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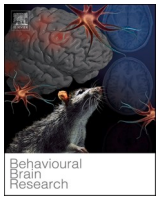

Research report

The role of sex on sign-tracking acquisition and outcome devaluation sensitivity in Long Evans rats

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ABSTRACT

Cues that predict rewards can trigger reward-seeking behaviors but also can, in some cases, become targets of motivation themselves. One behavioral phenomenon that captures this idea is sign-tracking in which animals, including humans, interact with reward-predictive cues even though it is not necessary to do so. Sign-tracking in rats has been studied in the domain of motivation and in how motivated behaviors can or cannot become excessive and habit-like over time. Many prior studies look at sign-tracking examine this behavior in male subjects, but there are few papers that look at this behavior in female subjects. Moreover, it is unknown where there might be sex-related variation in how flexible sign-tracking is when faced with changing reward values. Therefore, we asked if there were sex differences in the acquisition of sign-tracking behavior and if there were any sex differences in how sensitive animals were in their sign-tracking following reward devaluation. In contrast to previous reports, we found that males and females show no differences in how they acquire sign-tracking and in ultimate sign-tracking levels following training. Additionally, we found no difference in how quickly males and females learned to devalue the food reward, and we found no differences in sign-tracking levels by sex following outcome devaluation. We believe that this is primarily due to our experiment being performed in the Long Evans strain but also believe that there are many other factors contributing to differences between our study and previous work.

1. Introduction

Fundamental components of adaptive behavior include learning what cues predict rewarding items like food and responding to those cues with craving and reward-seeking; however, if those conditioned behaviors become overly persistent, such as resisting change when the outcomes are no longer desirable, they can be unhealthy. To ensure survival, individuals need to be able to change their behaviors to shifting circumstances in their environment. The role of sex in various Pavlovian behaviors is growing as a point of scientific interest. It is a salient one given the reasonable literature on sex differences in disorders characterized by maladaptive conditioned reward-seeking. For example, in Substance Abuse Disorder involving cocaine, women escalate more rapidly to the point of addiction than men do; women exhibit more unpleasant withdrawal symptoms than men during attempts to quit, and women also report greater craving induced by cues [1,2].

The effect that reward cues have on organisms is multifaceted, but one effect is to evoke "wanting" of the reward and even of the cue itself. A way to examine this effect behaviorally is by measuring sign-tracking in rodent models. Sign-tracking occurs when individuals are attracted to a cue that predicts a reward, and it is thought to reflect a process of incentive motivation to pursue rewards and their cues [3,4]. The alternative to sign-tracking is goal-tracking in which an individual is attracted to the reward itself rather than the cue [3]. In essence, sign-tracking is a manifestation of "wanting" or "craving" an outcome upon presentation of a cue paired with a reward. As a result, it needs to be determined if sign-tracking behavior itself, or the "wanting" or "craving" of a reward upon the presentation of a cue with which the reward is paired, shows sex differences that match what we see in human disorders.

Sign-tracking behavior has already been proven to be a useful readout for kinds of reward-seeking that can be either compulsive or flexible depending on different conditions. For example, in animal models of addiction and of obsessive-compulsive disorder, sign-tracking has been linked to excessive and inflexible reward-seeking; however, sign-tracking can also be flexible when major changes in the outcome occur [5-7]. One assay is outcome devaluation, in which the reward is paired with lithium chloride to induce nausea. Animals that continue

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interacting with a cue for a now-devalued outcome may be regarded as compulsive, or overly habitual, in their behavior. Animals that reduce sign-tracking under these conditions instead might be regarded as more flexible and adaptive [8]. Depending on several factors, sign-tracking itself can be sensitive to this sort of outcome devaluation procedure as well as similar outcome-related manipulations [3,4,8,9]. Thus, much like the behaviors of addicts, cue-evoked "wanting" as manifested in sign-tracking can show signs of flexibility but can also be related to inflexibility or compulsion.

It remains unknown how the sex of individuals relates to conditions under which sign-tracking behavior is flexible or compulsive, which is an important issue to resolve given sex differences in the development of compulsive behaviors and of their brain mechanisms. Concerning the expression of sign-tracking itself, one study that looked at the role of sex in Pavlovian approach behavior in rats found females engage in more stimulus-directed Pavlovian approach behaviors than males [10]. Similarly, another study found that female Sprague Dawley rats acquire Pavlovian approach to lever directed behavior (i.e. sign-tracking) behavior more quickly than males [11]. However, given the possibility of genetic differences in the propensity to sign-track, as well as in the development of addictions, additional strains of animals require attention too.

It also needs to be determined if sex plays a role in compulsive sign-tracking behavior in adult rats. Subjecting animals to an outcome devaluation procedure to change the value of the learned reward can be one way to measure the continued compulsiveness of a behavior. With regards to sign-tracking, behavioral flexibility occurs when animals interact less often with a cue when it comes to predict a devalued reward. Conversely, compulsivity occurs when animals fail to stop their interactions with the cue. One previous study has shown that adult females do show an insensitivity to satiety-induced outcome devaluation when a food reward is devalued, and males do not show this effect [10]. Our study is different from this previous study in that the food reward will be devalued by conditioned taste aversion conducted in testing chambers, which we and others have found to be key for assessing both acute and enduring forms of behavioral compulsivity [3,9].

Considering the literature above, the current study attempts to resolve whether there are sex differences in a strain of rat that shows a major proclivity towards sign-tracking (Long Evans), and likewise to resolve whether there are sex differences in how flexible vs. habitual sign-tracking is when the reward is devalued. Specifically, we expect that female rats will acquire sign-tracking more quickly than males and may be more persistent in sign-tracking behavior following outcome devaluation than their male counterparts.

2. Materials and methods

2.1. Subjects

Subjects were experimentally naïve male and female Long Evans rats that were at least p90 in age and were obtained from either Charles River (wild-type) or from Dr. Bruce Hope at the NIH (fos-GFP+) ($n = 34$ total; $n = 18$ female, $n = 16$ male; Charles River). Rats weighed 250–580 g upon arrival. Female and male subjects were identified by their genitalia. There were also 6 separate rats that were eliminated from the study because they failed to acquire the sign-tracking behavior during acquisition; these rats were at least one standard deviation below the mean during the last three days of acquisition. The strain of rats included in these experiments was mostly wild-type, ($n = 28$), but there were some Rosa26-Fos-GFP ($n = 6$) subjects. The fos-GFP+ rats behaved like their wild-type companions, so we combined them for analysis (Table S1). Rats were pair-housed in a climate-controlled colony room illuminated from 7:00 P.M. to 7:00 A.M. Following an acclimation period of 7 days, animals were individually housed and put on a food-restriction schedule to maintain body weights at 85 % of their ad libitum weights for the duration of the experiment. Rats were fed between 5 and 21 g of LabDiet Laboratory Rodent Diet chow. The experiments were performed in accordance with the National Institute of Health's *Guide for the Care and Use of Laboratory Animals* and with the National Research Council's *Guide for the Care and Use of Laboratory Animals.* Protocols were approved by the Dartmouth College Institutional Animal Care and Use Committee.

2.2. Apparatus

Sign-tracking training and testing were carried out in eight identical operant conditioning chambers (24 × 30.5 × 29 cm; Med Associates), enclosed in sound-attenuating chambers outfitted with an exhaust fan to provide airflow and background noise (~68 dB). The chambers were illuminated by a house light on the back wall of the chamber. Each chamber contained a recessed food magazine in the center of the front wall. Retractable levers (Med Associates model: ENV-112CM) were positioned on either side of the food magazine. Lever deflections were automatically recorded, and magazine entries were recorded through breaks of an infrared beam. Data were acquired through MED-PC software (Med Associates).

