Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 75 (2003) 733–740
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effects of maternal exposure to picrotoxin during lactation on physical and reflex development, square crossing and sexual behavior of rat offspring

A.C.Z. Baso[a], F.C. Goulart[a], E. Teodorov[b], L.F. Felício[b], M.M. Bernardi[b,*]

[a]*Departamento de Farmacologia do Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil*
[b]*Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Orlando Marques de Paiva, 87, São Paulo, SP 05508-900, Brazil*

Received 12 July 2002; received in revised form 26 December 2002; accepted 2 May 2003

Abstract

The effects of maternal exposure during the first 10 days of lactation to picrotoxin (0.75 mg/kg sc) on maternal behavior, offspring physical and neurobehavioral development as well as sexual behavior were studied. Results showed that (1) dam food and water consumption, maternal behavior and body weight were not different between control and experimental animals, (2) male and female pup body weight and the development of physical landmarks did no differ between control and experimental groups, (3) negative geotaxis was improved in female experimental offspring and palmar grasp reflex did not differ between groups, (4) at 75 days of age the square crossing by female rats of the experimental group was increased in relation to the control group; no differences were observed between male control and experimental animals, (5) male experimental rats exhibited a significant increase in the number of mounts, intromissions and ejaculations parallel to a decrease in latency to first mount, intromissions and ejaculation as well as in the latencies of first postejaculatory mount and intromission and (6) the intromission frequency per minute (hit rate) was increased in these animals. These results suggest that postnatal exposure to picrotoxin improved the sexual behavior of rats. Three hypotheses were proposed to explain the mechanisms underlying this effect: (1) the development of subsensitivity of GABAergic receptors, (2) an interference with early receptor development or (3) with neurotransmitter balance, mainly involving the dopaminergic system.

Keywords: Picrotoxin; Rat; Lactation

1. Introduction

Beings such as mammals are usually vulnerable to chemical or environmental agents that can induce the appearance of congenital defects. These defects can be expressed by morphological, biochemical or functional alterations occurring when individuals are exposed to a determined teratogenic agent. The teratogenic response of rats is generally related to exposure to a teratogen during development. Some alterations are not recognized at birth but are observed during neonatal life. Examples of these alterations in rats include modification of sexually dimorphic patterns, such as sexual behavior and gonadotrophin release. Changes in sexual behavior can only be observed during adult life. These alterations may be due to changes in the maternal hormonal inner environment caused by the administration of chemical agents.

Several neurotransmitters have been proposed to intervene in the control of sexual behavior in the male rat. Most pharmacological studies have focused on the monoamine transmitter; dopamine (DA) is thought to be an excitatory neurotransmitter, whereas serotonin may exert an inhibitory role on this behavior (Ahlenius et al., 1980, 1981, 1987; Gessa and Tagliamonte, 1975; Malmnãs, 1973, 1976; Meyerson et al., 1979).

The amino acid, γ-aminobutyric acid (GABA), the major inhibitory brain neurotransmitter, is also involved in the control of male sexual behavior. Studies of the effects of intracerebral injection of GABAergic agonists and antagonists on male sexual behavior have suggested that this system may normally play an inhibitory role in sexual behavior (Fernández-Guasti et al., 1986; Paredes and Agmo, 1992; Agmo et al., 1987). GABAergic drugs administered during pregnancy produce both anatomic and neurologic

* Corresponding author. Fax: +55-11-3091-78-29.
E-mail address: bernarde@usp.br (M.M. Bernardi).

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00146-1

malformations in the offspring (Manning et al., 1971; Middaugh et al., 1975) as well as sexual dysfunction in later life (Cagiano et al., 1990; Fieder et al., 1984; Kellogg et al., 1980; Lyubimov et al., 1975).

A previous study from our laboratory on sexual brain differentiation of rats showed that maternal exposure to picrotoxin (0.75 mg/kg) during the prenatal and postnatal sexual differentiation period of the central nervous system led to sexual behavior injuries in male offspring, suggesting an occurrence of demasculinization (Silva et al., 1998). In the same study, behavioral (sexual behavior) and endocrine (testosterone levels) data were assessed in the offspring of picrotoxin-treated dams, showing a decrease in male sexual behavior as well as a decrease in plasma testosterone levels of adult male offspring. These results were attributed, at least in part, to the stress produced by drug administration. In another study (Teodorov et al., 2002), we evaluated the effects of perinatal picrotoxin (0.75 mg/kg) on heterosexual and homosexual behavior of male rats, sexually experienced or not. Data showed that heterosexual behavior was improved while picrotoxin treatment reduced the lordotic response of homosexual behavior in inexperienced male rats. The heterosexual experience with female rats inhibited homosexual behavior of both experimental and control animals. These results suggest that perinatal maternal picrotoxin exposure improved heterosexual behavior in male rats and that sexual experience revealed this effect.

