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PRENATAL PROGESTERONE TREATMENT INDUCES SEX-DEPENDENT ANXIETY AND DEPRESSIVE-LIKE BEHAVIOUR IN ADULT OFFSPRING

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Abstract

Exogenous treatment during pregnancy with steroid hormones (estrogens, androgens, or glucocorticoids) affects the development of the fetus and the sexually mature generation. In clinical practice, the hormone progesterone is used therapeutically in programmes for assisted reproduction, infertility treatment, threatened abortion and premature birth. The hormone has a key role in establishing and maintaining pregnancy through its endocrine and immunological effects. Despite the fact that progesterone is widely used during pregnancy, the long-term effects of fetal exposure to exogenous progesterone on child development have barely been investigated. The aim of the present study is to investigate sex-dependent changes in the emotional status of a generation of prenatally treated with progesterone offspring. Female pregnant rats were treated subcutaneously with progesterone (50 mg/kg) from gestational (G) period G0 to G10. Anxiety and depressive-like behaviour of male and female adult offspring were evaluated with an open field (OF) test, elevated plus maze test (EPM), light dark test (LDT), sucrose preference test (SPT) and forced swimming test (FST). Prenatal treated with progesterone male and female offspring exhibited lower horizontal and vertical activity compared to the male and female control rats in the open field test and decreased distance and time spent in the open

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arms compared to the matched controls in the EPM test. They demonstrated depressive-like responses with anhedonia in the SPT and increased immobility time in the FST compared to the matched controls. Prenatal treatment with progesterone significantly affected emergence latency, time spent and crossing to the lit compartment in LDT. In conclusion, our results suggest that prenatal treatment with 50 mg/kg progesterone exerts a detrimental effect on emotional behaviour in male and female offspring. Future studies are needed to ascertain the underlying mechanism associated with these sustained behavioural abnormalities due to prenatal hormonal treatment.

Key words: prenatal treatment, progesterone, sex differences, behavioural tests

Introduction. In clinical practice, the hormone progesterone (Pro) is used therapeutically in programmes for assisted reproduction, infertility treatment, threatened abortion and premature birth [1]. It is a steroid hormone involved in the menstrual cycle, pregnancy and embryogenesis in humans. It is synthesized in the adrenal glands, gonads (after ovulation in the corpus luteum), brain, and during pregnancy in the placenta. The hormone has a key role in establishing and maintaining pregnancy through its endocrine and immunological effects. Studies on the effects of exogenously administered Pro are mainly focused on the tissues, namely the developing reproductive system [2]. There is evidence that, apart from reproduction, endogenous Pro affects the immune system, cardiovascular system, renal function, adipose tissue, respiratory system, spermiogenesis, sperm capacitation/acrosome reaction, and testosterone biosynthesis in Leydig cells [3]. Although this hormone plays an essential role in the first trimester of fetal development, the intake of the hormone is often recommended for pregnancy problems. Evidence in the literature shows that Pro preparations increase the risk of developing hypospadias and cryptorchidism in the male generation of mothers who are treated with Pro during pregnancy [4].

Studies on the effects of Pro administered to pregnant animals and humans show that the hormone harms intrauterine development [9]. Effects on the developing female reproductive system in mice exposed to Pro as neonates are expressed in persistent vaginal estrus and altered sexual behaviour [4].

Studies show that 66% of boys with mild hypospadias and 40% of those with severe hypospadias have testicular defects in testosterone biosynthesis. A higher risk of hypospadias was also observed in children conceived in vitro compared to controls, possibly related to Pro therapy at IVF [5]. Progesterone is a competitive inhibitor in converting testosterone to dihydrotestosterone [6]. Hypospadias and reduced anogenital distance can be considered part of a syndrome of failure of differentiation of the male genitalia associated with the closure of the urethral fold and are sometimes referred to as "feminization".

Effects investigated in human and animal studies include altered genitalia in both male and female fetuses, cardiovascular malformations and other birth

defects. Other effects of animal prenatal Pro administration are intrauterine death and reduced birth weight. In addition, postnatal manifestations in animals include altered sexual behaviour in males [4]. Finally, mortality due to progesterone has also been the topic of some research [7].

