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Individual variation in the propensity to attribute incentive salience to a food cue: influence of sex

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Abstract

There is considerable individual variation in the propensity of animals to attribute incentive salience to discrete reward cues, but to date most of this research has been conducted in male rats. The purpose of this study was to determine whether sex influences the propensity to attribute incentive salience to a food cue, using rats from two different outbred strains (Sprague-Dawley [SD] and Heterogeneous Stock [HS]). The motivational value of a food cue was assessed in two ways: (i) by the ability of the cue to elicit approach towards it and (ii) by its ability to act as a conditioned reinforcer. We found that female SD rats acquired Pavlovian conditioned approach behavior slightly faster than males, but no sex difference was detected in HS rats, and neither strain showed a sex difference in asymptotic performance of approach behavior. Moreover, female approach behavior did not differ across estrous cycle. Compared to males, females made more active responses during the test for conditioned reinforcement, although they made more inactive responses as well. We conclude that although there are small sex differences in performance on these tasks, these are probably not due to a notable sex difference in the propensity to attribute incentive salience to a food cue.

Keywords

sign-tracker; goal-tracker; sex differences; conditioned reinforcement; Pavlovian conditioned approach

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1. INTRODUCTION

Natural rewards (e.g., food and water) are powerful objects of desire, presumably because they are necessary for survival. However, much behavior directed towards obtaining them is not controlled so much by the reward itself, but by previously neutral stimuli (conditioned stimuli, CSs) that have been associated with them – i.e., sights, sounds and places that predict the availability and location of rewards. That is, if CSs are attributed with incentive salience they acquire the ability to act as incentive stimuli, and as such, attract animals towards them, often bringing them into close proximity with the reward itself [1–3]. In addition, incentive stimuli can arouse complex emotional and motivational states that serve to instigate motivation for them, and their associated reward, again facilitating the probability a reward will be sought and procured [4–7].

Animals vary in their propensity to attribute Pavlovian conditioned motivational properties (‘incentive salience’) to discrete reward cues. It has been reported, using a so-called ‘autoshaping’ procedure, that a cue (lever) associated with a food reward becomes attractive - eliciting approach towards it - only in some animals (sign-trackers, STs) [8–15]. Upon presentation of a lever-CS other animals go to the location of impending reward delivery (these animals are called goal-trackers, GTs) [10]. Thus, the lever-CS acquires one property of an incentive stimulus, attractiveness, to a greater extent in STs than GTs, and this predicts whether a food cue acquires other properties of an incentive stimulus [16–18]. For example, discrete reward cues arouse greater conditioned motivation, and act as a more effective conditioned reinforcer, in STs than GTs [19–21]. It has been suggested, therefore, that although a discrete food cue acts as an effective CS in both STs and GTs (i.e., it attains predictive value for both), it is attributed with greater incentive salience in STs. Interestingly, the propensity to approach a food cue also predicts the extent to which discrete drug cues acquire incentive motivational properties [13, 20, 22].

Previous studies on individual variation in the propensity to attribute incentive salience to reward cues have been conducted using male animals (mostly rats). There is, however, a great deal of clinical and preclinical research indicating that females are more responsive to both nondrug [23, 24] and drug rewards than are males [25–33], and that this increased responsive may be due to circulating sex hormones [34]. Drug self-administration studies have shown that females respond more than males through extinction and reinstatement procedures [35]. With regards to nondrug reward, Hammerslag and Gully (2013) demonstrated that female rats more readily acquired a goal-tracking response to a tone-CS, and were initially more resistant to extinction, compared to males. These studies suggest, therefore, that female rats may be more prone to attribute incentive salience to a reward cue than males. To further explore this hypothesis we quantified the extent to which a discrete food cue (lever-CS) acquired two properties of an incentive stimulus: (i) the ability to attract and (ii) the ability to act as a conditioned reinforcer, in male and female rats of two different outbred rat strains, Sprague-Dawley (SD) rats and Heterogenous Stock (HS) rats.

