Impacts of Stress During Early Puberty on Tracking Behavior in Hamsters

Yahaira Scarlett Leon

Honors Research Thesis

Department of Psychology, The University of Texas at Austin

Faculty Advisor: Yvon Delville, PhD

May, 2020

Abstract

In classical conditioning paradigms, sign-tracking (ST) behavior is classified by incentive salience attributed to a reward-associated cue instead of the reward itself. Goal-tracking (GT) behavior on the other hand, is when incentive salience is attributed to the reward instead of the reward-associated cue. The study investigated any possible effect of chronic stress during puberty on tracking behavior during adulthood. Experimental male golden hamsters (n=10) were exposed to social stress during early puberty by placing them in cages of adult male hamsters once a day from Postnatal Day 28 to Postnatal Day 42, while those in the control group (n=10) were placed in new clean empty cages during the process of subjugation. During adulthood, hamsters were placed in chambers where they learned to associate the onset of two LED light cues to the release of a food reward at the food cup on the other side of the chamber. Contrary to what was hypothesized, stress during early puberty did not impact tracking behavior in adulthood, however it did seem to negatively impact learning behavior.

Keywords: stress, impulsivity, sign-tracking, goal-tracking

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Stress affects behaviors including aggression and impulsivity. Several studies have revealed that stress induced via social subjugation in animals during puberty impacts impulsivity, the development of agonistic behavior and aggression later in life (Delville, Melloni Jr, & Ferris, 1998; González-Martínez, D'Aigle, S. Lee, H. Lee, & Delville, 2017; Wommack & Delville, 2003). Besides its impact on behavior, social subjugation in animals during puberty has also been found to cause changes in several neural networks of the brain (Delville et al., 1998). There are also physiological changes induced by subjugation and social stress including changes in weight and hormone levels in animals (Delville et al., 1998; Foster, Solomon, Huhman, & Bartness, 2005; Wommack & Delville, 2003).

Due to the other effects stress has on the brain and behavior, it seems reasonable to think that it might impact tracking behavior. Tracking behavior consists of sign-tracking (ST) and goal tracking (GT). Animals expressing ST behavior tend to interact with a reward cue more often than the reward itself, while animals expressing GT behavior interact with the reward or the reward site more often. Lovic, Saunders, Yager, and Robinson (2011) found that impulsive action was more prevalent among rats that showed ST behavior, and González-Martínez et al. (2017) found that stress during puberty causes condition-dependent effects on impulsivity. Based on this, it is suggested that social subjugation during adolescence might have an impact in tracking behavior. The focus of my study is to investigate how stress via social subjugation behaviorally shapes and impacts the predisposition to sign tracking behavior. This is important given that sign-tracking behavior has been previously associated with impulsivity and vulnerability to substance abuse in an individual (Robinson et al., 2011; see Robinson et al., 2014 for a review). Individuals predisposed to ST behavior are likely to be more vulnerable to

substance abuse due to behavioral and neurobiological reasons which will be discussed later in

this review (see Robinson, Yager, Cogan, & Saunders, 2014 for a review; Saunders & Robinson,

2010; Saunders, Yager, & Robinson, 2013).

Unpublished data suggests that non-subjugated golden hamsters tend to be sign-trackers.

Based on this and the notion that stress during early puberty lowers waiting impulsivity, which is

associated with goal tracking behavior, it is thus suggested that GT behavior will be more

prevalent amongst subjugated animals.

Stress and Impulsivity

Impulsivity is composed of two different behavioral expressions, impulsive action and

impulsive choice (Brevers et al., 2012). Impulsive action is defined by low action inhibition and

high waiting impulsivity. Low action inhibition refers to having poor inhibition of a conditioned

response associated with a reward. For example, animals can show low action inhibition by

continuously pressing a lever associated with a reward even after extinction of the reward.

Waiting impulsivity refers to the inability to tolerate delayed rewards (Brevers et al., 2012;

González-Martínez et al., 2017). This can be seen in animals who continuously keep pressing a

lever associated with a reward even after delays in the reward have been introduced. Impulsive

choice on the other hand, is defined by impulsively choosing to receive an immediate suboptimal

reward over a delayed but greater reward (Brevers et al., 2012; González-Martínez et al., 2017).

González-Martínez et al. (2017) revealed that stress during puberty has context-

dependent effects on impulsivity during adulthood in hamsters. Heightened impulsive action was

seen in subjugated hamsters in tasks meant to test action inhibition. However, in the same study

after delays were introduced, subjugated hamsters were able to successfully inhibit a conditioned

response associated with a reward significantly faster than hamsters that were not subjugated.

These data might suggest that the control group of hamsters were more capable of inhibiting an action, but were not able to wait as long before initiating an action, as the subjugated hamsters. In other words, when compared to the control group, subjugated hamsters had poorer action inhibition but lower levels of waiting impulsivity. This suggests that social stress during early puberty lowers waiting impulsivity later in life (González-Martínez et al., 2017).

In a different study, hamsters exposed to stress during early puberty were more likely to attack non-threatening intruders than hamsters that were not socially subjugated (Delville et al., 1998). Besides an increase in aggression, these context dependent attacks could also be due to an increase in impulsivity, causing a decrease in the ability to inhibit aggressive behavior. This notion is further supported by a study showing that, unlike high-aggression hamsters (hamsters that showed more aggressive behavior than low-aggression hamsters), low-aggression hamsters were able to tolerate and adapt to a newly introduced delay in reward by successfully inhibiting lever pressing (David, Cervantes, Trosky, Salinas, & Delville, 2004). This reveals a positive correlation between highly aggressive hamsters and impulsivity. Based on these studies, we can conclude that hamsters that were stressed during puberty are more likely to have lower levels of action inhibition and waiting impulsivity.