Thus, these results appear to be controversial. However, in the first experiment (Silva et al., 1998), the dams received 0.75 mg/kg picrotoxin once on day 18 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. In the second experiment, the treatment was similar to that applied by Silva et al. (1998), but picrotoxin was administered 2 h after birth (after the dams had nursed their pups). Thus, perinatal exposure to picrotoxin may reduce or improve the sexual behavior of rats depending on the immediate postnatal period of exposure to the drug. In the present study, we tried to clarify the importance of maternal exposure to picrotoxin administered 2 h after birth and once daily during the following 9 days of lactation. The dose used here was the same as employed in these previous studies and does not induce maternal or fetal intoxication or convulsive symptoms (Silva et al., 1998; Teodorov et al., 2002).

2. Method

2.1. Animals

Male and female Wistar rats from our own colony, weighing 250–270 g and ~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 12:12 h light–dark 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.

2.2. Maternal and offspring studies

Sixteen 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) and pregnant females were immediately housed in individual cages. The dams received picrotoxin (0.75 mg/kg, experimental group) or saline (1 ml/kg, control group) 2 h after natural parturition and once daily during the following 9 days of lactation.

At birth, litters were divided into groups of eight pups, four males and four females, and the remaining pups were discarded. All litters were examined externally, sexed and weighed. Maternal body weight as well as food and water consumption were recorded throughout the treatment period. The maternal behavior was also studied on postnatal day 1 (PND1), PND3, PND5, PND7 and PND11 according to the scoring system proposed by Soderstein and Eneroth (1984). The dams were observed and scored in their individual cages as follows: 0 = absence of nest, 1 = presence of nest constructed from a sheet of paper towel available in each cage, 2 = presence of all pups inside the nest, 3 = all pups warm inside the nest and 4 = dam positioned over all pups in a nursing posture.

The following parameters were recorded to evaluate the physical development of offspring: body weight (PND1–PND10), pinna detachment (PND2–PND4), ear unfolding (PND2–PND5), incisor eruption (PND6–PND12), ear (PND11–PND17) and eye opening (PND11–PND17), testis descent (PND22–PND28) and vaginal opening (PND34–PND40). Animals were observed until 100% of them showed each parameter and the mean day of appearance was calculated. The anogenital distance was measured on PND1 and the anogenital index was calculated by the anogenital distance/body weight ratio. The following reflexes were assessed in one male and one female of each litter: negative geotaxis (a minimum 90° turn after being placed face down on a 45° inclined platform for 30 s, beginning on day 2) and palmar grasp reflex (pup grasps a paper clip with forepaws if stroked). All tests were carried out at the same time of day (9:00–11:00 a.m.), with the pups separated from the mothers at the moment of observation and then immediately returned to their home cages. The mean day of appearance of each of the above parameters was calculated. All data were analyzed considering the litter as the smallest unit. On PND21, the offspring were weighed and weaned and the littermates were separated and housed by sex until completion of the study. Square crossing by the animals was measured in a four-beam activity cage meter on PND15, PND21 and PND75 of age. For this procedure, the animal was introduced into a clear

acrylic cage, and when it interrupted one or more infrared beams, the number of crossings was recorded by an automatic counter system. The beams were arranged in an array of emitters on one side of the cage and detectors on another. Only one animal of each gender from each litter was used for the tests in infancy and adult age (developmental studies, square crossings in the activity cage meter and sexual behavior).

2.3. Sexual behavior studies

Sexually naive rats of the control and experimental groups were used for the mating tests at 120 days of age as previously described (Chiavegatto et al., 1989; Felicio and Nasello, 1989). Briefly, animals were maintained under controlled temperature conditions on a 12 h inverted light–dark cycle (lights on at 10:00 p.m. for at least 21 days before the experiments for adaptation to the cycle). To investigate sexual behavior, male rats were allowed to mount ovariectomized lure 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, intromissions and ejaculations over a period of 40 min and postejaculatory mount and intromission latencies after the first ejaculation. The intromission frequency was calculated by the quotient between the intromission frequency and the difference between ejaculation latency and intromission latency (Agmo et al., 1987). All sexual behavior tests were held 4–8 h after the beginning of the dark period. One animal of each litter was used for the sexual behavior tests.

