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Influence of maternal testosterone on the strategies in the open field behaviour of rats

Lucia Kršková & Alžbeta Talarovičová

Comenius University, Faculty of Natural Science, Department of Animal Physiology and Ethology, Bratislava, SLOVAK REPUBLIC.

Correspondence to: RNDr. Lucia Kršková, PhD.
Comenius University, Faculty of Natural Science,
Department of Animal Physiology and Ethology
Mlynská dolina B-2
842 15 Bratislava, SLOVAK REPUBLIC
TEL: 00421/02/602 96 680: EMAIL: krskova@fns.uniba.sk

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ORIGINAL ARTICLE

Abstract

OBJECTIVES: The purpose of the present study was to characterize the influence of testosterone administered to pregnant females on offsprings postnatal behavioral strategies in the open field.

METHODS: The influence of maternal testosterone on behaviour of 23 day old male and female offsprings was studied in a 20-minute open field test. A total of 9 behavioural events were compared between a control (male n=12, female n=8) and a testosterone group (male n=9, female n=9). Dynamics and patterns of association of these behavioural events were analyzed. The testosterone group was prenatally exposed to testosterone (a single intramuscular injection of 2.5 mg testosteroni isobutyras on gestation day 14).

RESULTS: Male offsprings exposed prenatally to testosterone displayed significantly high levels of ambulation ($P<0.05$), sniffing ($P<0.01$), sniffing the air ($P<0.05$), urination ($P<0.05$) and significantly lower level of vocalization ($P<0.05$) than control. Female offsprings exposed prenatally to testosterone displayed significantly higher level of sniffing the air ($P<0.05$) than control. Significant differences in the dynamics of habituation process were registered only in the group of male. They were from 5 to 10 min in ambulation ($P<0.05$), from 10 to 15 min in sniffing ($P<0.05$) and vocalization ($P<0.05$), from 10 to 15 min and from 15 to 20 min in sniffing the air ($P<0.05$) and from 0 to 5 min in defecation ($P<0.05$) and urination ($P<0.01$).

CONCLUSION: Our results suggest that maternal testosterone may influence especially male postnatal open field strategies.

Introduction

Prenatal exposure to androgen can influence the development of brain organization [12, 4]. Enhanced testosterone level exposure during the prenatal age modulates functional cerebral lateralization [3], affects gender role behaviours [4], cognitive abilities into adulthood [13, 2], reproductive behaviour [20], pup-oriented behaviour [11], some aspects of maternal behaviour [14], agonistic behaviour [19], aggression [21, 22], play [18], maze learning [10].

The open field behaviour tests have been used on measurement of emotionality and exploration activity in animals. Large numbers of excrements and little activity of rat in an unknown environment indicate a fearful individual [5, 6]. Higher levels of activity usually suggest lower levels of anxiety [1], reduced fear in the animals and active environment exploration [17]. Decrement of activity – the process of habituation as a result of repetitive stimulation [9] is important for cognitive development [16], organization of behaviour and showing also a stage of the CNS development.

The purpose of this study was to specify the effect of prenatal fetal exposure to higher level of maternal testosterone on the males and females strategies in the open field behaviour.

Material and methods

Tested premature rats consisted of progeny of four female Wistar rats mated repeatedly with the same males. This approach enabled reducing genetic variability in studied parameters and to test predominantly epigenetic effects of administered testosterone.

Progeny of the first mating (control – C) consisted of 20 offsprings (12 males and 8 females). They were tested at age 23 days and weighed 55.62 ± 1.78 g at this age.

Females were subsequently mated with the same males as in the first trial and on gestation day 14 pregnant females received a single intramuscular injection of 2.5 mg of testosteroni isobutyras in 0.1 ml of microcrystalline aqua suspension (Agovirin Depot, Biotika, Slovak Republic). Offsprings (testostosterone – T, 9 males and 9 females) underwent essentially the same test procedures as offsprings of the first mating and weighed 51.29 ± 2.17 g at day 23.

On the first day after birth the litters were adjusted to seven pups per nest. They were housed with their mothers in a plastic cage (57×37×19 cm) with wood shavings. Temperature in an animal room was kept at 21±2°C and 12h:12h light/dark cycle (lights on 07.00h–19.00h) was used. Rats had a free access to water and commercial food pellets through the study. Young rats were weaned at day 23 of their age (after observation). The methods and procedures of the present study have been approved by the local Ethics Committee.

