Sex and the Stimulus-Movement Effect: Differences in Acquisition of Autoshaped Responding
in Cynomolgus Monkeys

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Abstract

The stimulus-movement effect refers to the phenomenon in which stimulus discrimination or acquisition of a response is facilitated by moving stimuli as opposed to stationary stimuli. The effect has been found in monkeys, rats, and humans, but the experiments conducted did not provide adequate female representation to investigate potential sex differences. The current experiment analyzed acquisition of stimulus touching in a progressive series of classical conditioning procedures in cynomolgus monkeys (*Macaca fascicularis*) as a function of sex and stimulus movement. Classical conditioning tasks arrange two or more stimuli in relation to each other with different temporal and predictive relations. Autoshaping procedures overlay operant contingencies onto a classical-conditioning stimulus arrangement. In the present case, a neutral stimulus (a small gray square displayed on a touchscreen) functioned as the conditional stimulus and a food pellet functioned as the unconditional stimulus. Although touching is not required to produce food, with repeated stimulus pairings subjects eventually touch the stimulus. Across conditions of increasing stimulus correlation and temporal contiguity, male monkeys acquired the response faster with a moving stimulus. In contrast, females acquired the response faster with a stationary stimulus. These results demonstrate that the stimulus-movement effect may be differentially affected by sex and indicate that additional experiments with females are needed to determine how sex interacts with behavioral phenomena discovered and elaborated almost exclusively using males.

1. Introduction

Pavlovian, respondent, or classical conditioning procedures arrange for a predictive and temporal relation between a stimulus (conditional stimulus, CS) and a biologically relevant stimulus (unconditional stimulus, US). Under these procedures, responding to the CS is generated and maintained without a programmed operant-response contingency and such engendered responding (i.e., learning) has been demonstrated in rodents [1, 2], birds [3, 4], nonhuman primates [5], and many other species [e.g., 6, 7]. Classical conditioning processes are viewed as fundamental, and almost certainly served as a critical adaptation and subsequent selection criterion throughout Cambrian animal species diversification and evolution [8].

The predictive relation between the CS and US can vary across procedures ranging from backward conditioning (the US is delivered prior to CS onset), to delay conditioning (the US is delivered soon after or immediately upon CS offset), and trace conditioning (the US is delivered after a considerable delay following CS offset). Across these procedures, delay conditioning is typically more effective in producing and maintaining CS-directed responding than trace conditioning, and both are more effective than backward conditioning [9]. Random conditioning procedures present the CS and US irrespectively of each other, generating little or no CS-directed responding, thereby providing an important control procedure to demonstrate that conditioning is dependent not simply upon the presentation of these stimuli but on their temporal and predictive relations [10].

Autoshaping procedures (also referred to as positive automaintenance, or PAM, procedures) superimpose operant-response contingencies onto classical-conditioning procedures, and responses typically lead to the immediate offset of the CS and delivery of the US (often a

morsel of food in laboratory animal studies). Since its discovery [3], autoshaping has been used to quickly and easily generate a target response (e.g., lever pressing or key pecking in rats and pigeons, respectively). In many operant research laboratories, autoshaping has replaced “manual shaping” as a means of generating the target operant response because it allows for automation of the shaping process for multiple subjects at the same time, greatly reducing the time commitments of investigative personnel. In the current experiment, different CS-US arrangements were presented in a fixed order to determine when CS-direct touching was acquired by cynomolgus macaques (*Macaca fascicularis*) of different sexes. Here, however, we arranged for the CS to either remain stationary or move about the touchscreen during each trial.

In the majority of classical and operant conditioning research, stimuli are presented in fixed locations. This is primarily a restriction of the equipment available, as stimuli and operanda are often seated or housed inside a wall or panel opening in the chamber. Although other options for presenting moving stimuli are becoming more popular (e.g., a touchscreen), they are sometimes not suitable for certain species or tasks or may be too expensive or difficult to employ. Because of these factors, experiments investigating the effects of moving stimuli on acquisition and learning have been scarce. Research to date, however, has determined that moving stimuli may facilitate acquisition and performance in various tasks.

The stimulus-movement effect refers to the phenomenon in which stimulus discrimination or acquisition of a response is facilitated by the movement of a stimulus when compared to a stationary stimulus. Stimulus movement in these scenarios is the velocity of a stimulus within a trial, as compared to a stationary stimulus which has no velocity. Even though stimuli may appear in new locations from trial to trial, such relocation is not considered stimulus movement when discussing the stimulus-movement effect.

