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Translational Examination of Risk-Related Decision-Making as an Endophenotype for Alcohol Use Disorders

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Publication Date

2013

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UNIVERSITY OF CALIFORNIA

Los Angeles

Translational Examination of Risk-Related

Decision-Making as an Endophenotype
for Alcohol Use Disorders

A dissertation submitted in partial satisfaction

of the requirements for the degree

Doctor of Philosophy in Neuroscience

by

James Rowan Ashenhurst

2014

Abstract of the Dissertation

Translational Examination of Risk-Related Decision-Making as an Endophenotype for Alcohol Use Disorders

by

James Rowan Ashenhurst

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2014

Professor James David Jentsch, Chair

Alcohol use disorders, a cluster of symptoms centered around a loss of control over alcohol use resulting in negative consequences on health and well-being, continue to cause worldwide suffering and economic loss. While decades of research have advanced our understanding of the genetic and brain-based causes of alcohol use disorders, the complexity of the symptom cluster and the corresponding intricacy of the implicated neural circuitry complicates the development of interventions. Thus, instead of focusing on the disorder as a whole, psychiatric research has placed research emphasis on intermediate phenotypes, or 'endophenotypes' that lie between the genetic basis and the disorder as a whole (Ducci & Goldman, 2008, 2012; D. Goldman, Oroszi, & Ducci, 2005; Manji, Gottesman, & Gould, 2003)

A number of endophentypes have been explored, most notably craving or incentive sensitization (Robinson & Berridge, 2001, 1993), affect or mood (Ahmed & Koob, 2005; G. F. Koob & Le Moal, 1997), and aspects of executive function (R. Goldstein & Volkow, 2011) including impulse control (Jentsch & Taylor, 1999). Related most closely to executive function, this dissertation examines biases in risk-related decision-making as having explanatory value for understanding alcohol dependence at its various stages: from alcohol use initiation, to acquisition of the clinical syndrome, to recovery and abstinence. Data presented herein was

drawn from both human clinical populations, and animal models, leveraging a translational approach.

First, the neurobiology and genetics of addiction are examined, as is the methods used to measure risk-related decision-making, and how this phenotype might conceptually relate to alcohol use disorders. Next, two analyses of risk-taking data from a clinical and sub-clinical sample demonstrate that, counter to initial predictions, risk-taking as assessed by the Balloon Analogue Risk Task [BART; (C. Lejuez et al., 2002)] is negatively related to clinical symptomatology. The nature of this relationship is further probed, revealing that differences in alcohol problem severity predict differences in loss reactivity, such that those with greater severity take less risk after a big loss than those with lesser severity.

Next, the effects of acute and sub-chronic alcohol dosing regimens are explored in an rodent analogue of the BART. Results indicated that acute alcohol dose dependently decreased risk-taking in the rat-BART, an effect partially consistent with the human literature where alcohol and other drugs are shown to have few effects on behavior in the human task (Peacock, Bruno, Martin, & Carr, 2013). Furthermore, six weeks of sub-chronic administration of alcohol (versus a saline control) did not significantly alter performance of the rat-BART. Suggestions for future studies are explored.

Finally, one component of the definition of an endophenotype is that it is heritable, and thus regulated by genes (Ducci & Goldman, 2008, 2012; D. Goldman et al., 2005; Manji et al., 2003). In order to assess the heritability of risk-related decision-making, a panel of inbred strains was phenotyped, revealing that about 55% of the variability in performance is attributable to genetic effects. An attempt is made to identify regions of the rat chromosomes that are linked with rat-BART performance using an F2 intercross strategy and quantitative trait loci (QTL) analysis. Preliminary results indicated a candidate region on Chromosome 1 (between approximately 90.99 Mb and 129.99 Mb). However, these results should be interpreted with caution as higher density trait mapping is required to more narrowly define the QTL region, and this significant result was only found in one of six variants of the rat-BART subject to analyses.

In sum, the data presented here, in the context of the broader literature, indicated that future research must address differences in behavioral metrics of risk-related decision-making, as results obtained can be contradictory depending on the task used. Additionally, there are areas where future research is more likely to be clinically influential than others. In particular, more work should be done to examine risk-taking during adolescence, at recovery and during treatment. Interventions at those stages appear most likely to improve outcomes for alcohol dependence.

The dissertation of James Rowan Ashenhurst is approved.

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University of California, Los Angeles

2014

To Keith . . .

who's love and support has

motivated me to achieve my dreams

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ACKNOWLEDGMENTS

My time at UCLA has been one filled with friendships and collaborations across a range of specializations, resulting in a highly translational body of work. I could not have done this alone. First and foremost, I would like to thank Drs. Jentsch and Ray for their guidance in the development of these studies from beginning to end. Their expertise and advice has been critical to my development as a scientist. It has been quite satisfying to go back and forth between animal models and clinical populations, a rare opportunity for any researcher. Their keen insight into behavioral neuroscience, genetics, and clinical phenomenology has aided me at every turn. I owe them my deepest gratitude for their support during my tenure here at UCLA.

Next, I would like to thank my other committee members, Drs. Maidment and London, for their oversight of this project and contribution to my scientific research education. Dr. Maidment provided fantastic grant writing guidance as part of the Neuroscience IDP curriculum. Dr. London's oversight of the Translational Neuroscience of Drug Abuse training grant was very important in exposing me to a variety of topics in addiction neuroscience, and in challenging me to always strive to improve written work and presentations.

The friendships and assistance from fellow lab members has also been essential to my UCLA experience. To start, I would like to thank the Jentsch lab. I have always appreciated feedback and assistance in surgery and lab methods from current and past members: Dr. Stephanie Groman, Alex James, Zach Pennington, Jason Woods, Dr. Catalina Cervantes, Karen Taylor, Dr. Rick Laughlin and Dr. Emanuele Seu. This work could not have been completed without my undergraduate research assistants: Mitchel Seaman, Alexander Hua, Malav Patel, and Nathan Razbannia.

From the Ray lab I would like to thank current and past members: Katy Lunny, Spencer Bujarski, Guadalupe Bacio, Kelly Courtney, Nathasha Moallem, Dr. Daniel Roche, Emily Hartwell, Vincent Allen Jr., Iris Guzman, Andia Heydari, Anna Sheng, Eliza Hart, Jessica Webb, Ryan Arellano, Ellen Chang, and Pauline Chin-Chung. I could not have done this

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work without your feedback in lab meetings and excellent work in gathering data from challenging clinical populations.

Research could not continue without the DLAM staff! I would like to thank all of the animal care staff and vets for their assistance in keeping the rats healthy and happy.

Genomic analyses are not simple task, and I would like to thank several people for their assistance in the development of the quantitative trait loci analyses in this dissertation. Dr. Elizabeth Byrda graciously provided her insight into multiplex PCR procedures. Dr. Uma Dandekar and the UCLA Genotyping and Sequencing core were essential to running genotyping assays, and her advice on the use of Touch-Down PCR protocols was critical to the success of this project. I would like to thank Drs. Rebecca Doerge and Zhao-Bang Zeng for teaching me statistical genetics methods and for the development of the QTLCartographer analysis package. Finally, I would like to thank Dr. Rita Cantor for her advice on running and interpreting robust QTL analyses.

I would like to thank my parents, John and Yvonne Ashenhurst, my partner, Keith Hall, and my close friends Donna Werling, Jess Bonham, Richard LeBeau, John Hicks, Kiri Hagerman, and Lauren Tracy (Food = Love!) for their support, love and friendship throughout the Ph.D process.

Contribution of Co-Authors. I would like to thank my co-authors for their contributions to this work. Dr. Lara Ray contributed data analysis and text authorship in Chapters One, Two, and Three. Dr. J. David Jentsch contributed to data analysis and text author-

ship in Chapters One, Two, Three, Four, Five and Six. Spencer Bujarski contributed data analysis and writing in Chapter Three. Mitchell Seaman contributed data collection and analysis to Chapter Five. Alexander Hua, Malav Patel, and Nathan Razbannia contributed to data collection and analysis in Chapters Four and Six.

Permissions. A portion of this dissertation contains versions of previously published articles. Chapter Two was published as: Ashenhurst, J.R., Jentsch, J.D., and Ray, L.A. (2011). Risk-taking and alcohol use disorders symptomatology in a sample of problem drinkers. *Experimental and Clinical Psychopharmacology*, 19(5), 361-70. DOI:10.1037/a0024412.

Chapter Five was previously published as: Ashenhurst, J. R., Seaman, M., and David Jentsch, J. (2012). Responding in a test of decision-making under risk is under moderate genetic control in the rat. *Alcoholism: Clinical and Experimental Research.* DOI: 10.1111/j.1530-0277.2011.01701.x Permission for re-use was obtained from John Wiley and Sons on November 25, 2013. License Number: 3276050502154.

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Interactive Effects of OPRM1 and DAT1 Variation on Subjective Responses to Alcohol. (In Press) Ray, L.A., Squeglia, L.M., Bujarski, S., Ashenhurst, J.R., and Anton, R. Alcohol and Alcoholism.

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CHAPTER 1

Background and Significance

1.1 Alcohol and Drug Dependence

Widespread abuse and misuse of psychoactive substances continues to greatly impact human health and economic productivity. The Centers for Disease Control and Prevention has estimated that the total cost of alcohol, tobacco, and illicit drug abuse is over $600 billion annually due to the effects on healthcare, productivity, and crime (CDC, 2008; NDIC, 2011; Rehm et al., 2009). Research into the epidemiology and phenomenology of drug and alcohol use suggest that some individuals are more at risk than others to develop particularly costly behavioral patterns of substance misuse as a consequence of both genetic and environmental factors (Kendler, Jacobson, Prescott, & Neale, 2003; Wichers, Gillespie, & Kendler, 2013). Clinical psychology and psychiatry have identified maladaptive patterns of misuse characterized by a) compulsive seeking and use of the drug, b) a loss of control of intake, and c) the emergence of a negative emotional state when access to the drug is limited (G. Koob & Volkow, 2010). Often, this disorder of behavior and thought results in negative life consequences in terms of health and social function, and this symptom cluster is collectively defined as substance use disorders (APA, 2000). Considering the economic burden in addition to the mental health consequences for the global population, research into the biologic etiology (in addition to causal environmental factors) of substance use disorders and their potential treatment is a critical need.

This dissertation seeks to advance the literature by examining risk-related decision-making as potentially having explanatory value for alcohol use disorders, specifically. Biases

in decision-making under circumstances of risk may serve as a cause or consequence of problematic alcohol use (C. Lejuez et al., 2002; Bechara et al., 2001). Identification of the biologic determinants of risk-taking propensity – at the level of the genome and within structures of the brain – may provide targets for intervention and the development of treatment strategies to ameliorate the consequences of alcohol misuse. This project requires a close examination of biological correlates of risk-taking propensity in addition to investigation into the relationship between risk-taking and alcohol use disorders as clinically defined.

In this introductory chapter, I will first provide a brief overview of some evidence for the current brain-based and genomic models of alcohol dependence. This evidence provides a strong case for continued research on alcohol dependence using behavioral neuroscience methods. Next, I will provide a conceptual overview of 'endophenotypes', with the proposition that risk-taking propensity may serve as an endophenotype for alcohol dependence. In this section, the relationship of risk-taking to major models of alcohol dependence is described, as well as common metrics used to assess decision-making under risk. Next, I describe known neural circuitry of decision-making under risk, which are likely targets of dysfunction in alcohol dependence. Finally, this chapter will outline the specific aims of this dissertation by describing experiments implemented in order to further examine the relationship between alcohol use or problems and risk-taking behavior as well as the potential genomic causes of risk-taking propensity.

1.2   The Biology of Alcohol Dependence

Ethyl alcohol (called simply 'alcohol' in this dissertation) is a pharmacologic agent that upon entering the body and traveling to the brain induces changes in neuronal microbiological cellular processes. Importantly, all drugs of abuse – including alcohol – induce effects in the nucleus accumbens (NAc), a component of the ventral striatum that is thought to be a central player in the brain reward system (DiChiara & Imperato, 1985, 1988; Imperato, Mulas, & Di Chiara, 1986). This brain region is the target of dopaminergic axons originating from

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the ventral tegmental area (VTA), and collectively this circuitry is commonly referred to as the mesolimbic dopamine system. In addition to subcortical reward circuitry, alcohol and other drugs of abuse are thought to induce abnormalities in the prefrontal cortex, a brain region associated with higher order executive function including: attention, self-control, working memory, cognitive flexibility, and awareness (R. Goldstein & Volkow, 2011). This combination of abnormalities in reward function and executive control – and perhaps the interaction between these systems – provides compelling evidence that alcohol use disorders are caused, in large part, by a biological brain-based disease process. The following sections will briefly outline evidence that alcohol adversely impacts brain-based reward and executive control regions (G. Koob & Volkow, 2010; R. Goldstein & Volkow, 2011; Jentsch & Taylor, 1999), providing support for continued use of behavioral neuroscience approaches into alcohol use disorders.

1.2.1 Alcohol and the Brain

1.2.1.1 Alcohol and the Ventral Striatum

Alcohol and cues associated with the anticipation of alcohol both increase dopaminergic signaling in the mesolimbic dopamine system. Systemically injected alcohol has been shown to increase levels of extracellular dopamine in the NAc (Blomqvist, Engel, Nissbrandt, & Soderpalm, 1993; Diana, Pistis, Carboni, Gessa, & Rossetti, 1993; DiChiara & Imperato, 1985), as has voluntary alcohol consumption (Weiss, Lorang, Bloom, & Koob, 1993; Weiss et al., 1996), and anticipation of alcohol induced by associated cues in rodents (Katner, Kerr, & Weiss, 1996; Katner & Weiss, 1999; Löf et al., 2007; Melendez et al., 2002; Weiss et al., 1993). These results have been replicated in humans using neuroimaging techniques, confirming that alcohol induces an increase in extracellular NAc dopamine, and that this effect is related to the subjective experience of euphoria and stimulation (Boileau et al., 2003; Ramchandani et al., 2011; Urban et al., 2010; Yoder et al., 2007). The precise mechanism by which alcohol and associated cues induce dopamine release remains unresolved due to the

heterogeneous effects of alcohol as a pharmacologic agent; effects on nicotinic acetylcholine, glycine, serotonin, γ-aminobutyric acid type A (GABA), glutamate, opioid, and ghrelin receptors are all implicated in mediating dopamine release in the NAc (Söderpalm & Ericson, 2013).

Finally, the syndrome of alcohol dependence is associated with alterations in this mesolimbic dopamine system. In particular, alcohol dependent individuals show reduced availability of dopamine D2/D3 receptors in the ventral striatum (Martinez et al., 2005; Volkow et al., 2002, 2007, 1996), which correlate with craving and liability to relapse (Heinz et al., 2005). Thus, a major component of alcohol use disorders is likely alcohol-induced alterations in dopaminergic signaling in reward related brain structures; essentially, the reward structures have adapted to the presence of alcohol as part of homeostasis. The absence of alcohol in the brain, then, may result in an aversive state that drives continued alcohol seeking and use (Ahmed & Koob, 2005; G. F. Koob, 2003; G. F. Koob & Le Moal, 1997; Söderpalm & Ericson, 2013).

1.2.1.2   Alcohol and the Frontal Cortex

Beyond reward processes in the ventral striatum, abnormalities in frontal cortical brain regions implicated in executive function are also associated with alcohol (and other drug) use disorders. Executive function is a broad term that includes many higher-order functions of the brain – many of which are shown to be disrupted in addiction (R. Goldstein & Volkow, 2011; Jentsch & Taylor, 1999) – including self-control (*i.e.*, response inhibition, inhibitory control), emotion regulation, awareness, attention, working memory, reversal learning, decision-making, cue reactivity, craving, and salience attribution (R. Goldstein & Volkow, 2011; Mansouri, Tanaka, & Buckley, 2009; Schoenbaum, Roesch, Stalnaker, & Takahashi, 2009). A complete review of executive functional deficits associated with abnormalities in specific regions of prefrontal cortical circuitry is beyond the scope of this chapter. However, below I highlight some evidence for morphologic deficits that are potentially linked with important aspects of alcohol use disorders such as cue reactivity, craving, and impulse

control.

Morphologic and functional studies have provided evidence that alcohol impacts cortical integrity. Heavy long-term alcohol use is associated with decreases in white and gray matter in the brain, particularly in the frontal cortex (de la Monte SM, 1988; Jernigan et al., 1991; Pfefferbaum, Sullivan, Mathalon, & Lim, 1997). Positron emission tomography techniques have shown abnormal levels of glucose metabolism in prefrontal cortex in alcohol dependent individuals (R. Z. Goldstein et al., 2004). These structural and metabolic abnormalities, potentially resulting from alcoholic neurotoxicity or neuroadaptation, suggest that functions attributed to this brain area may also be compromised or abnormal in alcohol dependent individuals, potentially contributing to disorder etiology (Abernathy, Chandler, & Woodward, 2010; R. Goldstein & Volkow, 2011; R. Z. Goldstein et al., 2004; Jentsch & Taylor, 1999; Parsons & Stevens, 1986).

For example, such abnormalities have been identified in terms of cue reactivity and impulse control (among the many other executive functions mentioned above). Abnormally potent motivational drives to seek and use alcohol may arise from enhanced salience of alcohol-related cues (Robinson & Berridge, 2001, 1993) as a consequence of compromised frontal cortical circuitry. Alcohol-related cue exposure (visual or taste cues) results in increased activation of the PFC in alcoholics (Grüsser et al., 2004; Filbey et al., 2008; Heinz et al., 2007), whereas in non-dependent individuals, alcohol administration *reduced* PFC activation (King, McNamara, Angstadt, & Phan, 2010). This heightened cue salience may underlie strong feelings of craving that may drive alcohol seeking and subsequent use (R. Goldstein & Volkow, 2011; Robinson & Berridge, 2001, 1993).

Similarly, impulse control, defined as the suppression of pre-potent actions or of internal motivational drives in favor of adaptive responses or drives (Jentsch & Taylor, 1999), appears to be compromised in alcoholism. Evidence that the prefrontal cortex is involved in inhibitory control includes the fact that lesions in the prefrontal cortex in monkeys [e.g., (Dias, Robbins, & Roberts, 1996; P. Goldman, Rosvold, Vest, & Galkin, 1971; Iverson & Mishkin, 1970)] result in perseverative deficits measured in tasks of inhibitory control (Jentsch & Taylor,

1999). Recent evidence linking prefrontal cortical abnormalities to impulse control in alcohol dependence, specifically, includes findings that the anterior cingulate cortex is hypoactive in alcoholics versus controls in a task of motor impulse control, the Stop Signal Task (C. Li, Luo, Yan, Bergquist, & Sinha, 2009). Additionally, alcohol dependent individuals with greater severity show reduced functional connectivity between frontal areas and the dorsal striatum during response inhibition versus those with lesser severity (Courtney, Ghahremani, & Ray, 2013). The large body of evidence linking impulse control to prefrontal integrity and substance dependence more broadly – that is, including stimulants and other drugs (Jentsch & Taylor, 1999) – is beyond the scope of this dissertation. But, it is clear that evidence from humans and animal models support the idea that deficits in frontal regions and the associated problems in impulse control also explain, in part, the development of problematic patterns of alcohol use.

1.2.2   Alcohol Use Disorders and Genomics

Starting in the 1970's, data from adoption and twin studies indicated that susceptibility for alcohol dependence is partly explained by heritable factors (Cloninger, Bohman, & Sigvardsson, 1981; Goodwin, Schulsinger, Hermansen, Guze, & Winokur, 1973; Kendler, Heath, Neale, Kessler, & Eaves, 1992; Kendler et al., 2012). These types of studies leverage the fact that monozygotic twins share all of their genome, while dizygotic twins share about half of their genes; including adopted individuals in analysis can probe for environmental effects by examining twins raised together versus twins raised apart (Cloninger et al., 1981; Goodwin et al., 1973; Kendler et al., 1992). After decades of such studies, the general estimate is that about 50-70% of alcohol dependence variability is attributable to genetic factors, with the remainder due to environmental effects, gene by environmental interactions, and error (Enoch & Goldman, 2001; D. Goldman & Ducci, 2007; Kendler et al., 2012). This level of heritability indicates that causal genomic factors for alcohol dependence susceptibility can be identified, although the precise number of genes implicated and all of their identities are still unknown (Kendler et al., 2012). Further complicating the issue, as alcohol depen-

dence requires individual access to alcohol, environmental effects can be stronger or weaker depending on cultural factors such as religiosity and parental involvement (Kendler et al., 2012).

Thus far, genetic variation in several neurobiological systems in reward and impulse-control pathways have been associated with alcohol dependence (Enoch & Goldman, 2001). Population-based and twin studies in humans and alcohol-related trait mapping in animal models have implicated allelic variation in several major neurotransmitter systems. Genes implicated include those encoding: serotonin transporters (Schneider et al., 2012), the 5-HT1B receptor (Lappalainen et al., 1998), GABA receptors (Buck & Hood, 1998; Parsian & Cloninger, 1997), dopamine receptors (Ishiguro et al., 1998), and opioid receptors (Ray et al., 2012), to name a few. Whether some of these variations exert greater effects than others, and if certain combinations are particularly deleterious (epistatic effects) is under study across the field of addiction genomics.

These data, in combination with brain-based models of alcohol dependence described above, confirm the large role biology plays in the development of problematic alcohol use. Identification of these biological factors, from genes, to the brain, to behavior (Enoch & Goldman, 2001), can provide targets for pharmacotherapies for the treatment of alcohol dependence.

1.2.3 The Endophenotype Approach to Substance Dependence

The previous sections outlined some of the many biological and brain-based components linked to problematic alcohol use and its associated clinically defined disorder, alcohol use disorders (APA, 2000). As with many mental disorders, substance use disorders are defined and diagnosed by a complex and multicomponent group of symptoms, all of which are behaviorally or cognitively-based rather than physiological. As such, the causal biomarkers of disease liability or expression are likely more distantly related to the disorder as a whole as compared to intermediate phenotypes. Thus, researchers have advanced a model of studying

"endophenotypes" in order to better understand biological factors that may underlie substance use disorder symptomatology (Ducci & Goldman, 2008, 2012; D. Goldman & Ducci, 2007; Hines, Ray, Hutchison, & Tabakoff, 2005; Manji et al., 2003).

Endophenotypes are defined as heritable behavioral phenotypes or biomarkers that are quantitative indicators of disease expression or liability (Ducci & Goldman, 2008, 2012; Manji et al., 2003). As intermediate phenotypes between the core biology and full disorder symptomatology, they are thought to be less etiologically complex, and can used to identify mediating influences from the environment as well (Ducci & Goldman, 2012). Many of the factors explained above in the section on alcohol and the brain may serve as endophenotypes, including pre-morbid abnormalities in reward circuitry or executive function that may result in a higher incidence of alcohol dependence (R. Goldstein & Volkow, 2011; G. Koob & Volkow, 2010).

1.3   Risk-Related Decision-Making as an Endophenotype

Among several behavioral phenotypes identified as plausible endophenotypes for substance use disorders, risk-related decision-making is currently under vigorous study in both pre-clinical animal models and in clinical populations (Ashenhurst, Jentsch, & Ray, 2011; Ashenhurst, Seaman, & David Jentsch, 2012; Bechara et al., 2001; Bechara & Damasio, 2002; C. Lejuez et al., 2002; C. Lejuez, Aklin, Zvolensky, & Pedulla, 2003; C. W. Lejuez, Simmons, Aklin, Daughters, & Dvir, 2004; Zeeb, Robbins, & Winstanley, 2009; Zeeb & Winstanley, 2011). The following sections will describe how risk-taking is commonly defined and measured, will present evidence that risk-taking propensity is related to substance misuse (particularly in adolescents), and will outline the known biological mechanisms that regulate decision-making under risk.

1.3.1 Defining Risk Taking in Relationship to Substance Use Disorders

The clinical criteria for alcohol (and other substance) dependence focus heavily on continued use of the substance despite knowledge of the occurrence and risks of adverse health, legal or social outcomes (APA, 2000). Thus, researchers have posited that propensity for risk-taking, driven in part by genetic factors, may enhance liability for problematic substance use (Kreek, Nielsen, Butelman, & LaForge, 2005). While debate exists regarding the precise definition of risk-taking propensity, in this dissertation it is defined as pattern of maladaptive choice behavior produced under conditions where there exists a potential for reward but also an unknown probability of negative outcomes. This definition is consistent with the idea that risk-taking propensity may be a contributing factor to substance use initiation and dependence liability, as continued substance use represents a decision to engage in maladaptive risky behavior.

Abnormal biases in risky decision-making exacerbating drug dependence are consistent with prominent models of substance use disorders, including the allostatic dysregulation model (G. F. Koob & Le Moal, 1997), the incentive sensitization model (Robinson & Berridge, 2001, 1993), and broader models of executive dysfunction (Jentsch & Taylor, 1999). Each model will be briefly described, and how risk-related decision-making might play a role will be evaluated. Importantly, these major models of substance dependence are not necessarily mutually exclusive, and instead each addresses unique components of the syndrome.

1.3.1.1 Major Models of Alcohol Dependence

In the allostatic dysregulation model (G. F. Koob & Le Moal, 1997), neuroadaptation due to chronic drug use results in dysregulation of opponent processes governing mood and affect; in other words, the frequent presence of alcohol in the body will trigger reward circuitry (outlined above) that will adapt over time in order to maintain homeostasis. With time, baseline function of reward circuitry in the brain will depend on the presence of alcohol; in

its absence, negative feelings may dominate (Ahmed & Koob, 2005; G. F. Koob & Le Moal, 1997). Thus, continued alcohol use represents an attempt to avoid the negative effects of withdrawal and to achieve positive affect, which requires escalated substance consumption due to acquired tolerance (Ahmed & Koob, 2005).

In the incentive sensitization model (Robinson & Berridge, 2001, 1993), neural changes caused by the drugs of abuse serve to sensitize motivational circuitry, leading to the formation of strong connections between drug-related cues and motivation to use the drug or craving. Here, 'sensitization' is used to mean an enhanced effect after repeated exposure (Robinson & Berridge, 2001). In this model, "wanting" for the drug is abnormally enhanced despite a lack of commensurate enhancement of "liking" or hedonic experience of drug reward (Robinson & Berridge, 2001, 1993).

Finally, there is a broader model of executive dysfunction due to abnormalities in pre-frontal cortical circuitry and its interaction with subcortical structures like the dorsal and ventral striatum (R. Goldstein & Volkow, 2011). While 'executive function' is a broad term that includes many higher-order functions of cognition such as working memory and attention, a major emphasis in research is on impulse control (Jentsch & Taylor, 1999). In this view, pre-morbid deficits in executive function predicts liability for problematic alcohol use, and long-term use may, in turn, further compromise executive functions and self-control (R. Goldstein & Volkow, 2011; Jentsch & Taylor, 1999).

1.3.1.2   Risk-taking in Relationship to Major Models

In all of these models, a high risk-taking temperament may exacerbate the process of substance dependence at several stages. Given the taboos and legal restrictions against drug and alcohol use, initiation of use (particularly during adolescence) may be enabled in part by a heightened propensity for risk-taking. Thus, greater risk-taking may contribute to an individual starting down the path to dependence, regardless of the validity of any of the above models.

Next, all models concur that during the onset of dependence, an individual will use the substance despite the occurrence of negative consequences; they differ in their attribution of reasons for this maladaptive behavior. It could be a drive to avoid negative affect (G. F. Koob & Le Moal, 1997), abnormally enhanced motivation and craving associated with drug-related cues (Robinson & Berridge, 1993), or deficits in executive function and self-control (R. Goldstein & Volkow, 2011; Jentsch & Taylor, 1999). A heightened propensity for risk-taking is clearly most closely related to the latter model, as decision-making – under risk or not – is included under the broad umbrella of executive function. Nevertheless, abnormalities in risk-related decision-making are not inconsistent with either the allostatic dysregulation or incentive sensitization models. Indeed, compromised decision-making abilities under circumstances of risk could exacerbate maladaptive behavior driven by avoidance of negative affect or by sensitized craving.

Finally, the struggle to remain abstinent may be compromised by risk-taking propensity. In the first two models, motivation to use (driven by affect or cue-induced craving) in combination with abnormal risky decision-making could result in a momentary decision to lapse, potentially re-commencing a cycle of use. Similarly, impulse control problems (Jentsch & Taylor, 1999) in individuals with heightened risk-taking propensity could result in greater liability to relapse as such an individual may put themselves in more risky circumstances where alcohol or related cues are available.

Thus, taken all together, possessing a propensity for heightened risk-taking may represent a core endophenotype associated with substance dependence, and therefore may also be a target for intervention. In order to evaluate the potential of risk-taking propensity as an endophenotype, behavior must be examined in terms of its heritability, its value as a pre-morbid predictor of substance use initiation, and as a phenotype that may be impacted as a consequence of substance use. To accomplish this, several research groups have developed metrics to evaluate individual differences in risk-taking behavior, including both human and animal-model appropriate tasks.

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1.3.2 Measuring Risk-Taking Behavior

Past research into risk-taking propensity has relied heavily on efforts to develop self-report questionnaires that reliably capture risk-preference related aspects of personality. The personality constructs targeted were often more broadly defined, such as "venturesomeness" (Eysenck, Pearson, Easting, & Allsopp, 1985) or "sensation seeking" (Zuckerman, Eysenck, & Eysenck, 1978). Questions posed often asked participants to endorse or reject a preference for a dangerous hypothetical scenario such as, "Would you enjoy parachute jumping?" (Eysenck et al., 1985). Also, some questions directly ask participants to reflect on their own risk-taking propensity, e.g., "Do you quite enjoy taking risks?" (Eysenck et al., 1985).

While these sets of questions were generally found to load into a factor structure consistent with the idea that together they represent a latent personality construct (Eysenck et al., 1985), some have questioned the validity of research participants' insight into their own risk-taking behavior, particularly if they possess cognitive biases that shape their decision-making processes (Ladouceur et al., 2000; C. Lejuez et al., 2002). Additionally, if the field is to approach risk-taking as an endophenotype, it is reasonable to prefer behavioral metrics that may be more sensitive to individual genomic, proteomic, or systems-levels differences over self-report questionnaires.

1.3.3 Conventional Tests of Risky Decision-Making

In the following sections, I outline common human and animal-model based tasks of risky decision-making and evaluate similarities and important differences between them. It is critical to understand the structure of these tasks in order to interpret their relationship with substance use problems and with underlying neurobiology. In many cases, researchers have developed animal analogs for human tasks; these are presented with their respective partners.

1.3.3.1   The Iowa Gambling Task

The Iowa Gambling Task (Bechara, Damasio, Damasio, & Anderson, 1994) is a game wherein subjects may sequentially choose from any of four decks of cards. Each card is associated with a gain or a loss in points/money. Commonly, two decks yield larger rewards but are also subject to a high rate of large penalties, while the other two yield small rewards but fewer penalties. The payout schedules are arranged such that across the testing session, it is ultimately disadvantageous to select the high reward/penalty decks. The common dependent variable is the difference between the numbers of advantageous versus disadvantageous choices. Generally, control (unaffected) persons start out by selecting the disadvantageous deck but update their behavior appropriately as the task progresses and their experience with the outcomes accumulates. Thus, learning and sampling is a critical component to optimal task performance.

1.3.3.2   The Rat Gambling Task

The Rat Gambling Task (Zeeb et al., 2009) was designed to mimic the structure of the Iowa Gambling task. In the rat version, animals are given a limited amount of time to select from four options. As with the decks in the Iowa Gambling Task, there are two choices with larger potential rewards (more sugar pellets) but also the chance of a large penalty (a long time-out period). The other two choices deliver smaller rewards, but also have shorter time-out penalties. Since time to perform the task is limited, optimal performance involves selection of the smaller reward but shorter time penalty options.

1.3.3.3   The Risky Decision-Making Task

The Risky Decision-Making Task (Mitchell, Vokes, Blankenship, Simon, & Setlow, 2011; Simon et al., 2011) is a rodent task that operates similarly to the gambling task described above but that implements stronger aversive punishment. Here, rats must choose between either a small reward "safe" lever or a large reward "risky" lever, which sometimes results

in an electric foot shock. In some ways this is more naturalistic in the context of drug use, as drug seeking and use (the reward) presents increased risk of severe negative consequences external to the rewarding properties of the drug per se (e.g., incarceration, adverse health consequences).

1.3.3.4 The Probabilistic Discounting Task

The Probabilistic Discounting Task (St Onge & Floresco, 2009) also requires rats to choose between two levers. The small/certain lever guarantees the delivery of one food reward pellet, while the large/risky lever may deliver four pellets with a given probability. Typically, the probability of reward delivery for the large/risky lever descends across four trial blocks from certain (100%) to unlikely (12.5%), producing a shift in optimal choice across the session and allowing for a parametric assessment of risky decision-making.

1.3.3.5 The Betting Task

The Betting Task (Cocker, Dinelle, Kornelson, Sossi, & Winstanley, 2012) is designed to assess sensitivity to betting magnitudes when outcomes are actually probabilistically equivalent, a form of irrational choice bias in the face of risk. Here, rats may again choose between safe and risky levers. The latter delivers either twice the value of the safe lever or no reward at 50:50 odds; thus both levers have equal utility, but the size of the "bet" can be varied to identify wager-sensitive and insensitive rats.

1.3.3.6 The Balloon Analog Risk Task (BART)

The BART is a computerized task originally developed for use in young adults (C. Lejuez et al., 2002) that measures sequential economic risk-taking behavior. In this task, the subject is presented with a picture of a balloon on a computer screen and is given the option to press two buttons. One button inflates (or "pumps") the balloon, and each inflation results in the accrual of a small amount of reward (monetary or a points system). Subjects may

choose to press the other "cash out" button at any time to add the earned rewards to their guaranteed "bank". With every pump, however, there is a chance that the balloon will burst on the screen, and reward for that trial is forfeited. In the task, optimal performance consists of pumping the balloon enough to maximize reward, while avoiding over-accumulating risk. Usually, risk of explosion is a set uniform probability for a balloon of a specific color, with values ranging from 1/8 to 1/128 (C. Lejuez et al., 2002). There is also an "automated" version of the task that removes the motor component of repeatedly pressing a button to inflate a balloon. Instead, participants may enter the number of pumps they intend to produce, and the computer inflates the balloon correspondingly with the possibility of bursting (Pleskac, Wallsten, Wang, & Lejuez, 2008).

The main dependent variable studied is the mean number of pumps produced on non-burst trials, although some have examined variability of pumping behavior across the session (Ashenhurst et al., 2011), and reactivity to burst trials (Mata, Hau, Papassotiropoulos, & Hertwig, 2012). This dissertation also examines a novel method for examining trial-by-trial loss and reward reactivity in the BART (Chapter Three).

1.3.3.7 The Rat Balloon Analogue Risk Task

The Rat-BART (Jentsch, Woods, Groman, & Seu, 2010) operates similarly to the human version and is adapted for use in rodent operant boxes. Here, rats press on one lever to accumulate food rewards that can be cashed out and received at any time by pressing on a second lever. However, a certain risk is applied such that an additional accumulation press may result in forfeiture of accumulated reward for that trial and a time-out. Risk levels vary between 1/6 to 1/10, and rates of reinforcement can be modified (from 100% to 33% random ratio) to parametrically assess the effects of reinforcement schedules on task performance.

1.3.3.8 The Cambridge Gambling Task

The Cambridge Gambling Task (Deakin, Aitken, Robbins, & Sahakian, 2004; Rogers, Everitt, et al., 1999) is a computerized task wherein subjects are asked to place bets on the location of a hidden token. Unlike in other tasks, the odds of guessing correctly are presented to the subject explicitly by varying the ratio of colors among "boxes" that may contain the token, and subjects are free to choose the size of their wager. The bet placed is simply on guessing correctly the color of the box under which the token is hidden. Thus, if the color ratio is 1 Red:9 Blue, these are the corresponding odds of guessing correctly. The options for the amount of points to bet are presented as a sequence of percentages of the total available points earned thus far in the session. The sequence of potential bets in a trial can either ascend or descend in magnitude (e.g., from 5% to 95%, or the reverse), and the subject stops the sequence at the desired betting magnitude for that trial. Common outcomes measured include the speed of decision-making, frequency of making less probable choices, "risk tolerance" (the mean wager), and "risk adjustment" (the degree to which subjects vary their wager size based on the parametrically varied explicit odds).

1.3.4 Comparison of Risky Decision-Making Tasks

It is important to keep in mind critical similarities and differences between the structures of optimal behavior in these tasks to interpret findings related to brain circuitry, pharmacology, and drug use behavior. As these are complex behavioral tasks, individual differences in performance may be explained by other related phenotypes that might be more or less influential on behavior in specific tasks; these could include temporal discounting rate, magnitude of reward preference, motivation to participate in the game, sensitivity to losses and rewards, working memory capacity, or general intelligence. Additionally, some have argued that certain tasks are more naturalistic in the context of drug abuse (Mitchell et al., 2011).

First, The Iowa Gambling Task, Rat Gambling Task, Risky Decision-Making Task, and BART all require individuals to learn from and respond to feedback to guide their behavior;

the risk levels are unknown to the participant prior to sampling the task. On the other hand, in the Cambridge Gambling Task, decision-making can ostensibly be guided by the visible cues (the red:blue ratio), and outcomes measured focus more on patterns of response to known risk rather than ambiguous risk. Since risk levels are known, this task does not fit the definition of risk-taking propensity outlined above; in the context of drug use, uncertain risk may be more naturalistic, as explicit probabilities of harm are unknown to abusers.

Next, the nature of negative outcomes differs between some tasks. The Iowa Gambling Task, Rat Gambling Task, BART, and Cambridge Gambling Task all involve risk along a single dimension of monetary or food outcomes. So, behavior results in more or less reward, while the Risky Decision-Making Task involves punishment (a shock) separate from reward. Whether either strategy is more appropriate for studying risk-taking in the specific context of addiction remains to be resolved, although on face value, separating punishments (incarceration, health risk) from reward (obtaining and using drug) seems more realistic (Mitchell et al., 2011).

Finally, the nature of the relationship between strategic risk-taking choice and outcome utility can be monotonic or non-linear, complicating interpretation of behavior between the tasks. In the Iowa Gambling Task, Rat Gambling Task, and Risky Decision-Making Task it is always advantageous to select the less risky of the limited options; consistent safe choice is rewarded with the greatest possible monetary reward at the end of the session and with minimal negative outcomes in some cases (e.g., shock). In the Betting Task, however, the choices are functionally equivalent, so differences in choice are purely a function of irrational bias. In the Probabilistic Discounting Task and Cambridge Gambling Task, risk levels shift across the testing session, so strategies must change across the session to optimize outcomes. Lastly, in the BART, consistently "safe" behavior (i.e., low levels of balloon pumping) will actually result in a reduction of reward outcomes compared to some elevated risk-taking. This is because optimum performance in the BART involves balancing an increase in reward with an increase in risk, resulting in a non-linear function where the most one can earn is by pumping to the mid-point of possible pumps on every trial (Jentsch et al., 2010; C. Lejuez

et al., 2002).

