2018

Heritable Variation in Reward Sensitivity and Impulsive Action and Choice in a Genetically Diverse Inbred Mouse Panel

Lauren S. Bailey

Binghamton University--SUNY, lbailey2@binghamton.edu

Follow this and additional works at: https://orb.binghamton.edu/dissertation_and_theses

Part of the Psychology Commons

HERITABLE VARIATION IN REWARD SENSITIVITY AND IMPULSIVE ACTION
AND CHOICE IN A GENETICALLY DIVERSE INBRED MOUSE PANEL

BY

LAUREN S. BAILEY

BA Kenyon College, 2016

THESIS

Submitted in partial fulfillment of the requirements for
the degree of Master of Science in Psychology
in the Graduate School of
Binghamton University
State University of New York
2018

Accepted in partial fulfillment of the requirements for
the degree of Master of Science in Psychology
in the Graduate School of
Binghamton University
State University of New York

May 7, 2018

J. David Jentsch, Chair and Faculty Advisor
Department of Psychology, Binghamton University

Lisa Savage, Member
Department of Psychology, Binghamton University

David Werner, Member
Department of Psychology, Binghamton University

Abstract

Drugs of abuse, including alcohol and stimulants like cocaine, produce effects that are
subject to individual variability, and genetic variation accounts for at least a portion of
those differences. Notably, research in both animal models and human subjects point
towards reward sensitivity and impulsivity as being trait characteristics that predict
relatively greater positive subjective responses to stimulant drugs. Unfortunately, past
efforts have yet to yield convincing insights into underlying genetic influences on these
traits due to the characteristics of the mouse panels used. The Collaborative Cross (CC)
recombinant inbred mouse strains, their inbred founders, and the Diversity Outbred (DO)
mice that are derived from them are a powerful genetic reference panel that has potential
as a tool for revealing genetic contributions to cocaine abuse and related traits. Here we
describe use of the eight CC/DO founder strains to examine the heritability of reward
sensitivity and impulsivity traits, as well as genetic correlations between these measures
and existing addiction-related phenotypes. *Methods.* Founder strains were all tested for
activity in an open field and reward sensitivity (intake of chocolate BOOST®). Mice
were then divided into two counterbalanced groups and underwent reversal learning
(impulsive action) or delay discounting (impulsive choice). *Results.* The founder mice
demonstrate significant heritability for anticipatory responding within the reversal task,
k-value within delay discounting, locomotor activity, and reward sensitivity. Total trials
to criteria within reversal was positively correlated with ethanol intake in female mice.
This research was conducted within the broader, inter-laboratory effort of the Center for
Systems Neurogenetics of Addiction (CSNA) to characterize CC and DO mice for
multiple, cocaine abuse related traits. These data will facilitate the discovery of genetic

correlations between predictive traits, which will then guide discovery of genes and genetic variants that contribute to addictive behaviors.

Table of Contents

List of Tables

List of Figures

Heritable variation in reward sensitivity and impulsive action and choice in a genetically

diverse inbred mouse panel

Not all those who initiate drug or alcohol use will progress to a pathological state,

in which the user sustains personal damage and struggles to reduce or cease use. It has

been proposed that progression of drug seeking to drug addiction follows a multistep

process: recreational and/or sporadic drug use, intensified and sustained drug use, and

ultimately an uncontrolled substance use disorder (SUD) (Piazza & Deroche-Gamonet,

2013). Transition from recreational use to sustained use is often a shift in quantity of the

drug taken, while transition from sustained use to loss of control is a shift in quality, with

users primarily exhibiting goal-directed drug taking behavior and exhibiting difficult with

limiting or confining drug taking (Piazza & Deroche-Gamonet, 2013). Monozygotic and

dizygotic twin studies have been essential in laying the foundation of quantifying

susceptibility. Evidence from twin studies supports the idea that the majority of risk for

developing an SUD relates to a single common genetic factor, as well as less potent

environmental influences (Kendler et al., 2003). An essential aim of current drug studies

must be to understand the fundamental differences between users who are at risk for

developing an SUD, and those who do not develop a pathological pattern of using.

Addiction criteria in these circumstances is defined by the Diagnostic and

Statistical Manual of Mental Disorders, Fifth Edition (DSM-5, American Psychiatric

Association, 2013), which represents one effort to separate recreational drug use from

pathological forms of consumption. The DSM-5 lists eleven criteria that qualify an

individual for an SUD. Meeting 2-3 items is considered a mild SUD, 4-5 is moderate, and

6+ is severe. These criteria include factors such as taking the substance in larger amounts

and for longer than intended, continued use despite attempting to stop, and tolerance and withdrawal manifestation (American Psychiatric Association, 2013).

The simplest explanation for the initial development of drug use is that users *like* and/or *want* the drug (Wise & Bozarth, 1987; Robinson & Berridge, 1993), which is supported by physiological evidence. Drugs of abuse typically result in a great increase of dopamine and a slow habituation in response to the drug (Piazza & Deroche-Gamonet, 2013), thereby increasing salience and conditioning the user to continue usage. Recreational drug use is easily acquired in laboratory animals; conventional laboratory animal models will, in many cases, learn to self-administer a subset of drugs with abuse liability, and most humans use some form of drug with abuse liability (Piazza & Deroche-Gamonet, 2013). Escalation of use is likely a combination of desiring the drug and being unable to refrain, though this does not explain why some users are more resistant, and only a minority become addicts.

This progression to loss of control and qualification for and SUD is influenced by a myriad of genetic and environmental factors that interplay with drug consumption. Narrowing down individual variability that is responsible for the progression to sustained use requires the analysis of relevant variables. Multiple heritable phenotypes have been shown to be predictive for heightened likelihood to seek out and use drugs in laboratory animals, including locomotor response to novelty (Nadal, Armario, & Janak, 2002; Piazza & Deroche-Gamonet, 2013), locomotor response to acute dose of drug (Piazza et al., 1989), novelty preference (Belin et al., 2011; Molander et al., 2011), anxiety-related behaviors (Spanagel et al., 1995), circadian phenotypes (Logan, Williams, & McClung, 2014; Rosenwasser, 2010), and impulsivity. These behaviors are not exclusive and likely

have overlapping components, and by studying them in tandem can a better understanding be gained of the biomarkers that lead to these drug-related phenotypes.

Defining and Assessing Impulsivity

Impulsivity is the trait-like proclivity to engage in excessive, uncontrolled, or rash reward pursuit and consumption, called impulsive behaviors (Jentsch et al., 2014). Impulses are not necessarily maladaptive or pathological, and can even be seen as advantageous in evolutionary circumstances as they represent an organism's desire to obtain a highly salient reward (Jentsch et al., 2014). These behaviors can, however, be considered pathological when they are intrusive, disrupt normal life routines, cause clinical distress, or lead to harmful outcomes (Moeller et al., 2001). The DSM-5 includes a category for Disruptive, Impulse-Control, and Conduct Disorders, which include intermittent explosive disorder, pyromania, kleptomania, and conduct disorder. Pathological impulsive behaviors are also a symptom of a variety of other psychiatric disorders, including bipolar disorder, borderline personality disorder, suicide, and substance use disorder. Thus, impulsivity is a phenotype of broad importance to many diagnostic categories, including addictions. SUD features impaired control over impulsive drug use (Jentsch et al., 2014) in symptoms defined by the DSM-5 such as:

1. Taking the drug in larger amounts and for longer than intended

2. Wanting to cut down or quit but not being able to do it

3. Spending a lot of time obtaining the drug

4. Craving or a strong desire to use the drug

8. Recurrent use of the drug in physically hazardous situations

Impulsivity is also a defining factor in progressing through the stages of addiction, between drug seeking, escalation, and uncontrolled use (Jentsch et al., 2014).

The assessment of impulsivity is similar between humans and animal models. In human studies, impulsivity can be measured using self-report measures, which includes the widely used Barratt Impulsiveness Scale (BIS-11), developed in 1959 and now in its eleventh revision (Barratt, 1959). The BIS-11 is a 30 item self-report measure designed to quantify three subtypes of impulsivity: cognitive impulsiveness, motor impulsiveness, and non-planning impulsiveness. Participants rate each question from 1-4 depending on how much they agree or disagree that it describes them (e.g. I make-up my mind quickly). The BIS-11 has wide application and has been used to assess impulsivity in populations of cocaine users, ecstasy users, mood disorders, suicide attempters, and criminals, as well as been translated into 11 languages (Stanford et al., 2009). Behavioral tasks have also been developed to quantify impulsive phenotypes in humans, such as the Go/No-Go task, delay discounting, and the balloon analogue risk task, which have analogs for use in animal models.

A common test of impulsivity, delay discounting, was initially created to assess rats and pigeons (Evenden & Ryan, 1996), though is now used in both human and animal subjects with variations to the methodology. Delay discounting is a paradigm established to assess an individual's tendency to reduce (discount) the subjective value of a reward if it has to wait to receive it. Often high value rewards incorporating a delay are chosen less than lower value rewards that can be received immediately (Ainslie, 1975). The delay discounting procedure aims to establish how the subject therefore discounts the delayed reward, either by altering the volume of reward or the length of delay. A fundamental

aspect of delay discounting is that the subject's responses during the delay procedure do not affect the trial; that is, that the subject makes an action to choose either reward, and then must wait for the consequence, thus differing from other similar procedures such as the differential reinforcement task (Evenden, 1999), and measuring *impulsive choice*. In analyzing delay discounting data, an equation is derived to match the curve relating delay to subjective value and estimating a value called k, which represents the scaling factor of a delay; the effect of the delay to reduce subjective value is larger among subjects with a large k value, and smaller among subjects with a smaller k value (Odum, 2011).

Human and animal delay discounting studies have fundamental differences in paying out. Delay discounting studies have been conducted in laboratory animals for reinforcers such as food, liquids, and intravenous (IV) drug administration (Calvert, Green, & Myerson, 2013), and the subject actually receives the reward after the trial. Studies with human participants often use money as a reward incentive and follow the Hyperbolic Delay-Discounting Model (Reynolds, 2006), which measures the devaluing of a reward over increasing delay times. Hyperbolic delay discounting often incorporates adjusting delay or adjusting amount to identify the subject's indifference point: the difference in size/value the delayed reward has to be in order to be chosen equally to the immediate reward (Reynolds, 2006). Human delay discounting studies can be sorted into three different categories: hypothetical, real reward, and real time (Reynolds, 2006). Hypothetical experiments ask the subject to make choices between two rewards, one with a delay (ex. Would you rather have $5 now or $15 in 10 days?), though the subject does not actually experience the delay or the reward. Face validity is a concern in hypothetical studies, and real reward studies therefore honor one random decision during the course of

the experiment and pay out immediately or with the delay accordingly. Delay discounting is often used in affected populations, such as individuals who engage in pathological drug consumption, gambling, or overeating. Morbidly obese women showed greater delay discounting than control women (Weller et al., 2008), and pathological gamblers showed increased delay discounting compared to healthy controls (Alessi & Petry, 2003; Dixon, Marley, & Jacobs, 2003).

Another popular test of impulsivity is reversal learning, which revolves around changing reinforcement contingencies: an action (e.g. pressing a lever) is paired with an outcome (e.g. receiving a food reward) so that the subject learns the actions necessary to receive the reward, and then learn to discriminate between stimuli (e.g. only pressing the left lever, not the right, leads to the food reward) (Izquierdo & Jentsch, 2011). After reaching an accuracy criterion, the contingencies are reversed (e.g. only pressing the right lever, not the left, now leads to the food reward). Subjects must demonstrate cognitive and behavioral flexibility, or impulse control, by constraining their previous responses and discarding the initially learned rule. Greater difficulty with stopping or updating behavior during reversal learning has been suggested to reflect greater *impulsive action*. Studies have shown that this behavioral inflexibility is genetically linked to impulsivity (Franken et al., 2008; Izquierdo & Jentsch, 2011). Reversal learning is impaired in OFC-lesioned animals (Boulougouris, Dalley, & Robbins, 2007) and in humans with relatively low striatal baseline dopamine synthesis capacity (Cools et al., 2009).

The Five-Choice Serial Reaction Time Task (5-CSRTT) was initially developed to reflect the Connor's performance test, measuring attention and executive control in children with ADHD (Robbins, 2002), though has since been adapted for animal models.

The animal is presented with five horizontally arranged nose-poke holes, one of which briefly illuminates, and must make a response in the correct (illuminated) hole. Accuracy is regarded as a measure of attention capacity, and anticipatory responding (making a nose poke before the visual signal is delivered) is considered as a failure in impulse control (Bari, Dalley, & Robbins, 2008). Anticipatory responses are considered to be *waiting impulsivity*, or the inability to withhold response in anticipation of a reward-related cue (Dalley, Everitt, & Robbins, 2011). Due to the nature of the 5-CSRTT, it is said to have overlap with delay discounting, which also measures willingness to wait for a reward (Dalley, Everitt, & Robbins, 2011).

The Multidimensional Model of Impulsivity

These tests of impulsivity described above, as well as others, are thought to be measuring different facets of impulsivity. Evenden (1999) proposed the multidimensional model of impulsivity, stating that the concept of impulsivity covered a wide range of behaviors and was difficult to define precisely. He published multiple studies that used three tasks measuring conceptually different types of impulsivity: visual discrimination (preparation to respond), the fixed consecutive number schedule (FCN; behavior execution), and variable delay of reinforcement (assessment of outcome). Visual discrimination aims to test reflection impulsivity (Kagan, 1966), or the tendency to either deliberate (reflect before responding) or act without deliberation in situations of uncertainty. The FCN, like the 5-CSRT, measures waiting impulsivity by counting anticipatory responses made before the mandatory number of lever presses to receive a reward. The variable delay of reinforcement is similar to delay discounting in that it asks rats to choose between one pellet immediately or several pellets after a delay.

Rats in these studies were treated with amphetamine, haloperidol, imipramine, citalopram, 8-OH-DPAT, DOI, WAY-100635, or ritanserin, and subsequent behavioral effects (decreasing the impulsive behavior, increasing the impulsive behavior, or having no effect) were measured. A single drug did not have the same effect on each test; for example, ethanol had no effect on the unreliable visual discrimination or FCN, but increased impulsive behavior of the variable delay of reinforcement (Evenden, 1999). Some drugs even had opposing effects; for example, haloperidol was found to decrease impulsive behavior in the unreliable visual discrimination task but increase impulsive behavior on the FCN, while having no behavioral effect on the variable delay of reinforcement (Evenden, 1998). Therefore Evenden concluded that there were different facets of impulsivity, each having a unique set of pharmacological influences. Evenden provided examples of different types of impulsivity, including response inhibition, resistance to delay of reinforcement, timing, behavioral switching, motor impulsivity, cognitive impulsivity, preparation, execution outcome, premature responding, and lack of persistence. Evenden did, however, conclude that this research had its limitations. There could have been confounding factors such as only one procedure was used to measure each proposed type of impulsivity, as well as the impulsivity score changing without there being a true change in impulse control, such as how ethanol exposed rats would inexplicably choose the immediate lever repeatedly over the delayed lever, despite the delay being 0s. Evenden expressed that this is an example of a way behavior can be altered without impulsivity changing, perhaps due to drug side effects.

Evenden's research has been replicated since, and the multidimensional theory of impulsivity remains a popular one. For example, a study found that rats who had

forebrain levels of 5-HT depleted with 5,7-dihydroxytrytamine had increased premature responding in a 5-choice serial reaction time task and enhanced locomotor activity in response to conditioned food presentation, though no difference in impulsive choice behavior in delay discounting (Winstanley et al., 2004). No significant bivariate correlation between impulsive action (five-choice serial reaction time task) and impulsive choice (delay discounting) has been found in either lab rats or humans, and furthermore, in humans three factors reflecting statistically orthogonal measures of impulsivity were identified: self-report, impulsive action, and impulsive choice (Broos et al., 2012). One study of human pathological gamblers revealed impaired behavior on impulsive action (stop-signal task) but not impulsive choice (delay discounting) (Brevers et al., 2012), though separate studies have found increases in impulsive choice in pathological gamblers (Alessi & Petry, 2003; Dixon, Marley, & Jacobs, 2003). These findings may be attributed to different areas of the brain regulating each type of impulsivity. In rats, impulsive action was associated with reduced dopamine release in the nucleus accumbens core, while impulsive choice was associated with reduced dopamine release in the nucleus accumbens core, shell, and medial prefrontal cortex (Diergaarde et al., 2008). In rats again, lesions of the subthalamic nucleus increased impulsive action, though decreased impulsive choice (Uslaner & Robinson, 2006).

Despite proposed differences in the types of impulsivity, there is evidence for these facets being linked to similar underlying neural mechanisms in frontostriatal circuitry. Examining the neurological components of impulse control reveals that dopamine plays a heavy role in controlling impulsivity, even taking into consideration the different types. Blocking dorsomedial striatal dopamine D2-like receptors impairs

response inhibition in the stop signal task, while blocking D1-like receptors improves it (Eagle et al., 2011). This result is mirrored for waiting as well, as activating D1-like receptors in the medial prefrontal cortex results in reduced anticipatory responding in the five-choice serial reaction time task (Chudasama & Robbins, 2004). This similar underlying circuitry challenges the distinction of subgroups of impulsivity and the supposed strong categorical differences, imploring further research to empirically test the relationship between the conceptually distinct facets. Jentsch et al. (2014) postulated two theories on impulsivity's relationship to drug abuse. The first theory states that the forms of impulsivity share some mechanisms with one another but that they each relate to and predict addiction vulnerability through a set of distinct, unique biological mechanisms and pathways. The second theory states that the facets of impulsivity share a portion of mechanism with each other and that this common biology is what links them all to addiction. These common neural mechanisms may include relatively low D2 availability, orbital and ventromedial frontal cortical dysfunction, and/or altered serotonergic transmission.

Impulsivity and Drug Use

Regardless of the measure of impulsivity being evaluated, studies have identified a strong and reproducible connection between substance use and impulsivity in humans, specifically that impulsivity levels are both a predictor and outcome of substance use. A review by de Wit (2008) reported that greater delay discounting has been seen in opioid users (Kirby, Petry, & Bickel, 1999), cocaine users (Coffey et al., 2003), alcohol abusers, and cigarette smokers (Bickel, Odum, & Madden, 1999). Heroin users undergoing withdrawal had an increase in delay discounting for heroin and money (Giordano et al.,

2002), while abstinent cigarette smokers only showed greater discounting for cigarettes versus money (Field et al., 2006). Cocaine use severity correlated with gray matter volume and reversal learning deficits in a cocaine-dependent population (Moreno-López et al., 2014) and smokers showed greater activation of the ventrolateral prefrontal cortex during monetary loss in the reversal task compared to healthy controls (de Ruiter et al., 2009). Voon and colleagues (2014) developed a human analogue of the 5-CSRTT. Subjects had to hold down the spacebar on a touch screen when four boxes appeared, and after a specified period, a green circle appeared briefly in one of the boxes that the subject would then have to touch after releasing the space bar. Anticipatory responding was measured by the subject releasing the space bar before the target appeared. Alcohol, methamphetamine, and cannabis users showed greater anticipatory responding compared to healthy subjects, and smokers showed greater anticipatory responding compared to ex-smokers and nonsmokers (Voon et al., 2014). Taken altogether, this research demonstrates that either impulsivity is a predictor of drug use or an outcome.

