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Transient reversal of olfactory preference following castration in male rats: Implication for estrogen receptor involvement

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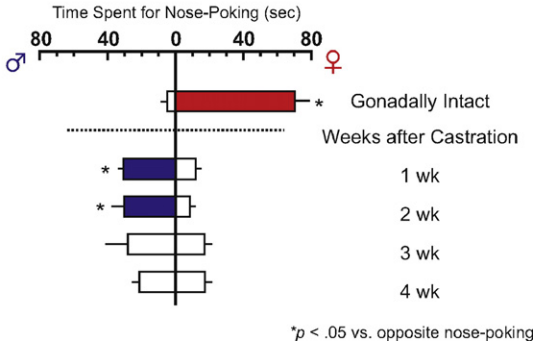
HIGHLIGHTS

- Either androgen or estrogen activates preference for female odor in male rats.
- Castration of male rats causes transient reversal of olfactory preference.
- Reversed preference following castration may be mediated by estrogen receptor.

GRAPHICAL ABSTRACT

Olfactory Preference Test
Subjects poke their nose into air-inlets in which airborne odors of stimulus animals (sexually active male vs. receptive female) flow through.



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ABSTRACT

We examined the effects of the sex steroid milieu on sexual odor preference of sexually-experienced male rats using an alternate choice paradigm after endocrine manipulations. Gonadally intact (GI) males showed a male typical preference, i.e. spent longer time sniffing estrous females than males or ovariectomized females. At 1–2 weeks after orchidectomy (ORx), the males exhibited a transient preference for sexually vigorous males, a female typical preference pattern, followed by a total loss of preference after 4 weeks. Subcutaneous implantation of a Silastic capsule containing formestane (4-OHA), an aromatase inhibitor, had no effect on the preference of gonadally intact rats, but successfully prevented the emergence of the female typical preference after ORx. Capsules containing testosterone (T), dihydrotestosterone (DHT), or estradiol benzoate (EB), but not those with cholesterol (CH), restored masculine typical preference in ORx males at 2 weeks after the placement. The feminine preference for males was observed at 2–3 weeks after removal of T or EB capsules, but not by the removal of DHT and CH capsules. The results suggest that either exogenous androgen or estrogen maintains the masculine typical odor preference. Estrogen itself or produced through aromatization of circulating T, induces a transient feminine typical preference at a certain decreased titer during its disappearance from the circulation. Estrogen at different titers might determine appearance of masculine or feminine typical olfactory preference in adult ORx rats.

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1. Introduction

Sexual behavior has been used as a dependable measure to identify brain sex in studies of sexual differentiation in experimental animals. In a sexual encounter, sexually mature male rats show robust mounts on females, whereas estrous females express lordosis posture in response to male mounts. These male and female behaviors are sexually dimorphic. Preceding sexual behavior, however, they also show clear sexual dimorphic choice of sexual partners, so-called sexual preference. In rats [1,2] and mice [3], sexually active males are attracted by receptive females rather than same-sex, males, and receptive females prefer sexually active males to same-sex, females. Such sexual preference is known to be influenced by sex steroids in early development (organizational effect) and circulating sex steroids in adult rats (activational effect) [4–8].

However, it has been reported that receptive female rats occasionally do not choose sexually active males when both sexually active males and females are available in a direct contact situation [9]. The female avoidance of male rats may be caused by an aversive component of sexual interaction since prevention of intromission by taping the vagina abolishes this female tendency and directs the females to sexually active male rats [9,10]. However, in this type of behavioral test, there still remains a serious problem for direct comparison between male and female behaviors. When placed a sexually active male and a receptive female in each side, male subjects may spend longer time with the female while female subjects may spend longer time with the male. If a subject spent same duration in both sides, are stimuli from stimulus male and female equivalent for the subject? Such difference of stimuli in quality makes it difficult to accomplish the quantitative comparison of sex difference. We have devised a new preference test apparatus to identify components limited to their choice behavior [1,11]. In our preference test, animals can access one of two odors by poking their nose into inlets of airborne odors of stimulus animals. This new preference test allows us to quantitatively compare sex differences in olfactory preference because the behavioral categories measured in the test are identical in females and males.