2.3. Behavioral testing

The sequence of training phases is presented in Fig. 1A. One day prior to start of training, rats were familiarized with grain pellets (Bio Serv, Product #F00165, 45 mg dustless precision pellets: Protein 21.3 %, Fat 3.8 %, Carbohydrate 54.0 %) in their home cages. All rats first received a single 30-minute acclimation session of magazine training during which these pellets were delivered freely on a random-time 30-second (RT30) schedule. Next, subjects underwent 12 daily, 60-minute sessions of Pavlovian associative training. During each training session, subjects received 25 conditioned stimulus (CS+) trials and 25 non-predictive stimulus (CS-) trials such that no more than two of the same trial type occurred sequentially. The average intertrial interval was 60 s. Each trial consisted of a 10-second lever presentation, but only CS+ trials presentation preceded delivery of two food pellets upon lever retraction. The assignment of left and right levers to CS+ and CS- identities was counterbalanced within groups of animals, but this assignment was held constant for each animal. Training was followed by one abbreviated predevaluation test session (5CS+, 5 CS- presentations) conducted in extinction conditions to establish a baseline level of responding to the stimulus without the reinforcing effects of the reward. The next day, subjects were given one reacquisition session (25 CS+, 25 CS- presentations) in which the reward was delivered like in earlier training sessions. Rats were fed their chow one hour after the end of these sessions to ensure maximum engagement with the task.

2.4. Outcome devaluation and post-devaluation testing

Rats were split into four groups based on mean CS+ lever press rats and standard error of the mean such that the groups had similar responding levels by Day 12 of training. This was done by averaging the mean lever press for each rat across Day 11 and Day 12 and then assigning rats into a group so that the groups were matched by this average. The groups were LiCl female (n=9), LiCl male (n=9), Saline female (n=9), and Saline male (n=7).

After group assignment, rats were exposed to outcome devaluation. Rats received between five to nine pairings of devaluation procedure. The pairings took place in the Med Associates conditioning chambers since it has been previously shown that sensitivity to devaluation is context-dependent [3]. Having pairings inside the context of the conditioning chambers enables better integration of LiCl learning into the conditioning context [9,12]. Rats were fed their chow at least one hour after pairings.

The pairings were spaced 48 h apart. For these pairings, pellets were delivered on the RT30 schedule previously used during magazine

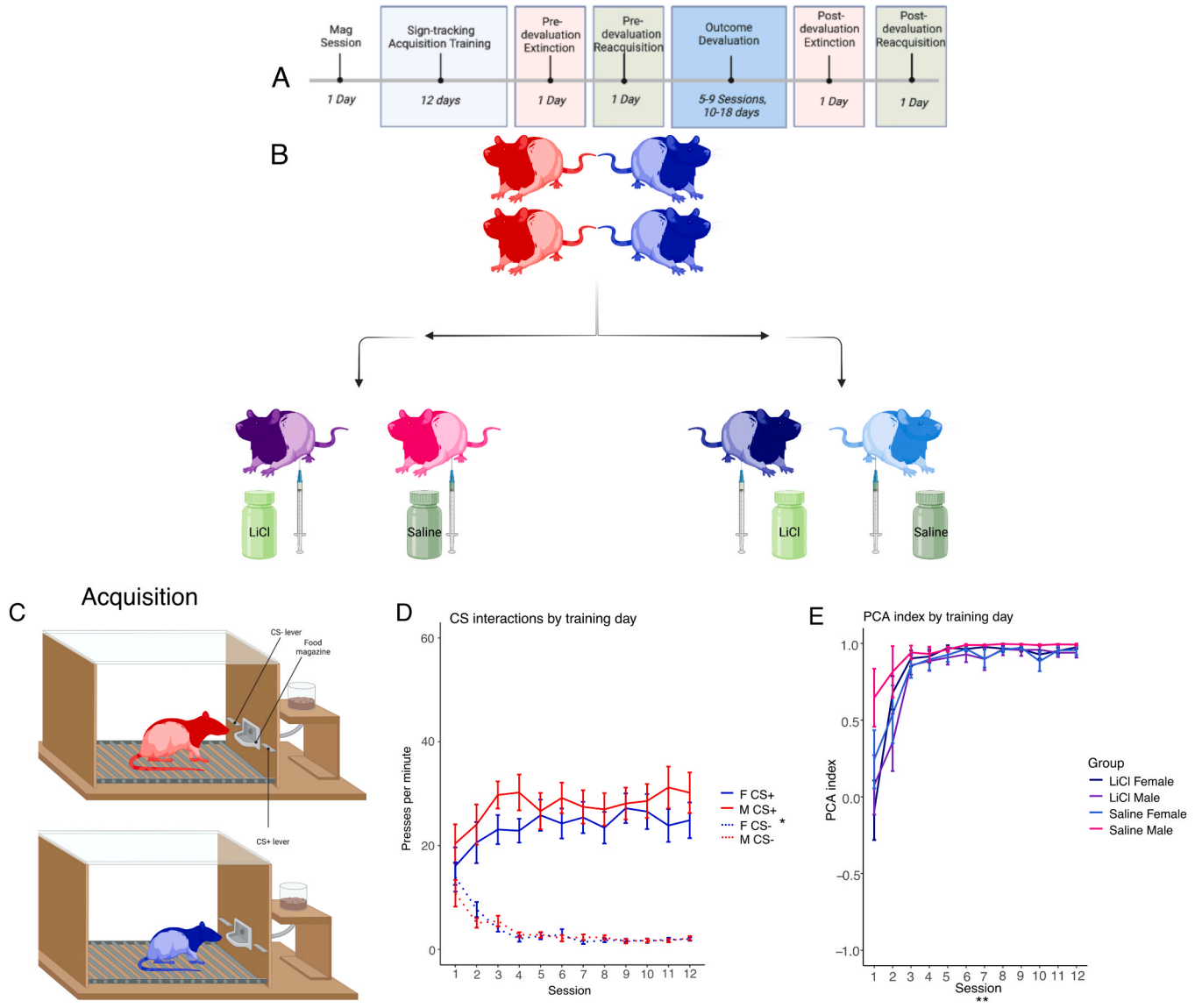

Fig. 1. Males and females show similar acquisition of sign-tracking behavior. A) Timeline of experimental procedures. B) Visualization of experimental groups. Males used in sign-tracking acquisition were split into two groups following day 12 of sign-tracking acquisition and received either LiCl or Saline during outcome devaluation sessions; the same was done with females. C) Schematic of groups and conditions in acquisition sessions. D) Mean presses per minute on CS levers with respect to sex during acquisition. There was a main effect of CS Type (P = 0.02*). E) Mean PCA index with respect to group during acquisition. There was a main effect of session (P = 0.00148**) [* indicate main effects].

training, but levers were not extended. To avoid clogging of the magazine by pellets, pairings 3–9 were successively shorter in length, as animals in LiCl groups rejected more pellets over time which increased the likelihood of rejected pellets backing up within the delivery tube. Specifically, subjects were given 30 pellets during the first two pairings, fifteen pellets during pairings three through five, and ten pellets for the remaining pairings. At the ends of these sessions, animals were removed from the conditioning chambers, held briefly in the plastic holding boxes, and the number of pellets consumed was recorded. Then, animals were injected with either 0.3 M LiCl in deionized water (10 mg/kg) or 0.9 % saline (10 mg/kg) based on their group assignment and allowed to rest for 20 min in the conditioning chambers. Once an animal in a LiCl group consumed 1 or fewer pellets during devaluation, it was advanced to post-devaluation probe sessions. Some animals reached this criterion earlier than other animals; some rats reached this criterion after only five pairings while others took up to nine pairings. Once a LiCl animal moved onto post-devaluation sessions, a matched Saline animal moved on with it. Post-devaluation sessions consisted of a brief extinction session (5 CS+, 5 CS- presentations) followed by an abbreviated, fully rewarded, reacquisition session (12 CS+, 13 CS-) to assess persistent sign-tracking in the following reward devaluation. Like previous pre-devaluation sessions, rats were fed their chow one hour after the end of these sessions to ensure maximum engagement with the task.

2.5. Behavioral measures and analyses

Lever deflections, magazine entries, and time spent in the magazine area were recorded via MED-PC. A simple PCA index was calculated for each rat via the following equation: (lever presses per CS+ trial – magazine entries per CS+ trial) / (lever presses per CS+ trial + magazine entries per CS+ trial). During outcome devaluation, pellets were counted before and after consumption to determine the percentage of pellets consumed. All statistical tests were performed using R (R Core Team 2016). All graphs were created through R (R: "ggplot2″) and designed with Affinity Designer. Schematics were made in Biorender and inserted into Affinity Designer. In order to extract the number

magazine entries during the 10 second CS+ presentation and the number of magazine entries during the 10 s following CS+ presentation, a python extraction protocol was used on the MedPC files.

