Pharmacology Biochemistry and Behavior, Vol. 60, No. 1, pp. 203–208, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00582-0

Perinatal Treatment with Picrotoxin Induces Sexual, Behavioral, and Neuroendocrine Changes in Male Rats

M. R. P. SILVA,* C. A. OLIVEIRA,† L. F. FELICIO,‡ A. G. NASELLO§ AND M. M. BERNARDI‡

*Departamento de Farmacologia do Instituto de Ciências Biomédicas, †Departamento de Reprodução
‡Departamento de Patologia da Faculdade de Medicina Veterinária e Zootecnia,
Universidade de São Paulo, 05508-900, §Departamento de Ciências Fisiológicas,
Faculdade de Ciências Médicas da Santa Casa de São Paulo, São Paulo, Brasil

Received 26 June 1997; Revised 1 September 1997; Accepted 7 October 1997

SILVA, M. R. P., C. A. OLIVEIRA, L. F. FELICIO, A. G. NASELLO AND M. M. BERNARDI. *Perinatal treatment with picrotoxin induces sexual, behavioral, and neuroendocrine changes in male rats*. PHARMACOL BIOCHEM BEHAV **60**(1) 203–208, 1998.—The effects of maternal exposure to picrotoxin (PT) during the prenatal and postnatal periods of sexual brain differentiation were studied. Behavioral (sexual behavior), physical (sexual maturation, body, and organ weights) and neurochemical (striatal and hypothalamic monoamine and respective metabolite levels) data were assessed in the offspring of PT-treated dams. The following results were obtained: 1) sexual maturation as measured by the day of testis descent and testis weight comparison was unchanged; 2) a decrease in male sexual behavior occurred, as well as a decrease in body, ductus deferens, and seminal vesicle weights and in plasma testosterone levels of adult male offspring; 3) striatal dopamine (DA) and homovanillic acid (HVA) levels were decreased and hypothalamic norepinephrine (NE) levels were increased. These results indicate that perinatal exposure to PT during the critical periods of male brain sexual differentiation has long-term effects on the reproductive physiology and behavior of male rats. © 1998 Elsevier Science Inc.

Perinatal stress GABA Dopamine Norepinephrine Sexual differentiation
Sexual behavior Testosterone

STRESS is part of normal life, so that, to a certain extent, some stressful situations such as physical exercise, various emotional states, and creative activity are usually considered healthy. However, prolonged and undesirable stress may induce noxious effects. It is now established that maternal stress has a demasculinizing effect on male sexual behavior in rats. Previous reports (3,50,52,53) have shown that stress during the last third of pregnancy severely reduces normal copulatory behavior in the adult male offspring of rats. In humans, maternal stress is thought to lead to increased occurrence of homosexuality in boys (18) and girls (7). However, recent studies have shown that changes in sex behavior induced by maternal stress are linked to the nature of the stressor (50).

Ward and Weisz (54) have hypothesized that prenatal stress disrupts the normal maternal hormonal milieu, resulting in the suppression of a surge of fetal testosterone on days 18 and 19 of gestation, which is necessary for later expression and maintenance of male sexual behavior. These authors have applied physical stress, a heat-light restraint stress, to induce effects on reproductive parameters. Studies concerning pharmacological stressors or perinatal stress-induced neurotransmitter as well as hormonal changes are new in the literature (31,34,37,51). Gamma aminobutyric acid receptor ($GABA_A$) antagonist induces stress. File and Lister (22) showed that subconvulsive doses of PT, a $GABA_A$ receptor antagonist, also induced several behavioral signs of anxiety correlated with increased corticosterone levels. Moreover, injections of $GABA_A$-receptor antagonists such as PT and bicuculline into the dorsomedial hypothalamus of rats elicit a constellation of physiological responses, including increased heart rate and plasma catecholamine levels, as well as behavioral responses such as increased locomotion activity and anxiogenic-like ef-

Requests for reprints should be addressed to Maria Martha Bernardi, Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Orlando M. Paiva, 87, São Paulo, SP, 05508-900, Brasil.

fects, measured in conflict, elevated plus-maze, and social interaction tests (28,43,44).

In a recent article (45) we reported that perinatal exposure to a subconvulsive dose of PT interferes with the sexually dimorphic behavior of male rats, measured in open-field and social interaction tests. GABAergic stimulation or inhibition also induces sex-related changes in adult offspring (21). Prenatal diazepam and phenobarbital treatments decrease brain and plasma testosterone levels during the fetal, neonatal, and adult periods (23) and impair copulatory activity in adult male offspring (8,27). The GABAergic system has also been reported to influence the development of the sexually dimorphic nucleus of the preoptic area (SDN-POA). Male rats perinatally treated with muscimol, a $GABA_A$ receptor-stimulating drug, showed significantly smaller SDN-POA volumes compared to controls (6). Fernandez-Guasti et al. (21) showed that GABAergic neurotransmission is involved in inhibitory processes underlying the masculine sexual behavior.