2. METHODS

2.1. Animals

Five-week old male (105–125 grams upon arrival) and female (90–110 grams upon arrival) SD rats were obtained from Harlan Laboratories (Indianapolis, IN, USA). These rats were acquired as litters, totaling 9 litters of male and female siblings ($n = 5\text{--}6$ each sex per litter, total $n = 48/\text{sex}$). Male (5–7 weeks old, 98–186 grams, $n = 24$) and female (5–7 weeks old, 86–143 grams, $n = 25$) HS rats from 11 different litters were acquired from the Medical College of Wisconsin. The National Institutes of Health (NIH)-derived HS rat colony at the Medical College of Wisconsin is comprised of outbred animals derived from a set of eight genetically and phenotypically diverse inbred founder strains: ACI/N, BN/N, BUF/N, F344/N, M520/N, MR/N, WKY/N, WN/N. These founder strains were selected because they represented a relative broad spectrum of laboratory rats' genomic differences and at least some were of independent origin [36, 37]. The HS rat colony has been maintained using a relatively large number of breeders (46 breeder pairs) and uses a circular breeding scheme in an effort to decrease inbreeding [37] which may, in part, explain their increased phenotypic variation.

Upon arrival, all rats were housed in same-sex pairs in Plexiglas cages and kept on a 12-h light/12-h dark cycle (lights on at 0800 h) with regulated temperature and humidity. Food and water were available *ad libitum*. The day prior to the first Pavlovian training session, SD males and females were 275–325 g and 135–165 g, and HS males and females were 210–310 g and 135–200 g, respectively. These weights correspond to roughly the same age in males and females. In order to determine stage of estrous cycling in a subset of SD females, (unstained) vaginal epithelial cells were examined daily via light microscope for at least 10 consecutive days beginning 2 days prior to onset of behavioral testing [38]. Males were handled daily during this time. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

2.2. Pavlovian Conditioned Approach

2.2.1. Conditioning Chambers—Med Associates test chambers (20.5 cm x 24.1 cm floor area, 29.2 cm high; Med Associates, St. Albans, VT) were used for Pavlovian training. Each chamber was equipped with a food receptacle located in the center of the 20.5 cm wall, 2.5 cm above the floor. A catch tray filled with corn-cob bedding was located underneath the floor, which was constructed from stainless steel rods. An illuminated retractable lever (Med Associates) was located approximately 2.5 cm to the left or right of the food receptacle, 6 cm above the floor. The side of the lever with respect to the food receptacle was counterbalanced across boxes to eliminate any side bias. A red house light was located on the wall opposite the food receptacle and remained on for the duration of training sessions. A white LED was flush-mounted on the inside of the lever and was used to illuminate the slot through which the lever protruded. The lever required a 10 g force to deflect, such that most contacts with the lever were recorded as a 'lever press'. The pellet dispenser (Med Associates) delivered one-45 mg banana-flavored food pellet (Bio-Serv®, #F0059, Frenchtown, NJ) into the food receptacle at a time. Head entry into the food receptacle was recorded each time a rat broke the infrared photobeam located inside the receptacle

(approximately 1.5 cm above the base of the food cup). Each conditioning chamber was located in a sound-attenuating enclosure and background noise was supplied by a ventilating fan to mask outside noise.

2.2.2. Pavlovian conditioning procedures—Rats were acclimated to the colony room for several weeks and handling occurred on a regular basis before training procedures commenced. All training sessions were conducted during the 12-h lights on period. Prior to the start of training ~20 banana-flavored food pellets were placed into the rats' home cages to familiarize the animals with this food. For pre-training rats were placed into the test chamber and red light remained on yet the lever remained retracted. Twenty-five food pellets were delivered on a variable interval (30 s) schedule in order to determine whether rats were reliably retrieving the pellets from the receptacle. By the end of the pre-training session, all rats consumed all of the food pellets. Pavlovian training began the following day using a procedure similar to that described previously [39]. During a Pavlovian training session, each individual trial consisted of the insertion of the illuminated lever (CS) into the chamber for 8 s, and immediately following the retraction of the lever the pellet dispenser was activated causing the delivery of a single food pellet (unconditioned stimulus, US) into the food receptacle. The intertrial interval (ITI) started immediately following the retraction of the lever. The CS was presented on a random interval 90 s schedule (i.e., one presentation of the CS occurred on average every 90 s, but the actual time between CS presentations varied randomly between 30 and 150 s). Each Pavlovian training session consisted of 25 trials, resulting in a 35–40 min session, and training was conducted over 8 consecutive days. We recorded the following events for each trial: (i) number of lever deflections, (ii) latency to first lever deflection, (iii) number of head entries into the food receptacle (referred to as magazine entries) during presentation of the CS, (iv) latency to the first receptacle entry following CS presentation, and (v) number of magazine entries during the ITI. All animals included in analysis consumed all food pellets during each training session. At the end of each training session the animals were returned to their home cages in the animal colony room.