In sum, the field has benefited greatly by the creative efforts of clinical and pre-clinical researchers to develop a variety of assessments for risk-taking propensity. As described above, elements of task design, even subtle ones, influence the nature of optimum behavior and the role of robust learning capacity for performance. Ultimately, it may be that these differences explain why task behavior as it relates to pharmacology, neurobiology, and clinical dependence is not uniform across these tasks, as is described in the next section.

1.4 Relationship of Risk-Taking to Substance Use and Dependence

Researchers have hypothesized that greater than average risk-taking propensity may serve as a risk factor for substance use disorders and related problems at each stage of dependence (Bechara et al., 2001; C. Lejuez et al., 2002; Rogers, Everitt, et al., 1999; MacPherson, Magidson, Reynolds, Kahler, & Lejuez, 2010; Peacock et al., 2013). In this section, the rationale for such hypotheses are presented, as well as data suggesting that the role of risk-taking propensity at each stage is not uniform.

1.4.1 Initiation of Substance Use in Adolescence

It is plausible that a pre-morbid risk-taking personality would promote engagement in drug seeking as one of many outlets of sensation seeking, particularly during adolescence (C. Lejuez et al., 2003). This developmental period is marked by a substantial increase in risk-taking and exploration; hypothetically, this results when maturation of the neural systems underlying approach or reward-seeking behavior precede those associated with executive functioning (prefrontal cortex) (Reyna & Farley, 2006; Galván, 2013; S. A. Brown et al., 2008).

Research on risk-taking and substance use initiation in younger populations has generally

supported a positive relationship. Among young adults and adolescents, greater risk-taking in the BART is associated with increased use of alcohol, tobacco, and MDMA (Fernie, Cole, Goudie, & Field, 2010; C. Lejuez et al., 2002; C. W. Lejuez, Aklin, Bornovalova, & Moolchan, 2005; C. W. Lejuez et al., 2007). Longitudinal assessment of BART performance during adolescence showed that greater increases in risk-taking over time predicted a larger odds ratio of alcohol use (MacPherson et al., 2010). Similar results were obtained with the Iowa Gambling Task in adolescent drinkers and tobacco users (Xiao, Koritzky, Johnson, & Bechara, 2013; Xiao, Bechara, et al., 2013). Lastly, pre-clinical data from the Risky Decision Making Task in rats suggests that adolescent risk-taking predicts later self-administration of cocaine (Mitchell et al., 2013). This self-administration behavior of a drug-of-abuse is a common rodent metric thought to approximate the drug-seeking component of substance use disorders. Overall, convergent data from multiple tasks and models show that adolescent risk-taking propensity does associate with or prospectively predict substance use initiation.

1.4.2 Effects of Acute Intoxication on Task Performance

Additionally, researchers may find that acute intoxication promotes risk-taking, particularly among those with current or a history of substance use disorders. This could be problematic, as disinhibition caused by stimulants or alcohol may result in poor choices with negative health or legal outcomes such as drinking and driving or binge drinking. The endorsement of legal or health consequences due to use is among the clinical criteria for substance use disorders (APA, 2000). Curiously, human and animal model data suggest the effects of acute intoxication to be minimal or again in the opposite direction as predicted (Chapter Four).

Behavior in these tasks has been examined under the acute effects of drugs of abuse themselves in the human laboratory, with few significant effects. Moderate alcohol doses (Peacock et al., 2013) and acute opioid administration (Zacny & de Wit, 2009) did not alter behavior in the BART. Alcohol and MDMA do not alter performance in the Iowa Gambling Task (Ramaekers & Kuypers, 2006). Acute alcohol did not impact risky decision-making

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in a visual line size estimation task (Farquhar, Lambert, Drummond, Tiplady, & Wright, 2002). While it remains possible that the lack of effects could be due to moderate dose ranges, at present it appears that behavior in these tasks is insensitive to (moderate) acute drug intoxication.

Data from rodent models examining the effects of acute drug of abuse exposure on risky decision-making have yielded mixed results. Morphine and ethanol did not have significant effects on behavior in the Risky Decision-Making Task, while nicotine and amphetamine decreased choice of the 'risky' lever (Mitchell et al., 2011). Acute administration of nicotine (Mendez, Gilbert, Bizon, & Setlow, 2012) or amphetamine (St Onge & Floresco, 2009) increased selection of the large/risky lever in the Probabilistic Discounting Task where the risk is for a time-out. On the other hand, a lower dose of nicotine and higher dose range of amphetamine *decreased* selection of the risky lever in the Risky Decision-Making Task, where subjects risk a foot shock (Mitchell et al., 2011; Simon, Gilbert, Mayse, Bizon, & Setlow, 2009). While non-linear effects of drugs on behavior are not atypical, it appears here that the nature of punishment (reward forfeiture versus active shock) may also modulate the effect of these drugs on choice behavior. Lastly, our own observations indicate that acute ethanol dose-dependently *decreased* risk-taking in the rat-BART (Chapter Four).

In sum, data from the literature suggests that the effects of acute intoxication on risk-taking propensity appear to be minimal or actually decrease risk-taking at moderate doses in these tasks. This suggests that if enhanced risk-taking is an important explanatory factor in substance abuse, it is not likely enhanced by the substances themselves in the acute term.

1.4.3   Comparing Clinical Populations and Controls Subjects

If drug use continues to the point of clinically-defined dependence, it is reasonable to hypothesize that the brain may have undergone neuroadaptive changes, including alterations in the brain circuits involved in maladaptive risk-taking. Decades of research have documented morphological and functional brain deficits in users versus controls particularly in the pre-

frontal cortex, an area associated with executive functions (R. Goldstein & Volkow, 2011; de la Monte SM, 1988; R. Z. Goldstein et al., 2004; Jernigan et al., 1991; Pfefferbaum et al., 1997; P. Thompson et al., 2004). Continued drug use in the face of negative consequences itself represents a pattern of maladaptive choice behavior, which may be partially explained by acquired abnormalities in decision-making. If the general hypothesis that drug users are more risk-prone as either a cause or consequence of drug use is true, quantified levels of risk-taking propensity should predict risk for problematic drug use and should discriminate between drug dependent individuals and healthy controls. With regard to this stage of dependence, data across the many risk-taking tasks obtained from adults is mixed, and in some cases counter to the predicted direction (see Chapters Two and Three).

The Iowa Gambling Task has been used to identify differences in decision-making between individuals with substance use problems and controls. In the Iowa Gambling Task, heavy users of cannabis, stimulants, alcohol and opiates tend to sub-optimally perform as compared to matched controls by failing to update their choice behavior and instead continuing to select disadvantageous "risky" decks across the test session (Bechara et al., 2001; Cunha, Bechara, de Andrade, & Nicastri, 2011; Kim, Sohn, & Jeong, 2011; Moreno et al., 2012; van der Plas, Crone, van den Wildenberg, Tranel, & Bechara, 2009; Verdejo-Garcia et al., 2007). This pattern of behavior may be linked with insensitivity to the future consequences of disadvantageous choices (Cantrell, Finn, Rickert, & Lucas, 2008; J. C. Stout, Busemeyer, Lin, Grant, & Bonson, 2004) or pathologically low sensitivity to negative reinforcement (L. L. Thompson et al., 2012). In terms of longitudinal data, early onset binge drinkers (versus non-binge drinkers and later onset binge drinkers) were more likely to select the disadvantageous decks (Goudriaan, Grekin, & Sher, 2007), and greater risk preference prospectively predicted problematic drinking in men, but not women (Goudriaan, Grekin, & Sher, 2011).

Similarly, in the Cambridge Gambling Task, stimulant, alcohol, marijuana and opiate abusers selected sub-optimal risky bets and had increased deliberation times in some circumstances compared to healthy controls (Fishbein et al., 2005; Rogers, Everitt, et al., 1999;

Schneider et al., 2012). However, there were no significant relationships between task performance parameters and severity of drug use except for deliberation times (Fishbein et al., 2005); as severity of use increased, so did the amount of time taken to make a choice, but only under circumstances of significant risk. These results indicate that potentially pathological differences in decision-making abilities are particularly compromised under high-risk circumstances.

Data from the Balloon Analogue Risk Task, however, is potentially counter-intuitive when examined among adult or young adult substance users. Studies have found that across the trajectory of the testing session, young adult tobacco users take *less* risk in the BART than non-smoking controls (Dean, Sugar, Hellemann, & London, 2011), and that among a large sample of adults with alcohol use problems, greater risk-taking in the BART predicted *less* severe clinical symptomatology [described in Chapter Two; (Ashenhurst et al., 2011; Courtney, Arellano, Barkley-Levenson, Glvan, et al., 2012)]. This negative relationship with clinical severity has been recently replicated in tobacco users (Ryan, Mackillop, & Carpenter, 2013). Thus, while these studies are predominantly cross-sectional, together they suggest that behavioral patterns in the BART are associated with substance use disorders in the opposite direction as predicted. Behavioral mechanisms underlying this effect (loss and reward reactivity) are examined in Chapter Three.

1.4.4 Relationship to Successful Recovery and Abstinence

Finally, recovery or successful abstinence may associate with decreases in risk-taking during detoxification and treatment, or with lower baseline levels of risk-taking that are closer to healthy population means. Abstinence requires that the individual continue to choose not to use the drug even under circumstances where drug-related cues that may trigger craving or drug seeking are present. Hypothetically, biases in decision-making could make an individual more susceptible to making the "risky" choice to use and relapse or to be involved in "risky" circumstances where such choices are even possible.

Data gathered from treatment studies suggest that risk-taking propensity does attenuate with detoxification and that lower risk-taking associates with better odds of recovery and abstinence in the short term (up to three months). Specifically, polysubstance-abusing patients in an in-patient treatment program showed decreases in risk-taking in the Iowa Gambling Task with time and greater risk-taking was associated with a higher rate of relapse at three months (De Wilde, Verdejo-García, Sabbe, Hulstijn, & Dom, 2013). Duration of successful abstinence from methamphetamine associated with decreased risk-taking (Wang et al., 2013). Performance of the Cambridge Gambling Task and Iowa Gambling Task at levels comparable to healthy controls (versus greater risk-taking) predicted a much greater odds ratio of abstinence at three months in opiate dependent patients (Passetti, Clark, Mehta, Joyce, & King, 2008). Among adolescents participating in a tobacco smoking cessation program, those who maintained abstinence for four weeks took less risk in the BART after a stress exposure than those who relapsed (Schepis, McFetridge, Chaplin, Sinha, & Krishnan-Sarin, 2011). Results are not uniform, however, as length of abstinence in heroin dependent patients was not associated with risky decision-making in the Iowa Gambling Task (X. Li et al., 2013).

While there have been few investigations into the role of risk-taking propensity in recovery and abstinence thus far, the preliminary data does suggest that this factor is related to clinical outcomes. Further investigation of the role of decision-making biases in treatment studies is warranted. In particular, to inform clinical practice, it would be highly useful to know if successful abstinence is predicted by a *change* in risk-taking behavior, as interventions could be targeted to induce such a change. Furthermore, if baseline performance of the task prior to or during detoxification can predict clinical outcomes to a meaningful degree, this suggests that assessment of risk-taking propensity at patient intake may be useful to help guide clinical practice.

1.5 Neural Circuitry of Risk-Related Decision-Making

Interpretation of neuroimaging data obtained from patients and controls during tests of risk-related decision-making is a significant challenge as broad neural networks are involved and the designs of some tasks limit their implementation in event-related fMRI analysis. However, animal models also offer the opportunity for controlled investigation of relevant circuitry. Below, I outline recent human and rodent data that implicates a frontal-striatal network in regulation of behavior in these tasks.

The Iowa Gambling Task was initially implemented in patients with ventromedial prefrontal cortex damage (Bechara et al., 1994); these individuals exhibit remarkably poor performance in the task, despite being unaffected in many other intellectual dimensions. Pharmacologic inactivation studies in rats demonstrated the roles of the basolateral amygdala and orbitofrontal cortex in performance of the Rat Gambling Task (Zeeb & Winstanley, 2011). Functional disconnection studies have also shown that communication between the basolateral amygdala and either the nucleus accumbens or prefrontal cortex regulate choice behavior in the Probabilistic Discounting Task (St Onge, Stopper, Zahm, & Floresco, 2012).

Similar regions are identified even when probabilities of outcomes are known, as in the Cambridge Gambling Task. Here, patients with ventromedial prefrontal cortex damage consistently bet more than healthy controls, and those with insula damage failed to appropriately decrease their bets as the odds of winning decreased (Clark et al., 2008). Furthermore, in healthy adolescents, increased risk-taking in the Cambridge Gambling Task is associated with diminished ventral striatal response to reward anticipation (Schneider et al., 2012).

Neuroimaging of control and substance-using subjects performing the BART has implicated a partially overlapping network of brain regions. Brain regions implicated in risk acceptance include anterior insula, anterior cingulate, dorsolateral prefrontal cortex, and de-activations of the ventromedial prefrontal cortex (Fecteau et al., 2007; Galvan et al., 2013; Schonberg et al., 2012). Activity in the amygdala was found to promote risk aversion after loss in the BART (Kohno et al., 2013). Pharmacologic inactivation studies in rats have

confirmed a role for the ventromedial prefrontal cortex and orbitofrontal cortex in aspects of risk-taking in the rat-BART (Jentsch et al., 2010). Furthermore, striatal D2/D3 dopamine receptor availability was negatively correlated with the degree to which dorsolateral pre-frontal cortex activation was modulated by risk-taking (Kohno et al., 2013), highlighting the interaction between these systems in updating potential reward values and guiding goal-directed behavior.

1.5.1 Endogenous Pharmacological Regulation

1.5.1.1 Serotonin

The hypothesized role of serotonin in the decision-making process involves its regulation of the affective and behavioral responses to negative feedback (Cools, Roberts, & Robbins, 2008). Serotonergic depletion produces a pattern of impaired decision-making in the Cambridge Gambling task similar to that observed in substance use disorders (Rogers, Everitt, et al., 1999). On the other hand, a serotonin receptor type 1A agonist (8-OH-DPAT) impaired performance in the Rat Gambling Task (Zeeb et al., 2009) while a selective serotonin re-uptake inhibitor (citalopram) had no effect (Baarendse, Winstanley, & Vanderschuren, 2013), suggesting that the relationship between serotonin and optimal decision-making is not necessarily simple nor linear.

1.5.1.2 Dopamine

Dopamine, however, may influence some non-affective aspects of decision-making related to learning and evaluating risk and reward levels in the frontal cortex and sub-cortical striatum. Although the effects are not uniform, pharmacologic stimulation and suppression of the dopamine system using systemic D1 and D2 receptor agonists and antagonists can bias choice behavior in some rodent tasks (Baarendse et al., 2013; Simon et al., 2011; St Onge & Floresco, 2009; Zeeb et al., 2009; Stopper, Khayambashi, & Floresco, 2013; Mitchell et al., 2013). Microdialysis measurement of dopamine efflux during the Probabilistic Discounting

Task in the prefrontal cortex and nucleus accumbens respectively suggests that the former encodes relative reward rate or availability while the latter encodes an integration of reward rate, uncertainty and preference, and decision information (St Onge, Ahn, Phillips, & Floresco, 2012). Additionally, striatal D2/D3 availability as assessed by micro-PET in rats was negatively correlated with wager-sensitivity in The Betting Task (Cocker et al., 2012) and lower striatal D2 mRNA expression was associated with greater risk-taking (Mitchell et al., 2013) in the Risky Decision-Making Task. These results imply that individual differences in dopamine receptor expression and function bias decision-making under risk, but the specific mechanism is yet to be resolved.

1.5.1.3   Norepinephrine

Finally, there have been few studies investigating the influence of the norepinephrine system on risk-taking. In particular, the norepinephrine reuptake inhibitor atomoxetine alone did not alter decision-making in the Rat Gambling Task, but it did increase choice of the disadvantageous lever when combined with the specific dopamine reuptake inhibitor GBR12909 (Baarendse et al., 2013).

Taken together, it is clear that decision-making under risk recruits broad neuromodulatory systems in multiple brain regions. Continued research into the precise mapping of signals at specific receptor subtypes in these networks onto specific decision-making related functions may provide targets that are also implicated in the neuroadaptive effects of repeated use of drugs of abuse. In turn, interventions designed to ameliorate or prevent these effects could be used to treat alcohol and other drug use disorders.

1.6   Aims of the Dissertation

While research efforts into the neural underpinnings of decision-making under risk have made considerable progress, the relationship between risk-taking propensity, clinical manifestations of alcohol use disorders, and the pharmacologic effects of alcohol itself on risk-taking

behavior are underexplored. Furthermore, as described above, different risk-taking tasks have provided inconsistent results. The following chapters of this body of work will describe human laboratory and pre-clinical animal model investigations into alcohol use and risk-taking using the Balloon Analogue Risk Task (BART) as a behavioral metric of risk-taking. Furthermore, as a proposed endophenotype, it is important to determine if risk-taking propensity (in the BART) is heritable, and subsequent investigation may identify positional genomic candidates that moderate risk-taking behavior. These two main translational components are the primary aims of this dissertation, as will be described below.

1.6.1   Aim 1: Alcohol Use Disorders and the BART

This first aim is to use a multi-species, integrated approach in order to determine the relationship between risk-taking behavior in the BART and alcohol use disorder symptomatology, acute alcohol intoxication, and chronic alcohol exposure. We make use of both human clinical populations and rat models in order to address these points while respecting the ethical boundaries of research in these two populations.

Chapter Two will describe analysis of the relationship between risk-taking behavior and clinical symptom count while also accounting for individual differences in working memory, general intelligence, and demographic variables. As already stated in the above review, the results demonstrate an unexpected negative relationship between risk-taking in the BART and alcohol use disorder symptom count; these findings serve as the impetus for the additional experiments, as these findings call into question the expected positive relationship between risk-taking and alcohol use disorders in adults.

In Chapter Three, we expand this initial analysis by implementing a trial-by-trial model that allows for dissection of reward and failure reactivity and their unique relationships to alcohol problem severity in order to further explain the findings in Chapter Two. Finally, Chapter Four describes animal-model experiments to determine the effects of both acute and chronic alcohol intoxication on behavior in the rodent analogue of the BART, with findings

largely consistent with the human literature.

1.6.2   Aim 2: Genetics and the Rat-BART

The second aim of the dissertation is to evaluate the heritability of rodent performance of the BART, and to attempt to identify quantitative trait loci linked with risk-taking propensity. To be an endophenotype, risk-taking propensity must be shown to be heritable, and therefore under genomic regulation (Ducci & Goldman, 2012; Manji et al., 2003). Animals that can easily be selectively bred, like rats, are ideal for studies of this kind. If the trait is shown to be heritable to at least a moderate degree, quantitative genomics methods can easily identify regions of the chromosome linked with risk-taking propensity (Lander & Green, 1987; Lander & Botstein, 1989). These genomic regions are likely to contain sites of variation that, in the broader population, also regulate decision-making under risk. Candidate genes can be identified using high density trait mapping in that area, and human homologues can be tested in healthy and clinical populations as being related to decision-making under risk, and with alcohol use disorders liability; this completes the pathway from gene, to endophenotype, to clinical disorder (Ducci & Goldman, 2008, 2012; Enoch & Goldman, 2001; D. Goldman et al., 2005; Manji et al., 2003).

Chapter Five describes an analysis of heritability conducted through examination of behavior across several inbred rat strains, and establishes that not only is risk-taking propensity heritable, but so are other sub-measures of the task like inter-trial variability and false starts, a kind of trial initiation error (Chapter Five Supplementary Analysis). Lastly, Chapter Six describes results from a genome-wide quantitative trait loci analysis on rat-BART behavior measured across a moderately large population of specially bred rats from an F2 intercross. It identifies a candidate QTL region on Chromosome 1 for risk-taking propensity, where future high-density trait mapping is warranted.

CHAPTER 2

Risk-taking and Alcohol Use Disorders Symptomatology in a Sample of Problem Drinkers

2.1   Abstract

Background: The relationship between risk-taking behavior and alcohol use disorder (AUD) symptoms is poorly understood. This study employed a modified version of a behavioral measure of risk-taking, the Balloon Analogue Risk Task (BART), to examine its relationship to alcohol use and related symptoms in a community sample of individuals with or at risk for AUD.

Methods: A total of 158 (71.9% male) participants completed a testing battery that included the BART, a structured diagnostic interview for AUD, and measures of alcohol use and related problems. Estimates of IQ and working memory were assessed as covariates.

Results: Results indicated that the relationship between risk-taking propensity, as assessed by the BART, and alcohol problems was significant and negative. Individuals with higher symptom count made fewer pumps per trial on the BART, indicating less risk-taking. Importantly, this relationship was attenuated when controlling for estimated IQ and working memory span. Further examination demonstrated that IQ and age mediated the relationship between risk-taking propensity and symptom count.

Conclusions: The main negative relationship observed between risk-taking on the BART and alcohol use and AUD symptomatology in this sample stands in contrast to the positive relationships observed in adolescent and non-clinical samples. Together, these find-

ings highlight the need to consider development and the course of addiction in order to fully elucidate the effects of risky-decision making on AUD liability. Furthermore, our results demonstrate the importance of inclusion of neurocognitive covariates (IQ) as well as demographic variables (age) when using this task.

2.2 Introduction

Previous research has suggested that risk-taking, as a personality trait, may serve as a liability factor for substance use disorders, including alcohol use disorders [AUD, e.g., (Bechara & Damasio, 2002; C. Lejuez et al., 2002; J. Stout, Rock, Campbell, Busemeyer, & Finn, 2005; Zuckerman, Ball, & Black, 1990)]. Risk-taking reflects the ability of an individual to weigh costs and benefits during decision-making with potentially negative outcomes. These processes are likely related to genetically-determined individual differences in psychological processes (Kuhnen & Chiao, 2009) such as sensitivity to reward and impairments of inhibitory (impulse) control (Kreek et al., 2005). Advances in assessment have resulted in objective, quantitative, behavioral measures of risk-taking tendencies, such as the Iowa Gambling Task (Bechara et al., 1994) and the more recently developed Balloon Analogue Risk Task [BART; (C. Lejuez et al., 2002)]. Unlike previously used self-report measures of risk-taking (Zuckerman et al., 1978, 1990), these behavioral tasks are thought to allow for the assessment of risk-taking as an objective behavior.

During performance on the BART, participants make decisions on each trial about the amount of risk they are willing to accept in order to inflate a computer-generated balloon and obtain a reward. They successively press a 'pump' button to accept risk in order to obtain larger reward accruals (and inflate the on-screen balloon) or a 'cash out' button to avoid further risk and to obtain the reward accrued thus far. If the risk function of the program is exceeded, participants will observe a trial failure (i.e., balloon explosion) with reward forfeiture. Across trials, the number of 'pump' presses made before 'cashing out' represents a behavioral measure of risk-taking, with individuals who are risk-prone pressing

a greater number of times on an average trial (C. Lejuez et al., 2002). As including data from trials in which the balloon exploded biases the mean (C. Lejuez et al., 2002), the commonly used metric in the literature and in this report is an adjusted mean. Performance on the BART appears to be trait-like as it has demonstrated test-retest reliability (White, Lejuez, & deWit, 2008) and moderate heritability in adolescent males (Anokhin, Goloshevkin, Grant, & Heath, 2009). A recent three year longitudinal study found that risk-taking propensity increased across development in adolescents, and that the magnitude of such increases was associated with alcohol use (MacPherson et al., 2010). These results indicate that BART performance may change across development and that the degree of increase in risk taking on the BART may be most predictive of "real-world" risk-taking behaviors, including alcohol use.

The association of the BART with "real world" risk-taking and substance use behaviors has been supported in several studies. Specifically, the average number of 'pump' responses made by subjects on the BART has been positively associated with substance use and other health-related risk-behaviors (C. Lejuez et al., 2002), such as having ever tried a cigarette (C. W. Lejuez et al., 2005) or having ever tried 'ecstasy' (Hopko et al., 2006). Alcohol consumption was positively related to greater 'pump' responses on the BART among individuals with low novelty seeking and low harm avoidance (Skeel, Pilarski, Pytlak, & Neudecker, 2008). Recently, performance on the BART predicted alcohol consumption in a college student sample over and above delayed reward discounting and response inhibition (Fernie et al., 2010). A recent study of adolescent/young adults found that smokers were less likely than nonsmokers to take risks on the BART, even in situations where risk-taking in the task is considered adaptive [i.e., low risk balloons; (Dean et al., 2011)]. However, no studies to date have examined the relationship between performance on the BART and clinical indices of AUD among clinical samples of individuals reporting AUD symptomatology. Should the relationship between risk-taking on the BART and AUD remain positive across development and after the onset of alcohol problems, higher pump responses on the BART are expected to be associated with higher alcohol use and problem severity. This would support the idea

that risk-taking contributes to the maintenance and severity of alcohol dependence. To that end, the present study will test the relationship between risk-taking, measured by the BART, and alcohol use and related problems in a large community sample of problems drinkers.

Although the BART represents a promising behavioral measure of risk-taking, several aspects of performance on the BART remain unexplored in the human literature. For example, pre-clinical research has demonstrated that inter-trial variability may be a distinct phenotype measured within the task (Jentsch et al., 2010); that is, some subjects choose to accept a more stable level of risk throughout the task, while others explore various levels of responding, or may be more reactive to recent trial outcomes. These two performance characteristics (average pumps and variability of pumps) were shown to rely on separate brain circuitry in the rat, further corroborating their distinction as separate factors (Jentsch et al., 2010). Although separate, they are both important components that underlie the utility of an organism's behavior when faced with decisions made under risk, with highly variable behavior nearly always detracting from ideal outcomes.

Another interesting metric that emerges from the task is the average number of pumps on trials immediately following a balloon explosion. Unlike the more general mean pumps measure typically used in analysis, this post-failure measure allows insight into specific influences of negative feedback on the decision-making process as it is occurring. One crucial diagnostic component of alcohol dependence consists of continued use of alcohol despite knowledge of adverse physical, emotional, or social consequences directly due to alcohol use. Thus, one might expect that compulsive alcohol use may be associated with reduced reactivity to negative feedback in the BART. With this in mind, the present study will examine dimensions of the BART beyond average number of pumps, including response variability and post-failure responding. In summary, the present study utilizes a clinical sample of adult non-treatment seeking problem-drinkers to: (a) test the association between decision-making under risk with AUD symptomatology in a clinical sample using the BART; (b) examine sub-dimensions of BART performance such as inter-trial variability and post-failure reactivity as they relate to AUD measures; and (c) account for neurocognitive variables likely

associated with performance on the BART, such as IQ and working memory. Together, these analyses seek to further elucidate the association between performance on the BART and risky alcohol use in the real world.

2.3 Methods

2.3.1 Participants

Non-treatment seeking heavy drinkers ($N = 158$) were recruited from the Los Angeles community through flyers, print, and online advertisements as part of a larger, ongoing alcohol administration study. Inclusion criteria were: (1) age between 21 and 65; (2) self-identification of "problems with alcohol"; (3) telephone endorsement of consuming a minimum of 48 standard drinks per month. Exclusion criteria were: (1) current treatment for alcohol problems, history of treatment in the 30 days prior to enrollment, or currently seeking treatment; (2) not having an alcoholic drink within 21 days of the telephone screening interview; (3) history of bipolar disorder or psychotic disorder, or positive evaluation for these disorders during a structured diagnostic interview. Participants were compensated $40 for participation in the face-to-face assessment procedure, as well as up to an additional $5 based on performance on the BART (outlined below). The average age of the sample was 30.29 ($SD = 10.49$, range 21 to 63), with a majority of participants being male (71.9%). The ethnic background of the sample was as follows: White (46.7%), African American (20.4%), Asian (6.6%), Latino (11.8%), Other/Mixed-Ethnicity (11.2%), and ethnicity not given (3.3%). The average number of years of education was 14.8 ($SD = 2.26$).

2.3.2 Procedures

Interested individuals called the laboratory and completed an initial telephone screening interview for the inclusion and exclusion criteria outlined above. During this telephone interview, participants were asked about quantity and frequency of drinking and if they

had ever been diagnosed with one of the exclusionary psychiatric disorders, namely bipolar disorder or any psychotic disorder. Participants were also asked whether they wanted to receive any treatment now or had received any treatment for alcohol problems (including formal treatment and/or use of self-help groups) in the past 30 days and were excluded for positive answers. Treatment seekers were excluded as the next phase of the study included an alcohol administration. Those who did indicate a desire for treatment were provided with a referral packet. Eligible participants were invited to a face-to-face assessment session, which included the BART, as well as the individual differences and neurocognitive measures described below. During the in-person testing session prior to the assessment procedures, all participants provided written informed consent upon receiving a complete explanation of the study. Blood alcohol concentration (BAC) equal to 0.00, as verified by a Breathalyzer test (Drger, Telford PA), was required before assessment commenced. All procedures were approved by the Institutional Review Board of the University of California, Los Angeles.

2.3.3   Measures

Demographic information was collected, including age, sex, ethnicity, and education. In-depth assessment of alcohol use and alcohol-related symptoms was performed, along with the BART and the following neurocognitive and individual differences measures.

2.3.3.1   Alcohol Use and Related Symptoms

Alcohol dependence and the exclusionary psychiatric diagnoses (i.e., bipolar disorder or psychotic disorder) were assessed using the Structured Clinical Interview for DSM-IV [SCID; (First, Spitzer, Gibbon, & Williams, 1995)] by bachelor's-level interviewers or graduate students under the training and supervision of a licensed clinical psychologist (last author). Alcohol abuse and dependence symptoms were recorded, for a total of 11 possible DSM-IV symptoms. The 30-day timeline follow-back (TLFB) interview (Sobell & Sobell, 1980) was used to assess drinking behavior including detailed data on the quantity and frequency of al-

cohol use over a 30-day period. An alcohol binge was defined as consuming 4 or more drinks on a given episode for a woman and 5 or more drinks for a man. The following measures of alcohol use were derived from the 30-day TLFB and used in the analyses: (a) total drinking days; (b) average drinks per drinking day; and (c) total binge drinking days.

2.3.3.2   The BART

A modified version of the BART (C. Lejuez et al., 2002) was administered as follows. Participants were presented with a picture of a balloon on the computer screen and could press two keys: one to inflate the balloon ('pump'), and one to end the trial ('cash out') and move on to the next trial. Each balloon trial began at a value of $0.01. With each pump, the participant could earn a small amount of money ($0.003) that was tallied continuously, and the balloon would near-instantly inflate on-screen by a small amount. Participants chose at each pump whether to continue to inflate the balloon or to press the 'cash out' key to end the trial, add the money to the guaranteed 'bank', and begin the next trial. However, a certain amount of risk is applied to each pump, such that inflation to a certain point will cause the balloon to visibly explode on the screen resulting in a loss of money earned so far on that trial. Risk of balloon explosion was distributed following a normal distribution with a mean at the midpoint of possible pumps (32 of 64 possible pumps) and with a standard deviation of 20; this value is half that of several previous studies employing the BART, which allowed a full range of 128 pumps. Each session consisted of 72 trials, which is more than used previously [e.g., (Fernie et al., 2010; C. Lejuez et al., 2002, 2003; MacPherson et al., 2010)]. These modifications were made to decrease the possibility of participant fatigue on individual trials, while still obtaining data from a large number of trials for reliability purposes. Because we were interested in pursuing sub-measures of the task (including the mean of pumps occurring after a trial failure), it was necessary to ensure that across the session enough explosions would occur to generate reliable data even among the more conservative participants. The task was administered in MATLAB (v7.5) on a Macbook laptop.

Participants earned up to an additional \$5.00 based on their performance on the BART. This amount was chosen as a function of the proportion of time spent on the task in comparison to the full assessment visit, as only about 12 minutes of the three-hour visit were spent on the BART. Because inclusion of pumps made in trials that resulted in explosions may negatively bias the mean, the adjusted mean pumps (AMP) was used as a primary variable of risk-taking propensity. This is the standard adjusted average used in other studies using the BART. In addition to AMP, a measure of response variability was derived using the within-subject inter-trial variability of pumps at 'cash out', divided by AMP (as variability will naturally increase with the mean). This measure was included based upon recent pre-clinical reports that within-subject response variability in the BART is an indicator of optimized performance that is mechanistically-dissociable from mean pumps per trial (Jentsch et al., 2010). Hereafter, this variability measure will be referred to as VARAMP. The average number of pumps on trials immediately following a trial failure (balloon explosion) was also calculated and will be referred to as post-failure mean pumps (PFMP).

2.3.3.3 Neurocognitive and Individual Difference Measures

The following neurocognitive and individual differences measures of high relevance to BART performance were administered: (1) The Shipley Institute for Living Scale (Zachary, 1986) is a brief self-report measure which provides both verbal, performance, and total IQ estimates that are moderately correlated with the WAIS-derived full scale IQ; (2) The Digit Span Task is a working memory task in which participants are asked to recall various sequences of numbers, either recalling the numbers from first to last (Digits Forward) or recalling the numbers from last to first (Digits Backward). This is a classic working memory task that captures individuals' abilities to cognitively retain and manipulate information. Norm-referenced scores from the Shipley scale and digit span task were used in the analyses as estimates of IQ and working memory, respectively.

2.3.4 Data Analytic Plan

Prior to the main analyses, all variables were tested for the distributional assumptions and were transformed, including norm-based transformations, as needed. For t-tests, comparisons that violated the homogeneity of variance assumption were remedied using the Satterthwaite corrected degrees of freedom. All comparisons and analyses were performed using SAS (v9.2) running on a PC. Primary analyses employed Pearson Product Moment Correlations and regression analyses using the General Linear Model to test the association of the individual dimensions of BART performance and measures of alcohol consumption (TLFB) and symptoms of alcohol abuse and dependence (Symptom Count). Age, sex, IQ, and WM were added as covariates to the models regressing alcohol use and symptoms on BART indices. These covariance models were introduced when significant associations between BART performance and alcohol measures were obtained in order to further probe for the validity of the univariate findings. As these four variables fall into two separate domains (demographic variables and neurocognitive variables), two separate models were tested. The first tested for the explanatory contribution of the demographic control variables (age and sex) to the main relationship between AUD symptomatology and risk-taking, while the second model tested inclusion of neurocognitive control variables (IQ and WM).

Given the number of variables being compared, corrections for multiple comparisons were made following the recommendations of Dar, Serlin and Omer (1994). To that end, we adjusted for the number of hypotheses being tested as opposed to the number of variables indexing these hypotheses. Since we are testing for the relationships between three dimensions of the BART (i.e., mean adjusted pumps, response variability, and post-failure mean pumps) and two levels of alcohol misuse (alcohol use and alcohol problems), we have corrected for p-value for 6 planned comparisons/hypotheses. Thus, the p value required for significance was $p = 0.0083$. Lastly, in order to further elucidate the multivariate nature of the associations among BART performance, alcohol use and problems, and the demographic and neurocognitive covariates, mediation and moderation models were examined where appropriate (Baron & Kenny, 1986). The Sobel test was used to determine mediation (Sobel,

1982) using available online software (Preacher & Leonardelli, March 2001).

2.4 Results

2.4.1 Descriptive Statistics

Six subjects were removed from the analyses as a result of positive assessments for either bipolar disorder or psychosis, as determined by the SCID, leaving a total of 152 subjects in the analyses reported herein. Of those, 72.3% met criteria for alcohol dependence, 15.5% met DSM-IV criteria for alcohol abuse only, 9.5% were diagnostic orphans (i.e., endorsed 1 or 2 dependence symptoms but did not meet diagnostic criteria for either alcohol abuse or dependence), and 2.7% did not endorse any symptoms of either alcohol abuse or dependence. Participants consumed an average of 6.49 standard drinks per drinking day ($SD = 4.5$, Range: 1.7 to 34.3), with an average of 128.4 drinks ($SD = 101.8$, Range: 5 to 549.4) over the past 30 days.

Means and standard deviations of BART performance and other study measures (e.g., norm-referenced IQ and digit span scores, alcohol consumption data) are presented in Table 2.1, along with correlations among them. Participants demonstrated a range of performance on the BART in terms of AMP ($M = 18.61, SD = 4.30$), with some being more variable between trials than others ($VARAMP, M = 1.23, SD = 0.89$). Nearly all participants were generally characterized as risk averse, falling on the lower half of potential pumps, consistent with previous samples (Hopko et al., 2006; C. Lejuez et al., 2002, 2003; C. W. Lejuez et al., 2004; Skeel et al., 2008; White et al., 2008). Participants earned on average \$3.48 ($SD = \$0.57$) during the task and took an average of 12.33 minutes ($SD = 3.75$ minutes) to complete it. Across all 72 trials, participants exceeded the risk function resulting in a balloon explosion an average of 17.18 times ($SD = 8.96$).

Table 2.1: Means, Standard Deviations, and Correlations Among Study Variables. * Indicates significance at $p < 0.0083$. ** at $p < 0.0001$. The first value was chosen as the corrected significance level based on the number of planned comparisons (see Data Analytic Plan)

Variable	Mean	SD	1	2	3	4	5	6	7	8	9
Demographic Information											
1. Age	30.01	10.41	-								
BART Performance Measures											
2. Adjusted Mean Pumps	18.61	4.32	-0.359**	-							
3. Variability of Pumps	1.25	0.84	0.038	-0.305**	-						
4. Post-Failure Pumps	17.92	3.86	-0.357**	0.873**	-0.114	-					
Individual Difference Measures											
5. IQ	100.2	20.56	-0.536**	0.449**	-0.164	0.395**	-				
6. Digit Span	10.38	3.07	-0.319**	0.345**	-0.151	0.318**	0.629**	-			
Alcohol Use and Pathology Measures											
7. AUD symptom count	5.36	2.93	0.457**	-0.228*	-0.022	-0.317**	-0.304*	-0.176	-		
8. Total Drinking Days	18.02	7.73	0.389**	-0.118	-0.068	-0.128	-0.238*	-0.126	0.416**	-	
9. Drinks per Drinking Day	6.95	4.52	0.212	-0.119	-0.085	-0.241*	-0.224*	-0.108	0.286*	0.068	-
10. Total Binge Drinking Days	12.3	8.52	0.440**	-0.096	-0.127	-0.188	-0.139	-0.049	0.547**	0.714**	0.413**

Sex differences in all study variables were considered in an independent samples t-test (Table 2.2). Men and women did not significantly differ in BART performance in terms of the mean adjusted pumps or post-failure mean pumps. The only measures for which there was a significant sex effect were number of drinking days in the past 30 days, and number of binge days, suggesting heavier drinking in males versus females.