The present study was designed to examine if maternal exposure to PT during the period of sexual brain differentiation in the offspring can affect the sexual and neuroendocrine parameters of male pups during adult life. Behavioral (sexual behavior), physical (sexual maturation, body and organ weights), and neurochemical (striatal and hypothalamic levels of monoamines and their metabolites) data were assessed in the male offspring of PT-treated dams. The dose of PT selected was a subconvulsive one that increased plasma corticosterone levels but did not induce myoclonic jerks in the rat.

METHOD

Animals

Male and female Wistar rats from our own colony, weighing 250–270 g and about 90 days of age were used. The animals were housed in polypropylene cages (32 × 40 × 18 cm) under controlled temperature (22–24°C), with a 12 L:12 D light schedule and free access to food and water. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

Statistical Analysis

The Student *t*-test was used when data were parametric, i.e., when Bartlett's test (28,30) showed the existence of homogeneity among data. For nonparametric data, the Mann–Whitney *U*-test was employed. In all cases results were considered significant for $p < 0.05$.

Radioimmunoassay

Testosterone concentration was measured in plasma samples using Coat-a-count kits (Diagnostic Products, LA, CA). Serum samples were assayed in duplicate and sensitivity to testosterone was 0.01 ng/ml. The intraassay and interassay variation was 0.4 and 4.5%, respectively.

Offspring Studies

Twenty sexually naive female rats were mated with males previously tested as fertile (two females and one male in each cage). The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears (day 0 of pregnancy). When pregnancy was confirmed, the female was immediately housed in an individual cage. The dams received PT (0.75 mg/kg) and saline (1 ml/kg) once on day 18th of pregnancy, immediately after parturition, i.e., during the first 10 min after delivery and before the dam started nursing, and once a day during the first 5 days of lactation. Because in rat the critical period for the organizational actions of gonadal hormones on sexual differentiation of the brain extends from approximately the last week of prenatal life through the first postnatal week (14,27), the rationale for choosing this schedule of drug administration was to expose mothers and pups to this unique stressor during this period of time. After delivery, eight pups (four males and four females) were left with each dam until weaning (21 days of age). No crossfostering was done. On postnatal day 1 all litters were examined externally, sexed, and weighed. After the lactation period, male animals were evaluated once a day for testis descent. The pups were also weighed at 1, 10, 21, and 60 days of age. All tests were carried out at the same time of day (0900–1100 h). On day 21 of lactation the offspring were weaned and the littermates separated and housed by sex.

Testis, Seminal Vesicle, and Ductus Deferens Weights

Experimental and control adult animals (120 days old) from dams treated as described above had their testes, seminal vesicles, and ductus deferens removed, separated from surrounding tissue and cleaned free of secretions. The organs were dried between two sheets of filter paper and their wet weight was determined. The organ weight/body weight ratio was calculated. The testes were analyzed for histopathological alterations.

Sexual Behavior Studies

Rats ages 120 days were used for the mating tests as described by Felicio et al. and Chiavegatto et al. (10,19). Briefly, animals were maintained under controlled-temperature conditions on a 12-h inverted light–dark cycle (lights on at 2200 h, for at least 21 days before the experiments). To investigate sexual behavior, male rats were allowed to mount ovariectomized females sexually activated with exogenous estradiol (50 μg/kg, SC, 54 h before the tests) and progesterone (2 mg/kg, SC, 6 h before the tests). The following parameters of male sexual behavior were recorded; mount, intromission, and ejaculatory latencies, number of mounts and intromissions until the first ejaculation, number of mounts, intromissions and ejaculations over a period of 40 min, and postejaculatory mount and intromission latencies after the first ejaculation. Also, copulatory efficiency (hit rate) was calculated by the following quotient: number of intromissions until first ejaculation divided by total number of mounts (incomplete mounts plus intromissions) until first ejaculation times 100. All sexual behavior tests were held 4 to 8 h after the beginning of the dark period.

Determination of Gonadal Hormone Levels

Control and experimental 120-day-old male rats were weighed and decapitated, and trunk blood was collected. Testosterone levels were measured by radioimmunoassay as described. The Mann–Whitney *U*-test was employed to test differences in hormone levels.