Individual linear mixed models (R; "lme4″) were used to analyze effects of different dependent variable responding (e.g., lever presses per minute, ppm) by fixed effects of experimental group, CS type, session, sex, and the interactions between these variables while accounting for random effects of differences in individual starting press rates and individual learning rates over sessions. Linear mixed models were fit by maximum likelihood, and the *t*-tests use Satterthwaite approximations of degrees of freedom (R; "lmerMod"). Models were analyzed with the package lme4 from CRAN [13]. Reported statistics include confidence intervals (95 % confidence intervals) and *P*-values [14]. When determining the role of sex in these models, linear mixed models with and without sex were assessed via ANOVA; this was done to determine to what extent sex was a factor in the dependent variable behavioral response of interest. Since there was no significant effect of sex observed in any of the models, we did not follow up with any post-hoc tests.

As percentage data are not normally distributed, a generalized linear mixed model was used to calculate the effects of devaluation and session on pellet consumption during outcome devaluation. Confidence intervals and *P*-values are reported.

Responses in the post-devaluation extinction session (e.g., ppm) were compared with responses in the predevaluation extinction session by creating individual linear mixed models to assess response rates by fixed effects of group, session, sex, and the interactions between these variables while accounting for random effects of individual starting points. Responses in the post-devaluation reacquisition session were similarly compared with responses in the predevaluation reacquisition session.

Pellets consumed during post-devaluation reacquisition sessions were recorded. These data were not normally distributed; therefore, a Wilcoxon signed-rank test with continuity correction was used to determine if animals differed in pellet consumption based on their group treatments.

3. Results

3.1. Acquisition

The mean presses per minute (ppm) over the course of training is presented in Fig. 1D. To compare rates of responding, a linear mixed model using ppm as the dependent variable and fixed effects of CS type, group, sex, and session as well as any interactions between CS type, group, sex, and session. Random intercepts for individual animals and learning curves were included.

There was no significant effect of group (estimate: –13.19 ppm; confidence interval (CI): –49.92–22.98; P = 0.49) which shows that the Saline and LiCl groups (collapsed by sex) did not differ on average in ppm. There was a trend of session (estimate: 3.08 ppm; CI: –0.20 to 6.32; P = 0.057) which shows that there was no statistical difference in lever presses over the course of sign-tracking acquisition, but there was a trend to increasing CS+ lever presses over sessions. There was a significant effect of CS type (estimate: 0.22 ppm; CI: 0.02–0.42; P = 0.021 *) such that rats preferentially pressed the CS+ lever over the CS- lever. There also was a nonsignificant trend of sex (estimate: 39.87; CI: –0.00 to 75.92; P = 0.059) which means that males and females (collapsed by group) did not differ on average in ppm, but there was a nonsignificant tendency for females to interact less with the CS+ than the males. There was also no interaction between session and sex (estimate: –0.67; CI: –4.92 to 3.77; P = 0.78), no interaction between session and group (estimate: 0.60; CI: –3.92 to 5.08; P = 0.78), and no three-way interaction between sex, group, and session (estimate= –0.01; CI: –5.92 to 6.44; P = 0.998).

We also sought to determine if there were any significant differences in Pavlovian Conditioned Approach (PCA) behavior during training since it is a common way to measure sign-tracking behavior. The PCA index is another way to measure sign-tracking since it quantifies both lever presses and magazine entry behavior during the CS+ period. The index was created in such a way that a score of + 1 aligns with exclusively sign-tracking rats while a score of –1 aligns with exclusively goal-tracking rats. The PCA index over the course of training is presented in Fig. 1E. A linear mixed model was created as explained previously, but the dependent variable in this model was PCA; CS Type was excluded from this model.

There was a main effect of session (estimate: 0.04; CI: 0.02–0.07; P = 0.0015 **) showing that subjects do increase their PCA index as sessions progress, but there was no significant main effect of sex (estimate: 0.24; CI: –0.08 to 0.58; P = 0.16) which mirrors what we observed in the model focusing on CS ppm, no significant main effect of group (estimate: –0.05; CI: –0.33 to 0.26; P = 0.76). Importantly, there was no interaction between sex and session (estimate: –0.02; CI: –0.06 to 0.01; P = 0.27), no interaction between group and session (estimate: 0.0081; CI: –0.025 to 0.04; P = 0.64), and no three-way interaction between sex, group, and session (estimate: 0.026; CI: –0.02–0.07; P = 0.32).

Thus, ppm began similarly with respect to CS+ and CS- levers; CS+ ppm non-significantly trended to slightly increase over sessions whereas CS- ppm fell which is indicative of learning. Together, these results indicate that animals readily discriminated between the CS+ and the CS- as shown by the number of lever presses made during presentations of these stimuli throughout training, and that there was no difference between in how the groups interacted with these levers. Importantly, there was also no difference in how males and females interacted with these levers which shows that males and females do not differ in how they acquire sign-tracking behavior and how much they sign-track once the association has been formed. Males and females also showed no differences in their PCA index during training which replicates what was observed when focusing on the lever pressing data; however, subjects did acquire the sign-tracking behavior as observed by the animals increasing their PCA index over the course of training.

3.2. Outcome devaluation

Rats received between five-to-nine devaluation pairings in the operant chambers. The mean percentage of pellets consumed by group over the course of these pairings is plotted in Fig. 2B. A generalized linear mixed model was created to analyze fixed effects of session, group, sex, and interactions between these factors with random intercepts for individual rats. Across the LiCl-reward pairings, there was a significant main effect of group on ppm (estimate: 18.08; CI: 3.80–32.83; P = 0.00905 **) which shows that the Saline and LiCl groups differed in the percent pellets consumed, but there were no significant main effects of day (estimate: 0.04; CI: –2.01 to 2.11; P = 0.97) or of sex (estimate: 0.12; CI: –13.12 to 14.25; P = 0.99). As expected, there was a significant interaction between group and session (estimate: –16.01; CI: –18.94 to –13.04; P = <2e-16 ***) while there were no significant interactions between session and sex (estimate: –0.08; CI: –2.88 to 2.85; P = 0.95) or three-way interactions between sex, group, and session (estimate: 0.23; CI: –3.91 to 4.16; P = 0.90).

These results indicate that the animals in the LiCl group consumed fewer pellets than the Saline group during devaluation sessions; specifically, the LiCl group learned to devalue and stop consuming the pellets while the Saline group, which didn't undergo conditioned taste aversion, continued consuming the pellets. Of note, sex had no relationship to the rate of conditioned taste aversion-learning.

3.3. Devaluation sensitivity in extinction

Responding during pre-devaluation and post-devaluation extinction sessions were compared. Sign-tracking rates by group and session are presented in Fig. 3B. A linear mixed model using CS+ response rates as the dependent variable and fixed effects of group, sex, session, and the