Based on the available literature data concerning the detrimental effects of Pro exposure during pregnancy, in the present study we evaluated the consequences of prenatal treatment with Pro on emotional behaviour (anxiety and depressive-like responses) in female and male adult offspring.

Materials and methods. ***Animals.*** Male and female adult Wistar rats (220–250 g) were housed at standard conditions (temperature $21 \pm 1\,^{\circ}\mathrm{C}$; 50–60% humidity; 12 h light/dark cycle; 3–4 per cage) with sawdust bedding and with food and water ad libitum. All experiments were performed in accordance with the European Communities Council Directives of 24 November 1986 (86/609/EEC). The project was approved by the Bulgarian Food Safety 276 Agency No 338/19.10.2022.

Prenatal treatment with progesterone. Rats were adapted for at least a week before starting breeding. Subsequently, female rats were mated with male breeders. The gestation day 0 (E0) was detected by the appearance of a positive vaginal smear. Pregnant females were randomly assigned to the control and group treated with Pro at a dose of 50 mg/kg subcutaneously (sc) from G0 to G10 (Pro50). Six dams from each group were used. Matched control dams were sc injected with vehicle. The dam with pups were kept together until weaning on postnatal (P)21. Rat pups of each litter with the same sex of nine were randomly assigned to control (C-veh) group and treated with Pro (Pro 50) at P60. For each test $n = 8$ offspring rats (P60) were used.

Behavioural tests. *Open field.* The tested rat was placed in the central quadrant of a grey polystyrene box ($100 \times 100 \times 60$ cm). The following parameters were measured: total distance travelled (cm) in the field, the time ratio in centre vs. total and number of rearings for 5 min.

Elevated plus maze. The apparatus consisted of two open arms (50×10 cm), two enclosed arms ($50 \times 10 \times 50$ cm), and a central platform (10×10 cm) elevated 50 cm above the floor level. At the beginning of the test, the rat was placed on the central platform facing an open arm. The test lasted 5 min. The parameters measured were as follows: total distance travelled (cm), the ratio of distance open arms vs. total (%) and time (s) spent in the open arms for a 5-min period.

Sucrose preference test (*SPT*). Anhedonia, considered a behavioural marker for depression, was estimated in single-housed rats as reported previously [8,9]. Preference for the sweet solution was calculated as a percentage of total sucrose consumed during a 24-h period. Each tested rat was placed in an individual cage and adapted to drink from two identical, graduated, and plastic bottles with tap water (100 ml) for a week. At the pretest performed for two days, water in one of the bottles was replaced by 1% sucrose. Taste preference was expressed as a percentage of the consumed volume of sucrose solution for a 24-h period.

Forced swimming test (*FST*). Depressive-like behaviour of rats measured as immobility time (s) was performed in an adapted version of the test, as was described earlier in a plastic container (height 60 cm, diameter 45 cm) filled to 30 cm with 21–24 °C water within a 5-min period [9].

Light dark test (*LDT*). The apparatus consisted of one open ($25 \times 50 \times 40$ cm) and one covered (dark) ($25 \times 25 \times 40$ cm) compartment, connected with a 7-cm door. The open part of the apparatus was illuminated by a bulb (80 lx) mounted over this area. At the start of the test, the rat was placed into the light compartment. The measured parameters were: latency crossing to light part of LD, crossing to light, total time spent in the lid compartment (s) and the number of explorations for 5 min.

Result and discussion. The influence of progesterone on the brain and on the behaviour of females is fairly well understood. However, less is known about the effect of Pro in males. Therefore, we examined the effects of Pro on sex-dependent changes in emotional status in male and female offspring, prenatally treated with the hormone.

The effect of prenatal treatment with Pro at a dose of 50 mg/kg on motor activity and anxiety behaviour in the Open field test is demonstrated in Fig. 1 and 2. Two-way ANOVA revealed a main Treatment effect [$F_{1,40} = 10.907$, $p = 0.002$] for the total distance travelled. Post-hoc analysis showed that prenatal treatment with Pro significantly reduced locomotion both in male and female offspring ($p = 0.05$), respectively, compared to the matched controls) (Fig. 1A).