To quantify the propensity of each individual rat to approach the lever-CS vs the food magazine during the CS period (i.e., sign-tracking or goal-tracking) a Pavlovian conditioned approach (PCA) index score was calculated, as previously described in detail [19]. This PCA index score consisted of averaging three measures of conditioned approach: (i) the *probability* of contacting either the lever-CS or food magazine during the CS period [$P(\text{lever}) - P(\text{magazine})$]; (ii) the *response bias* for contacting the lever-CS or the food magazine during the CS period [$(\# \text{lever-CS contacts} - \# \text{food magazine contacts}) / (\# \text{lever-CS contacts} + \# \text{food magazine contacts})$]; and (iii) the *latency* to contact the lever-CS or the food magazine during the CS period [$(\text{food magazine contact latency} - \text{lever-CS contact latency}) / 8$]. Averaging these three measures produces PCA index scores which are values on a scale ranging from -1.0 to +1.0, where +1 indicates an animal made a sign-tracking CR on every trial and -1 a goal-tracking CR on every trial, and 0 a 50:50 distribution of sign- and goal-tracking CRs. For the purpose of classification, rats with a PCA index score ranging from -1.0 to 0 were operationally defined as GTs (i.e., rats more likely to direct behavior towards food magazine than lever), and rats with a PCA index score between 0 and +1.0

were designated as STs (i.e., rats more likely to direct behavior towards the lever-CS than the food magazine).

2.3. Conditioned Reinforcement

The day after the 8th and final day of Pavlovian training, all rats were subjected to a 60 min test for conditioned reinforcement. Rats were tested in the same, yet reconfigured, chambers used for Pavlovian training. The food pellet receptacle was removed and the lever was moved into its central location where it was flanked by two nose-poke ports. Two nose-poke ports (2 cm diameter, 2 cm above floor) were equipped with photocells. One port was designated 'active' and the other 'inactive' in a counterbalanced manner. When a rat made a nose poke into the active port the lever was illuminated and inserted into the chamber for 2 s. A nose poke into the inactive port had no consequence. This test occurred under extinction conditions, such that no food pellets were delivered. Responses into the active and inactive ports were recorded.

2.4. Statistical Analysis

Repeated measures analysis of variance (ANOVA) were used to analyze training session data: PCA index scores, probability, contacts and latency. The SD and HS rats were never directly compared, as these were conducted as two separate experiments. Within strains, sex differences were examined for rats classified as STs using dependent variables related to lever (CS)-directed behavior, and for rats classified as GTs dependent variable related to goal-directed behavior were analyzed. A one-way ANOVA was used to determine the effect of estrus cycle/circulating sex hormones on PCA index score. A two-way ANOVA with the factors of sex (male and female) and port (active and inactive) was used to analyze data from the conditioned reinforcement test. Significant main effects and interactions were followed by planned pairwise comparison (Bonferroni t-test). Statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. Pavlovian conditioned approach behavior in male and female SD and HS rats

3.1.1. SD Female rats acquired a Pavlovian conditioned response faster than SD males, whereas HS females and males did not differ in Pavlovian conditioned response—