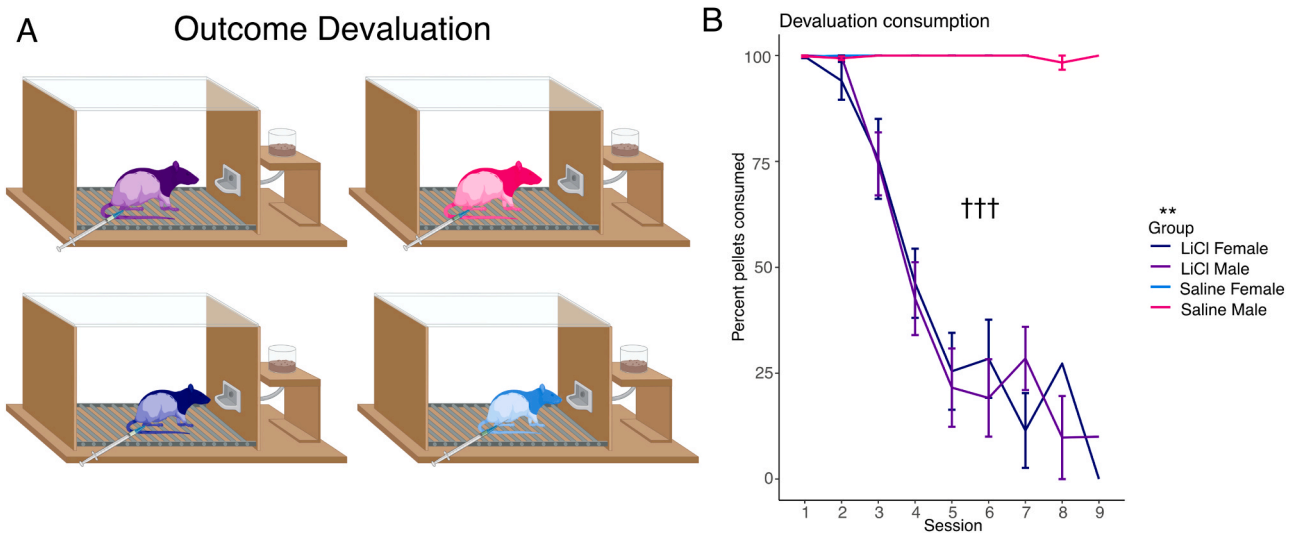

Fig. 2. Males and females are equally sensitive to outcome devaluation. A) Schematic of groups and conditions in outcome devaluation sessions. B) Mean percent pellets consumed with respect to group during outcome devaluation. There was a group by session interaction (P = <2e-16†††) and a main effect of group (P = 0.00905 **). [* indicate main effects], [† indicate interactions]. [The standard error increases from sessions 5–9 as rats reach criteria, so there are fewer numbers of animals contributing to these later sessions.].

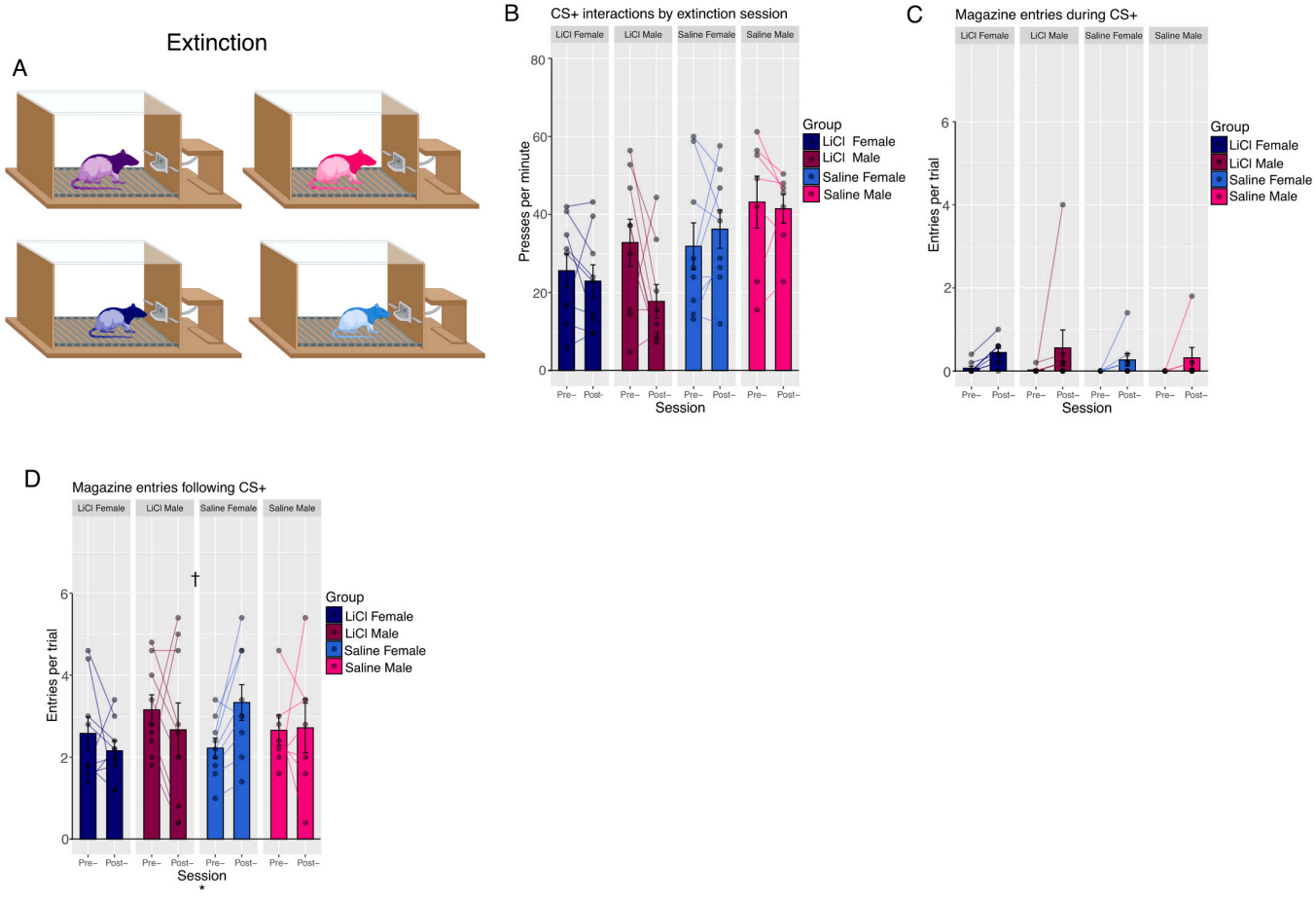

Fig. 3. Males and females show similar sign-tracking levels in extinction conditions following outcome devaluation. A) Schematic of groups and conditions in extinction sessions. B) Mean presses per minute of the CS+ lever by group across extinction sessions. C) Mean magazine entries during 10 s CS+ presentation by group across extinction session. D) Mean magazine entries in the 10 s following the CS+ presentation by group across extinction session. There was a group by session interaction (P = 0.038†), and a significant effect of session (P = 0.034 *). [* indicate main effects], [† indicate interactions]. [Transparent dots are data points for individual rats. Darker dots represent individual rats showing the same data point].

interactions between these fixed effects with random effects for individual rat starting points was used. This first sex-including linear mixed model was compared via ANOVA to a second mixed model using the same dependent variable and fixed effect inputs but eliminated sex as a factor. The sex-including model performed worse than the sex-excluding model on the ANOVA (sex-including model Akaike Information Criterion (AIC): 565.30; sex-excluding model AIC: 562.71). Because this difference was minor, we made our comparisons using the sex-including model despite the worse fit. See Table S2 for stats related to the sex-excluding model.

During post-devaluation testing under extinction conditions, groups did not differ in sign-tracking. The three-way interaction between sex and session and group was not significant (estimate: –6.286 ppm; CI: –24.97 to 14.37; P = 0.550). There was not a significant effect of group (estimate: –6.267 ppm; CI: –18.04 to 7.10; P = 0.346) or an effect of session (estimate: 4.400 ppm; CI: –4.58 to 15.27; P = 0.388) or an effect of sex (estimate: 11.333 ppm; CI: –2.13 to 26.19; P = 0.114). There were also no interactions between sex and session (estimate: –6.114 ppm; CI: –23.72 to 6.77; P = 0.427), between sex and group (estimate: –4.133 ppm; CI: –22.39 to 14.29; P = 0.670), or between day and group (estimate: –7.067 ppm; CI: –24.97 to 6.87; P = 0.327). There also were no significant effects when PCA index was examined (Supplemental Fig. 1).

