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Variation in Sign-Tracking and Goal-Tracking Behaviors in a Genetically Diverse Inbred Panel of Mice

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Examination of Genetic and Dopaminergic Differences in Sign-Tracking and Goal-Tracking Behavior in an Inbred Panel of Mice

Motivation is a critical aspect of life in all organisms, and is heavily implicated in survival behaviors that are goal-directed or threat-avoidant. In the mammalian brain, motivation is structurally and functionally linked to processes such as reward, drive, pleasure, and learning (Kringelbach & Berridge, 2016). While motivation has several components, incentive motivation focuses on the specific drive of goal-directed behaviors along with the level of persistence in reaching a goal or reward. Incentive motivation is highly associated with addictive and affective disorders involving maladaptive motivational impairment, dysfunction, or intensification such as Substance Use Disorder (SUD) and depression.

Individual differences in incentive motivation that may contribute to vulnerability to these conditions can be revealed in sign-tracking versus goal-tracking, which involves Pavlovian conditioned associations. Sign-tracking involves attraction to and interaction with reward-predicting cues, whereas goal-tracking involves cue-induced attraction to the site of reward delivery (Fraser & Janak, 2017). Both sign-trackers and goal-trackers respond to a learned CS-US association. However, sign-trackers designate motivational value to the CS, the cue, as opposed to the US, the reward delivery (Khoo et al., 2021). It has been found that sign-trackers may be more vulnerable to drug use, Substance Use Disorders (SUDs), overeating or binge eating, and relapse due to a difficulty in restraint (Holden, 2023). This could be due, in part, to the strength of the Pavlovian association between cues and appetitive rewards (Kelley & Berridge, 2002; Holden, 2023). As relapse is commonly instigated through associated cue exposure, sign-tracking may be a substantial contributor not only to relapse but to other addictive behaviors as well (Holden, 2023). Individual, genetic, and environmental differences contribute

to the occurrence of these behaviors and may explain the occurrence of sign versus goal-tracking. Both neurotransmitter release levels and neuronal activation vary in sign-trackers and goal-trackers, indicating heritable and environmental factors play a role in the emergence of these behaviors (Stringfield et al., 2019). If these individual differences manifest in psychopathology, understanding what gives rise to these differences in incentive motivation is a significant question and a critical step in the development of therapeutics for addictive disorders.

Disorders related to incentive motivation have been shown to be produced by dopaminergic regions including the striatum and midbrain. Studies have found that obese mice had significantly decreased striatal Dopamine D2 receptor (D2R) binding and that removing striatal D2Rs decreased physical activity in lean mice, supporting the idea that insufficient dopamine transmission in areas related to incentive motivation gives rise to motivational deficits seen in obesity (Friend et al., 2017). Additionally, it has been shown that engagement in motivated behavior was significantly attenuated given striatal dopamine depletions, suggesting a relationship between striatal dopamine and motivated behavior (Palmiter, 2008). Previous research such as this has illustrated that incentive motivation is necessary for healthy behaviors, but imbalance of these mechanisms may lead to inflexible patterns and behaviors, eventually allowing psychiatric disorders or potentially addictive drugs to hijack motivation networks (Sesack & Grace, 2010). Further research on the microcircuitry and dopaminergic function of areas involved in motivation such as the basal ganglia and midbrain may yield more effective therapeutics for these debilitating conditions.

Dopamine transmission between midbrain and striatal regions has been shown to have a major role in regulating motivated behaviors, specifically incentive motivation. Ongoing studies in this laboratory have shown that individual genetic differences contribute to the excitability of

dopamine systems, therefore implicating these individual differences in the occurrence of individual variations in motivated behavior. Phasic dopamine release in these structures allows for fluctuations in motivated behavior as activity increases and decreases in these areas, as there is a positive correlation between motivated behavior and phasic dopamine release. These phasic oscillations are due to reward achievement or omission (Schultz, Carelli, & Wightman, 2015). More specifically, research has connected the mesolimbic dopamine system, formed by the Ventral Tegmental Area (VTA) and Nucleus Accumbens (NAc), to motivation, addictive behaviors, reinforcement, and reward. NAc dopamine depletion through neurotoxic injections supports prior research on NAc dopamine function and motivated behaviors in that depletions affect the relationship between incentive value and behavioral output (Aberman & Salamone, 1999). Past studies have established the NAc core to be significant in incentive motivation of natural rewards such as food rewards, generating questions regarding the role of the NAc core specifically in sign versus goal-tracking.

Sign and goal-tracking behavior are associated with similar circuitry and neurotransmitter systems as incentive motivation. The neurobiology involved in sign-tracking behavior has been established as nearly identical to that of generalized motivational behavior, and is therefore highly dependent on dopamine levels (Fraser & Janak, 2017). Dopamine has been shown to be vital for the development and maintenance of sign-tracking responses, while it is not necessary for the development of goal-tracking responses (Flagel et al., 2011). Sign-tracking has additionally been shown to be specifically contingent on dopaminergic NAc core activity. Use of dopamine antagonists within the NAc core attenuated sign-tracking behavior, demonstrating a reliance of sign-tracking behavior on higher NAc core dopamine release (Fraser & Janak, 2017). Therefore, a decrease in NAc core dopamine subsequently decreases sign-tracking behavior.

Although both sign-tracking and goal-tracking are modulated by dopamine, sign-tracking has been shown to be more vulnerable to interference due to manipulation of dopamine than goal-tracking, suggesting a mechanistic difference between the two behaviors (Roughley & Killcross, 2019). While sign-tracking has been found to be driven by NAc core dopamine, goal-tracking has been more so attributed to frontal cortical mechanisms that may project to or from the NAc (Holden, 2023). The Paraventricular Nucleus of the Thalamus (PVT) has also been shown to regulate sign-tracking and goal-tracking behavior, as it has glutamatergic projections to the NAc that modulate dopamine release (Iglesias & Flagel, 2021). Neurobiological distinctions between sign-tracking and goal-tracking behavior also extend to sex differences, genetic differences, and individual differences.

Sex differences in the neural mechanisms of motivation have been studied generally, but considerably less so in reference to the NAc core. Sex differences in motivation have been known to induce differences in endogenous motivation, saliency, and incentive value of food and potentially addictive drugs between sexes in both rodents and humans (Song, Kalyani, & Becker, 2018). Gray matter volume differences between males and females in the NAc correlate positively with obesity and food-related motivation (Horstmann et al., 2011). In terms of addictive drugs, female rodents experience higher motivation for and escalation of drug taking than males. The same has been found in humans, along with greater withdrawal, cue-induced craving, and higher relapse levels in women than in men (Becker, 2016). Becker (2016) found a pattern of attenuated dopamine release in the NAc core in female rodents under habitual drug taking, plausibly leading to increased craving and drug-taking behavior as a result of reward prediction error. Males did not experience this attenuated dopamine response. This underlying sex difference in the NAc core is postulated to be a source of motivational sex differences in

addiction. However, in contrast to drug research, it has been found that male rodents have higher performance levels on motivation tasks involving food reward than females (Seu et al., 2014). The four core genotypes mouse model, which allows for the separation of sex chromosome complement from gonadal sex, demonstrated that the XY complement results in higher motivation behavior for food reward (regardless of gonadal sex) than the XX complement (Seu et al., 2014). Mice with an XY complement produced double the amount of lever presses than mice with an XX complement (Seu et al., 2014). This disparity in results suggests that further research in areas contributing to incentive motivation may be crucial in determining the neurobiological underpinnings of these sex differences.

Little research has looked at sex differences in sign-tracking and goal-tracking behavior, as the majority of these studies previously included only male subjects. With limited findings in rodent studies including both male and female subjects, it has been found that prior to any drug-taking, females exhibit higher levels of sign-tracking behavior than males (Stringfield et al., 2019). Additionally, females acquire sign-tracking more quickly than males. However, females also show increased goal-tracking compared to males. This suggests that the acquisition of motivational behavior, regardless of neurocircuitry and behavioral implications, occurs more quickly in females than males (Stringfield et al., 2019). In another study, females had both faster acquisition and poorer extinction of a Pavlovian approach task. Females were also found to be less sensitive to reward devaluation than males (Hammerslag & Gulley, 2013). It was concluded that females developed and maintained sign-tracking behavior more quickly and for longer than males. Current research suggests that there are sexual dimorphisms underlying sex differences in sign and goal-tracking behavior that may subsequently explain sexual dimorphisms in addictive, overeating, and binge eating behaviors. However, little investigation has focused on the circuitry

and neurotransmitter systems underlying these sex differences, particularly whether dopamine release in the NAc core could be responsible.

While there is a large body of research on the role of striatal dopamine systems in incentive motivation, there is much less research investigating the role of NAc core dopamine in sign and goal-tracking behaviors (Tomie et al., 2008). Consequently, sex differences in motivation have been studied, but there is a lack of research on sex differences, individual differences, and genetic heritability concerning sign and goal-tracking behaviors. Investigating sex differences strengthens the level of specificity and novelty of the current study. This study seeks to explore genetic, dopaminergic, and sex differences in sign-tracking and goal-tracking behavior, through Pavlovian conditioning tasks and Fast-Scan Cyclic Voltammetry (FSCV). FSCV has been used for decades in research to measure changes in neurotransmitter levels in the brain (Venton & Cao, 2020). Studies have utilized FSCV to monitor or measure dopamine levels in the NAc core using food rewards and found that motivational value of food cues modulate dopamine signals in the NAc core (Aitken, Greenfield, & Wassum, 2016). This technique has provided evidence that dopamine levels in the NAc core substantially impact incentive motivation (Aitken, Greenfield, & Wassum, 2016). It was hypothesized that genetic differences will be found in sign-tracking versus goal-tracking behavior. It was also hypothesized that higher dopamine release in the NAc core would correlate with increased sign-tracking versus goal-tracking behavior. In addition, given previous results in regard to sex differences in sign-tracking and goal-tracking, it was hypothesized that females would demonstrate increased sign-tracking and goal-tracking behavior in comparison to males, and would reflect higher dopamine release in the NAc core as well (Stringfield et al., 2019).

Methods

Subjects

In a previous study, five strains of inbred mice were examined for differences in incentive motivation using a progressive ratio task. This resulted in a spectrum of motivation with some strains showing higher numbers of lever presses for a palatable food reward than others. Based on these results, strains encompassing high, moderate, and low levels of incentive motivation were chosen for the current study.