Harlow and colleagues [11] were the first to report this effect with rhesus monkeys (*Macaca mulatta*) on two discrimination learning tasks. In experiment 1, the monkeys learned to discriminate between color (red [S+] vs. green [S-]) and movement (moving [S+] vs. stationary [S-]) in separate conditions. Following training, subjects were then given test conditions that reversed which stimulus was associated with reinforcement (i.e., red [S-] vs. green [S+] and moving [S-] vs. stationary [S+]). The monkeys initially performed better in the movement reversal, though after sufficient training color and movement discrimination performances were similar. In experiment 2, the discrimination was based on color, and the stimuli were either moving or stationary. In this experiment, movement was simply another aspect of the stimuli and provided no differentiating cues for reinforcement. Subjects reached asymptotic performance 40-50 trials earlier in the moving-stimulus group compared to the stationary-stimulus group, concordant with the stimulus-movement effect.

This experiment was followed by two experiments conducted by Kardos, da Pos, Dellantonio, and Saviolo [12, 13] wherein rats learned to exit a maze through a white door rather than a black door. The exit door was either stationary or swung continuously in a pendulum-like manner during each trial. Learning was enhanced with the moving exit door compared to the stationary one.

Washburn and colleagues then conducted a series of experiments with rhesus monkeys using computerized video tasks. The first of these experiments [14] presented four separate tasks in which measures of performance were enhanced by a moving stimulus: learning sets of stimuli, tracking reinforcement during reversal conditions, match-to-sample, and delayed match-to-sample tasks. The authors attributed these findings to attentional factors—moving stimuli commanded the attention of the subjects, so learning and performance were facilitated.

Washburn [15] replicated these basic findings with another pair of rhesus monkeys using the same learning set, tracking reversals, and match-to-sample tasks. Each of these tasks used stationary, moving, or flashing stimuli, with the assumption that the flashing stimulus would focus the attention of the rhesus monkeys without any movement. However, performances with stationary and flashing stimuli were identical on all three tasks, whereas performance was enhanced with moving stimuli. The stimulus-movement effect has also been documented in humans using tasks similar to those used with rhesus monkeys [16].

These findings were then extended to cynomolgus macaques by Bullock and Myers [17]. In their autoshaping (PAM) procedure, a small gray square (CS) was presented on a touchscreen for 8 seconds. Food delivery occurred at the offset of the stimulus, and a touch to the square would immediately terminate the CS and deliver a food pellet. Stimulus movement was examined with a stationary-stimulus group and a moving-stimulus group. The researchers found that monkeys in the moving-stimulus group acquired CS-directed touching more rapidly than those in the stationary-stimulus group, extending the stimulus-movement effect from rhesus macaques to cynomolgus macaques and from operant learning procedures to an autoshaping paradigm.

Most recently, Daniel and Katz [18] attempted to replicate the stimulus-movement effect in pigeons. They presented moving stimuli on a touchscreen using multiple stimulus velocities and delay lengths in a delayed match-to-sample task. The authors found a negative stimulus-movement effect in pigeons, which runs counter to what has been demonstrated with primates. For pigeons, accuracy was higher for stationary stimuli than moving stimuli. Movement is clearly an important sensory cue for organisms [19], but the underlying mechanism for the stimulus-movement effect has yet to be thoroughly explained. These recent results suggest that

the effect may vary across species and it is possible that the stimulus-movement effect may vary *within* a species as a function of other factors, such as age or sex.

The stimulus-movement effect has been found in rats, humans, and multiple species of monkeys. One particularly important shortcoming of these studies, however, is the almost exclusive lack of female subjects. In all but one study using monkeys, the subjects were either all male [14, 16, 17] or sex was not reported [11]. Only one study had a (single) female rhesus monkey [15]. In the human stimulus-movement research literature, both males and females are represented [16], but there is no indication that sex was ever included in any of the statistical analyses. Therefore, whether the stimulus-movement effect varies between sexes within a given species is currently unknown. The comparison between sexes is important because males typically score better on spatial problem-solving tasks than females in humans [20-22], non-human primates [23], rodents [24, 25], birds [26, 27], reptiles [28], and fish [29]. However, the type of spatial task that is used often determines the magnitude of the reported sex difference and in some species the sex difference is absent or opposite [e.g., 30, 31, 32]. For example, in shiny cowbirds (*Molothrus bonariensis*), females outperformed males in a spatial-cognition task [30], possibly due to species-specific pressures of the females to navigate to host nests. Although the comparison between moving and stationary stimuli is not a definitive spatial problem-solving task, past spatial-cognition research suggests possible sex differences in attention, responding, and conditioning to moving stimuli.