Studies using animal models have mimicked the relationship found in human participants, and animal subjects who had experienced various forms of exposure to drugs or alcohol were found to be more impulsive than controls. Krueger and colleagues (2009) injected mice daily with either cocaine or saline, waited two weeks, and then assessed them on reversal learning, three-choice serial reaction time task, and a delayed matching-to-position task, and found that cocaine-exposed mice had impairments in reversal learning and working memory. Similarly it was found that rats treated by an escalating dose of methamphetamine over four weeks and rats that received four weeks of saline with a single dose of methamphetamine showed impaired reversal learning as

compared to drug naïve rats (Kosheleff et al., 2012). In addition, chronic cocaine exposure caused reversal learning deficits in monkeys (Jentsch et al., 2002). Combined with human findings, a causal relationship has emerged with drug use resulting in high impulsivity across a variety of impulsivity-measuring tasks.

Animal models are more rigorously able to probe the consequence-outcome relationship of impulsivity and substance use better, and findings have indicated that highly impulsive subjects have a greater susceptibility for drug or alcohol seeking and consumption. Studies have shown that animal models that exhibit higher baseline impulsivity have increased sensitivity to intravenous (IV) drug self-administration, as well as acquisition and of instrumental IV drug self-administration (Jentsch et al., 2014). Rats identified as highly impulsive by the five-choice serial reaction time task have decreased D2/3 receptor availability and administer more cocaine (Dalley et al., 2007). In a separate study, highly impulsive rats identified by adjusting delay acquired self-administration faster, and females showed greater reinstatement at the highest dose (Perry, Nelson, Carroll, 2008), and impulsive rats identified by delay discounting displayed inelastic nicotine demand with consumption less sensitive to price increments (Diergaarde et al., 2012). Belin et. al (2008) demonstrated that high impulsivity can predict the switch to compulsive cocaine-taking, as more impulsive rats showed greater persistent and drug-taking in the face of adversity. A study using the recombinant inbred BxD mouse panel identified two strains as good reversal learners (good impulse control) and two strains as poor reversal learners (poor impulse control), and found that the poor reversal learning strains more rapidly acquired cocaine self-administration and administer cocaine at greater rates (Cervantes, Laughlin & Jentsch, 2013).

12

The Role of the Orbitofrontal Cortex

Studies have also demonstrated that the orbitofrontal cortex (OFC) and its outputs to the basal ganglia, are key mechanisms for impulse control. The OFC has been implicated in decision-making processes (Mar et al., 2011). It is further subdivided into lateral and medial regions that are cytoarchitecturally distinct and have different connections. The lateral region receives taste inputs and the medial region receives olfactory inputs, while both receive visual and somatosensory inputs, as well as projections from the amygdala and the mediodorsal thalamus (Elliot, Dolan, & Frith, 2000). The OFC is thus implicated as an area of convergence, which then projects to crucial areas such as the medial temporal cortical areas, hypothalamus, brain stem, and amygdala (Rempel-Clower, 2007). The OFC is able to target the intercalated nuclei of the amygdala to provide inhibitory influences (Rempel-Cower, 2007).

The medial and lateral regions of the OFC have been implicated in controlling different aspects of impulsive behavior. Primate studies have indicated that the medial OFC is associated with monitoring reinforcement contingencies and adjusting responses to varying incentive value of stimuli, and the lateral OFC is associated with punishment and suppression of responses (Kringelback & Rolls, 2004; Mar et al., 2011). Mimicking previous non-human primate findings, in a study using humans with frontal lobe legions, it was found that those with lateral damage had impaired credit assessment, and those with medial damage were more distracted by irrelevant options (Noonan et al., 2017). Rats with lesions to the medial OFC showed increased preference for larger-delayed reward and increased reversal learning, whereas rats with lesions to the lateral OFC showed decreased preference and retarded reversal learning (Mar et al., 2011), and

decreased dopamine transporter function in the OFC was associated with high impulsive

action in rats in the delay discounting task (Yates et al., 2016).

Disruptions in OFC function and dopaminergic transmissions lead to

susceptibility for drug self-administration and differences in addiction-related

phenotypes, such as impulsivity. Chronic drug exposure leads to epigenetic changes in

genes or their protein products, resulting in behavioral alteration such as heightened

impulsivity (Kreek et al., 2005), though animal studies have also demonstrated that

subjects can have a genetic predisposition for drug dependence.

Genetic Reference Populations

Genetic variation is an underlying mechanism associated with inter-individual

variation in impulse control, and in turn, addiction liability. The complexity of

impulsivity's relationship with frontostriatal circuitry and drug abuse is best viewed

through an empirical lens of being able to quantify this liability. Thus far, there have been

few studies that focus on genetic evidence of these traits, due in large part to the qualities

of the reference panels used. Genetic reference populations (GRP) are sets of inbred

strains derived from common founders with known, replicable genomes (Iraqi et al.,

2011) that are a powerful resource for the study of complex phenotypes and genotyping.

Mouse GRPs are typically recombinant inbred (RI) mouse strains that can be used to

analyze heritable phenotypes, circumventing limitations from human studies. Genotyped

strains in particular permit identification of quantitative trait loci (QTL) and association

with the genes of interest, thus capable of validating underlying genes responsible for

human disorders (Iraqi et al., 2011). RI lines are produced by crossing two inbred strains

together to create a F2 lineage, and then crossing that lineage via sibling mating to

produce genetically identical strains until approximately F22 (Broman, 2005). Existing RI panels are often derived from just two inbred strains and thus lack genetic diversity (Chesler et al., 2008), and panels such as the BxD have suffered from genetic drift (Chesler, 2014).

First proposed at the Edinburgh meeting of the International Mouse Genome Conference in October of 2001, the Collaborative Cross is a multi-parental RI panel. It avoids genetic bottleneck limitations by using five classical inbred strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HlLtJ) as well as three wild inbred strains (CAST/EiJ, PWK/PhJ, WSB/EiJ) to capture >90% genetic variation of lab mice strains (Odet et al., 2015). In addition to having high genetic diversity, the Collaborative Cross also has balanced allele frequencies and evenly distributed recombination sites (Aylor et al., 2011), with only chromosome 2 having overrepresentation of WSB/EiJ alleles and on chromosome X having a deficit of lines with CAST/EiJ alleles (Iraqi et al., 2011). The result of this is more statistical power and less spurious correlations, as well as the ability to develop correlations between multiple laboratories all using the Collaborative Cross mice as subjects. In addition to the Collaborative Cross, the eight founder strains were again used to develop the Diversity Outbred (DO) panel, which has the same allelic diversity as the CC strains and is a complementary resource for genetic mapping (Svenson et al., 2012).

Inbred mouse panels such as the CC permit estimates of heritability, which is operationally defined as the proportion of phenotypic variation that is explained by genetics. In inbred lines, heritability is estimated as the percent of phenotypic variance account for by strain. Past genetic reference population studies have considered this to be

an effective estimate of heritability, considering each mouse from each strain is, to the extent maximally possible, genetically identical to one another (Philip et al., 2009). Environmental and technical sources of variance are reduced within these panels, further increasing the ability to detect heritability of a trait over external influence and providing an advantage over human twin studies (Williams et al., 2004). The mathematical definition of heritability is the strain intra-class correlation (Philip et al., 2009):

$$h^2 = \sigma^2_{\text{Between Strain}} / (\sigma^2_{\text{Within Strain}} + \sigma^2_{\text{Between Strain}})$$

Present Study

The present study aims to examine genetic correlations of reinforcement learning, two tests of impulsivity (delay discounting and reversal learning), ethanol intake, and other catalogued addiction-related behaviors measured by others in the eight CC/DO founder strains. The tests of impulsivity are designed to measure three types of impulsivity: impulsive action (total trials to criteria in reversal learning), impulsive choice (k-value in delay discounting), and waiting impulsivity (anticipatory responses in reversal learning). We except to find some statistical evidence that the three measures of impulsivity are positively correlated with one another: if a strain rates high on impulsivity in one paradigm, it will rate high on impulsivity in the other paradigms. We also hypothesize that all tests of impulsivity will positively correlate with ethanol intake, which would support the concept that different types of impulsivity predict liability to exhibit escalated drug/alcohol intake. Phenotyping the founder strains will permit heritability analyses of these measures, as well as allow correlations to be run on other behavioral and biological phenotypes being studied in collaborating labs using the CC. Results from this study will additionally provide insight into the correlation between the

different types of impulsivity tested, and how they relate to ethanol intake, whether uniquely or similarly.

Methods

Mice

The study utilized the eight CC/DO founder mouse strains (A/J, Stock No. 000646 [n=18]; C57BL/6J, Stock No. 000664 [n=18]; 129S1/SvImJ, Stock No. 002448 [n=18]; NOD/ShiLtJ, Stock No. 001976 [n=18]; NZO/H1LtJ, Stock No. 002105 [n=18]; CAST/EiJ, Stock No. 000928 [n=18]; PWK/PhJ, Stock No. 003715 [n=18] and WSB/EiJ, Stock No. 001145 [n=18]). This study also includes two of the CC strains that are recombinant inbred strains resulting from the 8-way intercross (CC041/TauUncJ Stock No. 021893 [n=6], CC004/TauUncJ Stock No. 020944 [n=5]); these strains were selected into this study because our collaborative group revealed that these strains exhibit extremely different locomotor responses to cocaine, as well as different levels of cocaine self-administration. Currently, this study has completed evaluation of a total of 155 mice tested.

All animals were born at the Jackson Laboratory (Bar Harbor ME) and received via overnight shipping to Binghamton University between 35-49 days of age. Founder mice were delivered in three separate cohorts (N=48). Collaborative Cross mice were first present in cohort three (N=12), with three male and three female mice of each strain being represented in each cohort. All procedures involving animals were performed according to the Guide for the Care and Use of Laboratory Animals (NIH) in the AAALAC accredited program at Binghamton University with IACUC approval.

Upon arrival, mice were socially-housed in the same groups in which they were shipped, with three mice of the same strain and sex being grouped together in a cage. Animals were housed in a colony room with a 12-h/12-h illumination cycle (lights on at 0615 h) at an average of 68°F. All mice had access to water *ad libitum*, except during behavioral testing sessions (see below). At PND 60, mice were individually-housed in identical caging conditions, due to the aggression of the CAST, WSB, and PWK strains and the liability of injury when group-housed. They acclimated to the single house conditions for 10 days until PND 70 and were undisturbed during this time, except for weekly cage changes.

Upon receipt, and until PND 81, mice had *ad libitum* access to chow (Lab Diet 5001, ScottPharma Solutions), except during behavioral testing. All mice were then introduced to limited access to food to facilitate operant conditioning. Mice were weighed before food was removed, and that weight was recorded as their initial free feeding weight. During the limited access to food period, mice were fed once a day in the early afternoon, with non-wild mice initially receiving 3-g of chow per mouse and wild-derived mice (WSB/EiJ, PWK/PhJ, and CAST/EiJ) initially receiving 4-g of chow. Mice were weighed daily, and their weight was divided by their free feeding weight to obtain their percentage change in body weight. Chow quantity provided per day was titrated until mice reach 80-85% (non-wild) or 83-88% (wild-derived) of their free feeding weights. Once mice were approximately 85% of their free feeding weight, operant testing began (see Table 1 for mouse weights). If, at any point during the testing period, a mouse dropped below 80% of their free feeding weight, their daily chow quantity was increased.

If the mouse had two consecutive days being beneath 80%, they were temporarily

returned to *ad libitum* access to food until their weight had recovered.

Mice were handled by their tails, either with gloves (non-wild) or forceps (wild-

derived). During testing, all mice were removed from the operant box either by their tail

(non-wild) or by inserting the red tube from their home cage into the box, and removing

the tube when the mouse is inside (wild-derived).

Locomotor Response to Novelty and Habituation

At PND 70, mice were assessed for locomotor response in a novel open field

environment, during their light cycle. Mice were transported to a separate testing room on

a cart. Each mouse was individually placed in a 17 " L x 17" W x 12" H (43.2 x 43.2 x

30.5 cm) open field chamber fitted with infrared beams (Med-Associates MED-OFAS-

RSU; St Albans VT). All open field chambers were within sound attenuating cubicles

measuring 26" W x 22" H x 20.5" D (66 x 52.7 x 55.9 cm) at the interior with walls 0.75"

(1.9 cm) thick. Activity was recorded for 40-min, divided into eight 5-minute bins. The

primary dependent measure examined here was total distance traveled in centimeters.

After the session, mice were immediately removed and returned to their home cage.

Apparati were cleaned with a mixture of 10% Alconox detergent in water. The next

group of mice was brought into the room and placed in the apparatus. This was repeated

until all groups had been tested.

The following day at the same time, the mice were placed back in the same open

field chamber, and activity parameters were again recorded for 40-min. In addition to

total distance traveled, the difference in total activity on day 2, compared with day 1, was

also calculated (Day 2 – Day 1) to assess the degree of habituation of activity in the open field environment.

Palatable Food Consumption

One day after the conclusion of locomotor assessments, mice received home cage exposure to a highly palatable chocolate-flavored Boost solution (Nestle) from approximately PND 72-73. Boost was made available in the home cage in a plastic petri dish that was placed on top of the bedding. The solution was available continuously for this 48-h period, with the solution being refreshed at the 24-h time point.

On PND 74-80, mice were evaluated for Boost (and water) consumption in 7, consecutive, daily, 2-h lickometry sessions. All testing occurred during the light phase and took place inside dual lickometer Scurry boxes (Model 80822S, activity wheel removed; Lafayette Instruments). Each lickometer box is 35.3 x 23.5 x 20cm, and is fitted with a food hopper and two 50mL sipper bottles. No food was provided during the consumption test. One bottle was filled with Boost solution and the other was filled with water; the position (left or right) of the two solutions relative to one another was counterbalanced pseudorandomly across the testing days.

On testing days, mice were transported to the testing room on a cart. Room lights were on during testing and a room dehumidifier provided ambient background noise. A lickometer test was run prior to the mice being inserted to ensure that there were no technical problems with the box (including leakage of the solutions). At that point, the mice were placed, individually, into the lickometer boxes and were allowed to freely consume Boost and water for a 2-h period. Licks on each spout (per second) were counted by a computer. The number of licks was divided by the body weight of the

animal to account for variability attributed to body weight differences. After the daily sessions were completed, mice were transported back to the colony room and returned to their home cages.

Operant Conditioning – Reversal Learning

As described above, mice were transitioned to a limited food access schedule, once lickometer testing was completed. Once targeted reductions in body weights was achieved, half of all the mice from each sex of each strain were randomly designated for evaluation using an operant discrimination/reversal learning procedure. All operant testing took place in 8.5" L x 7" W x 5" H (21.6 x 17.8 x 12.7 cm) operant modular chambers (Model ENV-307W, Med Associates Inc.) with a stainless steel grid floor (Model ENV-307W-GFW, Med Associates Inc.) and within a sound attenuating cubicle. Mice were removed from their home cage by their tail and placed inside the operant box. A box test program evaluating the function of the house light, white noise, five nose-poke apertures, two response levers and reward delivery was conducted at the beginning of each testing day.

Each mouse was sequentially tested in a series of programs; mice transitioned from program to program individually, as they met criterion performance (see below). Mice underwent the following programs:

Stage 1: **Box habituation**. House light and white noise were active. No reinforcements were provided. The session lasted 1-h.

Stage 2: **Magazine training**. Again, the house light and white noise were active for the duration of the test, and 20-21µl Boost is dispensed every 30 seconds. The session ended after 1-h or after the mouse received 50 rewards, whichever came first.

Stage 3: Initial operant conditioning. During this stage, mice are trained to insert their nose into the center nose-poke aperture (hole 3 of 5). The session began with illumination of the house light and activation of the white noise generator; 10-s later, aperture 3 of 5 is illuminated (illumination of the hole was extinguished each time the mouse initiated a response in this hole). A behavioral response that broke the photocell in the aperture (usually, a nose poke) for at least 0, 100, or 200 msec (requirements varied from trial to trial) was reinforced by the delivery of 20-21ul of Boost solution; after each reinforcer was retrieved, a new trial was initiated 1.5-s later (signaled by illumination of the center nose poke aperture). If a response was initiated but was not sustained for the 0, 100, or 200 msec period, a time out period of 2-s occurred, during which time the central nose poke light and house light were extinguished. If a mouse did not voluntarily respond in the center hole for at least 15 minutes, the center hole was baited with a Boost-saturated cotton swab. Daily sessions lasted up to 1-h but were also terminated if an individual mouse completed 50 schedules. Each mouse was tested daily on this stage until it received at least 50 reinforcements in a single session, at which time it progressed to the next stage.

Stage 4: In this second stage of operant conditioning, mice were tested under the same basic conditions, except a minimum duration nose poke of 100- or 200-ms was required to trigger reinforcement. If a mouse did not respond in the center illuminated hole for 15 minutes, the center hole was baited with a Boost-saturated cotton swab. When the mouse completed 50 schedules in a single session, it progressed to Stage 5.

Stage 5: In this third stage of operant conditioning, mice were tested under the same basic conditions, except a minimum duration nose poke of 100-, 200-, or 300-ms was

required to trigger reinforcement delivery. If a mouse did not respond in the center illuminated hole for 15 minutes, the center hole was baited with a Boost-saturated cotton swab. When the mouse completed 50 schedules in a single session, it progressed to Stage 5.

Stage 6: **Discrimination learning stage**. As above, session onset is signaled by illumination of the house light and activation of the white noise generator; trial onset was signaled by illumination of the center nose poke aperture. As in stage 5, mice first completed an observing response into the center hole of 100-, or 200-ms duration. When this occurred, the two apertures flanking the central hole (hole 2 and 4) were immediately illuminated. A response into one of the two apertures (pseudorandomly assigned across strains) resulted in the delivery of a Boost reinforcer. Poking into the other hole - or not making any response within 30-s, triggered a time out, during which time the house light was extinguished. Responses into the reinforced hole were counted as correct trials; responses into the non-reinforced hole were counted as incorrect trials; and no response after trial initiation was counted as an omission. Daily sessions of 1-hr were conducted until learning criteria were met; this included a mouse completing at least 20 trials in a single session, and at least 80% running accuracy over the last 20 trials. Total time to reach criteria, total trials, total correct trials, total omits, average trial initiation latency, average reward retrieval latency, anticipatory trials in the correct flanking hole, and anticipatory trials in the incorrect flanking hole were recorded.