Subjects were purchased from the Jackson Laboratory (Bar Harbor ME). 50 mice of five different strains were included, with 10 mice per strain. These strains included: BALB/cJ, MRL/MpJ, C57BL/10J, C57Bl/6J, and A/J. There were consistently equal numbers of male and female subjects in each strain to allow for analysis of sex differences. Subjects were group housed at seven weeks of age and were given one week to become properly acclimated to the colony. Subjects were kept on a 12:12 light-dark cycle, and each cage was supplied with enrichment including: nestlets, tunnels, chew blocks, and bedding pucks. Prior to operant testing, subjects were food restricted to achieve body weights that were 85-90% of pre-restriction levels in order to ensure motivation for appetitive reward.

Procedures

Operant Testing

I. Habituation and Magazine Training

Three distinct stages of operant testing were utilized in this study. Subjects were first habituated to operant boxes for one 30-minute period. On the next day, subjects were put through one 45-minute magazine training program in which the association between the magazine and the chocolate “Boost” reward (~20 microliters per reward) was established. Intertrial Intervals

(ITIs) of 60”, 90”, 120”, 150”, and 180” were randomly assigned per trial and determined the time between reward deliveries. Reward retrieval latency was measured.

II. Autoshaping

Subjects moved into the autoshaping stage directly after magazine training. In this stage, the association between the audible insertion of a lever and the delivery of the reward was established. There was no contingency between interacting with the lever and receiving a reward. The same ITI values from the magazine training stage were used. Autoshaping was meant to measure sign and goal tracking behavior through a five-second offset between the appearance of the lever and the delivery of the ~20 microliter Boost reward. It measured reward retrieval latency, lever contacts per session (an indication of sign-tracking behavior) and magazine entries during the ITI, while the lever appeared, and during the CS and reward delivery periods (an indication of goal-tracking behavior). These were measured both within the five-second offset of each trial and holistically within each autoshaping session. Autoshaping sessions took place over 10 days, with one 60-minute session per day.

III. Contingency Degradation

After the autoshaping stage, subjects moved into the contingency degradation stage in which the association between the lever and the reward was broken. The same ITI intervals as both magazine training and autoshaping were utilized, along with a separate set of intervals which determined the time between lever presentations. Therefore, the times at which rewards were delivered and the times at which the lever was presented were not linked together. The same variables from the autoshaping stage were measured in the contingency degradation stage. Contingency degradation sessions took place over 10 days, with one 60-minute session per day.

IV. Extinction

The final stage of operant testing was extinction. This stage utilized the same protocol as contingency degradation and measured the same variables, with the exception that no reward was delivered at any time during extinction. Extinction sessions took place over 10 days, with one 60-minute session per day. After completion of the extinction stage, operant testing was over and data was analyzed.

Fast-Scan Cyclic Voltammetry

Fast-Scan Cyclic Voltammetry was conducted on subjects from the previously mentioned study consisting of the five strains utilized in the current study. Some of these mice had been tested on an operant conditioning progressive ratio task, and some had not. Tonic and phasic data from the NAc core of previous subjects were included in the current study in order to analyze genetic and dopaminergic differences between NAc core dopamine release and sign and goal-tracking behavior. No Fast-Scan Cyclic Voltammetry data was collected from the current subjects.

Brain tissue was collected through isoflurane-anesthetized decapitation on the testing date. Tissue was sliced at 300 microns on the vibratome. The NAc core was included on the chosen slices used for voltammetry. One slice was analyzed per brain. Once selected, slices were incubated on the voltammetry stage in oxygenated artificial cerebrospinal fluid (aCSF) for 30 minutes. Baseline measurements of the NAc core were recorded in five minute intervals for at least 10 measurements until stability was reached. The recording electrode was placed within the NAc core.

Amplitude of stimulation was consistently 3.5V for the core during baseline stimulation. 60 Hz was set as the stimulation frequency. One pulse was delivered per recording at baseline. Manipulation of signaling only occurred when three dopamine signals were reliably measured

within 10% of each other. Tonic firing measurements were accomplished using one pulse at a frequency of 60 Hz, while phasic firing was measured through multiple levels of stimulation. One pulse of 5 Hz, 10 Hz, 20 Hz, or 100 Hz was applied in five-minute intervals from lowest to highest stimulation. Both tonic and phasic firing were measured repeatedly using two stimulation pulses at a time. These procedures were then repeated twice for experimental data.

Statistical Analysis

A repeated measures Omnibus ANOVA was conducted examining the between subjects variables of strain and sex and the within subjects variable of session. The dependent variables consisted of reward retrieval latency, lever contacts per session, magazine entries during the ITI, magazine entries during the CS period (CS/Pre-US entries), magazine entries during the reward delivery (US entries), and magazine entries while the lever is presented (magazine entries during the CS period). Upon identification of significant differences, post hoc tests were run as appropriate.

Pearson correlations were conducted using strain means for lever contacts (sign-tracking) and CS entries (goal-tracking) in autoshaping along with dopamine release levels in the NAc core from the previous FSCV study.

Results

Lever Contacts

Autoshaping Lever Contacts

A day x strain interaction was found for lever contacts (Figure 1; $F(9, 360) = 1.775$ ($p = 0.005$)), suggesting that some strains modified their behavior as a function of day. The A/Js and MRLs performed the highest amount of lever contacts, showing no significant difference between each other ($SE = 2.738$, $p = 0.968$). Additionally, the BALBs, C5710Js, and C576Js

performed the lowest in lever contacts and did not show a significant difference between each other. No day x sex interaction was found for lever contacts. A main effect of strain was found with lever contacts, demonstrating that strains performed lever contacts differently (Figure 2). This was primarily driven by the MRLs and BALBs' increase in lever contacts. No sex x strain effect was found. A main effect of sex was found for lever contacts, showing that the two sexes performed lever contacts differently over time, as males performed more lever presses than females (Figure 3). See tables 1 and 4 for interactions and main effects, respectively.

Contingency Degradation Lever Contacts

A day x strain interaction was found for lever contacts (Figure 4; $F(36, 360) = 2.096$ ($p = 0.000$)), driven by the MRLs and A/Js executing more lever contacts than the other strains. The MRLs and A/Js showed no significant difference between each other ($SE = 12.846$, $p = 0.301$). The MRLs began with higher levels of lever contacts than the A/Js, but both strains reached about the same level by the end of the contingency degradation phase. The BALBs, C5710Js, and C576Js had no significant differences between each other, performed the lowest, and showed no trends in lever contacts. No day x sex or sex x strain interactions were found for lever contacts, along with no day x sex x strain interaction. A main effect of strain was found, demonstrating that contingency degradation occurs differently within strains (Figure 5). A main effect of sex was narrowly insignificant, although males performed more lever contacts than females throughout the entire phase of contingency degradation (Figure 6). Refer to table 2 for interactions and table 5 for main effects.

Extinction Lever Contacts

No day x strain interaction was observed in extinction for lever contacts.. No day x sex, sex x strain, or day x sex x strain interactions were found. A main effect of strain was found

during extinction (Figure 7). The C576Js and BALBs had no difference performance by sex, while the A/Js, C5710Js, and MRLs had significant differences. A main effect of sex was not found in extinction. See tables 3 and 6 for interactions and main effects, respectively.

Intertrial Interval Entries (ITI Entries)

Autoshaping ITI Entries

No day x strain, day x sex, or day x sex x strain interactions were found for ITI entries. No sex x strain interaction was found, and no main effect of sex was found. A main effect of strain was found, driven by the MRLs performing the highest number of ITI entries, showing a significant difference between all strains except the BALBs (SE = 5741.155, p = 0.248). A decrease in ITI entries and stabilization over time occurred for all strains except the C5710Js and C576Js, which exhibited no trend. See table 1 for interactions and table 4 for main effects.

Contingency Degradation ITI Entries

A day x strain interaction was found for ITI entries (F (36, 360) = 3.118 (p = 0.000)), showing that separate strains performed differently throughout contingency degradation. MRLs performed the highest number of ITI entries. No day x sex or sex x strain interactions were found. However, a day x sex x strain was found for ITI entries (F (36, 360) = 1.553 (p = 0.025)), with females continuously having higher levels of ITI entries compared to males and the MRLs having the highest ITI entries out of the strains. Both males and females trended down in number of ITI entries, then trended upward during the middle portion of contingency degradation, then back downward during the last portion. A main effect of sex was not found. A main effect of strain was found, as the MRLs performed the highest, showing a significant difference between all strains such as the A/Js (SE = 6288.691, p = 0.000), that performed the lowest out of all

strains, BALBs, C5710Js, and C576Js. Refer to tables 2 and 5 for interactions and main effects, respectively.

Extinction ITI Entries

A day x strain interaction was found with ITI entries (F (36, 360) = 3.450 (p = 0.000)), demonstrating that the sexes extinguished the ITI entry response at different rates. The MRLs performed the highest, showing a significant difference between all strains such as the BALBs (SE = 2644.613, p = 0.003), and A/Js, C5710Js, and C576Js (SE = 2644.613, p = 0.000). The MRLs and BALBs trended downward in ITI entries, while the A/Js, C5710Js, and C576Js did not have a change in ITI entries. A sex x strain interaction was also found (F (4, 40) = 3.874 (p = 0.009)), produced by the MRL males significantly performing more ITI entries than the females and a lack of sex differences in the other strains. A main effect of strain was also found due to the MRLs performing at higher levels of ITI entries than the other strains. No day x sex or day x sex x strain interactions were found. No main effect of sex was found. See tables 3 and 6 for interactions and main effects, respectively.

Conditioned Stimulus/Pre-Unconditioned Stimulus Entries (CS/Pre-US Entries)

Autoshaping CS Entries

A day x strain interaction was found for CS entries (Figure 8; F (9, 360) = 4.382 (p = 0.000)), demonstrating differing patterns in strain performance over time. The MRLs and BALBs performed the highest number of CS entries, showing no significant difference between each other (SE = 239.142, p = 0.894), while the A/Js performed the lowest out of all strains (SE = 239.142, p = 0.000). All strains, however, exhibited an upwards trend in CS entries over autoshaping. No day x sex interaction was found. A sex x strain effect was found for CS entries (Figure 9; F (4, 40) = 3.190 (p = 0.023)), suggesting a difference in sex performance per strain,

as females executed more CS entries than males in the C576Js and the MRLs while no significant sex disparities were found in the other strains. No day x sex x strain interaction was found. A main effect of strain was found, as the MRLs and BALBs performed more CS entries than the other strains (Figure 10). A main effect of sex was also found, as CS entries trended upwards and then stabilized by the end of autoshaping in both sexes, but females exceeded males in CS entries (Figure 11). Refer to table 1 for interactions and table 4 for main effects.