When assessing magazine entries during the ten-second period of CS+ presentation (Fig. 3C), there were not any significant effects of entries per trial (ept) when looking at fixed effect of sex (estimate: –4.95e-01 ept; CI: –0.57 to 0.47; P = 1.000), fixed effect of session (estimate: 2.67e-01 ept; CI: –0.24 to 0.74; P = 0.283) or fixed effect of group (estimate: 6.67e-02 ept; CI: –0.41 to 0.52; P = 0.788); there also was not a significant interaction between session and sex and group during this time period (estimate: 1.08e-01 ept; CI: –0.87 to 1.08; P = 0.832). There were also no interactions between sex and session (estimate: 4.76e-02 ept; CI: –0.68 to 0.74; P = 0.898), between sex and group (estimate: –4.44e-02 ept; CI: –23.65 to 15.31; P = 0.902), or between session and group (estimate: 1.11e-01 ept; CI: –0.55 to 0.79; P = 0.750). When isolating magazine entries during the ten-second period following CS+ presentation (Fig. 3D), the time period during which rewards would have been delivered, there was a significant interaction between session and group (estimate: –1.53 ept; CI: –2.85 to –0.11; P = 0.038 *) as animals with LiCl exposure tended to reduce such entries. There was a significant effect for session (estimate: 1.11 ept; CI: 0.15–2.08; P = 0.034 *) such that all animals generally increased going to the magazine during this time, but there was no difference of sex (estimate: 0.43 ept; CI: –0.87 to 1.66; P = 0.470) or group (estimate: 0.36; CI: –0.77 to 1.46; P = 0.527). There were also no significant interactions between sex and session (estimate: –1.05 ept; CI: –2.79 to 0.36; P = 0.174), between sex and group (estimate: 0.14 ept; CI: –1.49 to 1.86; P = 0.862), and between sex and group and session (estimate: 0.99

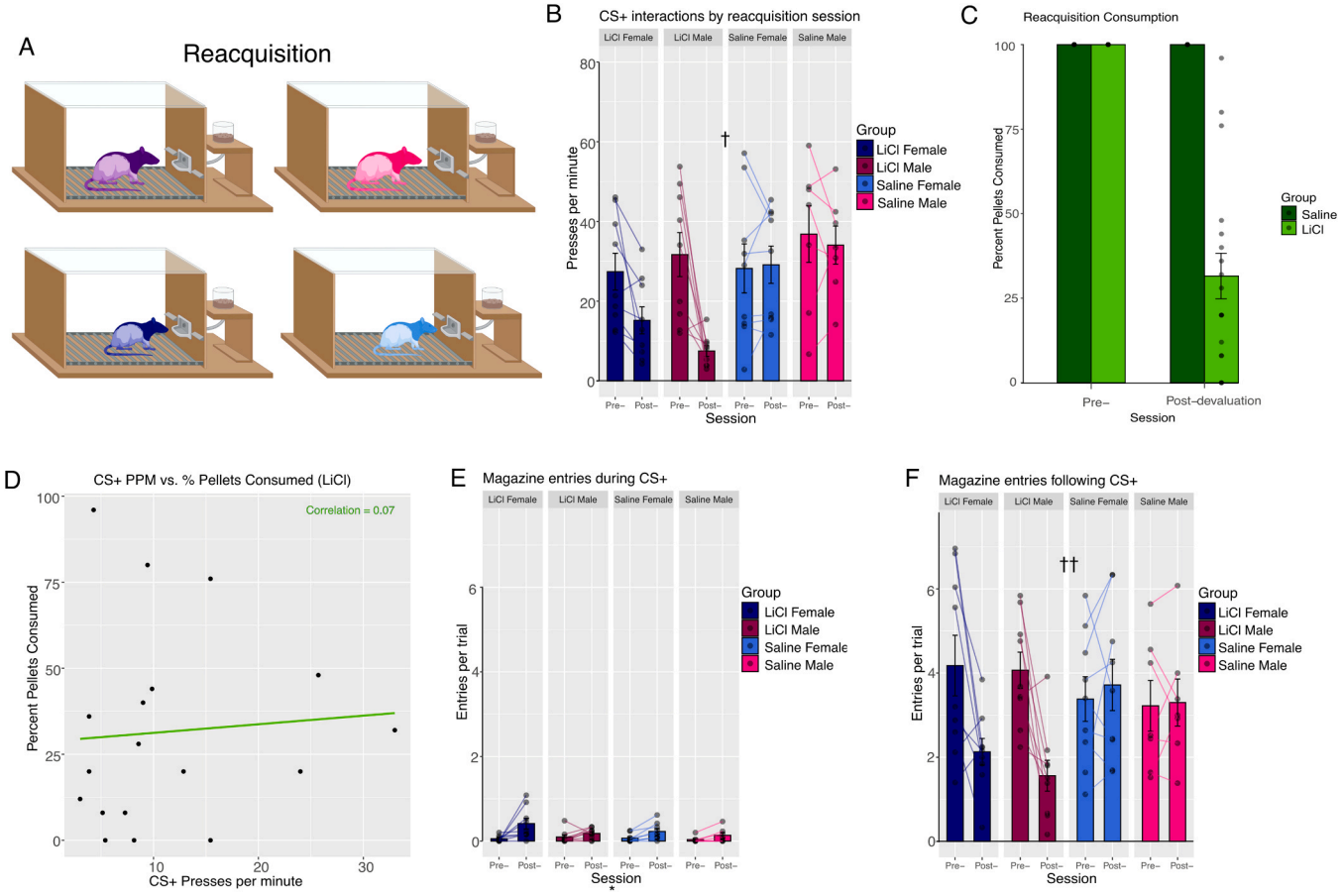

Fig. 4. Males and females show similar sign-tracking levels in reacquisition conditions following outcome devaluation. A) Schematic of groups and conditions in reacquisition sessions. B) Mean presses per minute on the CS+ lever by group across reacquisition sessions. There was a group by session interaction (P = 0.0498†). C) Mean percent pellets consumed across reacquisition session by group; animals in the LiCl group consumed fewer pellets than the Saline group (P = 0.0002108). D) Correlation plot between the percent pellets consumed and the CS+ presses per minute in the LiCl group in the post-devaluation reacquisition session. There was no correlation between these two behaviors (Pearsons= 0.07413183). E) Mean magazine entries in the 10 s CS+ presentation by group across reacquisition session. There was a main effect of session (P = 0.048 *). F) Mean magazine entries in the 10 s following the CS+ presentation by group across reacquisition session. There was a group by session interaction (P = 0.00437††). [* indicate main effects], [† indicate interactions]. [Transparent dots are data points for individual rats. Darker dots represent individual rats showing the same data point].

ept; CI: –1.00 to 3.03; P = 0.349).

In summary, these data indicate that sex is not a factor in sign-tracking behavior under extinction conditions after outcome devaluation. Although, surprisingly, under extinction conditions after outcome devaluation, sign-tracking to the CS+ failed so significantly integrate LiCl exposure in that group. Upon closer examination, there was a significant reduction in magazine entry behaviors in the LiCl group which suggests that animals integrated the outcome devaluation into their choices.

3.4. Devaluation sensitivity in reacquisition

Responding during pre-devaluation and post-devaluation reacquisition sessions were also compared. Sign-tracking rates by group and session are presented in Fig. 4B. A sex-including linear mixed model was compared to a second sex-excluding model via ANOVA as described above for reacquisition session rates. The sex-including model performed worse on the ANOVA than the sex-excluding model (sex-including model Akaike Information Criterion (AIC): 556.64; sex-excluding model AIC: 553.61). Because this difference was minor, we made our comparisons using the sex-including model despite the worse fit. See Table S2 for stats related to the sex-excluding model.

During the requisition test following devaluation, there was a significant interaction between session and group (estimate: –13.15 ppm; CI: –27.07 to 0.03; P = 0.0498 *). There was not a significant effect of group (estimate: –0.80 ppm; CI: –12.93 to 10.25; P = 0.90), of sex (estimate: 8.61 ppm; CI: –5.58 to 20.48; P = 0.205), or of session (estimate: 0.93 ppm; CI: –8.19 to 9.48; P = 0.840). There were also no interactions between sex and session (estimate: –3.69 ppm; CI: –17.42 to 9.99; P = 0.597), between sex and group (estimate: –4.34 ppm; CI: –22.55 to 12.92; P = 0.639), or between sex and session and group (estimate: –8.27 ppm; CI: –26.14 to 10.78; P = 0.389). When the PCA index was examined, there was a similarly significant interaction between group and session (estimate= –0.20; CI= –0.36 to –0.03; P = 0.025 *) (Supplemental Fig. 2).

For interaction with the CS- lever, there was also a significant interaction between group and session (estimate= –3.20 ppm; CI= –5.38 to –1.06; P = 0.003 **) as well as a significant main effect of day (estimate= 2.37 ppm; CI= 1.07–3.80; P = 0.002 **) (Supplemental Fig. 2). This effect reflected a greater amount of CS- interactions in control groups.; however, the overall amount of CS- interactions remained quite low.

