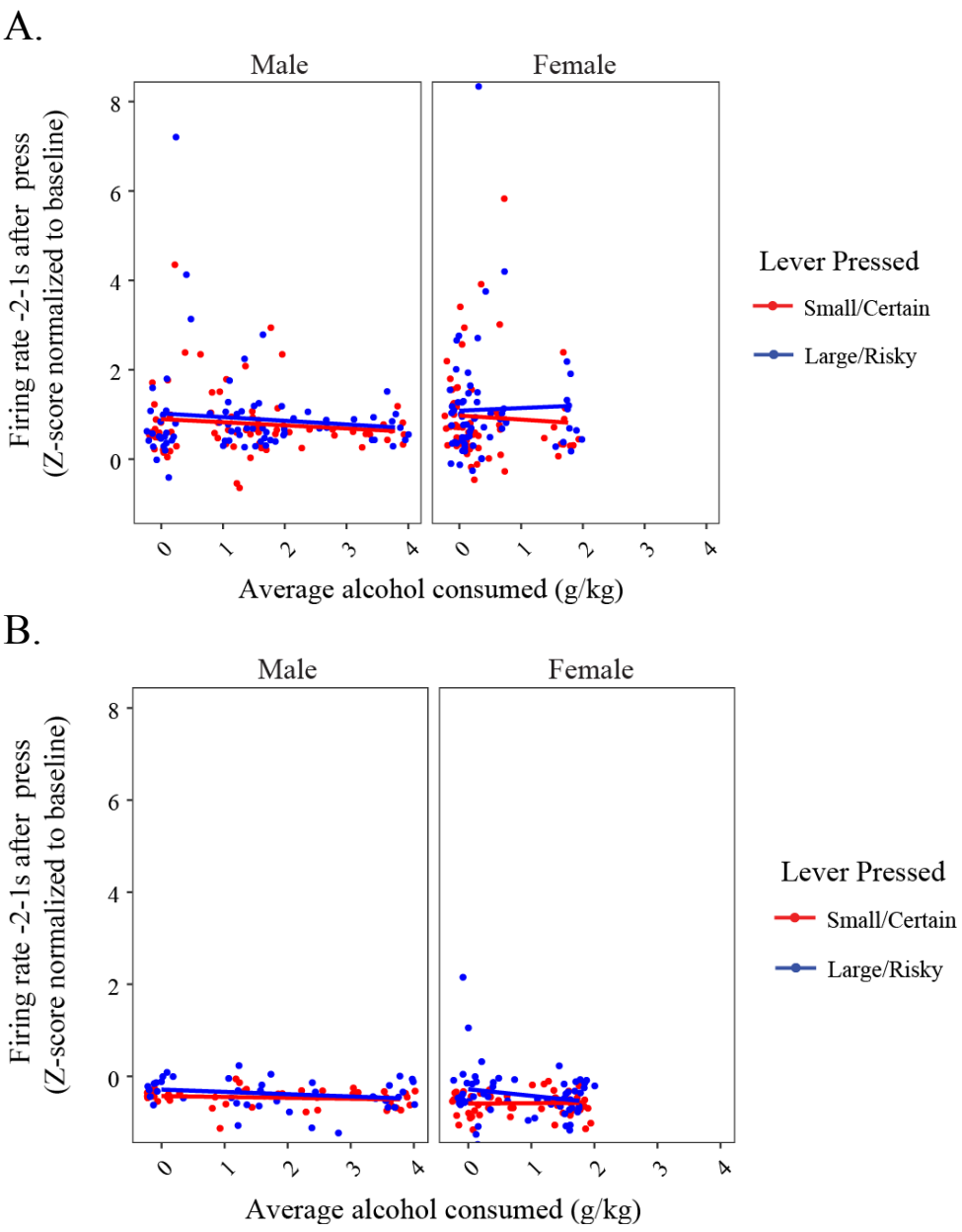


Figure 6.3 Patterns of neural activity in OPFC at the time of lever press as a factor of alcohol consumed in adolescence and lever pressed. (A) Change in normalized firing rate from baseline in neurons that on average exhibited an increase in firing rate at the time of lever press. (B) Change in normalized firing rate from baseline in neurons that on average exhibited a decrease in firing rate at the time of lever press.

Table 6.7

Summary of mixed linear regression analysis for variables predicting change in firing rate during the reward period in OFC PR neurons.

