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Effect of prenatal ethanol exposure on sexual motivation in adult rats

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ABSTRACT

Maternal alcohol use during pregnancy adversely affects prenatal and postnatal growth and increases the risk of behavioral deficits. The aim of the present study was to evaluate the effect of prenatal exposure to a moderate dose of alcohol on sexual motivation during adulthood. Rats were prenatally exposed to ethanol by feeding pregnant dams a liquid diet containing 25% ethanol-derived calories on days 6 through 19 of gestation. The controls consisted of pair-fed dams (receiving an isocaloric liquid diet containing 0% ethanol-derived calories) and dams with *ad libitum* access to a liquid control diet. The sexual motivation of offspring was evaluated during adulthood. The results revealed that the male and female pups of dams treated with alcohol exhibited reduced weight gain, which persisted until adulthood. Both male and female adult animals from dams that were exposed to alcohol showed a reduction in the preference score in the sexual motivation test. Taken together, these results provide evidence of the damaging effects of prenatal alcohol exposure on sexual motivation responses in adulthood.

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Introduction

During fetal development, the organism must work inside a delicate balance that depends on the uterine environment within which it grows and the placental connection between itself and the mother from whom it receives oxygen and nutrients. This balance can be directly affected by the substances that the mother ingests (Behnke & Smith, 2013; Streissguth & LaDue, 1987; Warren, 2015). In this context, harmful use of alcohol causes approximately 5.1% of the global burden of diseases such as fetal alcohol spectrum disorder that are attributable to alcohol use by women during their pregnancies (World Health Organization, 2014).

Studies have shown that alcohol consumption during pregnancy can be detrimental to the development of the fetus as well as to the continued growth and development of the child after birth and during adulthood (Mattson & Riley, 1998; Mattson, Crocker, & Nguyen, 2011). In addition to these alterations, prenatal alcohol exposure can produce a variety of altered behaviors during adulthood, such as interruption of maternal behavior (Pepino, Abate, Spear, & Molina, 2002), reduction of aggressive behavior probably related to lower testosterone levels (Lugo, Marino, Gass, Wilson, & Kelly, 2006), and feminized behavior in males and masculinized behaviors in females during the play behavior test (Meyer & Riley, 1986). Moreover, male rats prenatally exposed to alcohol show impaired masculinization and defeminization of sexually dimorphic copulatory behavior, characterized by female-typical lordosis behavior and failure to ejaculate (Ward, Ward, Winn, & Bielawski, 1994). Although there are data about the influence of prenatal alcohol exposure on behavioral changes and impaired reproductive function in adulthood, little is known about the effects of prenatal alcohol exposure on the potential changes in sexual behavior. Thus, the aim of this study was to evaluate whether sexual motivation of adult rats is affected by moderate prenatal exposure to alcohol.

Materials and methods

Animals

Adult virgin female (230–250 g) and male (280–300 g) *Wistar* rats were obtained from the Central Animal Facility of the Federal

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University of Alfenas. The animals were housed in a temperature- (22 °C) and humidity-controlled (40–60%) room on an automatic 12 h light/dark cycle (lights on at 7:00 AM). After 10 days of acclimation, the nulliparous females were mated by placing them with sexually experienced males. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears collected the next morning, which was designated as gestation day 0 (GD0). Pregnant females were then weighed and individually housed in standard plastic breeding cages (42 × 34 × 16 cm). All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas (509/2013).

Maternal alcohol exposure

Pregnant rats at GD6 were randomly assigned to one of three treatment groups, based on liquid diet. (1) Alcohol group – these animals were given free access to a liquid diet containing 25% alcohol-derived calories; (2) pair-fed group – animals were offered a liquid control diet with sucrose isocalorically replaced for ethanol, in an amount matched to that consumed by an ethanol-fed partner (g/kg body weight/day of gestation); and (3) *ad libitum*-fed control group – animals were offered *ad libitum* access to a liquid control diet. The liquid diet consisted of chocolate-flavored Sustagen 0.2 g/mL (Mead Johnson; Brazil) supplemented with 0.05 g/mL vitamins for rodents (Roevit; Brazil) in water.

The volume of diet that was consumed was measured daily. All animals received fresh diet daily within 1 h prior to lights-off to prevent a shift of corticosterone circadian rhythms, which occurs in animals that are on a restricted feeding schedule, such as pair-fed animals.

The liquid diets were discontinued after GD19 to decrease the risk of cannibalism by the dams at birth (McMurray et al., 2008). On GD19, the liquid diets were replaced by continuous access to lab chow and the breeding cages were checked daily for births. The animals were allowed free access to water during all experimental periods. The day of parturition (PND0) was recorded for each litter. To reduce variation in the growth of the pups, the litters were culled to 8 pups (4 males and 4 females) on PND1. The culled pups were visually examined for abnormalities, and then euthanized by inhalation of the anesthetic halothane.