Stage 7: **Reversal learning stage**. Testing was nearly identical to that described above in Stage 6, with the exception that the reinforcement contingencies associated with the two holes were switched. Testing progressed in daily sessions until animals once

again met the same learning criteria rule described above, and the same dependent variables were collected. After reversal was completed, mice were slowly adjusted back onto a free-feeding schedule.

Operant Conditioning – Delay discounting

As described above, mice were transitioned to a limited food access schedule, once lickometer testing was completed. Once targeted reductions in body weights was achieved, half of all the mice from each sex of each strain were randomly designated for evaluation using a delay discounting procedure. Mice are removed from their home cage and placed inside the operant box. A box test program evaluating the function of the house light, white noise, five nose-poke apertures, two response levers and reward delivery was conducted at the beginning of each testing day.

Each mouse was sequentially tested in a series of programs; mice transitioned from program to program individually, as they met criterion performance (see below). Mice underwent the following programs:

Stage 1: **Box habituation**. House light and white noise were active. No reinforcements were provided. The session lasted 1-h.

Stage 2: **Magazine training**. Again, the house light and white noise were active for the duration of the test, and 20-21µl Boost is dispensed every 30 seconds. The duration of testing was 1-h.

Stage 3: **Lever Press Training – FR1**. Session onset was signaled by illumination of the house light and activation of the white noise generator. On each trial, one lever (left or right) is inserted to the chamber and actuation of the lever by the mouse triggered delivery of 20-21 µl of Boost. Across trials, the lever that was inserted (left or

right) was pseudorandomly varied, such that each mouse actuated each lever a roughly equal number of times. Each daily session ended after 1-h or after 60 reinforcements were obtained.

Stage 4: **Lever Press Training – FR3.** In this stage of lever press training, a procedure nearly identical to that described in Stage 3, above, was used. The only difference was that 3 sequential responses on the inserted lever were required before the reinforcer was delivered. The program ended after 1-h or after 60 rewards were obtained.

Stage 5: **Trial Initiation Training**. In this stage of training, a procedure nearly identical to that described in Stage 4 was used. The only difference is that the mouse was required to complete an observing response (nose poke response into the center hole (aperture 3 of 5) on the opposite site of the chamber in order to trigger insertion of a lever. Responses on that lever were still reinforced on an FR3 schedule. The program ended after 1-h or after 60 rewards were obtained.

Stage 6: In this stage of training, a procedure nearly identical to that described in Stage 5 was used. The only difference was that the program ended after 1.5 hours, or after 80 rewards were obtained, whichever came first.

Stage 7: **Side bias**. Trials begin with both levers being presented, with a response on either on a FR3 schedule resulting in a delivery of 8-9 µl of Boost. After a 10-s inter-trial interval, both levers are again presented, but only a response on the other lever is rewarded. A trial is only counted if the mouse successfully presses the alternate lever. The program ends after 40 trials, or after 1.5 hours. The lever (right or left) on which each trial is initiated is recorded, and the dominant lever is considered the biased lever and is paired with the delayed lever.

After completing training, all mice are placed on a randomized Latin square of 0s, 3s, 6s, and 9s. Three consecutive days of testing are done at each delay. Reward is adjusted within trial, and delay is adjusted across sessions. Once the first Latin square is completed, mice receive a two-day break, and they undergo a second Latin square. Amount is measured for the immediate lever and the delayed lever and subtracted. The reward amount for each lever is averaged over the last 30 trials, and those values are averaged across the three days that delay was tested. These values are used to calculate a k-value for each animal: the scaling factor, or how much the subjective value of the reward is affected by the delay. The b-value is also recorded, which represents the animal's side bias, determined by dividing the average delayed amount by the average immediate amount for the 0s delay.

Ethanol intake

After reintroduction to an unlimited diet for two weeks (no food restriction), all reversal learning and delay discounting mice were again placed into Scurry lickometer boxes (Model 80822S; Lafayette Instruments Inc.; Lafayette IN), with access to a 20% ethanol and a tap water bottle (chow was also provided). Test sessions began one hour into the dark phase and lasted for 12 hours. Bottles were weighed before and after sessions for consumption analysis. Licks on both bottles were tracked for the whole session. Bottle positions were counterbalanced between groups and alternated between sessions. Mice were returned to the home-cage between sessions. All mice received 3 daily sessions.

Data Analysis

Data for each variable was analyzed by an ANOVA using SPSS Statistics, with number of licks for the lickometer task also analyzed using a linear mixed model. Data was first examined using a body plot and outliers two standard deviations from the mean were removed. Statistical significance was established to be a probability level of $p<.05$. Independent variables were strain and sex. All ten strains were included in the analyses for locomotor, reward intake, and ethanol intake, though the CC041/TauUncJ and CC004/TauUncJ strains were not included in reversal learning or delay discounting due to low subject numbers.

Heritability estimates were derived for each significant effect of strain using effect size, which is an estimate of the variance accounted for by the independent variable divided by the total amount of variance. Effect size is used to determine the magnitude of the result, or how much variability is explained by the independent variable. By using effect size for strain, the proportion of variance explained by strain, which translates to what portion of the variability is heritable from the strain's genotype. Despite the structure of this study not being inter-generational, heritability is a justified measure because of the nature of the founder strains. Each mouse within a strain is genetically identical to one another, mimicking the heritable rigor of monozygotic twin studies conducted in humans.

Results

To address strain level differences in body weight and/or in body weight change in response to food restriction, an ANOVA was conducted on free-feeding weight (grams) and percentage of free-feeding body weight at the start of testing and the average at each stage of operant training: acquisition and reversal for reversal learning, and the

first, second, third, and fourth delay periods for delay discounting. An interaction of sex by strain (F[7,143]=4.89, p<.001), main effect of strain (F[7,143]=284.737, p<.001), and main effect of sex (F[1,143]=145.744, p<.001) were unsurprisingly found for free-feeding weights due to the body mass variability of the founder strains. After food deprivation began, no strain or sex differences in percentage body weight were found at any point in operant experimentation for animals on reversal learning (Table 1) or delay discounting (Table 2), demonstrating that we successfully altered body weights to the same degree in each strain.

Locomotor Response to Novelty and Habituation

Total distance traveled in the open field chambers was assessed on days 1 and 2 of testing (Figure 1); these data were time-binned and were analyzed with a mixed model ANOVA. An interaction was found between time and strain (F[9,135]=3.683, p<.001, ηp^2=.197), though not time and sex, or time and sex and strain (F<1.6, p>.121). Two homogenous subsets emerged: strains with high ambulatory distance (C57BL/6J, NOD/ShiLtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ, CC004/TauUncJ) and strains with low ambulatory distance (CC041/TauUncJ, NZO/H1LtJ, 129S1/SvImJ, and A/J). A main effect was found for time bin (F[1,135]=135, p<.001, ηp^2=.116), strain (F[9, 135]=46.56, p<.001, ηp^2=.756) and sex (F[1,135]=4.276, p<.05, ηp^2=.031).

To evaluate the degree of habituation of the locomotor response occurring across the two days, a difference score was calculated by subtracting distance traveled on D1 from distance traveled on D2 for each mouse (Figure 2, 3); these results were analyzed using a two-way ANOVA. A main effect of strain was found (F[1,135]=3.683, p<.001, ηp^2=.197), though there was no main effect of sex nor an interaction between strain and

sex (F<1.602, p>.121). PWK and CC041 mice were the only strains to exhibit an increased ambulatory distance on the second day, evidence of sensitization rather than habituation.

Palatable Food Consumption

We examined data from the 7 consecutive days of Boost and water consumption conducted in the lickometer boxes. Considering only licks on the Boost spout, licks were analyzed in total, as well as adjusted for body weight (Licks/Body weight). Examining total licks, a linear mixed model demonstrated an interaction between strain and sex (F[9,1022.019]=5.373, *p*<.001), a main effect of strain (F[9, 1021.943]=38.069, *p*<.001; Figure 4), and a main effect of sex (F[1, 1031.775]=7.529, *p*<.01). When adjusted for body weight, a linear mixed model found an interaction between strain and sex (F[9, 1006.71]=6.143, *p*<.001), a main effect of strain (F[9, 1006.71]=77.209, *p*<.001; Figure 5), and a trending main effect of sex (F[1, 1034.197]=3.647, *p*=.056). No interaction was found for days by strain, days by sex, or days by strain by sex (F<.701, *p*>.938). Similarly, water licks were analyzed in total, and adjusted for body weight. For total licks, only a main effect of strain was found (F[9, 301.34]=4.144, *p*<.001; Figure 6). PWK/PhJ mice made more water licks than all strains except for NZO/HlLtJ, CAST/EiJ, and CC04/TauUncJ. Dividing total licks by body weight similarly revealed a main effect of strain (F[9, 508.33]=5.833, *p*<.001; Figure 7). PWK/PhJ and CAST/EiJ mice had significantly more licks adjusted for body weight than the other strains.

We next examined licking only on days 5-7 of testing to best estimate each animal's consummatory behavior at a point where licking behavior has stabilized. A between subjects ANOVA on unadjusted Boost licks again showed a main effect of strain

(F[9, 150]=12.464, $p<.001$, $\eta p^2=.463$; Figure 8, 9) and an interaction between strain and sex (F[9, 150]=2.059, $p<.05$, $\eta p^2=.125$), but no main effect of sex (F[1, 150]=.29, $p=.591$, $\eta p^2=.002$). CAST/EiJ mice had significantly more licks regardless of body weight than all strains except for the CC004/TauUncJ and A/J. For Boost licks adjusted for body weight, an interaction of strain by sex (F[9, 155]=1.97, $p<.05$, $\eta p^2=.116$) and a main effect of strain (F[9, 155]=19.164, $p<.0001$, $\eta p^2=.561$; Figure 10, 11) was found. CAST/EiJ mice lick significantly more than all other strains for their body weight. The same was done for average water licks on the final three days. For total unadjusted water licks, there was no main effect of strain or sex, or an interaction of strain by sex (Figure 12). For water licks adjusted for body weight, there was a trending main effect of strain (F[9, 155]=1.92, $p=.054$, $\eta p^2=.113$; Figure 13), with no interaction of strain by sex.

A preference score was next calculated for each animal by dividing the number of licks on the Boost spout by total licks (on the Boost and water spouts). This preference score was again averaged over the final three days of consumption and analyzed using a one-way ANOVA. No strain, sex, or strain by sex differences for preference was found ($p>.05$ for all); however, this is almost certainly a "ceiling effect" due to very high preference for Boost over water exhibited in all strains (Table 3).

Operant Conditioning – Reversal Learning

Reversal learning was analyzed examining the following at acquisition and reversal: total trials, correct trials, omits, total time, trial initiation latency, pellet retrieval time, average anticipatory correct responses, and average anticipatory incorrect responses. A MANOVA was first performed, identifying multivariate main effects of strain (F[72,250.89]=2.192, $p<.001$, $\eta p^2=.31$) and stage (F[8,40]=3.735, $p<.01$,

ηp^2=.428). A mixed model ANOVA was then run on the aforementioned variables. A main effect of stage (acquisition or reversal) was found for total trials (F[1,47]=5.123, p<.05, ηp^2=.098; Figure 14), anticipatory correct responses (F[1,47]=4.173, p<.05, ηp^2=.082; Figure 15), and anticipatory incorrect responses (F[1,47]=13.103, p<.001, ηp^2=.218; Figure 16). A main effect of strain was found for omissions (F[9,47]=5.471, p<.001, ηp^2=.512), total time (F[9,47]=3.547 p<.001, ηp^2=.404), trial initiation latency (F[9,47]=4.714, p<.001, ηp^2=.474), and average anticipatory incorrect responses (F[9,47]=2.621, p<.05, ηp^2=.334), with a trending effect for correct trials (F[9,47]=2.051, p=.054, ηp^2=.282). Neither main effects of sex nor an interaction of strain by sex were found. A strain by stage interaction was only found for correct trials (F[9,47]=2.268, p<.05, ηp^2=.303). NZO/HlLtJ had the highest average of anticipatory incorrect responses, significantly higher than the two lowest strains, CAST/EiJ and PWK/PhJ.

Operant Conditioning – Delay discounting

Variables k-value and b-value in delay discounting were analyzed with a between subjects ANOVA. A multivariate main effect of strain was identified (F[18,122]=2.906, p<.01, ηp^2=.307), as well as a main effect of strain for k-value (F[18,122]=3.304, p<.01, ηp^2=.331; Figure 17, 18) and b-value (F[18,122]=3.966, p<.01, ηp^2=.373). The interaction between strain and b violated homogeneity of regression slopes and an ANCOVA to isolate strain-dependent side bias was not conducted.

A Tukey post-hoc revealed 129S1/SvImJ mice as having the highest k-value, significantly higher than all other strains. CAST/EiJ mice had the lowest, significantly lower than 129S1/SvImJ and WSB/EiJ strains. 129S1/SvImJ mice additionally had the

highest b-value, significantly higher than all but the WSB/EiJ mice, who had the second highest.

Ethanol intake

A repeated measures ANOVA was run on each mouse's total lick consumption averaged over the three days of testing. A strain by sex interaction was found ($F[9,135]=3.195$, $p<.01$, $\eta p^2=.20$; Figure 19, 20) as well as a main effect of strain ($F[9,135]=26.252$, $p<.001$, $\eta p^2=.673$).

Correlations

A two-tailed bivariate Spearman's correlation was conducted on overall strain means to detect heritable relationships between phenotypes within the founders. Ambulatory distance traveled on D1 of the open field task was positively correlated with ambulatory distance traveled on D2 ($r_s=.952$, $p<.001$; Table 4 cell 1B; Figure 21), and average last three day Boost consumption was positively correlated with last three day Boost preference ($r_s=.714$, $p<.05$; Figure 22) as anticipated. Last three day Boost intake was positively correlated with last three day water intake ($r_s=.786$, $p<.05$; Figure 23), though Boost preference was not correlated with water intake ($r_s=.357$, $p=.385$). Last three day Boost intake was negatively correlated with k-value ($r_s=-.786$, $p<.05$; Figure 24).

Total trials to criteria at reversal was not correlated with total trials at acquisition ($r_s=.452$, $p=.260$; Table 4 cell 5F), though average anticipatory responding at reversal was correlated with average anticipatory responding at acquisition for a Pearson correlation ($r_s=.810$, $p<.05$; Table 4 cell 8G). None of the impulsivity measures were significantly correlated with each other: total trials to criteria at reversal and average

incorrect anticipatory responses at reversal (r_s=-.095, p=.823; Table 4 cell 6H); total trials to criteria at reversal and k-value (r_s=.381, p=.352; Table 4 cell 6I); average anticipatory incorrect responses at reversal and k-value (r_s=.571, p=.139; Table 4 cell 8I). K-value was, however, was positively correlated with average anticipatory incorrect responses at acquisition (r_s=.810, p<.05; Figure 25; Table 4 cell 7I).

Ethanol intake did not correlate with total trials to criteria, anticipatory responding, or k-value when collapsed for sex across strains. Analyzing each sex separately, however, revealed a positive correlation between ethanol intake and total trials at reversal for female mice (r_s=.810, p<.05; Figure 22) though not male mice (r_s=-.19, p=.651). A correlation was also found for female mice between ethanol intake and ambulatory activity on day 1 of locomotor response to novelty (r_s=762, p<.05).

Correlation values are shown in Table 4, which reports Spearman's Rho and Pearson's r.

Discussion

The goal of this study was to examine the heritability for addiction-related traits, and further understand the relationship different aspects of impulsivity have with each other and ethanol intake. Past studies have identified multiple dimensions of impulsivity each with a unique relationship to drug taking (Evenden, 1999), and using genetic reference population enables the examination of how these dimensions relate within homogenous strains. This study additionally marks one of the first efforts to phenotype the CC founder strains for these traits, and this study overall aims to provide a basis of information that can be compared with other laboratories using the CC founders.

Results show multiple heritable phenotypes emerging from the CC founder strains. Strains showed high heritability for locomotor activity in response to novelty, degree of habituation within the open field apparatus, reward sensitivity as measured by total Boost licks, waiting impulsivity in reversal learning, impulsive choice in delay discounting, and ethanol intake. Overall results demonstrate a genetic component of these phenotypes, with varying degrees of heritability as determined by the effect size associated with the strain level effect. Ambulatory distance in response to novelty and reward intake exhibited the highest degree of heritability. Two of the three measures of impulsivity (anticipatory incorrect trials and k-value) reached strain significance and each exhibited robust heritability even among only the eight founder strains phenotyped so far. Though total trials to criteria in acquisition and reversal did not exhibit statistically significant heritability, it may be that the heritability of impulsive action is lower than can be currently detected with the number of strains being used.

The results of this study thus far support those the theory proposed Evenden (1998), and imply that these three dimensions of impulsivity have separate components. We found no correlation between impulsive action, impulsive choice, and waiting impulsivity, indicating that a strain that scored high on one dimension of impulsivity did not necessarily score highly on another. These findings suggest one theory put forth by Jentsch and colleagues (2014), suggesting that there is not one unitary "impulsivity" and there exist different varieties each controlled by different mechanisms. While each of the types of impulsivity tested do share some mechanistic similarities within the prefrontal cortex, each does result in different cortical activation. Reversal learning, a test of action inhibition, requires function of the orbitofrontal cortex and dorsomedial striatum for

inhibitory control (Jentsch et al, 2014), also demonstrated by a MRI study in human adults (Ghahremani et al., 2010). Conversely, tests of impulsive choice and waiting impulsivity do not rely on the orbitofrontal cortex; impulsive choice is associated with the lateral portions of the frontal cortex and the hippocampus, and waiting impulsivity is associated with the ventromedial areas (Jentsch et al., 2014).

