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# DEVELOPMENTAL EXPRESSION OF SEXUAL DIFFERENCES IN OPEN-FIELD BEHAVIOUR AND PLASMA CHOLINESTERASE ACTIVITY IN MALE, FEMALE AND MASCULINIZED FEMALE RATS*

## *EXPRESSÃO COMPORTAMENTAL DAS DIFERENÇAS SEXUAIS NO CAMPO ABERTO E NA ATIVIDADE DA COLINESTERASE PLASMÁTICA DE RATOS MACHOS, FÊMEAS E FÊMEAS MASCULINIZADAS*

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**SUMMARY**

Sexual differences in behaviour and metabolism are well recognized. While some of these differences are related to testosterone exposure during neonatal life, others do not depend on the organizational action of androgens during early development. The objective of the following experiments was to study the development of sexual differences in plasma cholinesterase activity and to determine if these differences were related to testosterone exposure postnatally. Open-field activity was also recorded as a behavioral indicator of the actions of testosterone on sexual differentiation of the central nervous system. Three treatment groups of animals were used: normal male, normal female, and masculinized female rats (1 mg testosterone, SC, on day 2 of postnatal life). Open-field behaviour was measured on three consecutive days just after weaning (21-23 days of age), in association with the onset of puberty (30 - 36 days of age), or as adults (90 - 110 days of age); plasma cholinesterase activity was measured at 22, 30 - 36, and 90 - 110 days of age. As expected a sex difference in open-field activity was found between normal males and females. Postnatal androgen treatment in females decreased open-field activity in adulthood to levels similar to those found in normal males. Similar differences were observed just after weaning, but not at 30-36 days of age. In contrast, significant differences in cholinesterase activity were observed in adult animals, but not at days 22 and 30 - 33 of age. Masculinized female rats showed no differences in plasma cholinesterase activity when compared to normal female rats, both groups differing from males. These data suggest that sexual differences in plasma cholinesterase activity in adult rats, unlike differences in open-field behaviour, are not dependent on testosterone exposure during postnatal life. In addition, the results have shown that under stress (weaning) sex differences in open-field behaviour can be observed as early as at 21-23 days of age.

**UNITERMS**: Sex characteristic; Cholinesterase; Testosterone; Animal behaviour

## INTRODUCTION

Adult male rats are less active and defecate more than female rats when tested in the open field[1,11,14,17]. These differences persist even following gonadectomy [3,21], and are related to testosterone exposure during neonatal life[21]. At what age does this become apparent is less clear. The onset of these differences has been found to be present from the age of 30[24] to 50 - 60 days of age[3].

Peripheral sexual differences are also under early testosterone influence. Sex differences in acetylcholinesterase activity of skeletal muscles[22] and in plasma cholinesterase activity are well documented[8,20]. Plasma cholinesterase activity is higher in mature female rats than in males. Acetylcholinesterase activity is stimulated by estrogens, being high during physiological states related to high estrogen levels[20] and low after ovariectomy[8].

Brain cholinesterase activity can be altered by neonatal manipulations such as X-irradiation[16] and testosterone exposure[15]. In addition, sexual differences in the hypothalamic cholinergic system are neonatally modulated by testosterone[15].

Since most reports describe these sexual dimorphisms only in adult animals, the objective of the following experiments was to study the development of sexual differences in plasma cholinesterase activity using normal male and female rats and masculinized female rats during weaning, in association with the onset of puberty, and during adulthood, i.e., 21 - 23, 30 - 36 or 90 - 110 days of age. In addition, open-field activity was monitored in similarly treated animals as a behavioral measure of the effects of neonatal androgen exposure on this sexually dimorphic response.

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## MATERIAL AND METHOD

### Animals and Housing

All the experiments were made during 1989 (2nd semester), at the animal facilities and laboratories of the Departamento de Patologia, Faculdade de Medicina Veterinária, Universidade de São Paulo, Brasil.

A total of 242 Wistar rats from our colony were used. Lights in the animal room were on from 600 to 1800 h. Feed and water available "ad libitum" consumption. Room temperature ranged from 20 to 23°C. Experimental litters were culled to 8 - 10 pups per litter; each litter was adjusted to 4 male and 4 - 6 female pups. In half of the litters female pups were injected subcutaneously with 1 mg testosterone (Sigma) in 0.05 ml peanut oil on day 2 of postnatal life. The remaining pups received vehicle alone. All animals were weaned on day 21. All testosterone-treated female rats presented permanent vaginal estrous during adulthood and were also checked for cystic ovaries by the end of the experiment.