The current experiment was arranged to examine whether sex differentially affects acquisition of a CS-directed response to moving stimuli in cynomolgus macaques. Because previous literature suggests that the stimulus-movement effect is best revealed under acquisition, we arranged various CS-US pairing procedures (i.e., random, backward, trace, delay), ordered

from least to most likely to engender CS-directed responding. This approach provided weak stimulus conditioning arrangements first to best detect differences in acquisition as a function of stimulus movement. With each new condition, the correlation and temporal contiguity between stimulus presentation and pellet delivery became stronger and therefore more likely to engender stimulus touching. This is important because previous work from our laboratory [17] produced rapid acquisition under both autoshaping (PAM) and delay conditioning arrangements, but the study did not present any negative control conditions (e.g., random or backward) to naïve monkeys prior to response acquisition. Thus, it was not clear whether response acquisition would have occurred in the absence of a predictive and temporally contiguous CS-US relation. The present study, by arranging the sequence of conditions in order of increasing CS-US correlation and temporal contiguity, was designed to demonstrate control of response acquisition by these CS-US relations. Additionally, by doing so, we believed that the experiment would be more likely to detect differences in the emergence of response acquisition as a function of not only these variables, but also as a function of sex and stimulus movement. To this end, both male and female cynomolgus macaques were equally and randomly assigned into stationary- and moving-stimulus groups to determine whether acquisition of CS-directed responding would differ as a function of stimulus movement across these CS-US conditions.

2. Methods

2.1 Subjects

Sixteen (8 male, 8 female) young adult (approximately 5 years old) cynomolgus macaques (*Macaca fascicularis*) were acquired from a commercial vendor and allowed to acclimate to our facility for several months prior to the start of experimentation. All monkeys were experimentally naïve and pair-housed except during the behavioral session and feeding. A

standard primate diet (Harlan Teklad Formula 8714 15% monkey diet) was fed twice daily except on behavioral test days wherein the 80 food pellets (190 mg precision pellet, F0035, Bio-Serv, Flemington, NJ) delivered during the test replaced the morning feeding. Monkey weights were maintained within a relatively narrow range and approximated 90-100% of published growth curves for each sex of this species throughout the 3-month study [33 n.d.]. The afternoon feeding generally occurred at 1300, and behavioral testing began at 0930 (M-F), so a fasting period of approximately 18 hours preceded each behavioral test session, further enhancing food motivation. A different 30 kcal serving of fresh or frozen fruit or vegetable was given with the afternoon feeding as dietary enrichment.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense (USAMRICD) and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The USAMRICD is a research facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.2 Apparatus

The subjects were tested unrestrained in their home cages. Each monkey had a dedicated aluminum panel that was affixed to the front of the home cage during daily behavioral testing. During testing, the animal's home cage door was raised and secured to allow unhindered access to the touchscreen and food cup. Each panel consisted of a touchscreen monitor (38 cm diagonal, 22.86 cm tall by 30.48 cm wide, Surface Acoustic Wave flat panel LCD, Model 1547L, ELO, Inc., Menlo Park, CA, USA), a pellet dispenser that delivered a 190 mg calorically complete pellet as a US/reinforcer, a food cup illuminated with a white 12-V LED, a clear acrylic door

attached to a microswitch (requiring 1.7 N of force to operate), and a notebook computer (Dell Latitude E3400 running Windows XP) that controlled experimental events and collected data via a custom-written Visual Basic 6.0 (Microsoft Corp., Redmond, WA, USA) software program. A screen touch of greater than approximately 0.69 N of force was counted as a response. Pellet dispenser operation, LED illumination, and detection of food cup door switch movements (i.e., open or closed) were managed by a USB relay I/O interface (model ADU208; Ontrak Control Systems®, Sudbury, Ontario, Canada) connected to the computer.

Apparatus failures occurred in two separate incidences. In one case the touchscreen occasionally or completely failed to register responses for subject 25. The error was detected after six consecutive sessions and then subsequently fixed. The second incident involved a broken microswitch for the food cup door, which failed to record when the food cup door was opened or closed for subject 20. Data from both of these incidents were excluded from all analyses, and substitute sessions immediately prior to the apparatus failure were selected for analysis. Subject 15 was erroneously moved onto another experiment following the completion of the Stimulus Pairing condition and never experienced the PAM condition (but had already acquired CS touching).

2.3 Procedure

The behavioral sessions occurred five days per week (Monday – Friday) and began with a five-minute delay during which the screen was blank. Each session lasted approximately 90 minutes, but subjects were left undisturbed for a total of 3 hours (i.e., an additional 90 minutes post-session) to ensure that the behavioral session was completed well before the apparatus was removed and afternoon feeding could occur.