Dalley, Everitt, and Robbins (2011) suggested that anticipatory responding in the 5-CSRT and delay discounting paradigms have some overlap due to both tasks necessitating action restraint, though delay discounting differs due to the variable of relative reinforcing value. In the present study we found a correlation between average anticipatory incorrect responses at acquisition and k-value, though not average anticipatory incorrect responses at reversal. The lack of correlation at reversal indicates that k-value is not related to cognitive inflexibility, though may be related to a measure of action inhibition during the learning process. The acquisition stage of reversal is intended to act as a learning control; all mice are permitted to undergo testing as long as necessary until they meet criteria, to account for base strain differences in learning and memory. A strain difference of incorrect anticipatory responses at acquisition may indicate a lack of action restraint during training as well as a resilience to rule-learning, both of which correlate with a strain's relative value of a delayed reward. The lack of correlation of k-value with anticipatory incorrect responses at reversal could demonstrate the subjects modifying their behavior, or a smaller effect that cannot be detected with the current number of strains.

It also must be examined how the reinforcing value of Boost played into the results. Licks on the spout are a measure of palatability in subjects, which would appear

to correlate with impulsive choice, though not impulsive action or waiting impulsivity. We found a negative correlation between k-value and Boost licks in the reward sensitivity task, which could indicate that strains more incentivized to consume Boost are more willing to wait to receive it — finding Boost to be a higher value reward may be a moderator of the devaluing that comes with a delay. Testing the strains in the lickometer boxes provides essential information with regards to motivation and/or reward sensitivity. Past research has suggested sensitivity to reward is a singular trait capable of predicting motivation to seek out reinforcing stimuli (Davis & Fox, 2007), and presentation of food and drug cues result in activation of similar regions as well as activate similar gene expression programs (Kelley, Schiltz, & Landry, 2005). The fact that Boost licks are not correlated with ethanol intake would suggest that either sensitivity to food reward and ethanol reward are separate, or that another variable such as latency to consume may be a more accurate measure of reward sensitivity. In human studies, over-consumption can be a protective mechanism against drug dependence, as over-eating competes for the same binding sites as drug use (Kleiner et al., 2004; Warren, Frost-Pineda, & Gold, 2005). This has not been observed in animal models, and a negative correlation between Boost licks and ethanol licks in this study was not observed.

Ultimately only one dimension of impulsivity was correlated with overnight ethanol consumption, and the effect was sex-dependent. Only female mice demonstrated a correlation between total trials to criteria in reversal learning with amount of licks on the ethanol spout within the 12-hour consumption period. Waiting impulsivity and impulsive choice did not correlate with ethanol licks in either sex. Impulsive action's relationship to ethanol intake may be reliant upon function of the orbitofrontal cortex.

Lesions of the orbitofrontal cortex but not the medial prefrontal cortex result in slower stop-signal reaction times (Jentsch et al., 2014), suggesting that the orbitofrontal cortex is a fundamental component of action inhibition. Depletion of dopamine consistently results in higher impulsivity in reversal learning (Jentsch et al., 2014) and D2-like receptor stimulation increases inhibition and decreases impulsivity (Eagle et al., 2011), while results are variable for delay discounting and the 5-CSRTT (Jentsch et al., 2014). Similarly, individuals dependent upon alcohol, cocaine, heroin, and/or methamphetamine consistently demonstrate lower D2 receptor availability, and a relationship has been found in cocaine users between striatal D2 receptor density and OFC metabolic rates (Volkow et al., 2001). Alcoholism specifically is associated with decreased D2 receptors and mesolimbic dopamine transmission (Martinez et al., 2005) and it has been hypothesized that the OFC is responsible for reward modulation in response to striatal dopamine levels (Volkow et al., 2007). The relationship found in the present study may indicate the OFC's importance in modulating response inhibition, cognitive flexibility, and sensitivity to ethanol, while not correlated with impulsive decision-making or anticipatory responding. Sex differences specifically suggest that action inhibition is modulated differently in males and females.

One of the limitations of this study is that correlations are being conducted with only eight or ten strains. This relatively small sample size means that only correlations with large effects are capable of being detected, such as female mice on impulsive action and ethanol intake, or Boost licks and k-value. Until additional strains are added to this study, the statistics being run are not powered to detect strain correlations with a low or medium effect size. Therefore, ongoing work will accumulate phenotypes for an

increasingly large number of the CC strains, providing stronger powered to detect small to medium size effects.

For several phenotypes the strains exhibited clustering of scores. For example, Boost licks over the seven days (Figure 4) showed homogenous subsets of CAST/EiJ; NOD/ShiLtj, A/J, C57BL/6J, NZO/HILtJ, PWK/PhJ, and CC004/TauUncJ; and 129/SvlmJ, WSB/EiJ, and CC041/TauUncJ. This can also be seen in ambulatory distance (Figure 1), anticipatory incorrect responses (Figure 16), and k-value (Figure 17). While the founder strains were chosen due to their genetic diversity (Odet et al., 2015), this grouping of scores is anticipated as it demonstrates that the clustered strains have the same allele pairing on the gene for that phenotype. Considering base pairs are either A-T or C-G, this pattern of results demonstrates that while the strains themselves are genetically diverse, allelic similarity for a specific phenotype will result in clustering rather than a continuous spectrum of responses.

The inclusion of the wild-derived strains (CAST/EiJ, WSB/EiJ, PWK/PhJ) gives the CC more genetic diversity and ability to detect genetic correlations, though it introduces a dichotomy with the non-wild-derived strains. The other five inbred strains were selected from already existing inbred mouse lines. Bottlenecking has occurred since mice were kept by collectors and researchers and bred selectively for the ease of capture and handling, decreasing allelic diversity as they were inbred (Chesler, 2013). Conversely, the wild-derived founders were inbred but not selected for traits of docility or ease of handling. The end result is three strains that have heightened aggression in comparison to the other five and likely other behavioral traits that haven't been removed through selective breeding. Considering impulsivity's relationship with aggression has

been well documented in humans (Gvion & Apter, 2011; Manuck et al., 2000) and mice (Brunner & Hen, 1997; Lesch & Merschdorf, 2000), it must be considered that inbreeding may have reduced impulsivity in the non-wild-derived strains.

Another consideration is that all mice were shipped during adolescence (PND 35-49) and were individually-housed at the beginning of adulthood (PND 60). All efforts were made to attenuate the effects of shipping by allowing animals to acclimate untouched and group-housed for a week, and all strains underwent shipping. Single housing was necessary due to the aggression of the CAST/EiJ, WSB/EiJ, and PWK/PhJ strains, which become aggressive in both sexes during adulthood. As to not introduce the variable of single- versus group-housing, the decision was made to single-house all animals as late as possible, which was determined to be PND 60. One study isolated adult male rats (PND 63-91) and later tested them on cocaine IVSA, running two separate replicate studies to verify the reproducibility of results. The first group found that found that isolated rats acquired cocaine IVSA faster, though both groups self-administered similar levels by the fifth week of testing. The second group, however, had no differences, suggesting that other contextual factors may moderate the social housing effect (Bozarth, Murray, & Wise, 1987). Yet another study found that isolation-housing for 12 days did not alter the ethanol consumption of adolescent rats, though it did suppress consumption in adults (Doremus et al., 2006). It also must be taken into consideration that certain strains may be more sensitive to the effects of isolation housing, and the degree to which operant performance and ethanol intake is altered may vary.

A small amount of caffeine is present in the Boost reinforcer and may exert an effect, particularly if one or more strains was exceptionally sensitive. Chocolate Boost contains .62 mg of caffeine in one fluid ounce (Caffeine Informer). The maximum amount of caffeine a mouse could receive on average per day was calculated. Each reward delivery is approximately 20 μl, or .00068 fluid ounces, resulting in mice receiving .00418 mg caffeine per reinforcer. The maximum reinforcers a mouse received on average was 80 in delay discounting, with less rewards being received in reversal learning. Thus, a mouse could possibly receive at maximum of .3344 mg of caffeine per day. This was converted into a mg/kg dose for a low weight animal (12g) and a high weight animal (40g). Respectively the daily dose was calculated to be 10 mg/kg and 3 mg/kg (PO). A bolus dose of 15 mg/kg i.p. caffeine is described as being a moderate dose (Hnasko, Sotak, & Palmiter, 2005), and 1.5 mg/kg i.p. doses were found to be enough to produce conditioned place preference in mice (Patkina & Zvartau, 1998). Past studies have shown that .5-16 mg/kg i.p. dose of caffeine increases locomotor activity in mice (Kayir & Uzbay, 2004), and i.p. doses at 5mg/kg-15mg/kg increase wakefulness (Huang et al., 2005). Route of administration additionally plays a factor: oral consumption of caffeine decreased the amount of cocaine later self-administered, while 3 mg/kg i.p. injections increased it despite similar metabolite levels (Kuzmin et al., 2000). This information indicates that mice in the present study were receiving variable levels of caffeine that depended on weight and number of reinforcers received, though this was at maximum a low-moderate dose.

Altogether, the present study marks one of the preliminary attempts to phenotype the CC founder strains for ambulatory activity, reward sensitivity, three types of

impulsivity, and ethanol intake. Heritability for these traits has been identified, as well as a correlation between impulsive action and ethanol intake. Importantly none of the impulsivity measures were found to be correlated with one another, and the fact only one was related to ethanol intake indicates a potential sex-dependent effect of the orbitofrontal area of the cortex. Future research will include the CC strains, enabling correlations with low and medium effect sizes to be conducted, and eventually the identification of gene candidates within the striatum.

Mouse ID	Free-feed	Start	Acquisition	Reversal
NOD/ShiLtJ F	23.2g	85.3%	85%	90.8%
NOD/ShiLtJ M	29.5g	88.3%	83.5%	85%
129S1/SvImJ F	21.7g	91%	89.3%	88.8%
129S1/SvImJ M	25.3g	89.8%	88.6%	85.8%
A/J F	18.8g	89.6%	85.4%	85.2%
A/J M	23.5g	89.5%	83.0%	83.3%
C57BL/6J F	20.4g	89.3%	88%	87.8%
C57BL/6J M	26.3g	85.2%	85.4%	85.8%
NZO/H1LtJ F	36.3g	93%	86%	83.5%
NZO/H1LtJ M	41.4g	88.5%	89%	86.5%
CAST/EiJ F	13.1g	88.6%	86.2%	86.8%
CAST/EiJ M	16.0g	92%	88%	86%
WSB/EiJ F	16.9g	90%	84.3%	87%
WSB/EiJ M	17.5g	95%	84.5%	86.5%
PWK/PhJ F	15.4g	84.5%	86%	88%
PWK/PhJ M	17.8g	84.8%	86.4%	83.6%

Table 1. Free-feeding body weights and percentage of free-feeding weight for mice that underwent reversal learning, averaged by strain and sex. There were no differences between the strains in percentage body weight.

Mouse ID	Free-feed	Start	1st delay	2nd delay	3rd delay	4th delay
NOD/ShiLtJ F	21.8g	90.4%	89%	90.2%	88.8%	88.2%
NOD/ShiLtJ M	27.5g	84%	86.3%	88.8%	86.3%	83.8%
129S1/SvImJ F	20.4g	90.2%	87.8%	88.8%	86.6%	86.6%
129S1/SvImJ M	23.6g	91%	87.3%	88.3%	86%	87%
A/J F	20.4g	89%	86.8%	84.8%	83.8%	83.3%
A/J M	22.4g	88.2%	84.2%	86.6%	89.2%	86.4%
C57BL/6J F	20.5g	92%	88%	93%	88.6%	88%
C57BL/6J M	28.4g	86%	84.5%	86.5%	86.5%	85%
NZO/H1LtJ F	36.2g	90.3%	83.7%	85.3%	83%	82.7%
NZO/H1LtJ M	41.5g	89%	85.8%	84.3%	83.2%	83.5%
CAST/EiJ F	13.5g	87.8%	87.8%	86.5%	89.3%	87.3%
CAST/EiJ M	16.4g	88%	85.8%	86.3%	86.8%	84.5%
WSB/EiJ F	17.4g	90.3%	84.7%	92%	88%	92%
WSB/EiJ M	18.4g	95%	88.3%	89.8%	85.8%	89%
PWK/PhJ F	15.1g	89.2%	85%	88.8%	85%	87.8%
PWK/PhJ M	17.3g	89.5%	87.8%	85.8%	86.5%	86.8%

Table 2. Free-feeding body weights and percentage of free-feeding weight for mice that underwent delay discounting, averaged by strain and sex. There were no differences between the strains in percentage body weight.

Mouse ID	Mean	SD
NOD/ShiLtJ F	0.95	0.07
NOD/ShiLtJ M	0.9	0.13
129S1/SvImJ F	0.74	0.26
129S1/SvImJ M	0.94	0.11
A/J F	0.86	0.11
A/J M	0.9	0.15
C57BL/6J F	0.93	0.04
C57BL/6J M	0.91	0.12
NZO/H1LtJ F	0.91	0.08
NZO/H1LtJ M	0.9	0.06
CAST/EiJ F	0.93	0.14
CAST/EiJ M	0.91	0.09
WSB/EiJ F	0.83	0.18
WSB/EiJ M	0.85	0.08
PWK/PhJ F	0.87	0.14
PWK/PhJ M	0.91	0.08

Table 3. Means and standard deviations of Boost licks divided by total licks, averaged over the last three days of consumption.

	A	B	C	D	E	F	G	H	I	J
1.		r_s=.952 p<.001	r_s=-.527 p=.117	r_s=.115 p=.751	r_s=-.055 p=.881	r_s=-.285 p=.425	r_s=-.588 p=.074	r_s=-.539 p=.108	r_s=-.236 p=.511	r_s=.552 p=.098
2.	r=.982 p<.001		r_s=-.333 p=.347	r_s=.200 p=.580	r_s=.042 p=.907	r_s=-.297 p=.405	r_s=-.552 p=.098	r_s=-.661 p=.038	r_s=-.248 p=.489	r_s=.418 p=.229
3.	r=-.582 p=.130	r=-.416 p=.305		r_s=-.127 p=.726	r_s=-.091 p=.803	r_s=.442 p=.20	r_s=.333 p=.347	r_s=.224 p=.533	r_s=.03 p=.934	r_s=-.261 p=.467
4.	r=.117 p=.675	r=.205 p=.626	r=-.030 p=.943		r_s=.333 p=.347	r_s=-.127 p=.726	r_s=-.455 p=.187	r_s=-.588 p=.074	r_s=-.236 p=.511	r_s=.273 p=.446
5.	r=.021 p=.961	r=.021 p=.961	r=-.009 p=.983	r=-.057 p=.893		r_s=.139 p=.701	r_s=.018 p=.960	r_s=-.661 p=.038	r_s=.382 p=.276	r_s=-.709 p=.829
6.	r=-.314 p=.449	r=-.266 p=.525	r=-.365 p=.374	r=.065 p=.878	r=.459 p=.252		r_s=.055 p=.881	r_s=.103 p=.777	r_s=-.607 p=.855	r_s=.055 p=.881
7.	r=-.816 p=.012	r=-.819 p=.013	r=.445 p=.269	r=-.510 p=.197	r=-.173 p=.683	r=.199 p=.636		r_s=.491 p=.150	r_s=.721 p=.019	r_s=-.297 p=.405
8.	r=-.797 p=.018	r=-.850 p=.007	r=.172 p=.683	r=-.508 p=.119	r=-.289 p=.487	r=-.079 p=.852	r=.823 p=.012		r_s=.164 p=.651	r_s=-.188 p=.603
9.	r=-.406 p=.318	r=-.406 p=.318	r=.206 p=.624	r=-.629 p=.095	r=.536 p=.171	r=.219 p=.602	r=.448 p=.266	r=.285 p=.494		r_s=.152 p=.676
10.	r=.406 p=.318	r=.513 p=.194	r=.250 p=.550	r=.047 p=.991	r=-.094 p=.824	r=.144 p=.734	r=-.498 p=.209	r=-.443 p=.272	r=-.289 p=.448	

Table 4. Correlation table for strains collapsed by sex. *A priori* correlations are highlighted in green and significant *a priori* correlations are highlighted in yellow. Spearman correlations are reported on the upper right and Pearson correlations are reported on the lower left. The variables are as follows:

1/A. Ambulatory distance on D1 of locomotor

2/B. Ambulatory distance on D2 of locomotor

3/C. D2-D1 ambulatory distance of locomotor

4/D. Boost licks averaged over the final 3 days adjusted for body weight

5/E. Total trials to criteria in acquisition of reversal learning

6/F. Total trials to criteria in reversal of reversal learning

7/G. Average anticipatory incorrect responses in acquisition

8/H. Average anticipatory incorrect responses in reversal

9/I. k-value

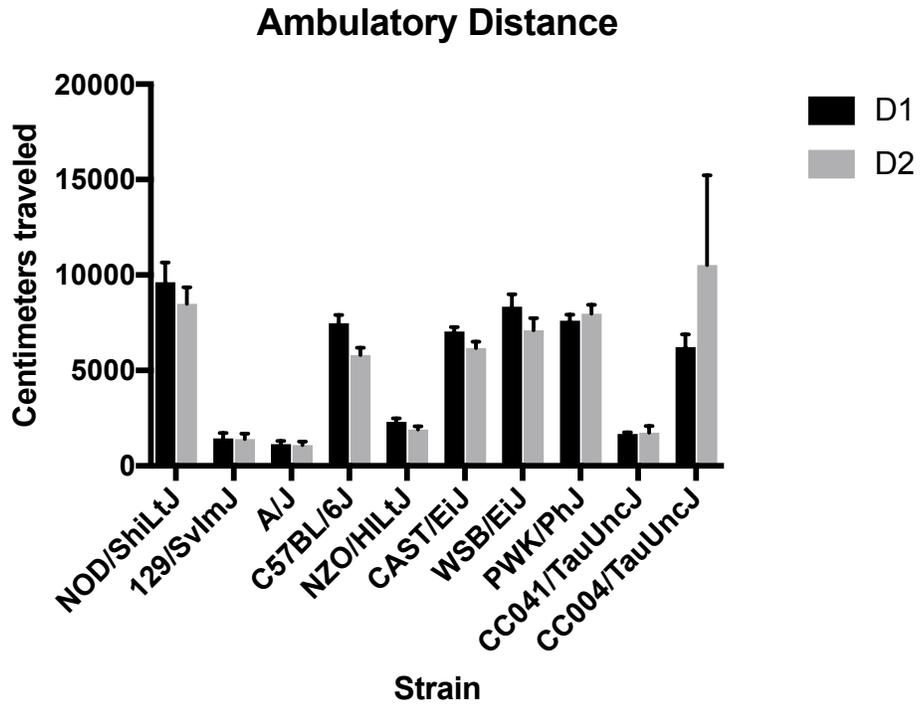10/J. Ethanol licks averaged over 3 days of consumption