Impulsivity and Tracking Behavior

In conditioning paradigms, cues paired with an unconditional stimuli (US) such as a food reward, become conditional stimuli (CS) when they induce a conditional response (CR). Incentive salience consists of motivational properties invoked by a reward. However, when incentive salience becomes attributed to cues associated with rewards, these cues start becoming alluring and desirable, turning into incentive stimuli which motivates behavior to invoke a conditional response (see Jones & Neria, 2019 for a review; Robinson et al., 2011). Animals

placed in conditioning chambers who pay more attention to the cue than the reward are considered sign-trackers (STs), while those that pay more attention to the reward instead of the cue are considered to be goal-trackers (GTs) (see Jones & Neria, 2019 for a review; Robinson et al., 2011).

In a previous study it was found that rats selectively bred to express high reactivity to a novel stimuli showed higher levels of sign-tracking and impulsive action, but lower levels of impulsive choice compared to rats that were bred to express low reactivity to a novel stimuli (Flagel, Waselus, Clinton, Watson, & Akil, 2010). Robinson et al. (2011) also revealed that sign-tracking rats were less capable of tolerating delayed rewards than goal-tracking rats, thus showing higher levels of waiting impulsivity, a form of action impulsivity.

Variations in tracking behavior have been found in humans as well as rodents. Morrow, Gheidi, Cope, and Billing (2019) translated a rat Pavlovian conditioning approach (PCA) task for human subjects using a lever (CS) as a cue for the release of a reward via a reward magazine in a different location. This PCA task was applied to twenty-one college students from the ages of eighteen to twenty-five in order to test sign tracking and goal tracking by tracking of eye orientation. Impulsivity measurements amongst these subjects were taken as well via a questionnaire-based system and compared to their tracking behavior in the PCA task. It was revealed that sign-trackers were more likely to have higher traits of impulsivity. In general, this supports a relationship between ST behavior and impulsiveness in humans as well as rats in similar conditioning contexts.

Numerous other studies have shown that impulsivity is more prominent amongst sign-trackers than goal-trackers in both animals and humans (e.g. Flagel, Akil, & Robinson, 2009; Morrow et al., 2019; Robinson et al., 2011). If waiting impulsivity is decreased in hamsters that

were stressed during puberty, those hamsters could be expected to have higher levels of goal-tracking behavior compared with hamsters who were not subjugated to stress during puberty.

Tracking Behavior and Substance Abuse Disorder

It is well established that sign-tracking behavior is associated with substance abuse disorders (e.g., Saunders & Robinson, 2010; Yager & Robinson, 2013; see Robinson et al., 2014 and Tomie, Grimes, & Pohorecky, 2007 for a review). For example, removal of a cue associated with cocaine decreased self-administration of cocaine in ST rats but not GT rats (Saunders & Robinson, 2010). This same study also found that ST rats showed more resistance than GT rats to an extinction process designed to diminish the association of a cocaine reward to a cocaine-associated cue, as seen by higher responses to the previously cocaine-associated cue in ST rats during the extinction phase. Reinstatement of a cocaine-associated cue was more prominent amongst ST rats than GT rats as well (Saunders & Robinson, 2010). These data suggest that sign-trackers could be more vulnerable to substance abuse disorders and relapse than goal-trackers, due to the incentive salience associated to the cue instead of the reward itself.

While most studies have found that cues associated with rewards, such as cocaine, opioids and food, are approached more often by ST rats than GT rats, one study found that nicotine associated cues were approached just as often by GT rats as ST rats (Yager & Robinson, 2015). These data suggest that nicotine-associated cues, unlike other reward-associated cues such as food, opioids or cocaine, are attributed to incentive salience to the same degree in both ST and GT rats. However, nicotine cues acted as a stronger conditioned reinforcer in ST rats than GT rats, suggesting that the incentive motivational properties of cues associated with nicotine are more prominent amongst ST rats than GT rats. This suggests that motivational properties and incentive salience attributed to reward cues can vary depending on the type of reward the cue is

associated with. These variations suggest that the association of sign-tracking behavior to substance abuse disorder is not limited to just behavioral phenotypes, but encompasses changes in neural networks associated with reward circuitries such as the mesolimbic pathway as well.

The Mesolimbic Pathway, Stress, and Tracking Behavior

The mesolimbic pathway is involved in the reward circuitry of the brain and includes dopaminergic projections in the ventral tegmental area (VTA) and the nucleus accumbens (NA). ST rodents and GT rodents are known to have differences in dopaminergic projections of the mesolimbic pathway (Gillis & Morrison, 2019; Saunders et al., 2013). Even when cocaine was co-administered with a shock punishment, ST rats were more likely than GT rats to seek cocaine self-administration when a cue associated with a cocaine reward was present (Saunders et al., 2013). This same study found that an injection of a dopamine (DA) antagonist into the nucleus accumbens (NA) core of rats significantly reduced self-administration of cocaine in the presence of cocaine-associated cues in ST rats. While cocaine-seeking behavior decreased in both GT and ST rats after the injection of the dopamine antagonist, the reduction was significantly greater in ST rats and was not statistically significant in GT rats. However, GT rats showed little and less cocaine-seeking behavior than ST rats overall.