2.4. Statistical analysis

All data were first analyzed by the Bartlett test (Johnson and Leone, 1974) to determine data homogeneity. Two-way ANOVA followed by the *S*-test was employed for the analysis of physical and reflexologic pup development data as well as of the data concerning activity in the automated cage. One-way ANOVA was used to analyze the food and water consumption by the dams during pregnancy. The Student's *t* test was used to analyze male sexual behavior. In all cases, results were considered significant for $P < .05$.

3. Results

Maternal picrotoxin exposure did not induce maternal toxicity because dams body weight or food and water consumptions were not modified by the treatment. Also, maternal care was not modified by picrotoxin exposure since maternal behavior was not different between control and experimental animals. In addition, because maternal behavior was not

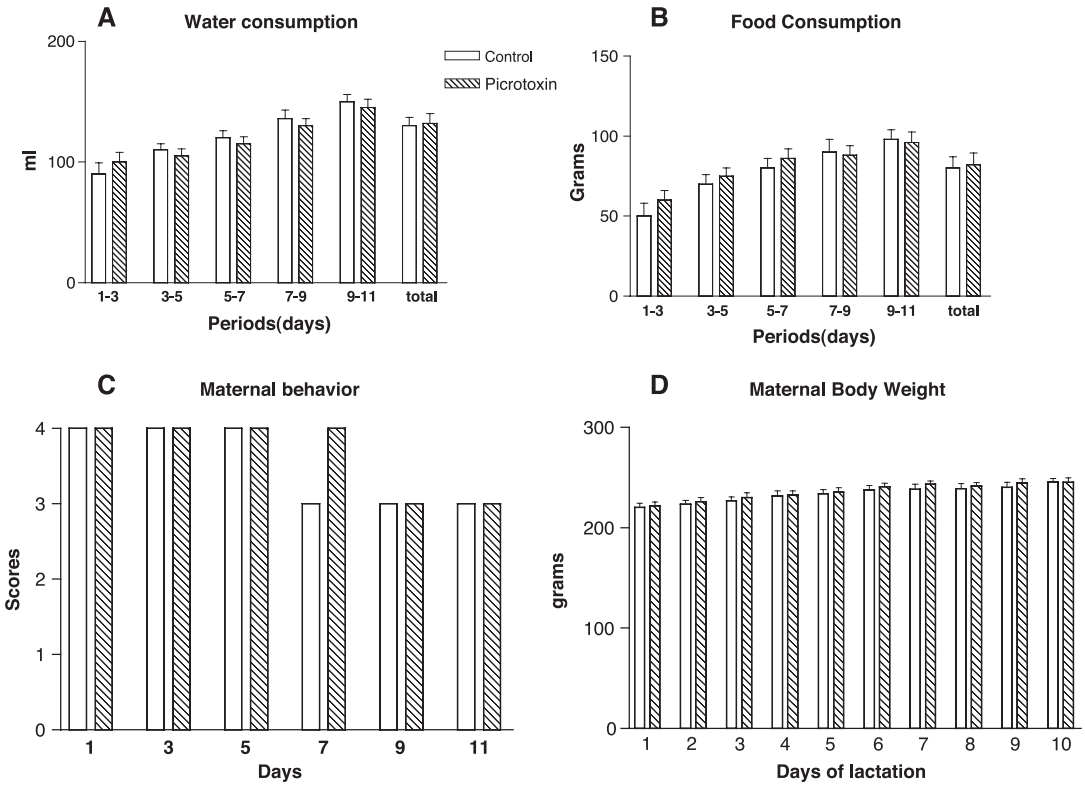

Fig. 1. Effects of maternal postnatal exposure to picrotoxin. (A) Maternal water consumption during treatment. (B) Maternal food consumption during treatment. (C) Maternal behavior during treatment. (D) Maternal body weight during treatment. Maternal food and water consumption as well as body weight are presented as means ± S.E.M. Maternal behavior is presented as median and range.