Table 2.2: Sex differences across study variables

Variable	Women (SD)	Men (SD)	df	t value
Age	28.52 (9.39)	30.65 (10.80)	143	-1.13
AMP	17.74 (3.14)	18.95 (4.67)	116	-1.81
VARAMP	1.30 (0.80)	1.23 (0.86)	140	0.45
PFMP	17.43 (2.64)	18.08 (4.24)	120	-1.1
IQ	98.56 (21.28)	101.25 (20.14)	142	-0.71
WM	9.82 (2.73)	10.65 (3.19)	142	-1.5
Count	4.66 (3.00)	5.66 (2.88)	144	-1.89
Drink Days	15.79 (6.87)	20.56 (7.74)	144	-2.40*
DPDD	6.00 (5.88)	7.37 (3.80)	59	-1.42
Binge Days	9.50 (6.83)	13.87 (8.72)	144	-2.96**

Participants' performance on the BART is illustrated in Figure 2.1, which relates AMP to money earned over the entire session. The optimal performance curve was calculated by determining the expected reward accrued given the expected percent of failed trials at any given level of risk-taking. As can be seen, most participants' mean responses fell to the left of the curve and many participants demonstrated suboptimal behavior by receiving much less money than would be expected. Split-half correlation of the means of pumps from the first and last half of trials showed that participants' behavior was consistent across the full session, $r(150) = 0.826, p < 0.001$. As an additional confirmation of within-subject reliability across the session, a paired samples t-test was conducted comparing AMP of the first and second halves; participants showed no significant difference between these two halves, $t(151) = 0.76, p = 0.45$.
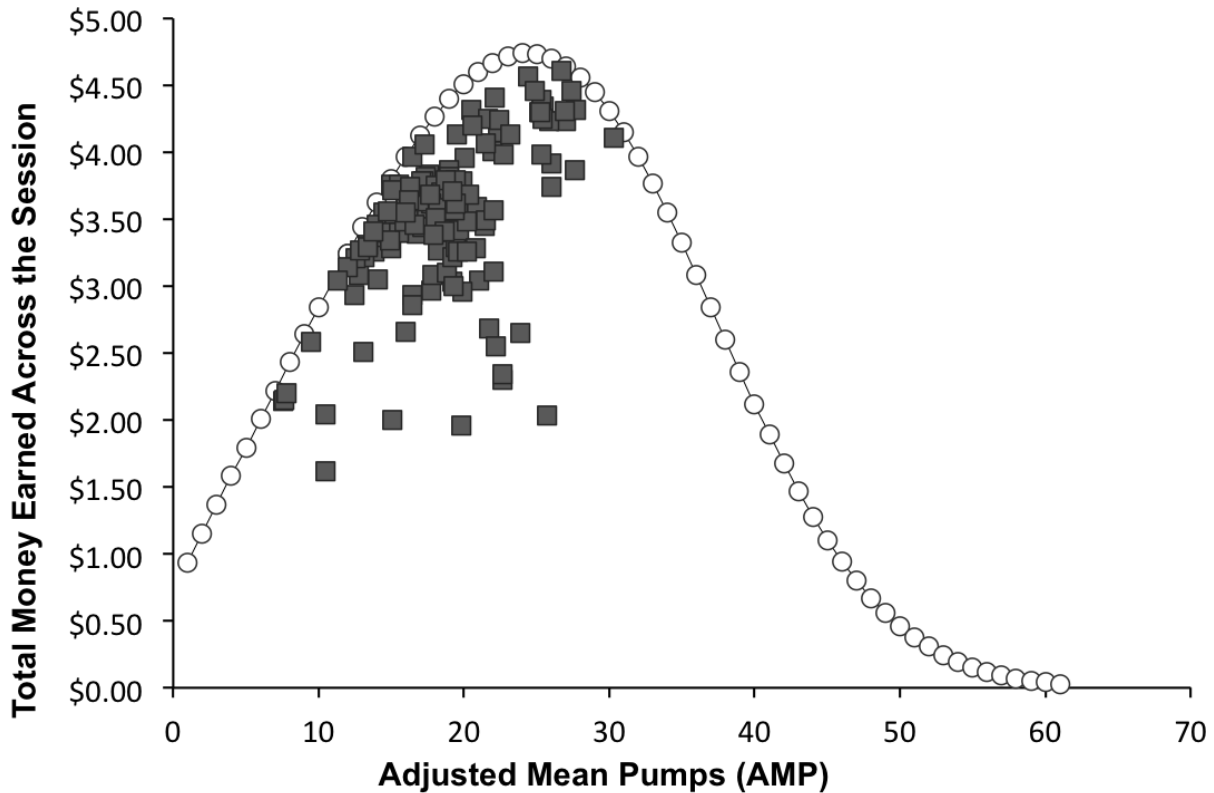
Figure 2.1: Individuals' Performance on the BART. Each dark square represents an individual participant. The curve represents an optimal function based on the chance of failure at any given mean of pumps and the expected amount of money accrued over an entire session.

2.4.2 BART Performance Indices and AUD Symptoms

AUD symptom count was significantly and negatively correlated with AMP (Table 2.1). The observed correlation falls in the range of medium effect size (Cohen, 1988, 1992). AMP was not significantly associated with number of drinking days, drinks per drinking day, or number of binge days. PFMP was also significantly and negatively associated with symptom count, as well as with average drinks per drinking day, but not number of binge days. VARAMP was not associated with any alcohol measures. Complete results are presented in Table 2.1.

2.4.3 Probing the Relationship between BART and AUD: Demographic and Neurocognitive Controls

Analyses were conducted to further probe the univariate associations obtained. Specifically, significant associations between dimensions of the BART and alcohol use disorders symptoms and alcohol use were investigated after controlling for relevant demographic (gender and sex) and neurocognitive (IQ and WM) variables (see Table 2.3). Results for demographic control variables revealed that adding sex and age to the regression model attenuated the negative relationship between symptom count and AMP. However, the association between PFMP and symptom count remained significant after controlling for age and sex. Additionally, PFMP remained significantly associated with drinks per drinking day when sex and age were added to the model. Analyses controlling for neurocognitive variables revealed that the negative relationship between symptom count and AMP was attenuated by adding estimated IQ and WM to the model. Unlike the relationship observed with AMP, the association between PFMP and symptom count remained significant when controlling for WM and IQ. Lastly, covariate analysis was conducted for the association between drinks per drinking day and PFMP. The relationship between PFMP and drinks per drinking day (DPDD) remained significantly, and negative in nature, even after controlling for IQ and WM indices.

2.4.4 Analyses of Mediation and Moderation Effects

In order to further elucidate the multivariate nature of the associations among BART performance, alcohol use and problems, and the demographic and neurocognitive covariates (i.e., age, sex, IQ, and working memory), mediation and moderation models were tested. Results revealed no significant moderation effects of age, sex, IQ, or working memory ($ps > .10$) in the relationships between performance on the BART and alcohol use and problems demonstrated above.

Mediation effects for age, sex, IQ and WM were examined as proposed by Baron and

Table 2.3: Regression models that included both demographic and neurocognitive covariates as control variables were tested. AMP = adjusted mean pumps; PFMP = post-failure mean pumps; WM = working memory. * indicates $p < 0.05$, ** indicates $p < 0.01$.

Dependent Variable: Symptom Count			
Demographic Controls	β	**Standard Error**	**p value**
Model 1:			
AMP	-0.06	0.06	0.27
Sex	0.84	0.49	0.09
Age	0.11	0.02	< 0.001**
Model 2:			
PFMP	-0.14	0.06	0.03*
Sex	1.02	0.47	0.03*
Age	0.11	0.02	< 0.001**
Neurocognitive Controls			
Model 1:			
AMP	-0.08	0.06	0.21
IQ	-0.04	0.02	0.01*
WM	0.05	0.1	0.64
Model 2:			
PFMP	-0.17	0.07	0.01*
IQ	-0.04	0.02	0.006**
WM	0.07	0.1	0.64

Dependent Variable: Drinks per Drinking Day			
Demographic Controls	β	**Standard Error**	**p value**
Model 1:			
PFMP	-0.25	0.11	0.02*
Sex	1.68	0.83	0.04*
Age	0.055	0.04	0.17
Neurocognitive Controls			
Model 2:			
PFMP	-0.22	0.11	0.04*
IQ	-0.05	0.02	0.05*
WM	0.12	0.16	0.45

Kenny (1986). In brief, this approach consists of examining univariate relationships between (a) the independent variable (IV) and the proposed mediator, (b) the IV and dependent variable (DV), and (c) between the DV and mediator. Should all univariate paths of the model be significant, a multivariate model is tested in which both the IV and mediator are simultaneously tested as predictors of the DV. Mediation is thought to occur when the magnitude of the IV to DV relationship is significantly attenuated by the mediator. A Sobel Test was used to formally test the mediation effects (Preacher & Leonardelli, March 2001; Sobel, 1982).

As can be seen in Table 2.1, all univariate paths are significant for the associations between BART performance (IV; measured by AMP and PFMD), age (mediator), and symptom count (DV). As such, mediation analyses were justified for these variables. Multivariate analyses revealed that the association between BART performance and symptom count is significantly attenuated by adding age to the model, providing initial evidence of mediation. Sobel tests confirmed that age significantly mediated the relationship between AMP and symptom count (Sobel Test $= -3.65, p < .001$) and the relationship between PFMP and symptom count (Sobel Test$= 3.61, p < .001$). Moreover, analyses of IQ as a putative mediator revealed that it significantly explained the relationship between AMP and symptom count (Sobel Test $= -3.22, p < .01$), between PFMP and symptom count (Sobel Test $= -3.05, p < .01$), and between PFMP and drinks per drinking day (Sobel Test $= -2.42, p < .01$). All univariate relationships can be seen in Table 2.1. Together, these analyses suggest that age and IQ serve as mediators of the relationships between performance on the BART and alcohol problems in this sample.

2.5   Discussion

This study sought to examine, for the first time, the relationship between performance on the BART and AUD symptoms in a community sample of problem drinkers. These analyses also included relevant covariates in order to more fully characterize the main effects

of BART performance on AUD symptomatology and drinking behavior. Analyses revealed that participants reporting more AUD symptoms were more conservative on the BART, as indexed by average pumps (AMP). The observed association was in the range of medium effect sizes (Cohen, 1988, 1992) and remained significant, albeit marginally, when controlling for working memory. Importantly, when controlling for IQ and demographic measures, the association between AMP and symptom count was no longer significant. Together, these results suggest that among individuals with AUD, higher risk-taking propensity, measured by the BART, is associated with lower symptom count, but that IQ and age account for a substantial proportion of the variance in BART performance and ultimately mediate this association. Thus, in order to fully understand the relationships between risk-taking and AUD symptomatology, age and IQ must be taken into account.

This finding is in contrast to studies of subclinical and adolescent samples reporting a positive relationship between BART performance and risk-taking behaviors, including substance use and abuse (Hopko et al., 2006; C. Lejuez et al., 2002; C. W. Lejuez et al., 2005; MacPherson et al., 2010; Skeel et al., 2008). However, these results are consistent with a recent report showing that adolescent smokers were more conservative on the BART than nonsmoking controls (Dean et al., 2011). Furthermore, AMP did not relate to alcohol consumption variables, such as drinking frequency, quantity, and binge drinking. It may be that there was not sufficient variability in drinking patterns to detect BART effects. However, there was considerable variability in the degree to which participants reported problems associated with alcohol use, which afforded greater statistical power to detect associations with alcohol use disorders symptomatology. The effects of age on BART performance are in contrast with the prospective study documenting increasing risk-taking in BART performance over time in an adolescent cohort (MacPherson et al., 2010). In the current study, age was inversely correlated with risk-taking on the BART and mediated the relationship between AMP and symptom count. The average age of the sample was 30 years old; therefore, the current findings, in conjunction with the existing adolescent literature, suggest that developmental and age considerations may be central to understanding the predictive utility of

risk-taking measured by the BART and alcohol use disorders.

2.5.1 Examination of Multiple Dimensions of the BART

The measure of inter-trial variability (VARAMP) was not associated with alcohol use and problems in this sample. This suggests that within this sample, variability in responding was not a useful phenotype for understanding AUD symptoms or drinking behavior. Although not predictive of alcohol problem severity among individuals currently exhibiting AUDs, these data support the hypothesis that the response variability phenotype is dissociable from the traditional AMP measure in human samples, which is consistent with recent pre-clinical findings (Jentsch et al., 2010). It remains to be seen if this lack of association is unique to this sample of problem drinkers or if this extends to social drinkers or younger samples of alcohol and drug users.

The measure of PFMP generally showed a larger effect size in relationship to alcohol use and related problems than did AMP. This includes a significant negative association with drinking behavior in terms of average alcohol units consumed per episode and number of binge days during the assessed period. Furthermore, this relationship survived control for demographics, IQ, and working memory. Indeed, IQ was also found to mediate this relationship. This indicates that responses to negative feedback (i.e., explosions) in the context of the BART may be a useful predictor of alcohol problems and consumption and that this association may be accounted for by differences in IQ. As with the main AMP measure, the relationship is negative, such that those who accepted more risk, on average, after a trial-failure had fewer problems and drank less. Thus, the ability to accept more risk after recent failures may be protective against alcohol problems, or more severe AUD symptomatology may associate with greater affective responses to failure. Interestingly, the recent study of adolescent smokers and nonsmokers also found a significant and positive association between verbal IQ and mean pumps on high risk balloons (Dean et al., 2011), although mediation analyses were not conducted.

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Given the cross-sectional nature of the study it is not possible to ascertain the direction of causality in these associations. For instance, it may be the case that the more risk-averse subset of heavy drinkers is more likely go on to develop AUD. This model would imply that in adulthood, risk-taking is a stable trait despite environmental influence, including alcohol use itself, and that a more conservative risk-taking profile represents a risk factor for the development of more severe AUD. Alternatively, it may be the case that the negative relationship between risk-taking on the BART and alcohol symptoms may be caused by the biobehavioral effects of extensive alcohol use itself. In other words, although higher risk-taking measured by the BART may be a liability factor for the onset of (heavy) alcohol use in a subset of individuals (C. Lejuez et al., 2002; MacPherson et al., 2010; Skeel et al., 2008), as alcohol use progresses, the relationship may reverse direction. Thus, the trait of risk-proneness as assessed by the BART may change across development and upon chronic alcohol exposure.

The plausibility of this hypothesis is supported by recent work demonstrating changes in risk-taking propensity across adolescence and young adulthood (MacPherson et al., 2010), confirming that this trait is liable to change. This conceptualization may also be consistent with the allostatic model of addiction in which binge-intoxication represents the early, impulsive stages of the disorder (G. F. Koob, 2003). This is also consistent with developmental psychopathology models of alcohol misuse, in which risk-taking propensity plays an important role in alcohol use initiation and transition to heavy drinking in youth (S. A. Brown et al., 2008).

Two possible mechanisms may underlie such a change in risk-taking as alcohol dependence progresses. The first is a direct pharmacological effect of repeated alcohol intoxication. The second is a negative feedback mechanism, such that those with more severe dependence then develop a more conservative temperament as a result of the negative consequences experienced during the onset and continuation of dependence. Both of these would be important hypotheses to test in future studies. Indeed, pharmacologically-induced neurocognitive effects of chronic alcohol use are plausible as IQ estimates were negatively associated with

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symptom count; participants who drank more and had more problems due to alcohol use tended to have lower estimated IQ scores and WM span. Although this is an association and the causal relationship cannot be determined here, this further supports the plausibility that chronic alcohol exposure may result in neurocognitive deficits with behavioral ramifications.

Despite these likely alcohol-related neurocognitive deficits, the inverse relationship between PFMP and symptom count remained significant even after controlling for WM span and IQ. Thus, over and above more global neurocognitive deficits associated with chronic alcohol use, it is possible that alcohol misuse itself may alter risk-taking behavior towards risk aversion in the BART context, possibly through a pharmacological mechanism. Lastly, an intriguing alternative explanation offered by Dean et al. (2011), consists of the notion that since most participants did not exceed the optimal reward/risk ration for the task (see Figure 2.1), participants who pumped more on the task often made more money, which in turn suggests that taking risks on the BART may represent an adaptive response. This conceptualization would be consistent with the mediational findings for IQ and suggest that individuals with higher IQ are better able to perform optimally on the BART (i.e., accept more 'adaptive risk'), which in turn is negatively associated with AUD outcomes. Delineating the optimal and even adaptive level of risk-taking clearly warrants further investigation.

2.5.2   Design Considerations and Future Directions

Regardless of the direction of causality, it appears that having a high risk-taking trait (at least as can be measured in the laboratory by the BART) may not contribute to AUD severity in the later stages of AUD development above and beyond its initial contribution to initiation of substance use. Prospective studies are certainly needed to empirically address the research questions raised by the study findings. These should consist of testing the pharmacological and negative feedback mechanistic hypotheses, as well as include age-matched non-AUD and abstinent community participants to consider the full range of alcohol use as it relates to the traditional AMP measure as well as the post-failure (PFMP) measure; these will greatly contribute to our understanding of how the risk-taking construct influences the development

of AUD symptomatology, from initiation of alcohol use to severe dependence, as well as how this reflects underlying AUD etiology. Future studies should also include assessments of IQ and WM, as these are clearly important control variables when evaluating behavior in the BART.

This study has a number of strengths and limitations. Study limitations include the cross-sectional design and a sample comprised of individuals already exhibiting alcohol problems who are not seeking treatment. This may hinder direct comparison to other at-risk and non-dependent samples already phenotyped for risk-taking propensity and alcohol-related behavior. Next, the imbalance of sex may have reduced the power of detecting a sex effect on BART performance or moderation effects of sex, BART and AUD symptoms. However, the only such effect reported in the literature was found in subjects under acute stress (Lighthall, Mather, & Gorlick, 2009).

As the version of the BART employed in this study differed from the traditional version of the task, it is important to consider if the effects observed might be specific to this task variant. The BART used here had a larger number of trials (72), a shorter range of potential pumps within a trial (64), and a low cash value for a single pump (0.03 cents) while previous versions have typically included 30 trials on one type of balloon and a range of 128 pumps [e.g., (Fernie et al., 2010; C. Lejuez et al., 2002; C. W. Lejuez et al., 2005; MacPherson et al., 2010; Skeel et al., 2008)]. Data from several variants of the task have already been published describing limited effects of task modifications. In the original task development study (C. Lejuez et al., 2002), balloons with potential ranges of 8, 32, and 128 pumps were concurrently tested and results revealed that the limited range balloons (8 and 32) showed no relationship with self-reported real-world risk behaviors as variability in performance was too limited. Furthermore, multiple task versions including modifications beyond number of trials and range of potential pumps have been published with no obvious or reported effects on participant behavior (Pleskac et al., 2008; Zacny & de Wit, 2009). While the issue of task variants should be kept in mind when comparing results across studies, participants do show consistently risk-averse behavior, and the effects of varying the task generally appear to alter

the variability observed in participant performance rather than altering the relationships per se.

Study strengths include the community sample of individuals with alcohol problems, including a large number of dependent individuals, as no studies addressing alcohol use and the BART published to date have sampled from a clinical population. In addition, this study employed a semi-structured diagnostic interview and included multiple relevant neurocognitive covariates, such as IQ and working memory, which appear to be critical to evaluating the relationship between risk-taking and AUD.

On balance, this study advances the understanding of risky decision making and AUDs by (1) employing a well-validated behavioral measure of risky decision making, the BART; (2) examining BART performance in a clinical sample phenotyped for diagnostic and neurocognitive measures of interest; and (3) considering multiple facets of BART performance, including response variability and post-failure reactivity. Results revealed a negative relationship between risk-taking on the BART and alcohol symptoms and problems, suggesting that although this behavior analog measure was positively associated with alcohol and substance use in non-clinical samples, the direction of the relationship may be reversed in clinical samples, such that individuals with more alcohol pathology are more risk averse. Importantly, IQ estimates were found to mediate the observed relationships between BART performance and AUD symptoms/alcohol use. Whether the observed reversal of the relationship between risk-taking and alcohol pathology reveals a cause or a consequence of chronic alcohol use, or rather reflects different stages of addiction, remains to be empirically determined.

CHAPTER 3

Modeling Behavioral Reactivity to Losses and Rewards on the Balloon Analogue Risk Task (BART): Moderation by Alcohol Problem Severity

3.1 Abstract

Background: The relationship between risk-taking behavior and substance dependence has proven to be complex, particularly when examining across participants expressing a range of substance use problem severity. While main indices of risk-taking in the Balloon Analogue Risk Task (BART) positively associate with problematic alcohol and drug use in adolescent and sub-clinical populations (e.g., (MacPherson et al., 2010)), the relationship is the opposite direction when examining behavior within adult substance using populations (Ashenhurst et al., 2011).

Methods: In order to examine potential mechanisms that underlie this negative relationship, we implemented multilevel regression models on trial-by-trial BART data gathered from 295 adult problem drinkers. These models accounted for participant behavior on trials following balloon bursts or cash outs as indices of loss and reward reactivity, respectively, and included control variables including age, IQ, and individual delay discounting rate.

Results: Results revealed that individual trial pumping was significantly predicted by trial number, and by whether or not the previous trial was a big burst or a big cash out (i.e., large magnitude of potential gains) in a manner consistent with a 'near-miss' effect. Furthermore, severity of alcohol problems moderated the effect of a previous trial big burst,

but not of a big cash out, on subsequent trial behavior such that those with greater severity demonstrated relative insensitivity to this 'near-miss' effect.

Conclusions: These results extend previous studies suggesting that alcohol abusers are less risky on the BART by specifying a mechanism underlying this pattern, namely, diminished reactivity to large magnitude losses.

3.2 Introduction

Alcohol use disorders are complex and multidimensional and may be understood, in part, by examining individual differences in endophenotypes, which are defined as heritable, biologically regulated behaviors or biomarkers that associate with disorder liability (Ducci & Goldman, 2008; D. Goldman et al., 2005). Given that clinical criteria for alcohol (and other substance) dependence focus heavily on continued use of the substance despite knowledge of the occurrence and risks of adverse health, legal or social outcomes (APA, 2000), researchers have posited that propensity for risk-taking, driven in part by genetic factors, may enhance liability for problematic substance use (Kreek et al., 2005). While debate exists regarding the precise definition of risk-taking propensity, we view it as a pattern of maladaptive choice behavior produced under conditions where there exists a potential for reward but also an unknown probability of negative outcomes. This definition is consistent with the idea that risk-taking propensity may be a contributing factor to substance dependence liability, as continued substance use represents a decision to engage in maladaptive risky behavior.

One increasingly popular task used to assess risk-taking propensity is the Balloon Analogue Risk Task (BART), which, as a behavioral task, is not subject to self-report bias (C. Lejuez et al., 2002). In this task, participants inflate a virtual balloon with a small potential payout per pump. However, the balloon may burst at any time, resulting in a forfeiture of earned money for that trial. Risk-taking is thus indexed by increased reward seeking in the face of greater potential loss. Consistent with behavior in this task being a potential endophenotype, twin studies and rat breeding studies have demonstrated that

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risk-taking behavior measured by the BART is moderately heritable (Anokhin et al., 2009; Ashenhurst et al., 2012).

Research using this task to examine the role of risky decision-making in substance use and misuse has yielded a complex picture, particularly when examining individuals expressing differences in substance use problem severity(Ashenhurst et al., 2011) and across stages of substance use involvement. In sub-clinical and adolescent populations, increased risk-taking in the BART is associated with increased problematic alcohol and drug use (Fernie et al., 2010; C. Lejuez et al., 2002; C. W. Lejuez et al., 2005; MacPherson et al., 2010). However, our group has demonstrated that risk-taking in the BART is negatively correlated with the severity of clinical alcohol dependence as defined by DSM-IV criteria in adults with a range of alcohol use problems (Ashenhurst et al., 2011). A similar negative relationship was also described in a sample of young tobacco smokers, whereby smokers were less risk-taking on the BART than non-smokers in terms of trajectory of balloon pumping across the test session (Dean et al., 2011). Additionally, among adult smokers, tobacco dependence was negatively correlated with risk-taking in the BART (Ryan et al., 2013). Together these studies suggest that behavioral patterns in the BART may change depending on the stage or severity of substance use problems, yet the specific mechanisms explaining these differences remain unclear.

Thus, the primary aim of this study was to implement a detailed trial-by-trial analysis of behavior in the BART to identify behavioral mechanisms that may explain why participants at greater levels of alcohol problem severity take less risk in the BART (Ashenhurst et al., 2011). In most analyses of the BART, data is tabulated as means across trials (calculating mean pumps on non-burst trials for a given administration of the BART), which fails to capture the trial-by-trial reactivity and learning that is occurring during the progression of the task. Indeed, powerful novel multi-level regression-based approaches to individual trial data have yielded interesting results associating dopamine transporter gene variation with behavior in the BART (Mata et al., 2012). Our first study aim was to replicate this model but instead of examining dopamine genetics, we examined whether alcohol problem severity

moderates reactivity to bursts on the BART.

The second study goal is to expand upon this modeling of trial-by-trial reactivity on the BART by including both reward and loss magnitude to see whether magnitudes of the previous gambles (e.g. bursts that resulted in a large forfeiture) influenced participant behavior. Furthermore, we sought to test whether the influence of reward and loss magnitude was moderated by alcohol problem severity. Considering findings from another risk-taking task, the Iowa Gambling Task (J. C. Stout et al., 2004), our initial hypothesis was that individuals with more alcohol-related problems would be less loss reactive (i.e. modulate their behavior less following balloon burst trials) and more reactive to cash outs (i.e. increase pumping more following large cash outs trials) than those reporting less severe alcohol problems.

The third study aim was to account for previously identified variables that influence performance of the BART, thus ruling out potential confounds. These control variables include demographic and neurocognitive indicators such as age, IQ, and working memory span (Ashenhurst et al., 2011). Furthermore, Dean et al. (2011) have suggested that the negative relationship between substance use and risk-taking in the BART may be due to the risk-taking being confounded with delay discounting since greater amounts of reward-seeking on trials in the BART (the outcome typically associated with more risky decisions) requires persistence and patience for a future (often small) reward. Consistent with this view, our group has shown, using a structural equation modeling approach, that risk-taking in the BART is negatively associated with delay discounting rates among problem drinkers. Importantly, performance on the BART and the DDT were both related to alcohol problems, but in opposing directions (Courtney, Arellano, Barkley-Levenson, Glvan, et al., 2012); risk-taking in the BART was negatively associated with greater alcohol problems, while delay discounting had a positive relationship. Thus, we included individual delay discounting rates as an additional control.

This study is the first, to our knowledge, to model both loss and reward reactivity on the BART directly. Additionally, this represents the first attempt to test if severity of alcohol problems is associated with behavioral reactivity to bursts and cash outs of differing

reward/loss magnitudes in the BART as assessed in a large community sample of heavy drinkers expressing a range of alcohol use problems. These analyses advance the literature by examining behavioral mechanistic explanations for the previously identified negative relationship between risk-taking propensity and alcohol problem severity (Ashenhurst et al., 2011) while controlling for potential confounding factors.

3.3 Methods

3.3.1 Participants

Non-treatment seeking heavy drinkers (N = 295) were recruited from the Los Angeles community through flyers, print, and online advertisements as part of a larger, alcohol administration study. A subset of these participants (approximately half) were included in a previous report (Ashenhurst et al., 2011). Inclusion criteria were: (1) age between 21 and 65; (2) self-identification of 'problems with alcohol'; (3) telephone endorsement of consuming a minimum of 48 standard drinks per month. Exclusion criteria were: (1) current treatment for alcohol problems, history of treatment in the 30 days prior to enrollment, or currently seeking treatment; (2) not having an alcoholic drink within 21 days of the telephone screening interview; (3) history of bipolar disorder or psychotic disorder, or positive evaluation for these disorders during a Structured Clinical Interview for DSM-IV (SCID; (First et al., 1995). Participants were compensated $40 for research participation as well as up to an additional $5 based on performance on the BART (outlined below). The average age of the sample was 30.78 ($SD = 10.31$, range 21 to 63), with a majority of participants being male (73.14%). The ethnic background of the sample was as follows: White (42.9%), African American (18.8%), Asian (5.9%), Latino (13.5%), Native American (1.4%), Other/Mixed-Ethnicity (15.3%). The average number of years of education was 14.0 ($SD = 3.92$). Descriptive statistics for alcohol use/problem indicators are presented in Table 1.

3.3.2 Procedures

Eligible non-treatment seeking individuals were invited to the laboratory for an in-person evaluation session, which included: the BART, the individual differences and alcohol problem severity measures described below, and a structured diagnostic interview. All participants provided written informed consent upon receiving a complete explanation of the study. Participants were required to have a blood alcohol concentration (BAC) equal to 0.000 g/dl, as verified by a Breathalyzer test (Drager, Telford PA), prior to the testing session. All procedures were approved by the Institutional Review Board of the University of California, Los Angeles.

3.3.3 Measures

Participants were given a demographic questionnaire including: age, sex, ethnicity, and education. Additional study measures included those used to compute an alcohol problem severity score (below), the Balloon Analogue Risk Task (BART), and the Delay Discounting Task.

3.3.3.1 Alcohol Problem Severity

Severity of alcohol problems was indexed via a principle component score capturing a number of alcohol problems scales described by our group previously (Moallem, Courtney, Bacio, & Ray, 2013; Ray et al., 2013). Components included in the severity factor were the following. We used the Structured Clinical Interview for DSM-IV (SCID; (First et al., 1995)) to identify symptoms of alcohol abuse and alcohol dependence. These were recorded for a total of 11 possible symptoms (4 of abuse and 7 of dependence). Alcohol withdrawal was assessed using the Clinical Institute Withdrawal Assessment – Alcohol Revised(Puz & Stokes, 2005). The Penn Alcohol Craving Scale (PACS) captured craving for alcohol during the previous week (Flannery, Volpicelli, & Pettinati, 1999). A total score was also calculated from the Alcohol Dependence Scale (ADS; (Skinner & Allen, 1982), a 25-item

scale that measures alcohol dependence symptoms over the past 12 months. The Drinker Inventory of Consequences [DrInC-2R; (Miller, Tonigan, & Longabaugh, 1995)] provided a baseline description of the number and frequency of various drinking consequences, which was summed into a single indicator of negative drinking consequences. Under a principal component analysis, these five indicator variables comprised a single meaningful factor that explained 55% of the variance in alcohol problem indicators, with each indicator loading on the single factor > 0.40.

In previous analyses from our research group, this alcohol problem severity construct was related to subjective response to alcohol in an alcohol challenge (Ray et al., 2013), to affective symptoms and motivation to change (Moallem et al., 2013), and with fronto-striatal functional connectivity during performance of the Stop Signal Task (Courtney, Arellano, Barkley-Levenson, Galvan, et al., 2012). For the present analyses, alcohol problem severity factor scores were centered and normalized to the sample.

3.3.3.2 The Balloon Analogue Risk Task (BART)

A modified version of the BART (C. Lejuez et al., 2002) was administered as described previously (Ashenhurst et al., 2011). Briefly, participants were allowed to 'pump' a virtual on-screen balloon and earn a small amount of money ($0.003) for each pump; these rewards are tallied continuously. At any point, the participant may stop pumping, add the earned reward to a guaranteed bank, and proceed to the next trial (a 'cash out'). However, a certain level of risk was applied such that additional pumping might result in an on-screen burst of the balloon and a forfeiture of money earned for that one 'burst' trial. Risk of balloon burst was distributed following a normal distribution with a mean at the midpoint of possible pumps (32 of 64 possible pumps) and with a standard deviation of 20. At the end of 72 trials, participants were compensated up to $5 based on their accumulated earned totals. We chose this compensation rate as only about 12 minutes of the three-hour visit were spent on the BART. Instead of collapsing all trial-by-trial data into single outcome measures as used previously (Ashenhurst et al., 2011; C. Lejuez et al., 2002), behavior on each trial was

tallied for each participant, and entered into a multi-level regression model similar to one published previously (Mata et al., 2012) as described below in data analysis.

3.3.3.3 Control Variables

In previous analysis from our group, we identified several neurocognitive variables that were related to performance in the BART and alcohol problems (Ashenhurst et al., 2011) including: (1) The Shipley Institute for Living Scale (Zachary, 1986) as an estimate of IQ; (2) The Digit Span Task as a classic working memory task that captures individuals' abilities to cognitively retain and manipulate information. Norm-referenced scores from the Shipley scale and digit span task were used in the analyses as estimates of IQ and working memory, respectively.

Lastly, we included scores from the Delay Discounting Task to account for differences in temporal discounting. Participants were presented with a series of 27 hypothetical monetary choices, and were asked to indicate their preferences between them. These choices were between small immediate rewards versus larger delayed rewards (e.g., \$31 today, or \$85 in seven days). The parameters of these options were selected from a previously validated measure of delay discounting (Kirby, Petry, & Bickel, 1999). Participants were not compensated based on their choices, but were asked to consider them as real.

Delay discounting rates were computed by analyzing choice patterns fitted to the hyperbolic discounting functions derived from the following equation: $V = A/(l + kD)$, where V is the present value of the delayed reward A at delay D, and k is a free parameter that determines the discount rate (Mazur, 1987). These k scores index the preference for smaller immediate rewards relative to larger delayed rewards. Three k variables were extracted from this measure, each pertaining to different magnitudes of reward: Means = \$25, \$55, \$85; (1) K-Small, (2) K-Medium, and (3) K-Large, respectively. The average of these was computed as K-Total and used as a control variable in all analyses (log-transformed for normality considerations).

3.3.4 Data Analytic Plan

Analyses were conducted using a multilevel regression-based framework (Singer, 1998) using Proc Mixed in SAS version 9.3 for Windows. For all analyses, trials (level 1) were nested within subjects (level 2) and number of pumps on a given trial was the outcome variable. In the first set of models examining the effect of previous trial burst Level 1 effects included: trial number (Trial; level 1; coded 2 to 72, trial 1 was excluded because data on previous trial could not be obtained), whether the current trial was a burst trial or not (Burst$_t$: coded success $= 0$, burst $= 1$), and whether the previous trial was a burst trial or not (Burst$_{t-1}$: coded previous success $= 0$, previous burst $= 1$). All level 1 effects were treated as random effects at the subject level with an unstructured variance/covariance matrix and Satterthwaite approximated degrees of freedom. Approximately a third of the variance in pumps was between-subject variance (ICC $= 0.35$), necessitating a multi-level nested approach to these analyses. The following set of equations was used to model behavior on the BART based on previously published models (Mata et al., 2012):

$$\text{Level 1: } pumps = \beta_{0i} + \beta_{1i}(\text{Trial}) + \beta_{2i}(\text{Burst}_t) + \beta_{3i}(\text{Burst}_{t-i}) + e_{it}$$
$$\text{Level 2: } \beta_{0i} = \gamma_{00} + \gamma_{01}(\text{Severity}) + \gamma_{N1}(\text{Covariates}^1) + u_{0i}$$
$$\beta_{1i} = \gamma_{10} + u_{1i}$$
$$\beta_{2i} = \gamma_{20} + u_{2i}$$
$$\beta_{3i} = \gamma_{3i}(\text{Severity}) + u_{3i}$$

In level 1, pumps on a given trial is predicted by a linear combination of the intercept (β_{0i}), the trial number (β_{1i}), whether that trial was a burst trial (β_{2i}), whether the previous trial was a burst trial, indexing reactivity to bursts (β_{3i}). These estimates then serve as outcomes at the subject level (Level 2) where severity of alcohol problems was allowed to predict intercept (simple effect of Severity, β_{01}) as well as reactivity to Bursts (Severity Burst$_{t-1}$, β_{31}), capturing whether alcohol problem severity moderated reactivity to Bursts.

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[1] For ease of presentation covariates (e.g. age, ethnicity, and IQ) are represented as a single variable.

In the second set of models examining the effect of cash out and burst magnitude, Level 1 effects included: Trial, $Burst_t$, whether the previous trial was a big burst (Big $Burst_{t-1}$; coded 0-75%ile pumped burst trial for a given participant = 0, top 25%ile pumped burst trial = 1), whether the previous trial was a typical burst (Typical $Burst_{t-1}$; reverse coded of Big $Burst_{t-1}$) and whether the whether the previous trial was a big cash out (Big Cash out$t$-1; coded 0-75%ile pumped cash out trial for a given participant = 0, top 25%ile cash out trials = 1). In this coding scheme, a typical cash out trial (i.e. bottom 75%ile of cash out trials) was the reference group. Given the added complexity of this model, a hierarchical modeling approach was employed where Level 1 effects were entered in Block 1 and in subsequent blocks, subject-level variables of interest were entered as both main effects and moderators of response to previous trial characteristics (e.g. Severity Big $Burst_{t-1}$; see Table 3.2) Again, all Level 1 effects were treated as random at the subject level with an unstructured variance/covariance matrix.

3.4   Results

3.4.1   Baseline Characteristics

Participants pumped on average 17.57 times per trial (SD = 5.95), and 23% of trials burst. See Table 3.1 for descriptive statistics on alcohol problem severity indicators.

3.4.2   Burst Reactivity

In a main-effects only model, there was a significant effect of trial number ($\beta = -0.017, SE = 0.003, p < 0.0001$) after controlling for age ($\beta = -0.04, SE = 0.02, p = 0.08$), ethnicity (*p*-value range: 0.01 to 0.48), working memory ($\beta = -0.06, SE = 0.08, p = 0.47$) and IQ ($\beta = 0.014, SE = 0.013, p = 0.30$). A significant main effect of $Burst_t$ was observed ($\beta = 0.39, SE = 0.19, p < 0.05$) as was a significant main effect of previous trial burst ($Burst_{t-1}$; $\beta = -1.26, SE = 0.12, p < 0.0001$). A significant main effect of alcohol problem

Measure	Mean	Std Dev
ADS	40.25	7.31
PACS	17.92	6.61
Symptom Count	5.23	2.81
DRINC-2R	40.9	22.12
CIWA-Ar	5.66	6.92

Table 3.1: Mean and standard deviation for the 5 indicators of alcohol problem severity. ADS: Alcohol Dependence Scale, PACS: Penn Alcohol Craving Scale, Symptom Count: Number of symptoms out of 11 (4 abuse and 7 dependence) from the SCID-IV, DRINC-2R: Drinkers inventory of Consequences -2 Revised, CIWA-Ar: Clinical Institute Withdrawal Assessment for Alcohol revised

severity ($\beta = -0.43, SE = 0.19, p < 0.05$) was observed. In a subsequent model a significant Severity Burst$_{t-1}$ interaction was observed ($\beta = -0.22, SE = 0.11, p < 0.05$) such that as Severity increased, participants pumped fewer times after a burst trial as compared to a cash out trial (see Figure 3.1). This moderated effect was unaffected by removal of covariates (Severity x Burst$_{t-1}$ in a model without covariates: $\beta = -0.20, SE = 0.10, p < 0.05$).