Saunders et al. (2013) observed self-administration of cocaine in the presence of cocaine-associated cues in ST and GT rats that had received injections of amphetamine in the nucleus accumbens to increase dopamine activity. Amphetamine affects dopamine's psychoactive function by blocking reuptake, allowing for dopamine to stay within the synaptic cleft for a longer period of time. Saunders et al. (2013) found that amphetamine increased cocaine-seeking behavior in ST rats by increased self-administration in the presence of cocaine-associated cues.

The findings described above suggest that craving for drugs by STs in the presence of a reward-associated cue is mediated by dopamine projections in the mesolimbic dopaminergic system. This in turn suggests that differences in the functioning of the mesolimbic dopaminergic system in STs from GTs cause STs to attribute incentive salience to a reward-associated cue instead of the reward itself. This could cause sign-trackers to be more vulnerable to relapse when exposed to cues associated with the abused substance and makes it more difficult to extinguish the association between reward cues and the actual reward (see Robinson et al., 2014 for a review; Saunders et al., 2013).

The mesolimbic dopaminergic system includes dopaminergic projections from the ventral tegmental area (VTA) to the NA. This neural network has been found to be associated with stress, tracking behavior and substance abuse (Berton et al., 2006; Tidey & Miczek, 1996; see Trainor, 2011 for a review). Tidey & Miczek (1996) conducted a study in which dopamine concentrations on the terminal areas of the mesolimbic dopaminergic system were measured in two groups of rats. One group of rats were exposed to stress via social defeat and the other group of rats (control group) were not exposed to social defeat. It was found that when rats were transferred to a novel or resident's cage, dopamine levels in the NA and prefrontal cortex (PFC) increased by about 130% of baseline levels. However, in rats that experienced social defeat, dopamine levels in the NA and PFC were about 160% of baseline levels. The greater increase in levels of dopamine concentrations found in the NA and PFC of animals that were stressed via social defeat could reflect increased attention to threatening stimuli and/or induced neurobiochemical changes in response to the social threat and stress of defeat.

Conclusions

Early exposure to stress or trauma can have long lasting consequences in humans. The purpose of my research is to study specific behavioral alterations associated with sign-tracking, impulsivity and substance abuse as a result of early stress. Sign-trackers (STs) are individuals who orient themselves to the conditioning cue. Goal-trackers (GTs) are individuals who orient themselves to the reward (Robinson et al., 2011). These tracking behaviors have been associated with the neurobiology of substance abuse, as the activity of dopaminergic neurons associated with reward differs between STs and GTs (Gillis & Morrison, 2019; see Jones & Neria, 2019 for a review; Saunders et al., 2013). In animal studies, substance abuse and impulsivity have been associated with sign-tracking and goal-tracking behaviors (Saunders & Robinson, 2010; see Robinson et al., 2014 for a review; Robinson et al., 2011). Under classical conditioning paradigms, animals learn to associate a cue with a reward. Furthermore, there is also an association with impulsivity, as STs are more impulsive than GTs, under tests addressing waiting impulsivity, a form of impulsive action (Robinson et al., 2011). As for substance abuse, the behavior is mediated by dopamine projections in the mesolimbic system from the VTA to the NA (Gillis & Morrison, 2019; see Salamone & Correa, 2012 for a review).

While several studies have shown long-lasting behavioral impacts in hamsters caused by stress, it is still unclear whether stress or trauma during early puberty directly impacts tracking behavior. For example, high waiting impulsivity was more prominent amongst hamsters that were not stressed and hamsters exhibiting sign-tracking behavior (González-Martínez et al., 2017; Robinson et al., 2011). Data collected from recent pilot studies suggest that previously stressed hamsters were more likely to be goal-trackers. However, the behavioral categorization of GTs in these pilot studies was potentially complicated by issues in the apparatus design which

are corrected in the present study. The purpose of this study is to thoroughly and adequately

investigate how early stress during puberty directly impacts tracking behavior which has been

found to be associated with impulsivity and substance abuse.

Methods

Design Overview

The experiment studied how stress induced during early puberty in hamsters alters tracking behavior later in life. It is hypothesized that stress induced via social subjugation during early puberty in golden hamsters causes hamsters to become goal-trackers later in life, while hamsters that are not stressed during early puberty are more likely to become sign-trackers. The experimental group consists of 10 male golden hamsters that were exposed to stress via social subjugation by an older hamster from Postnatal Day 28 (P28) to Postnatal Day 42 (P42). The control group consists of 10 male golden hamsters that were not subjugated to social stress. Later in adulthood, hamsters were placed in conditioning chambers and trained to expect a food reward to appear at the food cup whenever the light cues on the left turn on. Tracking behavior was quantified using pavlovian conditioned approach index (PCAI), latencies to arrive at the cue area and food cup area after cue onsets, and time spent around the food cup and cues. These measures serve as dependent variables that were compared between the control and experimental group (independent variable), over day, and between groups over day, in order to investigate how and to what degree stress during puberty impacts tracking behavior.

Subjects

A total of 20 golden hamsters were used that were bred in the laboratory. Breeder hamsters were derived from a colony obtained from Harlan Sprague–Dawley (Indianapolis. IN). Hamsters were kept in a reverse light cycle and housed with their dams until Postnatal Day 25 (P25). On Postnatal Day 7 (P7) each litter was culled to 4 males and 2 females. On P25 hamsters were weaned and placed in their own plexiglass cages. Ten male hamsters were randomly and evenly assigned to each group. Hamsters received food and water ad libitum and all behavioral

tasks were performed during the dark cycle. Hamsters were housed at the Animal Resource Center at the University of Texas at Austin, an AAALAC-accredited facility. The IACUC from The University of Texas at Austin approved of all the procedures in this experiment.