Figure 1 shows the change in mean ($\pm$ SEM) PCA index scores (a score of +1 indicates a ST response on all trials and -1 a GT response on all trials) as a function of the 8 consecutive Pavlovian training sessions for male and female SD (Fig 1A) and HS (Fig 1C) rats. As expected, there was a significant main effect of session in both rat strains (SD: $F_{(7,658)} = 97.228$, $p < 0.001$; HS: $F_{(7,329)} = 11.022$, $p < 0.001$). There was no main effect of sex for either strain (SD: $F_{(1,658)} = 2.199$, $p = 0.141$; HS: $F_{(1,329)} = 0.370$, $p = 0.546$), however, there was a significant interaction in SD rats (SD: $F_{(7,658)} = 2.502$, $p = 0.015$), but not HS rats ($F_{(7,329)} = 0.362$, $p = 0.924$). This interaction suggests that SD females acquired a PCA response faster than males, although this was not seen in the HS rats. Furthermore, even in the SD rats, a significant sex difference was only detected on day 3. In addition, when terminal PCA Index scores were calculated from the average of sessions 7 and 8, no sex differences were found in either strain (Fig 1B,D).

Figure 2 shows PCA index scores (averaged sessions 7 and 8) for every individual male and female rat tested. An average of the final 2 days (days 7 and 8) of Pavlovian training was selected for calculating PCA scores and used to compare between groups because PCA behavior was most stable at this point. As expected from the fact that there was no sex difference in the mean PCA Index scores in either strain (Fig. 1B, D), the distribution of the individual scores within the strains for male and female SD (Fig. 2A, B), and male and female HS (Fig. 2C, D) rats was very similar. However, it is evident that in the SD rats (Fig 2A, B) the distribution was shifted strongly towards sign-tracking - most index scores were positive - for both males and females. These rats were acquired from Harlan Laboratories and as described previously [40], PCA behavior varies significantly as a function of vendor and even individual source colonies of the same vendor. It is often the case that any particular 'batch' of rats can be skewed towards either a ST or GT CR [40], and the batch used here was strongly biased towards the former. In contrast, the genetically diverse HS rats were more uniformly distributed across the spectrum of potential index scores (−1.0 to +1.0) and this distribution was similar for males and females.

3.1.2. SD females acquired a sign-tracking response more rapidly than SD males, whereas HS females and male did not differ in sign- or goal-tracking responses—

To look in more detail at the different measures of Pavlovian conditioned approach behavior, animals were classed as STs or GTs based on their performance on Pavlovian training sessions 7 and 8. For SD rats, we only show data for STs because there were too few GTs ($n = 4/\text{sex}$) to make statistically meaningful comparisons between males and females. Thus, Figure 3 shows SD female and male STs across training sessions on 3 measures of lever (CS)-directed behavior: probability, contacts and latency. There was a significant main effect of session for all measures (3A, Lever Prob, $F_{(7,595)} = 52.323$, $p < 0.001$; 3B, Lever Contacts, $F_{(7,595)} = 49.540$, $p < 0.001$; 3C, Lever Latency, $F_{(7,595)} = 82.903$, $p < 0.001$), but no main effect of sex for any measure (3A, Lever Prob, $F_{(1,595)} = 2.345$, $p = 0.129$; 3B, Lever Contacts, $F_{(1,595)} = 0.516$, $p = 0.475$; 3C, Lever Latency, $F_{(1,595)} = 2.749$, $p = 0.101$). However, there was also significant sex $\times$ session interaction for all behavioral parameters (3A, Lever Prob, $F_{(7,595)} = 4.565$, $p < 0.001$; 3B, Lever Contacts, $F_{(7,595)} = 6.443$, $p < 0.001$; 3C, Lever Latency, $F_{(7,595)} = 3.476$, $p < 0.001$). This significant interaction indicates that SD females acquired a ST response more rapidly than males, as expected from analysis of the PCA scores (Fig. 1A). This sex difference was apparent only on the first few training sessions, as no sex difference in performance was detected on any measure from sessions 4 to 8 (Fig 3A–C).