3.4.3 Magnitude of Bursts and Rewards

To examine the influence of burst and reward magnitude, a series of multi-level models were conducted wherein the previous trial characteristic was coded as either a Big Burst, a Typical Burst, a Typical Cash Out (reference group) or a Big Cash Out (coding scheme above in Data Analytic Plan). Overall, 7% of trials were coded as Big Bursts, 17% as Typical Bursts, and 24% as Big Cash Outs, with the remaining 52% of trials serving as the Typical Cash Out reference group.

In Block 1 (i.e. Level 1 effects only) we observed significant main effects of Big Burst$_{t-1}$ ($\beta = 0.91, SE = 0.17, p < 0.0001$), Typical Burst$_{t-1}$ ($\beta = -1.13, SE = 0.11, p < 0.0001$), and Big Cash Out$_{t-i}$ ($\beta = 2.39, SE = 0.12, p < 0.0001$) such that pumping increased overall after big cash outs and big bursts and decreased after Typical Bursts (see Table 2).
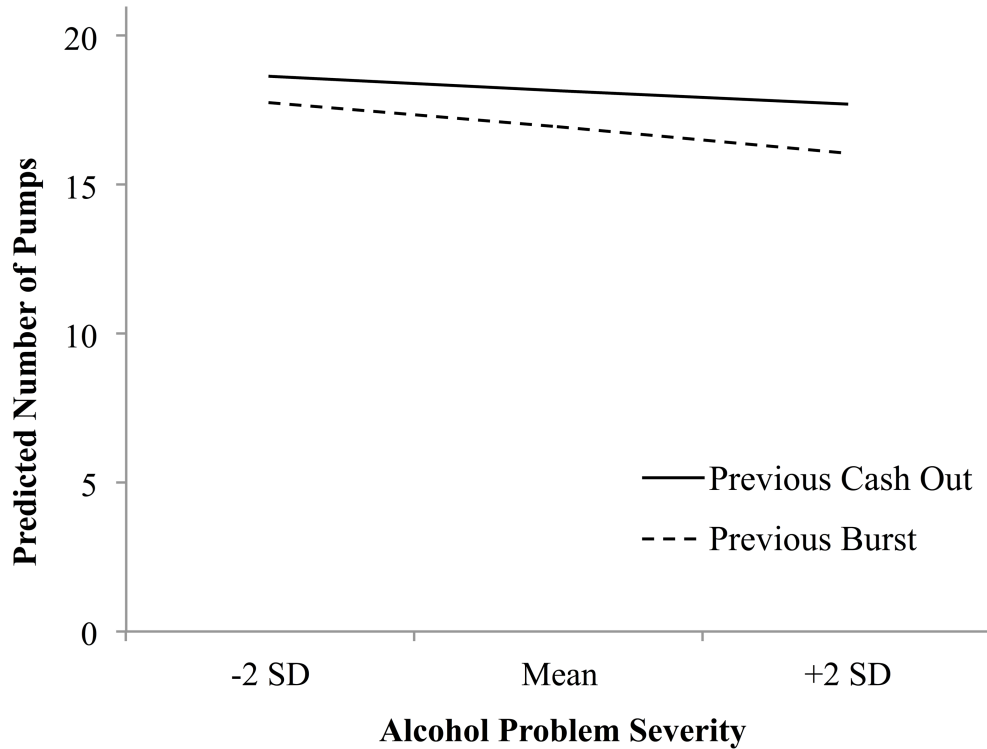
Figure 3.1: Predicted number of pumps based on a multilevel regression model examining the effect of alcohol problem severity on BART behavior. Predicted values are from a model without covariates and are shown for Trial number 32 (i.e. the middle of the task) and for non-burst trials (i.e. $\text{Burst}_t = 0$). Overall, participants pumped less after a burst trial as compared to after a cash out trial ($p < 0.0001$). Additionally, severity of alcohol problems was found to moderate reactivity to bursts ($p < 0.05$) such as level of alcoholism severity increased participants pumped fewer times after a burst trial as compared to a cash out trial.

Furthermore, severity of alcohol problems was found to moderate reactivity to big bursts only (Severity Big Burst_{t-1}: $\beta = -0.40, SE = 0.17, p < 0.05$). This effect was such that pumping increased following a big burst, at low-levels of alcohol use/problems only (Figure 2). Severity was not found to moderate response to big cash outs or to typical bursts ($ps > 0.29$). As is shown in Table 2, the significance or magnitude of these effects was not significantly impacted by the inclusion of subject-level covariates.
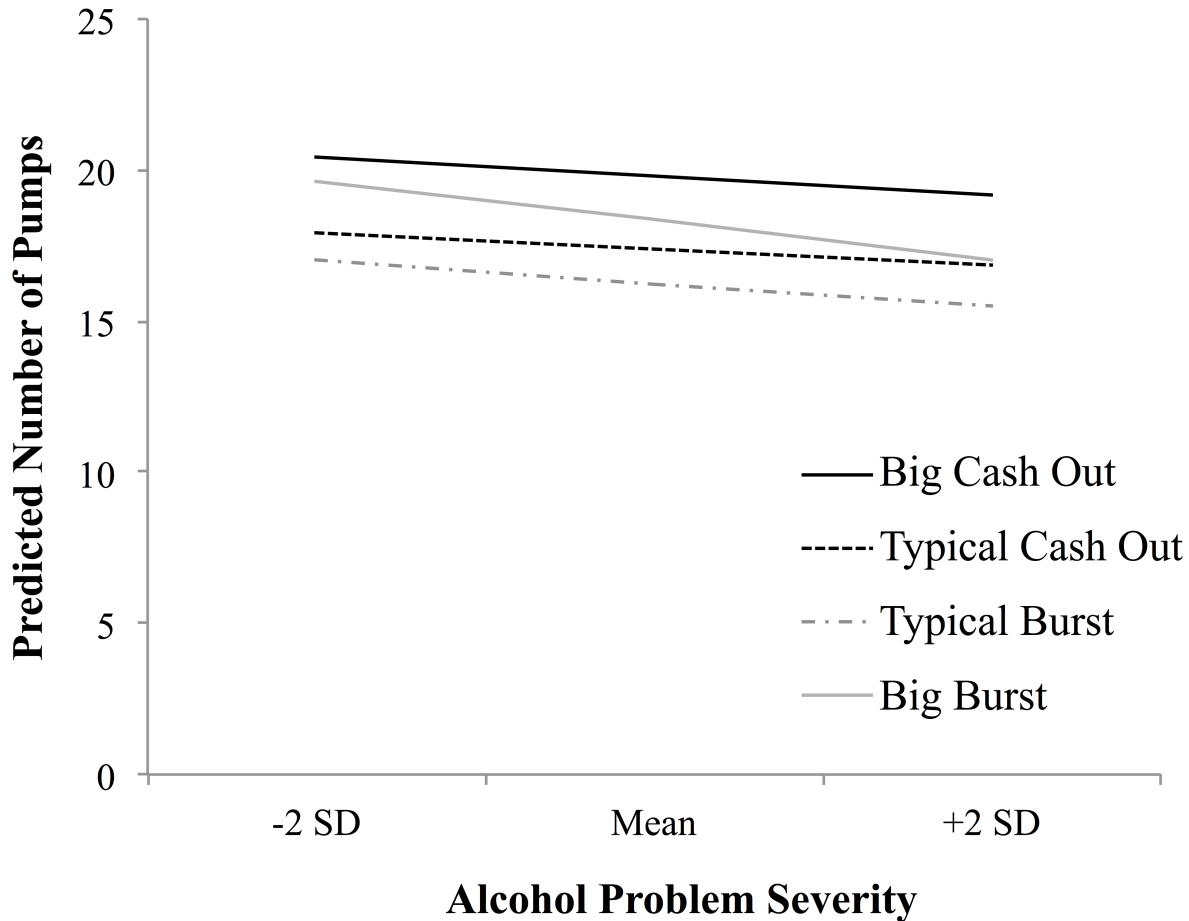
Figure 3.2: Predicted pump values based on a multilevel regression model examining the effect of alcohol problem severity on BART behavior. Predicted values are from a model without covariates and are shown for Trial number 32 (i.e. the middle of the task) and for non-burst trials (i.e. $\text{Burst}_t = 0$). Overall, previous trial characteristics were highly influential on BART behavior, such that pumping increased after both big cash outs and big bursts and decreased after a typical burst (all $ps < 0.0001$). Additionally, severity of alcohol problems was found to moderate reactivity to big bursts ($p < 0.05$) such that increased pumping following a big burst (i.e. the 'near-miss' effect) was only seen at low levels of alcohol problem severity. Alcohol problem severity did not moderate response to big cash outs or typical bursts.

3.4.4 BART Performance and Delay Discounting Rate

Controlling for delay discounting rate as both a main effect as well as a moderator of reactivity to bursts and cash outs did not substantively alter any of the results presented. Alone, delay discounting rate (log transformed) was significantly associated with number

of pumps on the BART ($\beta = -0.36, SE = 0.16, p < 0.05$) in the hypothesized direction, however this effect was not robust to controlling for age (K-Total:$\beta = -0.18, SE = 1.5, p = 0.23$; Age: $\beta = -0.11, SE = 0.02, p < 0.0001$). Furthermore, K-Total was not found to moderate responses to burst or cash out trials (either big or typical; all $ps > 0.15$).

3.5 Discussion

The goal of these analyses was to identify behavioral mechanisms underlying the previously observed negative relationship between risk-taking in the Balloon Analogue Risk Task (BART) and substance dependence (Ashenhurst et al., 2011; Dean et al., 2011; Ryan et al., 2013). This was accomplished by implementing a multilevel regression model to examine trial-by-trial behavior while taking into account behavioral reactivity to bursts (failures) and cash outs (rewards). Additionally, we sought to categorize such trials by magnitude of the gamble at stake and to control for important demographic and neurocognitive variables such as age, IQ, and individual delay discounting rate.

3.5.1 Greater Severity Predicts Greater Burst Reactivity

The parameterization of our first model closely follows one published previously (Mata et al., 2012). In this simpler model where magnitude of the gamble is not accounted for, our results show a similar effect of a previous burst trial to that of (Mata et al., 2012); that is, on trials following a balloon burst, participants tended to take less risk. Interestingly, the effect of previous trial bursts was significantly moderated by alcohol problem severity (Figure 3.1), indicating that participants expressing more problems took less risk following a prior burst than participants with lesser problem severity. This model suggests that the more severe participants were less risk-taking in part because they were more reactive to recent failure.

Counter to data from previous modeling (Mata et al., 2012), there was a small but significant effect of trial number such that across the testing session, participants took less risk as trials progressed. This inconsistent result may be due to different numbers of trials

between implementations of the task; Mata et al. (2012) used a 30 trial variant, while we used a version with 72 trials. Thus, we may have observed a small degree of participant fatigue. However, the magnitude of this effect was quite small, suggesting a predicted decrease in pumps across the session on the order of about less than two pumps.

3.5.2 Magnitude of Gambles and Alcohol Problem Severity

The extension of our primary aim of this analysis was to assess if alcohol problem severity was related to both burst and cash out reactivity taking into account the magnitude of the gamble in the previous trial. This analysis is the first, to our knowledge, to account for reactivity to both bursts and cash outs of differing magnitudes in a trial-by-trial analysis. Consistent with our expectations, overall, participants took less risk on trials following typical balloon 'bursts' (trial failures) and took more risk on trials following big cash outs (in the top 25th percentile; Figure 2). Intriguingly, participants tended to pump more on trials following big bursts (top 25th percentile of potential earnings) compared with typical cash outs. This observation is consistent with theory from the problem gambling literature on a 'near miss' effect (Reid, 1986). A 'near miss' is defined as a failure that comes close to being highly successful. Trials where participants successfully pumped to larger magnitudes but then were faced with a balloon burst may have been perceived as near misses. Near misses have been shown to increase motivation to voluntarily spend more time gambling and to bet more money in slot machine-like tasks (Cote, Caron, Aubert, Desrochers, & Ladouceur, 2003). A neuroimaging study indicated that neural responses to near misses in the striatum and the insula were similar to responses to wins, which may drive an increase in subsequent gambling despite the lack of actual reward delivery (Clark, Lawrence, Astley-Jones, & Gray, 2009). This activity may contribute to pathological gambling, as participants expressing greater severity of gambling problems show greater ventral striatal response to near misses (Chase & Clark, 2010). Although the BART was not designed to identify this effect, it is plausible that these near miss-like experiences in big burst trials encouraged greater levels of pumping on the following trial.

Results taking into account alcohol problem severity, however, were partially consistent with our initial hypotheses that greater alcohol problem severity would predict enhanced reward but reduced loss reactivity as has been demonstrated in analysis of the Iowa Gambling Task (J. C. Stout et al., 2004). Our results demonstrate that alcohol problem severity moderated reactivity to losses, but not to rewards. Specifically, participants with greater alcohol problem severity were less subject to a 'near miss' effect than participants with less severe alcohol problems; these severe participants did not increase their pumping after experiencing a big burst as compared to a typical cash out (Figure 3.2). Thus, these more severe participants did indeed demonstrate a blunted response to bursts, but only after trials with large gambles at stake. On the other hand, alcohol problem severity did not modulate the difference between reactivity to big cash outs versus typical cash outs. These results provide one potential mechanistic explanation for why greater alcohol problems are associated with less overall risk-taking in the BART (Ashenhurst et al., 2011), namely, a blunted near miss-like effect among those with greater alcohol problems.

3.5.3 Comparing the BART to Other Risk-Taking Tasks

Our primary model showed decreased pumping after burst trials and this effect appeared to be more robust in those with more severe alcohol problems. In subsequent models examining the impact of reward and loss magnitude, our results suggested that, while severity of alcohol problems did not moderate behavioral response to typical bursts, it was found to moderate response to big bursts. Thus, contrary to the tentative conclusions one would draw from the first model, namely that more participants with more alcohol problems were more responsive to losses, results from the second set of models demonstrate that severity of alcohol problems was negatively associated with magnitude of the near miss effect. This more specific effect related to large magnitude losses, then, explains the moderated relationship observed in the first set of models.

These findings stand in partial contrast to models of behavior in the Iowa Gambling Task (IGT), where substance users are found to be more reward sensitive and less loss reactive.

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The gap between the findings with the IGT and ours with the BART may be due to significant differences between the tasks, the populations studied, as well as the methods for analyzing behavior. While both tasks require sampling and learning to improve performance, the nature of optimal behavior does differ between them. In the IGT, less risk-taking is always a more advantageous choice, while in the BART, less risk-taking actually results in reduced economic utility; this is because optimum performance in the BART involves balancing an increase in reward with an increase in risk, resulting in a non-linear function (Jentsch et al., 2010; C. Lejuez et al., 2002). Next, our analysis is within a substance abusing population and does not compare abusers to healthy controls. Finally, we allowed loss and reward reactivity to operate independently in our statistical model, while models of the IGT restrict these two factors to being on one dimension represented by a single parameter (J. C. Stout et al., 2004). Still, recent co-administration of these two tasks in healthy controls demonstrated a positive relationship between risk-taking indicators in these tasks, although not among task-nave participants (Xu, Korczykowski, Zhu, & Rao, 2013). Future studies should co-administer the BART, IGT and other risk-taking tasks in clinical populations to evaluate the cross-task validity of risk-taking indicators as specific aspects of task design may subtly influence behavior.

Our results should be weighed with respect to the strengths and limitations of this study design. Our strengths included assessment of a large community sample of problem drinkers, extension of a previously published novel method to examine trial-by-trial behavior in the BART (Mata et al., 2012), and controlling for demographic variables, general intelligence, and temporal discounting rates in all analyses. Limitations included a somewhat restricted range of alcohol problem severity, as our sample does not include social drinkers and non-drinking controls and a cross-sectional research design, which precludes causal inferences; it is unclear if the moderation effect is either a cause or consequence of problematic alcohol use.

3.6 Conclusions and Future Directions

In sum, this study examined a multilevel regression analyses of trial-by-trial behavior in the BART (Mata et al., 2012) in a large sample of adults with a range of alcohol problem severity. We observed that participants with greater alcohol problem severity were less risk-taking in the face of a recent burst trial than participants with lesser severity. We extended this initial model by including parameterization of magnitudes of both cash out and burst trials to gauge behavioral reactivity in the BART. We found that with greater alcohol problem severity, participants were less subject to a 'near miss' effect, providing a more specific account for why more clinically severe participants take less risk, overall, in the BART (Ashenhurst et al., 2011; Ryan et al., 2013). Additionally, we confirmed a negative relationship between delay discounting and BART risk-taking as shown previously in SEM modeling (Courtney, Arellano, Barkley-Levenson, Glvan, et al., 2012) by using a different hierarchical regression approach and trial-by-trial modeling. Critically, our analyses survived controlling for previously implicated and theoretically important covariates of BART performance including delay discounting rate, IQ, and working memory span.

As others have observed a negative relationship between risk-taking in the BART and substance dependence in adult tobacco users (Ryan et al., 2013), future studies should examine trial-by-trial behavior to more fully evaluate behavior in the task. Decision-making under risk represents a complex cognitive process that is likely influenced by subtleties of task design. Still, observed differences in behavior within clinical populations and between substance dependent individuals and healthy controls are likely to indicate neurocognitive factors that partially explain liability to problematic substance use.

	Full Model		No Covariate Model	
Block 1: Level 1				
	Coefficient	**p-value**	**Coefficient**	**p-value**
Intercept	**17.86**	< 0.0001	**17.86**	< 0.0001
Trial	**-0.02**	< 0.0001	**-0.02**	< 0.0001
$Burst_t$	-0.06	0.708	-0.06	0.708
Big Burst$_{t-1}$	**0.91**	< 0.0001	**0.91**	< 0.0001
Small Burst$_{t-1}$	**-1.12**	< 0.0001	**-1.12**	< 0.0001
Big Cash Out$_{t-1}$	**2.39**	< 0.0001	**2.39**	< 0.0001
Block 2: Covariates				
Age	**-0.05**	**0.019**		
Black	**-1.5**	**0.013**		
Asian	0.97	0.213		
Latino	-1.29	0.051		
Native American	-1.18	0.411		
Mixed Ethnicity	-0.65	0.243		
IQ	0.01	0.459		
Working Memory	-0.02	0.786		
Block 3: Alcohol				
Severity*	**-0.45**	**0.026**	**-0.33**	**0.048**
Severity*Big Burst$_{t-1}$	**-0.44**	**0.02**	**-0.4**	**0.016**
Severity*Small Burst$_{t-1}$	-0.15	0.246	-0.12	0.293
Severity*Big Cash Out$_{t-1}$	-0.08	0.534	-0.06	0.627

Table 3.2: Parameter estimates (and p-values) from a set of hierarchical multi-level regression models predicting number of pumps as a function of trial characteristics (i.e. trial number [Trial] and whether that trial burst [Burst$_t$]), previous trial characteristics (i.e. whether previous trial was in the top 25% of burst trials [Big Burst$_{t-1}$], or in the bottom 75% burst trials [Typical Burst$_{t-1}$], or in the top 25% of payout trials [Big Cash out$_{t-1}$], relative to bottom 75% of payout trials). In subsequent blocks, subject-level variables of interest including alcoholism severity score [Severity] were entered as both main effects and moderators of response to previous trial characteristics (e.g. Severity Big Burst$_{t-1}$). Alcoholism severity was standardized. Multiple subject-level covariates were controlled for including Age, and Ethnicity, none of which were found to impact the significance of the results presented. Significant effects are bolded. Overall, behavior on the BART was found to be highly responsive to the characteristics of the previous trial (all $ps < 0.0001$). Additionally, the effect of previous trial big burst was found to be moderated by both alcoholism severity, ($p < 0.05$, see Figure 3.1). Neither alcoholism severity, nor delay discounting rate significantly moderated response to previous small burst or big cash out.

CHAPTER 4

Acute, but not Chronic, Administration of Alcohol Alters Behavior in Rats Performing a Version of the Balloon Analogue Risk Task

4.1   Abstract

Background: Heightened risk-taking propensity segregates with alcohol use disorders; it may serve as either a pre-morbid susceptibility factor or it may be consequence of alcohol use. Human studies on the effects of acute alcohol on risk-taking behavior have yielded few significant results, but this may be due to limited dose ranges studied. Furthermore, longitudinal studies on the effects of chronic alcohol are difficult to achieve in the human laboratory, a limitation that may be overcome with the use of animal models.

Methods: In the present study, rats trained to perform a variant of the Balloon Analogue Risk Task (rat- BART) were exposed to acute, intravenous doses of ethyl alcohol (n = 21) or subchronic, intermittently delivered doses of alcohol, versus control (n = 24). For the acute study (Experiment 1), rats received five alcohol doses (range 0.05 to 1 g/kg, counterbalanced order) or vehicle intravenously during sessions of the rat-BART. For the chronic study (Experiment 2), rats received six weeks of chronic intermittent gavage of an intoxicating dose of alcohol (3.5 g/kg at 20% w/v in saline, MWF) or saline only, and behavior during alcohol-free periods was assessed before and after treatment.

Results: In Experiment 1, alcohol *decreased* risk-taking indices in a dose-dependent fashion; performance of the task after administration of the highest dose was significantly

slower and was linked with with a greater rate of trial initiation errors (false starts). In Experiment 2, comparisons of pre- vs. post-alcohol exposure revealed no significant differences in behavior in the rat-BART as a function of alcohol exposure.

Conclusions: These data suggest that, in rats, acute moderate alcohol doses decrease willingness to accept risk in order to obtain food reward, while higher doses may disrupt abilities to optimally perform the task. Furthermore, any neuroadaptations elicited by six weeks of administration of a moderate, intoxicating dose of alcohol does not affect biases in decision-making under risk. These data, in combination with results from human administration studies, suggest that moderate acute doses of alcohol do not change risk-taking in the BART, but higher doses may decrease risk-taking propensity.

4.2   Introduction

Decades of research on the acute and chronic effects of alcohol on behavior and cognition have demonstrated that alcohol significantly impacts executive function, including aspects of psychomotor control and decision-making (Zoethout, Delgado, Ippel, Dahan, & van Gerven, 2011). Acute alcohol adversely impacts working memory, attention, and planning (Goodwin, Othmer, Halikas, & Freeman, 1970; Minocha, Barth, Roberson, Herold, & DA, 1985; Weissenborn & Duka, 2003; George, Rogers, & Duka, 2005), increases perseveration in the Wisconsin card-sorting task (Lyvers & Maltzman, 1991), and impairs performance of the Go/No-Go Task (Finn, Justus, Mazas, & Steinmetz, 1999), a test of impulsivity. Acute alcohol also impedes motor control (Connors & Maisto, 1980), likely due to increased extra-synaptic $GABA_A$ receptor activity in the cerebellum (Hanchar, Dodson, Olsen, Otis, & Wallner, 2005). Nevertheless, it remains unknown if acute or chronic alcohol alters risk-related decision-making, specifically.

Risk-taking, a sub-class of decision-making, may represent an additional contributing factor in increased liability for alcohol use disorders, as symptoms of substance use disorders include, in part, engagement in risky maladaptive behavior that may result in legal or health

problems (APA, 2000). In other words, it is clear that acute alcohol negatively impacts, say, the ability to drive a car. It is unclear, however, if alcohol may promote the *risky decision* to drive a car while intoxicated above and beyond baseline risk-taking levels for an individual. If acute alcohol does promote maladaptive risk-taking *per se*, then this may be one additional mechanism by which alcohol use can exacerbate negative consequences suffered as part of an alcohol use disorder.

Thus, the first aim of this study (Experiment 1) is to test for dose-dependent effects of acute alcohol on risk-taking behavior in rats as assessed by the rat-Balloon Analogue Risk Task (rat-BART). Previous attempts to detect an effect of acute alcohol on risk-taking behavior in humans have shown no significant results (Farquhar et al., 2002; Peacock et al., 2013; Ramaekers & Kuypers, 2006), possibly due to moderate dose ranges or individual differences in pharmacokinetics and metabolism of oral alcohol. Thus, for this study, intravenous delivery of a wide range of doses of alcohol in a within-subjects design allows for greater control and power. Despite the lack of effects of acute alcohol on BART performance in humans (Peacock et al., 2013), the original study hypothesis was that alcohol would increase risk-taking at the higher, intoxicating doses.

Chronic long-term alcohol use also associates with deficits in executive function and memory, with Korsakoff's Syndrome representing an extreme of memory dysfunction (Joyce & Robbins, 1991; Parsons & Stevens, 1986; Abernathy et al., 2010; R. Z. Goldstein et al., 2004). These executive control deficits are related to neuroadaptations and atrophy in the prefrontal cortex (R. Z. Goldstein et al., 2004). Imaging studies have revealed gross abnormalities in pre-frontal cortical morphology in chronic alcoholics in terms of reductions of grey matter (Jernigan et al., 1991) and white matter (de la Monte SM, 1988; Pfefferbaum et al., 1997).

Biases in decision-making under risk may emerge as a direct pharmacological effect of repeated alcohol intoxication and resultant neural atrophy (Jernigan et al., 1991; de la Monte SM, 1988; Pfefferbaum et al., 1997). Some studies have shown that alcohol dependent individuals (who have presumably been exposed to high levels of alcohol over time)

take greater risks than controls in the Iowa Gambling Task (Bechara et al., 2001), while our group has shown a *negative* relationship between alcohol use disorder symptomatology and risk-taking in the human BART [Chapter Two; (Ashenhurst et al., 2011)]. While these contrasting studies suggest a task-dependent potential relationship between chronic alcohol and risk-taking, the study designs are cross-sectional, limiting causal interpretation. Furthermore, as these studies assessed community populations, levels of chronic alcohol exposure are uncontrolled and potentially highly variable.

The second aim of this study (Experiment 2) is to assess the effects of chronic alcohol (versus saline) on risk-taking behavior in rats. To address the limitations of clinical study designs, rodent models can evaluate the impact of chronic exposure to alcohol (versus control solution) in order to detect differences in pre- versus post-exposure risk-taking. Furthermore, doses can be delivered in an intermittent fashion, which is thought to mimic binge intoxication patterns of alcohol use (Broadwater, Varlinskaya, & Spear, 2011). The original study hypothesis was that rats that received chronic alcohol exposure would exhibit a decrease in risk-taking as compared to pre-exposure baseline levels and saline-administered controls, consistent with human cross-sectional data (Ashenhurst et al., 2011).

4.3 Methods and Materials

4.3.1 Common Methods

4.3.1.1 Subjects

Adult male Long-Evans rats (Experiment 1, $n = 21$. Experiment 2, $n = 24$) were used in these studies. The animals were between p50 to p60 days of age and 190-200g in body weight at the inception of testing. An initial food restriction schedule was used to reduce body weights to 85% of free-feeding weight. In addition to the food rewards obtained during testing, the rats were supplied with a portion of standard laboratory rat chow (Purina) in their home cage after behavioral testing was complete.

4.3.1.2   Behavioral Training and Testing

Training and testing for both experiments were conducted in chambers fitted with a house light, internal stimulus lights, food-delivery magazine, and two retractable levers positioned to the left and right of the magazine (Med-Associates, St Albans VT). Training and behavioral testing was the same between experiments, except for drug manipulations. The boxes were controlled by a PC running Med-PC IV (Med-Associates). Subjects were first trained to respond on both levers in sessions using a fixed ratio (FR)-1 schedule of reinforcement for 45-mg dustless precision, purified diet pellets (Bio-Serv; Frenchtwon NJ). Subsequently, they were trained on an FR-3 and FR-10 on the designated 'add'- lever; subjects were progressed during this initial stage of training when they obtained at least 20 outcomes in a session. They were then familiarized with the general design of the task in a 50-trial 'forced' task in which only the add lever was presented until the rats responded between 2 and 15 times (randomly chosen from trial to trial); the add lever was then withdrawn and the cash-out lever was presented. A single cash-out response dispensed a number of pellets equal to the number of add-lever presses permitted on that trial.

Following this, the rats began daily testing on risk and no-risk variants of the actual task (all 50 trials long) with both levers presented. In the rat-BART, subjects can respond on the "add" lever until: (1) it causes the trial to fail by excessive responding (resulting in both levers being retracted and a 3-s time out signaled by lights off being enforced) or (2) it presses the cash-out lever and is presented with its earned reinforcement. Prior to surgery and alcohol administration, rats underwent several days of baseline assessment under conditions of 50% reinforcement (each two presses resulted in one pellet delivery on average) and 10% risk (1/10 chance that a lever press will produce trial failure) until performance was stable. The criterion for stability was no more than 20% change in performance over 3 days.

Both Experiment 1 and 2 were conducted under 50% reinforcement and 10% risk conditions. This reinforcement parameter was chosen in order to promote more lever pressing (Jentsch et al., 2010). Furthermore, this version of the rat-BART included an audible 'beep'

with each add lever press in order to indicate a successful press to the rat.

4.3.2   Experiment 1: Acute Alcohol

4.3.2.1   Dose-Response Testing Overview

After training and baseline assessment was completed, calorie restriction was suspended and the rats received jugular catheter implantation surgery. After full recovery seven to ten days later, rats were calorie restricted again to enhance motivation to perform in the rat-BART. Then rats received a "reminder" session in the rat-BART to reinforce task performance. Next, alcohol administration in the context of the rat-BART was used to determine a dose response curve. All rats received all doses in a latin-square type design, with individual doses tested on separate days. To avoid carry-over effects, drug administration days were separated by two days of washout.

4.3.2.2   Surgeries

Rats were first anesthetized with isoflurane (3-5% in an induction box followed by 2-4% by a nose cone) and then received jugular catheter implantation surgeries. Catheters (constructed in-house) were inserted using a guide cannula into the right jugular vein, and then secured with surgical sutures. The catheter was externalized caudal to the scapulae tips of the rat. Animals' catheter patency was maintained by daily flushing with the antibiotic ticarcillin clavulanate (Glaxo Smith Klein, Research Triangle Park, NC, USA) dissolved in heparinized saline (0.2mL during test and non-testing days). Catheter patency was validated prior to acute dosing by assessing response to propofol (0.15mL). An immediate response showed catheter patency, and a delay in response was noted as it indicated clogged catheters. Subsequent analyses were conducted without these animals.

4.3.2.3　Acute Ethanol

Rats received the freshly mixed ethanol dose consisting of diluted 95% ethanol in sterile isotonic saline at equal volumes of 1.0mL but at different concentrations to achieve the weight-corrected doses. Doses tested were: 0.05, 0.10, 0.25, 0.5, 1.0 g/kg body weight and vehicle saline. The maximum dose in this range was chosen as it produces significant intoxication without introducing ataxia that would completely impede performance of the rat-BART. Observation of the rats during the rat-BART confirmed that they were able to complete the task at this highest dose, but were noticeably slower; this effect is confirmed in analyses of timing of behavior in the task. An automated pump delivered alcohol solution over 1.5min beginning at 30sec after task commencement.

4.3.3　Experiment 2: Chronic Alcohol

4.3.3.1　Alcohol Administration

After rat-BART training, rats were balanced across two groups taking into consideration baseline performance in the rat-BART and weight. These groups were then given a regimen of chronic ethanol or saline administration (n=12 each). Administration was originally delivered by *i.p.* injection, intended to last six weeks. Prior to any ethanol *i.p.* administration, rats were given saline injections (1 ml, normal saline) *i.p.* once per day for 3 days in order to acclimatize the animals to *i.p.* injection procedures. Subsequently, ethanol (20% w/v in isotonic saline) was administered through (*i.p.*) injection (3.5g/kg w/v in 20% saline). However, initial dosing by this route caused peritonitis in several rats who were removed from the study (n=3). As an alternative to *i.p.* administration, oral gavage of ethanol solution was used for the remainder of the study (six weeks on Mondays, Wednesdays, and Fridays). Animals were restrained, and an 18G animal feeding cannula (Fisher Scientific) was inserted orally to dispense alcohol solution or an equivalent volume of saline. This chronic intermittent dosing regimen was chosen as it is thought to model binge intoxication patterns typical of problematic human alcohol use, and this procedure is known to alter response to

acute ethanol in tests of ethanol sensitivity such as the latency of righting reflex, a marker of acquired tolerance (Broadwater et al., 2011). Rats were given reminder sessions of the rat-BART up to once per week on non-ethanol days.

4.3.4   Statistical Analyses

4.3.4.1   Experiment 1

Repeated measures ANOVAs across the six doses tested were conducted separately for the following dependent measures from the BART: Mean lever presses (adjusted), the main risk-taking outcome measure, was the average number of lever presses at "cash out" for non-failure trials. Inter-trial variability was indexed as the within-session coefficient of variance (variability divided by the mean). Deliberation time was calculated as the average time (in seconds) between the final "Add" lever press and the "cash out". False starts, a trial error, were scored when the rat pressed on the "cash out" lever prior to pressing the "Add" lever. In post-hoc analyses to detect significant pair-wise differences between doses, a Bonferroni correction was applied. Since the infusion took place during the test session and the effects of alcohol may not have been uniform across the entire time period, data was binned into thirds of the total session. A 3 x 6 repeated measures ANOVA was conducted across these three time bins and the six doses tested; a significant interaction between time bin and dose would suggest that the time course of the effects of doses were different. All data was subject to tests for normality and sphericity. For measures with violations of the assumption of normality, Greenhouse-Geisser corrected degrees of freedom were used.

4.3.4.2   Experiment 2

A mixed repeated measures ANOVA was conducted to compare baseline rat-BART performance indices to values measured after six weeks (within subjects variable) of chronic alcohol or saline (between subjects variable). Baseline was computed as the average of the three days prior to alcohol or saline administration. Dependent variables from the rat-BART

included mean lever presses at cash out, inter-trial variability (the session coefficient of variance), false start rate, and deliberation times.

4.4   Results

4.4.1   Experiment 1: Acute Alcohol

Of the 21 rats who received successful catheter implantation surgery, five showed delayed or minimal responses to an infusion of propofol. Thus, these rats were excluded from all analyses, as their catheters were likely non-patent. This results in a final subject number of $N = 16$ rats.

4.4.1.1   Dose-Dependent Effects of Alcohol

There was a main effect of alcohol on mean lever presses at cash out, $F(5, 75) = 3.875, p < 0.01, \eta^2 = 0.186$, such that overall, increasing doses resulted in a decrease in risk-taking. Post-hoc tests for pair-wise differences between doses revealed significant differences between the highest dose (1 g/kg) and vehicle (Figure 4.1A). No other comparisons were significant. There was no significant effect of dose on inter-trial variability of lever presses, $F(5, 75) = 1.205, p > 0.05$, suggesting that alcohol did not influence the degree to which individual rats varied their risk-taking across the session (Figure 4.1B).

Analyses of false starts revealed a violation of sphericity, thus Greenhouse-Geisser corrected degrees of freedom were used. There was a significant main effect of dose on false starts, $F(1.73, 75) = 6.424, p < 0.01, \eta^2 = 0.30$, such that trial initiation errors increased with dose. Post-hoc analyses showed the 1g/kg dose was significantly higher than the 0.1g/kg dose, while all other comparisons were not significantly different (Figure 4.1C).

Lastly, there was a significant main effect of alcohol on deliberation times (the time between last add press and the cash-out press, Greenhouse-Geisser corrected), $F(1.244, 85) = 4.22, p < 0.05, \eta^2 = 0.22$. There were no significant post-hoc differences between doses,

however, likely due to high variability for this measure (Figure 4.1D).

4.4.2 Experiment 2: Chronic Alcohol

As described in the methods above, the original route of administration (*i.p.*) was suspended after three rats suffered ill health effects and were removed from the study. Instead, oral gavage was used for the duration of six weeks. Thus, the final sample size for this study was $N = 21$.

For the main index of risk-taking (Figure 4.3A), mean lever presses at cash out, there was a significant main effect of time, $F(1, 19) = 14.98, p < 0.001, \eta^2 = 0.441$, such that risk-taking decreased overall between the baseline and post-chronic administration period. There was no main effect of condition, $F(1, 19) = 0.003, p > 0.05$, nor was there an interaction between time and condition, $F(1, 19) = 0.783, p > 0.05$.

There was no main effect of time on inter-trial variability (Figure 4.3B), $F(1, 19) = 0.526, p > 0.05$. Nor was there a main effect of condition, $F(1, 19) = 1.05, p > 0.05$ or an interaction between condition and time, $F(1, 19) = 0.107, p > 0.05$.

Deliberation times were examined (Figure 4.3C). As with other measures, there was no main effect of time, $F(1, 19) = 0.824, p > 0.05$, and no main effect of condition, $F(1, 19) = 0.740, p > 0.05$. There was no significant interaction between time and condition, but this interaction approached trend level, $F(1, 19) = 2.378, p = 0.14$.

Finally, false starts (trial initation errors) were examined as well (Figure 4.3D). There was no main effect of time, $F(1, 19) = 1.74, p > 0.05$, nor condition $F(1, 19) = 0.235, p > 0.05$, nor an interaction between them, $F(1, 19) = 0.689, p > 0.05$.

4.5 Discussion

The goals of this study were two-fold. The first was to examine the effects of acute dosing of alcohol on risk-taking and other performance indices measured in the rat-BART.

In the second experiment, rats were given six weeks chronic intermittent administration of alcohol (or saline) to test for effects of chronic exposure on behavior in the rat-BART. The initial hypotheses were that acute alcohol should increase risk-taking at higher doses, and that chronic alcohol exposure should decrease risk-taking (compared to saline), consistent with human data showing a negative relationship between alcohol dependence severity and risk-taking in the human BART (Ashenhurst et al., 2011).

4.5.1   Acute Alcohol Decreases Risk-taking in the rat-BART

As seen in Figure 4.1, acute alcohol administration during the rat-BART *decreased* risk-taking but did not alter inter-trial variability. However, the size of this effect on risk-taking was small, and the only significant pair-wise comparison between doses was between the highest dose (1 g/kg) and saline. There were other significant effects on rat-BART performance also largely driven by this largest dose including an increase of deliberation times, and an increase of false starts (Figure 4.1 CD). These data are contrary to our initial expectation that acute alcohol would dose-dependently increase risk-taking in the rat-BART.

