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SEX-DEPENDENT DIFFERENCES OF EMOTIONAL STATUS IN A RAT MODEL OF PRENATAL STRESS

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Abstract

Untoward events during pregnancy negatively affect offspring mental development. We investigated sex-dependent differences of emotional problems in offspring with a history of prenatal stress (PNS). Female rats demonstrated decreased anxiety-like behaviour in comparison to male controls, shown in the light-dark test. Male and female offspring of prenatally stressed mothers were characterized by higher anxiety levels, compared to unstressed controls. PNS induced depression-like behaviour in both sexes without differences among them, indicated with decreased intake of sweet solution in the sucrose consumption tests and increased immobility time spent in the forced swim test. Control females showed higher plasma corticosteroid (CORT) concentrantions after acute stress and decreased recovery (120 min after the stressor) than control males. Both male and female PNS-offspring were with elevated levels of CORT in the plasma, which remained high 120 min after application of the stressogenic procedure. This study evidenced that the unfavourable effect of PNS on emotional state correlated with impaired feedback mechanism of the hypothalamic-pituitary-adrenal (HPA) axis function both in male and female offspring.

Key words: sex difference, anxiety, depression, rat, prenatal stress, corticosterone

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Introduction. While short-term increase of cortisol in the blood represents an adequate adaptive reaction against acute stress, sustained high levels of cortisol as a result of chronic stress are associated with multiple pathologies [1,2]. Stress exposure during pregnancy can cause serious disturbances in brain development with long-term consequences during the mature period [3–6]. Stress-induced high levels of cortisol during pregnancy predispose to late abnormal responses in offspring, including low birth weight, cardiovascular, metabolic, neuroendocrine and behavioural changes [7]. Moreover, offspring of stressed during pregnancy mothers are more vulnerable to pathological emotional responses and increased risk of attention deficit hyperactivity disorder, anxiety and depression [6]. The unstable emotional status of the future mother affects negatively the intrauterine development and predispose to severe depression, post-traumatic stress disorder and anxiety in the offspring [8,9]. There is a critical period during pregnancy to stress-induced pathology of offspring [9,10]. Clinical data suggest that the greater predisposition of females than males to psychiatric disorders, including anxiety and depression, correlates positively with the fluctuations in gonadal steroids and the hypothalamic-pituitary-adrenal (HPA) axis regulation across the menstrual cycle [11]. Experimental studies also support the assumption that exposure to the critical maternal and paternal stress periods might affect various emotional responses and regulatory mechanisms differently in male and female offspring [12]. Previous reports suggest that the impaired emotional responses of offspring positively correlate with disbalance in the HPA axis in a rat model of prenatal stress (PNS) [13,14]. In the present study, sex-dependent divergence in the development of impaired emotional responses in a mild prenatal stress rat model was explored.

Materials and methods. ***Subjects.*** Adult nulliparous Sprague Dawley rats (250 g) were group-housed at 5 per cage for estrous synchronization at 12-hour light/dark cycle with lights on at 08:00 hours, 21±1 °C temperature and 50–60% humidity with food and water ad libitum. Seven days later, female rats were individually housed with a male breeder for one night. Afterward, pregnant rats were allocated to PNS or undisturbed controls ($n = 8$/group) on a random basis.

Prenatal stress procedure. Every day between gestation days 7 and 21 (delivery) 1 daily and 1 overnight stressor were applied on pregnant rats as per the protocol of Sickmann et al. [15] with certain modifications. After weaning (22 days of birth), offspring of the same sex and mother were housed in pairs in one cage. Behavioural experiments began when the offspring reached sexual maturity (60 days) on control (C) or prenatally stressed (PNS) males and females (n=8 in each group). All experiments were performed in accordance with the European Communities Council Directives of 24 November 1986 (86/609/EEC).

Light-dark test. The apparatus consisted of two compartments: 2/3 light and open and 1/3 covered and dark area, connected with a 7 cm door. At the beginning of the 5 min test rats were placed into the light and illuminated com-

partment. The calculated standard measures were: (1) total time spent in the light area (s), which reflects anxiety-like behaviour; (2) the number of crossings to light and (3) the number of pokes in the light area, which reflect activity-exploration.

Sucrose preference test. Anhedonia response, considered a behavioural marker for depression, was explored with this test described in our previous study [16]. Preference for sweet solution was calculated as a percentage of total sucrose consumed during a 24-h period.

Forced swim test. This test was used for the study of depressive-like behaviour. Each rat was placed in a cylindrical container (height 60 cm, diameter 45 cm) and 25 °C water was poured up to 30 cm above the bottom, as described previously (Tchekalarova et al., [16]). The immobility time (s) (movements to keep the head above the water) for 5 min was recorded, indicating despair-like behaviour.

