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Female Hamster Preference for Odors is Not Regulated by Circulating Gonadal Hormones

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Abstract

Proceptive and receptive behaviors of female rodents, such as golden hamsters, are often regulated by changes in circulating levels of ovarian hormones. However, less is known about how ovarian hormones might regulate female hamster's attraction and preference for volatile odor from males. To evaluate this, we assessed female preference by recording investigation and proximity to male and female volatile odorants in a Y-maze across all days of the estrous cycle (Experiment 1 & 2) or following ovariectomy (Experiment 3). In Experiment 1, female subjects were tested four times, once on each day of their estrous cycle. Females showed a preference for male odors on diestrus day 1 and to a lesser degree on proestrus, but showed no preference on the day of behavioral estrus. Irrespective of cycle day, preference was apparent in the first few days of testing and disappeared by the fourth day, suggesting that repeated testing attenuated female preference. To avoid this problem, in Experiment 2 each animal was tested only on one day of the 4-day estrous cycle. Female preference for male volatile odors over those from females was observed on each day of their estrous cycle, including estrus. Moreover, following gonadectomy (Experiment 3) female hamsters still preferred male volatile odors to those of females. Taken together, this suggests that circulating levels of gonadal hormones do not influence preference for male volatile odors in female hamsters.

Keywords

Golden Hamster; Estrous Cycle; Olfaction; Scent; Attraction; Ovariectomy

1. Introduction

Female sexual behavior changes across the estrous cycle in a number of species, including golden hamsters, and is caused by cyclic variations in estrogen and progesterone secretion from ovaries [1]. In female golden hamsters both sexually receptive (lordosis) and proceptive or solicitational behaviors, such as vaginal marking, change across the estrous cycle. Vaginal marking is a behavior that deposits vaginal secretions, a potent source of sex attractants [2], and increases in response to male odors on all non-estrus days of the cycle with peak levels occurring on the day prior to behavioral estrus [3,4]. As these odors likely attract widely-dispersed males from a distance [5,6], females may deposit secretion in male-scented areas well in advance of behavioral estrus. If so, then females should be attracted to male odors over

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all or most days of the estrous cycle. However, the available data on this topic is conflicting. On one hand, female hamsters and rats tend to preferentially approach awake, behaving male conspecifics only during behavioral receptivity [7,8,9,10]. On the other hand, female hamsters investigate male odors at a similar level across the estrous cycle [4]. Consequently, the extent of cyclic variation of female preference for male odors, compared to female odors, is currently unknown, as is the degree to which this preference requires ovarian hormones.

For this reason, we measured the time that female golden hamsters spent in proximity to and investigating male or female volatile odors within an olfactory Y-maze across all days of their estrous cycle (Experiment 1 & 2) or following ovariectomy (Experiment 3).

2. General methods

2.1 Animals

Female subjects (4–7 months) and male and female stimulus animals (3–10 months) were sexually naïve golden hamsters (*Mesocricetus auratus*) either purchased from Charles River Laboratories (Wilmington, MA, USA) at three weeks of age or bred from a colony of Charles River Laboratories-derived animals. All hamsters were housed singly in solid-bottom plastic cages (36 × 30 × 16 cm), maintained on a reversed 14/10 light/dark cycle, and were allowed access to food and water ad libitum.

In Experiment 1 and 2, estrous cycles were determined for experimental subjects by briefly placing them in a cage daily with a male and observing the presence or absence of the sexually receptive (lordosis) posture. Once a female displayed lordosis, she was removed immediately from the male's cage to prevent copulation, and designated as being in estrus. These females were re-tested every four days (for two consecutive cycles) to determine if the female was maintaining a regular 4-day estrous cycle. If females did not display lordosis and/or attempted to attack the male, they were considered not to be in estrus and were tested on consecutive days until lordosis was observed in response to the male. The day following behavioral estrus was termed diestrus day 1 (D1), followed by diestrus day 2 (D2) and finally by proestrus (PE), the day prior to behavioral estrus (E). Subjects were re-tested for their cyclicity following behavioral testing; all animals displayed receptive behavior on the predicted day.