Determination of Striatal and Hypothalamic Monoamine and Metabolite Levels

Males aged 140 days, perinatally exposed or not to PT, were decapitated. Brains were dissected on dry ice and prepared as described previously (20). Briefly, the striatum and hypothalamus were weighed and stored at −70°C. During the weeks following sample collection, perchloric acid was added to the tis-

sues, which were then homogenized by sonication 1 week before the neurochemical evaluations. Dopamine (DA) and its metabolites, homovanillic acid (HVA) and 3,4 dihydroxyphenylacetic acid (DOPAC), noreprinephrine (NE) and its metabolite, vanilmandelic acid (VMA), and serotonin (5-HT) and its metabolite, 5-hydroxyindole acetic acid (5-HIAA), were measured by HPLC (Shimadzu, model 6A) with a C-1 column (Shimpak-ODS), an electrochemical detector (Shimadzu, model 6A), a sample injector (valve for 20 μl), and an integrator (Shimadzu, model 6A Chromatopac). Dihydroxybenzamine (DHBA) was used as the internal standard. Each sample was run for 28 min. The limit of detection was 2 pg for DA, DOPAC, NE, 5-HT, and 5-HIAA, and 20 pg for HVA.

RESULTS

Perinatal exposure to PT did not modify the time of testis descent (control group = 25.6 ± 0.5, n = 21; experimental group = 26.4 ± 0.4, n = 26). Also, no differences in body weight were observed between control and experimental pups throughout lactation and at 60 days of age (Table 1).

Male rats perinatally exposed to PT exhibited significant increases in the number of mounts until ejaculation, in number of mounts within 40 min and latency to first mount, and intromission compared to controls. Also, number of ejaculations within 40 min and copulatory efficiency were decreased significantly (Table 2).

Table 3 shows that body weight, testosterone levels, seminal vesicle, and ductus deferens wet weights were reduced by perinatal PT exposure, whereas testis wet weight did not differ between experimental and control animals.

PT-exposed animals exhibited lower striatal DA and HVA levels ($p < 0.05$). There were no differences in HVA/DA ratio between groups. Striatal 5-HT levels and the 5-HIAA and 5-HIAA/5-HT ratios were not different. The same was observed in relation to striatal NA, VMA, and VMA/NA (Table 4). Exposed animals showed higher hypothalamic NA levels. Hypothalamic VMA levels were undetectable in our detection system. There were no differences between groups in hypothalamic DA, HVA, DOPAC, 5-HT, 5-HIAA levels, or in DOPAC/DA and 5-HIAA/5-HT ratios (Table 4).

DISCUSSION

The present results reveal a marked disruption of sexual behavior and physiology induced by perinatal exposure to PT. It is known that interference with GABAergic neurotransmission as well as exposure to stress during the critical period disturbs the process of the sexual differentiation of the brain (3,8,13,18,26). However, in all of these studies, the drugs or the stress were administered during the prenatal critical period. The influence of these factors on the postnatal period or on both pre- and postnatal periods of sexual differentiation remains to be investigated. This fact is important because perinatal administration of GABAergic drugs influences the development of the sexually dimorphic nucleus of the preoptic area (SDN-POA). In fact, pregnant rats and their pups treated with the GABA agonist, muscimol, showed a significant reduction of SDN-POA volume in comparison to the sham-treated controls (6). In our study PT was administered to dams during both periods. The rationale for this experimental design is based on two effects of PT: (a) prenatal ad-

TABLE 1

BODY WEIGHT OF OFFSPRING ON THE FIRST, 10TH, 21ST, AND 60TH POSNATAL DAYS

Groups	n	Days			
		1st	10th	21st	60th
CM	27	5.9 ± 0.1	21.5 ± 0.4	34.9 ± 0.7	210.4 ± 3.4
EM	30	5.6 ± 0.1	22.4 ± 0.3	34.9 ± 0.6	211.7 ± 3.1
CC	24	5.5 ± 0.2	20.6 ± 0.4	33.9 ± 0.6	162.0 ± 2.5
EF	25	5.4 ± 0.1	21.0 ± 0.3	34.5 ± 0.6	164.1 ± 2.1

Mothers were treated with picrotoxin (0.75 mg/kg) or 0.9% saline, SC, on the 18th day of pregnancy and during the first 5 days of lactation. The values are means ± SEM. Control males (CM); experimental males (EM); control females (CF); experimental females (EF).