Contingency Degradation Pre-US Entries

A day x strain interaction was found for Pre-US entries ($F (36, 360) = 2.281$ ($p = 0.000$)), demonstrating strains performing differently over time, such as the MRLs. The MRLs performed the highest amount of Pre-US entries, showing significance between all other strains, such as the BALBs ($SE = 292.520$, $p = 0.002$) and the C5710Js, C576Js, and A/Js ($SE = 292.520$, $p = 0.000$). The BALBs, C5710Js, C576Js, and A/Js had no significant differences between each other. There was no trend in Pre-US entries by strain throughout contingency degradation, with the exception of the MRLs trending upward in Pre-US entries. No day x sex, sex x strain, or day x sex x strain interactions were found for Pre-US entries. A main effect of strain was found, as the MRLs drove a difference between the strains in performance of Pre-US entries. A main effect of sex was not found. See table 2 for interactions and table 5 for main effects.

Extinction Pre-US Entries

A day x strain effect was found for Pre-US entries ($F (36, 360) = 2.755$ ($p = 0.000$)). MRLs had significantly the highest amount of Pre-US entries compared to the BALBs ($SE = 101.449$, $p = 0.002$) and the C5710Js, C576Js, and A/Js ($SE = 101.449$, $p = 0.000$). The MRLS and BALBs once again decreased in Pre-US entries over time, while the C5710Js, C576Js, and A/Js had no trend across extinction. No day x sex interaction was found. A sex x strain

interaction was found for Pre-US entries ($F (4, 40) = 4.125$ ($p = 0.007$)), showing that the two sexes in various strains performed Pre-US entries differently. This was driven by the MRL males having significantly higher levels of Pre-US entries than females, and no other sex differences in strain being found. No day x sex x strain interaction was found. A main effect of strain was found for Pre-US entries, while a main effect of sex was not found in extinction. Refer to tables 3 and 6 for interactions and main effects, respectively.

Unconditioned Stimulus Entries (US Entries)

Autoshaping US Entries

A day x strain interaction was found ($F (9, 360) = 5.226$ ($p = 0.000$)), as the A/Js performed the lowest out of all strains in the measure of US entries ($SE = 113.641$, $p = 0.000$). No significant differences were found between strains in US entries with the exception of the A/Js, although all strains showed an upwards trend and stabilization of US entries over time. A day x sex ($F (9, 360) = 2.172$ ($p = 0.023$)) effect was found, demonstrating a difference in the performance of males versus females as a function of day. There was a slight difference in males executing more US entries than females. A sex x strain effect was found ($F (4, 40) = 2.934$ ($p = 0.023$)), as females carried out more US entries than males in three strains, showing a difference in the performance of sexes in each strain. No day x sex x strain effect was found. A main effect of strain was found, as the A/Js significantly performed lower than the other strains. No main effect of sex was found. Refer to table 1 for interactions and table 4 for main effects.

Contingency Degradation US Entries

A day x strain interaction was found ($F (36, 360) = 1.511$ ($p = 0.034$)), as the MRLs and BALBs performed the highest number of US entries and had no significant difference between each other ($SE = 136.364$, $p = 0.984$). No day x sex or sex x strain interactions were found. A

day x sex x strain effect was found (F (36, 360) = 1.654 (p = 0.012)), demonstrating that sex and strain both differed by day. Females consistently exceeded males in US entries throughout all of contingency degradation, and all strains had significantly different US entries by sex. Females performed more US entries than males in each strain. A main effect of strain was found, as the MRLs and BALBs performed higher numbers of US entries, and the A/Js performed the lowest (SE = 136.364, p = 0.000). All strains trended upward in US entries during contingency degradation. A main effect of sex was found, as females executed more US entries than males and both sexes increased US entries over time. Refer to tables 2 and 5 for interactions and main effects, respectively.

Extinction US Entries

Within the extinction phase no US was given. US entries in extinction were measured during what would have constituted the US period. A day x strain interaction was found (F (36, 360) = 3.971 (p = 0.000)), demonstrating a difference in the performance of US entries in various strains over time. MRLs were shown to have significantly the highest amount of US entries compared to the BALBs (SE = 110.446, p = 0.008) and the C5710Js, C576Js, and A/Js (SE = 110.446, p = 0.000). All strains trended downward in US entries during extinction.

A sex x strain interaction was found (F (4, 40) = 3.890 (p = 0.009)), driven by MRL males exceeding MRL females in US entries, along with no other strain sex differences being observed. No day x sex or day x sex x strain interactions were found. A main effect of strain was found, as the MRLs had higher levels of US entries than the other strains. No main effect of sex was found for US entries in extinction. See table 3 for interactions and table 6 for main effects.

Magazine Entries during the CS period (USL Entries)

Contingency Degradation USL Entries

In contingency degradation, no day x sex or sex x strain interactions were found. A day x strain interaction was found (Figure 12; F (36, 360) = 2.037 (p = 0.001)), in which the MRLs had the highest performance of magazine entries during the CS period but had no significant difference from the BALBs (SE = 446.523, p = 0.116). The A/Js performed the lowest, but had no significant difference from the C576Js (SE = 446.523, p = 0.392). A main effect of strain was also seen through similar means (Figure 13). Additionally, all strains trended downwards at the start of contingency degradation, but the MRLs trended back up. No day x sex x strain interaction was found. A main effect of sex was narrowly insignificant. However, females exceeded males in magazine entries during the CS period throughout all of contingency degradation (Figure 14). Refer to tables 2 and 5 for interactions and main effects, respectively.

Extinction USL Entries

A day x strain interaction was found (Figure 15; F (36, 360) = 2.586 (p = 0.000)), meaning that certain strains extinguished this behavior differently. For example, the MRLs were shown to have significantly more magazine entries during the CS period than all other strains, such as the BALBs (SE = 187.726, p = 0.015), C576Js (SE = 187.726, p = 0.001), and A/Js and C5710Js (SE = 187.726, p = 0.000). The MRLs and BALBs decreased over time, while the C5710Js, C576Js, and A/Js did not trend up or down during extinction. The main effect of strain is also demonstrated here, as each strain performed differently in comparison to the MRLs (Figure 16). No day x sex interaction was found. A sex x strain interaction was found (F (4, 40) = 2.966 (p = 0.031)), showing that the sexes performed differently per strain such as in the MRLs, in which males performed more magazine entries during the CS period than MRL females and no other strain sex differences were found (Figure 17). No day x sex x strain interaction was found. Lastly, no main effect of sex was found, although males consistently exceeded females in

magazine entries during the CS period throughout all of extinction. Refer to tables 3 and 6 for interactions and main effects, respectively.

Sign-Tracking and Goal-Tracking and Dopamine Release

Pearson correlations were run to compare strain means for lever pressing (sign-tracking) and CS entries (goal-tracking) to both tonic and phasic dopamine release. It was found that there were no significant correlations between these variables and tonic (1 pulse 60 Hz) or phasic release (2 or 5 pulse at 100 Hz). See table 7 for correlations.

Discussion

Incentive motivation and associated behaviors such as sign-tracking versus goal-tracking have been shown to be connected to Substance Use Disorders (SUDs), relapse specifically, along with food-related disorders such as overeating and binge eating (Holden, 2023). Sign-tracking has been shown to be a response implicated in higher vulnerability to relapse and SUDs (Holden, 2023). Individual and genetic differences play a role in this vulnerability, and it is plausible that these differences may lead to conclusions regarding the occurrence of sign-tracking versus goal-tracking. The relationship between sign-tracking and goal-tracking behavior and dopamine release has recently begun to be explored, with research showing that both sign-tracking and goal-tracking are modulated by dopamine release (Roughley & Killcross, 2019). Sign-tracking has been attributed to dopamine release in the NAc, specifically (Fraser & Janak, 2017). In the current study, it was hypothesized that genetic differences in sign-tracking versus goal-tracking behavior would be found in a panel of genetically diverse, inbred mice. It was also hypothesized that increased sign-tracking versus goal-tracking behavior would correlate to greater levels of dopamine release in the NAc core. Additionally, it was hypothesized that females would show higher performance of both sign-tracking and goal-tracking behavior compared to males given

previous literature on sex differences in sign-tracking and goal-tracking (Stringfield et al., 2019), and this difference would be reflected in higher levels of dopamine release in the NAc core as well. Importantly, in food deprivation, strains did not significantly differ in percentage weight loss across stages. Genetic differences were found for sign-tracking and goal-tracking behavior. However, dopamine release levels were not found to be correlated with sign-tracking or goal-tracking. Although goal-tracking behaviors neared significant correlation with NAc core dopamine release, a larger sample size is needed to assess this. Sex differences in sign-tracking behavior were found to conflict with the prior hypothesis, as males were found to perform higher levels of sign-tracking than females. Sex differences in goal-tracking behavior supported the hypothesis, as females were shown to perform more goal-tracking than males.

The principal measure of sign-tracking in this study was lever contacts. It was found both in autoshaping and contingency degradation that the various strains performed lever contacts differently over time, giving evidence of learning. In autoshaping, the Pavlovian association was learned, and in contingency degradation, the association was broken. Each strain learned differently over time, showing a genetic difference in the acquisition of sign-tracking behavior, along with a genetic difference in sign-tracking behavior upon the severing of a contingency. For example, the MRLs responded at higher levels in contingency degradation as opposed to autoshaping, whereas the A/Js maintained the same level of sign-tracking in both phases. Additionally, MRLs decreased in responding during contingency degradation whereas the A/Js remained consistent. These differences could be due to genetic disparities in learning. The MRLs exhibited patterns of learning more quickly, whereas the A/Js may have a genetic inability to learn as quickly. Therefore, these behavioral disparities demonstrate genetic differences in sign-tracking behavior. In extinction, this effect was not found, showing that this genetic

difference is not present in the extinction of sign-tracking behavior. It was also found that in autoshaping, males performed more lever contacts than females. This demonstrates that sign-tracking behavior is acquired differently depending on sex. In contingency degradation, this effect was narrowly insignificant, but males continued to perform more sign-tracking behavior than females. The extinction of sign-tracking behavior was not found to be dependent on sex. While genetic differences were found in sign-tracking behavior, results on sex differences contrast with the original hypothesis as males performed more sign-tracking behavior than females. This finding is inconsistent with prior research, as it has been found that females acquire and maintain sign-tracking responses both faster and for longer than males (Hammerslag & Gulley, 2013). This may be due to the specific strains selected in this study. These strains have not been studied by sex differences for these traits, thus males in these strains may be genetically predisposed to respond more than the females. This could also be due to overall activity. For example, MRL males exhibit hyperactivity while MRL females do not. This difference in activity may have produced higher levels of sign-tracking in males than females. However, the finding of genetic differences in sign-tracking behavior is supported by previous literature.