Figure 1. Ambulatory distance calculated by total centimeters on day 1 and day 2 in the open field chamber.
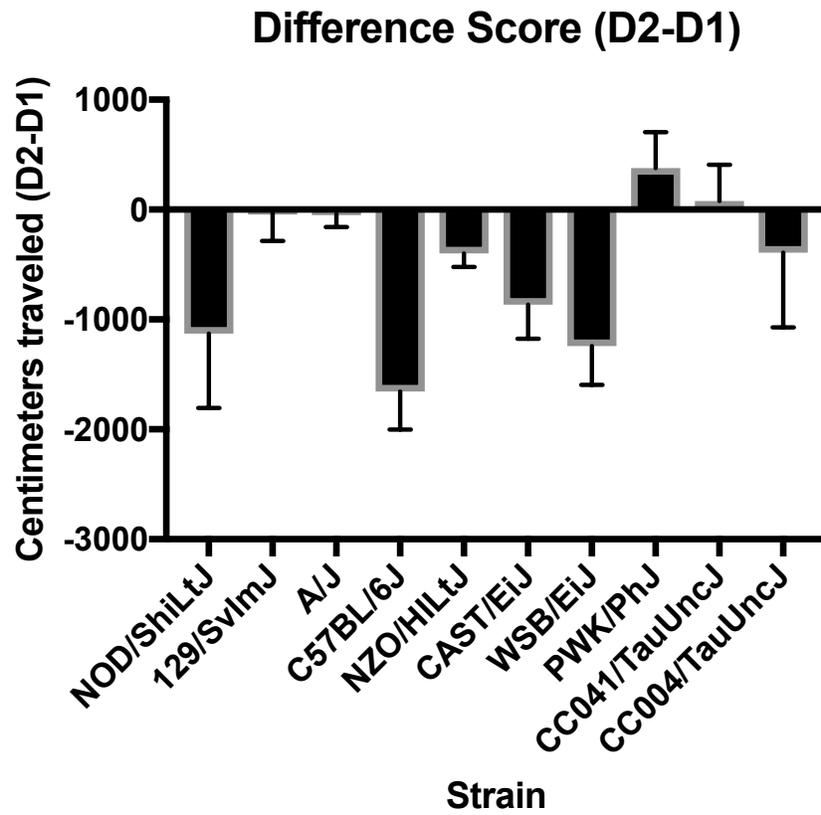
Difference Score (D2-D1)

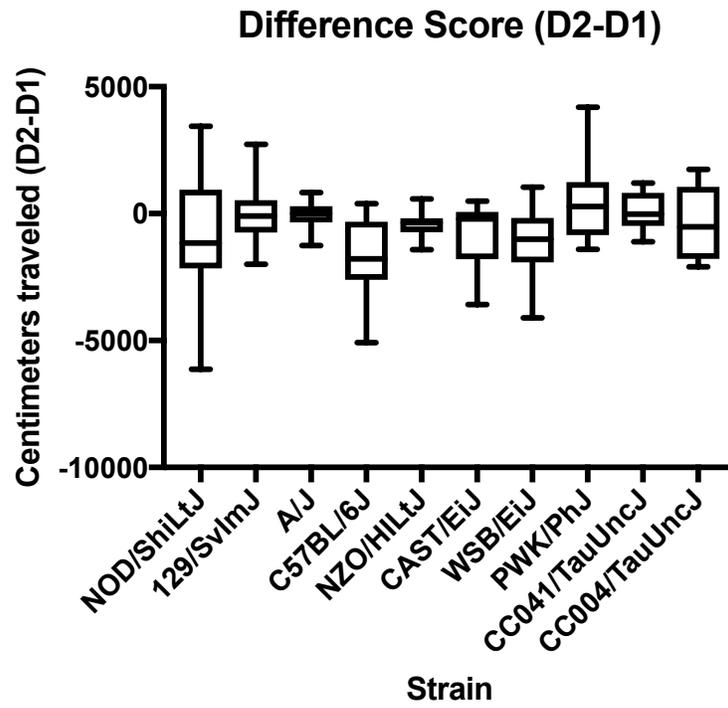*Figure 2.* The total centimeter difference between day 2 and day 1 in the open field chamber.

Figure 3. The total centimeter difference between day 2 and day 1 in the open field chamber displayed as a box plot.
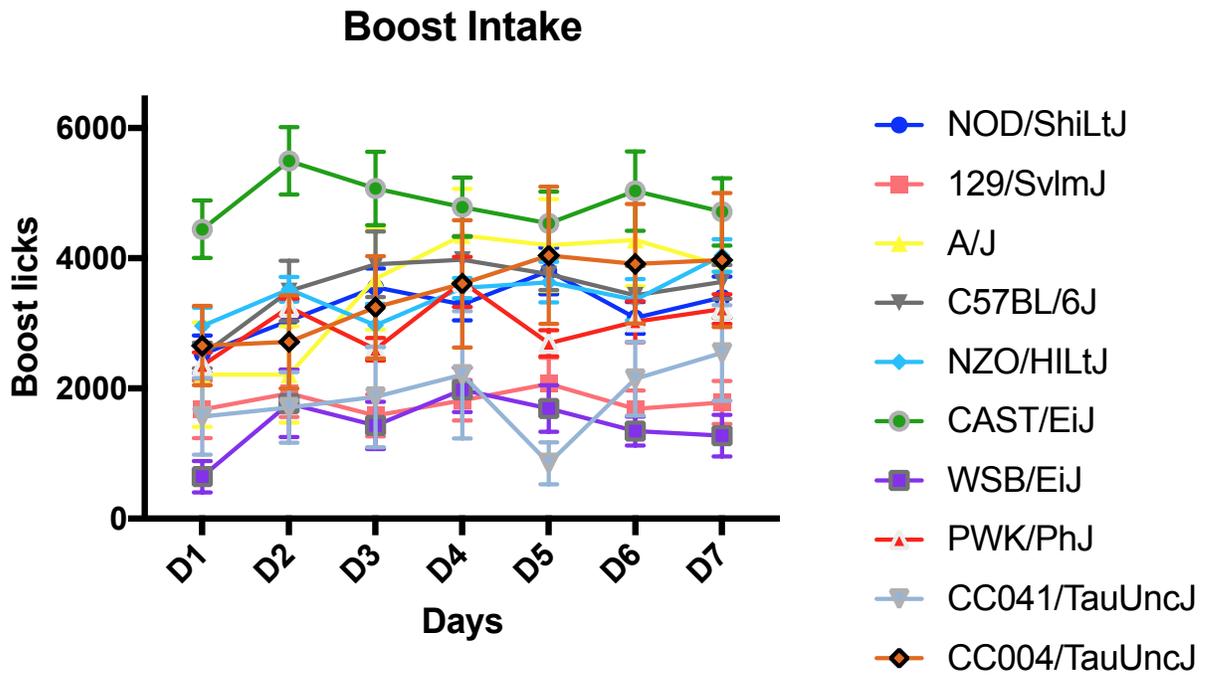
Figure 4. Average total number of Boost licks for each strain daily across seven
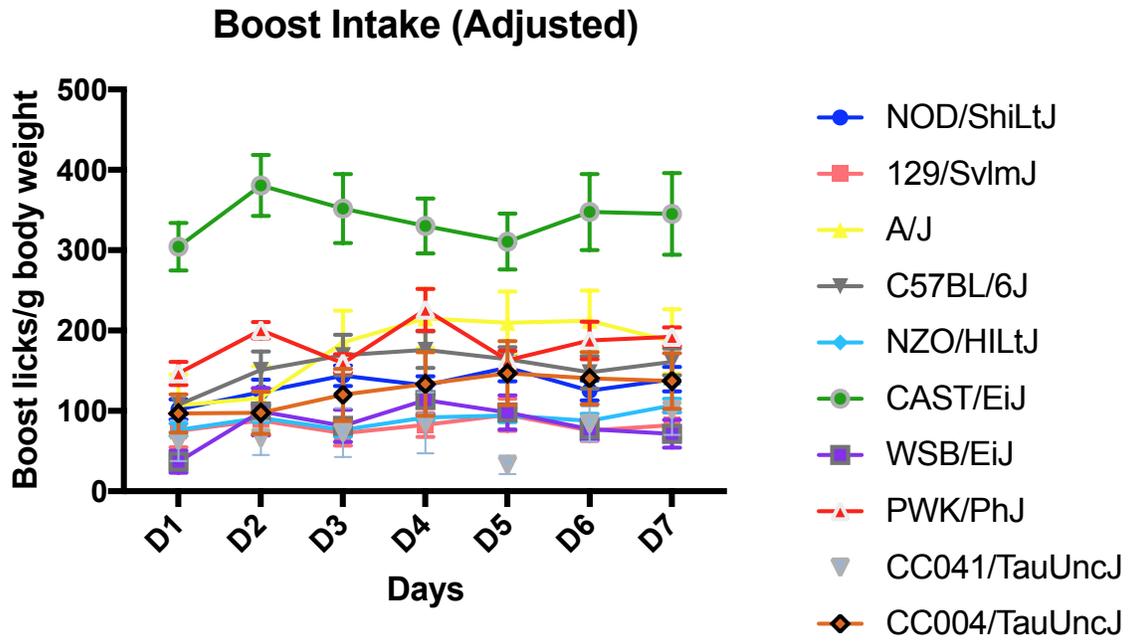consecutive days of testing.

Figure 5. Average total number of Boost licks adjusted for body weight (Licks/g body weight) for each strain daily across seven consecutive days of testing.
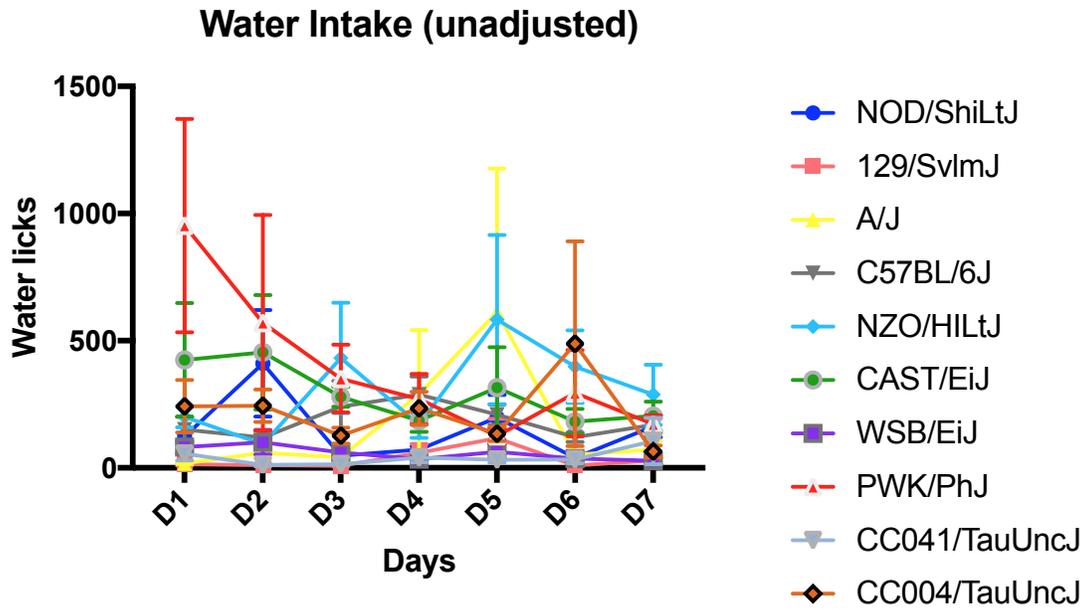
Figure 6. Average total number of water licks for each strain daily across seven consecutive days of testing.
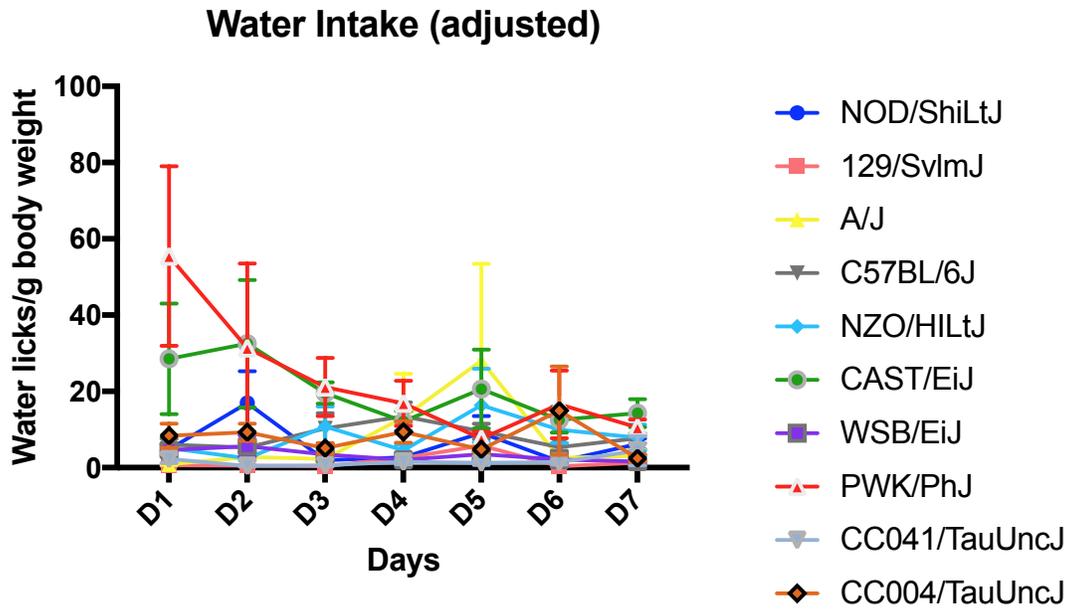
Figure 7. Average total number of water licks adjusted for body weight (Licks/g body weight) for each strain daily across seven consecutive days of testing.

Average Licks D5-7 (unadjusted)
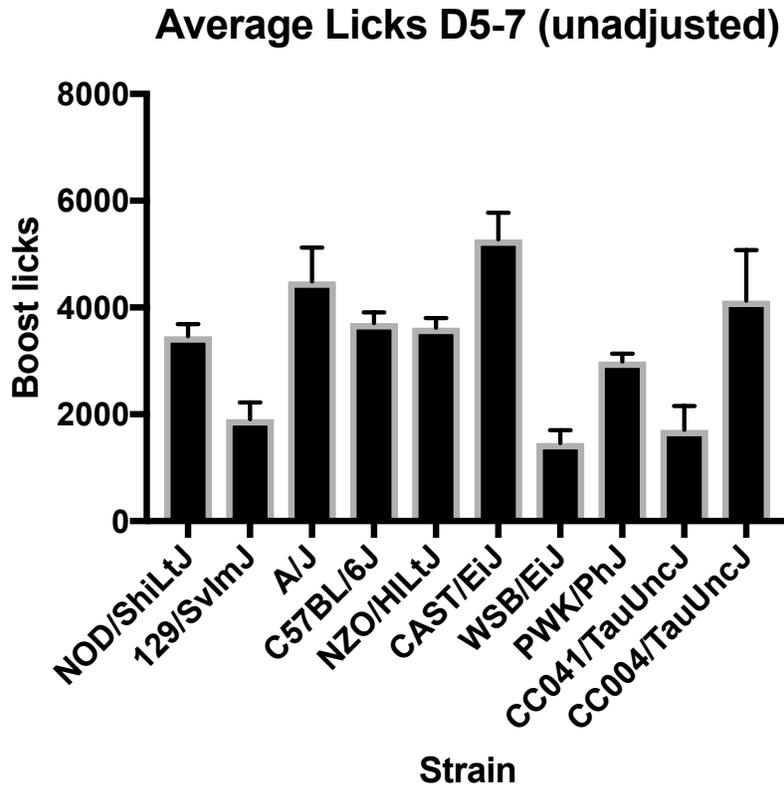
Figure 8. Average of the Boost licks on the last three days of testing (D5-D7) across strains.
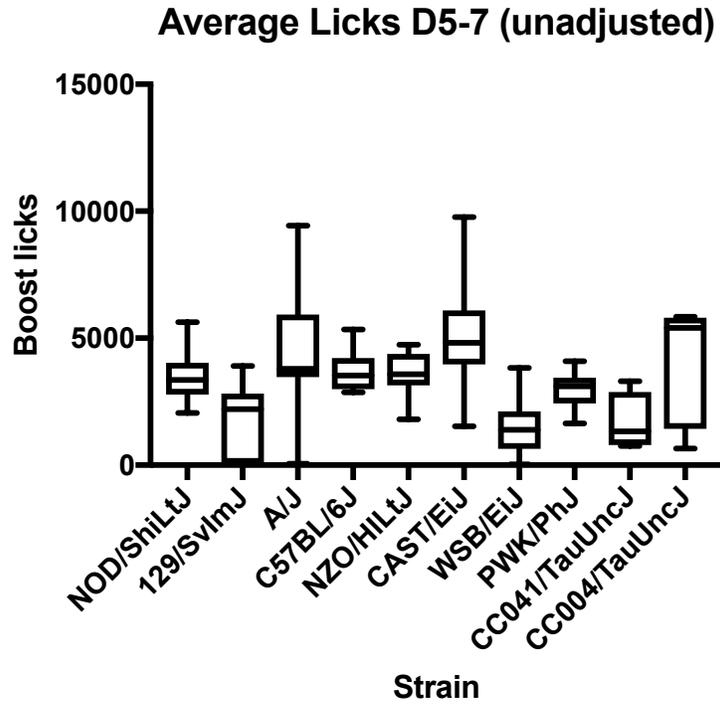
Figure 9. Average of the Boost licks on the last three days of testing (D5-D7) across strains displayed as a box plot.
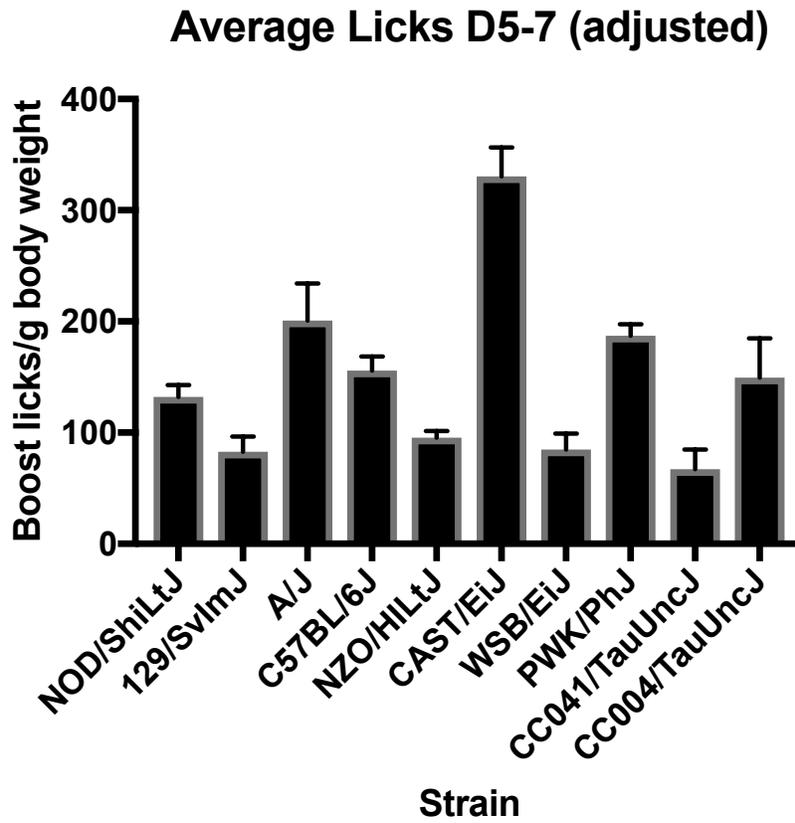
Figure 10. Average of the Boost licks on the last three days of testing (D5-D7) across strains, adjusted for body weight (licks/g body weight).