The distance travelled in the centre vs. the total distance in the OF apparatus assessed the anxiety level.

Two-way ANOVA showed a main Treatment effect [$F_{1,37} = 30.318$, $p < 0.001$]. Exposure to prenatal treatment with Pro increased the level of anxiety, which was demonstrated by a shorter time spent in central zone vs. total (male: $p < 0.001$, female: $p < 0.001$ compared to C-veh group male and female rats, respectively) (Fig. 1B).

Prenatal treatment with Pro [$F_{1,49} = 62.349$, $p < 0.001$], significantly affected the vertical activity (number of rearing). These results suggest that prenatal progesterone treatment decreased both horizontal and vertical activity in male and female groups compared with controls ($p < 0.001$) (Fig. 1C).

For the control group, distance and time spent in open arms in EPM strongly correlated with the percentage of time spent in the inner parts of the OF. Anxiety-like behaviour was operationally defined as decreased time spent in the open arms relative to controls. Like in the OF, the prenatal Pro treatment induced an anxious-like response with a diminished preference to stay in the aversive open arms of the apparatus. Male and female rats, treated with Pro 50 showed lower locomotion than controls, similar to the OF test. Two-way ANOVA showed a main Treatment effect for: total distance [$F_{1,33} = 10.506$, $p = 0.003$], distance open vs. total [$F_{1,31} = 29.991$, $p < 0.001$] and for time open arms vs. total [$F_{1,34} = 14.940$, $p < 0.001$].