TABLE 2

EFFECTS OF PERINATAL EXPOSURE TO PICROTOXIN ON SEXUAL BEHAVIOR OF MALE RATS

Parameters/Groups	Control	Picrotoxin
Latency to first mount(s)	10.1 ± 1.5	25.2 ± 6.5*
Latency to first intromission(s)	32.4 ± 9.8	100.3 ± 25.2*
Ejaculation Latency(s)	712.6 ± 90.4	901.4 ± 76.9
No. of mounts until ejaculation (NM)	22.4 ± 2.1	31.2 ± 2.4*
No. of intromissions until ejaculation (NI)	17.1 ± 11.2	20.3 ± 1.3
Postejaculatory mount latency(s)	355.6 ± 11.2	358.7 ± 25.3
Number of mounts in 40 min	48.1 ± 2.4	59.0 ± 4.4*
Number of intromission in 40 min	37.8 ± 2.3	40.1 ± 1.9
Number of ejaculations in 40 min	3.2 ± 0.2	2.6 ± 0.16*
Postejaculatory intromission latency(s)	355.6 ± 11.2	358.7 ± 25.3
Copulatory efficiency = NI/NM × 100	76	66*

Picrotoxin (0.75 mg/kg) or 0.9% NaCl solution was administered to dams on day 18 of pregnancy, immediately after parturition and during the first 5 days of lactation. Rats were tested for sexual behavior at 120 days of age. The values are means ± SEM for ten rats per group.

*$p < 0.05$ compared to the control group (Student's t-test).

TABLE 3

PLASMA TESTOSTERONE LEVELS, BODY WEIGHT, TESTIS, DUCTUS DEFERENS, AND SEMINAL VESICLE WET WEIGHT OF RATS PERINATALLY EXPOSED OR NOT TO PT

Parameters/Groups	Control	Picrotoxin
Testosterone plasma levels (ng/ml)	1.25 (0.81–1.02)	0.62 (0.29–1.63)*
Body weight (g)	381.5 ± 10.6	342.0 ± 9.9†
Testis wet weight (g)	3.34 ± 0.08	3.21 ± 0.11
Ductus deferens (g)	1.93 ± 0.08	1.29 ± 0.10†
Seminal vesicle (g)	0.30 ± 0.01	0.25 ± 0.01†

Plasma testosterone levels are presented as medians and respective limits; and the remaining parameters are presented as means ± SEM. Six animals/group were employed for the determination of plasma testosterone levels and 10 animals/group for the determination of the remaining parameters.

*$p < 0.05$ compared to the control group (Mann–Whitney U-test).

†$p < 0.05$ compared to the control group (Student's t-test).

TABLE 4
STRIATAL AND HYPOTHALAMIC MONAMINE LEVELS IN MALE RATS TREATED WITH PICROTOXIN DURING THE PERINATAL PERIOD

Groups	DA	HVA	DOPAC	DOPAC + HVA/DA	5-HT	5-HIAA	5-HIAA/5-HT	NE	VMA	VMA/NE
Hypothalamus										
Saline ($n = 7$)	0.265 ± 0.033	—	0.155 ± 0.034	0.597 ± 0.077	0.65 ± 0.079	0.803 ± 0.079	1.280 ± 0.121	1.808 ± 0.134	—	—
Picrotoxin ($n = 6$)	0.295 ± 0.029	—	0.163 ± 0.028	0.583 ± 0.132	0.926 ± 0.139	0.918 ± 0.062	1.069 ± 0.118	2.243* ± 0.087	—	—
Striatum										
Saline ($n = 9$)	9.709 ± 0.340	1.048 ± 0.103		0.106 ± 0.008	0.463 ± 0.067	0.734 ± 0.082	1.782 ± 0.308	0.204 ± 0.054	0.081 ± 0.008	0.596 ± 0.131
Picrotoxin ($n = 9$)	8.099* ± 0.364	0.767* ± 0.056		0.094 ± 0.004	0.447 ± 0.064	0.640 ± 0.098	1.509 ± 0.175	0.274 ± 0.081	0.103 ± 0.015	0.610 ± 0.135

Dopamine (DA); homovanillic acid (HVA); 3,4-dihydroxyphenylacetic acid (DOPAC); serotonin (5-HT); 5-hydroxyindoleacetic acid (5-HIAA); norepinephrine (NE); vanilmandelic acid (VMA). Data are means ± SEM.
*$p < 0.05$ compared to the control group (Student's t-test); n = number of animals.