modified, reduced maternal care was probably not responsible for the long-term effects observed here. Dam food and water consumption (Fig. 1A and B), maternal behavior (Fig. 1C) and body weight (Fig. 1D) were not different between control and experimental animals. Also, male and female pup body weight during treatment did not differ between experimental groups and controls (Fig. 2A and B). Physical landmarks of development such as pinna detachment, incisor eruption, testis descent and eye and vaginal openings of these animals did no differ between control and experimental groups (Table 1). With respect to the reflexes, two-way ANOVA showed no differences between groups in palmar grasp and that treatment had no effect on the results of negative geotaxis [$F(1,28)=0.60$, $P=.412$], whereas sex had a significant effect [$F(1,28)=9.11$, $P=.0054$] with interaction between factors [$F(1,28)=13,61$, $P=.0001$]. The *S*-test showed that the negative geotaxis of experimental female offspring was improved in relation to male experimental offspring as well as in relation to female control offspring. (Table 1).

Fig. 3 shows the square crossings of pups on PND15, PND21 and PND 75. Two-way ANOVA showed that the activity of experimental female rats was increased only at 75 days of age compared with control; no differences in activity were observed between control and experimental males [Treatment $F(1,280)=2,49$, $P=.128$; Sex $F(1,28)=17.38$, $P=.0003$; Interaction $F(1,28)=10.28$, $P=.0003$]. The *S*-test showed that the number of crossing of experimental females at 75 days of age differed from the other experimental and control groups.

Table 1
Effects of maternal postnatal picrotoxin exposure (0.75 mg/kg) on landmarks indicative of physical and reflexologic parameters of development in rats

Parameters	Groups			
	Male		Female	
	Control	Experimental	Control	Experimental
PD	3.00 ± 0.15	3.00 ± 0.16	3.00 ± 0.13	3.00 ± 0.13
IE	11.18 ± 0.22	11.40 ± 0.26	11.18 ± 0.21	11.30 ± 0.29
EO	15.09 ± 0.28	15.01 ± 0.17	15.00 ± 0.25	15.30 ± 0.21
TD	26.09 ± 0.09	26.10 ± 0.23	–	–
VO	–	–	37.63 ± 0.23	37.70 ± 0.15
NG	16.12 ± 1.33	17.06 ± 1.79	20.12 ± 1.57	10.73 ± 0.64 *,#
PG	2.06 ± 0.22	4.22 ± 1.24	1.90 ± 0.17	1.53 ± 0.04

Physical development data are presented as mean ± S.E.M. number of days. Physical parameter data: PD = pinna detachment; IE = incisor eruption; EO = eye opening; VO = vaginal opening; TD = testis descent. Reflexes: PG = palmar grasp reflex; NG = negative geotaxis. The means ± S.E.M. for eight rat pups per group were used to evaluate physical landmark parameters. * $P<.05$, compared with the respective control group.
* $P<.05$, compared with the experimental male group.
# $P<.05$, compared with the female control group.

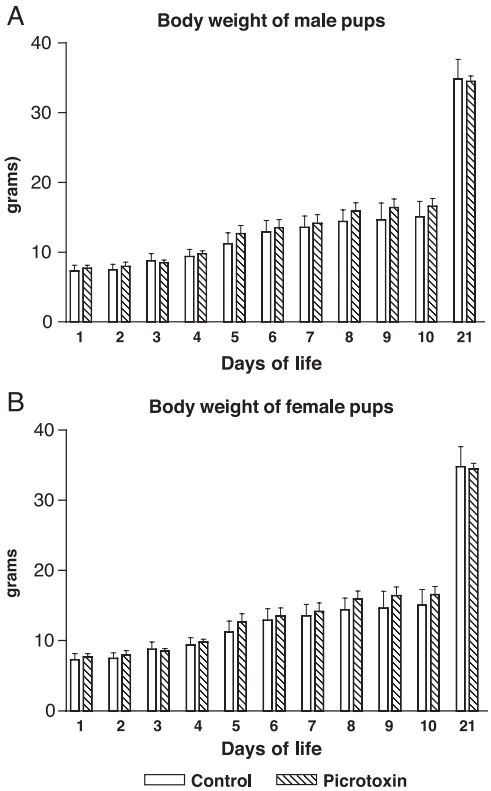

Fig. 2. Body weight of male (A) and female (B) pups on PND1–PND10 and at weaning. Mothers were treated with picrotoxin (0.75 mg/kg sc) or control solution subcutaneously from PND1 to PND10. The values are means ± S.E.M. of litter values.