The main measure of goal-tracking in this study was head entries into the magazine during the CS presentation. It was found in all three behavioral phases that strain performance varied as a function of time. This reflects acquisition of goal-tracking behavior occurring differently in the various strains, along with contingency severing and extinguishing of goal-tracking behavior occurring differently per strain. This demonstrates genetic differences in learning of goal-tracking behavior, supporting the original hypothesis. In both autoshaping and contingency degradation, it was found that females performed higher levels of goal-tracking behavior than males. Although there was narrow insignificance in contingency degradation,

females consistently performed more magazine entries during the CS period than males. This not only establishes a sex difference in the acquisition of goal-tracking behavior, and continuance of this difference upon the breaking of a contingency but supports the hypothesis that females would perform higher levels of both sign-tracking and goal-tracking behavior than males. Further, it is consistent with prior literature indicating that acquisition and maintenance of sign-tracking and goal-tracking responses occur more quickly in females, along with motivated learning in general (Stringfield et al., 2019). However, investigation of the extinction of these responses have not yielded the same sex differences. It was found in the present study that no sex differences were observed in extinction.

The main measure of reward responses was US entries, or magazine head entries during the delivery of the reward. In all three behavioral phases, it was found that strains performed US entries differently as a function of time, reflecting that the strains learned to perform or not to perform the reward response differently in all three behavioral paradigms. This demonstrates genetic differences in the acquisition, contingency breaking, and extinction of the reward response. In autoshaping, the acquisition of the reward response occurred differently per sex, as males executed more US entries than females. This contrasts with the hypothesis of females performing both more sign and goal-tracking behavior than males because the reward response is critical to the learning of, severing of, and extinguishing of the contingency. However, in contingency degradation, females performed more US entries than males in every strain, and throughout the entire phase. This shows that the reward response acquisition and contingency severing may not have uniform sex differences. This aligns with the hypothesis that females are higher sign-trackers and goal-trackers than males. Additionally, this is compatible with literature

on the subject because although males began performing in higher numbers than females, the females maintained the responses after the contingency was broken for longer than males. As females have been shown to sign-track and goal-track at higher levels than males and maintain these responses for longer, these results support the original hypothesis (Stringfield et al., 2019). Extinction of the reward response was not found to be dependent on sex. It was also found that both sex and strain differ by day, demonstrating that there were concurrently genetic and sex differences in learning.

It was found through correlations with FSCV data on these same strains that dopamine release in the NAc core was not correlated with sign-tracking or goal-tracking behavior. This does not support the original hypothesis that higher levels of dopamine release in the NAc core would correlate with higher levels of sign-tracking and goal-tracking behavior. These findings are also inconsistent with prior literature. It has been shown that upon administration of dopamine antagonists within the NAc core, sign-tracking behavior was attenuated (Fraser & Janak, 2017). This establishes a dependence of sign-tracking behavior on NAc core dopamine release. However, this connection of dopamine release to sign-tracking behavior seen previously was not found in this study. In following, no dopaminergic relation to goal-tracking was found. It has been shown that in response to reward-predicting cues, goal-trackers undergo phasic dopamine release within the NAc, just as sign-trackers do (Flagel et al., 2011). However, a more highly-powered study is required to rule out this relationship.

Several limitations exist within the present study. One limitation involves the power of the study. The correlations performed on the relationship between NAc core dopamine release and sign-tracking and goal-tracking behavior did not reach significance, yet exhibited high correlation values. This demonstrates that a lack of statistical power may have prevented

significance from being reached. Additionally, previous studies have found dopamine release in the NAc shell to be the driver of sign-tracking versus goal-tracking behavior as opposed to the NAc core (Flagel et al., 2011). This may be an explanation for the absence of significant correlations in this study between dopamine release in the NAc core and sign-tracking versus goal-tracking, as the NAc shell may have a more prominent role in these behaviors. Another limitation includes differences in behavioral tasks. In the prior study from which FSCV data was acquired, a progressive ratio task was carried out as opposed to the autoshaping, contingency degradation, and extinction paradigms in the present study. The difference in behavioral tasks between these subjects could plausibly produce differences in tonic and phasic dopamine release in the NAc core. FSCV data from subjects having directly experienced the sign-tracking and goal-tracking tasks in this study may yield more accurate results on the relationship between NAc core dopamine release and sign-tracking and goal-tracking behavior.

From these findings, future research directions can address several areas. One such direction includes analyzing sex differences in NAc core dopamine release and their relationship to sex differences in sign-tracking and goal-tracking behavior. As sex differences in behavior were investigated in the current study, but were not analyzed in relation to NAc core dopamine release, future research in this area may provide results critical to the understanding of how sex impacts these behaviors. Literature has suggested that although sign-tracking is contingent upon NAc core dopamine release, goal-tracking may be mechanistically distinct and may involve frontal mechanisms (Fraser & Janak, 2017; Holden, 2023). It has been shown that although phasic dopamine release in the NAc core is involved in both sign-tracking and goal-tracking, only sign-trackers experience a dopamine release in response to a cue, as well as a lessening dopamine response to the reward delivery over time (Flagel et al., 2011). As goal-trackers do not

experience this, mesolimbic dopamine may only be necessary for acquisition of sign-tracking and not goal-tracking. As other mechanisms have been shown to play a role in goal-tracking, future research could examine these circuits in addition to NAc dopamine in order to achieve a comprehensive understanding of the neurological underpinnings of sign-tracking and goal-tracking behaviors. Secondly, a replication of the current study with the addition of a FSCV component performed on ongoing subjects may be advantageous in evaluating the conflicting results seen in the present experiment. Additionally, it may be the case that supplementing this methodology with additional strains may increase the statistical power of this study, possibly resulting in the finding of significant correlations between NAc core dopamine release and sign-tracking and goal-tracking behavior along with a better exemplification of the genetic and sex differences that exist within these strains. As a relationship between NAc core dopamine release and sign-tracking and goal-tracking behavior has been found in previous studies, a study eliminating limitations seen here may lead to clearer results.

The present study hypothesized that genetic differences would be found in sign-tracking and goal-tracking behavior, sex differences would be found in that females would perform higher levels of both sign-tracking and goal-tracking than males, and increased NAc core dopamine release would be correlated with increased sign-tracking and goal-tracking behavior. Genetic differences were found, as both sign-tracking and goal-tracking behavior occurred differently by strain. However, sex differences in sign-tracking behavior showed males performing at higher levels than females, contrasting with the original hypothesis. Sex differences in goal-tracking supported the original hypothesis, as females performed more goal-tracking behavior than males. NAc core dopamine was also found to have no correlation to sign-tracking and goal-tracking, which disputes the hypothesis and findings in previous research. Overall, these results suggest

that further research is needed to identify the neurological nature of these relationships, along with the genetic and sex differences in sign-tracking and goal-tracking behavior.

Tables

	Measure	df	F	Sig
Day x Sex	LP	9	1.645	0.101
	ITI	9	0.177	0.996
	CS	9	1.656	0.098
	US	9	2.172	0.023
Day x Strain	LP	36	1.775	0.005
	ITI	36	1.123	0.294
	CS	36	4.382	0.000
	US	36	5.226	0.000
Sex x Strain	LP	4	2.228	0.083
	ITI	4	1.902	0.129
	CS	4	3.19	0.023
	US	4	2.934	0.032
Day x Sex x Strain	LP	36	1.150	0.260
	ITI	36	0.949	0.556
	CS	36	0.855	0.709
	US	36	0.584	0.975

Table 1. Interactions in Autoshaping.

	Measure	df	F	Sig
Day x Sex	LP	9	0.582	0.812
	ITI	9	1.111	0.354
	Pre-US	9	0.980	0.456
	US	9	1.215	0.285
	USL	9	1.217	0.283
Day x Strain	LP	36	2.096	0.000
	ITI	36	3.118	0.000
	Pre-US	36	2.281	0.000
	US	36	1.511	0.340
	USL	36	2.037	0.001
Sex x Strain	LP	4	1.267	0.299
	ITI	4	0.287	0.885
	Pre-US	4	0.364	0.833
	US	4	0.411	0.800
	USL	4	0.437	0.781
Day x Sex x Strain	LP	36	0.802	0.787
	ITI	36	1.553	0.025
	Pre-US	36	1.168	0.239
	US	36	1.654	0.012
	USL	36	1.362	0.086

Table 2. Interactions in Contingency Degradation.

	Measure	df	F	Sig
Day x Sex	LP	9	1.763	0.074
	ITI	9	0.481	0.887
	Pre-US	9	0.539	0.846
	US	9	0.348	0.958
	USL	9	0.195	0.995
Day x Strain	LP	36	1.308	0.117
	ITI	36	3.450	0.000
	Pre-US	36	2.755	0.000
	US	36	3.971	0.000
	USL	36	2.586	0.000
Sex x Strain	LP	4	2.114	0.097
	ITI	4	3.874	0.009
	Pre-US	4	4.125	0.007
	US	4	3.89	0.009
	USL	4	2.966	0.031
Day x Sex x Strain	LP	36	1.172	0.236
	ITI	36	1.324	0.106
	Pre-US	36	1.157	0.252
	US	36	1.316	0.112
	USL	36	0.937	0.577

Table 3. Interactions in Extinction.

	Measure	df	F	Sig
Day	LP	9	2.924	0.002
	ITI	9	7.004	0.000
	CS	9	115.488	0.000
	US	9	710.468	0.000
Sex	LP	1	5.649	0.022
	ITI	1	0.031	0.860
	CS	1	4.69	0.036
	US	1	2.499	0.122
Strain	LP	4	8.761	0.000
	ITI	4	8.467	0.000
	CS	4	30.757	0.000
	US	4	53.419	0.000

Table 4. Between-Subjects Main Effects in Autoshaping

	Measure	df	F	Sig
Day	LP	9	1.386	0.193
	ITI	9	14.467	0.000
	Pre-US	9	3.948	0.000
	US	9	19.890	0.000
	USL	9	34.263	0.000
Sex	LP	1	3.563	0.066
	ITI	1	1.144	0.291
	Pre-US	1	0.157	0.694
	US	1	12.260	0.001
	USL	1	3.589	0.065
Strain	LP	4	5.552	0.001
	ITI	4	13.263	0.000
	Pre-US	4	14.608	0.000
	US	4	20.779	0.000
	USL	4	9.903	0.000

Table 5. Between-Subjects Main Effects in Contingency Degradation.