### Experimental Groups

Subjects were tested for general activity in an open-field apparatus at 21 - 23 days of age (16 males [M]; 12 females [F] & 12 masculinized females [MF]); 30 - 36 days of age (11M; 12 F & 12 MF); or as adults between days 90 - 110 (24 M; 9 F & 22 MF). Plasma cholinesterase activity was measured 24 h after weaning (i.e. 22 days old rats 11 M; 12 F & 12 MF) on 30 - 36 days of age ( 11 M; 12 F & 12 MF) and from 90 - 110 days (16 M; 9F & 17 MF).

### Activity Testing

General activity levels were measured in circular open-fields. The open-field arena used for adult animals was 80 cm in diameter and 30 cm in height, having three lamps of 50 W each, similar to that described by BROADHURST[4](1960). The open-field used for 21 - 23 and 30 - 36 days old rats was smaller (diameter: 40 cm, height: 40 cm)[4,9]. In both test situations hand-operated counters and stopwatches were employed to score ambulation frequency (number of floor units entered) and rearing frequency (number of times the animal stood on hind-legs).

The open-field was washed with a 5% ethanol solution before each behavioral test to eliminate possible influences of odors left by previous subjects. To minimize possible circadian influences on open-field behavior, masculinized females, normal male, and normal female rats observations were intermixed. Observations were made between 700 and 1200 h, and animals were tested in the same order every day (3 min/day, 3 consecutive days).

### Cholinesterase Measurement

Plasma was obtained by collecting blood from the portal hepatic vein of ether anesthetized animals, using a heparinized syringe, and was centrifuged immediately (11,000 g for 10 min). Plasma cholinesterase activity was measured as decribed by ELLMAN et al.[7] (1961). Briefly, the enzyme activity is measured by following the increase of yellow color produced from thiocholine when it reacts with the dithiobisnitrobenzoate ion. The reaction rates were recorded with a Beckman spectrophotometer. Data was recorded as change in absorbance per minute ($\Delta$ A).

### Statistical Analyses

For all experiments, Friedman's two-way analysis of variance followed by Duncan's test was used to detect possible differences among the groups[23].

## RESULTS

### Open-field activity

*21 - 23 days old rats*

All behavioral parameters of the three different groups were compared. Results are outlined in Tab. 1. Both male and masculinized female rats presented a lower ambulation frequency 24 h after weaning (day 22; $p < 0.05$). Male rats presented lower rearing frequencies on days 22 and 23 ($p < 0.05$).

**TABLE 1**

Open-field behavior of 21 - 23 days old male, female and masculinized female rats. São Paulo - SP, 1989.

| Group | Parameters [a] | Day 21 [b] | Day 22 | Day 23 |
|---|---|---|---|---|
| Female (12) | AF | 31±7 [c] | 42± 7 | 35±6 |
| | RF | 11± 3 | 13±2 | 16±3 |
| Male (16) | AF | 41± 5 | 24±4 * | 33±6 |
| | RF | 11± 2 | 7±1* | 7 ±2* |
| Masculinized Female (12) | AF | 35± 8 | 25±3 * | 30±4 |
| | RF | 8± 2 | 9±2 | 6±1 |

[a] Ambulation Frequency (AF) and Rearing Frequency (RF)
[b] The first test session was immediately after weaning and the other testing occurred 24 and 48 hours after weaning.
[c] Means (± SE).
Number in parenthesis are the N for each group.
* $p < 0.05$ compared to females.

*30 - 36 days old rats*

Tab. 2 shows the behavioral responses of the three groups for three consecutive days between 30 and 36 days of age. There were no differences between the groups on any day in any parameter observed.

**TABLE 2**

Open-field behaviour of 30 - 36 days old male, female and masculinized female rats. São Paulo - SP, 1989.

| Group | Parameters [a] | Day 1 [b] | Day 2 | Day 3 |
|---|---|---|---|---|
| Female (12) | AF | 61±7 [c] | 45± 8 | 41± 7 |
| | RF | 23±4 | 15±3 | 13±3 |
| Male (11) | AF | 58±0 | 43±5 | 29±5 |
| | RF | 23±3 | 13±2 | 9±2 |
| Masculinized | | | | |
| Female (12) | AF | 49±5 | 36±4 | 28±4 |
| | RF | 20±3 | 10±1 | 7±1 |

[a] Ambulation Frequency (AF) and Rearing Frequency (RF)
[b] The interval between sessions was 24 h
[c] Means (±SE)
Number in parenthesis are the N for each group.

*90 - 110 days old rats*

Open field behavior of adult rats tested on three consecutive days is shown on Tab. 3.