2.2 Odor stimulus

Male and female odors were collected from the soiled (10–14 days post-cleaning) cages of odor donor as well as from the animals themselves (male, $n = 30$; female, $n = 33$). The odor stimuli collected from the cage consisted of soiled cotton bedding (4 Nestlets, ANCARE, Bellmore, NY) 10 ml of soiled litter, and one moistened gauze pad that was used to wipe the inner walls of the cage. Additional odors were collected from the odor donor by rubbing a moistened (with distilled water) gauze pad on its flank scent gland region (10 times per side) and by rubbing one additional gauze pad on the anogenital region (ten times). In addition, vaginal secretion was collected from females in behavioral estrus by manually inducing the female into a receptive posture (lordosis) and gently palpating the vaginal area with a disposable cotton swab. Care was taken that subjects were only exposed to odors from unrelated and unfamiliar odor donors. The bedding, litter, and gauze squares were sealed in a 100 ml plastic bag, and stored at 4° C until twenty minutes before use. An identical amount of clean cotton bedding, litter and gauze pads was shredded and placed in the odor ports during testing with clean stimuli. In all cases, experimenters wore latex gloves while handling odor stimuli in order to prevent contamination with human odor cues.

2.3 Behavioral Testing

Preference for odor stimuli were assessed by testing subjects in an enclosed, acrylic Y-maze (Figure 1) that consisted of a stem arm (length = 61cm) and two side arms (length = 68cm); all arms of the Y-maze were 10 cm wide and 10 cm high. The side arms angled off at 120° from the stem and bent back inward 120° at half their length. Each side arm had an odor stimulus chamber (length = 20cm) at its distal end that was separated from the rest of the maze by a door with a single, 1.8 cm diameter hole (odor port; Experiments 1 and 2) or by a perforated door (Experiment 3). This arrangement prevented subjects from contacting the odor source and would, therefore, be exposed only to volatile components of the odor stimulus. At the distal end of the stem was a start chamber (length = 20cm) separated from the rest of the maze by a removable, perforated door. Air was pulled from the distal odor chambers throughout the entire length of the Y-maze by an electric fan located behind the start chamber. The top of the maze was secured with a clean acrylic top to allow for overhead video recording of subjects' behavior.

Subjects were first tested in a clean condition in which only clean bedding was located in each of the stimulus arms of the Y maze. This was done both to familiarize subjects with the apparatus as well as to determine changes in motor activity across conditions. Following control testing, subjects were tested for their preference for male vs. female odors in the Y-maze (odor condition). Each test was started by placing subjects in the closed start chamber; one minute later the door was removed and subjects were allowed nine minutes to explore the maze. All testing was done within the first 6 hours of the dark phase, and was conducted under dim lighting conditions. The Y-maze was cleaned with 50% ethanol and allowed to dry between each subject. To conserve odor stimuli, male and female odors were re-used once before being discarded. The location of male or female odors was alternated between stimulus chambers following each test to control for any side bias in investigation.

Video recordings of each test were digitized onto a computer and scored by an observer blind to the condition of the subject (see below) using the Video Pro Observer 5.0 program, (Noldus Information Technology B.V., Wageningen, The Netherlands). To measure preference for male over female odors, we recorded the time spent investigating both stimulus doors as well as the time spent in each stimulus arm. For the manually scored data animals were considered to be investigating when their noses were within 1cm of the odor port, and were considered to be in proximity to the odor when they were in the arm in which the odor was located. For the photo-cell data animals were considered to be investigating when the infrared beam in the odor port was broken; proximity was determined by the breaking of infrared beams dividing the stem arm from the stimulus (side) arms (Figure 1). In addition, in both the clean and odor conditions, we recorded the total number of entries into the two stimulus arms as well as the stem arm as a measure of general activity. In Experiment 1 and 2, the scorer was blind to which arm contained the male or female odors and the subjects' estrous cycle status. In addition, in Experiment 3, the scorer was blind to the hormone condition of the subject.