These results are largely consistent, however, with data from the human literature demonstrating a lack of effects of alcohol and other drugs of abuse on performance of the BART. Moderate acute alcohol (Peacock et al., 2013) and opioid administration (Zacny & de Wit, 2009) have previously been shown not to alter human choice behavior in the BART. Similarly, alcohol and MDMA do not alter performance in the Iowa Gambling Task (Ramaekers & Kuypers, 2006), and acute alcohol did not impact risky decision-making in a visual line size estimation task (Farquhar et al., 2002).

Other rodent gambling tasks, on the other hand, have observed some effects of acute drug intoxication. Morphine and ethanol did not have significant effects on behavior in the Risky Decision-Making Task, while nicotine and amphetamine decreased choice of the "risky" lever (Mitchell et al., 2011). Acute nicotine administration (Mendez et al., 2012) and amphetamine (St Onge & Floresco, 2009) increased selection of the large/risky lever in

the Probabilistic Discounting Task where the risk is for a time-out. However, a lower dose of nicotine and higher dose range of amphetamine *decreased* selection of the risky lever in the Risky Decision-Making Task, where subjects risk a foot shock (Mitchell et al., 2011; Simon et al., 2009).

Overall, it appears that acute alcohol has few effects on risk-taking behavior as modeled either in humans or rodents. Thus, it appears unlikely that acute alcohol intoxication increases risk-taking *per se*. Thus alcohol may not influence decision-making above and beyond baseline individual levels in a manner that promotes maladaptive risky decision-making with potentially deleterious consequences.

4.5.2 Chronic Alcohol and Risk-taking Behavior

Of all the comparisons made, there was only a significant effect of time on risk-taking behavior. This effect was not explained by either chronic alcohol or saline administration, but by time alone (Figure 4.3A). There were no significant effects on inter-trial variability, false starts, or deliberation times. There is some hint that chronic alcohol (versus saline) may increase deliberation times, although this interaction is not significant (Figure 4.3C) Post-hoc power analysis indicated low power to detect such an effect (0.31).

These data stand in contrast to executive function deficits observed in chronic alcoholics. These include deficits in memory and decision-making (Joyce & Robbins, 1991; Parsons & Stevens, 1986; Abernathy et al., 2010; R. Z. Goldstein et al., 2004). Past research as observed specifically an increase in risk-taking in the Iowa Gambling Task among chronic alcoholics versus controls (Bechara et al., 2001), and a decrease of risk-taking in the BART with an increase in alcohol dependence symptomatology [Chapter Two; (Ashenhurst et al., 2011)]. These acquired deficits may take more exposure and higher doses than administered here. Future studies may find significant results with higher dosing over a longer period, perhaps by using a vapor chamber method for inhaled alcohol administration.

4.5.3   Strengths and Limitations

There are several strengths and limitations to consider when interpreting the results of this study. For Experiment 1, strengths included the use of acute alcohol dosing via an intravenous jugular catheter, as this allowed for near-instantaneous and well-controlled administration of alcohol. Furthermore, dose order was controlled for, delivered in a within-subjects design, and two days of wash out were allowed between doses to avoid carry-over effects. In experiment two, the ultimate route of administration (oral gavage) is more naturalistic in the context of alcohol use disorders and intermittent exposure is known to strongly induce neuroadpatation processes (Broadwater et al., 2011; Liang et al., 2006; Zahr et al., 2011).

Weaknesses included limited variability in lever presses, and low levels of risk-taking overall. Thus, floor effects may have reduced the ability to detect significant differences between acute doses tested, and to detect a change in behavior after chronic alcohol versus saline. Next, while the Long-Evans strain is an outbred strain that learns and performs the rat-BART well (Jentsch et al., 2010), it may be that this strain is more or less resistant to the effects of alcohol, limiting the extension of these data to humans. Future studies should test other strains, including those specifically bred to prefer or disprefer alcohol. Additionally, it is unclear if rats decreased their risk-taking at the highest dose merely as a consequence of sedation. The fact that deliberation times and false starts were both significantly higher suggests that task performance was impeded.

Lastly, in Experiment 2, six weeks of exposure at the dose chosen may not have been sufficient to induce neuroadaptive changes that bias decision-making behavior under risk, and sample sizes may have been insufficient to detect significant effects.

4.5.4   Conclusions

This study is the first to ascertain the effects of both acute and chronic alcohol exposure on rats performing the rat-BART. Results were contrary to those originally hypothesized. Acute

alcohol *decreased* risk-taking at the highest dose, while chronic alcohol (versus saline) had no detectable effect on behavior. These data stand in partial contrast to findings from the human literature which suggest that acute alcohol has no effect on risk-taking in the BART, and that greater alcohol problem severity, which is likely associated with greater chronic alcohol exposure, is *negatively* correlated with risk-taking in the BART. These findings advance our understanding of the potential etiologic role of risk-taking in alcohol use disorders or in the occurrence of negative consequences due to alcohol misuse. In particular, risky decision-making *per se* is likely not increased by acute alcohol. Furthermore neuroadaptive effects due to chronic alcohol may not underlie differences in risk taking observed across alcohol problem severity (Ashenhurst et al., 2011), although six weeks of alcohol administration may have failed to induce neuroadaptation consistent with that observed in chronic alcoholics.
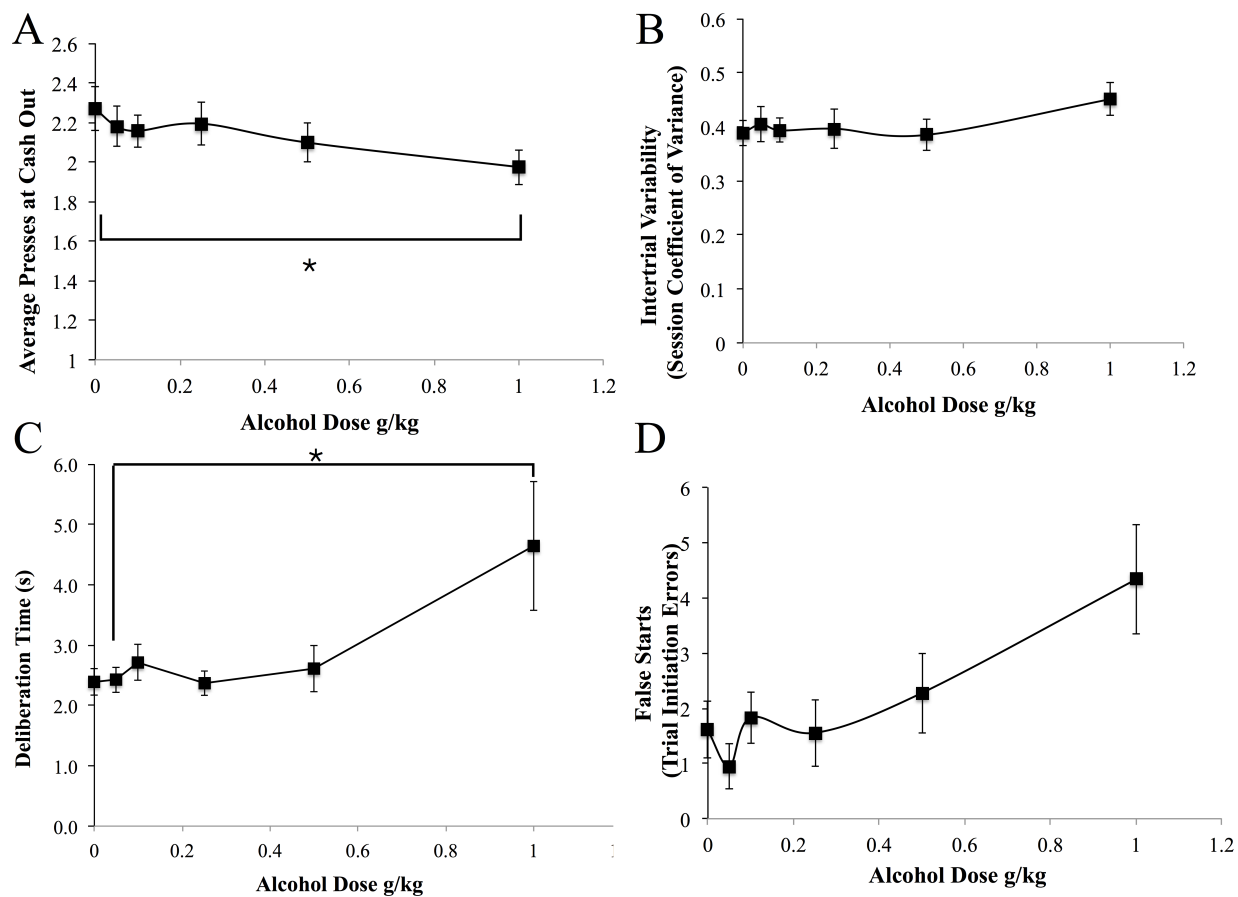
Figure 4.1: Dose-dependent effect of alcohol on risk-taking behavior. A) There was a main effect of dose such that greater alcohol decreased lever presses at cash out. Post-hoc comparisons with a Bonferroni correction revealed that only the highest dose (1 g/kg) and saline were significantly different. B) Alcohol did not have any significant effect on trial-to-trial variability. C) Alcohol increased the amount of time spent between the final 'add' lever press and the 'cash out' lever press. D) There was a main effect of dose such that greater alcohol increased false starts, which are trial initiation errors. This may reflect the impact of alcohol on task orientation or impulsivity.
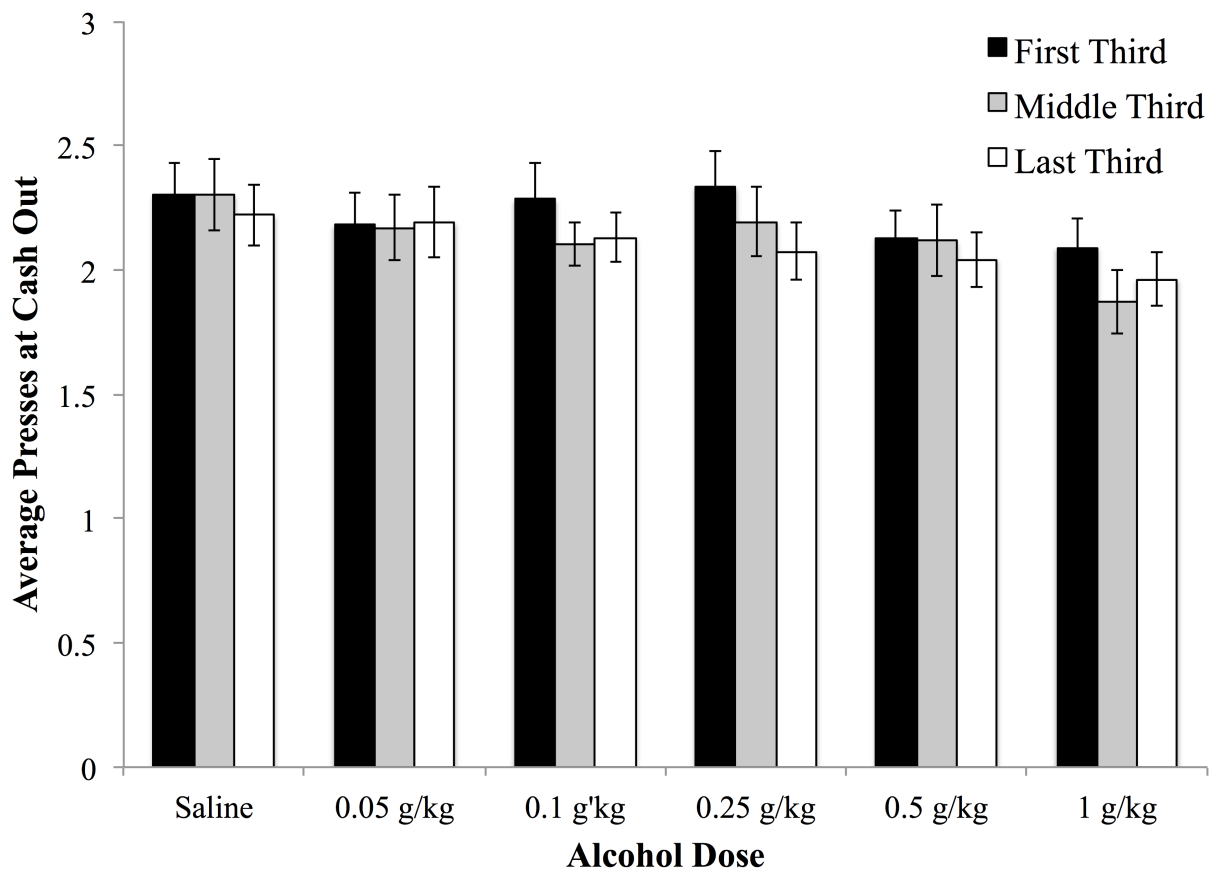
Figure 4.2: Session Time Bins Across Doses Tested. There was no significant interaction between time bin and dose, suggesting that the effects of alcohol across the duration of the session did not differ by dose. There was a main effect of time, however, such that overall, risk-taking decreased across the session. Post-hoc analyses did not reveal any significant simple effects for each dose.
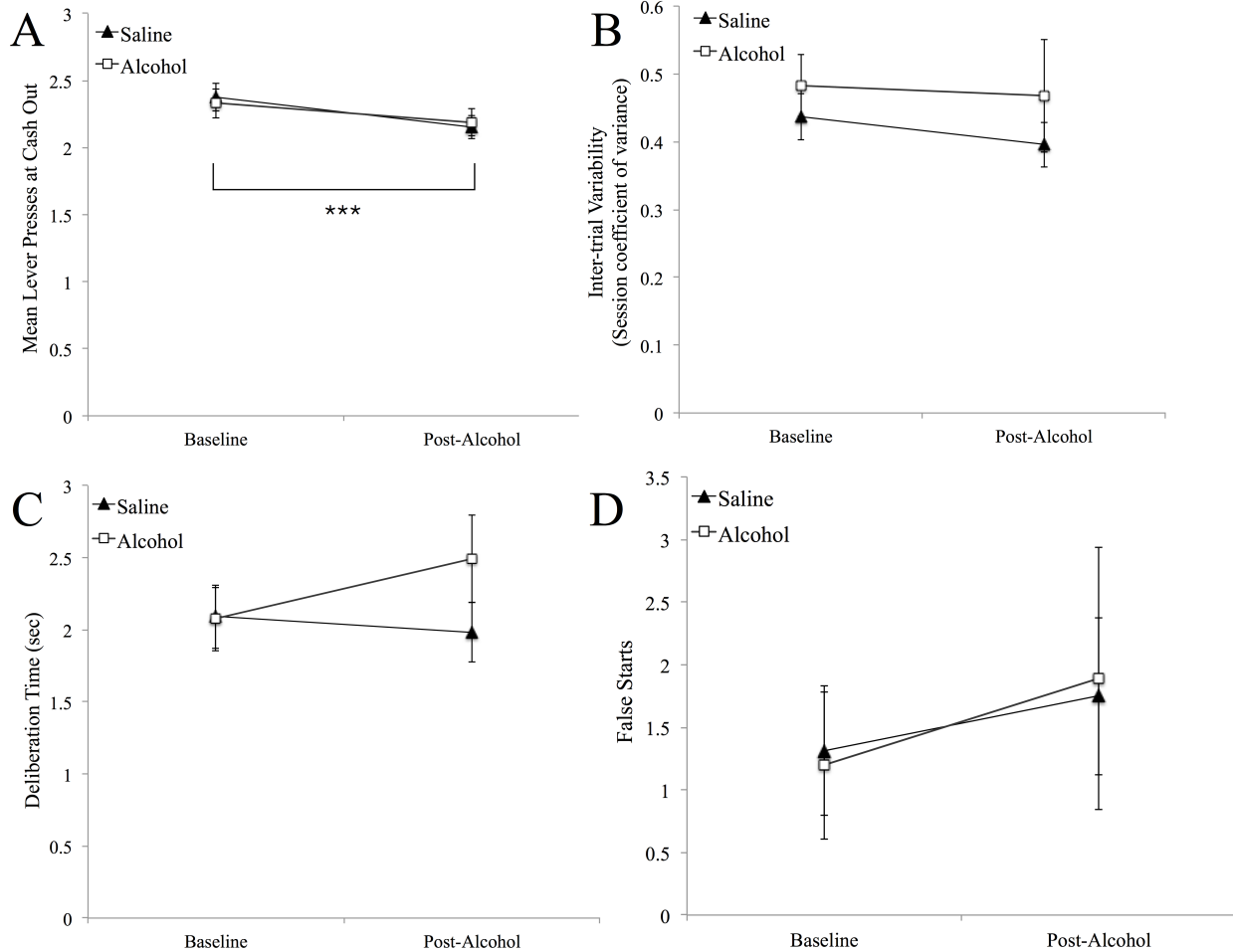
Figure 4.3: Effects of Chronic Alcohol Vs. Saline on the rat-BART. A) There was a significant effect of time, but no significant effect of alcohol versus saline condition, or interaction between condition and time. Overall, rats decreased their risk-taking over the duration of the experiment ($p < 0.001$). B) There were no effects of time or condition on trial-to-trial variability. C) There were no effects of time or condition on deliberation times. However, the interaction between them is not significant ($p = 0.14$). D) There were no main effects of time or condition on false starts.

CHAPTER 5

Responding in a Test of Decision-Making Under Risk is Under Moderate Genetic Control in the Rat

5.1 Abstract

Background: Risk-taking, measured with laboratory tasks such as the Balloon Analogue Risk Task (BART), is associated with real-life manifestations of risky behaviors, which may be an important component of inherited liability to alcohol use disorders. To identify genomic factors that influence these traits, the current study a) characterized performance of a rodent version of the BART in multiple inbred rat strains, b) tested the degree to which performance was under genetic control, c) explored sex-differences in performance, and d) evaluated the risk-taking behavior of F1 progeny of high risk- and low risk-taking strains to examine modes of inheritance.

Methods: Male and female rats (N=100) from five inbred strains (Wistar-Furth, Fischer-344, Lewis, Spontaneously Hypertensive, Brown Norway) and Wistar-Furth x Fischer-344 hybrids were tested in the rat-BART, as well as in tests of locomotor activity, sucrose preference and general motivation.

Results: About 55% of the variance in risk-taking behavior was attributable to heritable factors. The Fischer-344 strain was the most risk-taking, and the most variable in responding. The mating of low risk-taking Wistar-Furth and Fischer-344 rats produced progeny that behaved most like the Fischer-344 strain. Consistent with prior research in this laboratory (Jentsch et al., 2010), all rats were sensitive to changes in both risk and reinforcement parameters in the rat-BART; rats decreased voluntary risk-taking in the face of increasing

risk and increased lever pressing when reinforcement probabilities were reduced.

Conclusions: Our results endorse a moderately heritable pattern of risk-taking behavior in rats. The behavior of the hybrid progeny suggests a polygenic model with most gene effects transmitted by mode of dominant inheritance. The identification of high-risk and low-risk strains allows for isolation of quantitative trait loci associated with task performance and for probing the relationships between risk-taking and dimensions of alcohol use disorders.

5.2   Introduction

Efforts to understand the genomic influences on substance use disorders (SUD), including alcohol dependence, have grappled with the complexity of the multi-dimensional diagnostic phenotypes and the corresponding need to identify intermediate phenotypes to bridge the gap to underlying causal biological factors (Ducci & Goldman, 2008; D. Goldman et al., 2005; Hines et al., 2005; Manji et al., 2003). Several temperament phenotypes have already been identified that index susceptibility for SUDs and alcohol use disorders, including propensity for risk-taking (C. Lejuez et al., 2002; J. Stout et al., 2005) and impulsivity (Jentsch and Taylor, 1999). To most powerfully identify genotype-phenotype relationships, it is likely best to use objective and quantitative measures of risk-taking (e.g., (Bechara et al., 1994; Petry, 2001; Rogers, Owen, et al., 1999)) including the Balloon Analogue Risk Task (BART; (C. Lejuez et al., 2002)).

In the BART, subjects produce key-press responses to earn rewards; although each response is associated with a potential increase in the size of a desired outcome, it is also probabilistically related to trial failure and reward forfeiture. Therefore, subjects must weigh, on a response-by-response basis, their desire to accept risk to obtain greater rewards vs. their desire to avoid risk. Behavior in this task relates strongly to real world risk-taking and SUD phenotypes (Aklin, Lejuez, Zvolensky, Kahler, & Gwadz, 2005; Daughters et al., 2005; Hopko et al., 2006; C. Lejuez et al., 2003; C. W. Lejuez et al., 2004).

Risk-based decision-making in the human BART is substantially heritable in adolescent

males (Anokhin et al., 2009). That being said, almost nothing is known about how particular genomic influences relate to this phenotype. The discovery of these genetic determinants may be aided by rapid-scale whole-genome linkage studies in non-human animals. Our laboratory has recently developed a rodent behavioral task (rat-BART) that captures many key features of the human BART (Jentsch et al., 2010). These initial studies have demonstrated that, like humans, rats are risk-averse and exhibit performance that is predictably sensitive to changes in both risk and reward value. Additionally, these previous studies have highlighted that overall risk-taking and trial-to-trial variability in risk-taking are distinct constructs with dissociable underlying neural circuitry (Jentsch et al., 2010).

Lastly, meta-analysis has shown that men are more likely to engage in risky behaviors than women (Byrnes, Miller, & Schafer, 1999); however, sex differences in the BART only emerge under certain conditions, such as low risk (C. Lejuez et al., 2002) or while under stress (Lighthall et al., 2009). It is unknown if these differences exist in rodents, and this study offered the opportunity to test for them.

With these concepts in mind, the specific goals of this study were a) to characterize performance of a rodent version of the BART in multiple inbred rat strains, b) to test the degree to which performance was under genetic control using an approach previously employed to determine heritability of complex phenotypes such as delayed discounting (Wilhelm & Mitchell, 2009), behavioral inhibition (Gubner, Wilhelm, Phillips, & Mitchell, 2010), c) to explore sex-differences in performance, and d) to evaluate the risk-taking behavior of F1 progeny of high risk- and low risk-taking strains to examine modes of inheritance.

5.3   Methods and Materials

5.3.1   Animals

Animals (N total = 100) included Wistar-Furth (WF/NHsd, n=11), Spontaneously Hypertensive (SHR/NHsd, n=16), Fischer F344 (F344/NHsd, n=20), Lewis (LEW/SsNHsd,

n=11), and Brown Norway (BN/SsNHsd, n=7) strains born on site from pregnant dams (Harlan, Indianapolis IN); in phase two of the study, WF and F344 pairs were mated to produce four cohorts of hybrid F1 progeny (n=34) from three breeding pairs. Animals studied included both males (n=47) and females (n=53). These strains were selected because they are well-established inbred lines that were all available for timed-pregnancy delivery with no a priori hypotheses about task performance. To eliminate strain-specific rearing effects, all newborn pups were cross-fostered to Long-Evans (LE) dams that had given birth within 24 hrs. For each strain, subjects were derived from at least two separate litters from separate dams, with new foster LE dams for each litter. Pups were weaned at three weeks-of-age and placed into group housing (two or three individuals of single-sex and single strain) in acrylic shoebox cages (10" X 18") on a 14L:10D schedule.

Pre-training testing commenced at 60 days old. Before operant training began, a food restriction scheme reduced baseline body weights to 85% of free-feeding weight. In addition to the food rewards obtained during testing, all rats were supplied with a portion of standard laboratory rat chow (Purina) in their home cage after testing.

5.3.2 Behavioral Training and Testing

All rats completed the following series of behavioral assays, in the listed order: locomotor activity, sucrose preference (vs. water), rat-BART training, rat-BART battery and a progressive ratio (PR) test.

5.3.2.1 Control Tasks for Breeding Pair Selection

Locomotor activity, sucrose preference, and progressive ratio tests were given to control for aspects of rodent behavior that are likely under genetic influence and that may also impinge upon performance in the rat-BART; these control data were used to determine which strains would be mated to generate the F1 progeny. Testing for locomotor activity and sucrose preference was conducted before food restriction commenced.

Sucrose preference and locomotor activity measures were collected over a 7-day period. Activity counts were gathered in acrylic shoebox cages (10" X 18") placed into an infrared grid with 16 beams spaced every inch along the long sides of the box (Columbus Instruments, Columbus OH). During the first two days of exposure to the testing environment, the number of beam breaks were collected over a 15-min initial period and locomotor activity was scored as the average beam breaks of these two days. For sucrose preference testing, after the 15 min of habituation, bottles were presented for an additional hour in the same cages. On the first two days of locomotor assessment, a single bottle of sucrose (2% w/v in water) was presented. On the third day, rats were exposed to sucrose solution in their home cages for 8hrs (no locomotor testing); the first two days accustomed the rats to sucrose availability in the testing environment, while the third eliminated any lingering neophobia. After this home cage day, the rats completed four days of sucrose preference testing.

Sucrose preference was conducted by counterbalanced (left vs. right) presentation of two bottles (water and 2% sucrose solution) after the completion of a 15-min habituation period in the testing environment. Sucrose preference was determined by the relative consumption (in mL of solution per kg of body weight) of fluid during the 60-min period, averaged across the four days.

At the completion of testing on the rat-BART (described below), breakpoints were measured using a progressive ratio task in two consecutive daily testing sessions. Progressive ratio testing was done in the same context as the rat-BART. During these sessions, the 'add' lever (described below) was present and responding on it led to delivery of a pellet on a progressive-ratio schedule of reinforcement. The increase in response requirement was non-linear (Richardson & Roberts, 1996) [e.g., 1, 2, 4, 6, 9, 12, 15, 20, 25...]. If the rat did not respond to the lever for two minutes, the session was completed and break-point tabulated. The number of ratios completed over the two days of testing was averaged, to produce the calculated dependent measure.

5.3.2.2 Rat-BART task and training

Training and testing for the rat-BART were conducted in chambers fitted with a house light, internal stimulus lights, food-delivery magazine and two retractable levers positioned to the left and right of the chamber wall opposite the magazine controlled by a PC running Med-PC IV (Med-Associates, St Albans VT). Training and testing was conducted during the early portion of the light cycle (7am-11am). Reward obtained during training and testing was 45-mg dustless precision, purified food pellets (Bio-Serv; Frenchtown NJ).

Training and testing was conducted as previously described (Jentsch et al., 2010). Briefly, in the rat-BART task, animals are presented with two levers: one (the 'add' lever) leads to an increase in the amount of reward pellets obtained per trial and the other permits subjects to 'cash out' that reward and end the trial. Pressing this 'cash out' lever (after the 'add' lever has been pressed at least once) results in immediate withdrawal of both levers and presentation of the accrued reward for consumption. Because there is a certain chance that each 'add' press will lead to trial failure with forfeiture of reward, the animal must make real-time decisions about accepting and avoiding risk during reward-seeking.

5.3.2.3 Rat-BART Battery

After initial training, rats were presented with a battery of 9 different sets of task conditions over 9 consecutive days, orthogonally manipulating the risk applied to the task (0, 10 or 16.7% risk) and the probability of gaining additional reinforcement per add press (100%, 50%, 33%). The purpose of these conditions was to evaluate sensitivity to risk and reinforcement probability across strains. Conditions where there was any risk that add presses would lead to trial failure were signaled by illumination of the house light from the onset of testing; conditions where there was no such risk were signaled by illumination of an internal stimulus light that was distinct from the house light. The sequence was presented in a cyclic Latin square-type design to account for testing order effects, with a no-risk version occurring in each 1/3 of the sequence of nine task versions. Dependent variables collected for analysis

included mean lever presses of trials with successful cash-outs (analogous to mean adjusted pumps in human studies (C. Lejuez et al., 2002), within-subject inter-trial variability of lever presses normalized by the subject's mean (henceforth referred to as variability) and the number of pellets obtained in a session (Jentsch et al., 2010). Each session of the rat-BART consisted of 50 individual trials, with an inter-trial interval of 3s. Sessions were limited to one-hour duration, after which rats were removed from the testing chambers; in general, almost all rats completed all 50 trials within 20 min.

5.3.3   Parental Strain Selection and Breeding

A high risk-taking strain and a low risk-taking strain were bred to produce F1 progeny. Criteria for selecting the high risk-taking strain were: exhibiting the greatest mean lever presses/trial across all versions of the rat-BART. The low risk-taking strain was the strain exhibiting statistically significantly lower mean lever presses/trial than the high risk-taking strain in the rat-BART battery, but otherwise matching the high risk-taking strain for the three control tasks. This presents the possibility that the low risk-taking strain selected may not show the actual lowest mean lever presses in the BART of all of the strains; but, it is critical for future genetics work that the two parental strains be as similar as possible except for the risk-taking trait. Furthermore, considering that the selection emphasizes decision-making under risk, strain ranking was checked when performance under 0% risk conditions were removed from consideration (and ultimately this did not alter strain choice). The F1 progeny (n=34) were derived onsite from crosses of inbred rats that were phenotyped in our assessments (n=1 pair), as well as some that were purchased from the vendor and were never phenotyped (n=2 pairs). The breeding pair selected from the rats that were phenotyped was chosen because the two rats exhibited the most extreme risk-taking (or averse) behavior but were also the most closely matched on the control tasks. The first pair consisted of a F344 dam crossed with a WF male that were drawn from the phenotyped inbred groups. The latter two pairs were crosses of WF dams with F344 males, none of which were phenotyped prior to breeding.

5.3.4   Data Analysis

All analyses were conducted on a PC running SPSS (v15). All data were subject to evaluation of normality and homogeneity of variance. Violations of sphericity were adressed by using Greenhouse-Geisser adjusted degrees of freedom. The general design for estimating heritability consisted of a repeated measures analysis of variance (ANOVA) for mean lever presses and variability (variance/mean) presses across the 9 versions of the rat-BART with strain and sex as between-subjects factors. The proportion of variance attributable to the factor of strain serves as an estimate of heritability; this method has been used previously (e.g., (Gubner et al., 2010; Isles, Humby, Walters, & Wilkinson, 2004; Liu & Gershenfeld, 2001; Reed, Bachmanov, Beauchamp, Tordoff, & Price, 1997; Wilhelm & Mitchell, 2009). Post hoc comparisons were performed when necessary with the Bonferroni correction. ANOVAs were conducted for average beam breaks, sucrose preference, and progressive ratio breakpoints across strains and sexes.

5.4   Results

5.4.1   Inbred Strains Comparisons

5.4.1.1   Breeding Selection Control Tasks.

An ANOVA was conducted with sucrose preference as a dependent variable, and strain and sex as independent variables. Across all strains, males preferred sucrose ($M = 88.7\%, SD = 11.3\%$) more than females ($M = 76.8\%, SD = 23.6\%$). Analysis revealed significant main effects of strain ($F(4, 55) = 8.79, p < 0.01, \eta^2 = 0.39$) and sex ($F(1, 55) = 4.83, p < 0.05, \eta^2 = 0.081$) with no significant interaction ($F(4, 55) = 1.08, p > 0.05$). See Figure 5.1A for post-hoc comparisons of strains.

A similar ANOVA was conducted for locomotor activity, with beam breaks as the dependent measure. Females ($M = 2124.15, SD = 670.23$) were more active than males ($M = 1473.45, SD = 385.04$), and this proved to be a significant effect ($F(1, 55) = 24.75, p <$

0.05, $\eta^2 = 0.31$). There was no effect of strain ($F(4, 55) = 0.522, ns$), but there was a significant strain x sex interaction, ($F(4, 55) = 6.817, p < 0.05, \eta^2 = 0.331$). Figure 5.1B shows that the SH and the BN strains exhibited the most robust sex differences. Estimates of heritability of these phenotypes from this and previous studies can be compared in Table 1. An ANOVA with progressive ratio breakpoint as the dependent variable, and strain and sex as independent variables, was conducted. There were significant effects of strain ($F(4, 55) = 12.75, p < 0.05, \eta^2 = 0.481$) and sex ($F(1, 55) = 37.52, p < 0.05, \eta^2 = 0.406$). Across strains, males exhibited higher break points ($M = 11.01, SD = 1.55$) than females ($M = 8.48, SD = 2.69$). The interaction between strains and sex was also significant ($F(4, 55) = 3.41, p < 0.05, \eta^2 = 0.199$). Figure 5.1C demonstrates that the interaction was driven largely by the BN strain.

Importantly, the WF and F344 strains did not significantly differ in post hoc analyses of any of these breeding selection control tasks. Sucrose preference for WF ($M = 90.2\%, SD = 5.9\%$) was slightly higher than for F344 rats ($M = 84.5\%, SD = 12.1\%$), but this difference was not significant ($t(29) = 1.00$). As for locomotor activity, WF rats were less active ($M = 1670.2$ beam breaks, $SD = 308.5$) than F344 rats ($M = 1696.2, SD = 470.0$), but not significantly so ($t(29) = -0.11$). Finally, WF rats had a marginally lower progressive ratio break point ($M = 10.1$ ratios completed, $SD = 1.84$) than F344 rats ($M = 10.3, SD = 1.87$), and again this difference was not significant ($t(29) = -0.33$).

From these analyses, heritability estimates are as follows: sucrose preference 39%, locomotor activity, 4%, progressive ratio, 48%. These values are lower than obtained in previous rodent studies. Estimates for sucrose are up to 50% (Reed et al., 1997), and for locomotor activity, as high as 75% (Isles et al., 2004). In both cases, our lower estimates may be attributed to differences in sample size and the variety of methods used to calculate behavior. We are unaware of any published data that are comparable to progressive ratio data shown here.
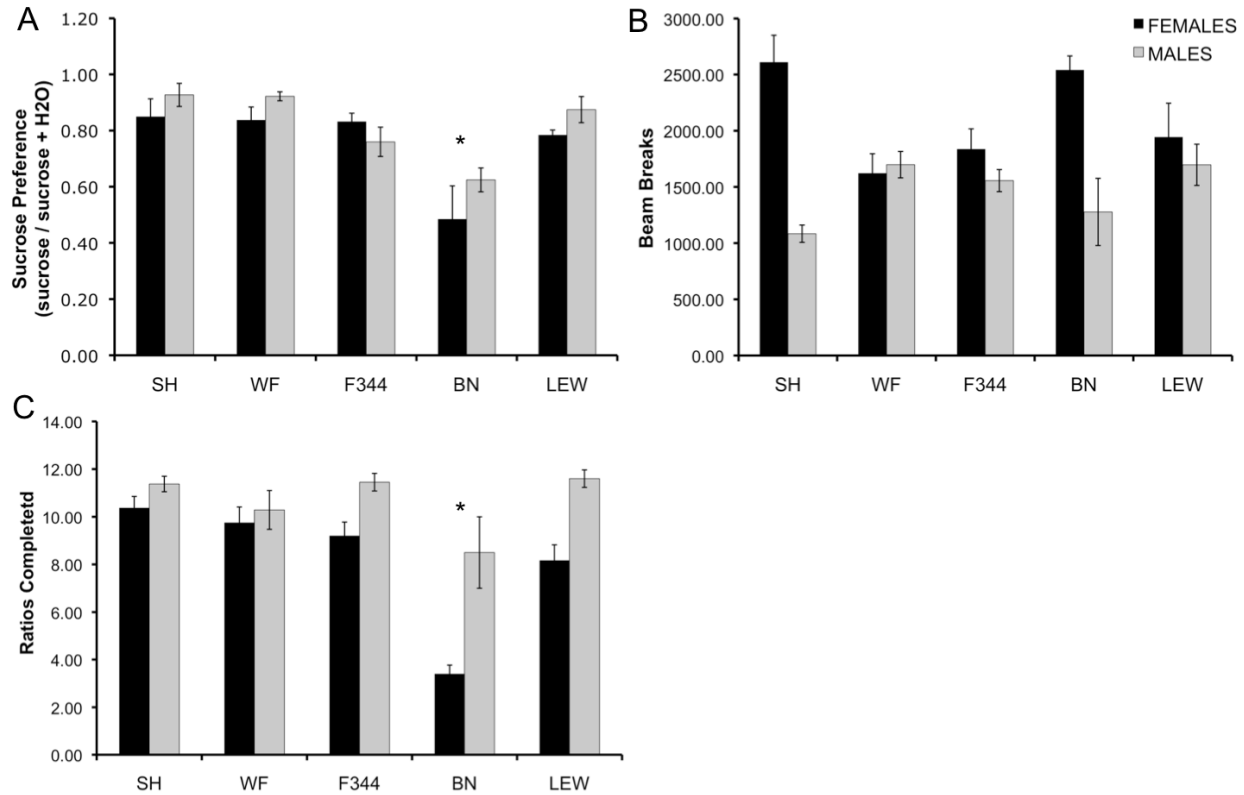
Figure 5.1: Breeding Selection Control Tasks. A) Sucrose preference (vs. water) of all strains across four days of testing calculated as relative consumption. The only strain that differed from others in post hoc testing was the BN strain. B) The number of beam-breaks over 15 min. No strain significantly differed in beam breaks (although there were some sex x strain interactions). C) Average number of ratios completed in the progressive ratio task across two test sessions. Most strains (except BNs) performed similarly. Most importantly, the F344 and WF strains showed no statistical difference in all three control tasks. Error bars are S.E.M. and * indicates a significant ($p < 0.05$) strain contrast in post hoc analysis with the Bonferroni correction.

5.4.1.2 Rat-BART Battery

A mixed 3x3 repeated-measures ANOVA (3 reinforcement conditions x 3 risk conditions) with strain and sex as between-subjects factors was conducted for the measures of mean lever presses per trial and the measure of within-session, within-subject variability. In terms of within-subject effects on mean lever presses across all strains (Figure 5.2), animals were sensitive to the changes in reinforcement conditions ($F(2, 110) = 6.18, p < 0.01, \eta^2 = 0.10$) as

well as to the changes in risk conditions ($F(1.59, 110) = 52.58, p < 0.01, \eta^2 = 0.49$). Namely, as risk of responding increased, mean leaver presses decreased, indicating less willingness to accept high-stakes gambles. On the other hand, as reinforcement probability decreased (e.g., from 100% to 50%), lever pressing correspondingly increased, indicating that rats were sensitive to the reward outcome of lever pressing; both of these results are consistent with previous work using outbred strains (Jentsch et al., 2010). Next, the interaction between risk and reinforcement conditions was significant ($F(4, 220) = 1.62, p < 0.05$). Variability of responding (calculated as variance across the session / mean of lever presses at cash-out) did not change as a function of reinforcement ($F(1.62, 110) = 0.93, ns$), though it was sensitive to changes in risk ($F(2, 110) = 40.85, p < 0.01, \eta^2 = 0.49$). Specifically, as risk applied to the task increased, inter-trial variability generally decreased, indicating that the degree to which rats alter their decision-making from trial to trial is dependent on the amount of risk in the session. Finally, the interaction between risk and reinforcement was not significant ($F(3.4, 220) = 0.39, ns$).