	Model PRo1	Model PRo2
Fixed Effects		
Intercept	0.191* (0.085)	0.240** (0.088)
Sex	0.262** (0.083)	0.097 (0.116)
Average EtOH	-0.018 (0.034)	-0.048 (0.036)
Reward 0 v Reward 1	0.272*** (0.067)	0.272*** (0.067)
Reward 0 v Reward 3	0.606*** (0.067)	0.606*** (0.067)
Reward 1 v Reward 3	0.334*** (0.067)	0.334*** (0.067)
Average EtOH*Sex		0.179* (0.089)
Random Effect		
N group	173	173
Observations	519	519

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

Table 6.8

Summary of mixed linear regression analysis for variables predicting change in firing rate during the reward period in OFC PR neurons continued.

	Males		*Females*	
	Model PRo.M1	Model PRo.M2	Model PRo.F1	Model PRo.F2
Fixed Effects				
Intercept	0.244** (0.087)	0.099 (0.102)	0.331** (0.111)	0.393** (0.129)
Average EtOH	-0.048 (0.033)	0.042 (0.047)	0.131 (0.089)	0.051 (0.123)
Reward 0 v Reward 1	0.300*** (0.085)	0.494*** (0.126)	0.239* (0.104)	0.080 (0.155)
Reward 0 v Reward 3	0.564*** (0.085)	0.807*** (0.126)	0.656*** (0.104)	0.629*** (0.155)
Reward 1 v Reward 3	0.264*** (0.085)	0.313*(0.126)	0.417 *** (0.104)	0.548*** (0.155)
Average EtOH* Reward 0 v 1		-0.120* (0.058)		0.203 (0.146)
Average EtOH* Reward 0 v 3		-0.150* (0.058)		0.035 (0.147)
Average EtOH* Reward 1 v 3		-0.030 (0.058)		-0.169 (0.147)
Random Effect				
N group	94	94	79	79
Observations	282	282	273	273

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

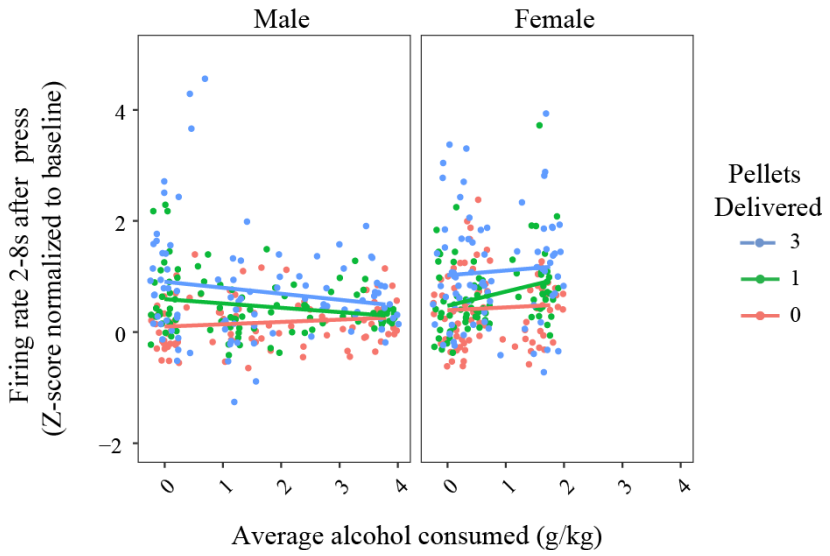
Table 6.9

Summary of hierarchical regression analysis for variables predicting change in firing rate during the reward period in OFC NR neurons.

	Model NRo1	Model NRo2	Model NRo3
Fixed Effects			
Intercept	0.049 (0.051)	0.055 (0.054)	-0.084 (0.054)
Sex	-0.094^ (0.049)	-0.107^ (0.061)	-0.094^ (0.049)
Average EtOH	-0.009 (0.024)	-0.015 (0.028)	-0.053 (0.034)
Reward 0 v Reward 1	-0.379*** (0.043)	-0.379*** (0.043)	-0.431*** (0.054)
Reward 0 v Reward 3	-0.607*** (0.043)	-0.607*** (0.043)	-0.660*** (0.054)
Reward 1 v Reward 3	-0.228** (0.043)	-0.228** (0.043)	-0.229 *** (0.054)
Average EtOH*Sex		-0.020 (0.053)	
Average EtOH*Reward 0 v 1			0.065 (0.041)
Average EtOH*Reward 0 v 3			0.066 (0.041)
Average EtOH*Reward 1 v 3			0.001 (0.041)
Random Effect			
N group	152	152	152
Observations	456	456	456

Note: ^ $p < 0.1$ * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$

A.



B.