ministration of PT releases corticosterone (22). In fact, it has been reported that stressful situations during the last period of pregnancy induce changes in adult reproductive behavior (33). These changes are characterized by a decrease of male sexual behavior (demasculinization) and facilitation of lordosis behavior induced by estrogens (feminization). Ward has suggested that the increase of adrenal steroids in the stressed mother will suppress the surge of androgens from fetal testes, which is critical for the male brain differentiation (52), and that prenatal maternal stress alters plasma testosterone in fetal males (54). In addition, treatment of pregnant rats with hydrocortisone from day 14 after conception until parturition resulted in demasculinization of male offspring (13). (b) Postnatal administration of PT may disrupt sexual behavior by two mechanisms. First, by a reduction in the release of newborn testosterone during the postnatal critical period because it was reported that GABA has a functional role in neuroendocrine control of the male gonad (40). Second, by interference with the content of bioactive substances of mother's milk. In the postnatal critical period for male brain differentiation (29), the milk gonadotropin-releasing hormone (GnRH) of early lactation is thought to play an important role in this process in the rat. Carlos et al. (9) showed that pups deprived of early lactation milk (ELM) exhibited, as adults, reduced fertility, decreased weight of ductus deferens and seminal vesicle, reduced levels of fructose in the seminal vesicle and prostate gland, as well as altered sexual performance. Thus, disruptive sexual effects caused by perinatal exposure to PT in male rats might have been due to interference with the content of bioactive substances in milk. Alternatively, because stress during pregnancy and lactation affects the interactions between dams and their offspring, these interactive effects may have contributed to the expression of demasculinization seen in adulthood (41). To separate prenatal influences from subsequent maturation and development, fostering (exchanging offspring with similarly treated mothers) and crossfostering (exchanging treated progeny with control mothers and vice versa) procedures should be employed prior to nursing. Such manipulation would clarify some aspects about these questions. However, Chiavegatto and Bernardi (11) showed that this procedure led to a decrease in pup body weight when mothers and offspring were submitted to different treatments. It was suggested that crossfostering reduced weight of crossfostered litters by interfering with postnatal mother–offspring interaction.

Such long-term organizational effects have also been reported for exploratory behavior as well as for social interaction. In a recent article, we reported that the offspring of female rats exposed to a subconvulsive dose of PT (0.75 mg/kg) on day 18 of pregnancy, immediately after parturition and daily during the first 5 days of lactation, after reaching adult age showed a lack of classical sexual dimorphic response in the open field; in the anxiety test, exposed male rats showed a behavioral response similar to that of female control rats (45). Those data suggest that perinatal exposure to PT may interfere with normal male masculinization, rather than increasing anxiety in male rats.

Drugs that interfere with offspring growth might directly or indirectly affect the subsequent sexual maturation of neonates (47). In this study, perinatal treatment with PT did not produce significant effects on the physical development of pups, i.e., no differences were observed in body weight or in day of testis descent between control and experimental animals. These results suggest that PT had no toxic effects on general development during the neonatal–infant period.

There was a marked reduction in the sexual behavior of animals perinatally exposed to PT. The increase in number of

mounts and concomitant decrease in copulatory efficiency induced by the perinatal treatment suggest that motor copulatory events were modified. In agreement with these results, Velazquez-Moctezuma et al. (50) showed that maternal stress induced by immobilization, unavoidable electric footshocks, and rapid eye movement sleep deprivation decreased copulatory efficiency, although water immersion had no effect. On the other hand, they observed that only electric footshock increased the latencies to first mount and intromission in a manner similar to that observed in our study, a fact suggesting a reduced motivational drive. These changes may also be due to a perinatal stress-induced decrease in luteinizing hormone (LH) release in male offspring (34). In fact, LH has been used as an index of sexual arousal in a variety of studies (12,25,49).

In the present study, sexual behavior, testosterone levels, and body, seminal vesicle, and ductus deferens weights were reduced in the adult offspring of PT-treated dams. These findings are consistent with a disruption of the androgenic milieu during the perinatal period (4,15,24,38). In addition, a reduction in androgen or estrogen levels during this period has also been shown to reduce the volume of the sexual dimorphic nucleus of the medial preoptic area in adult males (5,16), as observed for certain hypothalamic areas of homosexual men (35,48).

PT was administered during both periods of sexual brain masculinization. Thus, it is not possible to exactly specify the perinatal period when the drug had its demasculinizing effect. Further studies are needed to clarify this point. In addition, our experimental design did not allow to distinguish direct effects of PT on hormonal effects mediated by sex steroids or corticosteroids. Thus, two studies are currently underway in our laboratory to specifically investigate the prenatal and the postnatal effects of PT administration without employing the crossfostering procedures. Prenatal administration of PT increased male sexual index and the number of mounts and intromissions and decreased the number of ejaculations in 40 min, suggesting a disruption of male sexual behavior. These results were quite similar to those reported by Ward (52) after prenatal exposure to stress and to those obtained in the present investigation. Postnatal studies are currently underway.

In accordance with the concept of the critical period, sex differences in monoamine levels and metabolism begin to appear perinatally in rats (36,55). Experimental manipulation of the perinatal hormonal environment (23,38,42,46) as well as maternal stress (2,51) may lead to persistent changes in brain biochemistry.