	Measure	df	F	Sig
Day	LP	9	0.485	0.885
	ITI	9	9.270	0.000
	Pre-US	9	5.866	0.000
	US	9	58.398	0.000
	USL	9	6.338	0.000
Sex	LP	1	1.020	0.319
	ITI	1	2.363	0.132
	Pre-US	1	2.754	0.105
	US	1	0.585	0.449
	USL	1	1.635	0.208
Strain	LP	4	2.834	0.037
	ITI	4	14.107	0.000
	Pre-US	4	14.095	0.000
	US	4	15.448	0.000
	USL	4	9.934	0.000

Table 6. Between-Subjects Main Effects in Extinction.

Pulse, Hz	Measure	R	Sig
1, 60 (tonic)	LP	-0.428	0.472
	CS	0.817	0.091
2, 100 (phasic)	LP	-0.233	0.706
	CS	0.813	0.095
5, 100 (phasic)	LP	-0.215	0.728
	CS	0.789	0.113

Table 7. Pearson Correlations of Sign-tracking and Goal-tracking Behavior with NAc core DA Release.

Figures

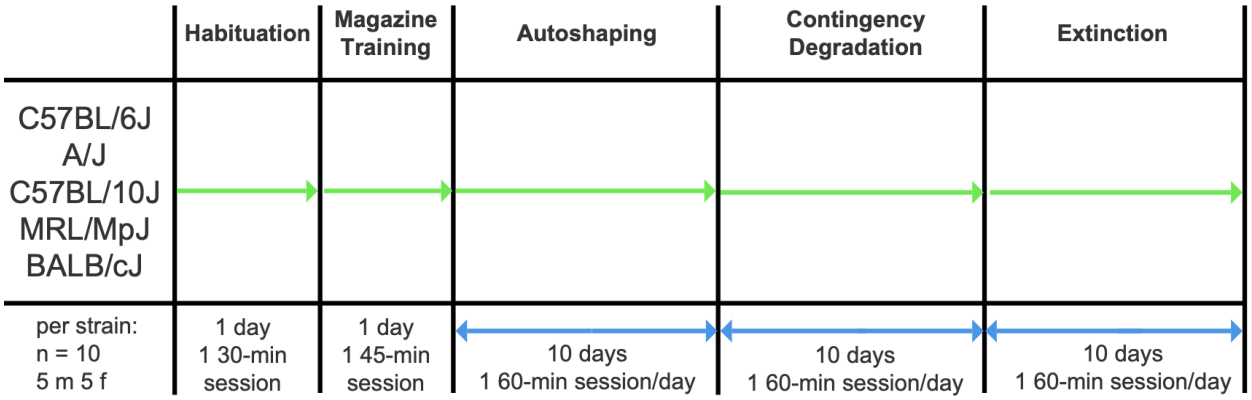

Figure 1. Experimental Timeline.

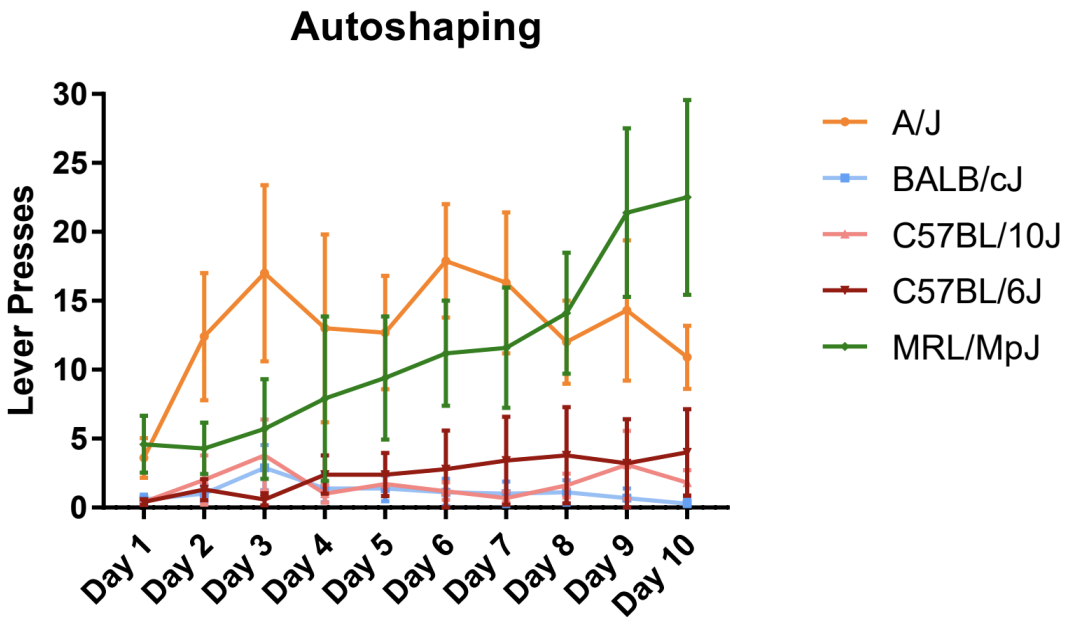

Figure 2. Day x Strain Lever Contacts in Autoshaping.

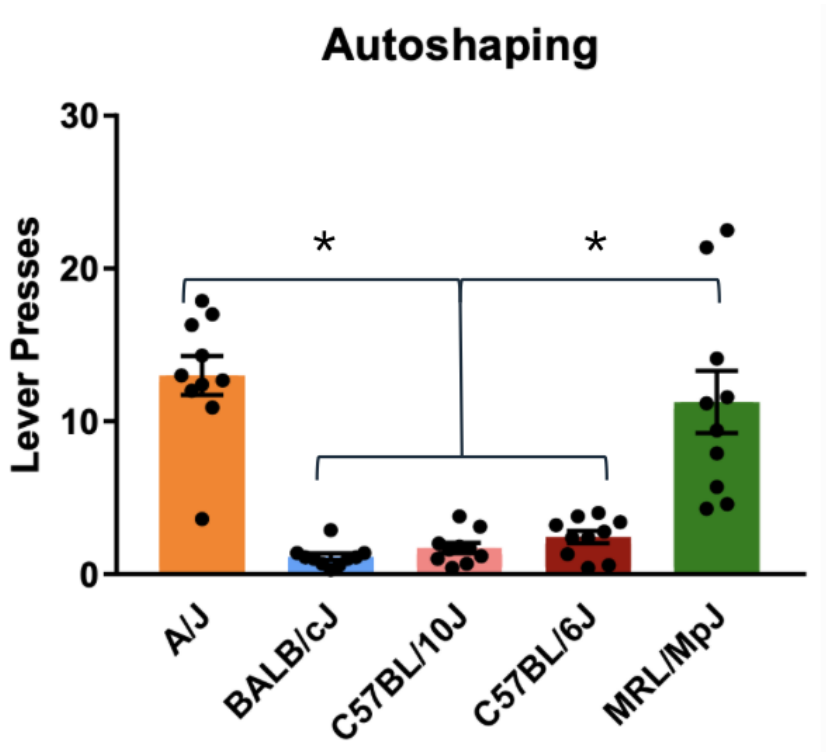

Figure 3. Main Effect of Strain on Lever Contacts in Autoshaping.

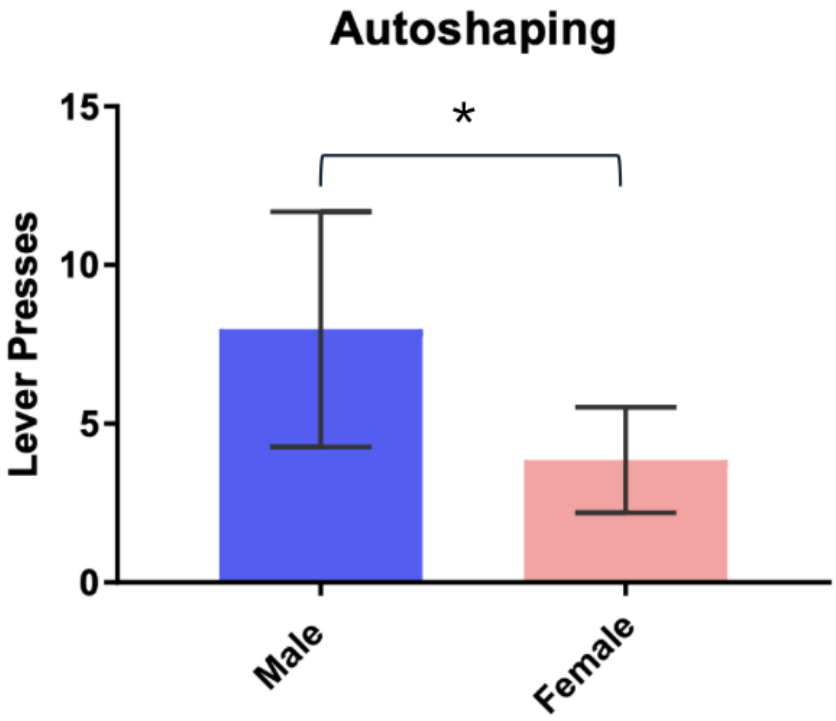

Figure 4. Main Effect of Sex on Lever Contacts in Autoshaping.

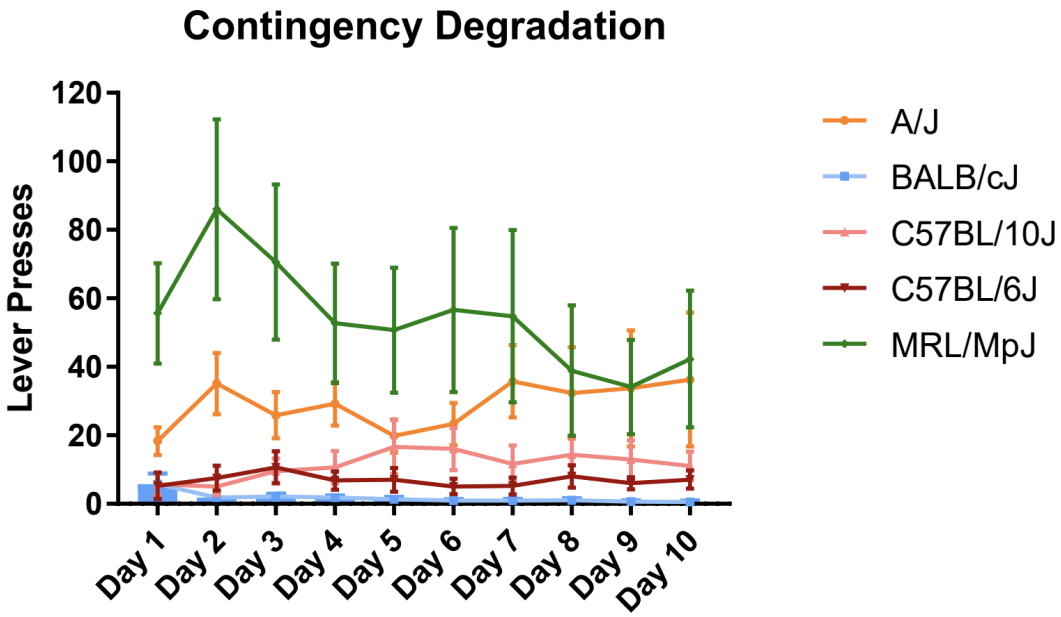

Figure 5. Day x Strain Lever Contacts in Contingency Degradation.

