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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 (Experiments 1 and 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 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 [7–10]. 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 (Experiments 1 and 2) or following ovariectomy (Experiment 3).

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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 Experiments 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 20 min 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 was assessed by testing subjects in an enclosed, acrylic Y-maze (Fig. 1) that consisted of a stem arm (length=61 cm) and two side arms (length=68 cm); 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=20 cm) 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=20 cm) 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 versus female odors in the Y-maze (odor condition). Each test was started by placing subjects in the closed start chamber; 1 min later the door was removed and subjects were allowed 9 min to explore the maze. All testing was done within the first 6 h 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

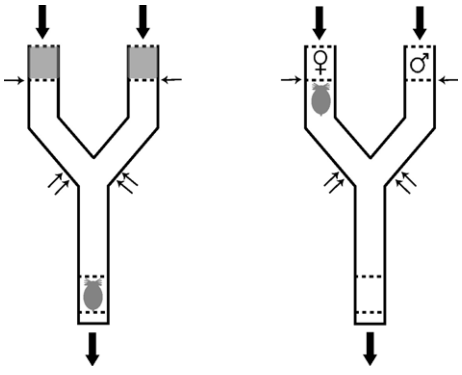

Fig. 1. Y-maze apparatus used in clean condition (left) and odor (male, female) condition (right). The small arrows represent the position of photo-cells and the large arrows represent flow of air. The dashed lines indicate chambers (top: odor stimulus; bottom: start) separated from maze by perforated doors.