Social Subjugation.

Hamsters in the experimental group were exposed to stress via social subjugation from P28 to P45, while those in the control group were not subjugated to stress. Stress via social subjugation was induced by placing the experimental hamsters in randomly selected cages of bigger adult male golden hamsters during the first half of the dark cycle. In order to control for any behavioral stress that might have been induced by the transfer of cages, hamsters in the control group were transferred to clean empty new cages. Offensive responses by resident adult hamsters were classified by attacks and bites. Submissive responses by the juvenile intruders were measured by on-back postures and tail-up displays, while avoidance behavior was classified by running away from the resident. Flank marks by residents expressing territorial behavior were also observed. Intruders were surveyed for any injuries after each subjugation session. If any major injuries were observed in an animal, they were removed from the study. Subjugation procedures were performed under dim red light. When hamsters were transferred from one room to another, their cages were covered with black plastic bags in order to reduce light and sound.

Conditioning Methods

Materials. Behavioral tasks were executed in commercially available individual conditioning chambers (Coulbourn Instruments, Allentown, PA) with stainless steel rod floors, clear acrylic front and back walls, aluminum ceiling and sidewalls (Fig. 1). Chambers contained five circular openings at the bottom of the left sidewall and a magazine on the right sidewall that

delivers food pellets (Dustless Precision Pellets®, 45 mg, Primate Purified Diet, Banana flavor, Bio Serv, Flemington, NJ) onto a food cup easily accessible to the hamsters. Each opening, and the food cup was equipped with an infrared beam in order to detect nose pokes. Light and sound reducing boxes were used to individually enclose each conditioning chamber. Each chamber was equipped with a camera for video recording as well. Openings were equipped with a light that when turned on acted as a cue signaling reward. Chambers were controlled by Graphic State software (Coulbourn Instruments, Allentown, PA). BORIS software was used for video and behavioral analysis.
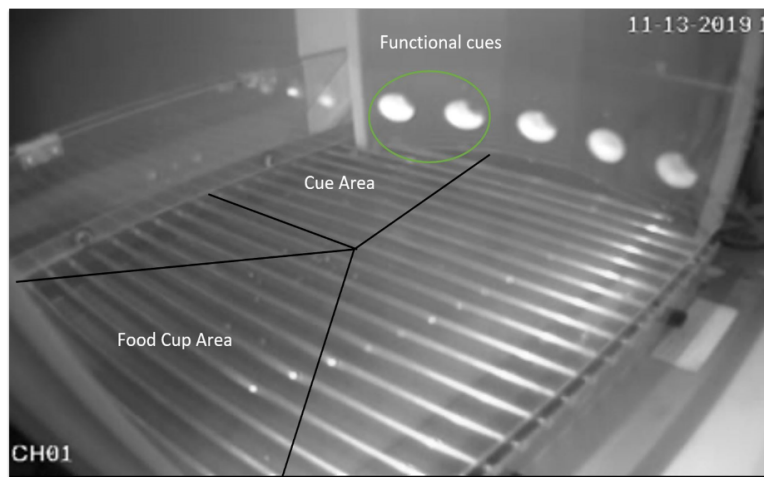

Figure 1. Areas of the conditioning chamber are depicted.

Conditioning. Hamsters were placed in the conditioning chambers for 20 minutes with the light and sound attenuating boxes closed in order to reduce light and noise. Hamsters underwent conditioning magazine training for the first two days. During magazine training 20 pellets were deposited onto the food cup over random variable intervals of 60 seconds in order for the hamsters to associate the food cup and conditioning chamber to a food reward. After magazine training, the main task was performed in the conditioning chambers with light and noise attenuating boxes closed. Each test subject underwent magazine training once a day for two days during the first half of the dark cycle. After magazine training, each test subject

underwent the main task once a day for five days during the first half of the dark cycle. During the main task the two reward-associated cues on the left lit up for 8 seconds as food dropped from the magazine onto the food cup. All behavioral tasks were performed during the first half of the dark cycle inside chambers enclosed by light and noise reducing boxes.

Behavioral Measures. Tracking behavior was assessed by area orientation and latency measures. Area orientation was identified by time spent around a specific area. Chambers were divided into two areas, the cue area and food cup area, refer to Figure 1. The two openings on the left served as reward-associated cues that predicted food pellets at the food cup on the opposite side of the chamber when lit up. The area around the left two openings was considered to be the "cue area". The opening in the middle did not light up. The area around the food cup on the opposite side of the openings was considered to be the "food cup area". Food cup area orientation, and cue area orientation were measured by the percentage of time the test subject spent in a specific area.

Latency was measured from the time that cues lit up to the time the test subject approached a certain area of the chamber. Cue to cue latency was measured from the time of onset of the two left cues to the time the test subject approached the cue area on the left. Cue to food cup latency was measured from the time of onset of the two cues on the left to the time the test subject approached the food cup area. All measures were recorded and logged using video playback via BORIS software.

Sign-tracking behavior is classified by an increase in cue area orientation relative to food cup area orientation, along with shorter cue latencies relative to food cup latencies. Goal-tracking behavior is classified by an increase in food cup area orientation relative to cue area orientation, along with shorter food cup latencies relative to cue latencies.