Figure 4 shows the performance of HS STs and GTs of both sexes across training sessions. STs were compared on lever (CS)-directed behavioral parameters (Fig 4A–C), whereas GTs were compared on goal-directed behavior parameters (Fig 4D–F). As expected, there was a significant main effect of session indicating that STs and GTs acquired their respective CRs (4A, Lever Prob, $F_{(7,195)} = 34.934$, $p < 0.001$; 4B, Lever Contacts, $F_{(7,195)} = 21.425$, $p < 0.001$; 4C, Lever Latency, $F_{(7,195)} = 7.833$, $p < 0.001$; 4D, Mag Prob, $F_{(7,119)} = 16.459$, $p < 0.001$; 4E, Mag Contacts, $F_{(7,119)} = 19.827$, $p < 0.001$; 4F, Mag Latency, $F_{(7,119)} = 2.989$, $p = 0.006$). However, there was no main effect of sex or a significant sex $\times$ session interaction for any measure (Figure 4A, Lever Prob, $F_{(1,195)} = 0.664$, $p = 0.422$; 4B, Lever Contacts,

$F_{(1,195)} = 0.464$, $p = 0.501$; 4C, Lever Latency, $F_{(1,195)} = 2.895$, $p = 0.100$; 4D, Mag Prob, $F_{(1,119)} = 3.362$, $p = 0.083$; 4E, Mag Contacts, $F_{(1,119)} = 2.714$, $p = 0.116$; 4F, Mag Latency, $F_{(1,119)} = 2.112$, $p = 0.162$) or (4A, Lever Prob, $F_{(7,195)} = 0.694$, $p = 0.677$; 4B, Lever Contacts, $F_{(7,195)} = 0.130$, $p = 0.996$; 4C, Lever Latency, $F_{(7,195)} = 0.374$, $p = 0.917$; 4D, Mag Prob, $F_{(7,119)} = 1.346$, $p = 0.235$; 4E, Mag Contacts, $F_{(7,119)} = 0.856$, $p = 0.544$; 4F, Mag Latency, $F_{(7,119)} = 0.883$, $p = 0.522$), indicating that in HS rats there was no sex difference in the acquisition of either sign- or goal-tracking CRs.

3.1.2. Estrous cycle did not affect Pavlovian Conditioned Approach behavior

—Figure 5 shows the PCA index scores for a subset of female SD rats, in which the estrus cycle was monitored using microscopic analysis of unstained vaginal epithelia cells for 10 consecutive days, including the time period during which Pavlovian training occurred. Of the 32 monitored females, 28 displayed consistent and conclusive patterns allowing for estrus cycle mapping, which permitted inclusion in analysis. PCA index scores from sessions 5–8 were re-ordered according to the determined estrus cycle phase: proestrus, estrus, metestrus or diestrus. Prior to estrus cycle phase grouping PCA index scores for training sessions 5–8 did not significantly differ, and after re-ordering there still was not a significant effect of estrus cycle on PCA index scores ($p = 0.722$).

3.2. Test for Conditioned Reinforcement in male and female SD and HS rats

3.2.1. Females made more responses in active and inactive ports compared to males

—Figure 6 shows mean (+SEM) active and inactive nose pokes during a test for conditioned reinforcement in SD (Fig 6A) and HS (Fig 6B) male and female rats. There was significant main effect of sex for both strains (SD: $F_{(1,190)} = 27.513$, $p < 0.001$; HS: $F_{(1,94)} = 7.837$, $p = 0.006$), indicating that females made more responses overall (both active and inactive) than males. There was also a significant effect of port (SD: $F_{(1,190)} = 256.273$, $p < 0.001$; HS: $F_{(1,94)} = 27.686$, $p < 0.001$) in both strains, indicating that both males and females made more responses in the active port than the inactive port. For SD rats there was a significant sex x port interaction ($F_{(1,190)} = 4.292$, $p < 0.040$), but no such interaction was found for HS rats ($F_{(1,94)} = 0.792$, $p = 0.376$). Pairwise comparisons revealed a significant difference between sexes for not only active responses (SD, $p < 0.001$; HS, $p < 0.001$), but also inactive responses (SD, $p = 0.026$).