2.4 Statistical Analysis

As the data were not normally distributed with different variances between conditions, non-parametric statistics were used for analysis. Preference for male over female odors was assessed by comparing investigation and proximity times of the male and female odors using Wilcoxon signed-ranks tests. Comparisons were deemed significant if $p < 0.05$ using one-tailed tests. One-tailed tests were used because we had strong a priori reasons for expecting that females would prefer male odors to those of females [11–13,14] rather than the reverse. Differences in levels of (a) investigation, (b) proximity and (c) total number of arm entries (clean and odor conditions) across the estrous cycle (Experiment 1 and 2) or testing days (Experiment 1) were assessed using Friedman (Experiment 1) or Kruskal-Wallis tests (Experiment 2). The effect of

ovariectomy on total number of arm entries in Experiment 3 was determined using a Mann-Whitney test. In addition, in Experiment 2 we correlated (Spearman's rho) manually-scored preference behavior (male – female investigation and proximity time) with preference behavior scored using photo-cell occlusion in the Y-maze. All statistical analyses were done using SPSS (12.0; SPSS Inc., Chicago, IL) statistical software. As data was analyzed using non-parametric statistics, information in graphs and table are presented as medians with 1st and 3rd quartiles as the measure of variability.

3. Experiment 1: Preference for male odors across the estrous cycle with repeated testing

3.1 Methods

The goal of this experiment was to determine if female Syrian hamsters' preference for volatile odors from male vs. female conspecifics changes across the estrous cycle. Each subject was tested on all four days of the estrous cycle. However, as there was little evidence of consistent preference for male vs. female odors across the estrous cycle and especially on E (see below), we tested the hypothesis that repeated testing had attenuated subjects' response to sexual odors. This was done by re-analyzing preference data by day of testing irrespective of estrous cycle day.

The estrous cycles of 21 female hamsters were determined and subjects were divided into four sub-groups (5–6 animals each) that each started testing on a different day of the estrous cycle. Subjects were first tested on four consecutive days of their estrous cycle in a Y-maze containing only clean bedding (clean condition). Three days later subjects began testing for their preference for male versus female odors (odor condition) in the Y-maze over four consecutive days of their estrous cycle.

3.2 Results and Discussion

Based on the total number of arm entries, subjects' activity level in the clean condition did not differ across days of the estrous cycle but did decrease across days of testing (Table 1) indicating that females were less active with repeated testing in the Y-maze. There was, however, no effect of either estrous day or testing day on motor activity in the Y-maze in the odor condition (Table 1).

When tested with male and female odors, female subjects showed marginally significant variation in their investigation of male odors across the estrous cycle ($\chi^2(3) = 7.63$; $p = .054$) but did not alter their investigation of female odors across the estrous cycle ($\chi^2(3) = .646$; $p = .886$). This pattern is reflected in the inconsistent preference for investigating male odors over female odors; preference for male odors was only observed on D1 and PE (Figure 2a). Similarly, subjects did not significantly change the amount of time spent in the male ($\chi^2(3) = 2.20$; $p = .532$) or female ($\chi^2(3) = 1.29$; $p = .733$) stimulus arms. Indeed, females only preferred to spend more time in the male odor stimulus arm than in the female arm on D1 (Figure 2b). Contrary to previous results [11, 12, 13, 14] females did not prefer male over female odors during estrus, measured either by investigation or proximity time.

Repeated testing of subjects resulted in a decrease in investigation of male ($\chi^2(3) = 22.25$; $p < .001$) and female odors ($\chi^2(3) = 8.77$; $p < .05$) over testing days (Figure 2c). Females showed a significant preference to investigate male odors only on the second and third days of testing; by the fourth day of testing no preference for these odors was evident (Figure 2c). Subjects preferred to spend similar amounts of time in the male ($\chi^2(3) = 3.23$; $p = .358$) and female ($\chi^2(3) = 5.57$; $p = .134$) stimuli arm across the four days of testing (Figure 2d). In fact, females preferentially spent time near the male odor only on the first day of testing (Figure 2c). Taken

together, it appears that female hamsters only expressed a preference for male odors early in the testing sequence; by the fourth test, females displayed no preference for male odors over female odors. This pattern of results suggests that repeated testing may have interfered with expression of normal odor preference across the estrous cycle.