Statistical Analyses

Area orientation in both groups of hamsters was quantified by the percentage of time spent around a specific area, while latencies were measured from the time of cue onset to the time the cues, or food cup was approached. The pavlovian conditioned approach index (PCAI) was calculated by subtracting the average cue latency from the average food cup latency, then dividing the subtraction by the response time. This measure is an indicator of incentive salience attributed to the cue. A relatively high PCAI indicates ST behavior. The average response time for a hamster is 20 seconds. As mentioned before, PCAI, latency, and area orientation measures were analyzed. Area orientation measures included time spent around the food cup area (food cup orientation) and time spent around the cue area (cue orientation). Latency measures included cue to cue area latency (cue latency) and cue to food cup latency (food cup latency). BORIS was used to quantify both area orientation and latency measures by recording the time lapse between the onset and offset of a state event. During procedural tasks, videos were recorded and then analyzed using BORIS by manually recording the onset and offset of a state event to get a time lapse for area orientations and latencies. Each state event was specific to either an area orientation or latency measure.

When analyzing area orientation, the chamber was divided into two different areas as shown in Figure 1 and the onset of a specific state event was logged as soon as a subject entered that specific area. The offset of that specific state event was logged as soon as the subject left the specific area. For each specific area orientation measure, the time lapses of each state event associated with the specific measure were added then divided by the total amount of time the session constituted of in order to get the percentage of time the subject spent around that specific area. Each session constituted of one specific test subject on one specific day. The amount of

time each session lasted was recorded using BORIS by assigning a "start/stop session" state event to each session. The start of a session was manually recorded by logging the onset of that specific state event as soon as the light and sound attenuating boxes were closed. The end of a session was manually recorded by logging the offset of the "start/stop session" state event as soon as the light and sound attenuating boxes were opened. The time lapse of a "start/stop session" state event determined the amount of time a session constituted of.

For latencies, state events were also used to indicate the time lapse from the onset of the cues to the time the subject reached the cue area or food cup. To do this, the onset of both "cue latency" and "food cup latency" state events were manually logged as soon as the light cues turned on; the offset of a specific latency state event was logged as soon as a subject entered the area associated with the specific latency measure. If a subject did not arrive to any area within 20 seconds, the logged onset of the state events were discarded. Because of this, the response rate was not recorded. Each session constituted of one specific test subject on one specific day. "Start/stop session" state events were also logged for all sessions as previously explained. The average cue and food cup latencies were recorded for each hamster per day by taking the mean of all recorded cue and food cup latencies for each session.

For both area orientation measures, the percentage of time spent around a specific area was taken for each subject, each day. Data was then sorted out into the control and experimental group, and the mean and standard error of each area orientation measure was calculated for each group, each day. This was done for each of the 5 days. For latency measures, the average food cup and cue latencies were taken for each subject, each day. Averages for each latency measure were then sorted out into the control and experimental group, then the mean and standard error of each latency measure was calculated for each group, each day. This was done for each of the 5

days. PCAI was calculated for each hamster, each day, using the average cue and food cup latencies for each hamster, each day. PCAI measures were then sorted into the control and experimental group, and the mean and standard error was taken for each group, each day. This was done for each of the 5 days. As previously stated, PCAI is calculated by subtracting the average cue latency from the average food cup latency, then dividing the subtraction by the average response time of 20 seconds.

Linear mixed-effect models (LMM) were applied to test the effects of chronic stress during puberty on PCAI, cue latency, food cup latency, cue orientation, and food cup orientation, between groups, overtime, and between groups overtime. Individual two-tailed t-tests were applied to each day to test for differences between groups in cue orientation, food cup orientation, cue latency, food cup latency and PCAI. After an increase in PCAI was observed from days 1-4 to day 5, in depth linear mixed-effect modeling post hoc tests were applied to cue latency and food cup latency measures on specific time frames to find any differences between groups, over specific time frames, and between groups over a specific time frame.

R v3.6.0, a data science program used to conduct in depth statistical analysis, was used for all statistical analyses in this study (R Core Team, 2014). "lme4" and "lmerTest" packages were used to perform all linear mixed-effect models . The random effects for all models were the measurement data of the test subjects. Group, day, and group by day effects were fixed to allow for analysis of measurements between groups, over time, and between groups over time. In order to determine the best fitting model for each LMM analysis, the model with the lowest Akaike information criteria (AIC) value was chosen. LMM results were presented as (b = beta coefficient $\pm$ standard error; n = number of observations in analysis; p = p value). Satterthwaite's method using an alpha of 0.05 (two-tailed) was used to derive all p values. Individual two tailed

t-tests were conducted on each day using an alpha level of 0.05 to find any statistically significant differences between groups. T-test results were presented as [Control: mean ± standard deviation; Experimental: mean ± standard deviation; t(df) = t value with degrees of freedom; p = p value].