For mean lever presses, there was no main effect of sex ($F(1, 55) = 0.54, p > 0.05$) but the effect of strain was significant ($F(4, 55) = 16.66, p < 0.001, \eta^2 = 0.55$). This proportion of variance explained by the factor of strain is the measure of heritability; in this case, 55% of the variability in performance across all task versions was explained by strain, and therefore genomic, differences. The interaction between strain and sex was not significant ($F(4, 55) = 1.62, ns$). For the variability measure, across all task versions, there was no main effect of sex ($F(1, 55) = 0.08, ns$) but there was a main effect of strain ($F(4, 55) = 8.33, p < 0.001, \eta^2 = 0.377$). The interaction between strain and sex for this measure was not significant ($F(4, 55) = 1.8, ns$).

5.4.2 F1 and Parental Strain Comparisons

As the F344 strain was the most risk-taking among the strains (whether data obtained in the 0% risk variants were considered or not), this strain was selected as the high-risk parental strain. The WF strain was selected as the low-risk parental strain as rats exhibited

less risk-taking than the F344s, but were indistinguishable from the F344s in the control tasks. Analysis comparing these parental strains and progeny followed exactly as with the comparison of the five inbred strains.

5.4.2.1 Control Tasks

An ANOVA comparing sucrose preference scores as the dependent variable, with strain and sex as independent variables was conducted. There was no main effect of strain ($F(2, 60) = 0.55, ns$) or sex ($F(1, 60) = 0.98, ns$) for this measure (Figure 5.3A). A similar ANOVA was conducted for locomotor activity as a dependent variable. There was a significant main effect of strain ($F(2, 60) = 5.68, p < 0.05, \eta^2 = 0.159$) but not of sex ($F(1, 60) = 2.31, ns$), and there was no interaction between these variables ($F(2, 60) = 0.97, ns$). Post-hoc analyses demonstrated that the only pair-wise comparison of strains that significantly differed was between the F1 and F344 strains, with the F1 strain having the lowest activity overall (Figure 5.3B).

Analysis of breakpoints in the progressive ratio paradigm in an ANOVA revealed no main effect of strain ($F(2, 60) = 2.01, ns$) but a significant main effect of sex ($F(1, 60) = 12.18, p < 0.05, \eta^2 = 0.169$). In terms of schedules completed, females showed a lower breakpoint ($M = 9.84, SD = 1.49$) than males ($M = 11.30, SD = 1.49$). The interaction between strain and sex was not significant ($F(2, 60) = 1.20, ns; Figure 5.3C$)

5.4.2.2 BART Battery

To compare these parental strains and their progeny, a mixed repeated measures ANOVA was conducted exactly as for the five inbred strains. In terms of within-subject effects on mean lever presses per trial (Figure 5.4), animals in these three strains were sensitive to changes in risk conditions ($F(2, 98) = 53.26, p < 0.05, \eta^2 = 0.521$), as well as to changes in reinforcement probabilities ($F(2, 98) = 4.12, p < 0.05, \eta^2 = 0.078$), consistent with and in the same directions as the data across all inbred strains reported above. The interaction

between risk and reinforcement conditions was not significant ($F(4, 196) = 1.54, ns$). As for variability of responding, among these three strains, variability was sensitive to risk ($F(2, 98) = 50.85, p < 0.05, \eta^2 = 0.509$), but not to reinforcement probability ($F(2, 98) = 0.83, ns$), again consistent with data reported above. Finally, for the variability measure, the interaction between risk and reinforcement was not significant ($F(4, 196) = 0.76, ns$).

In terms of between-subjects effects for the measure of mean lever presses per trial, there was a significant main effect of strain ($F(1, 49) = 21.34, p < 0.05, \eta^2 = 0.303$) but not sex ($F(2, 49) = 0.004, ns$). The interaction between strain and sex was not significant for this measure ($F(1, 49) = 2.51, ns$). Post hoc analyses demonstrated that the F1 strain showed similar mean lever presses to the F344 strain and that these means were not statistically different. However, the F1 strain did significantly differ from the WF strain in terms of mean lever presses (Figure 5.4).

For the variability measure there was no between-subjects main effect of sex ($F(1, 49) = 0.11, ns$) but there was a main effect of strain ($F(1, 49) = 10.81, p < 0.05, \eta^2 = 0.181$). The interaction between strain and sex for this measure was not significant ($F(1, 49) = 3.25, ns$). Post hoc analyses showed that as with the mean lever presses measure, the F1 and F344 strains did not significantly differ in inter-trial variability, yet the F344 and WF as well as the WF and F1 pairs did significantly differ (Figure 5.4D-F).

5.5   Discussion

This study sought to determine the degree to which responding in a test of decision-making under risk is explained by genetic, heritable factors using a recently developed rodent version of the Balloon Analogue Risk Task (Rat-BART; (Jentsch et al., 2010)) that captures the core components of the human task by asking subjects to make decisions while balancing potential gains and losses (C. Lejuez et al., 2002). As in human subjects (C. Lejuez et al., 2002), all rodent strains were generally risk-averse, falling on the lower half of potential responses. Also consistent with human behavior, the greater the riskiness of the task, the

less behavioral output subjects will make to obtain potential rewards (C. Lejuez et al., 2002). No implementations of the human BART have used random ratio schedules of reinforcement as in our task versions; thus direct comparison with human sample data of the effects of changes in reinforcement schedule is not possible.

Across all of the strains, the rats were sensitive both to changes in the reinforcement and risk parameters, with a larger effect size for the latter. The main effect of reinforcement demonstrates that a decrease in the reinforcement probability generally results in an increase of lever pressing, although with small effect; also, increases in the amount of risk applied to the task generally results in less lever pressing. This is consistent with previous data obtained by our laboratory using out-bred strains (Jentsch et al., 2010). Omnibus analysis of all inbred strains across all risk and reinforcement conditions found significant main effects of strain for mean responses and variability, suggestive of a robust genetic influence on risk-related responding; in fact, about 55% of the variance in the measure of mean lever presses was attributable to heritable, genetic factors across these strains. The variability measure was heritable to a more moderate degree, explaining about 36% of the variance. Neither one of these phenotypes appeared to relate either to between strain differences in palatability preference, locomotor activity or motivation to complete an instrumental task for reward. The remaining 45% and 64% (respectively) of the variance in these traits is likely due to environmental influences and/or to measurement error.

Sex differences emerged only in the control tasks. Across all strains, males had higher sucrose preference, lower locomotor activity, and higher breakpoints in the progressive ratio task. Some strains were more sexually divergent than others, with a statistically significant interaction between strain and sex in the progressive ratio task. This interaction appears to be generated by the BN strain, which generally had the most sexual divergence among all the control tasks. Neither mean lever presses nor variability had any relationship with sex in the rat-BART across the strains and across all task versions. This is consistent with the human literature in that sex differences in the BART may only emerge under certain conditions, such as while under acute stress (Lighthall et al., 2009). However, the power to

detect sex differences within a specific strain is reduced due to the statistical design of the study.

5.5.1 Strain-Differences in Risk-Related Responding

The F344 strain exhibited relatively high mean presses per trial as well as high intra-subject variability of responding (Figure 5.2). Notably, this phenotype may be indicative of relatively poorer function of the medial prefrontal cortex, as this laboratory showed that inactivation of this brain region produces a highly variable response profile associated with reward loss (Jentsch et al., 2010). We hypothesize that F344 rats carry gene alleles that predispose them to sub-optimal high risk-taking (higher gambles and high variability).

It is important to note that the F344 rats exhibited, on average, higher responding across risk conditions (from 0 to 16%). Because 0% conditions are a minority of all sessions, it is possible that many rats view all test conditions (including the 0% condition) as being somewhat risky and that strain differences in risk-taking propensity are present even in the objectively non-risky test settings. Since the F344 rats did not show greater amounts of lever pressing in the progressive ratio test, it is unlikely that their phenotype is one only of greater instrumental action or motivation to obtain reinforcers. In the future, this question may be resolved using a between subjects design in which rats are trained only under no risk or only under risk conditions.

In contrast, the WF strain showed relatively conservative behavior, made few high-risk gambles and demonstrated much more regulated and consistent responding; still, this strain did not perform differently in the other behavioral tasks (locomotor activity, sucrose prefer-ence, progressive ratio) as compared to the F344 strain. Thus, Fischer-344 and Wistar-Furth breeding pairs were matched, and F1 progeny produced.

Analysis comparing the F1 progeny and their parental strains showed that in the rat-BART overall, the F1s behave more like the high risk-taking F344s, but are different from the WFs in terms of mean lever presses and inter-trial variability. Although it is unlikely that

such a relatively complex behavior is under the influence of only one gene, it is apparent that many of the potential genes of effect exert their influence in an additive dominant fashion. In other words, it may be that a certain subset of gene alleles more powerfully influence risk-taking behaviors in this task more than others, and that single copies from the high-risk F344 strain is sufficient to influence behavior.

Future efforts to elaborate the associations between risk-taking, genes, and substance use phenotypes should include endeavors to identify quantitative trait loci (QTL) that determine performance in the rat-BART using an F2 strategy. Importantly, the number and effect sizes of genes involved in regulating risk-taking behavior will limit the efficacy of this approach. Given the complexity of the behavior, it seems unlikely that a single polymorphic gene is at work. The moderate heritability estimate of 55%, however, suggests that with a larger F2 sample size, this method may produce valuable results by identifying a small set of risk genes.

5.5.2   Limitations and Future Directions

This study exhibits several strengths and weaknesses. The most thorough method would have been to phenotype all known inbred strains with the rat-BART in order to increase the power of isolating the most relevant QTL for future studies. For a single site, this is clearly an implausible challenge. Although inbred rat pups were cross-fostered to the same outbred strain there remained potential effects due to environmental differences in utero. Also, the F1 cohort of rats was generated in-house, while the inbred strains were born from pregnant females acquired from a vendor; thus, pre-natal shipping stress is not controlled for in the comparisons between F1 and parental strains. Additionally, group sizes were imbalanced because of the rodent acquisition and rearing methods, weakening power and statistical reliability. Nevertheless, cross-fostering was a major strength of our approach, as it is clear that the behavioral phenotypes exhibited by the inbred strains are not due to rearing effects. Also, this study represents the first publication of rat-BART data for female rats, which appear to not significantly differ in performance of the rat-BART in these strains. Other

study strengths included the use of control tasks to further isolate trait differences specific to the rat-BART to the exclusion of related behaviors like general motivation and appetite for palatable rewards, though behavior in the sucrose preference and locomotor activity tasks does not account for effects due to food-restriction, which could possibly alter the rank-order of the strains in these control tasks.

In summary, these inbred rat strains significantly differ in their behavior in the rat-BART, both in terms of mean lever presses and inter-trial variability, and these traits are under approximately 55% and 36% genetic influence, respectively. Progeny produced by crossing F344 X WF strains showed that the high-risk taking trait is 'dominant' in the sense that the F1 generation was statistically indistinguishable from the F344 strain for both risk-taking and variability traits. These results further our understanding of the genetic control of decision making by establishing the proportion of this trait that is under such control, and by establishing a strain of rat in which QTL analysis may be pursued in future studies.

5.6 Supplementary Analysis of False Start Data

5.6.1 Introduction

The original analyses presented above and published previously (Ashenhurst et al., 2012) did not include analysis of false start data (trial initiation errors) across these inbred strains and F1 hybrids. This metric was evaluated in subsequent analyses of F2 rats (Chapter Six) and in behavior of rats exposed to acute and chronic regimens of alcohol exposure (Chapter Four). For completeness, the goal of this supplemental analysis was to ascertain the heritability of this trait across the inbred rat panel, and to examine behavior of the F1 generation as compared to their parental F344 and WF strains.

5.6.2 Data Analysis Plan

Animals included in these analyses are the same as reported above. The dependent variable examined here was mean false starts across the nine rat-BART variants tested. False starts are scored whenever a rat presses the cash out lever prior to pressing an add lever.

In order to determine the heritability of this metric across the inbred rat strains, mean false starts were entered into an ANOVA with strain and sex as independent variables. Variance explained by strain status serves as an estimate of heritability. Next, mean false starts between the F1 and parental strains were compared in an ANOVA to examine potential mode of heritability. Strain and sex were entered as independent variables, and post-hoc pairwise comparisons with a Bonferroni correction were used to assess the nature of heritability (e.g., Mendelian or quantitative additive trait). Finally, effects of the dam of origin were examined within the F1 generation, as a subset of F1 rats were born from WF dams crossed with F344 males, and a subset were born from the opposite cross. False start data within the F1s was entered into an ANOVA with sex and dam of origin coded as independent variables.

5.6.3 Results

Recorded data was determined to violate normality using a Shapiro-Wilk test, $W(65) = 0.86, p < 0.0001$), so a Box-Cox transformation was used (Box & Cox, 1964). Subsequent testing of normality after transformation ($\lambda = -0.069$) revealed that the transformation to normality was successful, $W(65) = 0.981, p = 0.43$. All analyses presented are on transformed data.

5.6.3.1 Inbred Strain Panel

Mean false start rate across strains and sexes as well a significant post-hoc strain comparisons are presented in Figure 5.5A. Analyses revealed that there was a significant effect of strain on false starts, $F(4, 55) = 7.27, p < 0.0001, \eta^2 = 0.346$, as well as an effect of sex,

$F(1, 55) = 8.82, p < 0.0001, \eta^2 = 0.138$. There was no significant interaction between sex and strain on false starts, $F(4, 55) = 0.615, p > 0.05$. Post-hoc comparisons with Bonferonni correction showed a significant difference between F344s and all other strains, but no differences between the other strains.

5.6.3.2 Parental Strains and F1s

Across the parental strains and F1s, there was a significant effect of strain, $F(2, 60) = 5.81, p < 0.001, \eta^2 = 0.162$, and sex, $F(1, 60) = 17.09, p < 0.0001, \eta^2 = 0.222$, but not a significant interaction between them, $F(2, 60) = 1.18, p > 0.05$. Post-hoc pairwise comparisons showed significant differences for all strain comparisons, except for between F344 and F1 rats (Figure 5.5B). Consistent with comparisons across the inbred rat panel, females had a higher number of false starts than males.

Analyses within the F1 rats found a significant effect of sex, $F(1, 31) = 26.39, p < 0.0001, \eta^2 = 0.46$, and dam of origin $F(1, 31) = 8.76, p < 0.001, \eta^2 = 0.22$, but no significant interaction between them, $F(1, 31) = 0.15, p > 0.05$. As seen in Figure 5.5C, animals originating from WF dams made fewer false starts than those originating from F344 dams.

5.6.4 Supplementary Discussion

Analyses of false starts across the inbred strain panel (accounting for sex) revealed that this trait is somewhat heritable ($h^2 = 0.346$), but also that females made more false start errors than males overall. The same strain that was the most risk-taking in the task, was also the strain that made the fewest false start errors (F344). Consistent with other measures from the rat-BART (Figure 5.4), the F1 generation most closely resembled the F344 rats, suggesting additive and dominant quantitative gene effects.

While there was a sex effect overall, the most marked sex difference was within the F344 strain. The pattern of females making more errors than males was consistently observed among the F1 rats as well (5.5BC). These data suggest that the trait of false starts within

the rat-BART may be sex linked. The fact that dam of origin had a significant effect on F1 behavior suggests the presence of maternal effects. The pattern of false starts within the F1 generation accounting for dam-of-origin is not consistent with an entirely X chromosome linked trait. The males born of the WF dams must have received their X chromosome from the WF strain, and vice-versa for those males born of F344s. If the trait were fully X-linked, one would predict that these two sets of males should differ in the trait; these males do not differ in false starts (5.5C). Importantly, these F1 rats were raised by their birth dams and not cross-fostered to another strain as done for the inbred strains in the above analyses. Thus, rearing effects could also account for the dam-of-origin effect.

These results resemble sex effects previously found in a two-choice serial reaction time task where female Sprague-Dawley rats made more premature responses than males (Burton & Fletcher, 2012). However, sex effects across tests of impulsive action are not consistent as under some circumstances and in some strains, males are more 'impulsive' than females (Anker, Gliddon, & Carroll, 2008; van Hest, van Haaren, & van de, 1987; Jentsch & Taylor, 2003)

Of clear importance is a sound interpretation of what this metric represents, as there are several possibilities. First, false starts may indicate deficits in inhibitory control. During the task, the rat is required to withhold responding on the most reward-proximal lever (the cash out lever) and instead must direct its behavior to the add lever; failure to withhold responding immediately on the cash out lever, in this view, represents a failure to inhibit responses oriented towards a salient stimulus. Second, false starts could indicate deficits in working memory. The rat-BART is a multi-step task that likely requires representations of task progression within working memory. Limited working memory capacity, therefore, may promote errors in task sequence execution. Additional tests of working memory (spatial and non-spatial) and of response inhibition (such as a Go/No-Go Task) in the parental strains and F1s may reveal a similar sex-linked effect, suggesting that either of these may be the underlying deficit.

In sum, these supplementary results support the notion that false starts in the rat-BART

represents a somewhat heritable trait that is clearly modulated by sex. It is unclear why females make consistently more trial initiation errors than males in this task, but not in others. Additionally, these analyses identified maternal effects on false starts, as evidenced by the F1 rats born of dams of different strains.
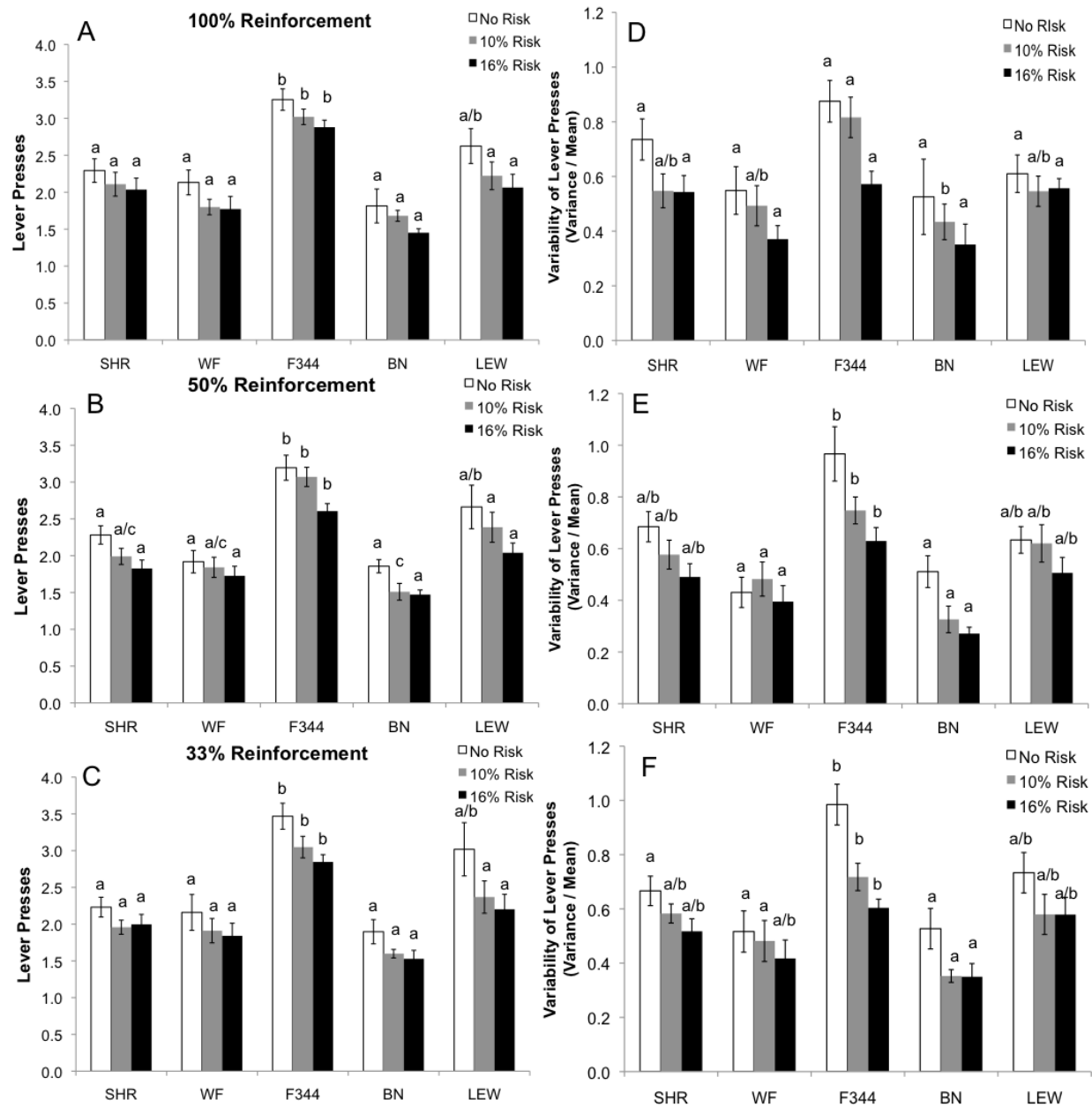
Figure 5.2: Performance of Inbred Strains in the rat-BART. A-C: Mean lever presses at 'cash out' of the inbred strains across three reinforcement and three risk conditions. The F344 strain showed the highest number of 'accept risk' responses across all condition. There was no effect of sex in any strain (not shown). D-F: Comparison of the inter-trial within-session variability of strains (computed as variance / mean) across all task variants. The F344 strain was again the most variable between trials in the rat-BART. Error bars are S.E.M. Bars marked by different letters were significantly different in between-subjects post hoc comparisons in the same task variant (same bar color) with a Bonferroni correction. Significance of within-subject comparisons is not presented here.

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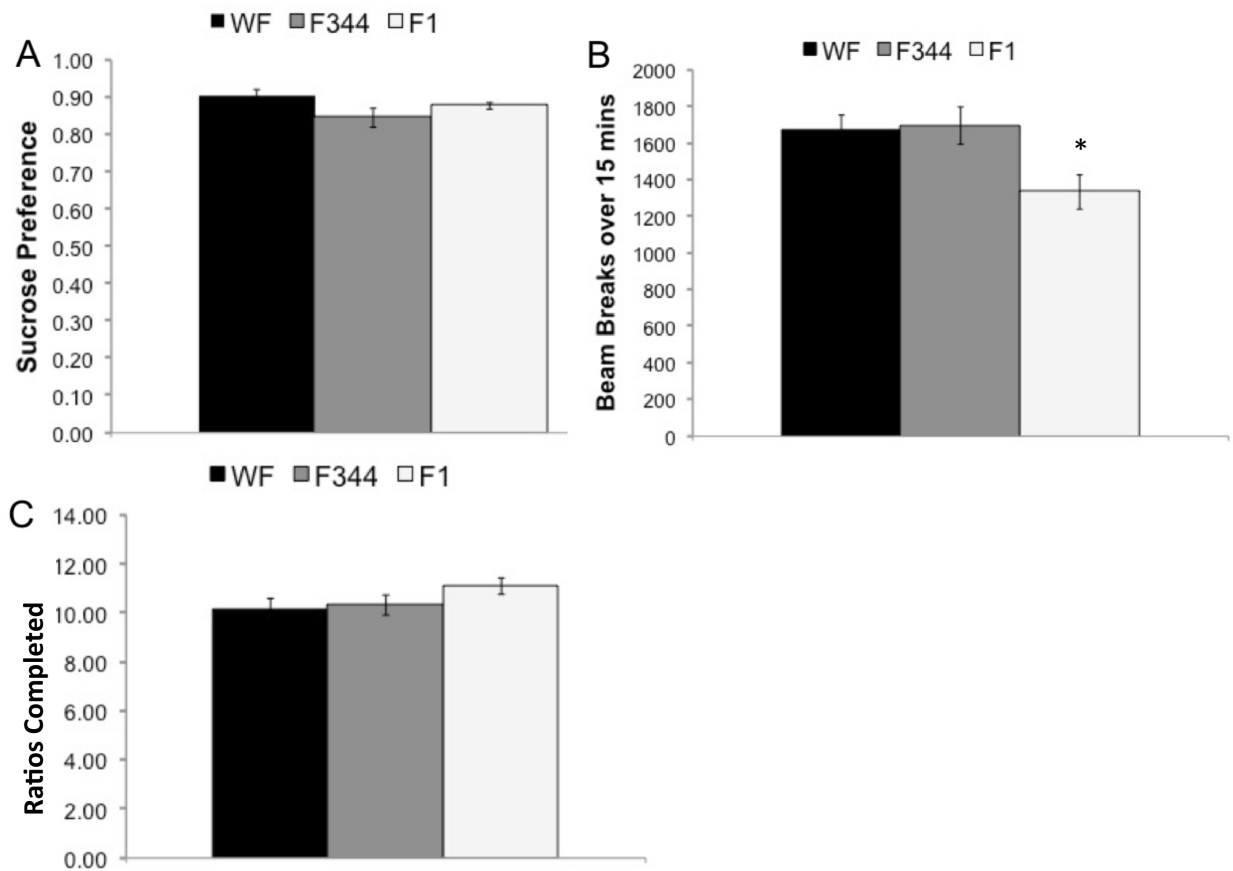
Figure 5.3: Control Task Behavior for F1 and Parental Strains. A) Sucrose preference (vs. water) for the F1 strain and the parental strains. There was no main effect of strain. B) Locomotor activity in response to a new environment in terms of beam breaks over 15 mins. There was a main effect of strain, driven by the F1 strain, which had the lowest activity overall. C) Data from the progressive ratio breakpoint test analyzed as number of reinforcements achieved. There was no main effect of strain, but there was an effect of sex (not presented). Error bars are S.E.M. and * indicates a significant ($p < 0.05$) strain contrast in post hoc analysis with the Bonferroni correction.
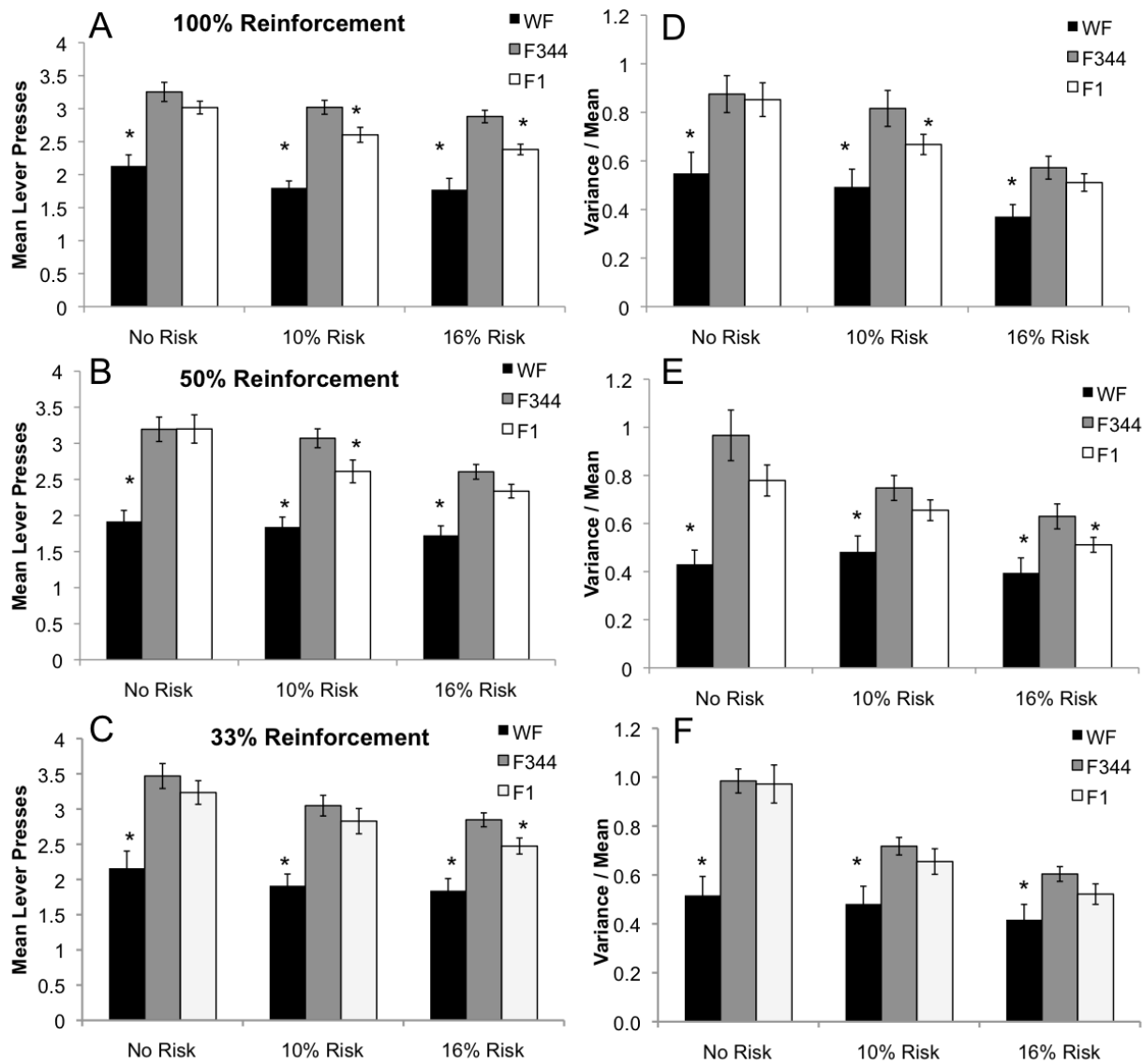
Figure 5.4: Comparison of F1 and Parental Strains. A-C: Mean lever presses at 'cash out' of the F1 and parental strains across three reinforcement and three risk conditions. The performance of the F1 strain generally resembled the Fischer-344 (F344) strain, with several exceptions where they expressed an intermediate phenotype. D-F: Comparison of the inter-trial within-session variability of strains (computed as variance / mean) across all task variants. Again, the F1 progeny more closely resembled the F344 parental strain in terms of variable behavior. Error bars are S.E.M. and * indicates a significant ($p < 0.05$) strain contrast in post hoc analysis with the Bonferroni correction.
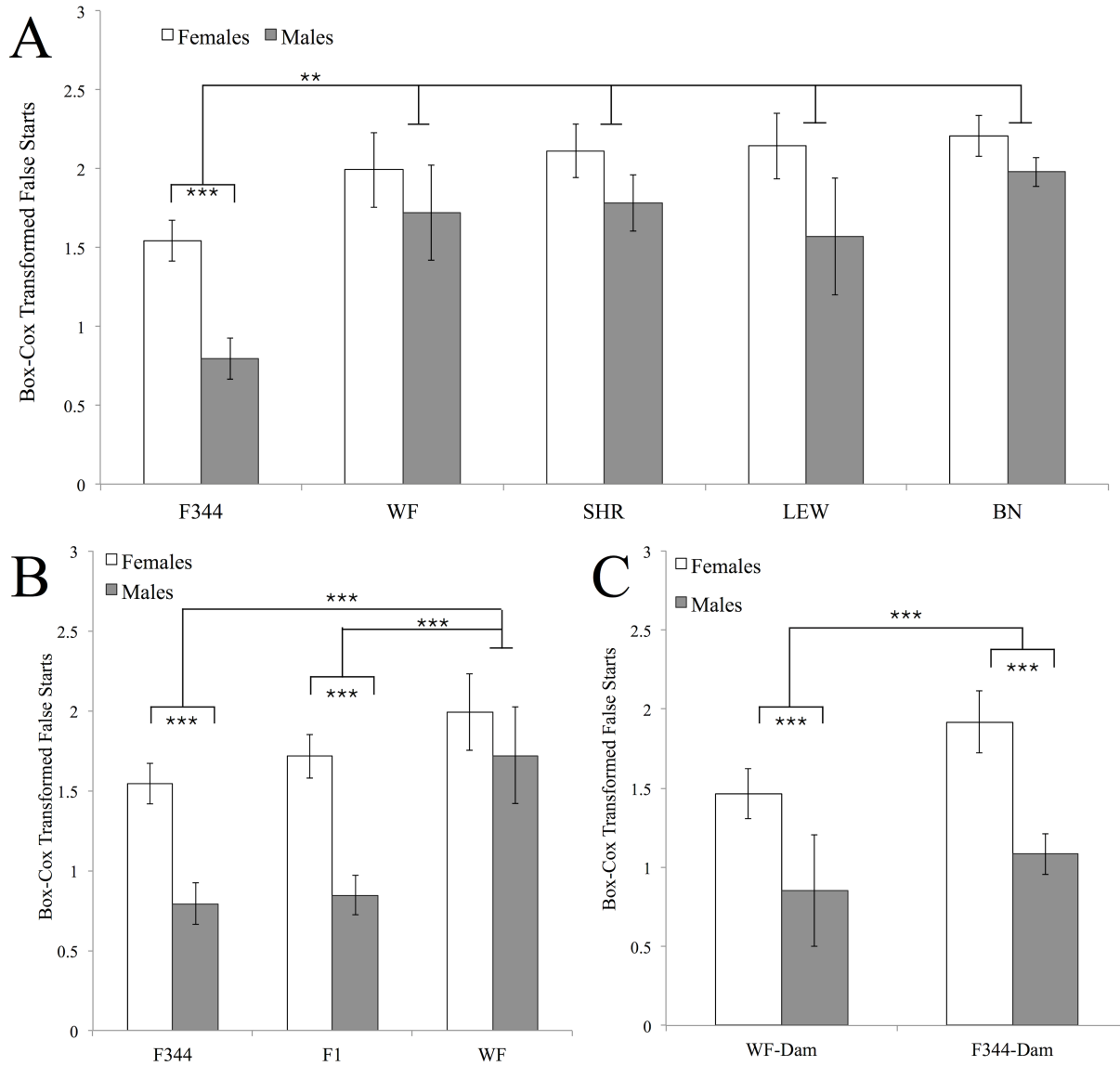
Figure 5.5: Examination of False Starts (Box-Cox Transformed). A) False starts across the inbred strains demonstrated a pattern of heritability estimated by the main effect of strain (approx. $h^2 = 0.346$). There was also a main effect of sex across all the strains, but no interaction between strain and sex. Simple effects post-hoc comparisons within strains revealed that there was a significant sex difference for the F344 strain only. B) Comparison of parental F344 and WF strains with the hybrid F1 progeny. There was a significant effect of strain and sex, but no significant interaction between them. Post-hoc strain comparisons showed that the WF strain made significantly more false starts than both F344s and F1s; the comparison between F344 and F1s was not significant. Looking at simple effects within strains, there was a significant effect of sex for the F344s and F1s but not for the WF strain. Error bars are S.E.M. and ** indicates a significant effect ($p < 0.01$) and *** indicates ($p < 0.001$) contrast in post hoc analysis with the Bonferroni correction.

CHAPTER 6

Modeling Quantitative Trait Loci Linked with Risk-Taking Behavior in the Rat

6.1 Abstract

Background: Recent investigation into risk-taking propensity as ascertained by the Balloon Analogue Risk Task (BART) and its rodent analogue have demonstrated moderate heritability. This suggests that selective breeding methods combined with genome-wide analysis may identify quantitative trait loci (QTL) that are linked with aspects of risk-taking behavior.

Methods: In the present study, an F2 intercross strategy was used to cross previously identified high risk-taking (Fisher-344) and low risk-taking (Wistar-Furth) inbred strains. These two founding strains were bred to produce an F1 generation, and subsequent F1 sibling pairs generated an F2 generation (n = 140). Rat genomic DNA was genotyped for a panel of microsatellite markers (60 total) with coverage on each of the 20 rat autosomes. Two methods of genotyping were used for each half of the full panel. The first set used a multiplex PCR and fluorescently-labeled primer strategy, while the second set used a standard singleplex and agarose gel electrophoresis method. Rat behavior was phenotyped in the rat-BART in terms of risk-taking, inter-trial variability, and trial initiation errors (false starts). Six parametric variations of the rat-BART that had two levels of risk (10% and 16%) and three levels of reinforcement ratio (100%, 50%, and 33%) were included in analyses. Quantitative trait loci analysis was performed using interval mapping methods available in QTL Cartographer software, with significance thresholds empirically determined by a 1000X permutation test.

Results: There was one significant QTL for the main risk-taking phenotype in the 100% reinforcement, 16% risk variation of the rat-BART, but not in the other task variants. This significant QTL was on Chromosomes 1 at approximately 103.2 Mb from the left telomere, with the 2-LOD support region including the area between 90.99 Mb and 129.99 Mb. There were no significant QTL for the other traits examined: inter-trial variability or false starts.

Conclusions: While preliminary, these results indicate that there may be genetic variation within this region on Chromosome 1 that regulates decision-making under risk, with primarily dominance effects. This region contains about 388 genes as identified in the Rat Genome Database. Potential candidates are highlighted. These results indicate that higher density trait mapping on Chromosome 1 is warranted in order to further narrow the QTL region and to advance candidate genes that are associated with risk-related decision-making in the context of the rat-BART. Additionally, human homologues should be explored.

6.2   Introduction

Biases in decision-making under circumstances of risk associates with susceptibility for a number of externalizing disorders. Research has indicated greater levels of risk-taking in some behavioral tasks among those with substance use disorders (Compton, Thomas, Conway, & Colliver, 2005; Kreek et al., 2005; Verdejo-Garcia, Lawrence, & Clark, 2008; Bechara et al., 2001; Cunha et al., 2011), bipolar disorder (Holmes et al., 2009; Reddy et al., 2013), and conduct disorder (Crowley, Raymond, Mikulich-Gilbertson, Thompson, & Lejuez, 2006; Fairchild et al., 2009) compared to unaffected individuals. Emerging evidence suggests that adolescence, in particular, is a period when heightened risk-taking propensity is particularly important for substance use initiation (Fernie et al., 2010; C. W. Lejuez et al., 2005; MacPherson et al., 2010; Xiao, Koritzky, et al., 2013; Xiao, Bechara, et al., 2013), as several studies have shown a negative relationship between risk-taking behavior and substance dependence severity in adults (Ashenhurst et al., 2011; Ryan et al., 2013).

Investigations into the biological determinants of risk-taking propensity have highlighted

the heritability of this trait (Kreek et al., 2005; Verdejo-Garcia et al., 2008). Twin studies in male adolescents have shown moderate heritability ($h^2 = 0.55$) of behavior in the Balloon Analogue Risk Task (BART) (Anokhin et al., 2009). Consistent with this finding, our group has demonstrated that performance in a rodent analogue (the rat-BART) is also heritable to the same degree ($h^2 = 0.55$) as determined by behavior across an inbred rat panel (Ashenhurst et al., 2012). While environmental influences are still important to consider, these data suggest that some genetic variation pre-disposes an individual to greater levels of risk-taking.