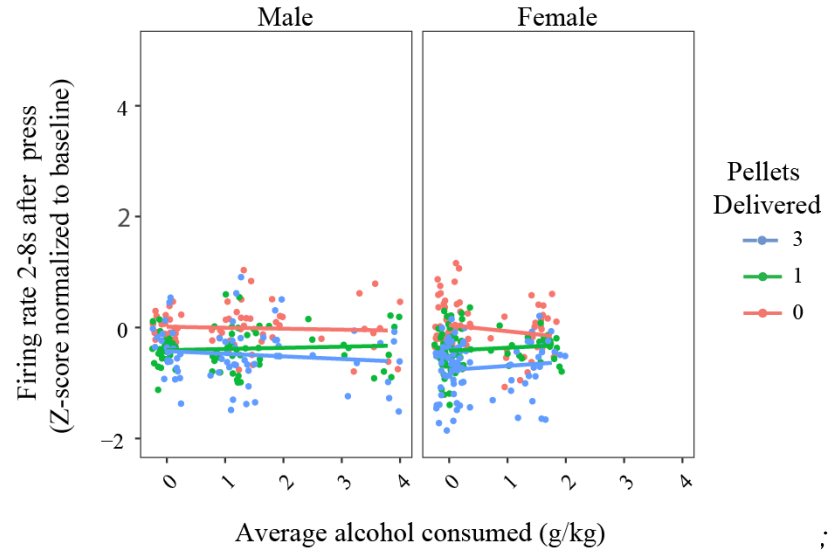


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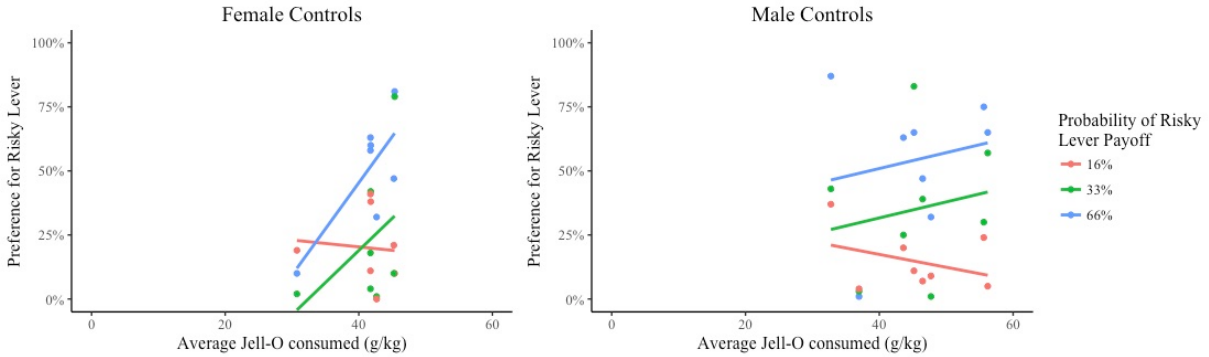
Figure 6.4 Patterns of neural activity in OFC during the reward period as a factor of alcohol consumed in adolescence and lever pressed. (A) Change in normalized firing rate from baseline in neurons that on average exhibited an increase in firing rate during the reward period. (B) Change in normalized firing rate from baseline in neurons that on average exhibited a decrease in firing rate during the reward period.

A.



B.

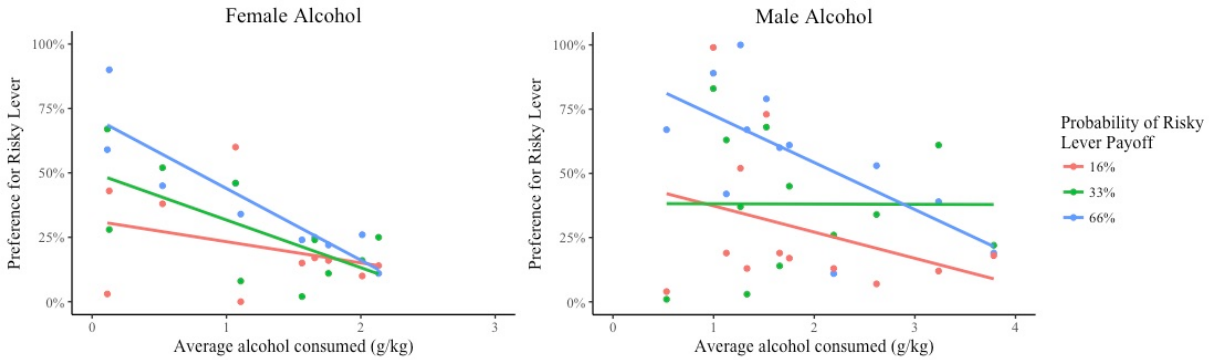


Figure 6.5 Simple slopes of the probability of risky lever payoff as a predictor of preference for the risky lever. (A) Control animals' performance on the probabilistic risk task as predicted by probability of risky lever payoff and gelatin consumed in adolescence. (B) Alcohol animals' performance on the probabilistic risk task as predicted by probability of risky lever payoff and prior alcohol use.