2.4 Behavioral Conditions

For the first session, the behavioral test panel was hung on the cage, and 60 pellets were delivered to the food cup at a rate of one per second. Each monkey was allowed 100 minutes to consume all pellets. Equal numbers of males and females were matched according to the number of screen touches emitted during the first session and then randomly assigned to either the moving- or stationary-stimulus group to ensure each movement group had equal representation. The classical conditioning study began the next day. On each trial, the CS (a 4.57 cm gray square) appeared in a random location on the screen. In the stationary-stimulus group, the stimulus did not move. In the moving-stimulus group, the stimulus moved at a velocity of approximately 3.3 cm/s in a horizontal or vertical direction (randomly selected on each trial). Whenever the stimulus reached the edge of the screen the direction of movement simply reversed, remaining in the horizontal (left-right) or vertical (up-down) plane throughout a given trial. The CS was presented for 8 s, and the time between presentations ranged from 10 to 110 s, selected randomly. Pellet delivery (the US) was always accompanied by a 0.25 s period during which the screen turned white and the food cup light was illuminated.

For all conditions, there were 80 CS and US presentations over the 90.66 min session. Figure 1 provides a graphical representation of the conditions. The first condition (Random) arranged for the two stimulus events (CS and US) to occur randomly and irrespective of each other. The interval between US presentations ranged from 18 to 118 s. In the second condition (Backward), the US preceded the onset of the CS. In the third condition (30-s Temporal), the CS was presented at regular temporal intervals of 68 s, and the US was presented exactly 30 s after CS offset. In the fourth condition (30-s Trace), the US followed CS offset by exactly 30 s, but the interval between US presentation and the next CS onset varied from 5 to 55 s. The fifth condition (15-s Trace) was identical to the fourth except that the time between CS offset and US

presentation was reduced to 15 s, and the interval between US presentation and the next CS onset varied from 5 to 85 s. In the sixth condition (Stimulus Pairing), US presentation always coincided with CS offset, and the CS was presented 10 to 110 s after US presentation. Responses to the CS produced no programmed consequence during any but the final condition. The final condition arranged a typical positive automaintenance (PAM) procedure and was identical to the Stimulus Pairing condition, but a response to the CS terminated it immediately and produced the US. The order of conditions was arranged identically across all subjects because obtaining such a cohort of naïve cynomolgus macaques is difficult and costly and further subdivisions of group assignment would have greatly decreased the sample size, compromising the ability to detect potential sex differences.

3. Results

The primary dependent measure to assess learning is the proportion of trials in which at least one CS touch (hit) occurred, which was calculated across sessions. Figure 2 shows individual subject data for female monkeys, and figure 3 shows individual subject data for male monkeys. The bottom row of panels for both figures displays the associated group means and standard error of the mean. The stationary-stimulus group is shown in the panels on the left and the moving-stimulus group is shown in the panels on the right. Acquisition was defined as three consecutive sessions wherein the proportion of trials with at least one hit was above 50%. Males typically acquired CS-directed touching faster than females, regardless of the stimulus-movement condition to which they were assigned. Furthermore, males in the moving-stimulus group acquired the target response faster than males in the stationary-stimulus group. In contrast, females in the stationary-stimulus group typically acquired CS-directed touching faster than females in the moving-stimulus group. The difference in acquisition for males as a function of

stimulus movement was less pronounced than that observed in the females because CS-directed touching was more frequent and occurred earlier in the males.

To statistically verify that the rate of acquisition varied as a function of the experimental factors, a 2x2x7 mixed-model ANOVA was conducted using proportion of trials with at least one hit as the dependent measure. Sex ($n=2$) and movement group ($n=2$; stationary or moving) were used as between-subjects factors and condition ($n=7$; Random, Backward, 30-s Temporal, 30-s Trace, 15-s Trace, Stimulus Pairing, and PAM) was used as a within-subjects factor. The ANOVA revealed differences in rate of acquisition between conditions, $F(6,66) = 41.06$, $p < .001$, $\eta_p^2 = .79$, a condition $\times$ movement interaction, $F(6,66) = 2.29$, $p = .046$, $\eta_p^2 = .17$, a sex $\times$ movement interaction, $F(1,11) = 5.23$, $p = .043$, $\eta_p^2 = .32$, and a sex $\times$ movement $\times$ condition group interaction, $F(6,66) = 4.12$, $p = .001$, $\eta_p^2 = .27$. The difference between conditions was expected, as the proportion of trials in which a hit occurred always began at some near-zero level and increased to a much higher level when acquisition occurred. Later conditions typically had higher hit-trial proportions than earlier conditions. A lack of a main effect for condition would indicate no acquisition occurred during the entirety of the experiment. Of course, the majority of animals acquired CS-directed responding, but the condition in which acquisition occurred varied across individuals.

