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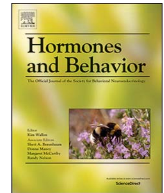
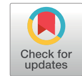


Gonadal hormones, but not sex, affect the acquisition and maintenance of a Go/No-Go odor discrimination task in mice



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ABSTRACT

In mice, olfaction is crucial for identifying social odors (pheromones) that signal the presence of suitable mates. We used a custom-built olfactometer and a thirst-motivated olfactory discrimination Go/No-Go (GNG) task to ask whether discrimination of volatile odors is sexually dimorphic and modulated in mice by adult sex hormones. Males and females gonadectomized prior to training failed to learn even the initial phase of the task, which involved nose poking at a port in one location obtaining water at an adjacent port. Gonadally intact males and females readily learned to seek water when male urine (S+) was present but not when female urine (S−) was present; they also learned the task when non-social odorants (amyl acetate, S+; peppermint, S−) were used. When mice were gonadectomized after training the ability of both sexes to discriminate urinary as well as non-social odors was reduced; however, after receiving testosterone propionate (castrated males) or estradiol benzoate (ovariectomized females), task performance was restored to pre-gonadectomy levels. There were no overall sex differences in performance across gonadal conditions in tests with either set of odors; however, ovariectomized females performed more poorly than castrated males in tests with non-social odors. Our results show that circulating sex hormones enable mice of both sexes to learn a GNG task and that gonadectomy reduces, while hormone replacement restores, their ability to discriminate between odors irrespective of the saliency of the odors used. Thus, gonadal hormones were essential for both learning and maintenance of task performance across sex and odor type.

1. Introduction

Numerous mammalian species ranging from rodents to primates rely on the main olfactory system to detect and discriminate between different volatile environmental odorants that provide critical information about the presence of food as well as dangerous, toxic chemicals in the environment. Volatile chemosignals from conspecifics that are detected by the main olfactory system also complement the action of pheromones detected by a parallel, vomeronasal-accessory olfactory system in signaling the sex and social status of conspecifics. Over the past several decades a large literature has examined the existence of sex differences and related effects of circulating sex hormones on aspects of olfactory function (Dorries, 1992; Kass et al., 2017). For example, early studies by Carr et al. (1962) used a thirst-motivated operant task to determine that prepubertal castration of male rats failed to disrupt their later ability to detect diminishing concentrations of urinary volatiles from estrous female rats or to discriminate between urinary volatiles from estrous vs anestrous females (Carr and Caul, 1962). In another early study using rats in a thirst motivated operant task (Pietras and Moulton, 1974) the ability of females to detect several non-social volatile odorants (e.g., eugenol) was maximal when subjects were in vaginal estrus and was much diminished at vaginal diestrus or after ovariectomy. In a pioneering series of studies Dorries and co-workers extended these early findings to the domestic pig by using a thirst-motivated operant task to show that gonadally intact (GI) females were significantly more sensitive than males to diminishing concentrations of the putative volatile boar pheromone, androstenone (Dorries, 1991; Dorries et al., 1995). A similar sex difference (female > male) in the capacity to detect very low concentrations of male as well as female urinary volatiles was seen in gonadectomized (GDX) mice that were tested using a simple habituation/dishabituation paradigm (Baum and Keverne, 2002). A similar sex difference (female > male) was also seen in a food motivated operant sand digging task in which ovariectomized female mice were better able than castrated males to detect diminishing concentrations of male urinary volatiles when estradiol was administered to both sexes (Sorwell et al., 2008). In several instances, these animal results have been extended to humans. Thus prepubertal children of both sexes were able to detect the volatile human male axillary

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secretion, androstenone, whereas after puberty women were significantly more likely than men to detect this odorant (Dorries et al., 1989). More recently, Dalton et al. (2002) reported that the ability of diminishing concentrations of several odorants (e.g., benzaldehyde) to be detected after repeated exposure trials was significantly greater in young adult women than in men. No such sex differences were seen in prepubertal children or when post-menopausal women were compared to men, suggesting that circulating ovarian sex hormones may augment odorant detection.

Nearly all of the above-mentioned animal and human studies concerning the effects of subjects' sex and/or circulating sex hormones on main olfactory system function have assessed odorant detection thresholds instead of subjects' capacity to discriminate different odorants. An exception is a study (Wesson et al., 2006) that used a hunger motivated, Go/No-Go (GNG) task to compare odorant discrimination among male and female wild type mice as well as in mice with a null mutation of the CYP19 gene (aromatase knockout; ArKO), which encodes aromatase, the enzyme that synthesizes estradiol from testosterone. In that study all mice were GDX and treated daily with estradiol throughout the experiment. The main behavioral findings were that wild type and ArKO males as well as ArKO females were significantly better than wild type females in discriminating pairs (male vs estrous female; testes-intact vs castrated male) of urinary odors as well as a pair of non-social odorants (amyl acetate vs butyl acetate). This outcome points to a possible early developmental role of estradiol, acting in the female, to disrupt brain mechanisms controlling olfactory discrimination. However, these behavioral results are surprising given earlier studies (reviewed above) showing that the capacity for odorant detection is normally greater in female than in male rodents. Also, the study of Wesson et al. (2006) did not assess the potential role of circulating sex steroids in modulating olfactory discrimination. We conducted the present experiments to assess more thoroughly the possible activational role of sex hormones in both male and female mice, first in the acquisition of a GNG task for assessing olfactory discrimination capacity and second in maintaining accurate discrimination of socially relevant as well as non-social pairs of volatile odorants.