Each young rat was individually tested for 20 minutes in the open field experiment. The experiments were performed in the testing chamber (72×34×39 cm). The bottom was demarcated into 32 squares (8x4 configuration) of equal dimensions by painted dark lines. The rats were tested during the light phase of the day (14.00h–17.00h). The data from the open field test were registered in protocols by shorthand.

Totally 9 behavioural events were recorded during observation in the experimental groups of C and T males and group of C and T females tested using the

TABLE 1. Behavioural traits of male offspring of female rats nontreated (C) and treated (T) on day 14 of pregnancy by testosterone (2.5 mg/animal).

Events	Control (n=12)	Testosterone (n=9)
Ambulation (number of squares visited/20 min)	340.8 ± 22.1	452.3 ±35.5*
Rearing (occurrence frequency/20 min)	78.2 ± 6.7	77.1 ± 6.7
Creeping (number of time intervals/20 min)	2.0 ± 0.4	2.7 ± 0.8
Sniffing (number of time intervals/20 min)	17.9 ± 0.3	19.4 ±0.2**
Sniffing the air (number of time intervals/20 min)	16.3 ± 0.8	19.0 ± 0.5*
Self grooming (duration in seconds)	160.2 ± 17.2	142.8 ± 15.8
Defecation (occurrence frequency/20 min)	3.3 ± 0.8	4.9 ± 0.4
Urination (occurrence frequency/20 min)	1.3 ± 0.3	2.9 ± 0.5*
Vocalization (occurrence frequency/20 min)	5.8 ± 2.2	1.7 ± 1.7*

Data are given as means per 20 min ± S.E.M. Asterisk indicates significant differences between control and testosterone group (*P<0.05, ** P<0.01).

TABLE 2. Behavioural traits of female offspring of female rats nontreated (C) and treated (T) on day 14 of pregnancy by testosterone (2.5 mg/animal).

Events	Control (n=8)	Testosterone (n=9)
Ambulation (number of squares visited/20 min)	356.1 ± 45.3	351.7 ± 45.7
Rearing (occurrence frequency/20 min)	61.9 ± 7.5	81.6 ± 18.9
Creeping (number of time intervals/20 min)	1.5 ± 0.5	2.6 ± 1.1
Sniffing (number of time intervals/20 min)	17.8 ± 0.4	17.7 ± 0.5
Sniffing the air (number of time intervals/20 min)	18.4 ± 0.5	19.6 ± 0.3*
Self grooming (duration in seconds)	136.4 ± 9.8	133.2 ± 8.9
Defecation (occurrence frequency/20 min)	3.6 ± 0.7	4.2 ± 0.6
Urination (occurrence frequency/20 min)	0.9 ± 0.2	1.9 ± 0.5
Vocalization (occurrence frequency/20 min)	0.9 ± 0.6	1.7 ± 0.9

Data are given as means per 20 min ± S.E.M. Asterisk indicates significant differences between control and testosterone group (*P<0.05, ** P<0.01).

Table 3. Differences between control and testosterone male group for the behavioural events in 5 min intervals.

Events	Time interval (min)	Control (n=12)	Testosterone (n=9)
Ambulation	0–5	127.8 ± 12.0	156.9 ± 13.9
	5–10	93.2 ± 9.7	128.4 ± 11.3*
	10–15	66.4 ± 8.8	80.7 ± 14.6
	15–20	53.3 ± 7.1	86.3 ± 14.0
Sniffing	0–5	4.9 ± 0.1	5.0 ± 0.0
	5–10	4.7 ± 0.1	4.9 ± 0.1
	10–15	4.3 ± 0.2	5.0 ± 0.0*
	15–20	4.0 ± 0.3	4.6 ± 0.2
Sniffing the air	0–5	4.3 ± 0.2	4.9 ± 0.1
	5–10	4.1 ± 0.3	4.4 ± 0.3
	10–15	3.8 ± 0.3	4.8 ± 0.2*
	15–20	4.1 ± 0.3	4.9 ± 0.1*
Defecation	0–5	1.3 ± 0.4	3.6 ± 0.4*
	5–10	1.6 ± 0.4	1.2 ± 0.4
	10–15	0.0 ± 0.0	0.0 ± 0.0
	15–20	0.4 ± 0.3	0.1 ± 0.1
Urination	0–5	0.1 ± 0.1	1.0 ± 0.3**
	5–10	0.4 ± 0.2	0.7 ± 0.2
	10–15	0.3 ± 0.1	0.7 ± 0.3
	15–20	0.5 ± 0.3	0.6 ± 0.2
Vocalization	0–5	0.7 ± 0.3	1.1 ± 1.1
	5–10	2.2 ± 1.3	0.4 ± 0.4
	10–15	1.3 ± 0.6	0.0 ± 0.0*
	15–20	1.7 ± 1.1	0.1 ± 0.1