Concerning pellet consumption, a Wilcoxon signed rank test revealed that only animals in the LiCl group significantly decreased their pellet consumption between pre- and post-devaluation sessions (V= 171, P = 0.0002108) (Fig. 4C). Because there appeared to be variation in the percent of pellets LiCl-exposed animals consumed during reacquisition, we examined the correlation between the percentage of pellets consumed and the ppm on the CS+ lever for the LiCl group. We ran a Pearson correlation coefficient calculation on the CS+ ppm and the percentage of pellets consumed on LiCl treated animals following outcome devaluation to determine if there was any relationship between those two behaviors. There was a no positive correlation coefficient (0.07413183) (Fig. 4D) indicating that there was no relationship in how much animals sign-tracked and how many pellets were consumed by this group during reacquisition.

When isolating magazine entries during the ten-second period during CS+ presentation (Fig. 4E), there was a significant fixed effect of session (estimate: 0.16 ept; CI: 0.01–0.31; P = 0.048 *) but no fixed effect of group (estimate: –0.01 ept; CI: –0.18 to 0.14; P = 0.872) or of sex (estimate: –0.03 ept; CI: –0.20 to 0.13; P = 0.715); there also was no significant interactions between sex and group and session (estimate: –0.21 ept; CI: –0.54 to 0.08; P = 0.192), between sex and session (estimate: –0.06 ept; CI: –0.26 to 0.20; P = 0.620), between sex and group (estimate: –0.07 ept; CI: –0.15 to 0.32; P = 0.551), and a nonsignificant trend between session and group during this time period (estimate: 0.20; CI: –0.02 to 0.40; P = 0.078). When isolating magazine entries during the ten-second period following CS+ presentation (Fig. 4F), there was a significant interaction between session and group (estimate: –2.38; CI: –4.03 to –0.88; P = 0.00437 **), but there were no significant effects for session (estimate: 0.34 ept; CI: –7.25 to 1.41; P = 0.547), for sex (estimate: –0.16 ept; CI: –1.54 to 1.38; P = 0.828), or for group (estimate: 0.80 ept; CI: –0.49 to 2.08; P = 0.25). There were also no significant interactions between sex and group and session (estimate: –0.20 ept; CI: –2.41 to 2.12; P = 0.863), between sex and session (estimate: –0.26 ept; CI: –0.20 to 1.56; P = 0.759), and between sex and group (estimate: 0.05 ept; CI: –0.19 to 2.08; P = 0.962).

In summary, these data indicate that there was a significant change in sign-tracking levels after outcome devaluation in reacquisition conditions with respect to group but not with respect to sex. Additionally, while both groups decreased their sign tracking following outcome devaluation, the LiCl group had a marked decrease compared to the saline group. The LiCl group also consumed fewer pellets during reacquisition following outcome devaluation. With respect to magazine entries, the LiCl group decreased magazine entries following outcome devaluation whereas the saline group did not have this same decrease. During CS+ presentations, both groups increased magazine entries following outcome devaluation; however, following CS+ presentations, the LiCl group decreased magazine entries while the saline group maintained a similar level of magazine entries.

4. Discussion

4.1. The role of sex in sign-tracking acquisition

Much of the research on sign-tracking behavior has included primarily male animals. Research into the acquisition and maintenance of sign-tracking in females, as well as in the flexibility of sign-tracking, needs to be more thoroughly examined. A few studies have examined the role of sex in the development of similar but non-identical cue-directed behaviors. For example, one study examined the role of age and sex in sign-tracking-like behaviors and found that adult female rats exhibit more of these behaviors than adult males in early training sessions [10]. This was also the case with adolescent females compared with adolescent males, but this separation of sex occurred during later training sessions rather than during early ones. Additionally, a study examined the role of sex and strain in PCA behavior to a lever-cue (i.e. sign-tracking) and found that Sprague Dawley female rats acquired sign-tracking more rapidly than male rats [11]. Interestingly, this sex difference was absent in the Heterogeneous Stock rats used in that study.

The conclusions from our study are inconsistent with these previous studies in that we found no differences between sex throughout sign-tracking acquisition or in final sign-tracking levels, and, in fact, including sex as a factor in our statistical model reduced its explanatory power for behavioral variance. One key difference in our study compared to these prior ones is that we used Long Evans rats. Male and female Long Evans rats may show no differences throughout sign-tracking acquisition similar to the Heterogenous Stock rats seen in Pitchers et al. [11] even though, ultimately, adult Sprague Dawley rats exhibit no sex differences by the end of training [10,11,15,16]. One study examining sign-tracking and goal-tracking behaviors in mice directly supports the idea that the strain of the rodent being studied can impact whether sign-tracking exhibits sex differences [17]. Specifically, male CAST/EiJ mice, male NOD/ShiLtJ mice, and male C57BL/6 J mice all exhibited increased sign-tracking levels as measured via number of lever presses or duration of lever contact and which was lacking in the females of those strains [17]. Female AJ mice exhibited increased sign-tracking levels relative to the male AJ mice as measured via increased lever contact duration [17]. The 129S1/SvImJ mice showed no sex differences in sign-tracking behavior, but also seemed to not acquire sign-tracking behavior generally [17]. In short, while females can exhibit more sign-tracking than males in certain strains of rodent (e.

g., Sprague Dawley rat, AJ mice), females and males do not differ in other strains (e.g., 129S1/SvImJ mice, Long Evans rat as used here). Studies interested in biological sex as a variable in motivated behaviors will need to keep animal strain, and even vendor, as a point of interest [18], but a study directly comparing sign-tracking acquisition in Long Evans and Sprague Dawley rats will need to be performed to confirm these claims.

Another relevant factor could be the intertrial interval (ITI). Previous studies have shown that increasing the ITI during training increases the bias of rats to develop sign-tracking over goal-tracking in both female Long Evans rats and in male and female Wistar rats [19,20]. Specifically, these studies found that female Long Evans rats engage with the CS+ at higher levels when the ITI is longer, and that, while male Wistar rats engage with the CS+ more than females over all ITIs tested, females do engage more with the CS+ as the ITI is increased [19,20]. While no studies have looked at the effects of different ITIs on both male and female Long Evans rats, it is possible that our relatively low ITI of 60 s could be masking sex differences that could be seen at a higher ITI. Both studies mentioned previously had higher ITIs ranging from 90 s to 150 s, so increasing the ITI of our experiment to somewhere within the 90–150 s range could produce a sex difference in which females exhibit more sign-tracking behavior like these previous studies [10,11].

4.2. The role of sex in the sensitivity of sign-tracking to outcome devaluation

In addition to the acquisition of sign-tracking behaviors, it is useful to determine if there are sex differences in the sensitivity of sign-tracking to outcome devaluation. This sensitivity is one way to examine habitual behavior which is of interest in many human disorders and pathologies, and, with the exception of two studies to our knowledge, most of the research examining this effect was performed in males. These two studies found that adult females were insensitive to outcome devaluation in their behavior, whereas males were devaluation sensitive [10,21]. In the first study, this lack of devaluation effect on CS+ -approach behavior was seen as adult females having an increased CS+ approach behavior relative to adult males immediately following satiety-mediated devaluation and during early extinction sessions following devaluation [10]. In the second study, this was seen as increased “lever approach” behavior in females compared with males after reward devaluation during which some subjects had their basolateral amygdala-to-nucleus accumbens core projections inactivated and during which some rats were satiated while others were not [22].

The conclusion from our study conflicts with these results in that we found no sex differences in the sensitivity of sign-tracking to outcome devaluation. As above, animal strain is likely to be a factor. Additionally, another reason why our data differs from prior work could be due to the method of outcome devaluation that was performed. We conducted conditioned taste aversion-mediated outcome devaluation which introduces a taste aversion to devalue the reward. The LiCl injection makes subjects nauseous following consumption of the reward, and the nauseous state becomes associated with the previously consumed reward. This form of outcome devaluation depends on an innate mammalian form of aversion learning during which a nauseous state is associated with something consumed as opposed to other types of stimuli present in the environment. The previous studies used satiety-mediated outcome devaluation during which animals have unrestricted access to the reward to devalue that reward. This satiety method takes advantage of the fact that subjects that are satiated will decrease the reward value of that specific reward. Satiety-mediated devaluation could allow for maintenance of sign-tracking behavior in females compared to males whereas females and males might similarly decrease maintenance of sign-tracking behavior following conditioned taste aversion-mediated devaluation. It is hard to draw too many conclusions due to the paucity of work on sign-tracking and devaluation sensitivity across animal sex, but variables of interest to cross-examine would likely be animal strain as well as devaluation methodology.