Norepinephrine (NE) innervation to the hypothalamus has a unique relationship with GABAergic system development and can be altered by benzodiazepine treatment during the course of maturation of these neurons (32). Hypothalamic evaluation of NE, dopamine, serotonin, and their metabolites showed an increase in NE levels without any alteration in the levels of the other amines or in their metabolites. The higher levels of NE observed here are in accordance with a demasculinizing effect of PT because it has been observed that hypothalamic NE levels are higher in females than in males. In addition, Donoso et al. (17) showed that castration of male rats produced an increase in NE in the anterior hypothalamus. The increase in hypothalamic NE levels may be due to the lower plasma testosterone levels observed in male rats perinatally exposed to PT.

Other authors have reported that prenatal stress results in a deficit in DA neurotransmission in the ventral striatum (51). Because striatal dopaminergic neurotransmission is important for the expression of male sexual behavior (14), this deficit may impair male sexual behavior in rats (14,51). The present results show a reduction in striatal DA and HVA levels in male rats perinatally exposed to PT. In addition, analysis of the sexual behavior of these animals showed a reduction in copulatory efficiency. The expression of this variable depends on the integrity of the striatal system which controls motor execution. Agmo et al. (1) showed that there is no relation between reduced copulatory efficiency and alterations in locomotor activity in an open field. However, this variable is reduced whenever motor execution is impaired in a rotarod. It is possible that the reduction in copulatory efficiency observed here was due to interference with the central motor system as a consequence of the decrease in striatal DA and HVA levels. On the other hand, we also observed increases in mount latency, which depends on another central mechanism and on brain areas involved with motivational aspects of male sexual behavior. In addition, because the DOPAC/DA and HVA/DA ratios were not affected by PT treatment, the role of the striatal dopaminergic system in this situation deserves further detailed investigation.

In summary, the above data indicate that perinatal exposure to PT during the critical period of male brain sexual differentiation has long-term effects on the reproductive physiology and behavior of male rats. These data do not provide information about the underlying mechanism by which a GABAergic antagonist influences male sexual differentiation. The possibility that the PT-induced phenomena described here were due to changes in the offspring hypothalamus–hypophysis–adrenal system will be our working hypothesis in future experiments.

ACKNOWLEDGEMENTS

This research was supported by a fellowship from Funação de Amparo à Pesquisa do Estado de São Paulo-FAPESP (Proc. 96/04273-4) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq to Maria Martha Bernardi (Grant 352189/96-7).

REFERENCES

1. Agmo, A.; Paredes, R.; Fernandez, H.: Differential effects of GABA transaminase inhibitors on sexual behavior, locomotion activity, and motor execution in male rat. Pharmacol. Biochem. Behav. 28:47–52; 1987.
2. Alonso, S. J.; Navarro, E.; Rodriguez, M.: Permanent dopaminergic alterations in the n. accumbens after prenatal stress. Pharmacol. Biochem. Behav. 49:353–358; 1994.
3. Anderson, R. H.; Fleming, D. E.; Rhees, R. W.; Kinghorn, E.: Relationships between sexual activity, plasma testosterone, and volume of the sexually dimorphic nucleus of the preoptic area in prenatally stressed and non-stressed rats. Brain Res. 370:1–10; 1986.
4. Arnold, A. P.; Breedlove, S. M.: Organizational and activational effects of sex steroids on brain and behavior: A reanalysis. Horm. Behav. 19:469–498; 1985.
5. Arnold, A. P.; Gorski, R. A.: Gonadal steroid induction of structural sex differences in the central nervous system. Annu. Rev. Neurosci. 7:413–422; 1984.
6. Bach, F.; Flügge, G.; Wuttke, W.: GABAergic influence on the development of sexually dimorphic nucleus of male and female rats. Brain Res. 573:341–344; 1992.
7. Bailey, J. M.; Willemam, L.; Parks, C.: Arch. Soc. Behav. 20:277–293; 1991. In: Swabb, D. F.; Hofman, M. A., eds. Sexual differentiation of the human hypothalamus in relation to gender and sexual orientation. Trends Neurosci. 18:264–270; 1995.