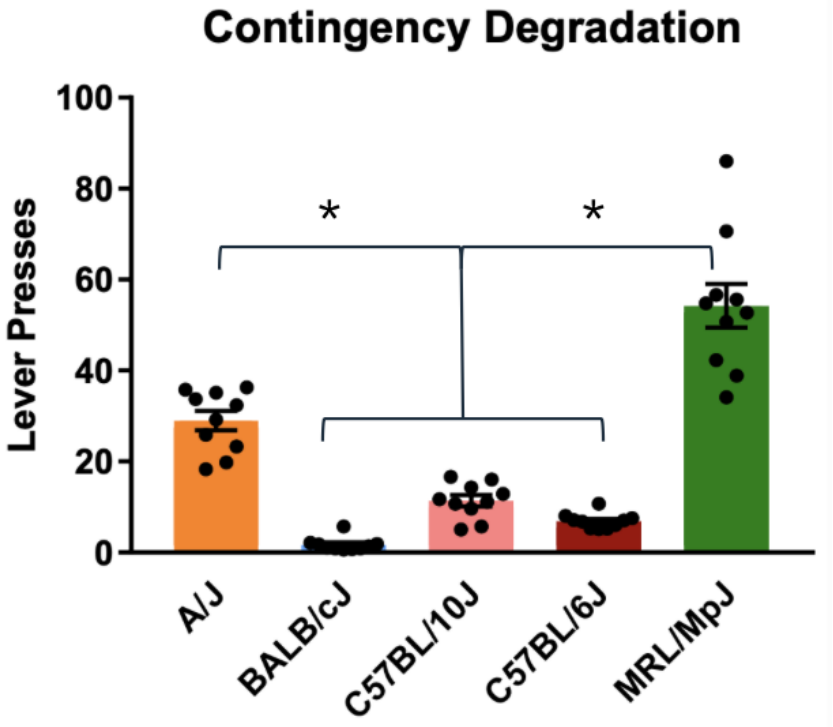

Figure 6. Main Effect of Strain on Lever Contacts in Contingency Degradation.

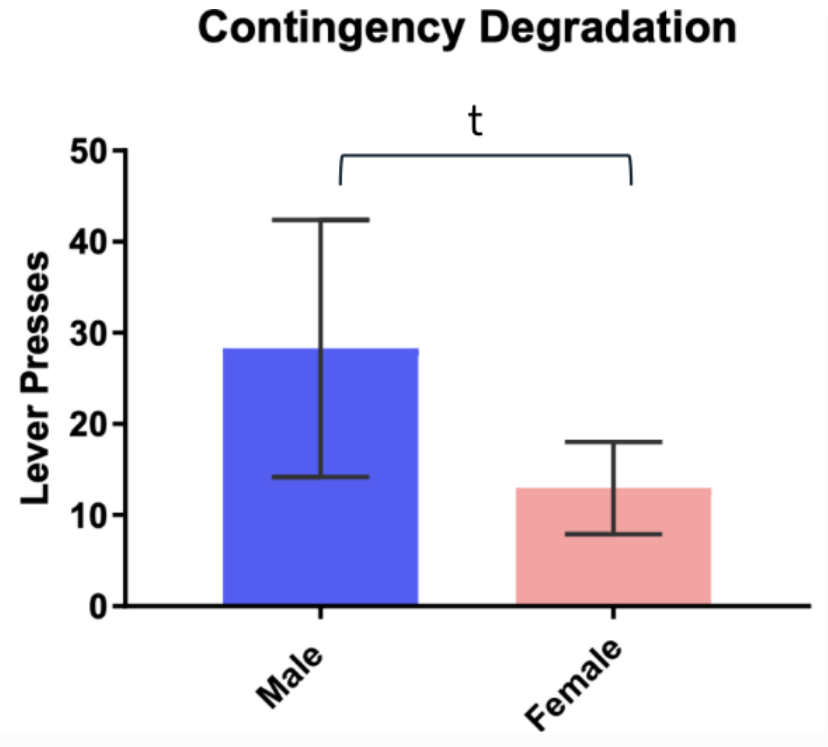

Figure 7. Main Effect of Sex on Lever Contacts in Contingency Degradation.

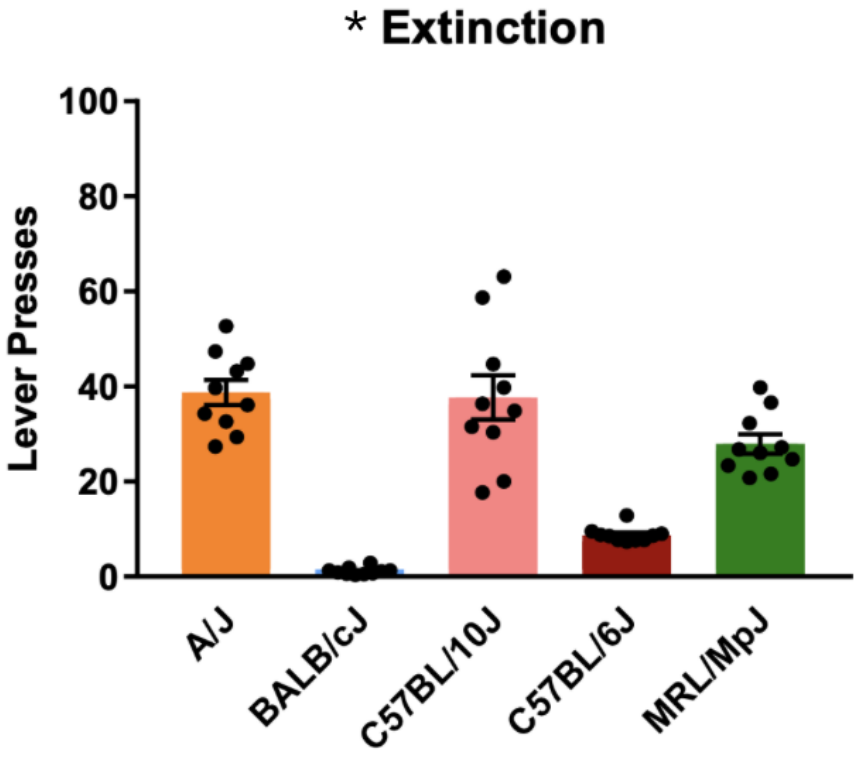

Figure 8. Main Effect of Strain on Lever Contacts in Extinction.

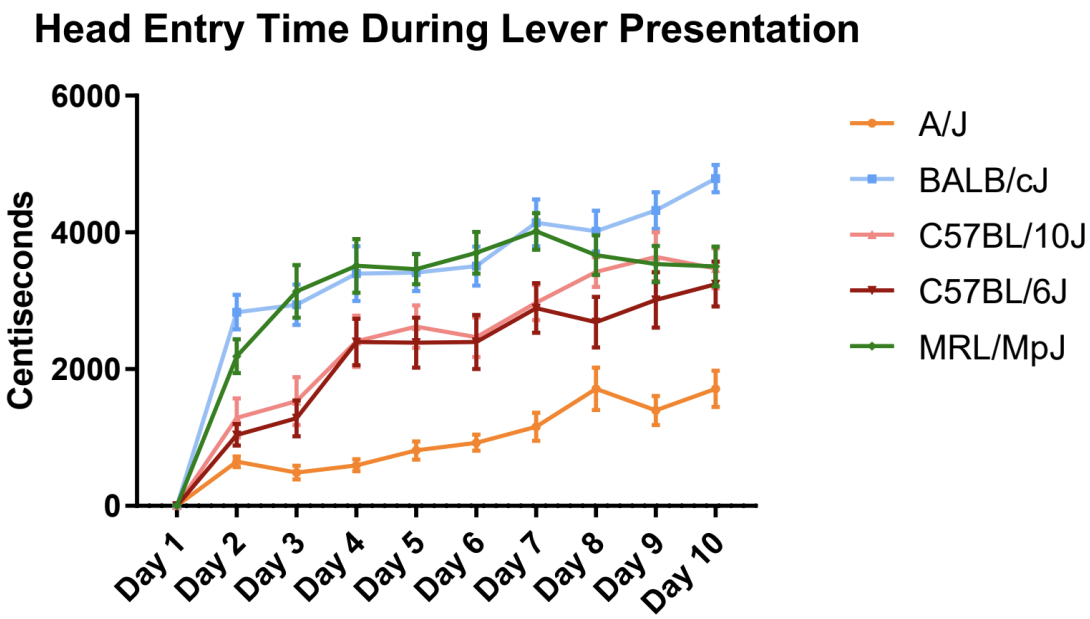

Figure 9. Day x Strain CS Entries During Lever Presentation in Autoshaping.