4. Experiment 2: Preference for male odors across the estrous cycle with single day testing

4.1 Methods

In Experiment 1, repeated testing of subjects in the Y-maze on every day of the estrous cycle may have impaired our ability to detect variation in preference across the cycle. Consequently, Experiment 2 was designed to more clearly test for effects of the estrous cycle on preference by testing each subject on only one day of their cycle. In addition, we tested the accuracy of our manual behavioral scoring by installing infrared photo-cell sensors to detect the hamster's movement into the two stimulus arms as well as its investigative behavior at the odor stimulus ports.

Estrous cycles were established for a new set of 41 female hamsters and subjects were then randomly assigned to one of four testing groups; D1 (n = 10), D2 (n = 10), PE (n = 10), and E (n = 10). On the appropriate day of the cycle, subjects were each tested once in the clean condition and once in odor condition. These tests were conducted over two successive estrous cycles. Fifteen male and 18 female hamsters provided odor stimuli for testing.

The Y-maze was modified by installing six infrared photo-detectors/emitter pairs (hereafter referred to as photo-cells) within the maze. One photo-cell was placed within each of the odor ports, and two were placed at the entrance of each side arm (Figure 1). One photo-cell at the arm entrance was placed 2 cm from the maze bifurcation and the other 5 cm further down the arm. This configuration ensured that entries were registered only when the hamster's body occluded both pairs of sensors. Custom computer software (Parallel Port Logger, Center for Behavioral Neuroscience) monitored the subjects' movements throughout the maze by recording all occlusions of photo-cells.

Due to computer error in measuring photo-cell input, data from two animals in the clean condition and four animals in the odor condition were eliminated from the analysis. This left thirty-eight animals in the clean condition (D1 = 9; D2 = 10; PE = 9; E = 10) and thirty-six animals in the odor condition (D1 = 10; D2 = 8; PE = 9; E = 9).

4.2 Results and Discussion

Females' activity level in the clean (manual: $\chi^2(3) = 2.13$; $p = .546$; photo-cell: $\chi^2(3) = 2.71$; $p = .439$) or odor condition (manual: $\chi^2(3) = 4.64$; $p = .20$; photo-cell: $\chi^2(3) = 0.22$; $p = .999$) did not differ across days of the estrous cycle (data not shown) indicating that females did not significantly alter their motor activity across the estrous cycle. The total number of entries recorded manually and via photo-cell occlusion were strongly correlated ($r(38) = .628$; $p < .001$).

In the clean condition there was no difference in investigation time of either right or left clean odor ports when recorded manually or via photo-cell occlusion across the four days of the estrous cycle (Table 2). There was no effect of estrous day on investigation of male odors (manual: $\chi^2(3) = 1.26$; $p = .737$; photo-cell: $\chi^2(3) = 4.19$; $p = .242$) or female odors (manual: $\chi^2(3) = 2.96$; $p = .398$; photo-cell: $\chi^2(3) = 0.97$; $p = .809$) (Figure 3a, Table 3). Based on manual scoring, females preferred to investigate male odors on PE and E and not on the two diestrus days (D1: $z = 0.968$, $p = .16$; D2: $z = 1.478$, $p = .07$; Figure 3a). However, using data collected

by photo-cell occlusion, investigative preference was apparent on D1 ($z = 2.395$, $p = .008$), D2 ($z = 2.201$, $p = .014$), and on E ($z = 2.380$, $p = .008$) but not PE ($z = 1.120$, $p = .13$) (Table 3). Thus, by combining the two methods of scoring odor investigation, it is apparent that females demonstrate a preference for male odors over female odors on each day of the estrous cycle (Figure 3a, Table 3).