Average Licks D5-7 (adjusted)
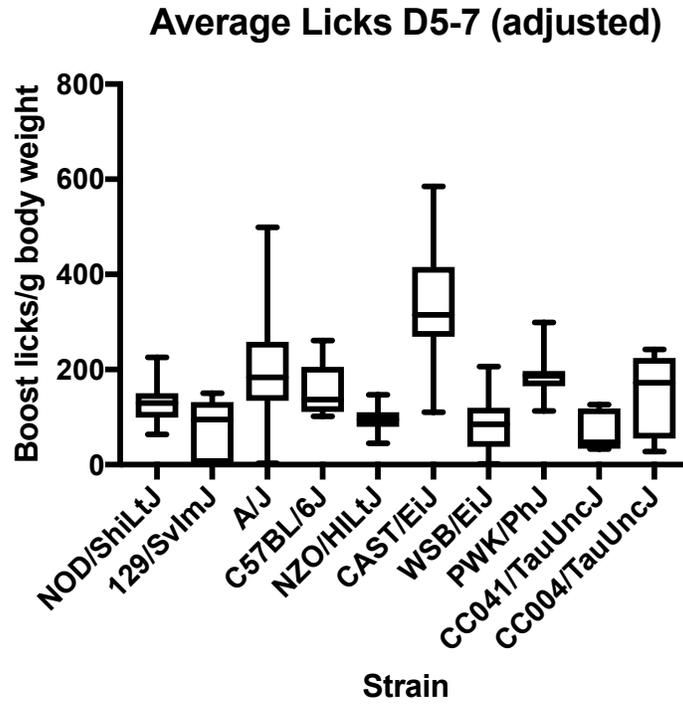
Figure 11. Average of the Boost licks on the last three days of testing (D5-D7) across strains, adjusted for body weight (licks/g body weight) displayed as a box plot.
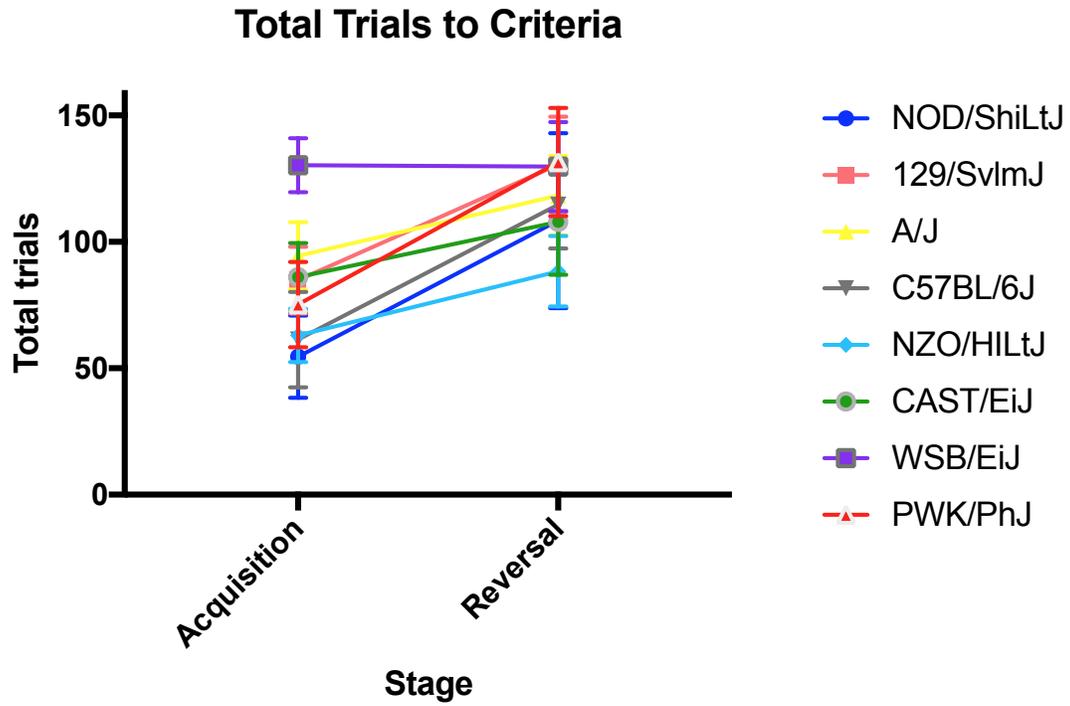
Average Water Licks D5-7 (unadjusted)
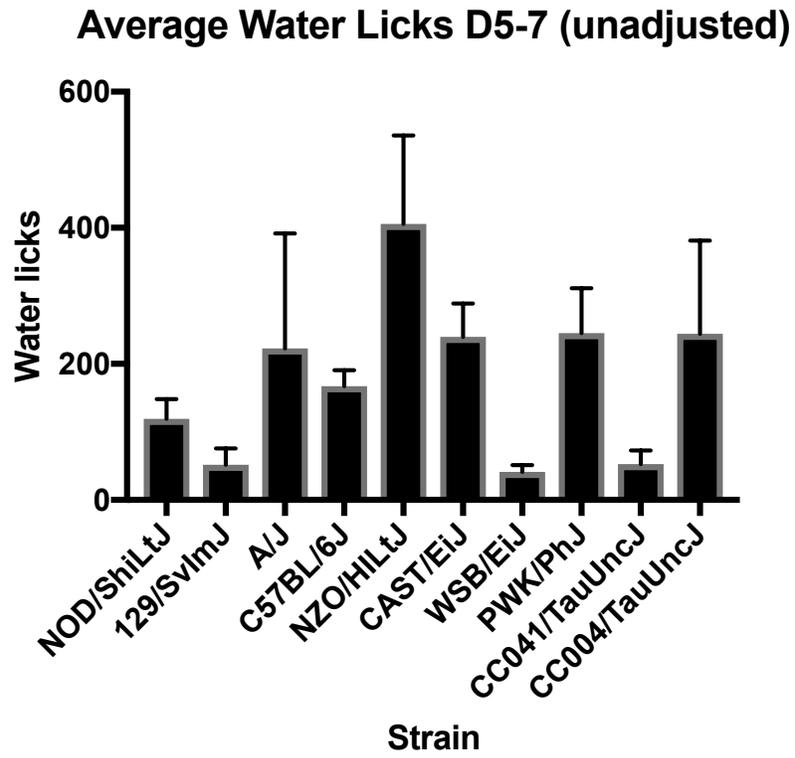
Figure 12. Average of the water licks on the last three days of testing (D5-D7) across strains.

Average Water Licks D5-7 (adjusted)
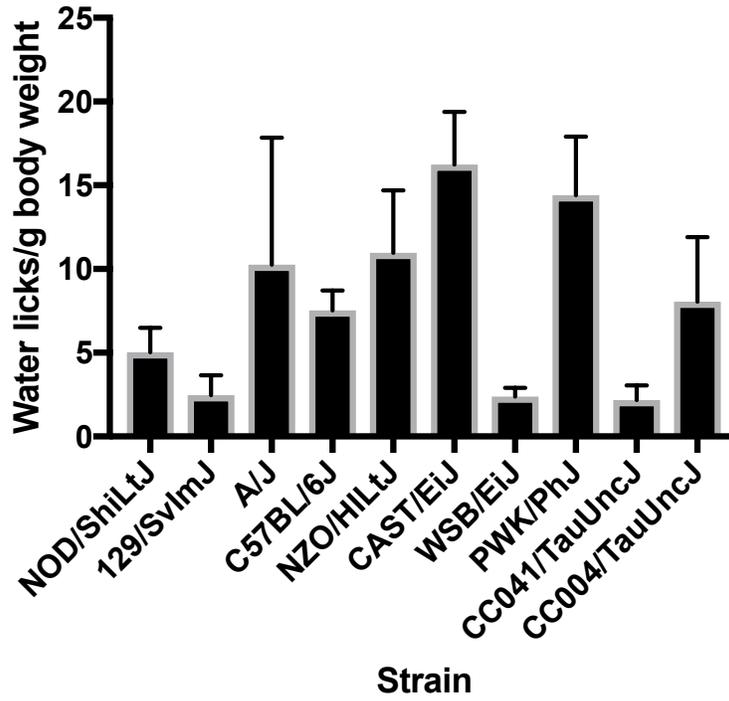
Figure 13. Average of the water licks on the last three days of testing (D5-D7) across strains, adjusted for body weight (licks/g body weight).

Figure 14. Average total trials in acquisition and reversal stages across strain. An increase from acquisition to reversal demonstrates cognitive inflexibility and impulsive action.
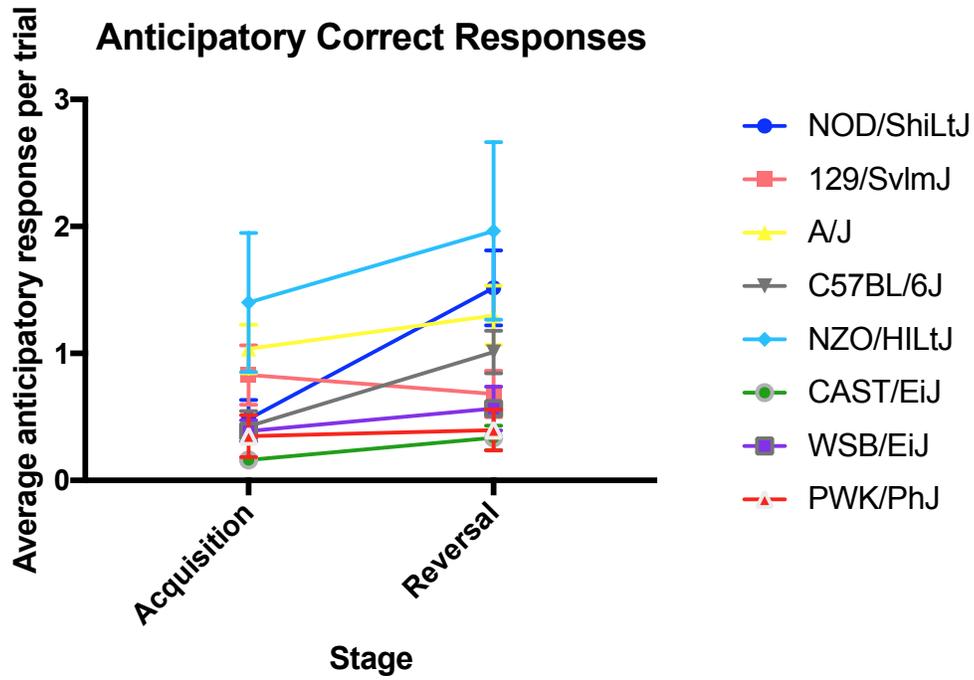
Figure 15. Average anticipatory correct response per trial (total correct anticipatory responses/total trials) in acquisition and reversal stages across strain.
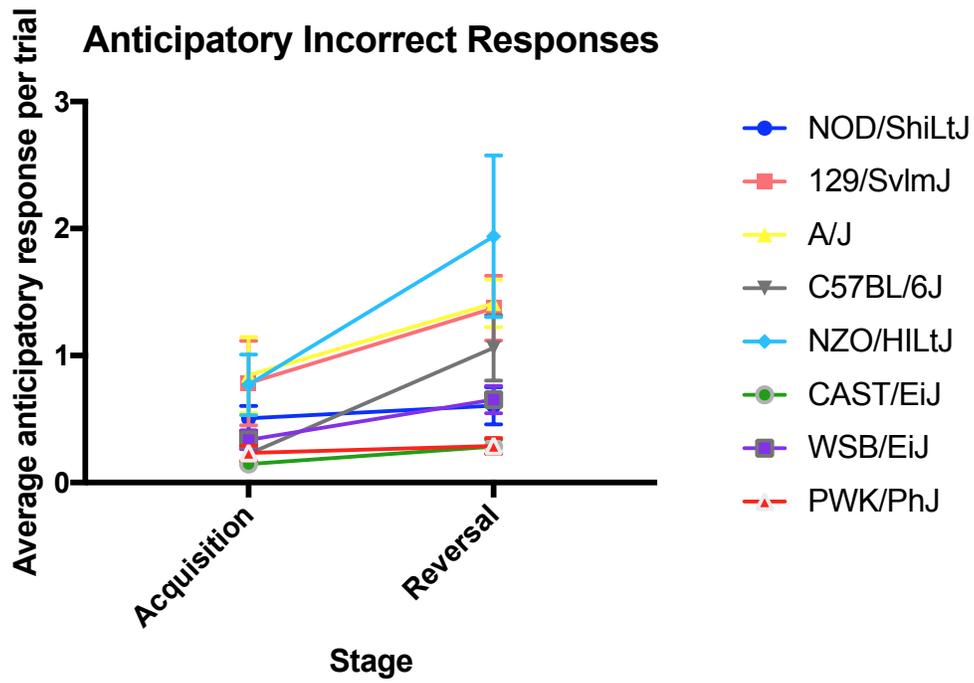
Figure 16. Average anticipatory incorrect response per trial (total correct anticipatory responses/total trials) in acquisition and reversal stages across strain.
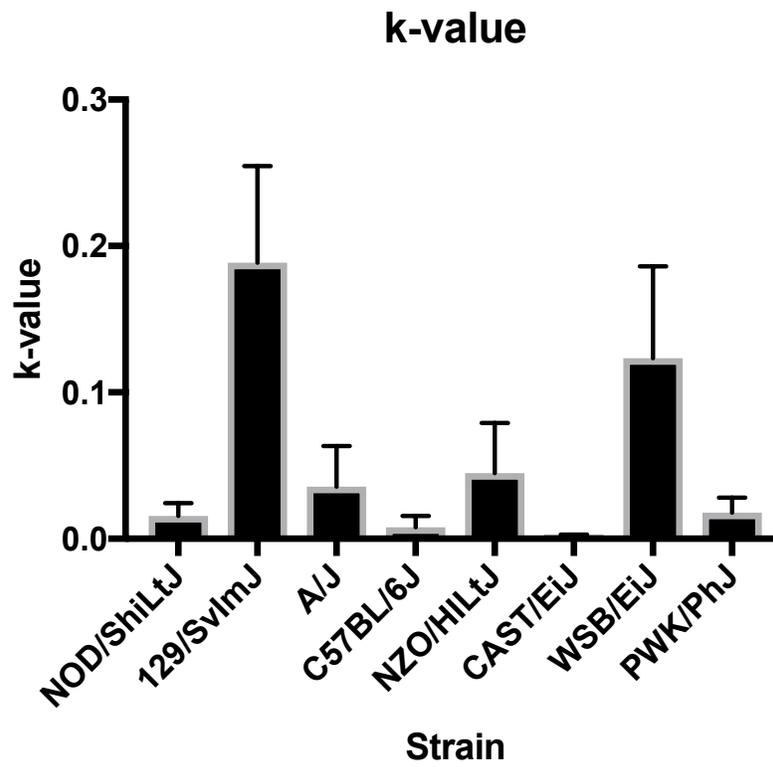
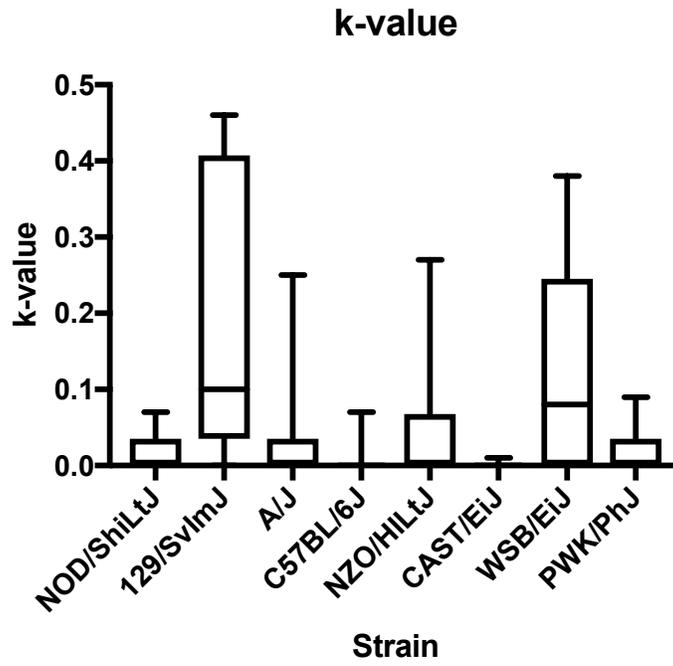*Figure 17.* Average k-value in delay discounting across strain.