It should be noted that, even when sex is not a factor, there is inconsistency within the literature of the effects of outcome devaluation on sign-tracking. Some initial studies that used only males have found that sign-tracking is completely resistant to the effects of outcome devaluation, and that only goal-tracking can be affected by outcome devaluation [23]. Other initial studies that also used only males but ensured that devaluation was occurring within the task environment found that sign-tracking is sensitive to the effects of outcome devaluation [3,9,24]. In our own study here, we made sure that the devaluation was occurring within the task environment. We also found differing results on the conclusion of if outcome devaluation had a significant impact on sign-tracking behavior under different conditions with regard to the continued presence of the reinforcer (reacquisition) or its absence (extinction). As a result of the differences that we see in the literature and within our own study, it seems that devaluation happening in the context under which Pavlovian testing occurs is critical to seeing an impact of outcome devaluation on sign-tracking behavior. Additionally, our study implies that the conditions carried out following outcome devaluation (i.e. under extinction or under reacquisition) could be another potential factor that could lead to a outcome devaluation having a statistical impact on sign-tracking behavior when taking into consideration sex and other variables.

Concerning variation in the devaluation sensitivity of sign-tracking that was unrelated to sex, some rats that were treated with LiCl consumed a high percentage of pellets in reacquisition. Pellets were classified as consumed if they were missing from the magazine, and some rats had many pellet pieces or even full pellets that were sitting in the pan of the operant chamber. These pellets were counted as “consumed pellets”, and they are the source of this variability. It is this variability that could have led to a lack of correlation between CS+ ppm and percent pellets consumed in the LiCl group during reacquisition; however, it is interesting that some rats would continue interacting with and, in some instances, biting the devalued reward.

On this point, our study was conducted in a nearly identical manner to one previously, in which it was found that rats reduced sign-tracking immediately after LiCl-induced devaluation if the devaluation was done in the task context rather than outside of the task context [3]. In that study, only male Long Evans rats from Charles River were used [3]. This was similar to an earlier study observing devaluation sensitivity in Long Evans rats from Charles River as well [9]. In the study here, both male and female rats were used, and we found no significant difference in sign-tracking under extinction conditions to a devalued CS+ but did find concurrent, significant change in magazine-directed behavior. This reduction of sign-tracking became significant in the subsequent reacquisition sessions. It is possible that having both sexes represented affected this result, but there were statistically no sex differences which precludes post-hoc analyses on sex. One can qualitatively observe that males in the current study resembled males in our prior study, and when examining only the male subjects of our study, we see trends like the previous study where males treated with LiCl decrease their CS+ ppm following outcome devaluation in extinction whereas males treated with Saline don’t alter their ppm [3]. Our female subjects displayed a more intermediate phenotype in both groups such that females who received LiCl barely decreased their CS+ ppm whereas females who received Saline barely increased their CS+ ppm relative to males. In other words, LiCl-exposed males pressed the least following extinction and Saline males pressed the most following extinction whereas the LiCl-exposed females pressed more than the LiCl-exposed males and followed by the Saline females. In other words, there might be a sex-by-experimental condition interaction which led to our LiCl group showing successful outcome devaluation as measured by the CS+ with the continued presence of the devalued reward (reacquisition condition) but not showing successful outcome devaluation as measured by the CS+ in the absence of the devalued reward (extinction condition). Given the precedent outlined by the previous experiment in which only males were

used, it is possible that females are driving this difference in extinction vs. reacquisition, although this was not able to be quantified statistically [3]. This finding highlights the importance of considering sex and the experimental conditions in which sign-tracking occurs following outcome devaluation (i.e. under extinction or reacquisition) in future research and the potential influence that it may have on observed outcomes.

4.3. Sex and other variable interactions on sign-tracking flexibility

While we have discussed possible reasons for the difference in our study when compared to prior work, animal strain, ITI, and method of outcome devaluation certainly are not the only sources of variation in sign-tracking behaviors. Differences in the details of the task design could interact with sex in sign-tracking acquisition and in its flexibility vs. habit-like nature as well. For example, the modality of the cue stimulus (e.g. auditory tone, lever insertion, *etc*.) that predicts a reward is not consistent across studies. Modality differences are not just limited to the cue; some studies have different reward types and amounts as well (e.g. liquid sucrose vs. pellets) (e.g., [10,21]). As another example, some studies use both a CS+ which predicts a food cue and a CS- which does not predict a food cue to control for differing activity levels across subjects, while other studies only have a CS+ to avoid the learning requirement of CS discrimination (e.g., [10,21,22]). Sign-tracking studies also split rats into "sign-trackers" and "goal-trackers" and focus on the extremes of a behavior that exists on a spectrum while other studies consider the full range of sign-tracking behavior before doing any experiments or manipulations (e.g., [10,11]). Sign-tracking studies also differ in how many acquisition or training sessions rodents perform (e.g., [10,21]); this could lead to differences in sign-tracking behavior because subjects could have stronger or weaker associations between the cue and the reward across studies [25]. Some studies that manipulate sign-tracking behavior following training do so under extinction conditions while other studies continue using the presence of the food reward which actively reinforces the sign-tracking behavior [10,26]. Interestingly, one study found that female sign-tracking rats had reduced microbial alpha diversity compared to male sign-tracking rats and goal-tracking rats more generally, so factors that could impact microbiome diversity, like home cage chow, could potentially be a factor in sign-tracking behavior when it does differ by sex [27]. Many effects of these individual sources of variation could be subtle, but they could manifest as large significant differences in sign-tracking when compounded.

For this study, as in our prior studies, we included a CS- for several reasons. Firstly, a CS- can control for generalized behavioral activity that could differ between experimental groups. Secondly, we wanted to ensure that learning was targeted to the CS that is predictive of the reward; in showing animals do not sign-track much to a CS-, we gain confidence that the CS+ itself (and not any item in the task chamber) gains a Pavlovian predictive relationship with the reward. Finally, having a CS- takes a step towards real-world relevance in the sense that discriminative learning about which stimuli do and do not relate to desired outcomes is ubiquitous. While we decided to include a CS-, some studies do not include a CS- [22]. We speculate that the logic is to avoid introducing a discrimination requirement to the learning conditions. While no studies examining how the inclusion or exclusion of a CS- impacts sign-tracking behavior have been performed, it is possible that the addition of a CS- biases the subject's attention to the CS+ because of this discriminative learning process which could render these subjects to sign-track more than in the absence of a CS-.

4.4. Applications

The results of this carry implications for understanding human motivated behaviors, particularly those that sign-tracking is thought to mode. Sign-tracking is just one of several reward-motivated behaviors that can be measured in rodent models. Although our study found that sex did not play a role in the acquisition of sign-tracking behavior or in its maintenance following outcome devaluation with our specific experimental paradigm, it remains to be seen if this lack of a sex-dependent effect applies to other similar behaviors. Theoretically, it is possible that other behaviors that capture aspects of "craving" or "wanting" motivation to food reward would also not be affected by the sex of the participant such as conditioned reinforcement or breakpoint on a progressive ratio task [28,29]. With regard to addiction, sign-tracking has been previously shown to be predictive of several drug-seeking behavioral measures including drug-seeking reinstatement and increased likelihood to endure punishment in order to obtain reward [30]. While an experiment directly comparing sign-tracking in the two genotypes still needs to be performed, Sprague Dawley rats seem to exhibit sex differences while Long Evan rats do not, Sprague Dawley rats might be a good animal model to use to model human disorders which exhibit sex differences such as compulsive eating and Cocaine Abuse Disorder in which females seem to be more at risk for the development and continued maintenance of these disorders [1,2,31]. Additionally, it is difficult to directly compare sign-tracking levels in strain via PCA index across studies, but it does look like Long Evans rats could potentially interact with a lever-cue more than Sprague Dawley rats [11,21]. Examining Pavlovian Approach behaviors in Long Evans rats may bias research towards sign-tracking instead of goal-tracking which could be desired or undesired based on if the research focus is on cue approach or on reward approach. With respect to human research, initial work looking at Value Modulated Attentional Capture (VMAC), a measure of attentional capture to cues that predict rewards and behavioral analogue to sign-tracking in humans, there were no sex differences when examining VMAC regarding risky alcohol behavior [32]. Even more relevantly, a recent study explicitly used a sign-tracking paradigm in human children; this group didn't find sex differences in sign-tracking like our findings [33]. Pending results from future experiments, this so far implies that Long Evans rats might be a good animal model to model human disorders that lack sex differences and to model human compulsivity, craving, and wanting behavior more generally in the absence of a specific disorder.