For the sex $\times$ movement and sex $\times$ movement $\times$ condition interactions, the ANOVA revealed that females in the stationary-stimulus group ($M = .275$, $SE = .067$) acquired CS touching faster than females in the moving-stimulus group ($M = .121$, $SE = .058$), as was expected based on visual analysis. In contrast, males in the moving-stimulus group ($M = .326$, $SE = .058$) acquired CS touching earlier than those in the stationary-stimulus group ($M = .213$, $SE = .058$). Thus, the condition $\times$ movement interaction is best explained by the different trajectories

of acquisition between males and females and the different movement conditions in which acquisition occurred. While males showed acquisition more rapidly in the moving condition and the females showed facilitated acquisition in the stationary condition, these trajectories are not symmetrical and therefore do not cancel each other out. As such, the condition $\times$ movement interaction is duplicative of the sex $\times$ movement $\times$ condition interaction, and the important finding here is that the acquisition trajectories differed by both sex and stimulus movement.

Following the proportion of hit trials analysis, it was important to determine how overall activity levels might differ between sexes, movement groups, and conditions. It is possible that subjects with a higher overall level of screen-directed activity would interact more with the touchscreen, fostering initial contact with the stimulus and producing earlier acquisition. Screen-directed activity can be captured by examining screen touches during the CS or the intertrial interval (ITI). During the CS, touches can be categorized as hits (touching within the 4.57 cm stimulus) or misses (touching anywhere else on the screen during CS presentation). The totality of all three categories of screen touching provides a measure of “screen-directed activity.” Figures 4 and 5 show the total number of hits, misses, and ITI responses that occurred in a session for females and males, respectively, and the bottom row of panels shows the group means. Across subjects, screen-directed activity varied at the start of the experiment, though increases in screen-directed activity typically coincided with acquisition. Across all sessions in which at least one response occurred, misses outnumbered hits in 91.6% of them. Hits and misses also appeared to covary (i.e., increase or decrease together), $r(1127) = .82, p < .001$, suggesting that these responses were part of the same functional class. Visual analysis of the data also suggested a possible sex difference in screen-directed activity. Males typically responded more than females, as shown by the individually scaled y-axes for all panels. Across all

conditions, females touched the screen more than 500 times in only 126 sessions. This is in contrast to the males, wherein this occurred in 219 sessions. This apparent sex difference in screen-directed activity could help explain the disparity in rate of acquisition. To investigate this possible sex difference, we ran a 2x2x7 ANOVA, as described above, using the total number of screen touches across sessions as the outcome variable. The analysis revealed a difference across conditions, $F(6,66) = 7.66, p < .001, \eta_p^2 = .41$, but not across movement groups ($p = .28$). Visual analysis suggested that males touched the screen more, though the sex $\times$ condition effect was not statistically significant, $F(6,66) = 2.11, p = .063, \eta_p^2 = .16$. This outcome should be viewed cautiously, however, because of the high variability within and between subjects, and a potential sex difference in screen-directed activity may underlie sex differences and should be considered in this and other studies.

To determine if the moving stimulus was more difficult to accurately touch, an independent-samples t-test compared the ratio of hits to misses across the stationary- and moving- stimulus groups for all sessions, excluding those where no screen touches occurred. If the moving stimulus was more difficult to touch, one would expect to observe a lower ratio of hits to misses in the moving-stimulus group. The t-test revealed a significant difference as a function of movement group, $t(938) = 2.47, p = .013$, indicating that accuracy was lower for the moving-stimulus group.

To examine potential baseline differences in screen-directed activity, a comparison of total screen touches (hits, misses, and ITI responses) was made using data from only the first session of the first condition (i.e., the Random condition). This analysis showed no differences as a function of sex ($p = .202$) or movement group ($p = .671$), and no interaction between the two (p

= .342). Thus, any differences in screen-directed activity that arose did so during the experiment and were not apparent at the very outset of the study.

In addition to the screen-touching comparison, the total number of times the food cup was accessed during a session was calculated. Figure 6 shows the data for female monkeys, and figure 7 shows the data for male monkeys. The bottom row of panels in both figures shows the associated group means. Much like the number of screen touches, there appeared to be a possible sex difference and a possible sex $\times$ condition interaction. Females appeared to access the food cup fewer times in a session than males, although this difference was less pronounced than the screen touches previously discussed. Interestingly, females in the stationary-stimulus group accessed the food cup more times in a session than those in the moving-stimulus group. While the stationary-stimulus group had sessions where the food cup was accessed over 1000 times (except for subject 15), this was rarely the case for the moving-stimulus group. Instead, this group typically accessed the food cup less than 400 times in a session. For males, no clear visual difference was evident between the two movement groups.