8. Cagiano, R.; De Salvia, M. A.; Persichella, M.; Renna, G.; Tattoli, M.: Behavioural changes in the offspring of rats exposed to diazepam during gestation. Eur. J. Pharmacol. 177:67–74; 1990.
9. Carlos, C. P.; Lemonica, Y. P.; Kempinas, W. G.; Pereira, O. C. M.: Does the male reproductive performance depend on the early lactation milk in rats? Physiol. Behav. 59:147–152; 1996.
10. Chiavegatto, S.; Bernardi, M. M.; De-Souza-Spinosa, H.: Effects of prenatal diphenhydramine administration on sexual behavior of rats. Braz. J. Med. Biol. Res. 22:729–732, 1989.
11. Chiavegatto, S.; Bernardi, M. M.: Prenatal versus postnatal effects on offspring weight gain of rats exposed to diphenhydramine: A critical evaluation of fostering procedures in rats. Comp. Biochem. Physiol. 99:219–221; 1991.
12. Cocquelin, A.; Bronson, F. H.: Episodic release of luteinizing hormone in male mice. Antagonism by neural refractory period. Endocrinology 109:1605–1610; 1981.
13. Bahlof, L. G.; Hard, E.; Larsson, K.: Sexual differentiation of offspring of mothers treated with cortisone during pregnancy. Physiol. Behav. 21:673–674; 1978.
14. Damsma, G.; Pfaus, J. G.; Wenkstern, D.; Phillips, A. G.; Fibiger, H. C.: Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: Comparison with novelty and locomotion. Behav. Neurosci. 106:181–191; 1992.
15. Döhler, K.D.: The pre- and postnatal influence of hormones and neurotransmitters on sexual differentiation of mammalian hypothalamus. Int. Rev. Cytol. 131:1–57;1991.
16. Döhler, K.D.; Cocquelin, A.; Davies, F.; Hines, M.; Shrne, J. E.; Gorski, R. A.: Differentiation of the sexually dimorphic nucleus in the preoptic area of the brain is determined by the perinatal hormone environment. Neurosci. Lett. 33:295–298;1982.
17. Donoso, A. O.; Stafano, F. J. E.; Biscardi, A. M.; Cukier, A. J.: Effects of castration on hypothalamic catecholamines. Am. J. Phisiol. 212:737–739; 1967.
18. Ellis, L.; Ames, M. A.; Peckham, W.; Burke, D.: Sexual orientation of human offspring may be altered by severe maternal stress during pregnancy. J. Sex. Res. 25:152–157; 1988.
19. Felicio, L. F.; Palermo-Neto, J.; Nasello, A. G.: Perinatal bromopride treatment: Effects on sexual behavior of male and female rats. Behav. Neural Biol. 52:145–151; 1989.
20. Felicio, L. F.; Florio, J. C.; Sider, L. H.; Cruz-Casallas, P. E.; Bridges, R. S.: Reproductive experience increases striatal and hypothalamic dopamine levels in pregnant rats. Brain Res. Bull. 40:253–256; 1996.
21. Fernandez-Guasti, A.; Larsson, K.; Beyer, C.: GABAergic control of sexual behavior. Pharmacol. Biochem. Behav. 24:1065–1070; 1986.
22. File, S. E.; Lister, R. G.: Does the reduction in social interaction produced by picrotoxin and pentylenetetrazole indicate anxiogenic action? Neuropharmacology 23:793–796; 1984.
23. González, M. I.; Leret, M. L.: Extrahypothalamic serotonergic modification after masculinization induced by neonatal gonadal hormones. Pharmacol. Biochem. Behav. 41:329–332; 1992.
24. Goy, R. W.; McEwen, B. S.: Sexual differentiation of the brain. Cambridge, MA: MIT Press; 1980:J-36
25. Graham, J. M.; Desjardins, C.: Classical conditioning: Induction of luteinizing hormone and testosterone secretion in anticipation of sexual activity. Science 210:1039–1041; 1980.
26. Gupta, C.; Yaffe, S. J.: Prenatal exposure to phenobarbital permanently decreases testosterone and causes reproductive dysfunction. Science 216:640–642; 1982.
27. Hernández-Alvarez, L. A. I.; Martínez-Vargas, A.; Victoria-Romero, B.; Márquez-Orozco, A.; Márquez-Orozco, M. C.: Permanent effects of prenatal exposure to diazepam on sexual behavior of male mice. Soc. Neurosci. Abstr. 22:2073; 1996.
28. Ixart, G.; Cryssogelou, H.; Szafarczyk, A.; Malaval, F.; Assenmacher, I.: Acute and delayed effects of picrotoxin on the adrenocorticotropic system of rats. Neurosci. Lett. 43:235–240; 1983.
29. Jacobson, C. D.; Gorski, R. A.: Neurogenesis of the sexual dimorphic nucleus of the preoptic-area in the rat. J. Comp. Neurol. 196:519–529; 1981.
30. Johnson, N.; Leone, F.: Statistics and experimental design. In: Engineering and physical sciences. New York: John Wiley; 1974.