Results

Area Orientation

Linear mixed-effect models were applied to test the effects of chronic stress during early puberty on each area orientation measure, and no significant differences in cue or food cup orientation were found between groups or groups over time. However, based on these models, overall food cup orientation significantly increased overtime (b = 1.17 ± .34; *n* = 10; p < .001), and overall cue orientation significantly decreased overtime (b = -1.2 ± .532; *n* = 10; p < .05).
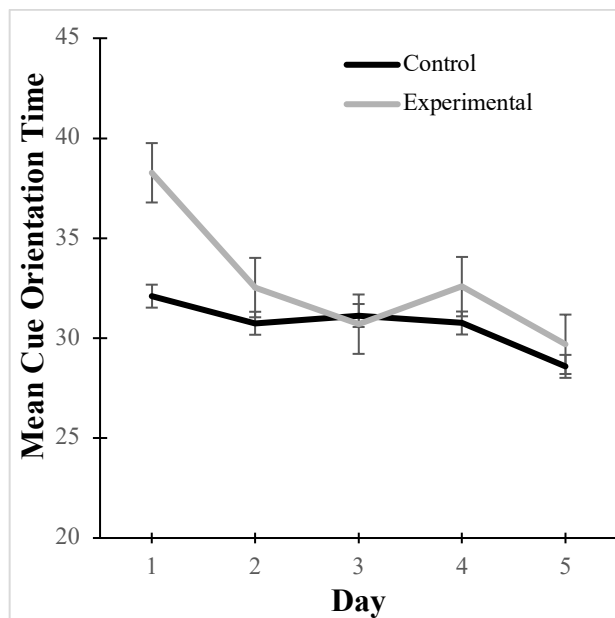
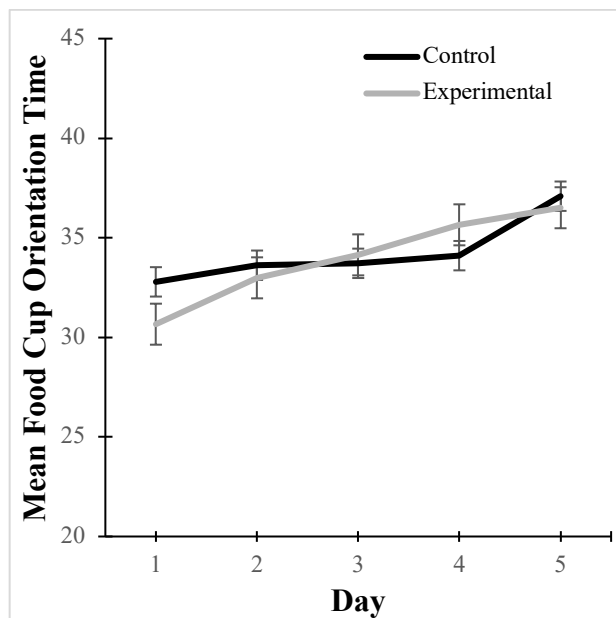Figure 2. Overall, mean cue orientation significantly decreased overtime (b = -1.2 ± .532; *n* = 10; p < .05). Data are M ± SE.

Figure 3. Overall, mean food cup orientation significantly increased overtime (b = 1.17 ± .34; *n* = 10; p < .001). Data are M ± SE.

Individual T-tests were used on each day to test for differences between groups on cue orientation and food cup orientation measures. After Bonferroni corrections were applied, no significant differences were found between the control and subjugated group on any day.

Latency Measures

Linear mixed-effect models were applied to test the effects of day, stress (group), and stress over day (group by day) on each latency measure. No statistically significant differences in

cue or food cup latency measures over time, between groups, or between groups over time were found.
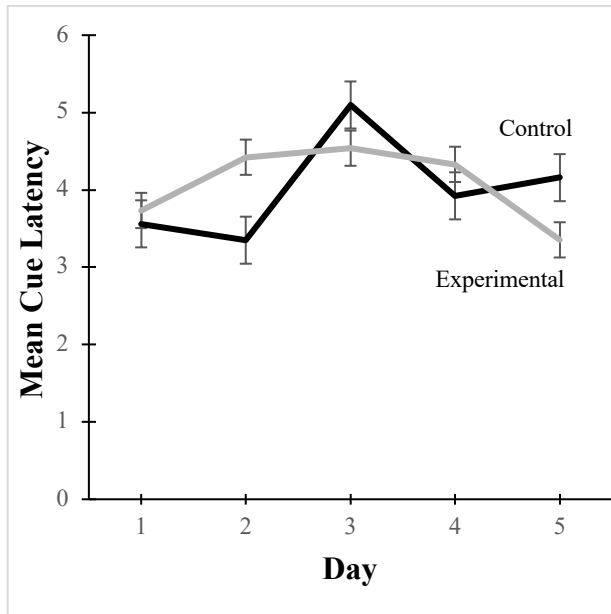

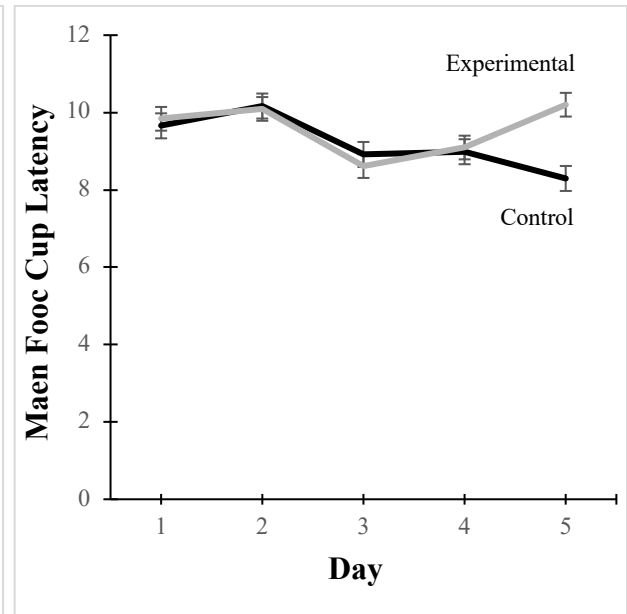Figure 4. No main effects of day, group, or group by day on cue latency measures were found. Data are M ± SE.