Some have already advanced candidate genes that may regulate behavior in risk-taking tasks, but have done so at the risk of neglecting other potentially linked loci. In particular, variation in the dopamine D4 receptor gene, *DRD4*, associates with risk-taking in an investment task (Kuhnen & Chiao, 2009), as does variation in the dopamine transporter gene *DAT1* with behavior in the BART in humans (Mata et al., 2012). While these candidate gene analyses are indicative of a potential role for dopamine genetics in decision-making under risk, these analyses did not examine the full genome. Targeted analyses may have missed other genomic loci that are linked with risk-taking behavior.

Thus, the goal of this study was to conduct a genome-wide quantitative trait loci (QTL) analysis in order to identify candidate genomic regions that may be pursued in future association studies with candidate genes. We sought to accomplish this by implementing our own F2 intercross strategy originating from the high risk-taking (Fischer-344) and low risk-taking (Wistar-Furth) strains previously identified in our heritability analyses (Ashenhurst et al., 2012). Our results, while preliminary, advance the field by examining the full genome, and by identifying regions where high-density genotyping is warranted.

6.3　Methods and Materials

6.3.1　Animals

6.3.1.1　Parental Strain Selection and Breeding

Previously, five inbred strains (Wistar-Furth, Fischer-344, Brown Norway, Spontaneously Hypertensive Rat, and Lewis) were assessed in the rat-BART, for sucrose preference, for spontaneous locomotor activity, and for motivation to perform operant tasks in a progressive ratio task (Ashenhurst et al., 2012). These additional phenotypes were ascertained in order to identify strains that differed in risk-taking, but not in other aspects related to task performance like preference for sweets or general motivation to perform an operant task. Of these five strains, the Fischer-344 and Wistar Furth strains significantly differed in terms of risk-taking behavior in the rat-BART, but did not differ in any of the these three control tasks (Ashenhurst et al., 2012). Next, three pairs of high risk-taking rats (Fischer-344 female; F344) and low risk-taking rats (Wistar Furth male; WF) were mated to produce an F1 generation.

6.3.1.2　F2 Generation

F1 sibling pairs were mated in order to produce an F2 generation (N = 140). Pups were born in house and reared by their F1 dams. Pups were weaned at three weeks of age and placed into group housing (two or three individuals of single-sex and single strain) in acrylic shoebox cages (10" X 18") on a 14L:10D schedule. Both males and females were used in analysis as we have observed no sex differences in main indicators of risk-taking in any conditions tested (Ashenhurst et al., 2012).

6.3.2　Rat-BART Task and Training

Training and testing for the rat-BART were conducted in chambers fitted with a house light, internal stimulus lights, food-delivery magazine and two retractable levers positioned

to the left and right of the chamber wall opposite the magazine controlled by a PC running Med-PC IV (Med-Associates, St Albans VT). Reward obtained during training and testing was 45-mg dustless precision, purified food pellets (Bio-Serv; Frenchtown NJ).

Training and testing was conducted as previously described (Ashenhurst et al., 2012; Jentsch et al., 2010). Briefly, in the rat-BART task, animals are presented with two levers: one (the 'add' lever) leads to an increase in the amount of reward pellets obtained per trial and the other permits subjects to 'cash out' that reward and end the trial. Pressing the 'cash out' lever (after the add lever has been pressed at least once) results in immediate withdrawal of both levers and presentation of the accrued reward. Because there is a certain chance that each add press will lead to trial failure with forfeiture of reward, the animal must make real-time decisions about accepting and avoiding risk during reward-seeking. Thus, the core components of this rodent task are analogous to the human BART (C. Lejuez et al., 2002).

Subjects were first trained in the following sequence with the goal of shaping responses to both add and cash-out levers: training on a fixed ratio (FR)-1 schedule of reinforcement for the add lever and then the same on the cash-out lever. Next, subjects were trained using an FR-3, followed by an FR-10, schedule on the add lever. Criterion for progression through these early stages of training was earning at least 20 pellets within an hour-long session (with the maximum being 100 pellets). Animals were then familiarized with the general nature of the rat-BART using a 50-trial 'forced' task in which only the add lever was presented until the rats responded between 2 and 15 times (randomly chosen from trial to trial); when the subjects completed the mandatory number of responses, the add lever was withdrawn and the cash-out lever was presented.

Animals were next trained - over six consecutive days - on versions of the task itself; this phase also introduced animals to the possibility of trial failures and to partial reinforcement conditions. Thus, animals encountered conditions wherein the probability that each add press would lead to an additional accrued pellet was set to 100%, 50% or 33%, while the risk that each add press would lead to trial failure was set at 0% (no risk) or 10% risk; it

is important to note, however, that the first add press was always 'safe'. During this phase, animals were able to choose when to cash out and obtain reward. In doing so they are able to avoid the risk associated with add presses; in other words, the rats were able respond on the add lever on each trial until: 1) it caused the trial to fail by excessive responding (resulting in both levers being removed and a 3-s time-out being enforced) or 2) it pressed the cash-out lever and was presented with its earned reinforcement.

Conditions where there was any risk that add presses would lead to trial failure were signaled by illumination of the house light from the onset of testing; conditions where there was no such risk were signaled by illumination of an internal stimulus light that was distinct from the house light. This lighting signal was maintained during the testing phase of the study. During this final training phase, there was no criterion to determine progression of animals on an individual basis; all subjects were moved through the same sequence as a group.

6.3.2.1 Rat-BART Battery

After initial training, rats were presented with a battery of 9 different sets of task conditions over 9 consecutive days, orthogonally manipulating the risk applied to the task (0, 10 or 16.7% risk) and the probability of gaining additional reinforcement per add press (100%, 50%, 33%). The sequence was presented in a cyclic Latin square-type design to account for testing order effects, with a no-risk version occurring in each 1/3 of the sequence of nine task versions. Dependent variables collected for analysis included: mean lever presses of trials with successful cash-outs (analogous to mean adjusted pumps in human studies (C. Lejuez et al., 2002), within-subject inter-trial variability of lever presses normalized by the subject's mean (the session coefficient of variance) and the number of trial initiation errors, or 'false starts'. False starts were scored whenever a rat pressed the 'cash out' lever prior to pressing the add lever. Each session of the rat-BART consisted of 50 individual trials, with an inter-trial interval of 3s. Sessions were limited to one-hour duration, after which rats were removed from the testing chambers; in general, almost all rats completed all 50 trials within

20 min.

6.3.3   Marker Panel Development and Validation

6.3.3.1   DNA extraction and purification

Genomic DNA was extracted from ear tissue from parental strains, F1s, and all F2 rats. Small clips of tissue were subject to cell lysis and digest by Proteinase k (Roche, Indianapolis, IN) in 300 μl of a lysis buffer (20 mM Tris-Cl pH 8.0, 5 mM EDTA, 400 mM NaCl, 1% w/v SDS) at 55°C overnight. Digests were centrifuged at 13,000 RPM for 3 min to separate protein from other soluble cellular contents including DNA. Isopropanol (500μl, 99%, Sigma-Aldritch, St Louis, MO) was added to the supernantant in order to precipitate DNA from the solution. This mixture was centrifuged at 13,000 RMP for 10 min, and DNA pellets were rinsed with 200μl 70% ethanol solution in order to remove salts and other polymerase chain reaction inhibitors. DNA pellets were re-suspended in 100μl tris-EDTA (pH 8.0, Fisher Scientific, Waltham, MA) for storage. Samples were diluted to 50-100 ng/μl concentrations in PCR-Grade water (Roche) as determined by a BioPhotometer (Eppendorf, Hamburg, Germany) prior to genotyping assays.

6.3.3.2   Marker Selection and Validation

A panel of 60 microsatellite (simple sequence length polymorphic) markers was developed with the goals of: 1) approximately 30 mb resolution coverage of all 20 rat autosomes and 2) maximal fragment length differences between the WF and F344 strains. About half of the markers used were previously identified for use in a panel suitable for multiplex-PCR (Bryda & Riley, 2008). The second half were identified using the Rat Genome Database GeneScanner tool (http://rgd.mcw.edu/, n.d.). Forward and reverse primer sequences were obtained from the Rat Genome Database (Laulederkind et al., 2013) and custom oligos (a subset fluorescently labeled) were commercially synthesized and purchased from Applied Biosystems, Inc (Foster City, CA).

Validation of the markers was conducted as follows. Three each of F344, WF and F1 rat genomic DNA samples were subject to PCR for each marker individually. Peaks/bands obtained after electrophoresis were compared to expected sizes from the Rat Genome Database (Laulederkind et al., 2013). A marker was considered valid if it reliably discriminated between F344 and WF rats with peaks/bands of different sizes that closely matched those from the Rat Genome Database, and if both peaks/bands were present in the F1 samples (see Figures 6.1 and 6.2 for examples). Of the 60 markers tested, four failed because of a lack of discrimination between parental strains or the lack of heterozygous peaks/bands in the F1 samples (see Table 6.1). For validated markers, base pair fragment lengths were all within 10bp of those given in the Rat Genome Database (Laulederkind et al., 2013). PCR parameters used for validation are the same as those used for genotyping, described below.

6.3.4 F2 Generation Genotyping Assays

Genotyping was conducted in two ways for the first and second halves of the marker panel. For the first half, fluorescent-labeled primers were used in a multiplex-PCR design. For the second half, un-labeled primers and singleplex PCR was used. Specific parameters of these assays are described below. PCR done for validation of these marker sets (outlined above) was conducted using these same parameters for F2 genotyping.

6.3.4.1 Set One: Multiplex-PCR

In order to reduce the number of individual PCR reactions needed for genotyping, a multiplex PCR strategy was used (Bryda & Riley, 2008). Four dye colors available from Applied Biosystems were used to label the 5' end of the forward primers: 6-FAM (blue), NED (yellow), PET (red) and VIC (green). Thirty markers were grouped into pools of seven or eight (four pools total) and assigned fluorescent labels under the following criteria: 1) no fragment lengths of either parental strain overlapped and were labeled with the same color dye within a given pool and 2) sequence overlap between primers is minimal within a pool.

Table 6.1: Full Marker Panel across all rat autosomes. Fragment lengths for Wistar-Furth (WF) and Fischer-344 (F344) strains are provided as well as the difference between them. Markers which failed to pass validation are indicated by (†). These markers were not included in the quantitative trait loci analysis. Markers for which segregation distortions were detected are indicated by (❖). Modeling was conducted with and without these markers.

Chromosome	Marker Name	WF Size	F344 Size	Difference	Chromosome	Marker Name	WF Size	F344 Size	Difference
1	D1Rat5 †	184	163	21	11	D11Rat63	252	206	46
	D1Rat261	141	156	15		D11Rat10	158	172	14
	D1Rat29	145	169	24		D11Mit2	229	261	32
	D1Rat149	190	210	20	12	D12Rat43	247	320	73
	D1Rat126	154	118	36		D12Rat14 †	156	188	32
2	D2Rat18	180	166	14		D12Rat53	136	123	13
	D2Rat174 ❖	181	165	16	13	D13Rat60	129	131	2
	D2Rat47	178	158	20		D13Rat142	196	202	6
	D2Rat250	146	180	34	14	D14Rat72	176	156	20
3	D3Rat277	236	250	14		D14Rat95 ❖	140	176	36
	D3Rat73	205	207	2	15	D15Rat116	166	144	22
	D3Rat15 ❖	151	121	30		D15Rat29 †	122	153	31
4	D4Rat3	150	148	2	16	D16Mgh9	266	298	32
	D4Rat14	186	178	8		D16Rat97	250	248	2
	D4Arb35 ❖	146	158	12		D16Rat15	146	144	2
5	D5Rat128	284	240	44	17	D17Mgh1	131	143	12
	D5Rat17	122	126	4		D17Rat151	179	154	25
	D5Rat33	118	132	14		D17Rat65	460	228	232
6	D6Rat62 ❖	200	232	32	18	D18Rat44	206	200	6
	D6Rat100	188	182	6		D18Rat8	164	154	10
	D6Rat3	115	146	31		D18Rat61	222	246	24
7	D7Mgh9	154	124	30		D18Rat116	235	225	10
	D7Rat74	135	129	6		D18Rat111	245	223	22
	D7Rat94	113	117	4	19	D19Rat47	256	209	47
8	D8Rat163	177	191	14		D19Rat11	141	120	21
	D8Rat41 ❖	123	153	30		D19Rat58	107	137	30
	D8Mgh4	156	159	3	20	D20Mit4 ❖	200	216	16
9	D9Rat132 †	216	134	82		D20Rat55	225	195	30
	D9Mit3	176	174	2					
10	D9Rat104	186	216	30					
	D10Rat130	199	207	8					
	D10Rat135	162	140	22					

The design of these four multiplex pools is displayed in Table 6.2.

Multiplex PCR was then conducted for each of the four multiplex pools similarly to as done described (Bryda & Riley, 2008). Each 20μl PCR reaction contained between 50 to 100ng rat genomic DNA, 2μl 10X Reaction Buffer (ThermoPrime, Thermo Scientific), and the following final concentrations of these reagents: 1.5mM MgCl$_2$, 0.2mM of each dNTP (TheremoScientific), 0.5 U ThermoPrime *taq* Polymerase, and 0.2 to 0.5 μM of each labeled primer. Each reaction was brought to the final reaction volume (20μl) with sterile PCR-grade water (Roche).

Touchdown PCR amplification (Don, Cox, Wainwright, Baker, & Mattick, 1991) was performed in an Applied Biosystems 2720 Thermal Cycler. In this method, annealing temperature is decreased by one half degree with each cycle in a first round of cycles in order to amplify multiple targets with primer sequences that have different optimal melting/annealing temperatures as is the case in multiplex PCR. The following cycle parameters were used: one cycle at 94°C for 2 mins for initial denaturation, followed by 14 cycles of 94°C for 20s, one step at 60°C to 53°C (decreasing by half a degree with each cycle), and an extension step at 72°C for 1 min. Following this touch-down protocol, fragments were amplified under the following parameters for 30 cycles: 94°C for 20s, 53°C for 30s, 72°C for 1 min. A final extension period of 9 mins at 72°C was given to complete any incomplete fragments.

PCR product was processed by the UCLA Genotyping and Sequencing Core and run through an Applied Biosystems 3730 capillary sequencer instrument to detect fluorescent peaks representing DNA fragments. Fragment lengths were standardized against GeneScan 500 LIZ Size S Standard (Applied Biosystems), which was mixed into each sample set. Data was visualized and allele sizes were called using GeneMapper software (v4, Applied Biosytems).

Table 6.2: Mutiplex PCR pools. This table displays the multiplex PCR design, with the markers in each of the four multiplex pools outlined. Included is the fluorescent dye label used on the 5' end of the forward primer, the chromosome, and the sizes (base pairs) of the Fisher-344 and Wistar-Furth alleles respectively. Pools were designed such that no markers tagged with the same color fluorescent dye had overlapping allele sizes.

Marker	Pool	Dye	Chromosome	F344 Size	WF Size
D18Rat61	1	fam	18	246	222
D8Rat41	1	ned	8	153	123
D18Rat44	1	ned	18	200	206
D5Rat17	1	pet	5	126	122
D9Mit3	1	pet	9	174	176
D5Rat33	1	vic	5	132	118
D18Rat8	1	vic	18	154	164
D13Rat142	2	fam	13	202	196
D16Rat97	2	fam	16	248	250
D8Mgh4	2	ned	8	159	156
D4Rat14	2	pet	4	178	186
D7Rat94	2	pet	7	117	113
D17Mgh1	2	pet	17	143	131
D1Rat29	2	vic	1	169	145
D1Rat149	2	vic	1	210	190
D3Rat73	3	fam	3	207	205
D3Rat277	3	fam	3	250	236
D16Rat15	3	ned	16	144	146
D20Mit4	3	ned	20	216	200
D2Rat174	3	pet	2	165	181
D7Rat74	3	pet	7	129	135
D4Arb35	3	vic	4	158	146
D18Rat116	3	vic	18	225	235
D4Rat3	4	fam	4	148	150
D6Rat100	4	fam	6	182	188
D13Rat60	4	ned	13	131	129
D14Rat72	4	ned	14	156	176
D3Rat15	4	pet	3	121	151
D15Rat116	4	vic	15	144	166
D10Rat130	4	vic	10	207	199

6.3.4.2 Set Two: Singleplex PCR

For the second set of markers, each forward/reverse primer set was subject to individual PCR at a total reaction volume of 10μl. Each PCR reaction contained: 50-100ng genomic DNA, 5μl 2X concentration Thermo Scientific ThermoPrime Plus MasterMix (1.5 mM MgCl$_2$), 0.5 μM each for the forward and reverse primers, and PCR-grade water (Roche). PCR amplification was performed in an Applied Biosystems 2720 Thermal Cycler under the following conditions: 1 cycle at 94°C for 2 mins, then 37 cycles of 94°C for 20s, 54°C for 30s, and 72°C for 1 min. The final extension time was 7 mins at 72°C.

Genotyping was conducted by agarose gel electrophoresis. Gels were made at a concentration of 2% by heating 200 mL of TAE [made from 50X stock TAE solution (Bio-Rad, Hercules, CA) in dd-H$_2$O] mixed with 4g agarose (Invitrogen UltraPure™) to boiling. After this, 10μl ethidium bromide (10mg/ml, Bio-Rad) was added to the molten gel, and then gels were cast in a 2x30 or 2x20 well format and placed in a electrophoresis gel box (Sub-Cell® GT, Bio-Rad) containing TAE solution. PCR samples were mixed with 1μl loading dye (BlueJuice™ gel loading buffer 10X, Invitrogen) and 10μl of this mixture was loaded into individual wells. A 100bp fragment size standard (GeneRuler™, Fermentas, Vilnius, Lithuania) was used to verify fragment lengths against those in the Rat Genome Databse. Gels were run at 100V for 1.5hrs, after which DNA bands were visualized on a UV lightbox (UV Transilluminator, UVP Inc., Upland, CA). An example of marker validation by these methods is presented in Figure 6.2, and an example of F2 genotyping by these methods is presented in Figure 6.3.

6.3.5 Data Analysis Plan

6.3.5.1 Behavioral Data Analysis

All analyses of trait data were conducted on a PC running SPSS (v15). All data were subject to evaluation of normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965) and for violations of the assumption of homogeneity of variance where necessary. Traits that

are not normally distributed across the F2 generation were transformed using the Box and Cox transformation (Box & Cox, 1964). Data across the six versions of the rat-BART that included risk (10% and 16%) were averaged to produce single dependent variables for task performance indices. Dependent variables subject to QTL analyses included mean lever presses at cash out, the main indicator of risk taking, inter-trial variability, and average false start rate.

6.3.5.2 Quantitative Trait Loci Analysis

QTL were identified using QTL Cartographer (v2.5.011). First, observed allele frequencies in the F2 generation are compared to the expected 1:2:1 AA:AB:BB Mendelian ratio. Significant segregation distortions (χ^2 test) are indicative of problematic allele calls, interference during meiosis cross-over events (causal segregation distortion loci), and gametic or zygotic selection (Xu, 2008). Significant segregation distortions were detected for several markers (indicated in Table 6.1). Models were run with and without these data, and this did not alter the significance of the one QTL identified (Figure 6.5).

Next, data from only the task variants where risk was applied (e.g., 10% and 16% risk) was subject to interval mapping. The method used by the software compares the likelihood of the data at points along each chromosome either assuming the presence or absence of a QTL, resulting in a log-of-odds (LOD) score (Lander & Botstein, 1989). Interval mapping allows for analysis of points *between* genotyped markers by estimating the likelihood distribution of genotypes at 1 cMorgan steps between known markers with a known distance between them. This strategy is essentially like computing a linear regression with missing data but with known probability distributions for the missing independent variables (Lander & Botstein, 1989). To address this, Expectation-Maximization algorithms (EM) are used for maximum likelihood estimation (Dempster, Laird, & Rubin, 1977; Lander & Green, 1987). The boundaries of any identified QTL were defined as the 2-LOD support region around the peak likelihood ratio statistic (LRS) (Lander & Botstein, 1989). As this F2 population contained both males and females, sex was entered as a covariate in all models.

Significance thresholds were empirically determined by a 1000X permutation test (Churchill & Doerge, 1994). Essentially, observed data is permuted 1000 times and QTL interval mapping analysis is conducted on each permutation. Maximum LOD statistics for each permutation are recorded and ordered from lowest to highest. For a study-wise error rate of $\alpha = 0.05$, an empirical threshold is drawn at the 950th highest ordered test statistic (Churchill & Doerge, 1994). Empirically determined LOD thresholds for mean lever presses are shown in Table 6.3. There were no LOD scores above 3 found for inter-trial variability or false start measures, so (computationally intensive) empirical thresholds were not determined.

	100% Reinforcement		50% Reinforcement		33% Reinforcement	
Risk Level	10%	16%	10%	16%	10%	16%
LR	16.09	17.24	21.17	17.9	20.69	16.3
LOD	3.49	3.74	4.59	3.88	4.49	3.54

Table 6.3: Empirically Determined Significance Thresholds for Mean Lever Presses for all Risk Variants. A 1000X permutation test was used to score empirical thresholds for significance in QTL analyses. Values shown are for mean lever presses measured in each of the six rat-BART task variants that were subject to QTL analysis. LR = likelihood ratio statistic. LOD = log-of-odds statistic.

6.4   Results

6.4.1   rat-BART behavior and sex effects

Across the F2 population and across all rat-BART tasks, the mean of lever presses was $M = 2.38, SD = 0.53$, while the mean of inter-trial variability was $M = 0.59, SD = 0.14$, and the mean of false starts was $M = 4.78, SD = 4.8$ (all values untransformed).

The distributions of average lever presses at cash out (risk-taking) and inter-trial variability were consistent with a normal distribution as assessed by the Shapiro-Wilk test (Shapiro & Wilk, 1965). However, false starts, $W(140) = 0.712, p < 0.001$, significantly deviated from normality. Thus, false start data were subject to a Box-Cox transformation (Box &

Cox, 1964). The resultant transformed data ($\lambda = -0.205$) was consistent with a normal distribution, $W(140) = 0.994, p > 0.05$.

Next, behavioral data was subject to analyses for sex effects in a one-way ANOVA (males n = 67, females n = 73). There was no effect of sex on risk-taking, $F(1, 138) = 1.82, p > 0.05$, or inter-trial variability, $F(1, 138) = 0.119, p > 0.05$. There was, however, a significant effect of sex on false starts, $F(1, 138) = 5.96, p < 0.01$. On average, females made more false starts (untransformed $M = 5.77, SD = 5.8$. transformed $M = 1.33, SD = 0.51$) than males (untransformed $M = 3.72, SD = 3.15$. transformed $M = 1.13, SD = 0.46$)

6.4.2 QTL analyses

Separate interval-mapping QTL models were run in QTL Cartographer on risk-taking, inter-trial variability, and false start data from the six risk versions of the rat-BART. Across all of the rat-BART versions tested, there was only one QTL that surpassed the empirical significance threshold in the 100% reinforcement, 16% risk condition on Chromosome 1 (Figure 6.5). Otherwise, there were no significant QTL in other task variants for the main index of risk-taking (Figures 6.6-6.7), or for inter-trial variability or false starts (Figures 6.8-6.9).

The location of the significant QTL is on Chromosome 1, at approximately 103.2 Mb from the left telomere, with the 2-LOD support region including the area between 90.99 Mb and 129.99 Mb. This region is near the rat microsattelite marker D1Rat129. Genes in this area are discussed below.

6.5   Discussion

The goal of these analyses was to identify quantitative trait loci (QTL) linked with behavioral metrics observed in the rat-BART in a moderately sized F2 population bred from parental strains that significantly differed in risk-taking propensity. This was accomplished by designing and implementing a genome-wide microsatellite marker panel using both mul-

tiplex and singleplex PCR methods.

6.5.1   Identification of a Potential QTL

The results of interval mapping analyses using QTL cartographer revealed only one significant QTL that surpassed the empirically estimated significance thresholds. This was observed in the 100% reinforcement and 16% risk condition. Importantly, however, subthreshold peaks in the same region on Chromosome 1 are present in all of the task variants analyzed. The location of this QTL was on Chromosome 1 at approximately 103.2 Mb from the left telomere, with the 2-LOD support region including the area between 90.99 Mb and 129.99 Mb.

Searching for genes within this region in the Rat Genome Database (Laulederkind et al., 2013) yielded 388 genes. Given this large number, no single candidate should be advanced as potentially regulating decision-making under risk. However, there are several genes present in this region that are expressed in the brain and are likely to have major effects on neural function. This includes *Chrna7*, a gene coding the nicotinic acetylcholine receptor α-7. Variation in this gene has been shown to modulate nicotine reward phenotypes in mice (Harenza, Muldoon, Biasi, Imad-Damaj, & Miles, 2013), and is proposed to associate with some cases of schizophrenia and may be a novel target for therapeutic intervention (Freedman, 2013). Another gene is *Igf1r* which encodes an insulin regulated growth factor that is implicated in axonal growth (Dupraz et al., 2013). Finally, the region includes an aldehyde dehydrogenase gene *Aldh16a1*, which is implicated in alcohol metabolism and this enzyme class is a target of therpeutic treatment (Koppaka et al., 2012).

Finally, this region was subject to a search for traits previously found to be linked to this area in QTL analyses using the Rat Genome Database (Laulederkind et al., 2013). There were 71 QTL records identified, with most pertaining to blood pressure, cardiac weight, and bone mineral density. QTL potentially related to the risk-taking trait, alcohol, and brain function more broadly include two for alcohol response in the loss of righting reflex

test (RGD ID: 1641897, 2317833), two for anxiety (RGD ID: 738022, 2313402), and two for stress response (RGD ID: 1578770, 1358189).

6.5.2 General Discussion

Given the moderate level of heritability for the main trait of risk-taking ($h^2 = 0.55$), this lack of significance is likely due to insufficient sample size or insufficient density of markers used during genotyping. Both of these reduce the power to detect QTL. Typical F2 QTL designs that have detected significant results for behavioral traits in rats have sample sizes in the range of 200 (Ruiz-Opazo & Tonkiss, 2006) to over 900 (Fernández-Teruel et al., 2002) individual F2 rats.

Furthermore, as several markers did not pass validation (Table 6.1), there was a chromosome (Chr15) for which genotype data was available for only one marker, eliminating the utility of interval mapping techniques for that chromosome (single marker analyses for this one Chr15 marker were also not significant). Additionally, segregation distortions in several markers (Table 6.1) may indicate inaccuracies in genotyping. Notably, five of the seven markers for which segregation distortions were significant were ascertained using multiplex PCR methods and GeneMapper allele calling algorithms. This suggests that complications arising from potential cross-marker primer interference or inaccuracies in allele call algorithms may have hindered the reliability of these analyses. Removal of these markers from analyses did not improve the results.

Analyses did reveal, however, a significant effect of sex on false starts, a finding consistent with previous analyses (Chapter 5 Supplement). This replication in a heterogeneous F2 population derived from the WF and F344 parental strains in combination with the findings that this trait may be sex linked suggests that development of a marker panel on the sex chromosomes is warranted. Subsequent analyses may identify sex-linked QTL.

6.5.3 Future Directions and Limitations

Future analyses should include more markers, particularly on chromosomes that were underrepresented, and around loci with sub-threshold peaks. Furthermore, given that these rats were phenotyped on nine versions of the rat-BART that parametrically varied the amount of risk and the reinforcement ratio, additional behavioral traits could be examined. For example, individual differences in risk or reward sensitivity, as determined by individual risk and reinforcement slopes from regressions, may also be heritable and subject to QTL analyses.

There are several strengths and notable limitations to this study. The strengths include an F2 intercross design originating from an inbred strain cross that significantly differed in terms of risk-taking, but did not differ in terms of sucrose preference or motivation to perform in an operant task (Ashenhurst et al., 2012). The use of interval mapping techniques also enhances this study by allowing for estimation of loci between genotyped markers, and although there were no significant differences on the main risk-taking trait by sex, this variable was controlled for in all QTL analyses. Limitations included a limited marker set and relatively small F2 population for studies of this kind. Additionally, multiplex PCR may have resulted in unreliable allele calls due to cross-primer interference or the limitations of GeneMapper software algorithms.

6.5.4 Conclusions

The purpose of this preliminary study was to attempt to locate quantitative trait loci linked with heritable performance indices from the rat-BART including risk-taking, inter-trial variability, and false starts, using an F2 intercross strategy. There was only one significant QTL identified on Chromosome 1 for the main measure of risk-taking and in only one task variant. There were no QTL identified for other task variants or other traits examined. There was a significant effect of sex on false starts, replicating findings in the parental strains. Future endeavors with more F2 rats and markers may indicate significant loci from which positional candidates can be identified and examined.
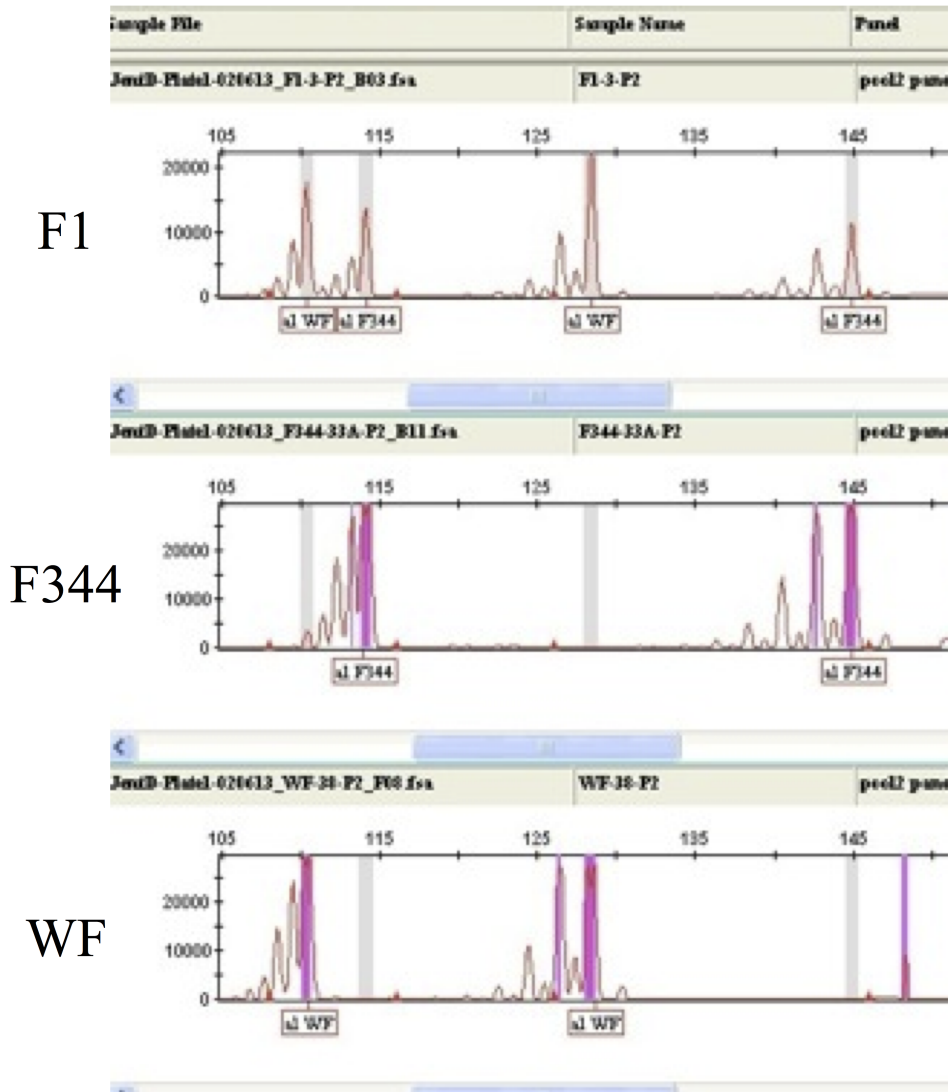
Figure 6.1: Screen capture showing multiplex genotyping validation using GeneMapper software. The three window panels show fluorescent peaks (red - PET) detected by GeneMapper software for F1 (heterozygous), Fischer-344 and Wistar-Furth rats for two separate and non-overlapping markers in multiplex pool 2 (D7Rat94 and D17Mgh1). These panels show that single allele peaks appear only in either F344 or WF rats for each marker, representing their homozygous alleles. For heterozygotes (F1), on the other hand, all peaks are present for the two markers.
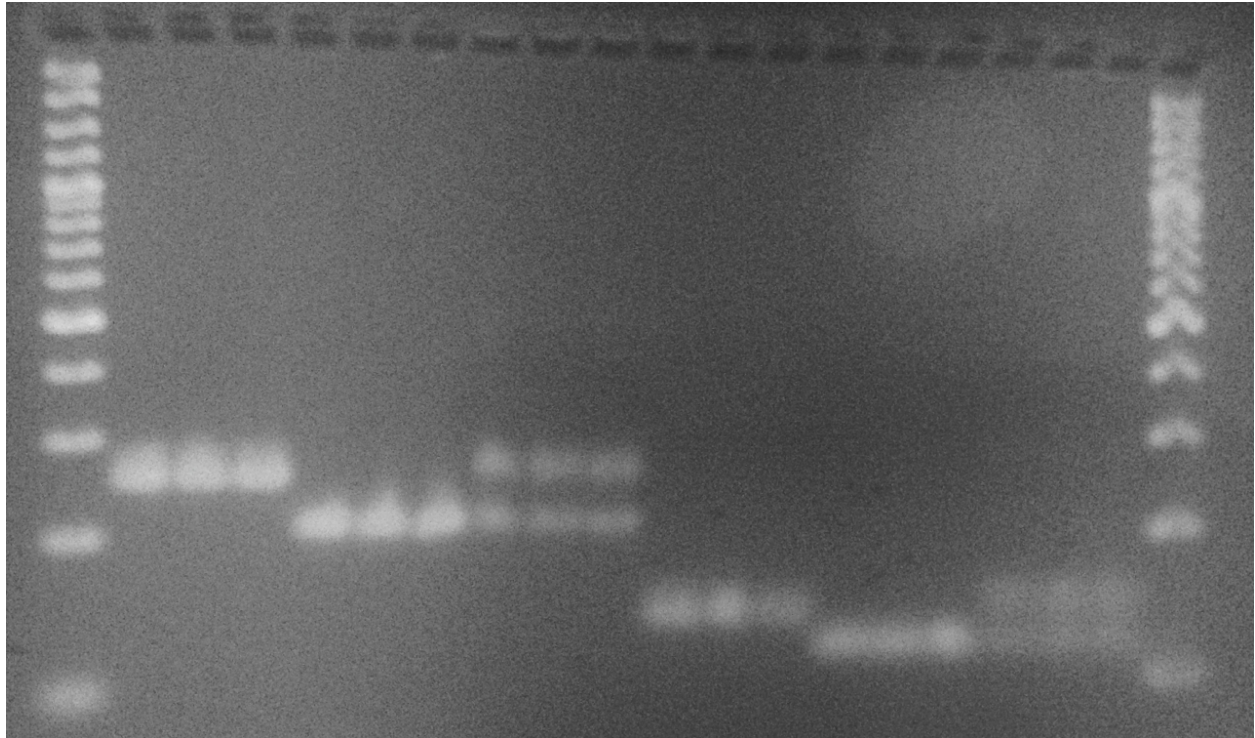
Figure 6.2: Gel agarose photo showing validation of two markers. At left is D19Rat47, and at right is D19Rat11. Bands represent WF, F344, and F1 rats genotyped in triplicate.
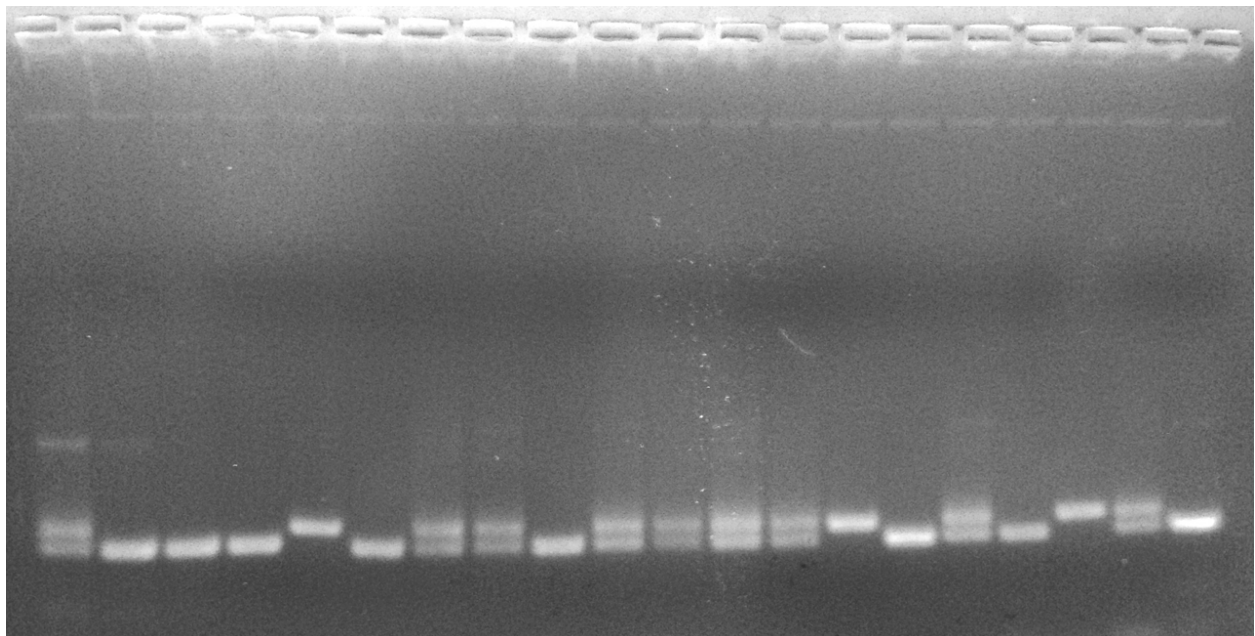

Figure 6.3: Gel agarose photo showing PCR product from F2 strains for marker D11Mit2. Bands clearly show WF (229 bp), F344 (261 bp), or F1 (heterozygous) genotypes.
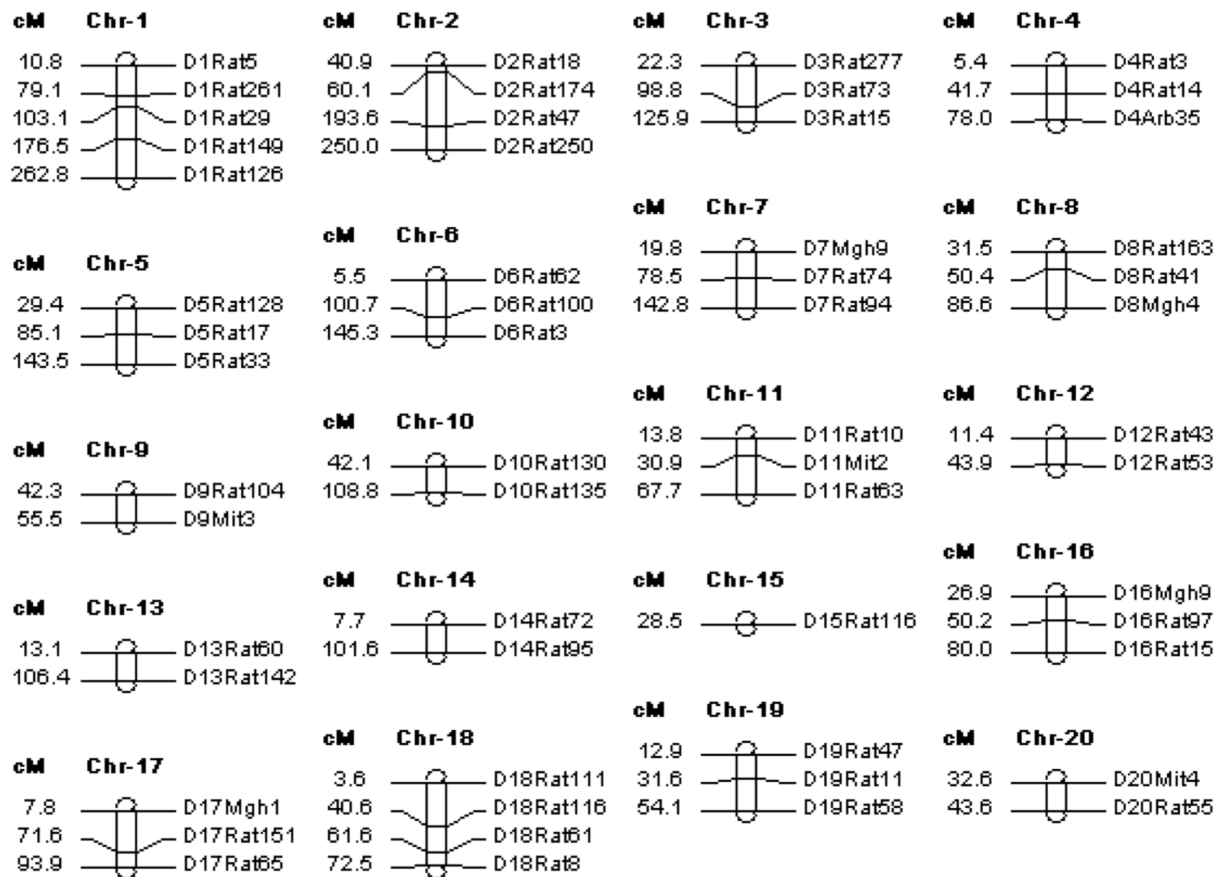
Figure 6.4: Map of validated markers across all 20 autosomes. Distances are in centi-Morgans, derived from data provided in the Rat Genome Database.
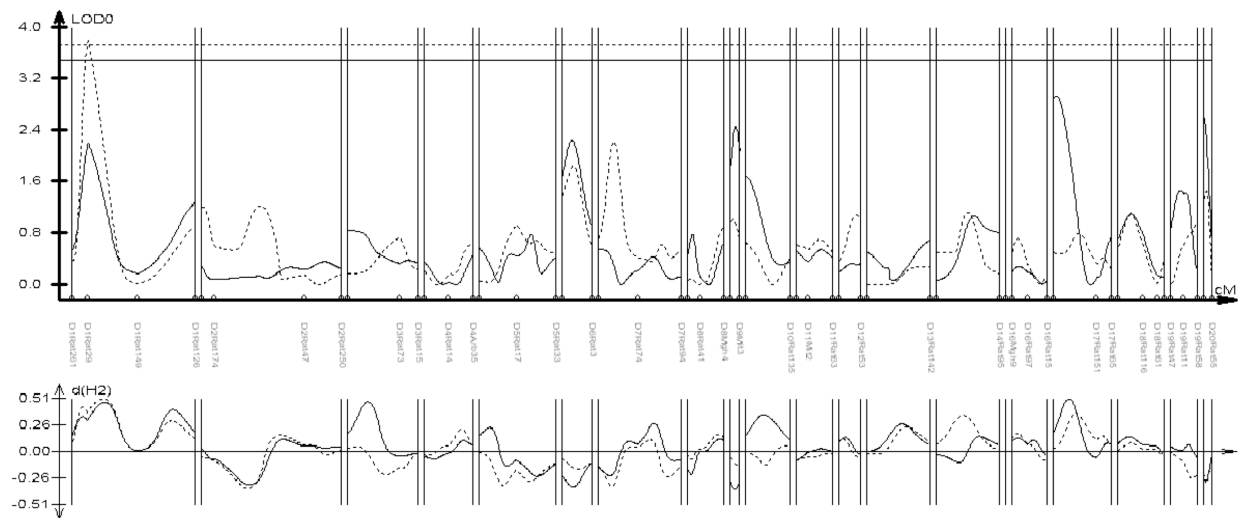
Figure 6.5: 100% Reinforcement Condition - Mean Lever Presses: Results of QTL interval mapping from QTL cartographer for the main indicator of risk-taking in the rat-BART, mean lever presses at cash out, in the 100% reinforcement condition. Solid line = 10% risk, dotted line = 16% risk. Displayed are LOD scores at 1cM steps between genotyped markers along the 20 rat autosomes. Dominance effects are shown below. There was one QTL that surpassed the empirical significance threshold found on Chromosome 1 in the 100% reinforcement, 16% risk condition. Of note, sub-threshold peaks appear in a similar region in all task variants used.

