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Peripubertal exposure to male odors influences female puberty and adult expression of male-directed odor preference in mice

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ABSTRACT

Testosterone-dependent olfactory signals emitted by male are well known to accelerate female puberty in mice (Vandenbergh effect). However, it remains unclear whether these chemosignals also influence adult expression of male-directed odor preference. Therefore, we exposed female mice to intact or castrated male bedding (vs clean bedding as control) during the peripubertal period (postnatal day (PD) 21–38) and measured male-directed odor preference in adulthood. At PD45 or PD60, females exposed to intact male odors, and thus showing puberty acceleration, preferred to investigate odors from intact males over females or castrated males. Females exposed to castrated male odors did not show puberty acceleration but preferred male (intact or castrated) over female odors. Finally, control females did not show any odor preference when tested at PD45, although a preference for male odors emerged later (PD60). In a second experiment, females that were exposed to intact male odors after pubertal transition (PD36–53) also preferred intact male over castrated male odors. In conclusion, our results indicate that peripubertal exposure to male odors induced early expression of male-directed odor preference regardless of puberty-accelerating effect and that induction of male-directed odor preference is not specific to the peripubertal period.

Introduction

Puberty is the transition between the juvenile stage and adulthood during which animals become physiologically and behaviorally capable of sexual reproduction (Sisk and Foster, 2004). In rodents, sexual behaviors are mediated by the perception of olfactory stimuli from opposite-sex conspecifics that become attractive during pubertal transition (Kelliher and Wersinger, 2009). For example, prepubertal female mice avoid adult male odors, while they prefer to investigate adult male odors after puberty (Drickamer, 1989; Drickamer and Brown, 1998).

In female mice, peripubertal exposure to male odors can profoundly impact reproductive physiology as prepubertal females exposed to male urine from weaning show early onset of puberty, measured through three events: vaginal opening, first estrus and/or uterine growth (Vandenbergh effect; Vandenbergh, 1967, 1969). This effect is mediated by olfactory chemosignals since puberty acceleration disappears after lesioning the accessory olfactory system, but not in females deprived of vision or hearing (Bronson and Maruniak, 1975; Lomas and Keverne, 1982). Olfactory stimuli responsible for puberty acceleration are released in male urine under the influence of testosterone since castrated male urine does not accelerate female puberty onset (Lombardi et al., 1976; Vandenbergh, 1969).

In addition to their physiological effects, male odors regulate also sexual behaviors in female mice. Indeed, expression of male-directed odor preference in adulthood requires functional olfactory systems (Keller et al., 2006a, 2006b) and depends upon previous experience with male odors (Moncho-Bogani et al., 2002; Martinez-Ricos et al., 2007, 2008). Indeed, adult female mice reared without adult male do not prefer to investigate intact male odors over female or castrated male odors in adulthood. However, they display male-directed odor preference after adult exposure to intact male-soiled bedding for 4 days.

Whether peripubertal exposure to male odors is also efficient to induce an adult male-directed odor preference is poorly known. For instance, female mice exposed to an adult male behind a wire mesh partition for 4 days after weaning did not show preference for intact male odors over castrated male odors in adulthood (Hayashi and Kimura, 1978). Therefore, we decided to explore in more details the effect of peripubertal exposure to male odors on male-directed odor preference and puberty acceleration in female mice exposed to intact or castrated male-soiled bedding.

Materials and methods

Animals

Experiments were conducted on Swiss mice purchased from R. Janvier Breeding Center (Le Genest Saint-Isle, France) and bred in

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our animal facility. A total of 237 female mice coming from 53 litters were used. One week before birth, pregnant females were housed in a clean room deprived of any male odors. At postnatal day (PD) 2–3, newborn pups were sexed and litters were equalized to 5 females and 5 males. At PD21, female mice were weaned and housed in individual cages (23 × 16 × 14 cm) containing 50 g of clean bedding (blend of Sterilabo® and Copolabo®, Sisca, Alfortville, France). Animals were kept under a constant 14:10 h light:dark cycle and ambient air temperature was maintained at 20 ± 1 °C. Water and pellet food (Safe, Augy, France) were provided ad libitum.

All the procedures were conducted in accordance with the European directive 2010/63/EU on the protection of animals used for scientific purposes and approved by an ethical committee for animal experimentation (CEEA VdL, Tours, France, n°2012-10-2).

Soiled bedding collection

Male mice were castrated under isoflurane anesthesia three weeks before soiled bedding collection. During the whole period of stimulation, soiled beddings were collected daily from several intact and castrated males (n = 8–12 according to experimental sessions) housed in a separate room than experimental female mice. At the day of preference test, soiled beddings were collected from the same stimulus males and several females (n = 8–12 according to experimental sessions). All beddings soiled by the same type of stimulus animals (intact male, castrated male or female) were mixed.