Blood alcohol concentration

In order to determinate the maximal or near maximal blood alcohol concentration achieved by alcohol-consuming rats, blood samples were taken on GD19 by decapitation ($n = 7$ dams/group), 2 h after lights-off, which typically follows a major eating bout. The ethanol was quantified in total blood samples by using a gas chromatograph (model GC1000, Ciola & Gregori, S. Paulo, SP, Brazil), equipped with a flame ionization detector and a capillary column HP Innowax (length 30 m, internal diameter 0.53 mm, film thickness 1.0 μm). The operational conditions were injector temperature: 150 °C, detector temperature: 180 °C, column temperature: 120 °C. The carrier gas was nitrogen (flow rate of 8.0 mL/min). Hydrogen, synthetic air, and nitrogen were used in the detector at 0.80, 0.95 and 0.70 bar, respectively. The standard calibration curve was calculated by using a rat blank blood sample fortified with ethanol from 0.2 to 4.0 g/L, using butanol at 1.0 g/L as internal standard. For each analysis, 0.5 mL of each analytical standard and 0.5 mL of the butanol solution were added into a 5 mL glass flask, which was sealed and put into an oven at 80 °C for 10 min. Afterward, 0.1 mL of the headspace was injected into the chromatograph for the analysis. The rat samples were submitted to the same analytical procedure.

Physical development

One male and one female from each litter (control, $n = 10$; pair-fed, $n = 8$; and alcohol, $n = 10$ litters) were weighed at PND1, PND3, PND7, PND10, PND14, PND21, PND28, PND35, PND42, and PND49. The testicular descent (descent of both testes to the scrotum) and vaginal opening were assessed in one male and one female from each litter. A digital pachymeter was used to measure the anogenital distance (considered as the length from the anus to the genital tubercle) in millimeters, and the anogenital index was calculated as the ratio of the anogenital distance/body weight. One male and one female pup from each litter were marked with ink and used for all physical development assessments as well as for body weight follow-ups. On PND21, the offspring were weaned and the littermates were housed together, but separated according to sex (Smart & Dobbing, 1971). One male and one female pup from each litter were used for offspring evaluations to minimize potential confounding factors associated with the litter (Lazic & Essioux, 2013).

Sexual incentive motivation test

The sexual behavior of male and female adults was evaluated at 70 days of age. The experiments were adapted from methodology proposed by Ágmo (Ágmo, 1997; Ágmo, 2003). Sexual incentive motivation was evaluated in an oval open field (100 × 50 cm) surrounded by a 45 cm high wall. An incandescent light bulb provided dim red light (approximately 5 lux in the arena). The incentive animals (a receptive female for sexual incentive for males or social incentive for females, and a sexually experienced male for social incentive for males or sexual incentive for females) were located in cages placed outside the arena at diagonally opposed corners on each long wall. The incentive females (receptive) were ovariectomized under anesthesia about 2 weeks before the start of experiments. Sexual receptivity was induced by subcutaneous (s.c.) injection of 25 μg of estradiol benzoate about 52 h before tests and progesterone (1 mg, s.c.) injected about 4 h before tests. Both steroids were from Sigma (St. Louis, MO, USA) and they were dissolved in peanut oil and injected in a volume of 0.2 mL/rat. An opening (25 × 25 cm), located at floor level, in the arena wall allowed the test animals to communicate with each incentive animal cage, as previously described (Ágmo, 2003). A wire mesh separated the incentive animal from the experimental subject. A zone (20 × 30 cm) outside each incentive animal cage was designated the incentive zone (Fig. 1, Supplementary Video 1). To start the experiment, the subjects were habituated to the testing environment during three 10 min sessions. The incentive animals were not present during the habituation procedure. Immediately before each session, the arena and the incentive animal cages were cleaned with a solution of ethanol in water. The test was similar to the habituation procedure and lasted for 20 min, but in this case, an incentive rat was placed in each incentive animal cage. During the test, the subject could hear, see, and smell the incentive animals but no copulatory interactions were possible. The vaginal secretions of female rats' offspring of control, pair-fed, or alcohol-fed dams were collected every day (4:00 PM) and examined microscopically using a light microscope. The characteristics of the four phases (proestrus, estrus, metestrus, and diestrus) of the estrous cycle were determined by the proportion of the epithelial cells, cornified cells, and leukocytes in the vaginal smear. A female rat in proestrus phase was marked and selected for the sexual motivation test at 8:00 PM of the same day. We registered the number of visits and time that the experimental rats spent in the female incentive zone (FIZ) or male incentive zone (MIZ). In addition, a preference score (time spent in the female or male zone/time spent in both incentive zones) was

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