Figure 6.6: 50% Reinforcement Condition - Mean Lever Presses: Results of QTL interval mapping from QTL cartographer for the main indicator of risk-taking in the rat-BART, mean lever presses at cash out, in the 50% reinforcement condition. Solid line = 10% risk, dotted line = 16% risk. Displayed are LOD scores at 1cM steps between genotyped markers along the 20 rat autosomes. Dominance effects are shown below. There were no significant QTL in these conditions.
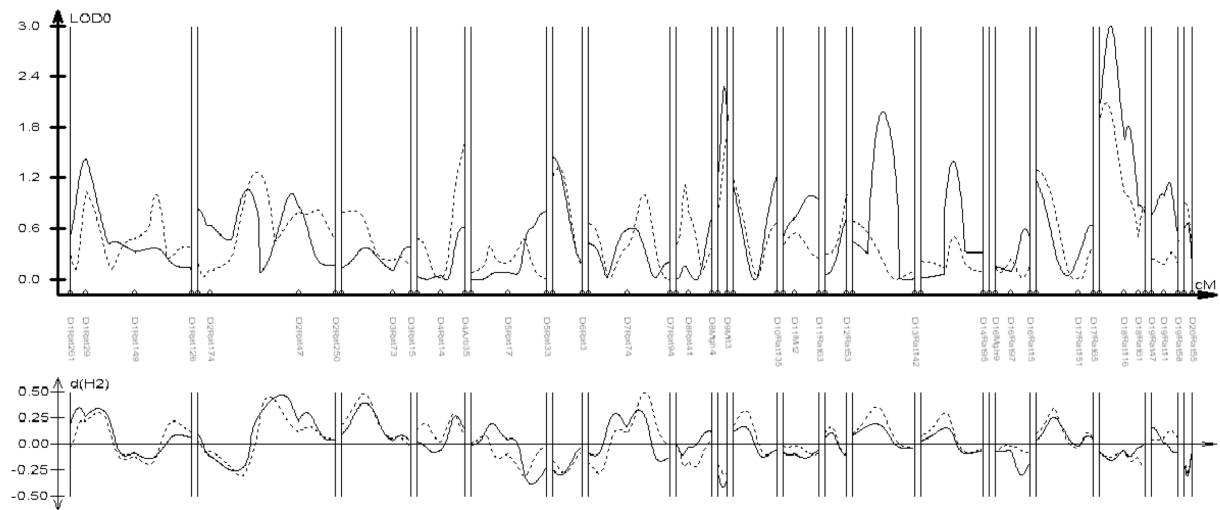
Figure 6.7: 33% Reinforcement Condition - Mean Lever Presses: Results of QTL interval mapping from QTL cartographer for the main indicator of risk-taking in the rat-BART, mean lever presses at cash out, in the 33% reinforcement condition. Solid line = 10% risk, dotted line = 16% risk. Displayed are LOD scores at 1cM steps between genotyped markers along the 20 rat autosomes. Dominance effects are shown 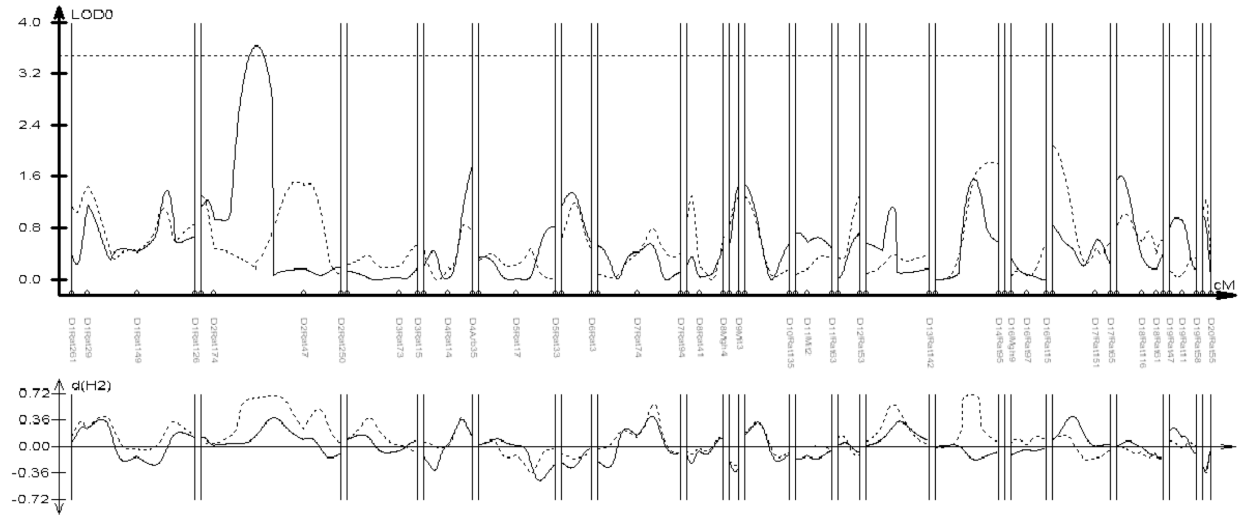below. There were no significant QTL in these conditions. The empirically determined threshold for the 10% risk condition (solid line) was 4.5 and is not shown.
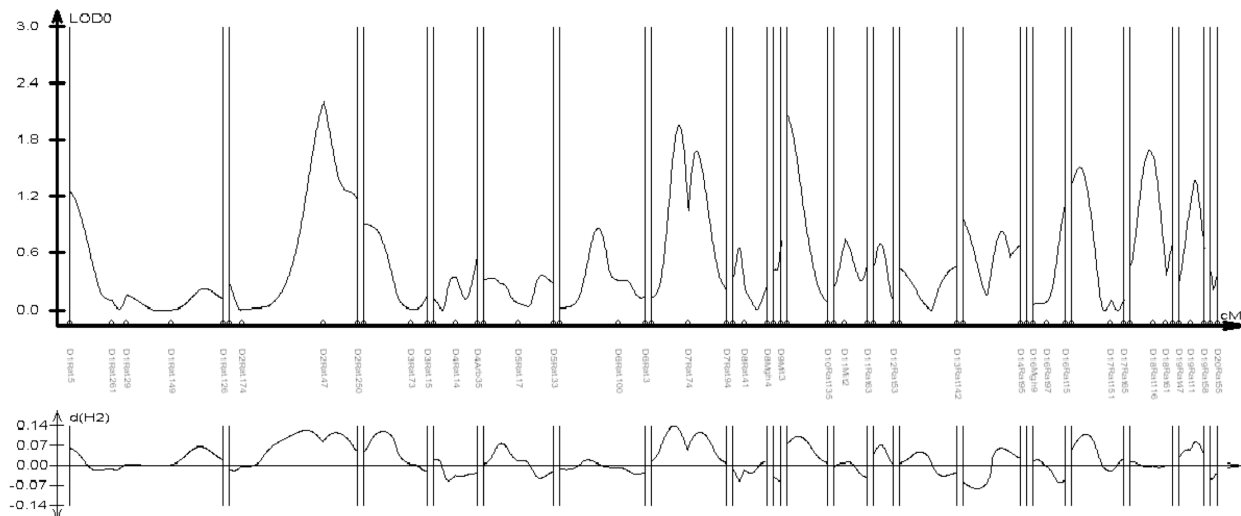
Figure 6.8: Mean Inter-trial Variability QTL: There were no significant QTL for inter-trial variability in any conditions. For simplicity, results for the mean of inter-trial variability across all task versions is presented. Displayed are LOD scores at 1cM steps between genotyped markers along the 20 rat autosomes. Dominance effects are shown below.

135
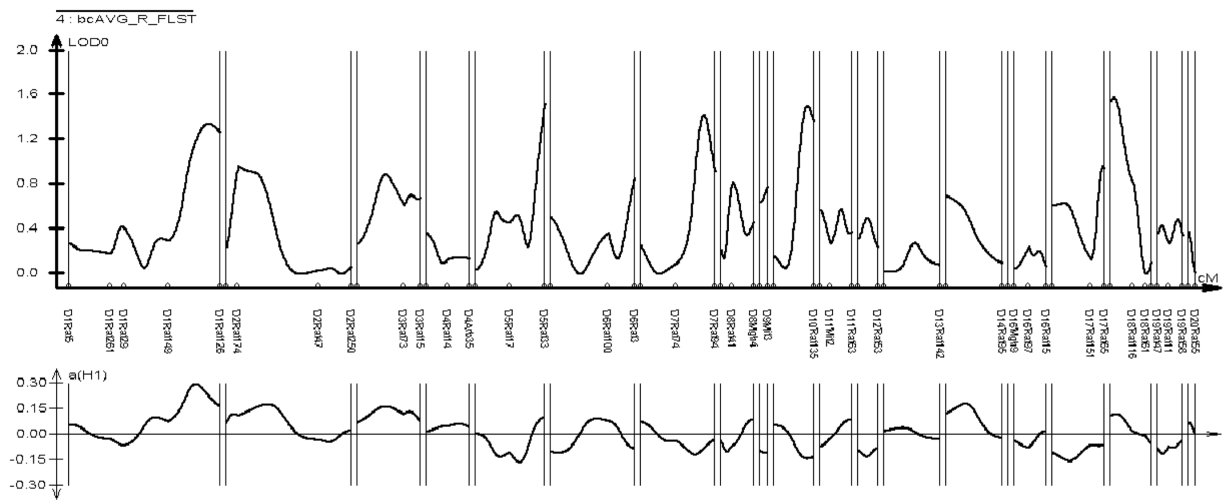
Figure 6.9: Mean Box-Cox Transformed False Start QTL: There were no significant QTL for Box-Cox transformed false start data. For simplicity, results for the mean of box-cox transformed false start data across all task versions is presented. Displayed are LOD scores at 1cM steps between genotyped markers along the 20 rat autosomes. Additive effects are shown below.

CHAPTER 7

Conclusions and Future Directions

7.1   Summary of Findings

The complex and multi-dimensional nature of alcohol (and other substance) use disorders complicates research into the biological underpinnings that may be targeted for treatment. This is particularly true of identifying causal genetic factors for disease liability, which are far upstream of proteomic and systems networks and behavior (Ducci & Goldman, 2008, 2012; D. Goldman et al., 2005). Thus, researchers have endeavored to identify 'endophenotypes' which lie between genes and the clinical disorder as a whole (D. Goldman & Ducci, 2007). Of many potential endophenotypes, research presented here has focused on risk-taking propensity as having explanatory value for alcohol use disorder etiology.

As a progressive disorder with potential for a lifetime of repeated relapse and recovery cycles, risk-taking propensity may play a greater role at some stages of alcohol dependence than at others. Evaluation of the literature indicated four areas where risk-taking may serve as an endophenotype: a) initiation of substance use in adolescence, c) the interface between acute alcohol intoxication and risk-taking, c) alcohol use disorder symptomatology and the effects of long-term chronic alcohol on risk-taking in adults, and d) recovery and liability to relapse.

Chapters of this dissertation evaluated risk-related decision-making as a potential endophenotype for alcohol use disorders at several levels and across species. This included examination of the relationship between risk-taking, reward and loss reactivity, and clinical symptomatology or severity in adults, as well as an attempt to model the effects of chronic

alcohol exposure (Chapters Two, Three and Four). Next, I evaluated risk-taking behavior in response to acute alcohol in a rodent model of risk-taking (Chapter Four). Finally, since heritability is a crucial component for identifying endophenotypes with underlying genomic causes (D. Goldman et al., 2005), rat risk-taking behavioral performance was subject to heritability and genome-wide quantitative trait loci analyses (Chapters Five and Six). The following sections will outline the findings in a broader general discussion, and will suggest directions for future research that may improve examination of this kind of decision-making, potentially resulting in identification of novel targets for treatment strategies.

7.1.1   Acute alcohol and Risk-Taking

Acute alcohol intoxication clearly interferes with executive function in terms of working memory, attention, and psychomotor control (Connors & Maisto, 1980; Finn et al., 1999; George et al., 2005; Goodwin et al., 1970; Lyvers & Maltzman, 1991; Minocha et al., 1985; Weissenborn & Duka, 2003). It is also possible that acute alcohol intoxication promotes risk-taking behavior by compromising decision-making processes through the aforementioned effects on executive function. If true, this could be one additional mechanism by which alcohol use may lead to negative consequences.

Chapter four described the effects of acute alcohol doses on behavior as modeled by rats performing the rat-BART, revealing that alcohol dose-dependently *decreased* risk-taking. The highest dose also slowed performance of the task, indicating that sedative effects of alcohol may underlie this decrease in risk-taking. Acute alcohol dosing in humans does not alter performance in the BART (Peacock et al., 2013), and neither does administration of opioids (Zacny & de Wit, 2009).

Together, these data suggest that risk-taking propensity is not sensitive to the acute effects of intoxicating drugs in the predicted direction. This is not entirely surprising for alcohol, given its sedative effects. However, the effects of stimulants on behavior in the BART have not been examined. It may be that cocaine and amphetamines – given acutely

– could enhance risk-taking, as some doses of amphetamines have increased risk-taking in the rodent Probabilistic Discounting Task (St Onge & Floresco, 2009). Future efforts with alcohol, however, appear unlikely to find any effects on risk-taking propensity *per se*.

7.1.2 Risk-Taking and Alcohol Use Disorder Severity

Chapters Two and Three described data from a large community sample of individuals with a range of severity or clinical symptomatology of alcohol use disorders. The goal of this analysis was to determine the relationship between risk-taking propensity, as ascertained in a behavioral task [the Balloon Analogue Risk Task; BART; (C. Lejuez et al., 2002)], and alcohol use disorders as clinically defined. Modeling was conducted on two levels: overall task performance, and trial-by-trial performance. The former tested for associations between the main indicator of risk-taking (mean pumps in the BART) and symptom count from a clinical diagnostic interview [the SCID; (First et al., 1995)]. The latter implemented a hierarchical regression on trial-by-trial data categorized by the magnitude of wins and reward forfeitures, probing for moderating effects of alcohol problem severity indexed by a robust metric derived from multiple component measures.

Findings from the first – more global – analysis indicated that unlike findings using the Iowa Gambling Task (Bechara et al., 2001), severity of alcohol dependence was overall *negatively* correlated with risk-taking in the BART. This relationship was shown to be mediated by IQ, working memory span, and age, indicating that individual differences in cognitive capacity and demographics are important to consider when interpreting data from the BART. Importantly, the direction of this finding has been replicated in adult tobacco users, which also show a negative relationship between tobacco dependence severity and risk-taking in the BART (Ryan et al., 2013).

The second analysis, which probed more deeply into behavior in the task, identified a specific mechanism by which alcohol problem severity moderates risk-taking in response to wins and losses. Individuals with greater alcohol problem severity were more *conservative*

after a loss of a big potential reward compared to those with lesser problem severity (Figure 3.2). Individuals with fewer problems, interestingly, actually took *more* risk on trials following a big loss than a typical win, showing a pattern somewhat like a 'near miss' effect (Chase & Clark, 2010; Clark et al., 2009; Reid, 1986). These results indicate that more severe participants may have taken less risk overall as a function of their increased loss sensitivity. Importantly, these results survived controlling for IQ, working memory, and demographic variables, suggesting that this deeper effect is not mediated by these variables, as is the more global relationship.

These results run counter to findings among alcohol and stimulant dependent individuals in the Iowa Gambling Task (J. C. Stout et al., 2004; J. Stout et al., 2005; Bechara et al., 2001; Bechara & Damasio, 2002), where dependent individuals are found to be more risk-seeking, and *less loss sensitive* compared to controls. Ultimately, differences in the nature of optimum behavior in the task and the methods used in modeling may explain this discrepancy. As outlined in the introduction where risk-taking tasks are compared, the BART requires an individual to balance an increase in risk with an increase in reward (C. Lejuez et al., 2002), while in the Iowa Gambling Task, it is always optimal to choose the safe option (Bechara et al., 1994). In fact, behavior between the Iowa Gambling Task and the BART were not found to correlate on initial co-administration in two studies; instead, risk-taking indices were not significantly associated until the second and third repeated administration of the task or in behavior measured only in later phases of the tasks (Xu et al., 2013; Upton, Bishara, Ahn, & Stout, 2011). This relationship that only appears in repeated administrations of the tasks may be due to the fact that adaptive responding in these tasks requires learning about the reward and loss probabilities. Thus, construct validity is achieved only after learning effects are controlled for (Xu et al., 2013).

In sum, looking across data from multiple tasks, the relationship between risk taking propensity and alcohol use disorders in adults remains unresolved. Findings are task-dependent and in opposing directions, suggesting that either A) these tasks do not measure the same construct, or B) specific cognitive/executive deficits – that are related to alcohol use

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disorders – are more influential on performance of some tasks, but not others, and perhaps in opposing directions. Suggestions for the further development of risk-taking assessments and investigations into cross-task validity are discussed below (Future Directions in Risk-taking Assessment).

7.1.3   The effects of Chronic Alcohol on Risk-Related Decision-Making

In Chapter Two, I described several hypotheses that might explain the negative relationship between the risk-taking in the BART and alcohol use disorder severity. Among them is the suggestion that chronic alcohol exposure might result in neuroadaptations that produce a brain with what appears as risk-averse biases in this task. A review of the literature suggests that, indeed, chronic alcohol exposure does appear to result in significant neural atrophy of both white and gray matter (Jernigan et al., 1991; de la Monte SM, 1988; Pfefferbaum et al., 1997), and in significant deficits in executive function and cognition (Abernathy et al., 2010; R. Z. Goldstein et al., 2004; Parsons & Stevens, 1986). While analyses in Chapter Three survived controlling for IQ and working memory span, these factors were shown to mediate global BART performance across participants in Chapter Two. Indeed, alcohol use disorder symptom count was negatively correlated with norm-referenced IQ estimates (Table 2.1). While these results are cross-sectional and years of alcohol exposure were not examined, this association is consistent with the idea that chronic alcohol can adversely impact cognitive capacity or executive function, which are important factors in a complex decision-making task like the BART.

Thus, Experiment Two in Chapter Four modeled the effect of chronic alcohol exposure on risk-taking in the BART in rats. This was accomplished with six weeks of intermittent oral administration of a fairly intoxicating dose of alcohol. Contrary to expectations, alcohol (versus saline) did not alter performance in the BART. This may indicate that biases in risk-taking do not result from chronic alcohol exposure *per se.* However, procedural difficulty (a switch from *i.p.* to oral gavage), the length of the study (six weeks), and the dose administered may have limited the translational validity of this study. There was a hint of

an effect of alcohol on deliberation times, but it was not significant.

Future efforts using animal models should employ stronger dosing regimens and robust routes of administration such as the alcohol vapor chamber. Still, oral dosing is more ecologically valid than inhaled vapor, so perhaps higher doses of oral alcohol could suffice. Additionally, human clinical studies could attempt to measure the magnitude of alcohol exposure over a participant's life – in a manner perhaps like pack-years used in the tobacco literature (Bernaards, Twisk, Snel, Van Mechelen, & Kemper, 2001) – to detect associations between chronic alcohol exposure and risk-taking in the BART. Structural MRI could also be used to associate specific deficits in neural integrity – caused by long-term alcohol toxicity – with risk-taking performance in the BART or other risk-related decision-making tasks.

7.1.4 Genetics of Risk-Taking

The plausibility of risk-taking propensity as an endophenotype (Ducci & Goldman, 2008, 2012) is enhanced by assessments of heritability and identification of causal genomic loci and eventually genes that regulate this trait. Rodent models are particularly useful for this kind of research given the ease of selective breeding, and the growing informatics resources for rodent genomics (Laulederkind et al., 2013). Chapter Five established the heritability of this trait in rats, and Chapter Six identified quantitative trait loci linked with risk-taking performance in the rat-BART.

7.1.4.1 Risk-Taking is a Heritable Trait in Rats

Risk-taking propensity is a heritable trait, with a moderate level of the variance explained by genomic factors. Human twin studies indicated that about 55% of the variance in performance in the BART is attributable by genetic factors (Anokhin et al., 2009), as was replicated in a study of inbred rats presented here [Chapter Five (Ashenhurst et al., 2012)]. In addition to this main measure of risk-taking, moderate heritability was described for inter-trial variability of performance ($h^2 = 36\%$), and for false starts (trial initiation errors, $h^2 =$

35%; Chapter Five Supplementary Analysis). Inbred rat strains that were at the extremes risk-taking were identified, and crosses between them demonstrated that transmission of this trait was mostly, but not entirely, dominant (Figure 5.4).

7.1.4.2 Genomic Loci Regulating Risk-Taking

Potential genomic loci linked with risk-taking were identified in Chapter Six. The F1 rats generated from crossing the high and low risk-taking strains were sibling-mated, and a fairly large population of F2 rats was generated (N=140). A genome-wide panel of markers was used to link genomic variation with variation in risk-taking, with indication of loci on Chromosomes 1 at approximately 103.2 Mb from the left telomere, with the 2-LOD support region including the area between 90.99 Mb and 129.99 Mb. Although these results are preliminary and would benefit from the addition of several more markers and more F2 animals, these results advance our understanding of the genetic regulation of decision-making under risk.

Searching for genes within this region in the Rat Genome Database (Laulederkind et al., 2013) yielded 388 genes and 71 previously identified QTL. Given these large numbers, no single candidate should be advanced as potentially regulating decision-making under risk. Nonetheless, several genes present in this region that are expressed in the brain and are likely to have major effects on neural function including: a) *Chrna7*, a gene coding the nicotinic acetylcholine receptor α-7, b) *Igf1r* which encodes an insulin regulated growth factor that is implicated in axonal growth (Dupraz et al., 2013), and c) an aldehyde dehydrogenase gene *Aldh16a1*, which is implicated in alcohol metabolism and this enzyme class is a target of therpeutic treatment (Koppaka et al., 2012). While most of the 71 QTL records identified pertained to blood pressure, cardiac weight, and bone mineral density, several previously identified QTL had behavioral effects that could be potentially related to the risk-taking trait, alcohol, and brain function more broadly. These included two for alcohol response in the loss of righting reflex test (RGD ID: 1641897, 2317833), two for anxiety (RGD ID: 738022, 2313402), and two for stress response (RGD ID: 1578770, 1358189).

Future analyses extending from this work could include further modeling of the behavioral data, and could use targeted genotyping in order to confirm this QTL and more narrowly define its boundaries. In terms of behavioral data, since rats were phenotyped in nine parametric variants of the rat-BART, one could examine individual differences in risk and reinforcement sensitivity among the F2 population. Further genomic study should include higher density genotyping in the area on Chromosome 1, and additional coverage on underrepresented chromosomes like Chromosome 15, and potentially the sex chromosomes as well. This work might identify positional gene candidates, and natural variation within these genes and their human homologues could be associated with risk-taking propensity. The relationship between these variants and alcohol use disorders more broadly could also be examined. This would complete the pathway from gene, to endophenotype, to disorder (D. Goldman et al., 2005; D. Goldman & Ducci, 2007; Manji et al., 2003).

Lastly, human studies have found some signal from dopamine related genes. This includes an association between variation in the dopamine D4 receptor gene *DRD4* and behavior in a gambling/investment task (Kuhnen & Chiao, 2009), and variation in the dopamine transporter gene *DAT1* with behavior in the BART (Mata et al., 2012). Examination of variability in the rodent homologues of these genes may reveal similar associations with risk-taking behavior. Importantly, *DRD4* variation has also been associated with alcohol dependence (Le Foll, Gallo, Le Strat, Lu, & Gorwood, 2009) and novelty seeking (Lahti et al., 2005).

7.2   Future Directions

7.2.1   Neural Regulation of Decision-Making Under Risk

Future studies should continue to probe specific regions of the prefrontal cortex, such as the dorsolateral prefrontal (DL), ventromedial prefronal (VM), or orbital prefrontal (OF), where neural deficits due to alcohol or other substance abuse could associate with specific deficits in decision-making under risk. Critically, these studies should also examine sub-

components of executive function such as attention, working memory, cognitive flexibility, and overall learning. Attempts to find such regions have been made using the Iowa Gambling Task, where dysfunction of the VM prefrontal cortex (Bechara et al., 2001) was found to associate with greater risk-taking. This study compared patients with bilateral lesions in the ventromedial prefrontal cortex with substance dependent individuals, including cocaine, methamphetamine, and alcohol. A larger portion of those with substance dependence (61%) performed the Iowa Gambling Task in a manner like patients with VM damage than did healthy controls (32.5%). However, there are major limitations to the interpretation of this study. Most problematically, the substance dependent individuals were not shown to exhibit structural deficits in the VM cortex (Bechara et al., 2001). Second, due to the structure of the Iowa Gambling Task, it is unclear if enhanced selection of the risky option is due to a risk preference *per se*, or if it is due to compromised learning abilities. It may be the case that the substance dependent individuals, as well as the VM-lesioned patients, simply fail to learn to adaptively prefer the low risk options after sampling. Assessment of these patients in other risk-related decision-making tasks may clarify if decision-making deficits due to VM damage are specific to maladaptive risk preferences.

Data examining the DL and OF cortex are also suggestive that these regions play specific roles in decision-making under risk. Inactivation of the likely rodent functional analogue of the DL, the medial prefrontal cortex (V. Brown & Bowman, 2002; Preuss, 1995), increases the variability of responding in the rat-BART, while inactivation of the OF decreased risk-taking (Jentsch et al., 2010). In the Cambridge Gambling Tasks, patients with OF damage showed greater deliberation times and were less likely to choose the more probable outcome (Rogers, Everitt, et al., 1999). Glucose metabolism in OF in polysubstance abusers is abnormal compared to healthy controls (Stapleton et al., 1995), suggestive of a link between OF dysfunction, substance use, and compromised decision-making under risk. Future studies should continue to tease apart the specific deficits caused by OF damage in lesion patients by using multiple tests of decision making under risk while also accounting for potential differences in working memory, cognitive flexibility, and perseveration. It may be that ab-

normal decision-making under risk is explained by deficits in specific components of executive function resulting from damage to specific loci within the OF cortex.

Finally, components of the striatum (and its interaction with prefrontal cortex) are also implicated in decision-making under risk (Kohno et al., 2013; Rao, Korczykowski, Pluta, Hoang, & Detre, 2008), particularly in terms of reinforcement learning and risk assessment (Kohno et al., 2013) and, more broadly, inhibitory control (Courtney, Arellano, Barkley-Levenson, Galvan, et al., 2012). Importantly, striatal D2/D3 dopamine receptor binding potential is related to the modulation of striatal activation by pumping in the BART as measured by functional magnetic resonance imaging [fMRI; (Kohno et al., 2013)]. Kohno and colleagues (2013) propose that individuals with higher D2/D3 binding potential have blunted cortical inhibition of reward-driven responses, and are more sensitive to reward. Continued research into the role of dopaminergic signaling in the striatum, and the functional interaction between the prefrontal cortex and striatum may provide a model for regulation of reward-driven decision-making under circumstances of risk. Neuroimaging studies should employ functional connectivity techniques, such as diffusion tensor imaging, or psychophysiological interaction (PPI) analyses in fMRI (Gitelman, Penny, Ashburner, & Friston, 2003). Analysis using PPI during performance of a task of inhibitory control (the stop signal task) showed that individuals with greater alcohol problem severity had weaker functional connectivity between the prefrontal cortex and putamen than those with lesser severity (Courtney et al., 2013). Consequences of this connectivity deficit may also include abnormal decision-making under risk.

7.2.2 Targeting Risk-Taking in Interventions and Treatment

Looking across the several stages of alcohol use disorders where risk-taking may serve as an endophenotype, it is apparent that the role of risk-taking is not uniform. It appears that risk-taking propensity is a good predictor of alcohol use initiation, particularly in adolescence (Fernie et al., 2010; C. Lejuez et al., 2002; C. W. Lejuez et al., 2005, 2007; MacPherson et al., 2010; Xiao, Koritzky, et al., 2013; Xiao, Bechara, et al., 2013). On the other hand, as

demonstrated here in rats (Chapter Four) and previously in humans (Peacock et al., 2013), alcohol itself may not increase risk-taking above baseline levels for an individual. If anything, acute alcohol decreases risk-taking. In terms of its relationship to clinical symptomatology, the relationship depends on the task used, with a positive relationship in the Iowa Gambling Task (Bechara et al., 2001), and a negative relationship in the BART [Chapter Two (Ashenhurst et al., 2011)]. Chronic exposure modeled here (Chapter Four) failed to alter risk-taking behavior. Finally, several studies have indicated that greater risk-taking propensity predicts lower odds of success in recovery and abstinence (De Wilde et al., 2013; Passetti et al., 2008). These promising results suggest that interventions targeted at reducing risk-taking during treatment may improve rates of recovery. However, the effect size of such an intervention, and thus its clinical utility, must be examined. Taken together, these data indicate that research focusing on adolescent use initiation and recovery programs may yield the most fruitful information. On the other hand, research on the acute effects of alcohol and the relationship with clinical symptomatology may not provide clinically impactful results.

7.2.3   Further Development and Cross-Validation of Risk-Taking Tasks

Progress in this field requires a closer examination of risk-taking tasks, and potentially development of tasks that are more specific for alcohol and drug using populations. Many research groups have developed a plethora of behavioral assessments of decision-making under risk for use in humans (Bechara et al., 2001; C. Lejuez et al., 2002; Rogers, Owen, et al., 1999) and in rats (Cocker et al., 2012; Jentsch et al., 2010; Mitchell et al., 2011; St Onge & Floresco, 2009; Zeeb et al., 2009). There are critical differences between them, however (Chapter One), and in some cases behavior between them fails to correlate (Xu et al., 2013), or they produce contrary results when assessed in clinical populations (Ashenhurst et al., 2011; Bechara et al., 2001).

Looking across the literature, there have been few attempts to cross-validate behavior between these tasks that are described as measuring the same construct (Xu et al., 2013). In order to determine if, in fact, these tasks are assessing the same risk-taking propensity

construct, future studies should co-administer these human tasks in healthy and clinical populations. Analyses using principal component or structural equation modeling methods could reveal that behavioral metrics of risk-taking across these tasks do represent a single factor. Alternatively, loadings into separate factors would indicate that risk-taking metrics from these tasks are not measuring quite the same thing. What these different factors represent, then, would be determined by the nature of differences in the tasks and how they load separately or together. Given that these are often complex tasks, it may be that conceptually more narrow components of executive function like working memory or attention are recruited to different degrees in different tasks, also explaining discrepancies.

Next, the overarching hypothesis proposed by researchers using these tasks is that greater risk-taking propensity is maladaptive (Bechara et al., 2001, 1994; C. Lejuez et al., 2002; Rogers, Owen, et al., 1999), but this is not always the case. In the BART specifically, individuals nearly always fall on the lower half of the optimum function (e.g., Figure 2.1); few participants, healthy or otherwise, actually reach optimal performance, and are instead quite risk-averse (Ashenhurst et al., 2011; Dean et al., 2011; Fernie et al., 2010; C. Lejuez et al., 2002; Pleskac et al., 2008). Rats in the rat-BART behave similarly, and rarely maximize the reward earned across the test session (Ashenhurst et al., 2012; Jentsch et al., 2010).Thus, greater risk-taking within the range of normal participant behavior is actually *adaptive*, as has been pointed out by others (Dean et al., 2011). This too may be the a reason why results obtained using the Iowa Gambling Task or Cambridge Gambling Task versus the BART are contradictory (Ashenhurst et al., 2011; Bechara et al., 2001; Rogers, Everitt, et al., 1999)

Finally, researchers have assumed that monetary reward accurately captures maladaptive risk-taking behavior that is guided towards drug or alcohol reward. This is problematic, as a study has shown that cocaine abusers do not show the same sensitivity to monetary gradients as do controls (R. Goldstein et al., 2007); this may also be true for alcohol dependent individuals. Thus, providing money as incentive to take risks may not accurately reflect risk-taking with the goal of obtaining and using alcohol. It would be worthwhile to develop a risk-taking task where the outcome is not monetary (for humans) or food (for rats) but

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is instead access to alcohol or drugs. While the ethics of a study of this kind should be carefully examined, particularly if implemented in a human clinical population, results may be more ecologically valid. Alcohol dependent individuals who are performing a BART-like gambling task in order to obtain alcohol rewards rather than monetary rewards may show the opposite results of those demonstrated here (Chapter Two). If so, this would suggest that outcomes in gambling tasks do matter, and that money is an inaccurate surrogate for alcohol reward. If results are the same, however, then this would support the idea that differences in risk-taking in the BART for money rewards are indeed ecologically valid.

7.3   Conclusion

In sum, this dissertation examined risk-taking propensity as an endophenotype for alcohol use disorders. This was accomplished by using a human risk-taking task and its rodent analogue to probe for a) relationships with alcohol use disorders, as clinically defined, b) the effects of acute and chronic alcohol on rat behavior, and c) the heritability and genomic loci associated with rat-BART behavior. The data presented here, in the context of the broader literature, indicated areas where future research that is most likely to be clinically influential is warranted. In particular, more work should be done to examine risk-taking during adolescence and at recovery. Lastly, there were indications of genomic loci that may regulate risk-taking on Chromosome 1. High density trait mapping may reveal a more narrow region, from which candidate genes can be identified, providing a more complete path from genes, to behavior, to disorder.

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