Similarly, subjects spent comparable amounts of time in the arm containing male (manual: $\chi^2(3) = 0.622$; $p = .891$; photo-cell: $\chi^2(3) = 1.02$; $p = .795$) and female odors (manual: $\chi^2(3) = 2.12$; $p = .548$; photo-cell: $\chi^2(3) = 1.31$; $p = .726$) across the estrous cycle (Figure 3b, Table 3). Importantly, with one exception (PE, photo-cell: $z = 1.48$, $p = .07$; Table 3), both manual (Figure 3b) and photo-cell occlusion (D1; $z = 2.090$, $p = .018$, D2; $z = 1.820$, $p = .034$, E; $z = 2.310$, $p = .01$; Table 3) data methods of measuring proximity to odor sources indicate that females preferred to spend more time near male odors than near female odors across all days of the estrous cycle.

There was a strong correlation between the manually-scored and photo-cell scored preference data for both duration of investigation of odor stimuli ($r(36) = .456$; $p < .001$) and for duration spent in stimuli arms ($r(36) = .734$; $p < .001$). However, the overall investigation time recorded is considerably shorter and more variable when the data is scored manually than when data is recorded via photo-cells. This is likely due the increased difficulty in determining whether or not the subject's nose was completely within the small odor port when observed from videotape.

5. Experiment 3: Effects of ovariectomy on preference for male odors

A new group of 26 female golden hamsters were randomly assigned to either an ovariectomy group (OVX; $n = 14$) or a sham-operated group (Sham; $n = 12$). OVX animals had their ovaries removed under 1–2% isoflurane anesthesia (100% oxygen) through two bilateral incisions in the dorsal surface; wound clips were used to close the incisions. Animals in the Sham condition received the identical surgical procedure except that the ovaries were not removed.

Eight weeks following surgery, OVX and estrous Sham animals were tested in an olfactory Y-maze identical to that used in Experiment 1 and 2, except that odor stimuli were located behind a perforated door rather than a door with a single hole. This was done to aid scoring of chemosensory investigation from videotape. Subjects were tested once in the clean condition followed four days later by a single test in the odor condition. Social odors were provided by thirteen males and thirteen females. Following testing, OVX females were injected with an overdose of sodium pentobarbital (Nembutal; 100 mg/kg) and sacrificed to determine if ovaries had been removed bilaterally. All OVX females were found to be missing both ovaries and were retained in the analysis.

5.2 Results and Discussion

OVX and Sham groups showed similar levels of activity in both clean ($U = 74.0$; $p = .631$) and odor conditions ($U = 65.0$; $p = .347$; data not shown). Both OVX and Sham females spent more time investigating male odors than female odors (Figure 4a) as well as spending more time in the arm containing the male odor stimulus than with female odors (Figure 4b).

6. General Discussion

We have demonstrated, using measures of chemoinvestigation and proximity, that female hamsters prefer male volatile odors over those from females across all days of the estrous cycle and not just during behavioral estrus. Furthermore, ovariectomy did not eliminate female preference for male odors. Unexpectedly, Experiment 1 demonstrated that female's preference

for male volatile odors diminishes with repeated testing. This pattern of results suggests that female preference for male volatile odors is not under the control of circulating gonadal steroids, and may require contact with odorants to maintain preference. Given that other aspects of the female hamster's sexual behavior are regulated by changes in gonadal steroids across the estrous cycle [1], it is somewhat surprising that preference for the volatile odors of male conspecifics appears to be under minimal steroidal control. In fact, previous research in rats and hamsters has demonstrated that hormones do control attraction and preference for male conspecifics. For example, female hamsters and rats spend more time near reproductively-active caged males than females or gonadectomized males during estrus but not during other days of the estrous cycle [7,8,9,10]. Ovariectomy eliminates or severely reduces this preference in hamsters, rats and ferrets and can be partially or completely restored by estradiol or testosterone treatment [7,9,15,16 but see 17]. However, in all of these cases, attraction to male and female conspecifics was assessed in a situation that allowed visual and auditory as well as olfactory stimulation. Consequently, the present results suggest that attraction to odor cues, especially volatile ones, may be normally under limited hormonal control, whereas approach to an active conspecific may depend more on gonadal steroids. Additional support for this idea comes from the finding that the overall levels of investigation of male odors does not vary across the estrous cycle of female hamsters or mice [4,18].