Compared to females, male rats displayed lower ambulation and rearing frequencies on days 2 and 3 ($p < 0.05$). Masculinized female rats displayed a lower ambulation frequency on days 2 and 3 ($p < 0.05$) plus a lower rearing frequency than normal females on day 3 ($p < 0.05$).

**TABLE 3**

Open -field behaviour of adult male, female and masculinized female. São Paulo - SP, 1989.

| Group | Parameters[a] | Day 1[b] | Day 2 | Day 3 |
|---|---|---|---|---|
| Female (9) | AF | 57±3 [c] | 83±6 | 78±5 |
| | RF | 27±3 | 23±3 | 24±3 |
| Male (24) | AF | 45±3 | 49±4* | 50±5* |
| | RF | 19±2 | 14±2* | 14±1* |
| Masculinized | | | | |
| Female (22) | AF | 48±3 | 54±4* | 44±4* |
| | RF | 22±2 | 18±2 | 15±2* |

[a] Ambulation Frequency (AF) and Rearing Frequency (RF).
[b] Animals were 90 - 110 days old. The interval between sessions was 24 h.
[c] Means (±SE).
Number in parenthesis are the N for each group.
* $p < 0.05$ compared to females.

**Comparisons of plasma cholinesterase activity of rats according to age**

The plasma cholinesterase activity of the weaning, prepubertal, and adult rats was measured in animals of the three treatment groups. As shown in Tab. 4, adult males exhibited lower cholinesterase activity than both adult females and adult masculinized females ($p < 0.01$). The enzyme activity increased significantly from days 30 - 36 to adulthood in normal and masculinized female rats ($p < 0.01$) . A decline in the cholinesterase activity was found in males between these ages in the same period ($p < 0.05$).

**TABLE 4**

Plasma cholisnesterase activity of male, female and masculinized female rats. São Paulo - SP, 1989.

| Group | Day 22 | Days 30 -36 | Days 90 - 110 |
|---|---|---|---|
| Female | 1.760±0.138 [a] | 1.717±0.066 | 5.250±0.209 |
| | (13) | (12) | (9) |
| Male | 1.502±0.078 | 1.691±0.081 | 1.436±0.054* |
| | (14) | (11) | (16) |
| Masculinized | | | |
| Female | 1.484±0.078 | 1.764±0.071 | 5.204±0.328 |
| | (17) | (12) | (17) |

[a] Means (±SE). Data are expressed as ΔA / min / ml.
Number in parenthesis are the N for each group.
* $p < 0.01$ compared to females.

## DISCUSSION

In the present experiment the hypotheses that the sexual dimorphism in plasma cholinesterase activity[8,21] is under the influence of testosterone during neonatal life was examined and the ontogeny of plasma cholinesterase activity was measured. The results suggest that neonatal exposure to testosterone does not influence the development of sexual dimorphism of enzyme activity, which becomes apparent only during adult life. The influence of postnatal testosterone treatment on the development of sexual differences in open-field behaviour was also observed. The behavioral results show sexual differences as early as 22 days of age.

Testosterone is known to exert an organizational influence on the developing central nervous system during restricted critical perinatal periods of neural differentiation at which time the brain is sufficiently plastic to respond permanently and irreversibly to this hormone. Studies in the rat indicate that the central nervous system is sensitive to the organizational action of physiological levels of this hormone until day 5 post-partum[2]. In the present experiments testosterone was injected on the second day of postnatal life to induce masculinization in female rats[2]. Female pups treated with testoster-

one in the present study, exhibited signs of masculinization (permanent vaginal estrous and cystic ovaries).