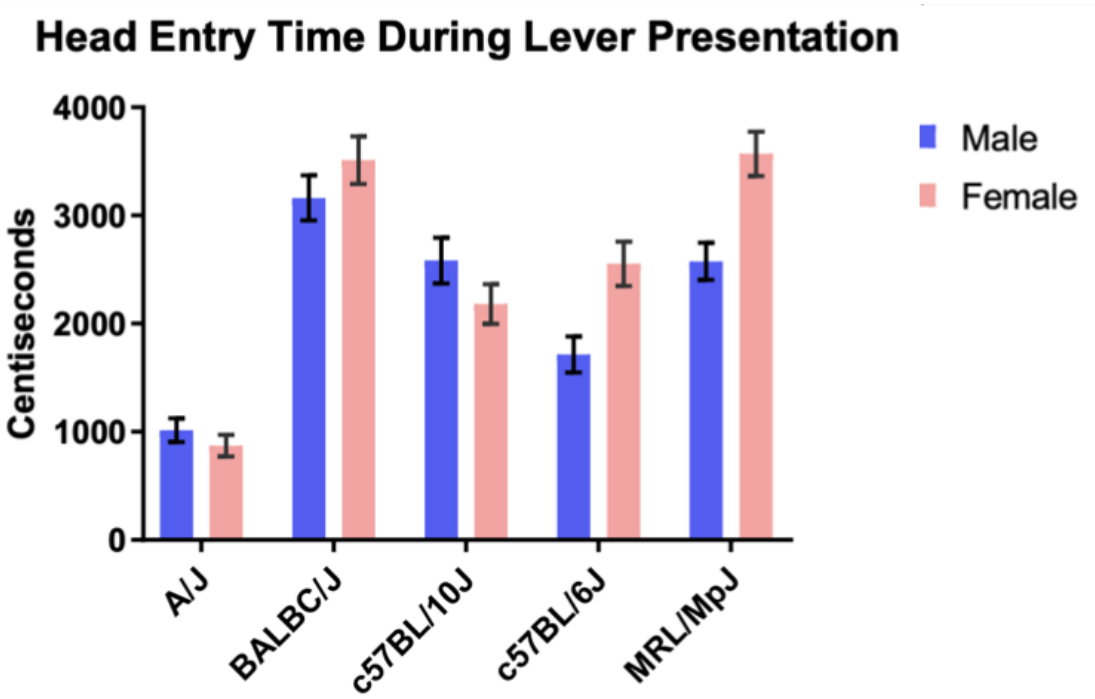

Figure 10. Strain x Sex CS Entries During Lever Presentation in Autoshaping.

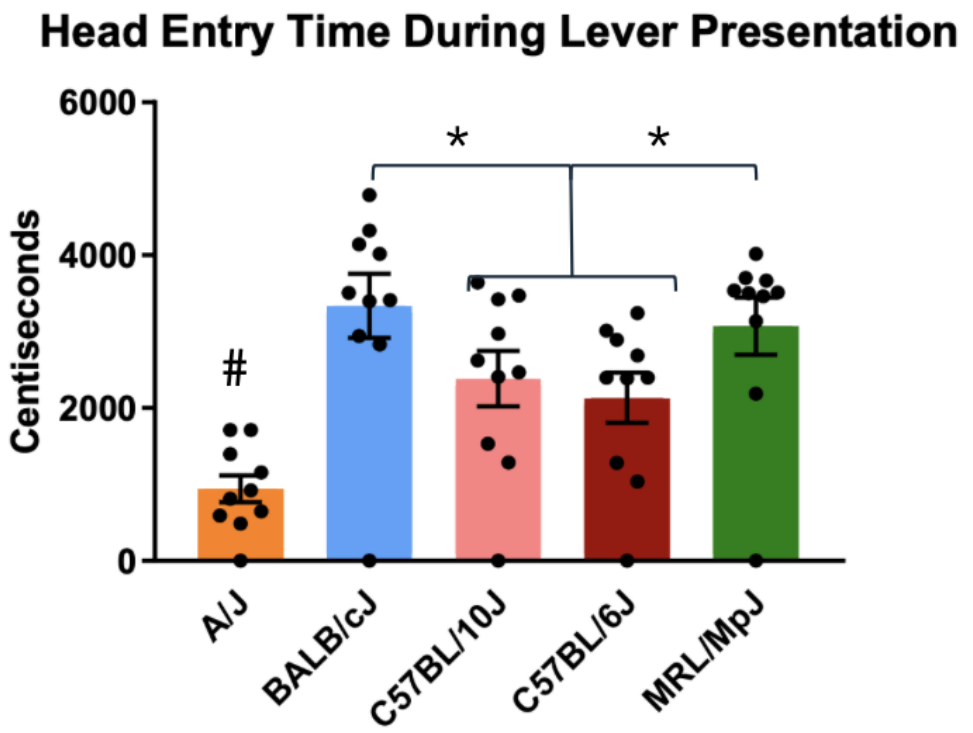

Figure 11. Main Effect of Strain on CS Entries During Lever Presentation in Autoshaping.

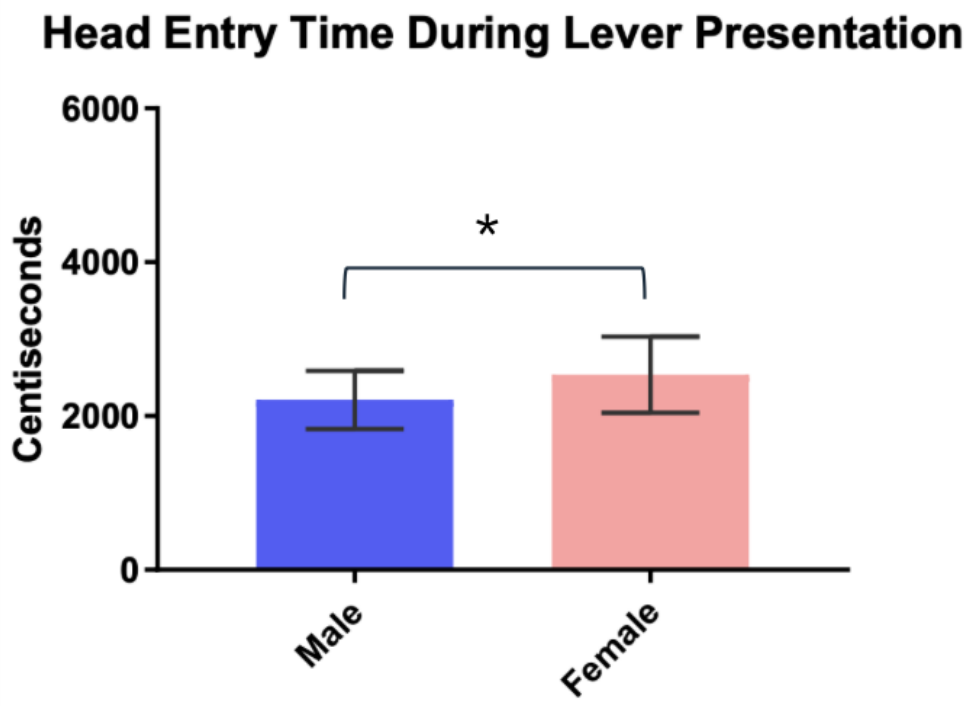

Figure 12. Main Effect of Sex on CS Entries During Lever Presentation in Autoshaping.

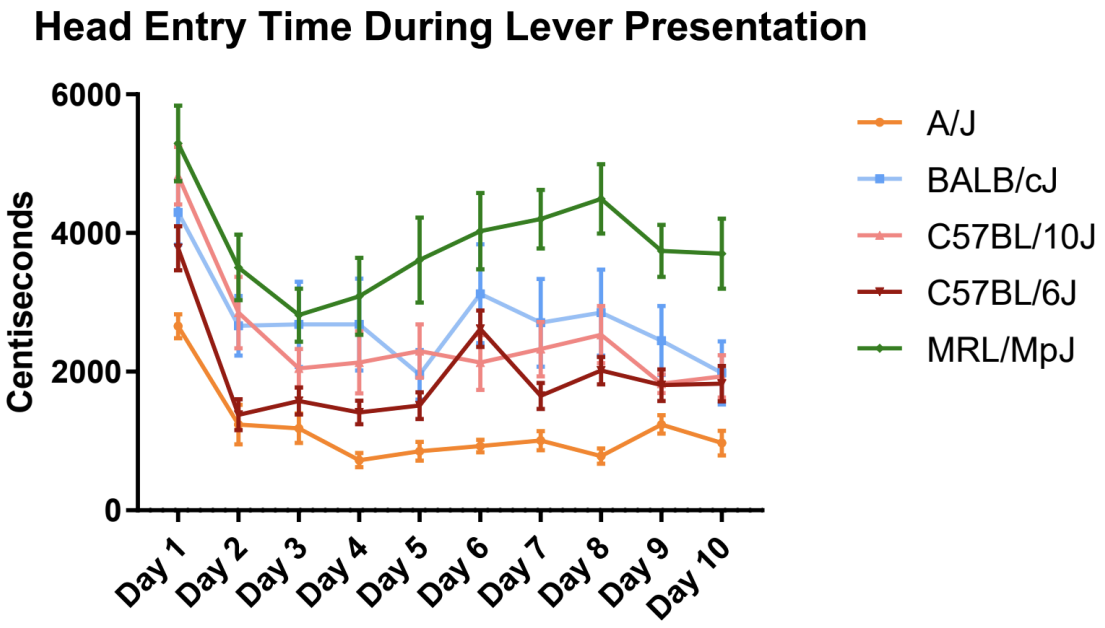

Figure 13. Day x Strain CS Entries During Lever Presentation in Contingency Degradation.

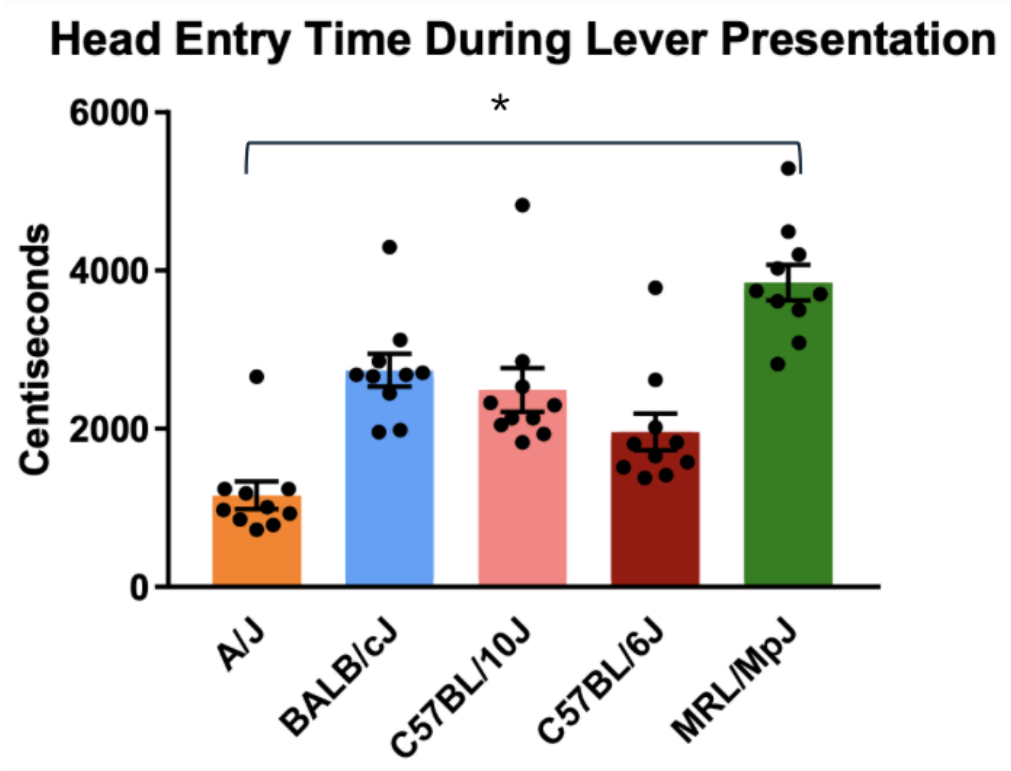

Figure 14. Main Effect of Strain on CS Entries During Lever Presentation in Contingency Degradation.