Data are given as means ± S.E.M. Asterisk indicates significant differences between control and testosterone group (*P<0.05, ** P<0.01).

open field method: ambulation (animals move actively within the observation area), rearing (animals rear up on hind legs), self grooming (self-grooming is predominantly demonstrated as washing), defecation, urination (marking), creeping (slowly move, with the body near the ground), sniffing (walls and ground), sniffing the air (inhaling with the head up, whiskers moving), vocalizations (audible vocalizations, from soft peeps and squeaks to loud shrieks).

Ambulation was estimated on the basis of numbers of squares visited. Rearing, defecation, urination and vocalizations were expressed as frequencies. Creeping, sniffing and sniffing the air were expressed as presence (absence) in observed one minute time interval. The self-grooming was expressed as duration in seconds.

Process of habituation was estimated in four five minutes intervals.

The significance of difference between C and T group of males or females was estimated by the non-parametrical Mann-Whitney test.

Results

Control and testosterone treated animals showed different patterns of behaviour in the open field observation. Significant differences between C and T male offspring rats were found in the ambulation behaviour. T males visited more squares than C males (P<0.05). Male offspring of females exposed to T displayed significantly higher occurrence of sniffing (P<0.01), sniffing the air (P<0.05), frequency of urination (P<0.05) and significantly lower frequency of vocalization (P<0.05) than C (Table 1). Significant differences between C and T females were found in sniffing the air (P<0.05). Testosterone treated animals expressed significantly higher frequency of this activity (Table 2).

Control and testosterone treated males showed differences in the habituation process of ambulation. It was from 5 to 10 min (P<0.05, Table 3). Habituation process of sniffing and vocalization was significantly different from 10 to 15 min (P<0.05, Table 3). Habituation process of sniffing the air was significantly different from 10 to 15 min and from 15 to 20 min (P<0.05, Table 3). Habituation process of defecation and urination is significantly different from 0 to 5 min (P<0.05, P<0,01, Table 3). No significant differences were found in the process of habituation between C and T females.

Discussion

The differences in the occurrence and frequency of some behavioural traits suggested significant differences in the strategies of a new environment exploration used by C and T animals. T males demonstrated a higher ambulation (horizontal activity – frequent repeated movements from side to side) in the open field than C males. High ambulation scores are used as an indicator of reduced fear in the animals and an active environment exploration [17]. Higher frequency of sniffing, sniffing the air and low level of vocalization in the male T group may be another indicator of reduced fear. These finding is in accord with conclusions of Lambadjieva [15] that testosterone stimulates exploratory motivation of male rats. The frequency of male urine marking positively correlates with plasma testosterone level [7]. A higher frequency of urination was found in our T males and might be related to the prenatal exposure to higher maternal testosterone level.

The female offsprings of pregnant rats exposed to testosterone displayed a significantly higher level of sniffing the air. It is connected with more active exploration behaviour. We recorded significant differences in the dynamics of habituation process only in the males in ambulation, sniffing, sniffing the air, vocalization and defecation. These differences may reflect differences in cognitive development [1].

Our results may support the Baron-Cohen´s [2] extreme male brain theory of autism. According to this theory the male brain is defined psychometrically as a more cognitive, significantly better systemising than emphathising. Intrauterine testosterone level is responsible for cerebral asymmetry [8]. Androgens present before birth influence cognitive abilities, at least for certain spatial abilities [13]. Increased frequency of repeated movements from side to side, sniffing, sniffing the air, urination, decreased vocalization and differences in dynamics of habituation in testosterone affected male rats in our experiment resembles some aspects of autistic children behaviour (motoric stereotype behaviour, sniffing people and objects, problems with communication, frequent urination, differences in cognitive processes).

Our data indicates that manipulation of testosterone level in a specific developmental intrauterine period should be used for creation of animal model to study some behavioural aspects of autism.

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