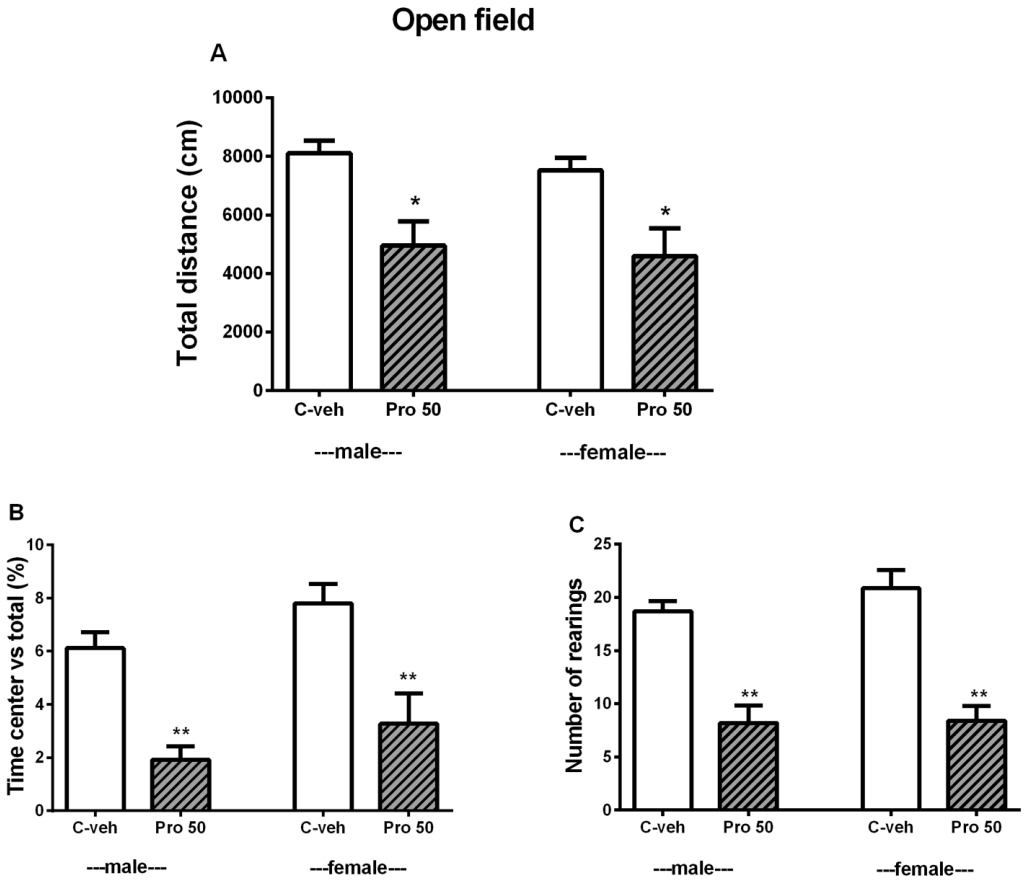


Fig. 1. Effect of prenatal progesterone (Pro) treatment in Open field test on total distance travelled (A), time centre vs. total (B) and a number of rearing (C) ($n = 8$). Data are presented as means ± SEM, $^{*}p < 0.05$, $^{**}p \leq 0.005$ vs. control of the same sex

In the literature, there is little data on the effect of prenatal Pro treatment on emotional development and depression in the offspring. Stress conditions induce the conversion of Pro or pregnenolone to cortisol. Effects on the developing embryo and fetus due to maternal exposure to Pro during pregnancy are also a concern in human medicine [10]. This steroid and lipophilic hormone readily cross the blood-brain barrier accumulating in a number of brain structures at concentrations higher than those measured in the serum of female rats [11].

Depressive-like behaviour of rats was investigated with a SPT and FST. Anhedonia developed as a sequence of prenatal treatment with Pro 50 was determined using the preference to drink sweet solutions. Two-way ANOVA showed a main Treatment effect [$F_{1,38} = 23.232$, $p < 0.001$]. Furthermore, the post-hoc test confirmed that both male and female offspring with prenatal treatment with Pro 50 showed a decreased preference for the consumption of sucrose (Fig. 3A).The