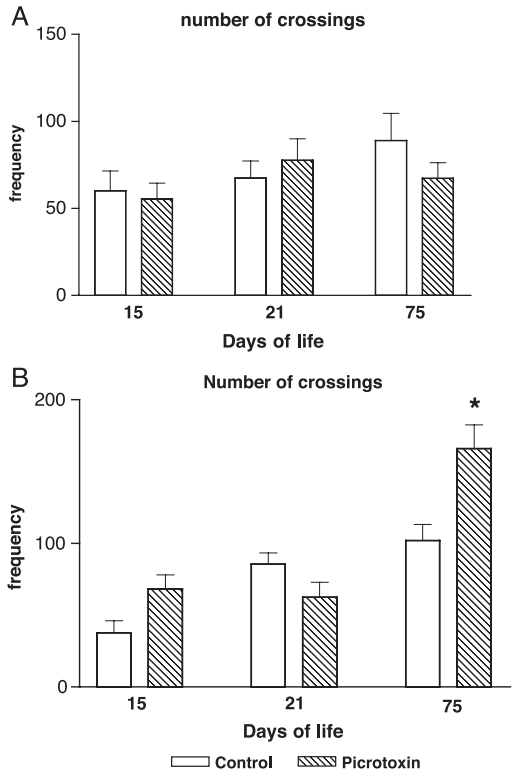

Fig. 3. Activity of male (A) and female (B) rat pups in an activity cage. Mothers were treated with picrotoxin (0.75 mg/kg sc) or control solution subcutaneously on PND1–PND10. The values are means ± S.E.M. for litter values. * $P<.05$, compared with the respective control group (two-way ANOVA followed by the *S*-test).

Table 2
Effects of maternal postnatal exposure to picrotoxin on sexual behavior of male rats

Parameters/groups	Control (8)	Experimental (8)
Mount latency	2.93 ± 0.54	1.19 ± 0.23 *
Intromission latency	3.78 ± 0.27	2.16 ± 0.46 *
Ejaculatory latency	11.27 ± 1.93	4.61 ± 0.28 *
Postejaculatory mount latency	5.02 ± 0.41	3.67 ± 0.46 *
Postejaculatory intromission latency	6.69 ± 0.48	3.67 ± 0.48 *
Number of mounts	19.00 ± 2.61	25.50 ± 1.98 *
Number of intromissions in 40 min	22.06 ± 1.28	12.5 ± 3.50 *
Number of ejaculations up to 30 min after the first intromission	1.12 ± 0.12	2.37 ± 0.53 *
Frequency of intromissions per minute	1.98 ± 0.22	4.63 ± 1.21 *

The dams received picrotoxin (0.75 mg/kg, experimental group) or saline (1 ml/kg, control group) at parturition and once a day during the first 9 days of lactation. Offspring rats were tested for sexual behavior at 100 days of age. Data are reported as means ± S.E.M. $n = 8$ per group. Latencies were measured in seconds.

* $P < .05$, compared with control (Student's t test).

Male rats postnatally exposed to picrotoxin exhibited a more intense reproductive behavior than control animals. A significant increase in the number of mounts, intromissions and ejaculations parallel to a decrease in the latencies to first mount, intromission and ejaculation as well as in the latencies to first postejaculatory mount and intromission was observed in experimental males compared with control. Also, the intromission frequency per minute was increased in experimental males (Table 2).

4. Discussion

The present results indicate that maternal postnatal picrotoxin exposure did not induce maternal toxicity, as shown by the lack of effects on dam body weight or food and water consumption. In addition, because maternal behavior was not modified, reduced maternal care was probably not responsible for the long-term effects observed here.

There are some data suggesting that drugs that interfere with offspring growth might directly or indirectly affect subsequent maturation and development of neonates (Smart and Dobbing, 1971). In our study, postnatal treatment with picrotoxin 2 h after parturition had no effect on postnatal physical development.