4. DISCUSSION

The NIH recently issued a policy statement indicating that they are going to require the inclusion of both sexes in preclinical studies [41]. We were inspired by this to report previously unpublished experiments, using two different outbred rat strains (Sprague-Dawley [SD] and Heterogeneous Stock [HS]), designed to determine whether sex influences individual variation in the propensity to attribute incentive salience to a food cue. Specifically, we estimated the motivational value of a food cue in two ways: (i) by the degree to which it came to elicit approach towards it and (ii) by its ability to serve as a conditioned reinforcer [13, 16, 17, 20, 42]. We were able to detect some sex differences, but they were very minor, only evident in one strain and dependent on the behavioral test employed. SD females acquired Pavlovian conditioned approach to a food cue (sign-

tracking) slightly faster than SD males, but there was no difference in asymptotic performance and no sex differences on this task in HS rats. On the test for conditioned reinforcement the food cue was a more effective conditioned reinforcer in SD females, although the effect was very small, and was not seen in HS rats. It is also notable that females of both strains made more inactive responses than males. Overall, although male and female rats differ to some extent in performing these tasks they do not appear to vary to an important degree in their propensity to attribute incentive salience to a food cue, at least as assessed by the measures used here.

We have previously reported that the propensity to approach a food cue predicts the extent to which discrete drug cues acquire incentive motivational properties, and instigate drug-seeking behavior [20, 22]. The role of sex differences and circulating sex hormones have been studied in all phases of drug abuse, including, but not limited to, acquisition, maintenance, escalation and reinstatement. Females appear to be more sensitive to drugs than males at each of these phases (for review see [43]; [26, 34, 44]) and most studies support a critical role of estrogen as the underlying factor. Most pertinent to our findings, females acquire drug self-administration of cocaine [45, 46], methamphetamine [47] and nicotine [48], and in some reports heroin [46], more rapidly than males. Regarding cues, females make more responses during signaled i.v. cocaine availability, whereas no sex difference was detected during non-signaled i.v. cocaine [49].

However, it is difficult to pinpoint the psychological mechanism(s) responsible for these sex differences. In the present study, the more rapid acquisition of Pavlovian conditioned approach in SD females was only evident for the first few days of training, and there was no difference between sexes in terminal performance, consistent with findings using sucrose [23]. Many factors could contribute to this sex difference in performance, including motivation [50], attention [51, 52], learning [53, 54], impulsivity [52, 55, 56] or even differences in activity/exploration [57–60]. For example, females have been reported to work more than males just for sensory stimulation[28], and this could have contributed to the increase in active nose pokes by females compared to males in the test for conditioned reinforcement – although it would not account for greater inactive responses as well in females. Whatever the case, the small sex differences we observed may very well be due to greater activity/exploration, in particular, as a result of environmental change (i.e., lever extension), rather than attribution of incentive motivational properties to the lever [28, 61, 62]. Given that we found no differences in PCA scores (except during session 3 in SD), no difference in terminal performance PCA, and the small difference in conditioned reinforcement was confounded because females were generally more active, we conclude that any sex difference in performance was probably not due to differences in the propensity to attribute incentive salience to reward cues. The small differences in performance are more likely due to one or more of the other factors mentioned above.

The current study utilized two different outbred strains of rats: SD and HS. Although there were only small sex differences within each strain, there was a substantial difference in PCA behavior between SD and HS strains. Differences in PCA behavior have been demonstrated previously between rat strains (i.e., Lewis and Fischer) [63], between vendors of similar strains, and between individual colonies from the same vendor [40]. Different phenotypes,

including PCA behavior, can result from environmental or genotypic factors, or a combination of both. For example, early life stress due to maternal separation results in adult rats exhibiting more ST behavior [64], whereas being reared in an enriched environment - compared to being raised in isolation - decreases ST behavior [65]. Furthermore, population bottleneck and random genetic drift can reduce genetic variation [66, 67], thus the occurrence of either event within a population could contribute to variation in PCA behavior. Here, we used 9 different litters of SD rats ($n = 5-6$ per sex per litter) and found a significant effect of litter (data not shown). Although this litter effect could represent a genetic contribution to PCA behavior, it could just as likely reflect an environmental contribution to PCA behavior (e.g., maternal care), since we did not monitor or control for early life experiences. It is interesting that the HS rats showed especially marked phenotypic variation in PCA behavior, suggesting these animals may be especially advantageous for studying the potential genetic basis of variation in the propensity to attribute incentive salience to reward cues (e.g., <http://ratgenes.org>).