Experiment 1. *influence of exposure to male odors during the peripubertal period on male-directed odor preference*

Peripubertal exposure to male odors (PD21–38)

During the peripubertal period, females were exposed daily either to intact male-soiled bedding (n = 68), castrated male-soiled bedding (n = 67) or clean bedding (n = 66). Bedding was introduced into the cages of experimental females (the 25 g of bedding present in a female's cage was replaced daily by 25 g of male-soiled bedding or clean bedding in order to refresh the olfactory environment). All bedding stimulations took place between 9:00 and 11:00 a.m. during the light phase. The exposure lasted 18 days, from PD21 until PD38 (preliminary data showed that this period fully covered pubertal transition; Figs. 1A, B). To prevent volatile odor contamination, females were housed in separate rooms according to their type of bedding stimulation for the whole period of stimulation. At PD39, all females were housed with clean bedding in the same room until male-directed odor preference tests were performed.

Evaluation of weight gain and puberty onset (PD21–38)

During peripubertal exposure to male odors (from PD21 to PD38), the growth of females was monitored by weighing animals every 2 days. Puberty onset was evaluated by assessing the age at vaginal opening through daily visual examination (Figs. 1A, B).

Male-directed odor preference test (PD45 or PD60)

Seven (PD45) or 22 (PD60) days after the last exposure to male odors (Figs. 1A, B), females were tested for their preference toward volatile odors emanating from two different soiled beddings. Three tests were performed with independent groups of females (n = 10–14 animals/test): (1) intact male vs female odors, (2) intact male vs castrated male odors or (3) castrated male vs female odors (Figs. 1A, B).

Male-directed odor preference was tested in a Y-maze apparatus for 5 min (previously described in Keller et al., 2006a, 2006b, 2006c). The Y-maze apparatus was in clear Plexiglas® and consisted of a start box (12.5 × 9 × 10 cm) connected to a stem (37 cm) dividing into 2 arms (50 cm) and ending in 2 goal boxes (12 × 8 × 10 cm). A fan generated an airflow coming from the goal boxes to the start box. The start and goal boxes were separated from the rest of the maze by doors containing several holes allowing the flow of volatile odor cues.

Three days before the test (i.e. at PD42 or PD57), females were accustomed to the Y-maze apparatus for 5 min in the absence of any odor stimulus. At PD45 or PD60, a glass dish containing soiled bedding (12 g) was placed behind the door at a 0.5 cm distance in each goal box. The order of stimuli presentation was randomized across subject. The female was first placed in the start box with the door closed to adapt for 1 min. The 5-min test began when the door was removed. The amount of time a female spent sniffing each odor stimulus was recorded. The maze was cleaned with 70% ethanol after each test.

Experiment 2. *influence of exposure to male odors after pubertal transition on male-directed odor preference*

Postpubertal exposure to male odors (PD36–53) and male-directed odor preference test (PD60)

After having checked that all females exhibited vaginal opening, females were exposed daily to intact male-soiled bedding (n = 12), castrated male-soiled bedding (n = 12) or clean bedding (n = 12) for 18 days, from PD36 until PD53 (Fig. 1C), using the same method as described in Experiment 1. At PD54, all females were housed on clean bedding in the same room until the male-directed odor preference test took place. Seven days (PD60) after exposure to male odors (Fig. 1C), females were tested for their preference to investigate intact male over castrated male odors in a Y-maze apparatus, as previously described.

Statistical analysis

As data were normally distributed (Shapiro–Wilk test), body weight, mean age at vaginal opening and time spent sniffing intact male odors were analyzed by one-way or two-way analysis of variance (ANOVA). Significant effects revealed by the ANOVAs were further explored using post hoc Fisher's LSD test. Time spent sniffing the two olfactory stimuli (mean ± SE) during odor preference tests was compared using paired *t*-test within experimental groups. Effect sizes for paired *t*-tests were further estimated by calculating the Cohen's *d* (d = M/SD, where M is the mean of differences and SD is the standard deviation of differences; $d = 0.2$ is considered as a small effect size, 0.5 represents a medium effect size and 0.8 a large effect size). Cumulative percentage of females showing vaginal opening at each PD was compared between experimental groups by the χ^2 test of Pearson. The threshold for significant difference was set at $p < 0.05$. All analyses were conducted using the software Statistica 10 (StatSoft, Tulsa, OK, USA).

Results

Experiment 1. *influence of exposure to male odors during the peripubertal period on male-directed odor preference*

Weight gain and puberty onset from PD21 to PD38

Two-way repeated-measures ANOVA with bedding stimulation as independent factor and age as dependent factor revealed no significant difference in body weight between females according to bedding exposure from PD21 to PD38. There was however, a significant effect of age on body weight ($F_{8,\ 197} = 8915.09$, $p < 0.001$, data not shown).

Females exposed to clean or castrated male-soiled bedding from PD21 to PD38 showed a similar vaginal opening pattern, distributed over 9 days (from PD24 to PD33; Fig. 2) whereas females exposed to