Although our results suggest that changes in ovarian hormones have little effect on female preference, exogenous treatment with gonadal steroids can alter preference for social odors in female hamsters. For example, treating neonatal [19], pubertal [20] or ovariectomized adult female hamsters [21,22] with testosterone increases their level of investigation of potent female sexual odor cues (e.g. vaginal secretion) to levels roughly comparable to those observed in male hamsters. This sensitivity to steroidal influence leaves open the possibility that females in our study were exposed to sufficient levels of estrogens, perhaps via phytoestrogens in their diet [23], to maintain preference in low estrogen conditions (Diestrus 1 and 2) and in ovariectomized subjects.

The finding that females decrease their preference for volatile odorants of males following repeated presentations, although surprising, is consistent with previous results showing that preference can be eliminated or altered by social exposure [24,25]. One possible explanation for why this preference extinguishes across repeated presentations is that our experimental subjects could not directly contact social odors. By eliminating contact with the odorant, we likely reduced or prevented the vomeronasal or accessory olfactory system from detecting less volatile odor cues in the stimulus that may mediate reinforcing or rewarding aspects of sexual odors [26]. This possibility is supported by previous findings showing that female mice only prefer volatile odorants from males if they have had prior experience with both volatile and non-volatile male odors [27,28]. Similarly, vomeronasal organ removal leads to a rapid extinguishing of odor investigation in guinea pigs [29] and odor-elicited ultrasonic vocalizations in mice [30]. If so, then direct contact with bedding should maintain preference for volatile male odors over repeated trials. Preliminary results from our lab suggest that sexual experience or exposure to non-volatile odorants can indeed maintain preference for volatile odorants in male hamsters [31]. Consequently, female hamsters may have maintained their preference if they were sexually experienced as female hamsters show conditioned place preference following copulation [32].

At a functional level, the decoupling of preference for volatile male odors from hormonal control observed in hamsters may represent an adaptation for organizing mating in a solitary, widely-dispersed species [33]. Female hamsters, unlike females from species that often live in high densities such as rats [34] and mice [35], advertise their impending receptivity for several days prior to ovulation by vaginal scent marking more frequently to male odors [3,4]. It is therefore likely that females need to locate areas frequented and scent-marked by males well

in advance of actual mating so that the female's deposited secretions can attract males [2,36] across relatively large distances [5,37]. Emancipating sex-typical odor preference from hormonal control in female hamsters may be one mechanism that allows adaptive coordination of reproduction in this solitary species.

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Figure 1.

Y- maze apparatus used in clean condition (left) and odor (male, female) condition (right). The small arrows represent the position of photo-cells and the large arrows represent flow of air. The dashed lines indicate chambers (top: odor stimulus; bottom: start) separated from maze by perforated doors.

Figure 2.

Median (1st and 3rd quartile) number of seconds females (n = 21) spent investigating odor ports containing male or female soiled bedding (a, c) as well as their time spent in the Y-maze arm containing male or female odor stimuli (b, d). Subjects were tested on every day of their estrous cycle (D1, D2, PE, E) but sequence of testing varied between subjects. (a) and (b) present data from each day of their estrous cycle collapsed across testing sequence. (c) and (d) present the same data organized by day in the testing sequence (Day 1–4) collapsed across estrous day. * indicates a significant difference (p < 0.05; Wilcoxon signed-ranks test) in response to male versus female odor stimuli.

Figure 3.

Median (1st and 3rd quartile) number of seconds females spent investigating odor ports containing male or female soiled bedding (a) as well as their time spent in the Y-maze arm containing male or female odor stimuli (b) across the estrous cycle. Subjects were tested on only one day of their estrous cycle (D1, D2, PE, E) and their behavior was scored manually. All groups: n = 10. * indicates a significant difference ($p < 0.05$; Wilcoxon signed-ranks test) in response to male versus female odor stimuli.

Figure 4.

Median (1st and 3rd quartile) number of seconds that OVX and estrus Sham females spent investigating odor ports containing male or female soiled bedding (a) as well as their time spent in the Y-maze arm containing male or female odor stimuli (b). * indicates a significant difference ($p < 0.05$; Wilcoxon signed-ranks test) in response to male versus female odor stimuli.