5. Conclusion

With this study, we aimed to determine if sex was a factor in the acquisition of sign-tracking behavior and if sex played a role in compulsive sign-tracking behavior as measured by sign-tracking levels following outcome devaluation. During acquisition, we found that both male and female animals were able to discriminate between the CS+ and the CS- lever and interacted with the CS+ lever more than the CS- lever; however, there was no difference between male and female rats in ultimate sign-tracking levels at the end of acquisition, and there was also no difference in the rate of acquiring the sign-tracking behavior. During conditioned taste aversion learning, we found that the groups that had been treated with LiCl both learned to devalue the LiCl-paired pellets as both groups rejected them when they were available, and we found that males and females learned this association similarly. Next, following this devaluation, the sexes again did not differ in how sensitive their sign-tracking behavior was to devaluation in a post-devaluation extinction test and subsequent post-devaluation reacquisition sessions. We conclude that Long Evans rats do not always exhibit sex differences in cue-directed motivation, nor in how sensitive it is to change following reward devaluation, in contrast to other rodent strains like Sprague Dawley rats and discuss potential sources of variation across studies.

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Submission verification

This work has not been published previously and is not under consideration for publication elsewhere.

Conflict of interest

None.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114656.

References

[1] T. Kosten, et al., Gender differences in cocaine use and treatment response, J. Subst. Abus. Treat. 10 (1) (1993) 63–66.
[2] H. Fox, et al., The effects of exogenous progesterone on drug craving and stress arousal in cocaine dependence: impact of gender and cue type, Psychoneuroendocrinology 38 (9) (2013) 1532–1544.
[3] K. Amaya, J. Stott, K. Smith, Sign-tracking behavior is sensitive to outcome devaluation in a devaluation context-dependent manner: implications for analyzing habitual behavior, Learn. Mem. 27 (4) (2020) 136–149.
[4] S. Chang, K. Smith, An omission procedure reorganizes the microstructure of sign-tracking while preserving incentive salience, Learn. Mem. 23 (4) (2016) 151–155.
[5] B. Van den Boom, et al., Behavioral flexibility in a mouse model for obsessive-compulsive disorder: impaired pavlovian reversal learning in SAPAP3 mutants (n/a. Web), Genes Brain Behav. 18 (4) (2019), e12557 (n/a. Web).
[6] B. Tunstall, D. Kearns, Sign-tracking predicts increased choice of cocaine over food in rats, Behav. Brain Res. 281 (2015) 222–228.
[7] F. Versace, et al., Heterogeneity in brain reactivity to pleasant and food cues: evidence of sign-tracking in humans, Soc. Cogn. Affect. Neurosci. 11 (4) (2016) 604–611.
[8] M. Robinson, K. Berridge, Instant transformation of learned repulsion into motivational "wanting, Curr. Biol. 23 (4) (2013) 282–289.
[9] R. Derman, et al., Sign-tracking is an expectancy-mediated behavior that relies on prediction error mechanisms, Learn. Mem. 25 (2018) 550–563.
[10] L. Hammerslag, J. Gulley, Age and sex differences in reward behavior in adolescent and adult rats: age and sex differences in reward behavior, Dev. Psychobiol. 56 (4) (2014) 611–621.
[11] K. Pitchers, et al., Individual variation in the propensity to attribute incentive salience to a food cue: influence of sex, Behav. Brain Res. 278 (2015) 462–469.
[12] M. Bouton, et al., Effect of context on the instrumental reinforcer devaluation effect produced by taste-aversion learning, J. Exp. Psychol.: Anim. Learn. Cogn. 47 (4) (2021) 476–489.
[13] D. Bates, et al., Fitting linear mixed-effects models using lme4, J. Stat. Softw. 67 (2015) 1–48.
[14] A. Kuznetsova, P. Brockhoff, R. Christensen, lmerTest package: tests in linear mixed effects models, J. Stat. Softw. 82 (2017) 26.
[15] A. Kucinski, Y. Kim, Basal forebrain chemogenetic inhibition disrupts the superior complex movement control of goal-tracking rats, Behav. Neurosci. 133 (1) (2019) 121–134.
[16] A. Kucinski, C. Avila, M. Sarter. "Basal Forebrain Chemogenetic Inhibition Converts the Attentional Control Mode of Goal-Trackers to That of Sign-Trackers." (Web) eNeuro 9.6 (2022) (Web).
[17] P. Dickson, et al., Sex and strain influence attribution of incentive salience to reward cues in mice, Behav. Brain Res. 292 (2015) 305–315.
[18] C. Fitzpatrick, et al., Variation in the form of pavlovian conditioned approach behavior among outbred male Sprague-Dawley rats from vendors and colonies: sign-tracking vs. goal-tracking (Web), PLoS ONE 8 (10) (2013) (Web).
[19] S. Mahmodi, S. Peck, G. Madden, Effects of inter-trial interval on sign-tracking and conditioned reinforcer efficacy in female rats (Web), Behav. Process. 210 (2023) (Web).
[20] F. Van Haaren, A. Van Hest, Van, N. de Poll, Acquisition and reversal of a discriminated autoshpahed response in male and females rats: effects of long or short and fixed or variable intertrial interval durations, Learn. Motiv. 18 (1987) 220–233.
[21] D. Kochli, et al., Basolateral amygdala to nucleus accumbens communication differentially mediates devaluation sensitivity of sign- and goal- tracking rats (Web), Front. Behav. Neurosci. 14 (2020) (Web).
[22] M. Naeem, N. White, R. Burwell, Parallel learning in an autoshaping paradigm, Behav. Neurosci. 130 (4) (2016) 376–392.
[23] S. Morrison, M. Bamkole, S. Nicola, Sign tracking, but not goal tracking, is resistant to outcome devaluation (Web), Front. Neurosci. 9 (2015) (Web).
[24] C. María-Ríos, et al., Effects of predictive and incentive value manipulation on sign- and goal-tracking behavior (Web), Neurobiol. Learn. Mem. 203 (2023) (Web).
[25] S. Ahn, A. Philips, Dopamine efflux in the nucleus accumbens during within-session extinction, outcome-dependent, and habit-based instrumental responding for food reward, Psychopharmacology 191 (2007) 641–651.
[26] M. Panayi, S. Killcross, Functional heterogeneity within the rodent lateral orbitofrontal cortex dissociates outcome devaluation and reversal learning deficits (Web), Elife 7 (2018), e37357 (Web).
[27] V. Peterson, et al., Sex-dependent associations between addiction-related behaviors and the microbiome in outbred rats (Web), EBioMedicine 55 (2020) (Web).
[28] S. Bacharach, et al., Cannabinoid receptor-1 signaling contribution to sign-tracking and conditioned reinforcement in rats, Psychopharmacology 235 (2018) 3031–3043.
[29] J. Beckmann, M. Bardo, Environmental enrichment reduces attribution of incentive salience to a food-associated stimulus, Behav. Brain Res. 226 (1) (2012) 331–334.
[30] B. Saunders, T. Robinson, Individual variation in resisting temptation: implications for addiction, Neurosci. Biobehav. Rev. 37 (9) (2013) 1955–1975.
[31] D. Ferriday, J. Brunstrom, How does food-cue exposure lead to larger meal sizes? Br. J. Nutr. 100 (6) (2008) 1325–1332.
[32] L. Albertella, et al., Persistence of value-modulated attentional capture is associated with risky alcohol use (Web), Addict. Behav. Rep. 10 (2019) (Web).
[33] J. Colaizzi, et al., The propensity to sign-track is associated with externalizing behavior and distinct patterns of reward-related brain activation in youth (Web), Sci. Rep. 13 (2023) 4402 (Web).