We first compared the ability of adult GI vs GDX male and female mice to acquire a thirst-motivated GNG odor discrimination task. We then trained GI males and females to discriminate between pairs of urinary volatiles (male vs estrous female) followed by a pair of non-social odorants (amyl acetate vs peppermint) using the same GNG procedure. We subsequently assessed the ability of these same mice to discriminate these two types of odorants several weeks after GDX and then again several weeks after replacement hormones were given (males received testosterone propionate; females received estradiol benzoate).

2. Methods

2.1. Subjects

Male ($n = 19$) and female ($n = 20$) CFW mice were purchased at 5–7 weeks of age from Charles River Laboratories (Wilmington, MA, USA). All the procedures involving animals were approved by the Boston University Institutional Animal Care Use Committee (IACUC). Animals were group-housed (4 per cage) in same-sex cages under a 12:12 h reversed light: dark cycle (lights off at 9 am). All behavioral tests were carried out during the dark phase of the cycle. All mice were sexually naïve and had no direct contact with members of the opposite sex after arriving in our vivarium. Food was given ad libitum but water was restricted beginning 24 h prior to day 1 of training. During the training period, the amount of water reward for each mouse was recorded every day. Most animals met all of their water needs from two daily 20-minute training sessions. The few animals that performed poorly during any particular test (i.e., received < 50 water rewards per test session) were given access to water for 20 min at the end of the 2nd training session. Subjects were weighed daily to ensure that body weight did not fall > 20% below the pre-water deprivation baseline.

2.2. Odors

We speculated that in mice of both sexes circulating gonadal hormones may more readily influence subjects' ability to discriminate pairs of salient social odors (such as urinary odors from conspecifics) than pairs of non-social odors. Accordingly, we compared subjects' ability to discriminate between male vs female urinary volatiles in one set of tests and between amyl acetate and peppermint (Sigma Aldrich, St. Louis, MO) in another series of tests. Urine was collected in metabolic chambers from 4 testes-intact male and 4 ovary-intact female mice that were not otherwise included in the study. No effort was made to link females' estrous cycle stage to the collection of urine. Urine collected over multiple days was pooled and stored at −80 °C. Male urine and amyl acetate were arbitrarily chosen as the rewarded (S+) odors; female urine and peppermint were used as the non-rewarded (S−) odors. Odors were placed in 25-ml glass vials at a total volume of 10 ml. Urine was diluted 1:10 in deionized water, while amyl acetate and peppermint were diluted 1:10 in mineral oil. At the end of each day's testing session the odors were discarded and fresh odors were prepared on the next day of testing.

2.3. Go/No-Go test box

The GNG testing box was a square Plexiglas box (26.5L × 20W × 30H cm) that contained two ports (1.5 cm in diameter 5 cm apart: a port in which odors were presented and an adjacent port that dispensed water). Both ports were equipped with infrared beams so that when a nose poke occurred the beam was interrupted. Odors were presented using a custom built olfactometer (Verhagen et al., 2007) that was controlled by an Arduino UNO microcontroller using Arduino software. The olfactometer was made of stainless steel manifolds, Teflon tubing and dedicated connector lines to individual 25-ml glass odor vials to avoid cross-contamination of odor streams. House air under pressure was filtered through activated charcoal and used to deliver odors from the odor vial headspace. Odors were sent to a manifold into which subjects could insert the snout (nose-poke) through a port to access odorized air in the chamber. When animals were not nose-poking, a vacuum removed odors from the manifold, preventing odors from exiting through the port. However, once odors were introduced in phase 3, nose-poking turned off the vacuum, allowing subjects to sample the odor. Nose removal initiated introduction of the odor for the next trial (S+ or S−) into the manifold, and at the same time the vacuum was restored until the next nose-poke.

2.4. Procedure

All mice underwent bilateral removal of the gonads under 2% isoflurane anesthesia. Animals were given analgesic on the day of surgery and for the next two subsequent days (carprofen, 5 mg/kg, s.c.). Mice in Group 1 ($n = 7$ males; $n = 8$ females) were gonadectomized prior to any behavioral testing and given 3 weeks to recover before GNG training began. Mice in Group 2 (n = 8 males and n = 8 females) were initially left gonadally intact through olfactory discrimination training and testing, first with urinary odors followed by non-social odors. Mice were then gonadectomized, and after 3 weeks of washout from gonadal hormones, mice were re-tested in the GNG task again using the same pairs of urinary followed by non-social odors. After these tests, males began to receive daily, subcutaneous injections of either testosterone propionate (TP; males; 3 mg/kg in sesame oil) or 17β-estradiol benzoate (EB; females; 1 μg in sesame oil) (Martel and Baum, 2009; Wesson et al., 2006). Injections were given for 7 days prior to resuming GNG testing, first with the pairs of non-social odors followed by the pairs of urinary odors. Daily steroid injections continued until testing

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