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Potential contribution of prenatal estrogens to the sexual differentiation of mate preferences in mice

Olivier Brock, Julie Bakker *

GIGA-Neurosciences, University of Liege, Avenue de l'Hôpital 1 (B36), 4000 Liege, Belgium

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ABSTRACT

The neural mechanisms controlling sexual behavior are sexually differentiated by perinatal actions of gonadal hormones. We recently observed using female mice deficient in alpha-fetoprotein (AFP-KO) and which lack the protective actions of AFP against maternal estrogens, that exposure to prenatal estrogens completely defeminized their potential to show lordosis behavior in adulthood. Therefore, we determined here whether mate preferences were also affected in female AFP-KO mice. We observed a robust preference for an estrous female over an intact male in female AFP-KO mice, which were ovariectomized in adulthood and subsequently treated with estradiol and progesterone, whereas similarly treated WT females preferred the intact male over the estrous female. Gonadally intact WT males preferred the estrous female over the male, but only when visual cues were blocked by placing stimulus animals behind opaque partitions. Furthermore, when given the choice between an intact male and a castrated male, WT females preferred the intact male, whereas AFP-KO females showed no preference. Finally when given the choice between an estrous female and an ovariectomized female, WT males preferred the estrous female whereas AFP-KO females preferred the ovariectomized female or showed no preference depending on whether they could see the stimulus animals or not. Taken together, when AFP-KO females are tested under estrous conditions, they do not show any male-directed preferences, indicating a reduced sexual motivation to seek out the male in these females. However, they do not completely resemble males in their mate preferences suggesting that the male-typical pattern of mate preferences is not solely organized by prenatal estrogens.

Introduction

When in breeding condition, male and female mammals usually seek out and mate with opposite sex conspecifics. It has been well established that the neural mechanisms controlling mate preference are sexually differentiated by the perinatal actions of sex steroid hormones (Bakker, 2003). Thus, a female-directed preference develops in male rats under the early (perinatal) influence of estradiol derived from neural aromatization of testosterone (Bakker et al., 1993, 1996a,b). Male rats treated neonatally with 1,4,6-androstatriene-3,17-dione (ATD), a specific inhibitor of the aromatase enzyme, failed to show a preference for an estrous female when given a choice between an estrous female and a sexually active male in a three compartment box, whereas normal males clearly preferred the vicinity of the estrous female. In fact, such neonatally ATD-treated male rats preferred the vicinity of the sexually active male, in particular following estradiol treatment in adulthood (Bakker et al., 1993, 1996a,b) suggesting that their mate preferences had not been masculinized.

We showed using aromatase knockout (ArKO) mice, which carry a targeted mutation in the *Cyp19* gene (Bakker et al., 2002a) and as a result cannot convert androgens into estrogens, that in the mouse, like in the rat, female-directed preferences seem to develop under the influence of estrogens (Bakker et al., 2004). Gonadally intact male ArKO mice failed to show a preference for an estrous female when provided with volatile body odors from an estrous female and a sexually active male in a Y-maze (Bakker et al., 2002a), whereas wild-type (WT) males preferred to investigate the estrous female box. Adult treatment of male ArKO mice with estradiol failed to induce a female-directed preference suggesting that mate preferences develop perinatally in male mice under the influence of estrogens (Bakker et al., 2004). Similar results have been obtained in male mice carrying a mutation in the estrogen receptor alpha gene (ERalphaKO; Wersinger and Rissman, 2000). Such ERalphaKO males failed to show a preference for an estrous female when given the choice between an anesthetized estrous female and an anesthetized intact male, further confirming the pivotal role of estrogens and ER in the development of female-directed preferences.

Interestingly, we recently observed female-typical (i.e., male-directed) preferences in female mice carrying a mutation in the *Afp*

* Corresponding author. Fax: +32 4 366 5953.
E-mail address: jbakker@ulg.ac.be (J. Bakker).

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gene (AFP-KO) which encodes the major fetal plasma protein alpha-fetoprotein that binds estradiol with high affinity (Bakker et al., 2007). This finding was quite unexpected since female AFP-KO mice are clearly defeminized with regard to their lordosis behavior (Bakker et al., 2006) as well as their GnRH/Kisspeptin system, i.e., no steroid induced preovulatory LH surges (De Mees et al., 2006; Gonzalez-Martinez et al., 2008). The observation of a robust male-directed preference in AFP-KO females would thus suggest that the development of mate preferences is postnatally influenced by ovarian estrogens. This finding is in line with our earlier study in female ArKO mice (Bakker et al., 2002b) that suggests feminizing effects of estradiol on the female brain since female ArKO mice showed reduced levels of lordosis behavior as well as no clear mate preferences in adulthood. Taken together, there is still some ambiguity about the role of perinatal estradiol signaling in the development of mate preferences in mice.

Therefore, in the present study, to further investigate the organizational role of prenatal estrogens in mate preferences, we extended our initial study to determine mate preferences in AFP-KO and WT female mice using a different testing paradigm, i.e., a three compartment box in addition to the Y-maze. The rationale for using a three compartment box was to offer the animals different choices between stimulus animals without the need to run from one arm to the other, since preferences measured in the Y-maze may be influenced by differences in exploratory activity of the subjects. Furthermore females were tested under estrous conditions, i.e., following treatment with estradiol and progesterone and not with estradiol alone as was done in our previous study (Bakker et al., 2007). We also determined mate preferences in a group of WT males that were left gonadally intact to determine whether AFP-KO females resembled males or not.