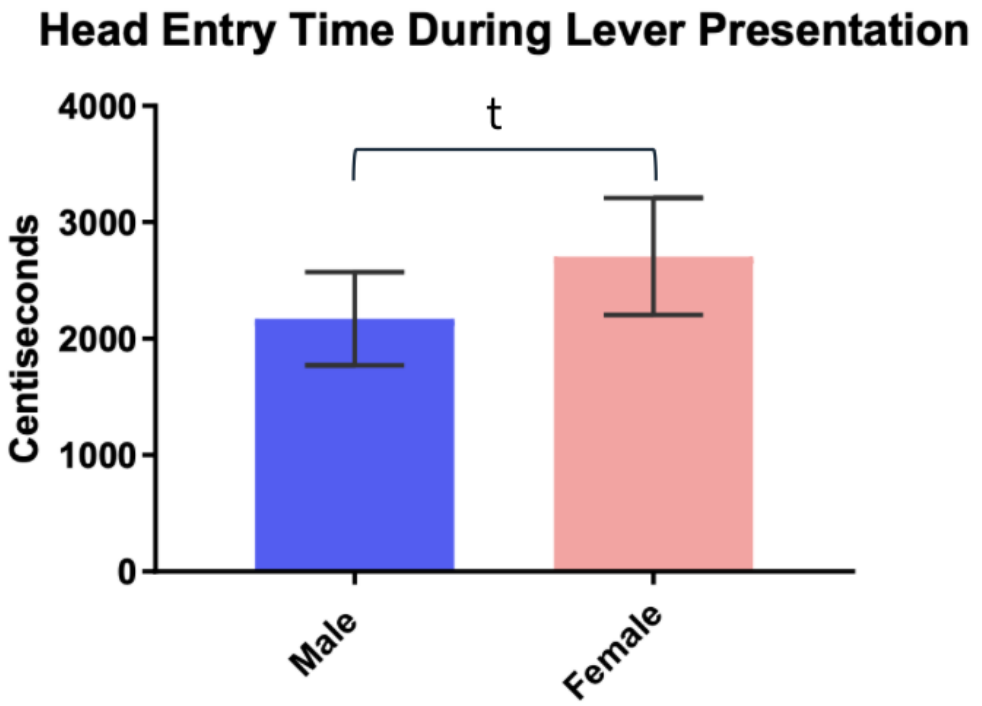

Figure 15. Main Effect of Sex on CS Entries During Lever Presentation in Contingency Degradation.

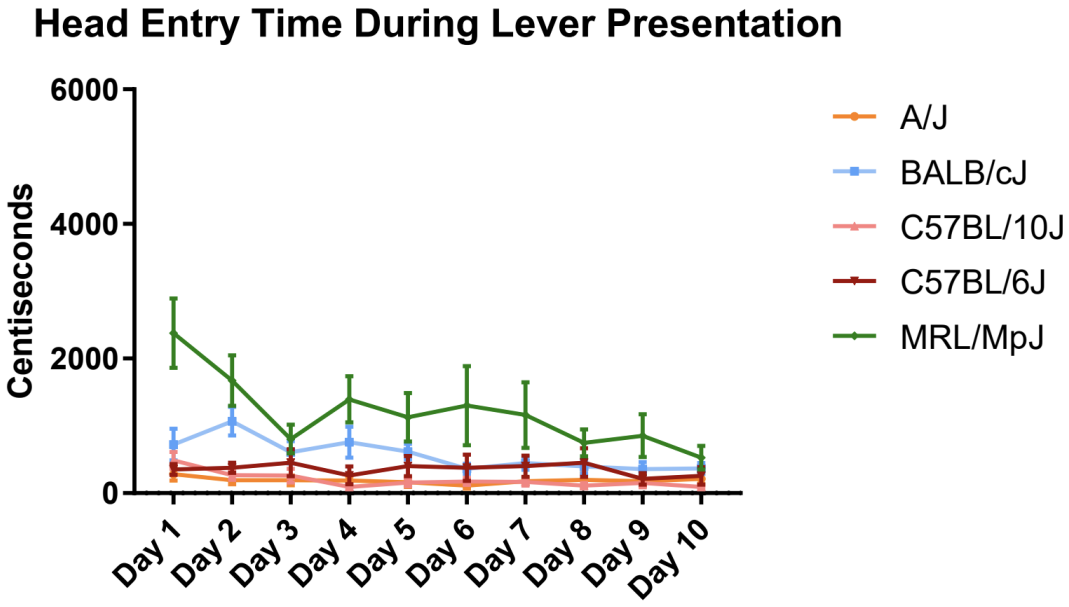

Figure 16. Day x Strain CS Entries During Lever Presentation in Extinction.

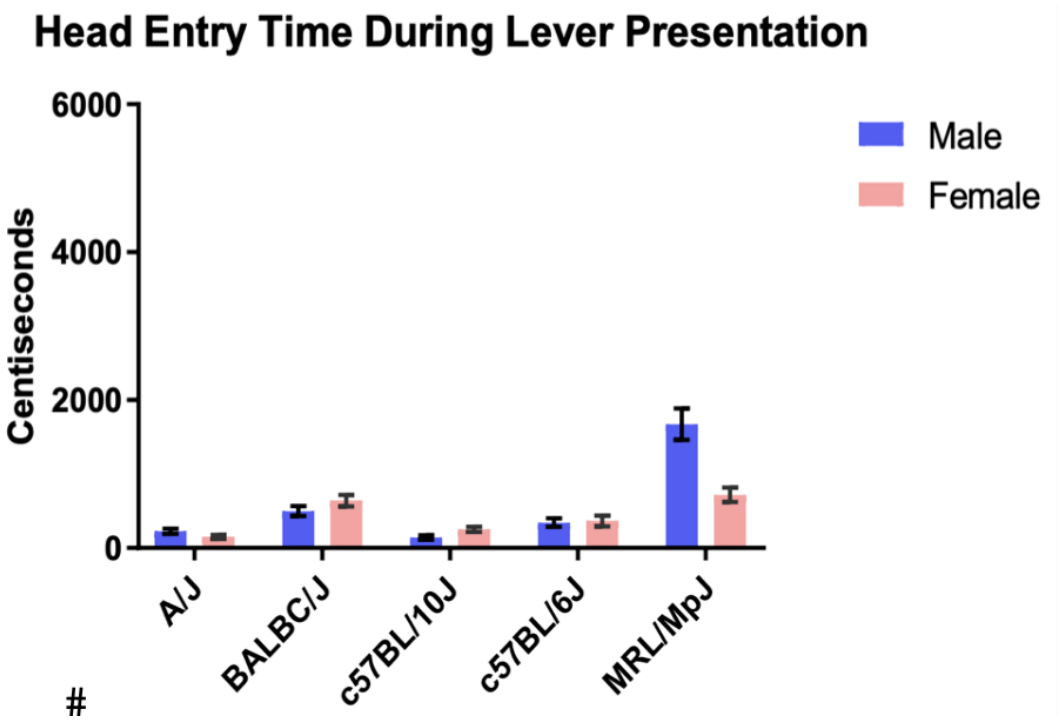

Figure 17. Sex x Strain CS Entries During Lever Presentation in Extinction.

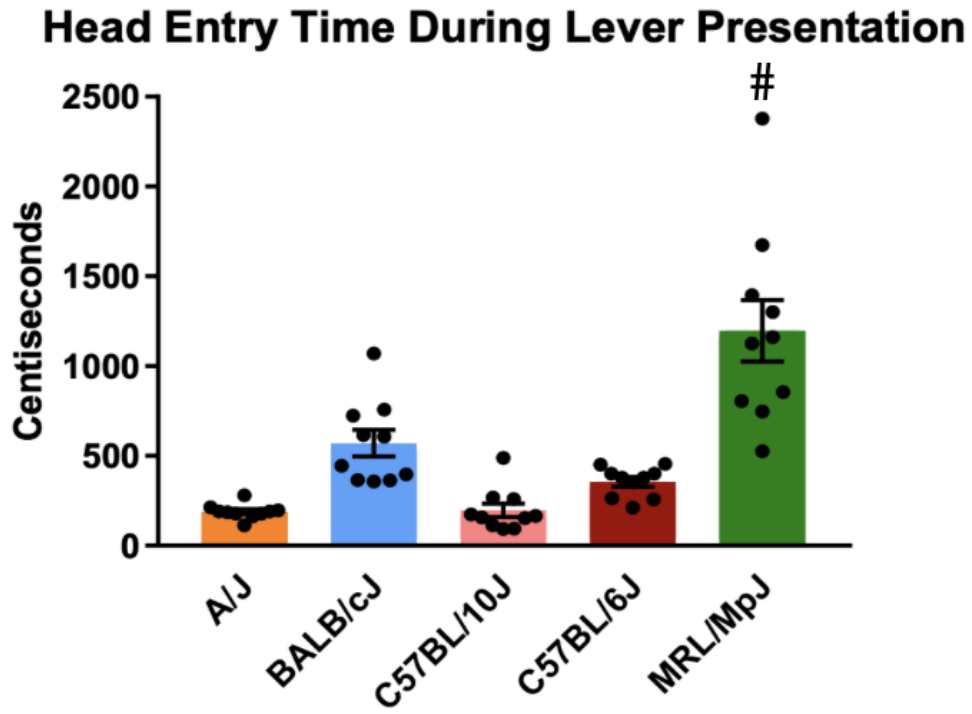

Figure 18. Main Effect of Strain on CS Entries During Lever Presentation in Extinction.

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