Figure 18. Average k-value in delay discounting across strain displayed as a box plot.
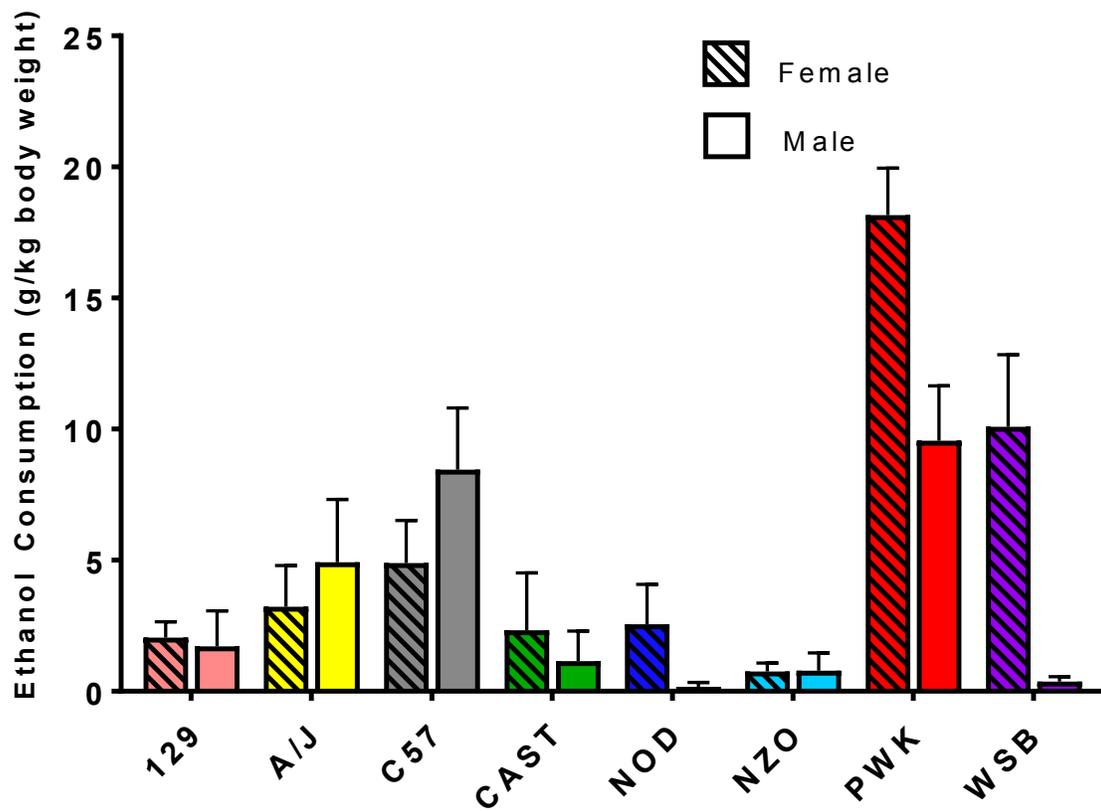
Figure 19. Average ethanol licks across the 3 days of testing, adjusted for body weight.
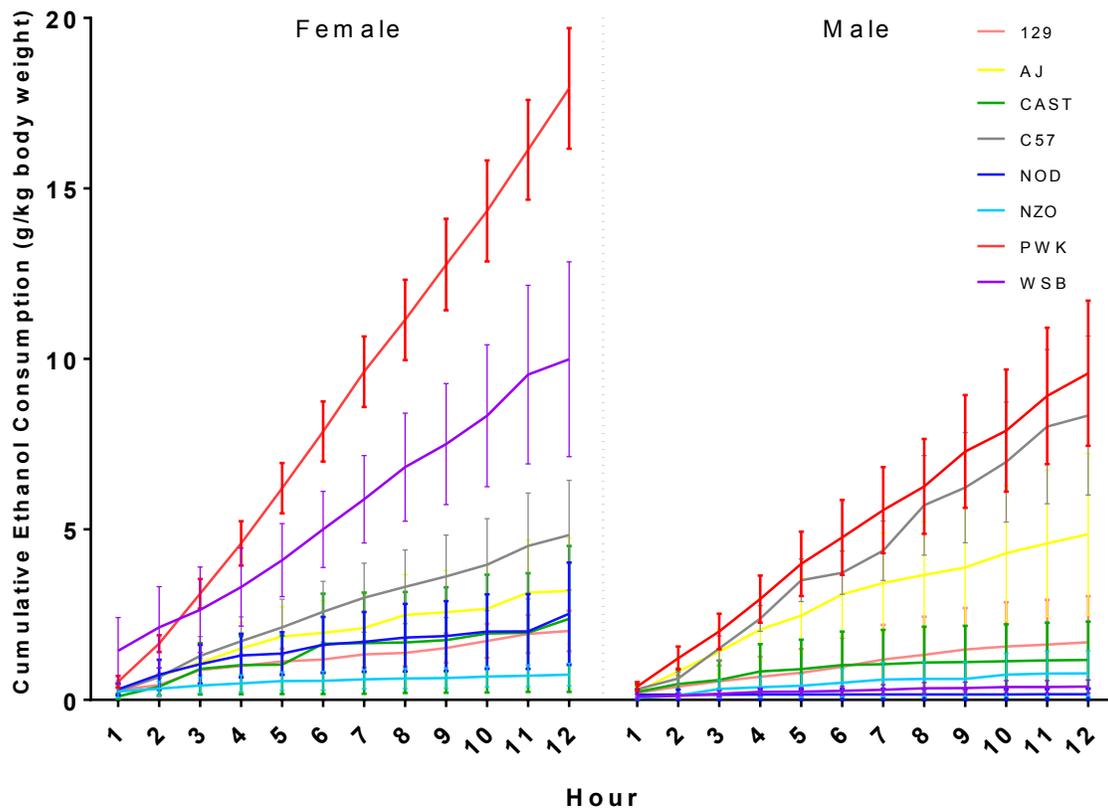
Figure 20. Averaged ethanol licks over 12 hours of testing, adjusted for body weight.
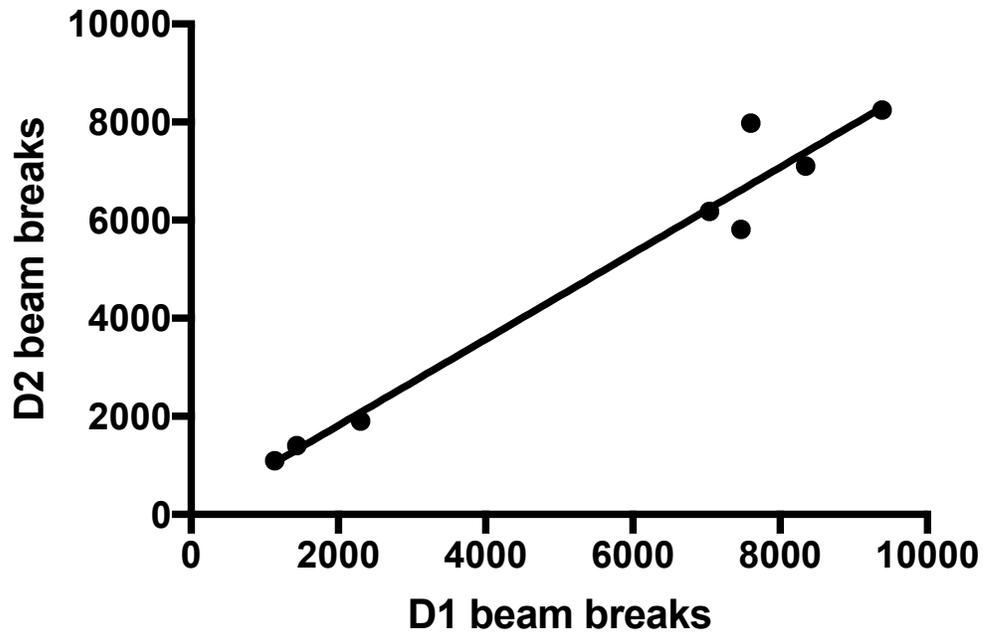
Figure 21. Positive correlation between ambulatory distance on D1 of locomotor testing and D2 of locomotor testing.

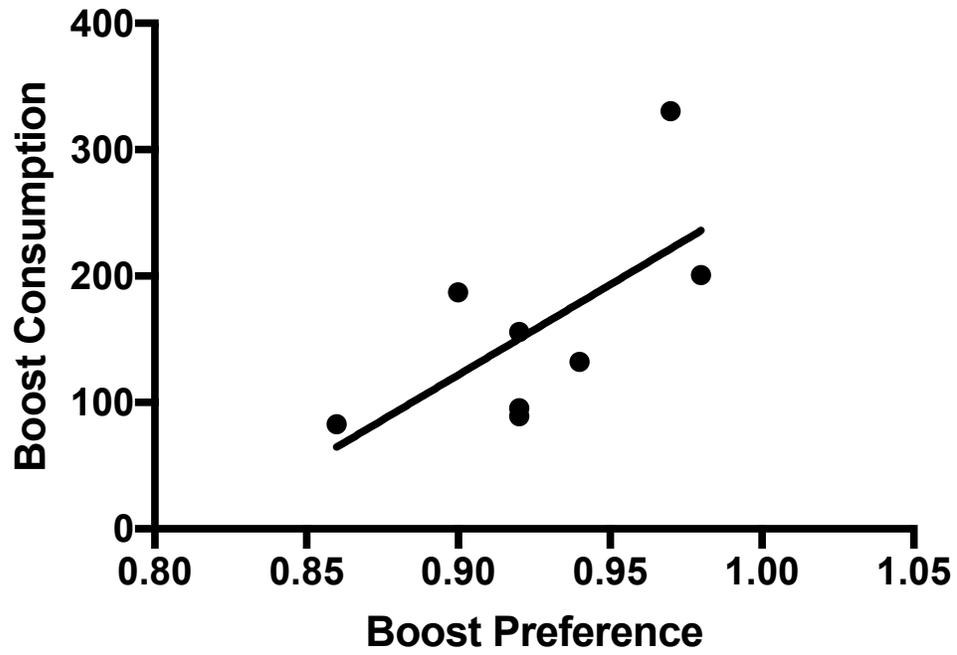*Figure 22.* Positing correlation between Boost licks adjusted for body weight averaged over the last three days of testing and Boost preference averaged over last three days of testing.
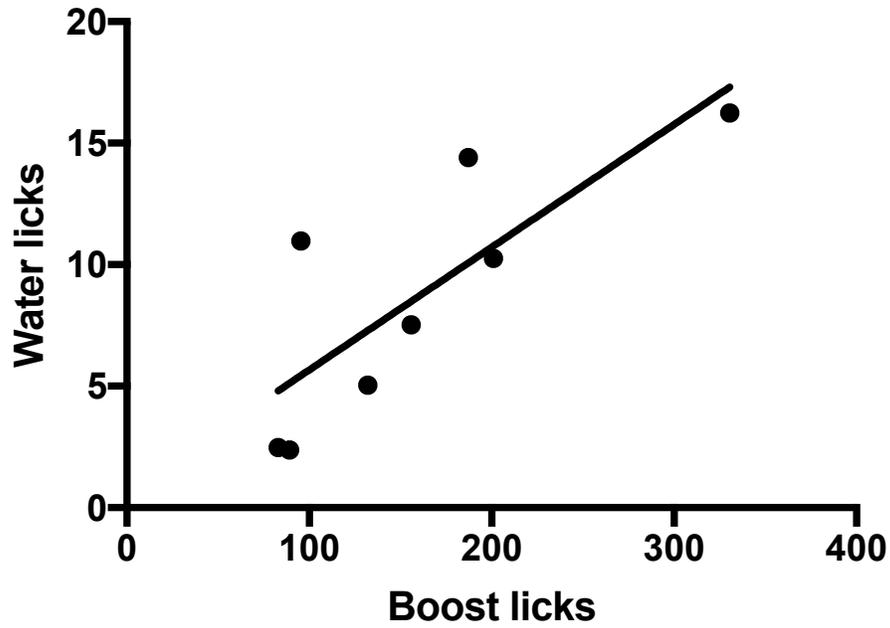
Figure 23. Positive correlation between Boost licks adjusted for body weight averaged over the last three days of testing and water licks adjusted for body weight averaged over the last three days of testing.

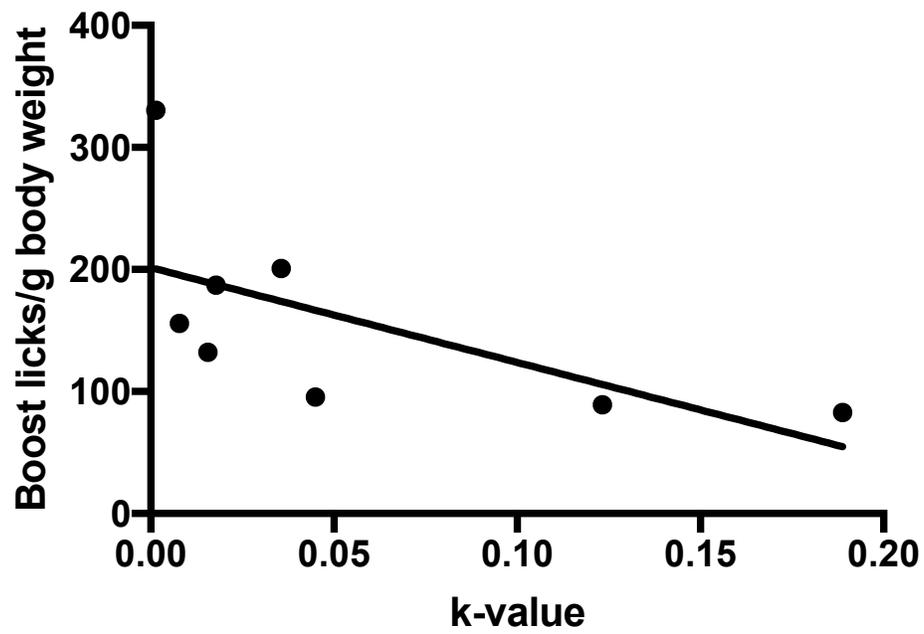*Figure 24.* Negative correlation between k-value and Boost licks adjusted by weight, averaged across the final three days of consumption.
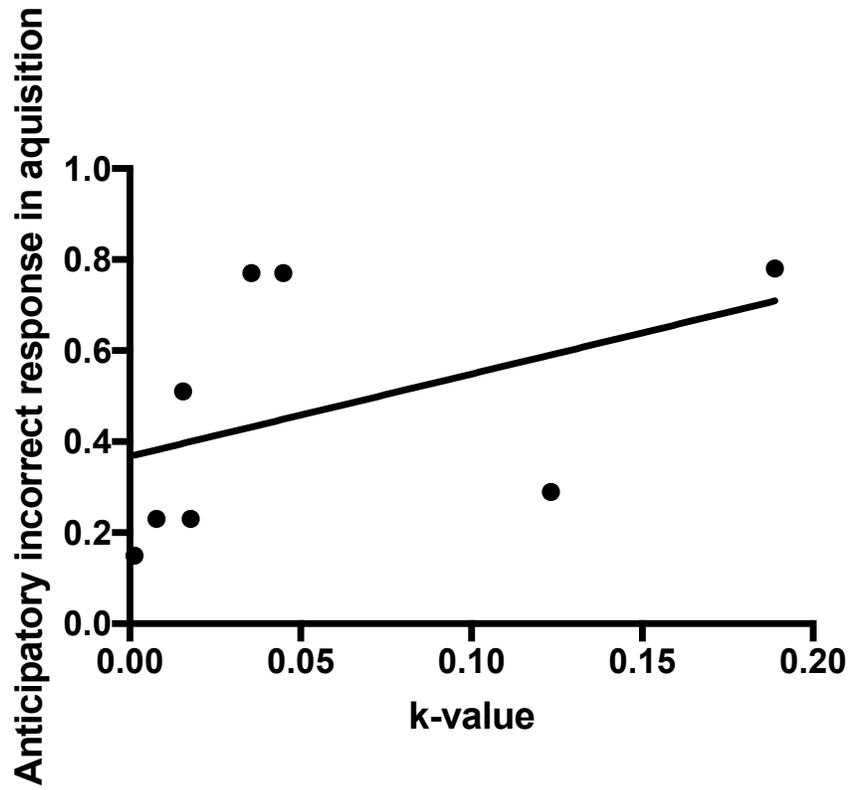
Figure 25. Positive correlation between k-value in delay discounting and average incorrect anticipatory responses in acquisition of reversal learning.
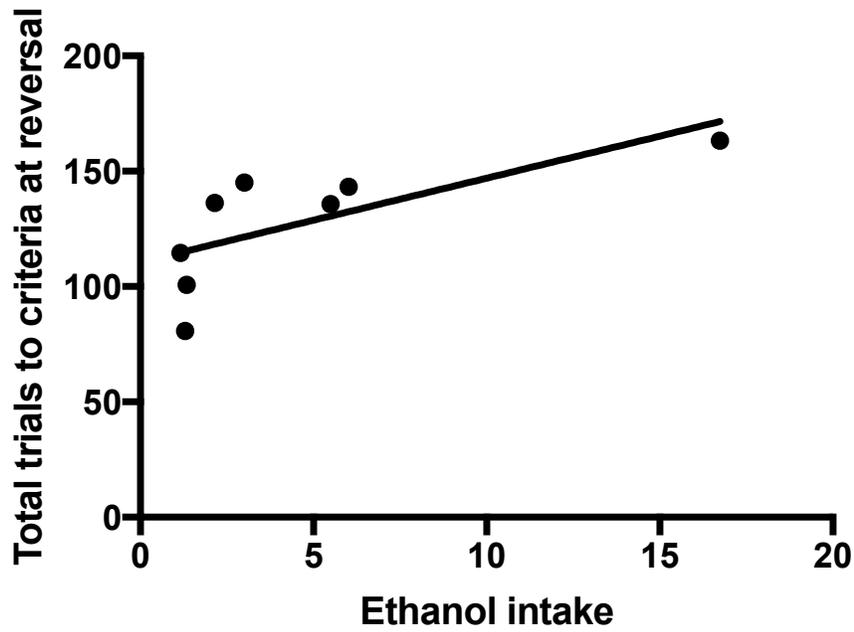
Figure 26. Positive correlation in female mice only of ethanol intake adjusted for body weight and trial to criteria in reversal learning.

References

Ainslie G. (1975). Specious reward: A behavioral theory of impulsiveness and impulse control. *Psychological Bulletin, 82*:463-496.

Alessi S.M., Petry N.M. (2003). Pathological gambling severity is associated with impulsivity in a delay discounting procedure. *Behav Processes, 64*(3):345-354.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.).* Washington, DC: Author.

Aylor D.L., Valdar W., Foulds-Mathes W., Buus R.J., Verdugo R.A., Baric R.S., Ferris M.T., Frelinger J.A., Heise M., Frieman M.B., Galinski L.E., Bell T.A., Didion J.D., Hua K., Nehrenberg D.L., Powell C.L., Steigerwalt J., Xie Y., Kelada S.N., Collins F.S., Yang I.V., Schwartz D.A., Branstetter L.A., Chesler E.J., Miller D.R., Spence J., Liu E.Y., McMillan L., Sarkar A., Wang J., Wang W., Zhang Q., Broman K.W., Korstanje R., Durrant C., Mott R., Iraqi F.A., Pomp D., Threadgill D., de Villena F.P., Churchill G.A. (2011). Genetic analysis of complex traits in the emerging Collaborative Cross. *Genome Res, 21*(8):1213-1222.

Bari A., Dalley J.W., Robbins T.W. (2008). The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nat Protoc., 3*(5):759-767.

Barratt E.S. (1959). Anxiety and impulsiveness related to psychomotor efficiency. *Perceptual and Motor Skills, 9*:191-198.