Figure 5. No main effects of day, group, or group by day on cue latency measures were found. Data are M ± SE.

Individual T-tests were then used on each day to test for differences between groups on cue latency and food cup latency measures. After Bonferroni corrections were applied, no significant differences were found between the control and subjugated group on any day.

PCAI Results

As previously stated, PCAI was calculated by subtracting the mean cue latency from the mean food cup latency, then dividing the subtraction by the average response time of a hamster (20 seconds). As stated in the statistical analysis section, PCAI was calculated for each subject, each day. The data was then sorted into the experimental and control groups and the mean PCAI was taken for each group, each day. Linear mixed-effect models were applied to test the effects of day, group, and group over day, on PCAI measurements. However, no main effects of day, group or group over day were found on PCAI measurements.
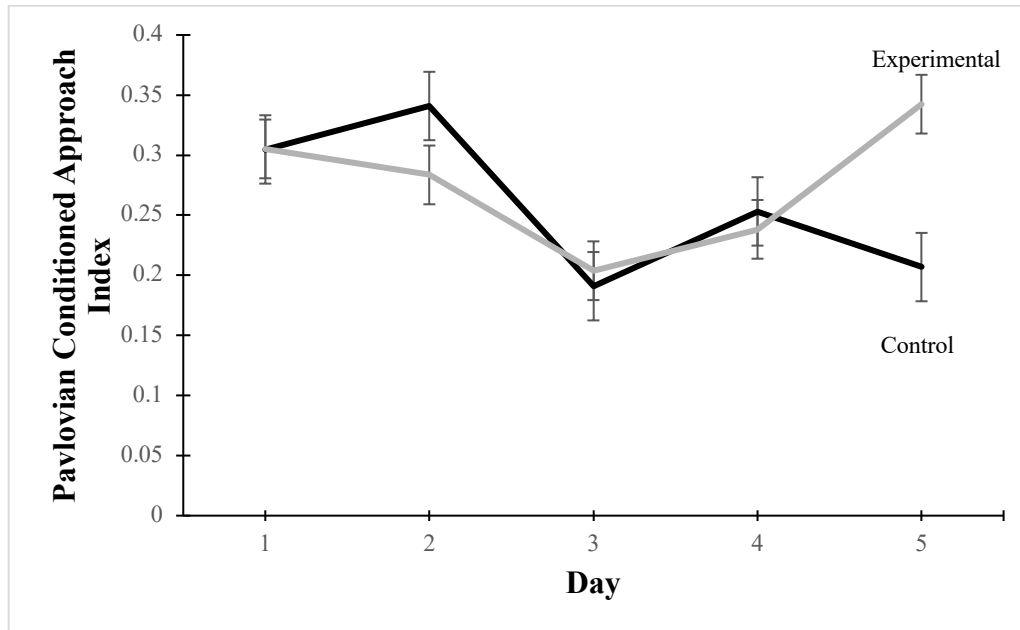
Figure 6. No main effects of day, group, or group by day on PCAI measures were found. Data are M ± SE.

Individual T-tests were then used on each day to test for differences between groups on PCAI measures. Only day 5 showed a statistically significant difference between groups. On day 5, the average PCAI was significantly higher for hamsters in the experimental group [Control: .207 ± .124; Experimental: .342 ± .119; $t(18)$ = -2.49; p = .023]. However, after Bonferroni corrections were applied, no significant differences were found between the control and subjugated group on any day.

Discussion

As stated in the results section, no significant differences in cue or food cup orientation were found between groups or groups over time, however overall food cup orientation significantly increased overtime, while overall cue orientation significantly decreased overtime. Regarding latency measures, no statistically significant differences in cue or food cup latencies between groups, over time, or between groups over time were found. No main effects of day, group, or group over day, on overall PCAI measurements were found in the applied linear mixed-effect model (LMM). Individual t-tests were applied to each day in order to find any statistically significant differences between groups. On day 5 the PCAI was significantly higher in the experimental group, however after application of Bonferroni corrections, no significant differences were found between the control and subjugated group on any day.

Because impulsive action is more prevalent among rats that show ST behavior, and stress during puberty has been found to decrease action inhibition and waiting impulsivity, it was hypothesized that animals stressed during early puberty were more likely to show signs of goal tracking behavior (González-Martínez et al. 2017; Robinson et al., 2011). This was further supported by running pilot studies in which subjugated hamsters appeared to be spending more time at the feeder than the cue while control hamsters appeared to be spending more time at the cue than the feeder. However, these pilot studies were problematic since the cue was a lever located next to the food cup, this could have introduced human error when analyzing area orientation during video analysis. I ran my study in order to properly investigate this hypothesis with a more accurate model in which the cues were LED lights located on a chamber wall opposite to the wall where the feeder was located at. Based on the hypothesis that stressed hamsters were more likely to become goal-trackers (GTs), I expected to see a higher food cup

orientation, lower cue orientation, shorter food cup latencies, longer cue latencies and lower

PCAI in the experimental group. However, based on the results, this was not exactly the case.