To investigate these potential group differences, a 2x2x7 mixed-model ANOVA was conducted in the manner described above using the total food cup events as the outcome variable. The analysis revealed no difference as a function of sex ($p = .237$) or movement group ($p = .506$). The number of food cup events did change as a function of condition, $F(6,66) = 4.10$, $p = .001$, $\eta_p^2 = .27$, and the movement $\times$ condition interaction was also significant, $F(6,66) = 3.89$, $p = .002$, $\eta_p^2 = .26$. This is similar to the hit proportion analysis in that some subjects showed increased or decreased food cup entry in different conditions. The increases or decreases appeared to be occasionally associated with acquisition of the response but also occurred at other

times. As such, this statistical difference appears to be a function of large shifts in food cup entry in particular individuals and does not represent group-level differences in food cup entries.

One potential explanation for differences in screen-directed activity and rates of acquisition is that motivational factors differed between sexes or movement groups. To investigate this possibility, we analyzed the latency to collect a food pellet following its delivery. If there were any differences in motivational factors, latencies should differ. However, a 2x2x7 mixed-model ANOVA revealed that neither sex ($p = .846$) nor movement group ($p = .221$) nor condition ($p = .081$) were significant, with this latter finding indicating that food cup latency did not greatly change when acquisition occurred. Instead, the latency to collect a pellet was largely stable throughout the experiment and motivational differences between sexes and movement groups were not apparent and, therefore, cannot explain the differences observed.

4. Discussion

The majority of both male and female cynomolgus macaques acquired CS-directed touching when exposed to a computerized classical conditioning procedure. The emergence of CS-directed touching was determined by an interaction of stimulus type (i.e., moving vs. stationary) and the subject's sex. For females, the stationary-stimulus group began touching the CS sooner than the moving-stimulus group. In fact, two of the four females in the moving-stimulus group (12 and 14) never exhibited any significant amount of CS-directed touching. The opposite was true for males: monkeys in the moving-stimulus group began touching the CS sooner than those in the stationary-stimulus group.

These findings replicate previous work in rhesus and cynomolgus macaques, where a moving stimulus enhanced learning or task performance [11, 14-17]. In the current experiment, the moving stimulus led to faster acquisition of CS-directed touching, but only in males. This is

particularly important because previous studies used males almost exclusively. Thus, our study may be the first to examine and reveal potential sex differences in learning as a function of stimulus movement.

It remains to be seen whether the sex difference found here is unique to these individuals, this species, or is part of a more universal phenomenon. At the very least, this finding underscores the importance of including both sexes in behavioral research. The sex differences seen here may also have implications beyond the acquisition of CS-directed touching. Specifically, the apparent visual difference in screen-directed activity between males and females may warrant additional replication (despite the fact that the ANOVA was not statistically significant, $p = .063$), such a sex difference in screen-directed activity would be an important factor to consider when addressing any number of experimental questions. Further investigating the present findings in a direct way may be difficult, however, because procuring the number of naïve monkeys needed for an acquisition study such as this one can be quite costly.

If a sex difference in screen-directed activity does exist, it also needs to be determined whether it subsides with additional training, persists indefinitely, or can be altered by specific training parameters. Importantly, no difference in screen-directed activity was observed on the first day of the experiment, when the apparatus was novel to all subjects. During the very first session, all subjects interacted with the touch screen at approximately the same rate, so the difference in screen-directed activity arose as a function of experience with the conditioning procedures. This finding may be important for future studies, as potential sex differences in screen-directed activity could affect responding in many ways, with more screen-directed activity likely leading to more rapid contact with changing contingencies. Also, higher and lower rates of screen-directed activity may be differentially sensitive to experimental manipulations

such as drug challenges, wherein the baseline rate of responding modulates the drug's effect [e.g., 34]. The changes in screen-directed activity seen in the present study appear to be related to experience and not attributable to initial differences between the sexes in these naïve subjects.

It should be noted that the order of conditions used here was fixed and arranged in such a way that early conditions were not likely to generate CS-directed touching. This arrangement could have produced a behavioral history that was differentially affected by sex. It is impossible to determine conclusively whether the sex difference found here was a product of the stimulus-movement effect or the order of conditions in the current experiment, but it seems more likely that a sex difference exists for moving stimuli than a history generated by the specific set of conditions used here.

We also determined that motivational differences did not explain observed differences in CS-directed touching, as both males and females interacted with the food cup similarly and collected all delivered pellets with similar latencies. However, there was a clear sex difference in how subjects behaved during the CS. Here, females demonstrated goal-tracking, whereas males demonstrated sign-tracking. Goal-tracking is responding to or approaching the location of the US (the food cup) during the CS, while sign-tracking is responding to the CS or its location (the touchscreen). The females displayed goal-tracking by interacting with the food cup throughout the CS presentation, even if acquisition of CS-directed responding was delayed or never emerged. The males also interacted with the food cup, but were more likely to show sign-tracking, that is, interacting with the CS and the touchscreen during CS presentation. So while monkeys of both sexes showed evidence of learning and rapidly acquired food cup-directed behavior, the predominant responses that occurred during the CS differed by sex and by stimulus-movement condition. Even though females did not acquire touchscreen responding as

readily as males, they still exhibited learning, just of a different kind. It may be the case that males are more attracted to the moving stimulus and therefore more likely to show sign-tracking, though it is unclear what mechanism(s) may underlie this sex difference.