Materials and methods

Animals

All breeding and genotyping were performed at the GIGA Neurosciences, University of Liège, Liège, Belgium. In the present study, male and female mice heterozygous for the allele Afptm1lbmm (in the *CD1* background strain; Gabant et al., 2002; Bakker et al., 2006) were bred to generate wild-type (WT) and homozygous-null (AFP-KO) offspring. Mice were genotyped by PCR analysis of tail DNA (for more detailed description, see Bakker et al., 2006, 2007). Subjects were weaned at 21 days and placed into individual cages under a reversed light–dark cycle (12 h:12 h light/dark; 20.00 h lights on and 8.00 h lights off) in special light and temperature controlled housing units. Food and water were always available *ad libitum*.

Stimulus animals (all of the *CD1* strain) were gonadally intact males, long-term gonadectomized male (gdx) and female (ovx) mice, and ovariectomized females brought into behavioral estrus by treatment with estradiol and progesterone. Gonadectomy was performed under general anesthesia after an intraperitoneal injection (i.p.) of a mixture of ketamine (80 mg/kg per mouse) and medetomidine (Domitor, Pfizer, 1 mg/kg per mouse). Mice received atipamezole (Antisedan, Pfizer, 4 mg/kg per mouse) subcutaneously (s.c.) at the end of the surgery in order to antagonize medetomidine-induced effects, thereby accelerating their recovery. Ovariectomized females that were going to serve as estrous female stimulus were at the same time implanted with a Silastic capsule containing 17β-estradiol (diluted 1:1 with cholesterol, for more details see Bakker et al., 2002b).

All experiments were conducted in accordance with the guidelines set forth by the National Institutes of Health "Guiding Principles for the Care and Use of Research Animals" and were approved by the Ethical Committees for Animal Use of the University of Liege.

Behavioral tests

Mate preferences using the three compartment box

To assess mate preferences using either visual, auditory, and/or olfactory stimuli, we used a box (60 × 13 × 30 cm) that was divided into three compartments by placing either opaque or transparent partitions. Each compartment was thus 20 cm in length. The partitions contained perforated holes at a height of 8 cm to facilitate the diffusion of odors from the two side compartments to the middle compartment. Tests were performed during the dark phase of the light cycle (6 h after lights out). Animals were habituated to the three compartment box only once on the day before the behavioral experiments by placing them in the middle compartment for 10 min (with no stimulus animals placed in the two side compartments). On the day of testing, stimulus animals were placed in the two side compartments with their own bedding to make the stimulus as odorous as possible. The subject was then introduced into the middle compartment containing no sawdust, and was observed for 9 min. The time spent poking its nose through the holes of the partition or actively sniffing the bottom of the partition was recorded with a stopwatch. Each test session was divided into 3 min intervals in order to determine whether investigation times would decrease during the test, since we have previously observed that subjects have the tendency to investigate more during the first minutes of the test. However, since we did not observe any decrease along the test session, we only present the total time spent investigating the two stimuli in the results section.

Thus 24 adult mice (WT: 8 females and 10 males; AFP-KO: 6 females) were used in the present experiments. All females were ovariectomized and implanted in the neck with a 5 mm long Silastic capsule containing 17β-estradiol (diluted 1:1 with cholesterol) and received an injection of 500 μg progesterone 3 h before each test. Subjects were tested between 10 and 17 weeks of age. All stimulus animals were awake and either presented behind transparent partitions (thus only physical contact was prevented, and mate preferences were based on visual, auditory and olfactory cues) or opaque partitions (to prevent any visual cues). We used the following experimental protocol to test mate preferences: the first series of preference tests were conducted using transparent partitions, thus allowing visual, auditory and olfactory cues. Subjects were first offered a choice between an intact male versus an estrous female. Next, they were offered a choice between an estrous female versus a long-term ovx female, and finally, a choice between an intact male versus a long-term gdx male. The second series of preference tests using opaque partitions, thus only allowing auditory and olfactory cues, was conducted the week after. Again, subjects were first offered a choice between an intact male versus an estrous female, then between an estrous female versus a long-term ovx female, and finally between an intact male versus a long-term gdx male. All tests were conducted on separate days and at least 2 days apart since female subjects were injected with progesterone before each preference test. The position (left versus right compartment) and presentation of the stimulus animals were not randomized to prevent variability due to possible residual odors in the compartments.

Mate preferences using the Y-maze

Following these tests in the three compartment box, subjects were tested once for their mate preferences in the Y-maze (for a more detailed description of the maze, see Bakker et al., 2002a) with as choice volatile odors from an anesthetized male versus those of an anesthetized estrous female (one AFP-KO female died between the three compartment box and the Y-maze tests). Briefly, before being tested for their mate preferences, all subjects were tested for 5 min in the Y-maze without any odor stimuli to adapt to the testing apparatus and to determine whether they would develop any side preferences. When subjects were tested for mate preferences using volatile body