It was observed that postnatal picrotoxin exposure decreased reflex development in female but not in male offspring. According to Altman and Sudarshan (1975), three peripheral systems may be involved in the regulation of postural adjustments, including vestibular, extereoceptive (e.g., tactile) and proprioceptive systems. The vestibular system functions at birth, though vestibular reactions are hampered by immaturity of the motor system. The righting reflex and geotaxic responses of young rats reflect both motor development and activity guided by the vestibular system (Altman and Sudarshan, 1975). In the present study, postnatal picrotoxin modified negative geotaxis in the female experimental group, showing interaction of sex and treatment. This fact indicated that motor differences could be modified by prenatal treatment and that females were more sensitive to the effects of the drug.

Although square crossing in a cage meter was measured during pup development (PND15 and PND21), only at PND75 did we observe an increased activity in female pups. Thus, picrotoxin exposure also modified the female motor system in different ways during development. Another fact is that in the present experiment we did not evaluate the estrous cycle phases of the female control and experimental groups. Female activity varies, depending on the estrous cycle phase. In this respect, female in the proestrus–estrus have higher levels of activity than in other cycle phases.

As already mentioned, perinatal exposure to picrotoxin, a GABAergic antagonist, could reduce or improve the sexual behavior of rats depending on the immediate postnatal period of exposure to the drug (Silva et al., 1998; Teodorov et al., 2002). Treatments were applied once a day on GD18 and GD21 as well as during the first 5 days of lactation (PND1–PND5). However, Silva et al. (1998) administered picrotoxin immediately after birth, before the dams had nursed their pups, and Teodorov et al. (2002) gave the first picrotoxin postnatal dose 2 h after parturition, i.e., after dams had nursed their offspring. These differences could be explained by an action of picrotoxin on different mechanisms related to sexual brain differentiation occurring during the perinatal period.

In fact, male brain differentiation occurs during two periods of time in rats, i.e., the prenatal period (on day 18 of pregnancy) and PND1–PND10 (Fritschy and Mohler, 1995), when the milk gonadotropin-releasing hormone (GnRH) of early lactation is thought to play an important role. The most important time to interfere with the postnatal period of brain masculinization seems to be the first 2 h after birth (Kacsóh et al., 1986; Carlos et al., 1996).

In the present investigation, there was marked improvement in the sexual behavior of male offspring of dams treated with picrotoxin 2 h postpartum and during the first 9 days of lactation. Latency to first mount, intromission and ejaculation were decreased in animals of the experimental group compared with control, suggesting an increased motivational drive in these animals. On the other hand, picrotoxin-exposed animals had a reduced number of mounts, intromissions and ejaculations, suggesting an increased sexual activity, as well as a higher hit rate.

Postnatal picrotoxin treatment shortened the ejaculatory latency and the interintromission interval (postejaculatory intromission interval). Fernández-Guasti et al. (1986) also showed in adult animals that GABAergic antagonists produced the same effects. The authors attributed these effects to an influence of these drugs not only on the GABAergic system but also on the cholinergic system. Further research, however, is required to test these interpretations.

The improvement on offspring sexual behavior might also be a consequence of picrotoxin release to the pups through

milk. Thus, some hypotheses can be raised to explain the present results.

First, early postnatal exposure of the offspring to picrotoxin increased the excitability of the central nervous system and then facilitated male sexual behavior in adult age. In this respect, acute exposure to the GABA antagonists bicuculline or picrotoxin into the medial preoptic area facilitates most aspects of male sexual behavior (Fernández-Guasti et al., 1985, 1986). This local blockade of GABA receptors produces neuronal excitation closely similar to that produced by electrical stimulation. Indeed, nonspecific excitation of the preoptic area facilitated sexual behavior in a way indistinguishable from that of bicuculline. However, systemic treatment or injections into the nucleus caudatus putamen of adult rats with two GABAergic drugs, muscimol and bicuculline, did not cause changes in mating pattern (Fernández-Guasti et al., 1986), showing that the preoptic area plays a critical role in controlling sexual behavior.