Belin D., Berson N., Balado E., Piazza P.V., Deroche-Gamonet V. (2011). High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology, 36*(3):569-79.

Belin D., Mar A.C., Dalley J.W., Robbins T.W., Everitt B.J. (2008). High impulsivity predicts the switch to compulsive cocaine-taking. *Science, 320*(5881):1352-1355.

Bickel W.K., Odum A.L., Madden G.J. (1999). Impulsivity and ciga-rette smoking: delay discounting in current, never, and ex-smokers. *Psychopharmacology (Berl) 146*:447–454.

Boulougouris V., Dalley J.W., Robbins T.W. (2007). Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat. *Behav Brain Res., 179*(2):219-228.

Bozarth M.A., Murray A., Wise R.A. (1989). Influence of housing conditions on the acquisition of intravenous heroin and cocaine self-administration in rats. *Pharmacol Biochem Behav, 33*(4):903-907.

Brevers D., Cleeremans A., Verbruggen F., Bechara A., Kornreich C., Verbanck P., Noël X. (2012). Impulsive Action but Not Impulsive Choice Determines Problem Gambling Severity. *PLoS One, 7*(11).

Broos N., Schmaal L., Wiskerke J. Kostelijk L., Lam T., Stoop N., Weierink L., Ham J., de Geus E.J.C., Schoffelmeer A.N.M., van den Brink W., Veltman D.J., de Vries T.J., Pattij T., Goudriaan A.E. (2012). The relationship between impulsive choice and impulsive action: A cross-species translational study. *PLoS ONE, 7*(5).

Brunner D., Hen R. (1997). Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. *Neurobiology of Suicide, 836*(1):81-105.

Calvert A.L., Green L., Myerson J. (2011). Discounting in pigeons when the choice is between two delayed rewards: implications for species comparisons. *Frontiers in Neuroscience, 5*(96).

Cervantes M.C., Laughlin R.E., Jentsch J.D. (2013). Cocaine self-administration behavior in inbred mouse lines segregating different capacities for inhibitory control. *Psychopharmacology (Berl), 229*(3):515-525.

Chesler E.J. (2014). Out of the bottleneck: the Diversity Outcross and Collaborative Cross mouse populations in behavioral genetics research. *Mamm Genome, 25*:3-11.

Chesler E.J., Miller D.R., Branstetter L.R., Galloway L.D., Jackson B.L., Philip V.M., Voy B.H., Culiat C.T., Threadgill D.W., Williams R.W., Churchill G.A., Johnson D.K., Manly K.F. (2008). The Collaborative Cross at Oak Ridge National Laboratory: developing a powerful resource for systems genetics. *Mamm Genome, 19*(6):382-389.

Chudasama Y., Robbins T.W. (2004). Dopaminergic modulation of visual attention and working memory in the rodent prefrontal cortex. *Neuropsychopharmacology, 29*(9):1628-1636.

Coffey S.F., Gudeklski G.D., Saladin M.E., Brady K.T. (2003). Impulsivity and rapid discounting of delayed hypothetical rewards in cocaine-dependent individuals. *Exp Clin Psychopharmacol, 11*:18–25.

Cools R., Frank M.J., Gibbs S.E., Miyakawa A., Jagust W., D'Esposito M. (2009). Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. *J Neuroscience, 29*(5):1538-1543.

Dalley J.W., Fryer T.D., Brichard L., Robinson E.S., Theobald D.E., Lääne K., Peña Y., Murphy E.R., Shah Y., Probst K., Abakumova I., Aigbirhio F.I., Richards H.K., Hong Y., Baron J.C., Everitt B.J., Robbins T.W. (2007). Nucleus accumbens D2/3

receptors predict trait impulsivity and cocaine reinforcement. *Science, 315*(5816):1267-1270.

Davis C., Fox J. (2008). Sensitivity to reward and body mass index (BMI): evidence for a non-linear relationship. *Appetite, 50*(1):43-49.

de Ruiter M.B., Veltman D.J., Goudriaan A.E., Oosterlaan J., Sjoerds Z., van den Brink W. (2009). Response perseveraton and ventral prefrontal sensitivity to reward and punishment in male problem gamblers and smokers. *Neuropsychopharmacology, 34*:1027-1038.

de Wit H. (2008). Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addiction Biology, 14*(1):22-31.

Diergaarde L., Pattij T., Poortvliet I., Hogenboom F., de Vries W., Schoffelmeer A.N., de Vries T.J. (2008). Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. *Biol Psychiatry, 63*(3):301-308.

Diergaarde L., van Mourik Y., Pattij T., Schoffelmeer A.N., De Vries T.J. (2012). Poor impulse control predicts inelastic demand for nicotine but not alcohol in rats.. *Addict Biol., 17*(3):576-587.

Dixon M.R., Marley J., Jacobs E.A. (2003). Delay discounting by pathological gamblers. *J Appl Behav Anal., 36*(4):449-458.

Doremus T.L., Brunell S.C., Rajendran P., Spear L.P. (2005). Factors influencing elevated ethanol consumption in adolescent relative to adult rats. *Alcohol Clin Exp Res., 29*:1796-1808.

Eagle D.M., Wong J.C.K., Allan M.E., Mar A.C., Theobald D.E., Robbins T.W. (2011). Contrasting roles for dopamine D1- and D2-receptor subtypes in the dorsomedial striatum but not the nucleus accumbens core during behavioral inhibition in the stop-signal task in rats. *J Neuroscience, 31*(2):7349-7356.

Elliott R., Dolan R.J., Frith C.D. (2000). Dissociable functions in the medial and lateral orbitofrontal cortex: evidence from human neuroimaging studies. *Cereb Cortex, 10*(3):308-317.

Evenden J.L. (1998). The pharmacology of impulsive behaviour in rats III: the effects of amphetamine, haloperidol, imipramine, chlordiazepoxide and ethanol on a paced fixed consecutive number schedule. *Psychopharmacology, 138*(3-4):295-304.

Evenden J.L. (1999). Varieties of impulsivity. *Psychopharmacology, 146*:348-361.

Evenden J.L., Ryan C.N. (1996). The pharmacology of impulsive behavior in rats: the effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology, 128*:161-170.

Field M., Santarcangelo M., Sumnall H., Goudie A., Cole J. (2006). Delay discounting and the behavioural economics of cigarette purchases in smokers: the effects of nicotine deprivation. *Psychopharmacology, 186*(2):255-263.

Franken I.H., van Strien J.W., Nijs I., Muris P. (2008). Impulsivity is associated with behavioral decision-making deficits. *Psychiatry Res., 158*(2):155-163.

Ghahremani D.G., Monterosso J., Jentsch J.D., Bilder R.M., Poldrack R.A. (2010). Neural components underlying behavioral flexibility in human reversal learning. *Cereb Cortex, 20*(8):1843-52.

Ghahremani D.G., Tabibnia G., Monterosso J., Hellemann G., Poldrack R.A., London E.D. (2011). Effect of Modafinil on learning and task-related brain activity in methamphetamine-dependent and healthy individuals. *Neuropsychopharmacology, 36*(5):950-959.

Giordano L.A., Bickel W.K., Loewenstein G., Jacobs E.A., Marsch L., Badger G.J. (2002). Mild opioid deprivation increases the degree that opioid-dependent outpatients discount delayed heroin and money. *Psychopharmacology, 163*:174-182.

Gvion Y., Apter A. (2011). Aggression, impulsivity, and suicide behavior: a review of the literature. *Arch Suicide Res.,15*(2):93-112.

Hnasko T.S., Sotak B.N., Palmiter R.D. (2005). Morphine reward in dopamine-deficient mice. *Nature, 438*(7069):854-857.

Huang Z.L., Qu W.M., Eguchi N., Chen J.F., Schwarzschild M.A., Fredholm B.B., Urade Y., Hayaishi O. (2005). Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. *Nat Neurosci, 8*(7):858-859.

Iraqi F.A., Churchill G., Mott R. (2008). The Collaborative Cross, developing a resource for mammalian systems genetics: a status report of the Wellcome Trust cohort. *Mamm Genome, 19*:379-381.

Izquierdo A., Jentsch J.D. (2012). Reversal learning as a measure of impulsive and compulsive behavior in addictions. *Psychopharmacology (Berl), 219*(2):607-620.

Jentsch J.D., Ashenhurst J.R., Cervantes M.C., James A.S., Groman S.M., Pennington Z.T. (2014). Dissecting impulsivity and its relationships to drug addictions. *Ann N Y Acad Sci., 1327*:1-26.

Jentsch J.D., Olausson P., De La Garza R., Taylor J.R. (2002). Impairments of reversal learning and response perseveration after repeated, intermittent cocaine administrations to monkeys. *Neuropsychopharmacology, 26*(2):183-190.

Kagan J. (1966). Reflection-impulsivity: The generality and dynamics of conceptual tempo. *Journal of Abnormal Psychology, 71*(1):17-24.

Kayir H., Uzbay I.T. (2004). Evidence for the role of nitric oxide in caffeine-induced locomotor activity in mice. *Psychopharmacology (Berl), 172*(1):11-15.

Kelley A.E., Schiltz C.A., Landry C.F. (2005). Neural systems recruited by drug- and food-related cues: studies of gene activation in corticolimbic regions. *Physiol Behav, 86*(1-2):11-14.

Kendler K.S., Prescott C.A., Myers J., Neale M.C. (2003). The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Arch Gen Psychiatry, 60*(9):929-37.

Kirby K.N., Petry N.M., Bickel W.K. (1999). Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls. *J Exp Psychol Gen, 128*:78–87.

Kleiner K.D., Gold M.S., Frost-Pineda K., Lenz-Brunsman B., Perri M.G., Jacobs W.S. (2004). Body mass index and alcohol use. *J Addict Dis, 23*(3):105-118.

Kosheleff A.R., Rodriguez D., O'Dell S.J., Marshall J.F., Izquierdo A. (2012). Comparison of single-dose and extended methamphetamine administration on reversal learning in rats. *Psychopharmacology (Berl), 224*(3):459-467.

Kreek M.J., Nielsen D.A., Butelman E.R., LaForge K.S. (2005). Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nat Neurosci, 8*(11):1450-1457.

Kringelbach M.L., Rolls E.T. (2004). The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Prog Neurobiol., 72*(5):341-372.

Krueger D.D., Howell J.L., Oo H., Olausson P., Taylor J.R., Nairn A.C. (2009). Prior chronic cocaine exposure in mice induces persistent alterations in cognitive function. *Behav Pharmacology, 20*(8):695-704.

Kuzmin A., Johansson B., Semenova S., FredholmB.B. (2000). Differences in the effect of chronic and acute caffeine on self-administration of cocaine in mice. *Eur J Neurosci, 12*(8):3026-3032.

Lesch K.P., Merschdorf U. (2000). Impulsivity, aggression, and serotonin: a molecular psychobiological perspective. *Behav Sci Law., 18*(5):581-604.

Logan R.W., Williams W.P. 3rd, McClung C.A. (2014). Circadian rhythms and addiction: mechanistic insights and future directions. *Behavioral Neuroscience, 128*(3):387-412.

Luijten M., Littel M., Frank I.H.A. (2011). Deficits in inhibitory control in smokers during a Go/NoGo task: An ivvestigation using event-related brain potentials. *PLoS ONE, 6*(4).

Manuck S.B., Flory J.D., Ferrell R.E., Mann J.J., Muldoon M.F. (2000). A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity. *Psychiatry Res., 95*(1):9-23.

Mar A.C., Walker A.L.J., Theobald D.E., Eagle D.M., Robbins T.W. (2011). Dissociable effects of lesions to orbitofrontal cortex subregions on impulsive choice in the rat. *J Neuroscience, 31*(17):6398-6404.

Martinez D., Gil R., Slifstein M., Hwang D., Huang Y., Perez A., Kegeles L., Talbot P., Evans S., Krystal J., Maruelle M., Abi-Dargham A. (2005). Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. *Biol Psychiatry, 58*:779-786.

Moeller F.G., Barratt E.S., Bougherty D.M., Schmitz J.M., Swann A.C. (2001). Psychiatric aspects of impulsivity. *Am J Psychiatry, 158*(11):1783-1793.

Molander A.C., Mar A., Norbury A., Steventon S., Moreno M., Caprioli D., Theobald D.E., Belin D., Everit B.J., Robbins T.W., Dalley J.W. (2011). High impulsivity predicting vulnerability to cocaine addiction in rats: some relationship with novelty preference but not novelty reactivity, anxiety or stress. *Psychopharmacology (Berl), 215*(4):721-731.

Moreno-López L., Perales J.C., van Son D., Albrein-Urios N., Soriano-Mas C., Martinez-Gonzalez J.M., Wiers R.W., Verdejo-García A. (2014). Cocaine use severity and cerebellar gray matter are associated with reversal learning deficits in cocaine-dependent individuals. *Addiction Biology, 20*(3):546-556.

Nadal R., Armario A., Janak P. (2002). Positive relationship between activity in a novel environment and operant ethanol self-administration in rats. *Psychopharmacology, 162*(3):333-338.

Noonan M.P., Chau B., Rushworth M.F.S., Fellows L.K. (2017). Contrasting effects of medial and lateral orbitofrontal cortex lesions on credit assignment and decision making in humans. *J Neuroscience, 37*(29):7023-7035.

Odet F., Pan W., Bell T.A., Goodson S.G., Stevans A.M., Yun Z., Aylor D.L., Kao C., McMillan L., Pardo-Manuel de Villena F., O'Brien D.A. (2015). The founder strains of the Collaborative Cross express a complex combination of advantageous and deleterious traits for male reproduction. *Genes, Genomes, and Genetics, 5*(12):2671-2683.

Odum A.L. (2013). Delay discounting: I'm a k, you're a k. *Journal of the Experimental Analysis of Behavior, 96*(3):427:439

Patkina N.A., Zvartau E.E. (1998). Caffeine place conditioning in rats: comparison with cocaine and ethanol. *Eur Neuropsychopharmacol, 8*(4):287-291.

Perry J.L., Nelson S.E., Carroll M.E. (2008). Impulsive choice as a predictor of acquisition of IV cocaine self- administration and reinstatement of cocaine-seeking behavior in male and female rats. *Exp Clin Psychopharmacol., 16*(2):165-177.

Philip V.M., Duvvuru S., Gomero B., Ansah T.A., Blaha C.D., Cook M.N., Hamre K.M., Lariviere W.R., Matthews D.B., Mittleman G., Goldowitz D., Chesler E.J. (2009). High-throughput behavioral phenotyping in the expanded panel of BXD recombinant inbred strains. *Genes, Brain, and Behavior, 9*(2):129-159

Piazza P.V., Deminière J.M., le Moal M., Simon H. (1989). Factors that predict individual vulnerability to amphetamine self-administration. *Science, 245*(4925):1511-1513

Piazza P.V., Deroche-Gamonet V. (2013). A multistep general theory of transition to addiction. *Psychopharmacology (Berl), 229*(3):387-413.

Rempel-Clower N.L. (2007). Role of orbitofrontal cortex connections in emotion. *Ann N Y Acad Sci., 1121*:72-86.

Reynolds B. (2006). A review of delay-discounting research with humans: relations to drug use and gambling. *Behavioral Pharmacology, 17*(8):651-667.

Robinson T.E., Berridge K.C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev., 18*(3):247-91.

Rosenwasser A.M. (2010). Circadian clock genes: Non-circadian roles in sleep, addiction, and psychiatric disorders? *Neuroscience & Biobehavioral Reviews, 34*(8):1249-1255.

Spanagel R., Montkowski A., Allingham K., Stöhr T., Shoaib M., Holsboer F., Landgraf R. (1995). Anxiety: a potential predictor of vulnerability to the initiation of ethanol self-administration in rats. *Psychopharmacology (Berl), 122*(4):369-373.

Stanford M.S., Mathias C.W., Dougherty D.M., Lake S.L., Anderson N.E., Patton J.H. (2009). Fifty years of the Barratt Impulsiveness Scale: An update and review. *Personality and Individual Differences, 47*(5):385-395.

Svenson K.L., Gatti D.M., Valdar W., Welsh C.E., Cheng R., Chesler E.J., Palmer A.A., McMillan L., Churchill G.A. (2012). High-resolution genetic mapping using the Mouse Diversity Outbred population. *Genetics, 190*(2):437-447.

Uslaner J.M., Robinson T.E. (2006). Subthalamic nucleus lesions increase impulsive action and decrease impulsive choice - mediation by enhanced incentive motivation? *Eur J Neurosci., 24*(8):2345-2354.

Volkow N.D., Chang L., Wang G.J., Fowler J.S., Ding Y.S., Sedler M., Logan J., Franceschi D., Gatley J., Hitzemann R., Gifford A., Wong C., Pappas N. (2001). Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *Am J Psychiatry, 158*(12):2015-2021.

Volkow N.D., Wang G.J., Telang F., Fowler J.S., Logan J., Jayne M., Ma Y., Pradhan K., Wong C. (2007). Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. *J Neurosci, 27*(46):12700-6.

Voon V., Irvine M.A., Derbyshire K., Worbe Y., Lange I., Abbott S., Morein-Zamir S., Dudley R., Caprioli D., Harrison N.A., Wood J., Dalley J.W., Bullmore E.T., Grant J.E., Robbins T.W. (2014). Measuring "waiting" impulsivity in substance addictions and binge eating disorder in a novel analogue of rodent serial reaction time task. *Biol Psychiatry, 75*(2):148-155.

Warren M., Frost-Pineda K., Gold M. (2005). Body mass index and marijuana use. *J Addict Dis, 24*(3):95-100.

Weller R.E., Cook E.W. 3rd, Avsar K.B., Cox J.E. (2008). Obese women show greater delay discounting than healthy-weight women. *Appetite, 51*(3):563-569.

Williams R.W., Bennett B., Lu L., Gu J., DeFries J.C., Carosone-Link P.H., Rikke B.A., Belknap J.K., Johnson T.E. (2004). Genetic structure of the LXS panel of recombinant inbred mouse strains: a powerful resource for complex trait analysis. *Mammalian Genome, 15*(8):637-647.

Winstanley C.A., Dalley J.W., Theobald D.E., Robbins T.W. (2004). Fractionating impulsivity: contrasting effects of central 5-HT depletion on different measures of impulsive behavior. *Neuropsychopharmacology, 29*(7):1331-1343.

Wise R.A., t M.A. (1987). A psychomotor stimulant theory of addiction. *Psychol Rev, 94*(4):469-492.

Yates J.R., Darna M., Beckmann J.S., Dwoskin L.P., Bardo M.T. (2016). Individual differences in impulsive action and dopamine transporter function in rat orbitofrontal cortex. *Neuroscience, 313*:122-129.