VII.Curriculum Vitae

Samantha D. Corwin

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EDUCATION

University of Illinois at Chicago — Chicago, IL
Ph.D., Neuroscience — September 2014- Present

University of California, Irvine — Irvine, CA
B.S., Biology — September 2010- June 2014

University of Sussex — Brighton, United Kingdom
June 2012- September 2012

RESEARCH EXPERIENCE

University of Illinois at Chicago — Chicago, IL

J. Roitman Laboratory — August 2015- Present
Dissertation Research

- Evaluated the long-term effects of adolescent alcohol use on decision-making in adulthood.
- Catechized the effects of alcohol on prefrontal cortex activity as they relate to the encoding of decision-related variables.

S. Lagenecker Laboratory — June 2015- August 2015
Research Rotation

- Analyzed resting state connectivity changes in relation to impulsivity and childhood abuse.
- Processed and built comprehensive first and second level fMRI models.

J. Larson Laboratory — September 2014- May 2015
Research Rotation

- Examined Fragile X hippocampal plasticity using in-slice electrophysiology.
- Conducted probabilistic reversal learning experiments in Fragile X using an odor discrimination paradigm.

University of California, Irvine — Irvine, CA

C. Gall Laboratory — September 2011- June 2014
Undergraduate Research Assistant

- Rebuilt computer animated 3D models of dendrites.
- Studied and the sub-anatomy of the hippocampus and their involvement in memory encoding.

Summer Research Fellowship — June 2013- September 2014

- Examined the effects of an enriched environment on neural activity in Fragile X using c-Fos.

AWARDS AND HONORS:

Laboratory of Integrative Neuroscience Travel Award 2018
Award that included travel funds to attend and present at a scientific conference.

Department of Psychology Travel Award 2016, 2017, & 2018
Merit-based award that included travel funds to attend and present at a scientific conference.

Undergraduate Summer Research Opportunities Fellowship 2013
Stipend that supported my individual project studying activated neural networks in an animal model of autism.

Order of Omega Honors Award 2014
Recognition for achieving and maintaining a GPA of 3.5 or higher.

PUBLICATIONS

Corwin, S.D., Roitman, J.D. (IN PREPARATION) Voluntary alcohol consumption in adolescent rats is affected by behavior of peers.

Quinn, M.E., Stange, J.P., Jenkins, L.M., **Corwin, S.**, DelDonno, S.R., Bessette, K.L., Welsh, R.C., Langenecker, S.A. (2018) Cognitive Control and Network Disruption in Remitted Depression: A Correlate of Childhood Adversity. *Social, Cognitive, and Affective Neuroscience* 1-10

Jenkins, L.M., Stange, J., Bessette, K.L., Chang, Y-S., **Corwin, S.D.**, Skerrett, K., Patrón, V.G., Zubieta, J-K., Crane, N.A., Passarotti, A., Pine, D.S. & Langenecker, S.A. (2018). Differential engagement of cognitive control regions and subgenual cingulate based on presence or absence of comorbid anxiety with depression. *Journal of Affective Disorders,* 241: 371-380

Corwin, S.D. (2017). Plyr Package. A Language, not a Letter: Learning Statistics in R. Retrieved from https://ademos.people.uic.edu/Chapter5.html

Cox C.D., Palmer L.C., Rex C.S., Babayan A.H., Pham D.T., **Corwin S.D.**, Trieu B.H., Gall C.M., Lynch G. (2014). A Map of LTP-Related Synaptic Changes in Dorsal Hippocampus Following Unsupervised Learning. The Journal of Neuroscience 34(8):3033-3041.

PRESENTATIONS AND ACADEMIC TALKS

Corwin, S.D., Roitman, J.D. Adolescent alcohol use alters encoding of decision-related variables in prefrontal cortex. Poster presented at: Annual Scientific Meeting of the Research Society on Alcoholism; 2018 June 16-20; San Diego, CA.

Patel, B.N., **Corwin, S.D.,** Jacobs-Brichford, E. The long-term effects of concerted alcohol and cannabinoid use on decision-making. Poster presented at: 4th Annual UIC Psychology's Cross-Program Conference; 2018 March 16; Chicago, IL.