Second, postnatal exposure to picrotoxin may induce specific alterations related to GABA neurotransmission. In fact, $GABA_A$ receptors are characterized by an extensive structural heterogeneity based on a family of at least 15 subunits encoded by different genes (Cherubini et al., 1991; Paysan et al., 1994). This heterogeneity might be of particular significance during ontogeny, because GABA has been suggested to exert neurotrophic functions in the immature brain (Chronwall and Wolff, 1980; Ma et al., 1992, 1993; Fritschy and Mohler, 1995; Hornung and Fritschy, 1996; Lauder et al., 1986; Laurie et al., 1992). The expression of $GABA_A$ receptor subunits is developmentally regulated, suggesting that neurons possess distinct receptor subtypes at various stages of brain maturation. A major switch in the expression of $GABA_A$ receptor subunits has been demonstrated in the developing rat brain, with receptors containing the α_2-subunit being replaced postnatally by receptors containing the α_1-subunit (Fritschy et al., 1994; Poulter et al., 1993). The α_1-subunit, one the most abundant subunits in the adult brain (Benke et al., 1991; Fisman et al., 1993; Poulter et al., 1993), has a delayed appearance, which coincides with the formation of inhibitory circuits. The functional significance of this switch in receptor subtypes remains unknown. $GABA_A$ is excitatory in the developing brain (Cherubini et al., 1991; Hornung and Fritschy, 1996) and is inhibitory postnatally (even by PND5) as well as in adult age (Hornung and Fritschy, 1996). Thus, the expression of distinct receptor subtypes during development provides a molecular basis for the changes observed here. In fact, although speculative, it is possible that early postnatal exposure to picrotoxin interfered with the switch of $GABA_A$ receptors, which remained excitatory. This explains the improvement of sexual behavior observed here because the GABAergic system normally inhibits sexual behavior (Fernández-Guasti et al., 1986).

On the other hand, the GABAergic system is in balance with other neurotransmission systems, so that the interference with one system could interfere with others, possibly causing the behavioral modifications observed here.

Central dopaminergic systems are neural substrates of male sexual motivation aspects and behavior (Meyerson et al., 1979; Mas et al., 1987; Ahlenius et al., 1987; Hull et al., 1986, 1999). There are several lines of evidence showing that the increase in GABAergic system activity induces central DA system inhibition (Perez de La Mora et al., 1975; Lloyd et al., 1980; Fuxe et al., 1975). Thus, it is possible that perinatal treatment altered the DA system activity through the GABAergic system. The picrotoxin administered to the dams reached the offspring through milk and blocked their GABAergic receptors during early development. These receptors might have been subsensitive and less responsive to endogenous GABAergic neurotransmitters in adult age, with the consequent facilitation of male sexual behavior observed. However, the central nervous system is complex and interactions between neurotransmitter systems are poorly understood. On this basis, the interference with one of these systems could change the activity of another system. Thus, the interpretation of these data is an initial step to understand the effect of perinatal exposure to drugs that change neurotransmitter systems.

Our animals had no previous sexual experience. Usually, sexual behavior improves with experience and the first test might not be a reliable index of the execution of the behavior (Hucke et al., 1998). Because our animals had no sexual experience, it is not possible to determine to what extent the results of the present investigation were really related to sexual behavior and to what extent they were due to the lack of sexual experience. In this respect, a previous study from our laboratory (Teodorov et al., 2002) showed that perinatal exposure of dams to picrotoxin also improves the sexual behavior of male rats and that sexual experience revealed this effect.

Finally, although GABAergic compounds inhibit sex behavior, most of them induce the inhibition at doses that have clear motor inhibitory effects. Agmo et al. (1987) showed that an impairment of motor execution and not of locomotion activity is related to the effects of GABAergic drugs on sexual behavior. In the present study, animal activity was measured in an activity meter and not in a rotaroad as used by Agmo et al. (1987). Thus, the interference of postnatal picrotoxin exposure and the offspring motor execution related to the sexual behavior alteration observed here need additional investigations.

Acknowledgements

This research was supported by a fellowship from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Proc. 96/04273-4 and 06690-1) and from Conselho Nacional de Desenvolvimento Científico e

Tecnológico-CNPq to Maria Martha Bernardi (grant 352189/96-7).

References

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 1987;28:47–52.

Ahlenius S, Larsson K, Svensson L. Further evidence for an inhibitory role of central 5-HT in male rat sexual behavior. Psychopharmacology 1980;68:217–20.

Ahlenius S, Larsson L, Svensson L, Hjorts S, Carlsson A, Lindberg P, et al. Effects of a new type of 5-HT receptor agonist on male rat sexual behavior. Pharmacol Biochem Behav 1981;15:785–92.

Ahlenius S, Carlsson A, Hillegaart V, Hjorth S, Larsson K. Region-selective activation of brain monoamine synthesis by sexual activity in the male rat. Eur J Pharmacol 1987;144:77–82.