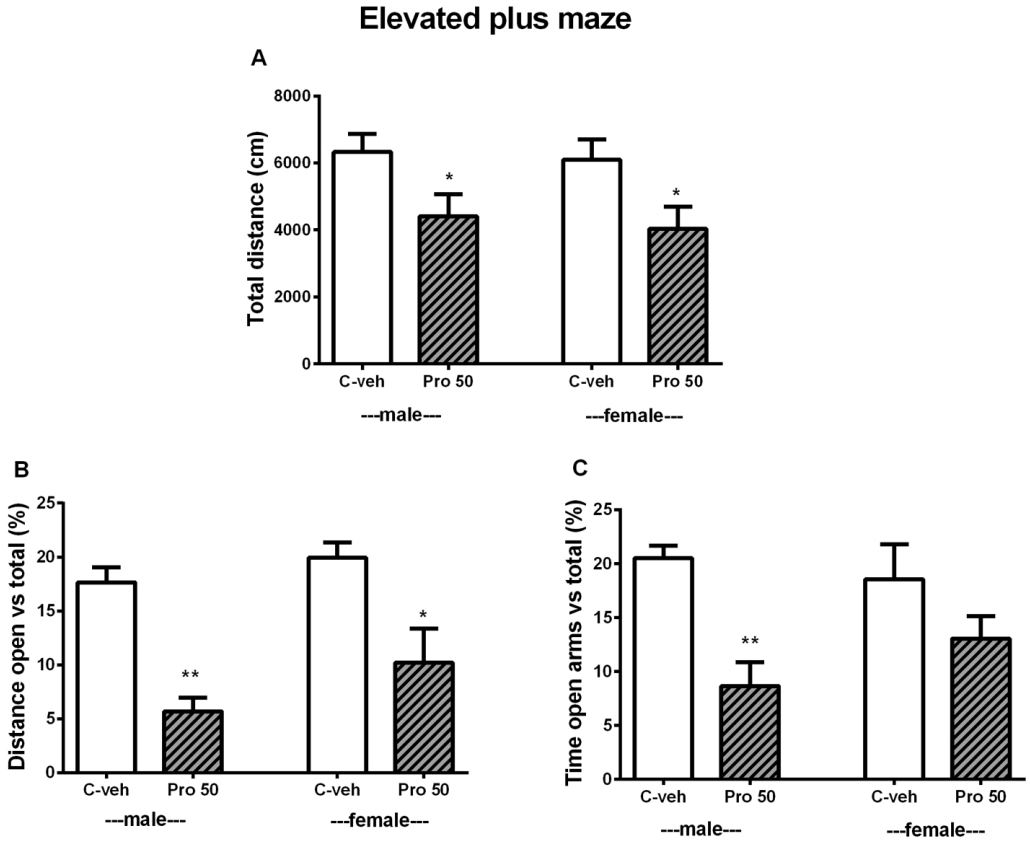


Fig. 2. Effects of prenatal progesterone treatment: on total distance (cm) (A), ratio of distance open vs. total (B) and the ratio time spent in the open arms vs. total (C) ($n = 8$). Data are presented as mean ± SEM, $^{*}p < 0.05$, $^{**}p \leq 0.005$ vs. control of the same sex

despair-like response was evaluated by measuring the immobility time in the FST. Two-way ANOVA showed a main Treatment effect [$F_{1,35} = 27.003$, $p < 0.001$]. Like in the SPT, the exposure to Pro 50 induced increased immobility in the two sexes and showed depressive-like behaviour ($p < 0.001$). The amygdala, followed by the cerebellum, nucleus accumbens, and hypothalamus is the brain region with the highest progesterone concentration in females [12]. Studies in ovariectomized female rats have shown that Pro can alleviate their depressive behaviour or facilitate the action of antidepressants [13].

The light/dark test in rats screens medications for possible anxiolytic and anxiogenic effects. In rodents, Pro and its metabolites rise in response to factors that cause stress [14]. Anxiety-like behaviour test was defined as decreased time spent in the lid compartment relative to controls. Our results show that prenatal treatment with Pro in a dose of 50 mg/kg significantly affected emergence latency (Two-way ANOVA revealed a main Treatment effect [$F_{1,40} = 9.784$, $p = 0.003$]),

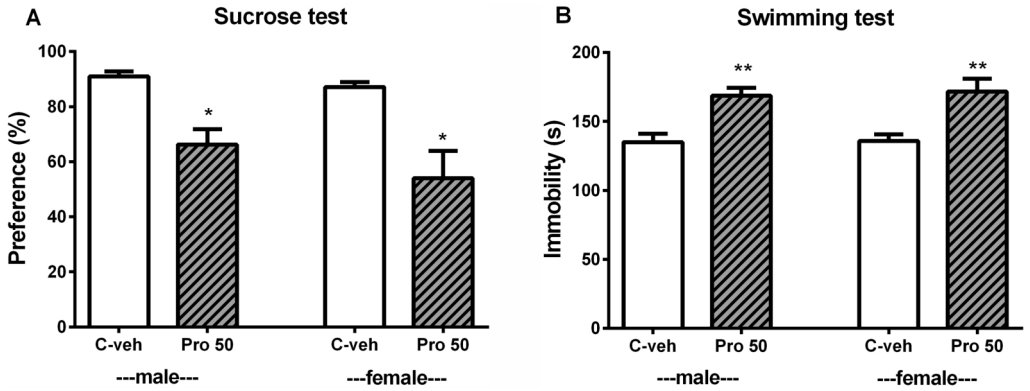


Fig. 3. Effect of prenatal progesterone treatment on (A) preference to sucrose solution (%) in the sucrose preference test, and (B) immobility time (s) in the forced swimming test ($n = 8$). Data are presented as mean ± SEM, $^{*}p < 0.05$, $^{**}p \leq 0.005$ vs. control of the same sex