The influence of exposure to gonadal steroids during early development on various kinds of behaviour in adulthood, such as stereotypy[10], avoidance, taste aversion, maze learning, and open-field behavior[1,14,17] is well known[9,19,25]. In the present study open-field activity was recorded as a behavioral expression of the sexual differentiation of the central nervous system. The behavioral differences observed for adult animals in open-field testing, i.e., lower ambulation and rearing frequencies of male and masculinized female rats, confirm the data from other authors[6,13,18]. Previous studies on the ontogeny of these differences indicated that they become apparent at 30 days of age[24], where as other authors[3] described that these differences emerge at the age of 50 - 60 days. Our results showed that those differences can be observed as early as 21-23 days of age. In addition, the differences are similar to those observed for adult animals, i.e., males and masculinized females exhibited lower ambulation and rearing frequencies than female rats. The discrepancy between the results obtained here and by other authors[3,24] may be due to the testing situation. In the present experiments young animals were observed for open-field behavior immediately after weaning (1$^{st}$ session) and 24 h and 48 h after weaning (2$^{nd}$ and 3$^{rd}$ sessions respectively). On day 21 the animals stayed with their mother until just prior to testing. On day 22, 24 h after weaning, ambulatory (AF), an established sex-related behavioral parameter for adult rats, was significantly higher in normal females and lower in the other groups. Similar differences were not observed on the other days. On days 22, the male's and female's AFs were pushed in opposite directions, emphasizing the sexual differences among the groups. In addition, the AF of masculinized females on day 22 was almost the same as that exhibited by males that day, both being the lowest AF over the three test days. These findings raise the following questions: are the rats responding to the possible stress of weaning more intensely in the second test session than in the others? Does the stress of weaning facilitate the expression of sexually dimorphic behaviours? Future experiments should be done to examine these questions. It is possible that weaning stress facilitates the expression of sexual differences observed in rats on day 22. Weaning stress also facilitates the expression of other events such as dopaminergic supersensitivity induced by perinatal treatment with dopamine antagonists which is expressed by an increase in ambulation frequency in an open-field[11]. Sexual differences in open-field behavior could be influenced in a similar manner by the weaning process. The negative results obtained for 30 - 36 days old rats appear to support this hypothesis, since these animals were not under the potential stress of recent weaning.

Testosterone exposure neonatally can influence cholinesterase activity both in the periphery[22] and in the central nervous system[15], inducing functional and morphological changes[5,12]. Moreover, both acetyl-and butyrylcholinesterase activity can be alterated in rats by other types of neonatal manipulations[16]. In the present experiment the hypothesis that the sexual dimorphism in plasma cholinesterase activity[8,21] is under the influence of testosterone during neonatal life was examined and the ontogeny of plasma cholinesterase activity was measured. The results suggest that neonatal exposure to testosterone does not influence the development of sexual dimorphism of the enzyme activity, which becomes apparent only during adult life. The high level of enzymatic activity in both female and masculinized female rats plus the low level in cholinesterase activity in males during adulthood are in agreement with earlier studies showing that plasma cholinesterase activity is depressed by testosterone and stimulated by estrogen in adulthood[8,20]. The present results suggest that the sexual dimorphism observed for the enzyme activity is not related to the sexual differences in open-field behavior. In addition, the results suggest that exposure to testosterone postnatally, at the time and dose reported here, does not influence enzyme activity in intact animals, although having an activational action in adulthood. Further studies are necessary to elucidate the mechanisms underlining the steroid influence on plasma cholinesterase activity as well as its ontogeny.

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## RESUMO

Sabe-se que algumas diferenças sexuais no metabolismo e no comportamento estão relacionadas com o efeito neonatal da testosterona e outras não sofrem esse tipo de influência. O objetivo desses experimentos foi estudar o desenvolvimento das diferenças sexuais na atividade da colinesterase plasmática e de determinar se essas diferenças seriam relacionadas com a exposição pós-natal à testosterona em ratos. A atividade geral no campo aberto foi também verificada como um indicador comportamental das ações da testosterona na diferenciação sexual do sistema nervoso central. Foram usados três grupos de animais: machos normais, fêmeas normais e fêmeas masculinizadas (1 mg testosterona, SC, no 2º dia de vida pós-natal). A atividade geral no campo aberto foi medida durante três dias consecutivos logo após o desmame (21 - 23 dias de idade), durante o início da puberdade (30 - 36 dias de idade) e nos adultos (90 - 110 dias de idade); a atividade da colinesterase plasmática foi medida aos 22, 30 - 36 e 90 - 110 dias de idade. Como esperado, foi encontrada uma diferença sexual no campo aberto entre machos e fêmeas normais. O tratamento pós-natal com andrógeno nas fêmeas diminuiu a atividade no campo aberto na idade adulta a padrões similares àqueles observados para machos normais. Foram observadas diferenças similares logo após o desmame, mas não aos 22 e aos 30 - 36 dias de idade. Em contraste, foram observadas diferenças significantes na atividade da colinesterase de animais adultos mas não nos dias 22 e 30 - 33 de idade. Quando comparadas às fêmeas normais, as fêmeas masculinizadas não apresentaram diferenças na atividade da colinesterase plasmática, sendo que esses dois grupos foram diferentes dos machos. Esses resultados sugerem que as diferenças sexuais na atividade da colinesterase plasmática de ratos adultos não são dependentes de exposição a testosterona durante o início da vida pós-natal. Além disso os resultados demonstram que sob estresse (desmame) as diferenças sexuais no comportamento no campo aberto podem ser observadas já aos 21 - 23 dias de idade.

**UNITERMOS:** Características sexuais; Colinesterase; Testosterona; Comportamento animal

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