31. Jones, M. T.; Hillhouse, E. W.: Neurotransmitter regulation of corticotropin-releasing factor in vitro. Ann. NY Acad. Sci. 297:536–560; 1977.
32. Kellogg, C. K.; Retell, T. M.: Release of [^{3}H] norepinephrine: Alteration by early developmental exposure to diazepam. Brain Res. 366:137–144; 1986.
33. Kinsley, C. H.; Krasnegor, N. A.; Bridges, R. S., eds. Prenatal and postnatal influences on parental behavior in rodents. In: Mammalian parenting. New York: Oxford Univ. Press; 1990: 347–371.
34. Kinsley, C. H.; Mann, P. E.; Bridges, R. S.: Diminished luteinizing hormone release in prenatally stressed male rats after exposure to sexually receptive females. Physiol. Behav. 52:925–928; 1992.
35. Levay, S.: A difference in hypothalamic structure between heterosexual and homosexual men. Science 253:1034–1037; 1991.
36. MacLusky, N. J.; Naftolin, F.: Sexual differentiation of the central nervous system. Science 211:1294–1303; 1981.
37. Matheson, G. K.: Effects of GABA agonists on ACTH release. Brain Res. Bull. 5 (Suppl. 2):447–452; 1980.
38. Moguilevsky, J. A.; Justo, G.; Justo, S.; Szwarcfarb, B.; Carbone, S.; Scacchi, P.: Modulatory effect of testosterone on serotoninergic controls of prolactin secretion in prepubertal rats. Neuroendocrinology 51:197–201; 1990.
39. Reisert, I.; Pilgrim, C.: Sexual differentiation of monoaminergic neurons—Genetic or epigenetic. Trends Neurosci. 14:468–473; 1991.
40. Ritta, M. N.; Campos, M. B.; Calandra, R. S.: Coexistence of gamma-aminobutyric acid type A and type B receptors in testicular interstitial cells. J. Neurochem. 56:1236–1240; 1991.
41. Sachs, B. D.; Meisel, R. L.: The physiology of male sexual behavior. In: Knobil, E.; Neill, J., eds. The physiology of reproduction. New York: Raven Press; 1988:1393–1485.
42. Sañudo-Peña, M. C.; Hernández-Tristán, R.: Effects of neonatal antiandrogenization upon hypothalamic monoamines and behavior. Neurosci. Res. Commun. 14:158–165; 1994.
43. Shekhar, A.; Hingtgen, J. N.; DiMicco, J. A.: GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats. Brain Res. 512:81–88; 1990.
44. Shekhar, A.; Katner, J. S.: Dorsomedial hypothalamic GABA regulates anxiety in the social interaction test. Pharmacol. Biochem. Behav. 50:253–258; 1995.
45. Silva, M. R. P.; Felicio, L. F.; Nasello, A. C.; Bernardi, M. M.: Is perinatal picrotoxin anxiogenic? Braz. J. Med. Biol. Res. 28:663–666; 1995.
46. Simerly, R. B.; Swanson, L. W.; Handa, R. J.; Gorski, R. A.: Influence of perinatal androgens on the sexually dimorphic distribution of tyrosine hydroxylase-immunoreactive cells and fibers in the anteroventral-periventricular nucleus of rat. Neuroendocrinology 40:501–510; 1985.
47. Spyker, J. M.: Assessing the impact of low levels of chemicals on development: Behavioral and latent effects. Fed. Proc. 34:1835–1844; 1975.
48. Swaab, D. F.; Hofman, M. A.: Sexual differentiation of the human hypothalamus in relation to gender and sexual orientation. Trends Neurosci. 18:264–270; 1995.
49. Taleisnik, S.; Caligaris, L.; Astrada, J. J.: Effect of copulation on the release of pituitary gonadotropins in male and female rats. Endocrinology 79:49–54; 1966.
50. Velazquez-Moctezuma, J.; Salazar, E. D.; Rueda, M. L. C.: The effect of prenatal stress on adult sexual behavior in rats depends on the nature of the stressor. Physiol. Behav. 53:443–448; 1993.
51. Wang, C. T.; Huang, R. L.; Tai, M. Y.; Tsai, Y. F.; Peng, M. T.: Dopamine release in the nucleus accumbens during sexual behavior in prenatally stressed adult male rats. Neurosci. Lett. 200:29–32; 1995.
52. Ward, I. L.: Prenatal stress feminizes and demasculinizes the behaviors of males. Science 175:82–84; 1972.
53. Ward, I. L.; Ward, O. B.; Winn, R. J.; Bielawski, D.: Male and female sexual behavior potential of male rats prenatally exposed to the influence of alcohol, stress, or both factors. Behav. Neurosci. 108:1188–1195; 1994.
54. Ward, I. L.; Weisz, J.: Differential effects of maternal stress on circulating levels of corticosterone, progesterone and testosterone in male and female rat fetuses and their mothers. Endocrinology 114:1635–1644; 1984.
55. Wilson, W. E.; Agrawal, A. K.: Brain regional levels of neurotransmitter amines as neurochemical correlates of sex-specific ontogenesis in the rat. Dev. Neurosci. 2:195–200; 1979.