Although the sex differences seen here have not previously been observed, other classical-conditioning tasks have produced noteworthy sex differences. In classical eyeblink conditioning, female rats learn the CS-US pairing before males [35-37]. However, in fear-conditioning procedures, female rats exhibit attenuated freezing and startle responses compared to their male counterparts [38, 39]. In many of these cases, the sex difference often emerges at the beginning of training and eventually disappears after sufficient training has occurred and behavior reaches some asymptotic level.

A similar example of sex differences appearing early in acquisition and subsequently disappearing after sufficient training has been observed with moving stimuli in monkeys, where the moving stimulus enhanced acquisition, but both the moving and stationary stimuli produced a similar asymptotic level of responding [11, 17]. In the current experiment, early CS-directed touching was different between males and females, but this difference typically diminished with continued exposure to the conditioning procedures, and the majority of subjects were touching the stimulus by the end of the experiment, irrespective of their sex and group assignment. Although both males and females eventually learned to touch food-associated stimuli with high probability, the potential for sex differences in screen-directed activity is a finding worthy of further study. Acquisition procedures appear to be particularly well-suited for revealing sex differences in learning, but the degree to which such differences may or may not lessen with extended experience is also an important topic of further study.

The putative cause of the sex difference observed here is not known, but hormones have been implicated in sex differences in rats, as is the case with estrogen in eyeblink conditioning [36, 37]. Female rats outperform male rats at any point in their estrus cycle, but conditioning is more rapid during the proestrus phase when estrogen levels are at their highest. However, ovariectomized females perform the same as males, and estrogen therapy at physiological doses is unable to recover the previously observed sex difference. Only estrogen therapy with levels much higher than physiological doses causes the sex difference to emerge in ovariectomized females.

Testosterone also has been found to play a role in eyeblink conditioning. Males that receive testosterone antagonists or are castrated show no differences in acquisition of an eyeblink response from control males. However, females that receive testosterone treatments perform the same as males. So while testosterone does not seem to affect the learning of males in this task, it does slow the learning of females. Taken together, hormones clearly affect learning in an eyeblink conditioning task, though the role of each and their individual and combined effects are still being investigated [40].

While hormones affect eyeblink conditioning in rats, we cannot say whether or not hormones played a role in the sex differences observed here. No hormonal data were collected, though such a data set could be of great importance and worth pursuing in future research. Evidence exists that higher estrogen levels can decrease performance in spatial tasks and that estrus and menstrual cycles cyclically change performance in a variety of spatial-cognition tasks [41-44]. In addition, ovariectomized female rhesus monkeys showed protection against age-related declines in spatial-cognition performance [45]. There is also tentative evidence for estrus state contributing to performance on a problem-solving task in chimpanzees [46]. However, it

may be the case that acquisition of CS-directed responding in the current study was not affected by hormones, as no evidence of cyclical responding in the female monkeys could be discerned.

One might be tempted to explain the present sex difference in CS-directed responding in terms of differences in neophobia between males and females of this species, however such an account is difficult to support based on available evidence. Neophobia is the fear of new things and in humans it is commonly investigated in relation to children's eating habits and food selection [47]. In animals, novelty-seeking behaviors (less neophobia) have been linked to habitat complexity [48], omnivorous diets [49, 50], and ecological plasticity [51, 52]. Findings are mixed on whether neophobia systematically varies by sex across species. In a neotropical raptor, the chimango caracaras (*Milvago chimango*), males and females equally approached novel objects [53]. Novel-object exploration was also equivalent between sexes in wild baboons (*Papio ursinus*) and geladas (*Theropithecus gelada*) [54]. In wild tufted capuchins (*Cebus paella*), females responded more readily than males to novel objects [55]. However, males responded more readily than females to novel objects in species as diverse as ravens (*Corvus corax*) [27], stump-tail macaques (*Macaca arctoides*), and spider monkeys (*Ateles geoffroyi*) [56].

In the current study, food-related neophobia was not a contributing factor to the reported sex difference as both sexes readily retrieved and consumed the food provided and, thus, no motivational differences (or differences in neophobia) were observed. Neophobia in this context might also be revealed as a reduced tendency to interact with the CS, as they had no prior experience with stimuli shown on touchscreens. On the surface, this difference would appear to help explain differences in total touchscreen-directed activity, but the observed differences in acquisition as a function of stimulus movement would then require one to accept the assumption that a moving stimulus invokes greater neophobia than a stationary stimulus in females only.

