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Prenatal stress and ethanol exposure produces inversion of sexual partner preference in mice

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ABSTRACT

The presence of a sexually receptive female behind perforated transparent partition induced sexual arousal and specific behavior in male mice so they spent more time near partition in an attempt to make their way to the female. Three-chambered free-choice model was used to evaluate sexual partner preference. The main pattern of sexual preference was the time spent by a male mouse at the partition dividing female (F-partition time) versus a partition dividing male (M-partition time). Pregnant mice were given ethanol (11 vol.%) for 1–21 gestational days, and were exposed to restraint stress (2 h daily for 15–21 day of the gestation). Control pregnant mice had free access to water and food and were not stressed. Adult male offspring of ethanol and stress exposed dams (E+S) showed decreased F-partition time and increased M-partition time. Whereas F-partition time in all control mice prevailed over M-partition time, 78% E+S mice demonstrated prevailed M-partition time. E+S mice were more active in social interaction with juvenile male. No significant differences between E+S and control mice in the open field and novelty tests were revealed. Therefore, E+S exposure during dam gestation inverted sexual partner preference in male offspring, suggesting that stress and alcohol in pregnancy produces predisposition to homosexuality.

In the early 80s Dörner et al. [8,9] hypothesized that prenatal stress might present a risk factor for homosexuality in man. Lately, research on prenatal stress and human sexual behavior produced inconsistent results. Some human studies have not been able to document any association between prenatal stress and human male homosexuality [2], while others [11,10] supported the idea that maternal stress during pregnancy may alter the sexual orientation of male offspring.

Concomitantly, a lot of experimental evidence demonstrated that prenatal stress during last week of gestation resulted in demasculinization and feminization of adult male offspring and in marked deficits in adult male rat mating behavior [14,22,26,31]. Decreased sexual motivation measured as sexual partner preference was revealed in prenatally stressed male offspring. Prenatally stressed male rats showed no preference for male or female, whereas control rats exhibited significantly higher number and duration of visits to female than male stimulus rats [30].

Another prenatal factor that may strongly affect the development of the offspring is alcohol. Fetal alcohol spectrum disorder (FASD) is a common neuropsychiatric disorder revealed in prenatal alcohol exposed subjects [3,4,13,16,24]. Neurophysiological impairments in FASD include physical, mental behavior and learning disabilities [24].

Prenatal alcohol exposure in the rat was shown to interfere with the neurobehavioral sexual differentiation of the male brain [21,20]. Adult male mice that had been exposed to ethanol prenatally showed a decreased preference for the opposite sex and increased preference for the same sex as a partner, although their physical development was apparently unaffected [34]. When tested for feminine sexual behavior in adulthood, ethanol-exposed male rats showed marked signs for feminization as evidenced by increased amount of lordosis responses [15]. No alterations in the masculine sexual behavior in the offspring of ethanol-treated dams were seen [5,15]. Regarding human, no evidence was found that prenatal exposure to alcohol impacted offspring sexual orientation of ether males or females [10].

The copulatory behavior of male rats exposed to ethanol combined with stress during prenatal development was studied [32]. It was shown that prenatal exposure to stress, alcohol, or the combination of alcohol plus stress increased the probability that males would display female lordotic pattern in adulthood. There were no significant differences between these groups in the expression of the lordosis, however, alcohol plus stress exposed rats showed severe failure in the ejaculation [33].

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The potential contribution of prenatal exposure to alcohol in combination with stress to sexual motivation and sexual partner preference is still not clear.

Our special interest was to determine whether there were distinct alteration in sexual partner preference in ethanol and stress combined exposed offspring (E + S). Sexual preference in adult male offspring was studied in the three-chambered free-choice model with a receptive female in one and a male in other of adjusting chamber. It was shown that a placement of a receptive female behind a transparent partition produced sexual arousal in male [1]. The main pattern of sexual preference was the time spent by tested male mouse at the partition in attempts to find his way to the female compared to the male partition time. We also studied the effect of E + S exposure on social interaction with juvenile male, open field and object recognition behavior in mice.

CBA/LacJ mice (3–4-month-old, 20–22 g) were housed in the plastic cages (37 cm × 21 cm × 15 cm) in four groups (three females and one male per group) under standard laboratory conditions with a temperature of 20 ± 2°C in a natural light dark cycle (07:30–19:00 light cycle) with free access to water and pellet food.

The females were inspected twice a day. On the first day of gestation, determined by the presence of a vaginal plug, female mice were rehoused in other cages and randomly assigned to one of two groups. The first group was accepted as a control one and had free access to water and food. They were not stressed. In the second group, water was replaced by ethanol (11 vol.%) dissolved in distilled water, and dams drunk the ethanol solution during all 21 gestation days until parturition. On average, a female consumed 2.5 ± 0.1 ml of ethanol solution per day. All pregnant mice were housed in groups consisting of 4 animals. On the 19th day of pregnancy each female was placed individually in a cage. After delivery, ethanol solution was replaced by the water. On the 15–21 days of pregnancy the dams were stressed for 2 h daily by placing in cylindrical restraint tube (D = 2.5 cm, L = 8 cm) under illumination (150 W on the distance 30 cm) [19,25,31]. Stress was emerged at light portion of the light–dark cycle at random times so that dams could not predict the time of the stress exposure. The exposure of pregnant female mice to unpredictable stress paradigm during the third week of pregnancy was based on numerous data showing vulnerability of rodent brain to environmental stress at this prenatal period of development [23,32].