Using this apparatus, we have previously described masculine and feminine typical response patterns toward various pairs of conspecific odors [1], and we have reported the effects of gonadectomy in male and female rats. Ovariectomized (OVx) female rats primed with estrogen and progesterone preferred the odor of sexually active males to that of females or sexual inactive males, while normal males with intact testes preferred the odor of receptive females to that of males or sexually inactive OVx females. After elimination of sex steroids, female rats rapidly lost their preference for any odor of conspecifics, whereas male rats temporarily showed a feminine pattern of olfactory preference for 2 weeks and thereafter completely lost their preference [1]. That was the first demonstration of the short-term effect of orchidectomy (ORx) on male sexual preference though several studies had reported decay of preference after long-term ORx [8,12,13]. This suggests that the male rat brain has residual neural circuits for feminine preference, which is normally concealed by explicit masculine preference.

This reversed olfactory preference is thought to be regulated by residual circulating hormones following ORx because the reversal is temporary, and decayed soon after. In the present study, we examined our hypothesis that the reversed olfactory preference appeared transiently after ORx is regulated by estrogen rather than androgen. First, we examined olfactory preference in ORx males treated with T, which activates both androgen and estrogen receptors, with DHT, which activates only androgen receptors, or with EB, which activates only estrogen receptors, by Silastic capsule implantation. Then, the effects of removing these capsules, to mimic ORx, were compared for olfactory preference and sexual behavior. In this study, males with intact testes were also treated with an aromatase inhibitor, to examine the effect of ORx without an estrogen effect.

2. Materials and methods

2.1. Animals

Male and female Long-Evans rats were obtained from the Institute for Animal Reproduction (Ibaraki, Japan). The animals were maintained under a controlled temperature (23 ± 2 °C) and a reversed illumination (lights on from 23:00 to 11:00). Food and water were available ad libitum. All experiments and animal housing adhered to the Guidelines for the Care and Use of Laboratory Animals of the Nippon Medical School, and have been approved by our institutional Committee for Animal Experimentation Ethics.

Before the experiment, all females were subjected to OVx under ether anesthesia. When used for the behavioral tests, some females were injected with estradiol benzoate (5 μg in 0.1 ml sesame oil) and progesterone (500 μg in 0.1 ml sesame oil) at 48 and 3–7 h prior to use, respectively, as receptive stimulus females, and others were used without injection as OVx stimulus females (no cross-use between receptive and OVx females). Several males that showed vigorous sexual activities were used as sexual stimulus males, and some were subjected to ORx under ether anesthesia, which was performed at least 3 weeks before use, as ORx stimulus males.

2.2. Apparatus and test for olfactory preference

The acrylic observation box used for alternate choice paradigm was described in our previous paper [1]. The box, 110 cm long (L), 12 cm wide (W), and 30 cm high (H), was divided into three compartments of equal width, divided by two opaque partitions. Three panels, with a 3 cm hole in diameter, bored at the center but at different heights from the floor, were assembled into a partition, which allowed the passage of air and sound but prevented visual and physical interactions. A 2 cm-deep, hollow transparent cylinder was attached to each hole located in the panels facing the middle compartment at a height of 2 cm. The side compartments had air-inlets (mesh-covered holes, 10 cm in diameter) at the ends, and the center compartment had an air outlet at the top. An electrical fan, attached to the air outlet via a flexible duct, introduced air (approximately 0.2 m^3/min) from the compartments on both sides into the center compartment through the hollow cylinders. Olfactory preference was determined by the time spent by the experimental animals poking their nose into the cylinders.

Before each test, the apparatus was cleaned with 70% ethanol (*v*/*v*) and bedded with fresh paper tips (Alpha-dri, Shepherd Specialty Paper, Kalamazoo, MI), and the experimental subject was placed in the middle compartment. After a period of 5 min to allow the rats to acclimatize to the apparatus, the test was initiated by introducing the stimulus pair into the side compartments: the positions of stimulus animals in each pair were counterbalanced between the individual preference tests. The behavior of the experimental animals was observed and recorded using a remote video system for 5 min. The time spent and the number of nose-pokings into the left and right transparent cylinders were measured with an event recorder on a personal computer.

2.3. Pairs of stimulus animals in preference test

On each test day, every experimental animal underwent three preference tests with different pairs of stimulus animals: 1) sexually vigorous male or receptive female, 2) receptive or OVx females, and 3) sexual or ORx males. The test order of the stimulus pairs was counterbalanced, and the interval between the tests was greater than 1 h.

2.4. Sexual behavior test

On each test day, sexual behavior with receptive females was also tested following a series of olfactory preference tests by 3 stimulus pairs. Each male was placed in a transparent observation