The lack of differences in area orientation between groups and groups over time suggest

that stress did not impact the amount of attention given to a specific area. However, the increase

in food cup orientation overtime, and decrease in cue orientation overtime, suggests that

hamsters learned to expect food at the food cup. While I expected a decrease in cue latency over

time in experimental hamsters, results showed lack of differences in cue latencies over time,

between groups, and between groups over time. This suggests that stress during puberty had no

impact on incentive salience attributed to the cue, failing to support the hypothesis that stress was

likely to decrease incentive salience associated to the cue. Regarding food cup latencies, I was

expecting to see an increase over time in hamsters that were stressed, however results showed no

differences in food cup latencies between groups, over time, and between groups over time. This

suggests that stress during puberty had no impact on incentive salience attributed to the food cup,

failing to support the hypothesis that stress was likely to increase incentive salience associated to

the food cup.

The Pavlovian Conditioned Approach Index is a measure of how well a subject is

conditioned. A high PCAI shows less conditioning and a low PCAI shows better conditioning,

but PCAI is also used to spot tracking behavior. Goal-trackers (GTs) are more likely to have

shorter food cup latencies and higher cue latencies, thus a lower PCAI; sing-trackers (STs) are

more likely to have shorter cue latencies, and higher food cup latencies, thus a higher PCAI.

Initially, I expected to see a decrease in PCAI over time in the stressed group because it was

hypothesized that stressed hamsters were more likely to be goal-trackers. In the applied linear

mixed-effect model, no main effects of group (stress), time (day) or group over time, were found

on PCAI, however t-test showed differently. Individual t-tests were conducted to look for any differences between groups on a specific day. On day 5, average PCAI of the experimental group was significantly higher than that of the control group, however after application of Bonferroni corrections, no significant differences were found between the control and subjugated group on any day. Normally, Bonferroni corrected p-values are usually the values considered when determining significance. However, because the data collected for PCAI measures were inconsistent in differences and variance between days, I decided to analyze t-tests for PCAI measures individually, meaning t-tests were applied to each day individually to analyze differences in PCAI between groups on a specific day only, and not how they relate to other days. Because t-tests were applied and analyzed individually for each day, Bonferroni corrections could be disregarded. As seen in Figure 6., the inconsistency in data variance between days is especially noticeable when graphed. On Figure 6., from day 1 to day 4, PCAI seems to be decreasing over time in both groups at the same pace, however on day 5 PCAI between both groups dramatically changes. From day 4 to day 5, PCAI in the experimental group increases at a faster rate, while PCAI in the control group decreases at a faster rate. This dramatic and inconsistent change in PCAI between days 1-4 and day 5, suggests that hamsters might have started showing differences in learning on day 5. This suggestion is consistent with collected food cup latency data. As seen on figure 5., latencies from onset of cues to arrival at food cup got slightly shorter over time at the same pace for both groups from day 1 to 4. However, on day 5, food cup latencies between the experimental and control group drastically changed. From day 4 to 5, food cup latencies increased at a faster pace for the experimental group, while they decreased at a faster rate for the control group. While at first glance these findings might suggest that the experimental hamsters were more likely to attribute incentive salience to the cue instead

of the reward, it is highly unlikely that this is what happened. As seen on figures 2. & 3., both

groups spent the same amount of time at the cue and food cup area. This observation is also

supported by linear mixed-effect models and t-tests, these statistical methods did not find any

differences in in area orientation between groups or over time between groups. The only effect

that was found to have an impact on area orientation was time. Over time, both groups of

hamsters spent more time around the food cup than they did around the cue area. This shows that

they did not deviate in attribution of incentive salience, and suggests that the difference seen in

PCAI and food cup latency between groups from days 1-4 to day 5, is due to a learning effect. I

can also conclude that this difference is not due to a motivational effect of hunger because on day

5, control hamsters on average weighed 143.2 ounces, with a standard error of 3.82 ounces, and

experimental hamsters weighed 152.4 ounces with a standard error of 3.33 ounces. If the

difference in PCAI measures on day 5 would have been due to a motivational effect associated

with hunger, the control group would have weighed more since they showed a lower PCAI. As

previously stated, PCAI is just a measure of how well an animal is conditioned. A high PCAI

shows poorer conditioning and learning; a low PCAI shows better conditioning, and learning.

Based on these concepts, the differences in PCAI between groups on day 5, suggest that hamsters

not exposed to chronic social stress during early puberty learn faster than those that were.

  While this study does show some surprising impacts of stress on learning, it did not go

without limitations. The response rate shows the percentage of times a subject responds to the

onset of cues, whether it is by approaching the cue or food cup. As mentioned before in the

statistical analysis section of the methods, the response rate was unfortunately not recorded since

I was expecting to analyze tracking behavior instead of conditioning and learning behavior.

Having a response rate to analyze could have been extremely useful to find any differences in

learning behavior between groups, over time or between groups over time. If a higher response rate was evident amongst the control group over time, it could have further confirmed our conclusion that stress during early puberty negatively affects learning in adulthood. Other useful data could have been the analysis of the number of food pellets left at the food cup after each session. This could have further confirmed whether or not, animals were actually poking into the food cup to receive the food reward instead of just relying on the videos of the subjects entering the food cup area. Because this experiment was designed to test tracking behavior, videos were not appropriately analyzed for learning and conditioning data collection. In the future it would be useful to reanalyze all videos for learning and conditioning data, including response rates and then statistically analyze response rate data. Another limitation of the study was time. Since significant differences in PCAI did not show up until day 5, it would have fared better to test the hamsters for a longer period of time in order to see how measurements would have changed between groups over time after day 5.

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