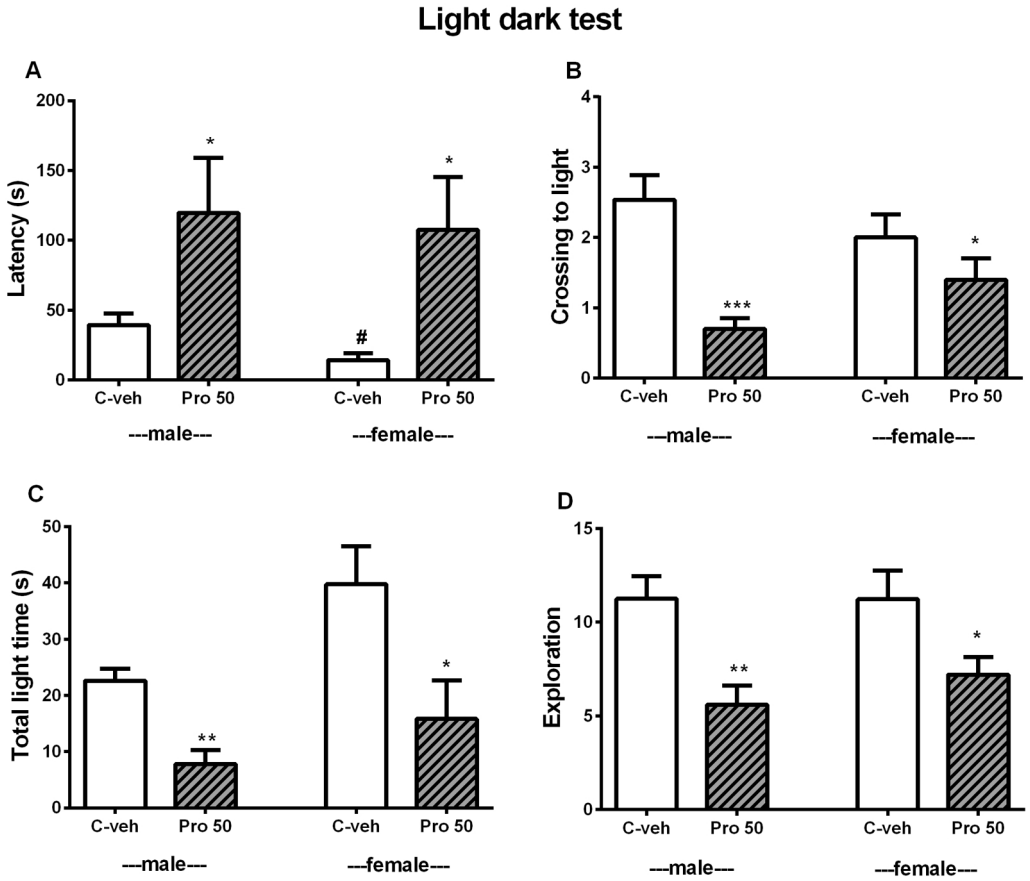


Fig. 4. Effect of prenatal progesterone treatment on (A) latency (s), (B) crossing to light, (C) total light time (s) and (D) exploration ($n = 8$). Data are presented as mean ± SEM, $^{*}p < 0.05$, $^{**}p \leq 0.005$ vs. control of the same sex, $^{\#}p < 0.005$ C-male vs. C-female

crossing to light part (Two-way ANOVA showed a main Treatment effect [$F_{1,40} = 15.320$, $p < 0.001$]), total light time (Two-way ANOVA showed a main Treatment effect [$F_{1,37} = 15.442$, $p < 0.001$]) and time spent in the lit compartment (Two-way ANOVA showed a main Treatment effect [$F_{1,40} = 16.501$, $p < 0.001$]). The post hoc test showed that all experimental groups (male and female) significantly decreased their latency first crossing to light, number of light crossings, time spent in lit compartment and exploration ($p < 0.005$) and exhibited more anxiety than control groups.

Conclusion. Our findings revealed that the male and female rats exposed prenatally to Pro produced elevated anxiety with concomitant low motor activity and depressive-like responses compared with control groups. The long-term detrimental impact on emotional behaviour in adult offspring of such hormonal treatment in pregnant rats suggests plastic changes in limbic brain structures associated with emotional responses, including amygdala and hippocampus. Although there are numerous clinical data revealing impaired functioning of the immune system, cardiovascular system, renal system, respiratory system as well as the reproductive system in male offspring, the experimental studies are few and the underlying mechanism related to the detrimental impact of prenatal Pro treatment on offspring is unclear. Therefore, the preliminary data of this study, supported by three tests for anxiety and two tests for depressive-like responses in rats give the background for further exploration of the signalling pathway involved in the effects of hormonal treatment during pregnancy, including the activity of the hypothalamus-pituitary-adrenal axis and changes in brain progesterone receptors.

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