"Adolescent alcohol use results in altered risk taking in adulthood" Laboratory of Integrative Neuroscience Symposium, University of Illinois, Chicago. 2017

Corwin, S.D., Roitman, J.D. Adolescent alcohol exposure alters neural encoding in OFC and mPFC affecting decision-making behavior. Poster presented at: Annual Scientific Meeting of the Research Society on Alcoholism; 2017 June 24-28; Denver, CO.

Corwin, S.D., Jacobs-Brichford, E., Roitman, J.D. Social influence on voluntary adolescent alcohol consumption: effects on risk preference and dopamine receptor expression. Poster presented at Society for Neuroscience's Annual Meeting; 2016 November 12-16; San Diego, CA.

Corwin, S.D., Roitman, J.D. Adolescent Alcohol Use Increases Risk Preference and Alters Dopamine Receptor Expression in OFC. Poster session presented at: Center for Alcohol Related Epigenetics 1st Annual Retreat; 2016 March 31; Chicago, IL

Corwin, S.D., Skerrett, K.A., Jenkins, L.M., Barba, A., Kreutzer, K., Hymen, E., Dion, C., Marshall, D., Passarotti, A., Langenecker, S.A. Childhood Trauma Alters Cognitive Control in PGNG Task and RDLPFC Resting State Connectivity. Poster session presented at: UIC's 6th Annual Research Extravaganza; 2015 September 16; Chicago, IL

Palmer L.C., Cox C.D., Pham D.T., **Corwin S.D.**, Hong B.S., Gall C.M., Lynch G. Methamphetamine has discrete effects on previously enriched rats placed in a novel, complex environment. Poster session presented at: Society for Neuroscience's Annual Meeting; 2013 November 9-13; San Diego, CA

Corwin S.D., Gall C.M., Lynch G. Memory Maps in the Hippocampus. Poster Presented at UCI's 20th Annual Undergraduate Research Opportunities Program Symposium; 2013 May 18; Irvine, CA

TEACHING EXPERIENCE

University of Illinois at Chicago Chicago, IL
Teaching Assistant
Abnormal Psychology: Fall 2015
Behavioral Neuroscience: Fall 2016
Behavioral Neuroscience Lab: Fall 2016, Fall 2017, Spring 2018
Cognitive Neuroscience: Spring 2016, Spring 2017
Cognitive Neuroscience Lab: Fall 2018

MENTORING

University of Illinois at Chicago Chicago, IL
Direct Supervisor of Undergraduate Student Researchers
J. Roitman Lab: 6 students

University of California, Irvine Irvine, CA
Undergraduate Student Researcher Trainer
C. Gall Lab: 3 students

WORK EXPERIENCE

Aspire Capital Chicago, IL

Biotechnology Analyst Intern September 2017 – June 2018

- Conducted diligence reviews on public, mid-cap, clinical-stage biotechnology companies to determine investment opportunities.
- Critically evaluated relevant scientific, clinical, and financial information to determine technological risk and potential investment return of lead product candidates.
- Presented detailed investment analyses to principal investors and summarized investment theses in written memorandum.

EnterpriseWorks Chicago, IL

Student Consultant August 2016 – December 2016

- Worked in a team to conduct market analysis and develop commercialization strategies for viable technology developed at University of Illinois at Chicago.
- Corresponded with lead scientists to fully understand mechanism of action and commercialization opportunity of the developed technology.
- Presented findings and investment recommendation to professors from various scientific fields, technology transfer coordinators, consultants, and potential investors.

VOLUNTEER SERVICE

University of Illinois at Chicago Chicago, IL

Organizing Committee, Laboratory of Integrative Neuroscience's Symposium 2017-2018

- Worked with 4 other graduate students and 2 faculty members to organize the day-long event that included talks by graduate students and 2 keynote speeches from prominent academics from outside the University.

Expanding Your Horizons (EYH) Chicago, IL

Parent Program Co-Chair 2017-2018

- Worked closely with other parent program co-chair and members of the EYH organizing committee to deliver a successful conference that encouraged middle school girls from underserved neighborhoods to pursue careers in STEM fields.
- Organized and curated a well-rounded event for parents attending the annual conference. Included hands-on science activities, a career panel, speakers from various science related organizations, and a keynote address by the senior vice president of Abbot.

Volunteer 2016-2017

- Assisted on the day of the annual symposium by helping to coordinate delivery of goods and directing attendees.

PAWS Chicago, IL

Foster Parent 2017-2018

- Cared for sick and injured dogs until they were healthy enough for surgery or adoption.
- Required communication and coordination with PAWS employees to ensure dogs were at all scheduled vet visits.