After weaning, 27-day-old male offspring were housed in groups of 4–5 animals from different litters. On the postnatal day 90, the testing of male offspring began. The mean weight of control animals was 27.4 ± 0.3 g, the one of E + S group was 27.9 ± 0.6 g ($p > 0.5$).

Three days before the experiment, the mice were isolated into individual cages to remove the group effects. The mouse behavior was registered by means of a digital video recorder and evaluated in EthoStudio software [17].

All experimental procedures were in compliance with "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council, 2003). All efforts were made to minimize the number of animals used and their suffering.

Tests were performed on 3-month-old sexually naive male mice. The experimental steel cage (45 cm × 15 cm × 10 cm) consisted of a three equal sections (15 cm × 15 cm × 10 cm) divided by perforated transparent partitions. To habituate to the testing cage, three days prior to the experiment E + S and control mice were placed individually in the middle chamber of an experimental cage. To study sexual partner preference in male mice, a receptive female representing an effective "producer of pheromones" was introduced into one neighboring section and a male mouse was introduced into other of the neighboring section of the cage. Estrus in the female was induced by pretreatment with estradiol benzoate (40 μg s.c. 48 h prior to testing) and progesterone (500 μg s.c. 4 h prior to testing) [28,29]. Sexual partner preference was registered for 10 min. The males would normally approach the partition, smell and touch it with one or two forepaws and hold or hang on to the partition. Furthermore, the males would trust their noses into the holes of the partition and even chew it. The main pattern of sexual partner preference was the time spent by a male mouse at the partition in attempts to find his way to the female (F-partition time) compared to the time spent by a male near the partition dividing him from male (M-partition time). The number of approaches to the partitions was also registered.

Open field behavior was studied in an open round arena (D = 40 cm) made of opaque plastic and illuminated by two halogen 12-W lamps. Each animal was placed near the wall of the chamber and tested for 5 min. The total path length and time spent in the center (%) were recorded automatically. Time in the center was counted automatically by computer as time in central region (D = 20 cm) of the open field arena.

Novelty test was carried out in an open round arena (D = 40 cm) made of opaque plastic and illuminated by two halogen 12-W lamps. Habituation consisted of a 5 min period in the empty cage the day before the test. In trial, mice were placed in arena and allowed to explore freely two identical objects (blue wooden cubes) for 5 min. A mouse was considered to be engaging in exploratory behavior if the animal touched the object with its forepaw or nose or sniffed at the object within a distance of 1.5 cm [36]. Novelty-induced exploration was defined as the total time spent exploring both of the identical objects and was considered as a reliable indicator of exploratory behavior [7].

Social interaction with juvenile male was studied in the plastic home cage (37 cm × 21 cm × 15 cm) of the resident mouse, with wood shavings as bedding. The test started with a cautious introduction of juvenile male 4-week of age to the resident's cage. Social behavior assessed in terms of the duration of the social contacts (sniffing, social grooming) during 10 min of the test.

The data are presented as means ± SEMs for quantitative attributes and as percentages for qualitative ones. Differences between experimental groups were analyzed by Fisher exact two-tailed criterion (2 × 2 contingency table) for categorical variables (share of mice with inverted sexual preference) and repeated measures ANOVA followed by Fisher LSD post hoc comparison (indices in the test for sexual partner preference) or one-way ANOVA (indices in the other behavioral tests) for numerical variables. The value of $p < 0.05$ was considered significant.

Control males produced considerably stronger behavioral response to a receptive female than to a male ($F_{1,9} = 23.79$, $p < 0.001$). Specifically, the male directed itself towards the female, stopped by dividing partition and stayed there for a long time trying to reach for the female. The F-partition time was almost twofold higher compared to M-partition the time (Fig. 1, control).

Repeated measure ANOVA revealed significant effect of interactions between group of animals and sex of partner located behind a partition ($F_{1,17} = 31.0$, $p < 0.001$) in sexual partner preference test. Sexual preference inversion in male E + S offspring was expressed in a significant increase in time spent at partition with male ($p < 0.001$) and in a reduction of time spent in attempts to penetrate through the barrier separating the receptive female ($p < 0.001$; Fig. 1). Sexual preference in male E + S offspring was also manifested as more frequent approaches to the partition separating them from males than from females ($F_{1,17} = 6.98$, $p < 0.01$; Fig. 1, E + S). In all mice of the control group, interest a receptive female prevailed over the interest a male, whereas in 78% E + S mice, the time spent at the M-partition exceeded the time spent at the F-partition ($\chi^2 = 12{,}31$; $df = 1$, $p < 0.001$).

Social interaction E + S males with juvenile male mouse significantly increased ($F_{1,28} = 21.75$, $p < 0.001$; Fig. 2A). In contrast, there