In summary, we conclude that if sex influences individual variation in the propensity to attribute incentive salience to a food cue, it is only a very minor effect, at least as indicated by the measures used here. An extension of this conclusion is that the greater vulnerability of females to addiction may not be due to a greater propensity to attribute incentive salience to drug cues. However, we have to quickly add that this latter hypothesis is tempered by the possibility that, unlike with males [13], in females, responsivity to a food cue may not predict the response to drug cues. Additional studies will be required to determine if this is the case.

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Highlights

- Two different outbred strains: Sprague-Dawley and Heterogeneous Stock
- Motivational value of the cue was assessed by two measures
- Female SD rats acquired PCA behavior slightly faster than males, but not in HS rats
- No sex difference or any impact of estrous cycle in asymptotic PCA behavior
- Females had more active/inactive responses in the conditioned reinforcement test

Figure 1. Pavlovian conditioned approach index scores in male and female SD and HS rats
PCA index scores (group mean $\pm$ SEM) across 8 daily sessions of training and mean PCA index ($\pm$ SEM) score over the last two sessions of training subdivided by sex (female, white; male, black) and strain: (A, B) Sprague Dawley (n = 48/ sex) and (C,D) Heterogeneous Stock (female, n = 24; male, n = 25). * indicates significant difference between sexes during training session (p < 0.05).

Figure 2. Individual variation in Pavlovian conditioned approach behavior

PCA index scores (−1.0 to +1.0) for every individual rat, averaged from the last two sessions of Pavlovian training. Sprague Dawley (SD) (A) females (n = 48) and (B) males (n = 48), and Heterogeneous Stock (HS) (C) females (n = 24) and (D) males (n = 25).

Figure 3. Lever (CS)-directed behavior (sign-tracking) during Pavlovian training in Sprague Dawley rats designated as sign-trackers

Group means $\pm$ SEM for Sprague Dawley female sign-trackers (white, PCA index score between 0 and 1.0, $n = 44$) and male sign-trackers (black, PCA index score between 0 and 1.0, $n = 44$) for: (A) probability of approaching the lever (CS) during 8 s CS period, (B) number of lever contacts, and (C) latency to first lever contact after CS presentation. For all measures, there was a significant effect of session ($p < 0.001$). * indicates significant difference between males and females during training session ($p < 0.05$).

Figure 4. Lever (CS)-directed behavior (sign-tracking) vs. food magazine-directed behavior (goal-tracking) during Pavlovian training in Heterogeneous Stock rats

Group means $\pm$ SEM for HS female sign-trackers (white circles, PCA index score between 0 and 1.0, $n = 16$) and goal-trackers (white squares, PCA index between -1.0 and 0, $n = 8$), and male sign-trackers (black circles, PCA index score between 0 and 1.0, $n = 14$) and goal-trackers (black squares, PCA index between -1.0 and 0, $n = 11$) for: (A) probability of approaching the lever (CS) during 8 s CS period, (B) number of lever contacts, (C) latency to first lever contact after CS presentation, (D) probability of approaching the food magazine during the 8 s CS period, (E) number of head entries into the food magazine during the 8 s CS period, and (F) latency to first head entry into the food magazine entry after CS presentation. There was no main effect of sex or a significant sex $\times$ session interaction, for any measure in the acquisition of either ST or GT CRs.

Figure 5. Pavlovian conditioned approach index scores within a subset of Sprague Dawley females throughout estrous cycle

PCA index scores (group mean + SEM) from training sessions 5 through 8 re-ordered based on estrous cycle phase (n = 28).

Figure 6. Test for conditioned reinforcement

Group means + SEM for active and inactive responses by females (white) and males (black) within strains: (A) Sprague Dawley ($n = 32/\text{sex}$) and (B) Heterogeneous Stock (female, $n = 24$; male, $n = 25$). * indicates significant difference compared to females within active or inactive responses ($p < 0.05$).