Measurement of corticosterone (CORT) levels. For testing of the HPA axis responsivity, trunk blood samples for CORT concentration analysis, after decapitation of the rats, were collected at 3 time points: 1) base levels "0" – the day before exposure to stressor – FST; 2) 10 min after being exposed to FST and 3) 2 h after the FST, and were centrifuged (10 min, 4 °C) at 4000 rpm. The plasma CORT (pg/ml) was measured by Elisa test kit (Enzo, Switzerland) following the instructions of the manufacturer.

Statistical analysis. Data were shown as mean ± SEM. Behavioural results were analysed using a two-way ANOVA and CORT levels by a three-way ANOVA, followed by a Bonferroni post hoc test. Statistically significant differences were accepted at $p \leq 0.05$.

Results and discussion. In the light-dark test, two-way ANOVA indicated 1) a main effect of Sex ($F_{1,33} = 3.2$, $p < 0.05$) and effect of PNS ($F_{1,33} = 19.2$, $p < 0.001$) on total time spent in the light area; 2) a significant effect of Sex ($F_{1,39} = 4.13$, $p < 0.04$) and effect of PNS ($F_{1,39} = 9.9$, $p < 0.003$) on the number of crossings to light, and (3), a significant effect of PNS ($F_{1,64} = 20.1$, $p < 0.001$) on the exploration activity. Sex differences were detected in the light-dark test, as female offspring were less anxious than males ($p < 0.05$) (Fig. 1A, B). Other researchers also showed that males have a higher anxiety level than females [15], while Zuena et al. [17] revealed the opposite. On the contrary, Van den Hove et al. [18] did not find any differences between males and females of the same strain in the elevated plus-maze test. The results might be associated with the impact of the female estrous cycle on this behaviour, as well as differences in the methods and the test procedure. Despite the fact that the level of anxiety did not differ between male and female offspring with a history of PNS, it was elevated significantly compared to that of control offspring ($p < 0.05$) (Fig. 1A, B). Moreover, these data were confirmed by a decreased exploration, as a signal of PNS-induced enhanced fear ($p < 0.05$) (Fig. 1C). While some authors reported PNS impact in a sex-dependent manner [17, 18], others showed no PNS influence

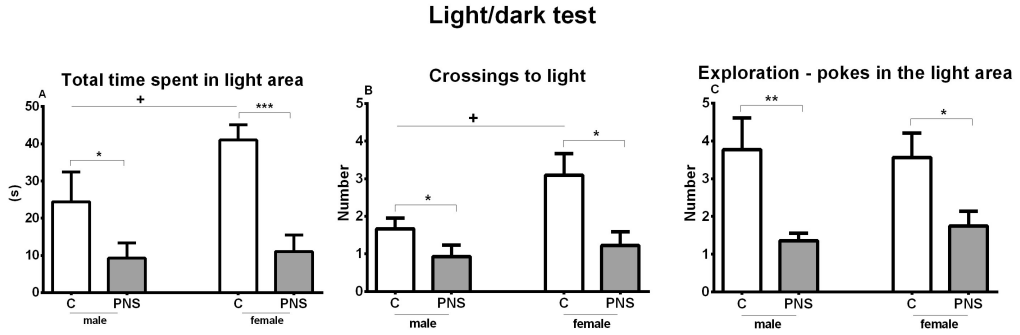

Fig. 1. The effect of prenatal stress (PNS) in male and female rats in the light-dark test: A) total time spent in the light area (s); B) the number of crossings to light; C) the number of pokes in the light area. Data are presented as means ± S.E.M. $^{*}p \leq 0.05$, $^{**}p \leq 0.005$, $^{***}p \leq 0.0005$; $^{+}p \leq 0.05$ vs. male

on anxiety [15]. The discrepancies among studies might be due to different types of stressor applied or age of the animals at the time of testing. The results suggest that the impact of sex on anxiety, as well as interactions between sex and PNS need to be further investigated [17].

Two-way ANOVA revealed a significant effect of PNS ($F_{1,66} = 34.6$, $p < 0.001$) on depression behaviour in the sucrose preference test. PNS significantly reduced the intake of the sucrose solution in the offspring of both sexes ($p = 0.04$) (Fig. 2A). These data were confirmed in the FST with a significant effect of PNS ($F_{1,47} = 112.275$, $p < 0.001$) shown by two-way ANOVA on immobility time spent with no interactions between sex and PNS factors. The time animals spent immobile was significantly increased in male and female PNS rats ($p = 0.05$)