While such an assumption may seem plausible, it remains purely speculative, and no available evidence suggests a consistent sex difference in neophobia in this macaque species. However, we are also hesitant to suggest that our results show that females are neophobic, as that would suggest that female cynomolgus macaques are hesitant to interact with stimuli across a range of contexts and such an assertion is well beyond the scope of this data set. Any appeals to neophobia must be made very carefully to avoid circular reasoning that simply recasts the observed findings (a sex difference) using this term.

Despite the inability to determine conclusively what underlies the sex difference in CS-directed touching, the present study stands as a clear example of why investigators should endeavor to include both sexes whenever possible as subjects in behavioral research. Recent NIH guidance requires researchers to consider sex as a biological variable when designing experiments [57], further bolstering the case for including female subjects. The present study advanced some of the stated goals by of this NIH guidance by using “adequate consideration of both sexes in [the experiment] and disaggregation of data by sex [to allow] for sex-based comparisons” thereby enhancing “the interpretation, validation, and generalizability of research findings.” Females are often not included in behavioral investigations for a variety of reasons. Behavior may change as a function of an estrus cycle [e.g., 58, 59], which adds another layer of complexity and requires more time and resources for proper experimental design and control. It may also be the case that the behavior of females is assumed to be similar to that of males, or similar enough that any sex differences are considered trivial or easily overcome by the experimental manipulation(s). The present study demonstrates the importance of including females in behavioral research and testing these assumptions, as the experimental manipulation

was differentially affected by sex, and past research used an insufficient number of female subjects to detect this interaction.

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Figure 1: Single column

Figures 2-7: Double column

Figure 1. Diagram of the various experimental conditions, displayed in the order of their use.

This depiction is an example of multiple CS and US presentations, so in conditions with variable CS-US or US-CS intervals, random lengths were chosen for demonstration purposes. The CS duration was 8 s in all conditions, but was terminated early in the Positive Automaintenance condition if a response to the CS occurred. The US duration was 0.25 s, but is displayed as longer for ease of readability and demonstration purposes.

Figure 2. Proportion of trials in which at least one hit occurred for female monkeys in each session across conditions. The number appearing in each panel corresponds to each monkey's identification number. Monkeys in the stationary-stimulus group are shown in the left column and the moving-stimulus group is shown in the right column. Data series change color and shape at each condition change. Black squares represent the Random condition, white squares represent the Backward condition, black triangles represent the 30-s Temporal condition, white diamonds represent the 30-s Trace condition, black diamonds represent the 15-s Trace condition, white circles represent the Stimulus Pairing condition, and black circles represent the Positive Automaintenance (PAM) condition. The two panels in the bottom row represent the group means, with the error bars showing the standard error of the mean.

Figure 3. Proportion of trials in which at least one hit occurred for male monkeys across conditions. Other details are as described in Figure 2.

Figure 4. Total number of screen touches for female monkeys in each session across conditions. The number appearing in each panel corresponds to each monkey's identification number. Hits are represented by black squares, misses represented by white diamonds, and intertrial-interval (ITI) responses by the gray circles. Condition changes are marked by a vertical dashed line and are labeled above the first row of panels. Monkeys in the stationary-stimulus group are shown in the left column, and the moving-stimulus group is shown in the right column. Note the individual y-axis scaling for each panel. The two panels in the bottom row represent the group means, with the error bars showing the standard error of the mean.

Figure 5. Total number of screen touches for male monkeys in each session across conditions. Data excluded for subject 25 because of an apparatus failure. Other details are as described in Figure 4.

Figure 6. Total number of food cup events for female monkeys in each session across conditions. Note the individual y-axis scaling for each panel. Other details are as described in Figure 2.

Figure 7. Total number of food cup events for male monkeys in each session across conditions. Note the individual y-axis scaling for each panel. Other details are as described in Figure 2.

MALES

Stationary Stimulus

Moving Stimulus

Sessions

FEMALES

Stationary Stimulus

Moving Stimulus

MALES

FEMALES

MALES

Stationary Stimulus

Moving Stimulus

Sessions

FEMALES

Stationary Stimulus

Moving Stimulus

Random

US-US Interval: 18 – 118 s

Backward

CS-US Interval: 10 – 110 s

30-s Temporal

CS-CS Interval: 68 s

CS-US Interval: 30 s

30-s/15-s Trace

CS-US Interval: 30/15 s

US-CS Interval: 5 – 55/85 s

Stimulus Pairing

US-CS Interval: 10 – 110 s

Positive Automaintenance

*Touches terminate the CS
immediately and commence
US delivery*