Table 1
Total Number of Arm Entries Across 4 Days of the Estrous Cycle and 4 Consecutive Days of Testing

Condition		Median (1 st and 3 rd Quartiles) Total Number of Arm Entries				χ^2 (3)	p
		Diestrus 1 Day 1	Diestrus 2 Day 2	Proestrus 3 Day 3	Estrus Day 4		
Clean	Estrous Day	17.0 (14.0, 20.0)	21.0 (17.0, 24.0)	18.0 (17.0, 24.0)	19.0 (17.0, 25.0)	5.8	.12
	Testing Day	24.0 (21.0, 25.0)	18.0 (14.0, 21.0)	19.0 (17.0, 23.0)	17.0 (15.0, 19.0)	12	.01
Odor	Estrous Day	18.0 (15.5, 22.0)	19.0 (15.5, 20.5)	18.0 (17.0, 21.0)	18.0 (15.0, 21.5)	0.7	.88
	Testing Day	18.0 (16.5, 20.5)	19.0 (17.0, 21.5)	18.0 (15.5, 20.5)	18.0 (14.0, 22.5)	0.4	.93

Table 2
Investigation and Proximity to Clean Stimuli Across the 4-Day Estrus Cycle

Scoring Method	Measure	Median (1 st and 3 rd Quartiles) Investigation Time and Time in Arm (Left, Right) Scored Manually and by Photo-cell Occlusion				
		Diestrus 1	Diestrus 2	Proestrus	Estrus	χ^2 (3) <i>p</i>
Manual	Investigation					
	Left	4.8 (2.7, 7.9)	6.7 (4.1, 14.5)	3.7 (2.2, 19.5)	3.5 (0.5, 9.2)	1.9 .61
	Right	11.2 (7.8, 17.0)	7.1 (4.5, 21.4)	5.3 (0.1, 22.0)	10.9 (8.4, 108.3)	2.8 .43
	Arm					
Photo-cell	Left	145.0 (126.4, 169.9)	162.1 (144.4, 195.5)	187.0 (155.9, 231.3)	135.1 (98.7, 176.9)	9.4 .02
	Right	187.0 (147.3, 208.9)	171.9 (140.1, 210.6)	159.1 (125.5, 213.7)	187.6 (138.0, 282.5)	0.7 .86
	Investigation					
	Left	4.3 (0, 12.1)	20.5 (1, 44.6)	0 (0, 33.1)	1.8 (0, 20.7)	3.4 .33
	Right	10.5 (0, 36.3)	8.1 (0, 41.0)	12.5 (0, 34.7)	1.3 (0, 29.3)	0.6 .89
	Arm					
	Left	117.7 (106.4, 142.5)	117.3 (96.1, 33.2)	157.8 (139.6, 171.6)	127 (97.3, 161.2)	10.4 .01
	Right	133.9 (113, 158.1)	136.2 (119.8, 175.1)	147.5 (112.4, 174.5)	127.4 (102.7, 172.4)	0.8 .85

Table 3
Investigation and Proximity to Odor Stimuli Across the 4-Day Estrus Cycle Scored by Photo-cell Occlusion

Measure	Median (1 st and 3 rd Quartiles) Investigation Time and Time in Arm (Male, Female)			
	Diestrus 1	Diestrus 2	Proestrus	Estrus
Investigation				
Male	51.9 (33.6, 65.7)	46.3 (7.8, 72.7)	26.5 (0, 54.8)	28.0 (14.1, 44.5)
Female	19.5 (0, 38.3)	22.5 (0, 36.1)	13.6 (0, 38.9)	14.8 (0, 17.8)
Arm				
Male	130.4 (109.5, 171.1)	142.6 (100.1, 222.6)	152.7 (126.1, 201.6)	160.2 (122.4, 187.7)
Female	85.7 (56.3, 122.6)	78.9 (72.6, 128.4)	107.7 (72.7, 162.4)	87 (39.4, 134.3)