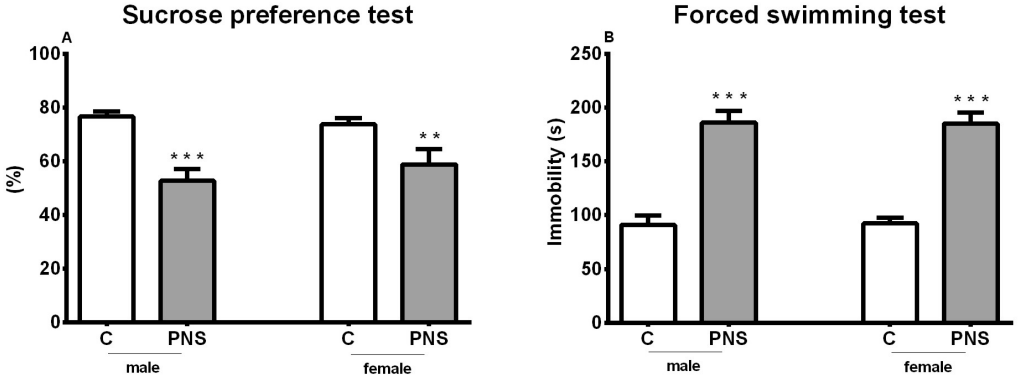

Fig. 2. The effect of PNS in male and female rats on: A) preference to sucrose solution (%) in the suscrose preference test; B) immobility time (s) in the forced swimming test. Data are presented as means ± S.E.M. $^{*}p \leq 0.05$, $^{**}p \leq 0.005$, $^{***}p \leq 0.0005$ vs. control of the same sex; $^{+}p \leq 0.05$ vs. male

(Fig. 2B). We found no differences between male and female control and PNS offspring in the immobility time spent in the FST and in the sucrose consumption. Concerning the PNS effect on both sexes literature data are controversial with papers, demonstrating depression only in males [18], and others, only in female rats [15]. However, the PNS-induced depressive and despair-like behaviours in both sexes determined in our study are in line with other investigations [19], confirming that maternal mental health is crucial for the development of emotional behaviour later in life.

A main effect of PNS ($F_{1,62} = 5.26$, $p < 0.025$) was revealed by three-way ANOVA on CORT levels, as well as effect of Time ($F_{1,62} = 7.28$, $p < 0.001$) and PNS x Time interaction ($F_{1,62} = 4.9$, $p < 0.010$). The levels of CORT at basal and stress conditions are shown in Fig 3. There were no sex differences in basal CORT levels of male and female control offspring ($p > 0.05$), which were not affaected by PNS in either sex. Furthermore, intact females were more sensitive to an acute stressor, showing higher plasma CORT concentration and decreased recovery (120 min after the stress application) than intact males ($p < 0.05$). These data suggest that females are more vulnerable to stress [11, 12]. Both male and female PNS-offspring were with elevated levels of CORT in the plasma, which remained high following 120 min after application of the stressed procedure ($p < 0.05$), suggesting an impaired HPA axis activity. The results of our study propose that the offspring of both sexes of prenatally stressed mothers are very susceptible to acute stress. Our results are in agreement with other literature data [15] and although they differ from those of other researchers [18], there is a consensus among authors that PNS induces disturbed inhibitory feedback regulation of the HPA axis in male and female rats.

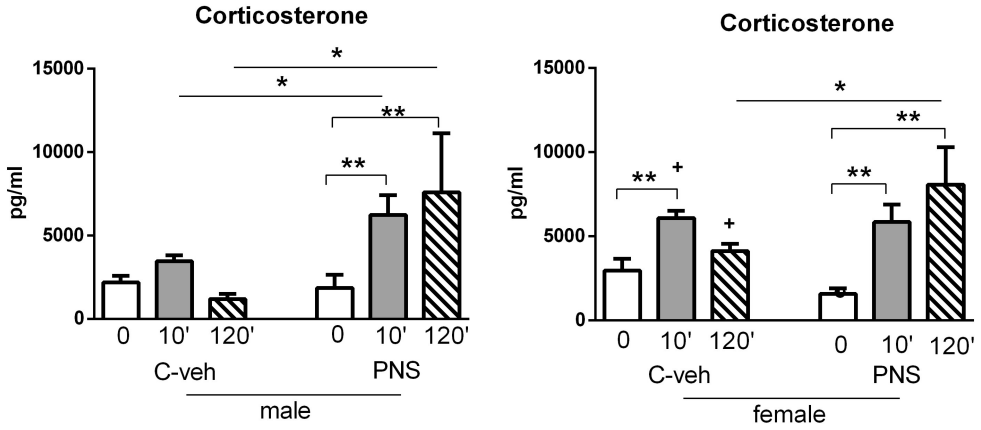

Fig. 3. The effect of PNS on plasma CORT levels at 3 time points: 1) base levels "0" – the day before exposure to sressor – FST; 2) 10 min after being exposed to FST; 3) 2 h after the FST in A) Male rats and B) Female rats. Data are presented as means ± S.E.M. $^{*}p \leq 0.05$, $^{**}p \leq 0.005$, $^{***}p \leq 0.0005$; $^{+}p \leq 0.05$ vs. male

Conclusion. Our data evidenced that PNS can affect the emotional status of both male and female offspring, illustrating a strong relationship between parental mental health and neurobiological physiological and behavioural problems among male and female offspring. An important factor of the abovementioned is the altered activity of the HPA axis following PNS.

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