Altman J, Sudarshan K. Postnatal development of locomotion in the laboratory rat. Anim Behav 1975;23:896–920.

Benke D, Mertens S, Trzeciak A, Gillessen D, Mohler H. $GABA_A$ receptors display association of γ2-subunit with α1- and β2/3 subunits. J Biol Chem 1991;266:4478–83.

Cagiano R, De Salvia MA, Persichella M, Renna G, Tattoli M, Cuomo V. Behavioral changes in the offspring of rats exposed to diazepam during gestation. Eur J Pharmacol 1990;177:67–9.

Carlos CP, Lemonica YP, Kempinas WG, Pereira OCM. Does the male reproductive performance depend on the early lactation milk in rats? Physiol Behav 1996;59:147–52.

Cherubini E, Gaiarsa JL, Bem-Ari Y. GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 1991;14:515–9.

Chiavegatto S, Bernardi MM, De-Souza-Spinosa H. Effects of prenatal diphenhydramine administration on sexual behavior of rats. Braz J Med Biol Res 1989;22:729–32.

Chronwall B, Wolff JR. Prenatal and postnatal development of GABA-accumulating cells in the occipital neocortex of rat. J Comp Neurol 1980;190:187–208.

Felicio LF, Nasello AG. Effect of acute bromopride on rat prolactin levels and sexual behavior. Braz J Med Biol Res 1989;22:1011–4.

Fernández-Guasti A, Larsson K, Beyer C. Comparison of the effects of different isomers of bicuculline infused in the preoptic area on male rat sexual behavior. Experientia 1985;41:1414–6.

Fernández-Guasti A, Larsson K, Beyer C. GABAergic control of masculine sexual behavior. Pharmacol Biochem Behav 1986;24:1065–70.

Fieder B, Epstein S, Grimm VE. The effects of exposure to diazepam during various stages of gestation or during lactation on the development and behavior of rat pups. Psychopharmacology 1984;83:51–5.

Fisman ML, Behar T, Lange GD, Smith SV, Novotny EA, Barker JL. GABAergic cells and signals appear together in the early post-mitotic period of telencephalic and striatal development. Dev Brain Res 1993; 73:243–51.

Fritschy JM, Mohler H. $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 1995;359:154–94.

Fritschy JM, Paysan J, Enna A, Mohler H. Switch in the expression of rat GABAA receptor subtypes during postnatal development: an immunohistochemical study. J Neurosci 1994;14:5302–24.

Fuxe K, Kölkelt T, Ljungdahl A, Agnati L, Johansson O, Perez de La Mora M. Evidence for an inhibitory GABAergic control of the mesolimbic dopamine neurons: possibility of improving treatment of schizophrenia by combined treatment with neuroleptics and GABAergic drugs. Med Biol 1975;53:177–83.

Gessa GL, Tagliamonte A. Role of brain serotonin and dopamine in male sexual behavior. In: Sandler M, Gessa GL, editors. Pharmacology and biochemistry. New York: Raven Press; 1975 (117 pp.).

Hornung JP, Fritschy JM. Developmental profile of $GABA_A$-receptor in the marmoset monkey: expression of distinct subtypes in pre- and postnatal brain. J Comp Neurol 1996;367:413–30.

Hucke EE, Cruz-Casallas PE, Florio JC, Felicio LF. Reproductive experience reduces striatal dopaminergic responses in freely moving female rats. Neuroreport 1998;9:3589–93.

Hull EM, Bitran D, Pehek EA, Warner RK, Band LC, Holmes GM. Dopaminergic control of male sex behavior in rats: effects of an intracerebrally-infused agonist. Brain Res 1986;370:73–81.

Hull EM, Lorrain DS, Matuszewich L, Lumley LA, Putnam SK, Moses J. Hormone–neurotransmitter interactions in the control of sexual behavior. Behav Brain Res 1999;105:105–1116.

Johnson N, Leone F. Statistics and experimental design. Engineering and physiological sciences. New York: Wiley; 1974. p. 241–4.

Kacsóh B, Nagy GY, Veress Z, Toth BE, Kanyicska B, Csernus V, et al. Data suggesting that milk of early lactation period might be involved in sexual differentiation of rat brain. Endocrinology 1986;20:155–66.

Kellogg C, Tervo D, Ison J, Parisi L. Prenatal exposure to diazepam alters behavioral development in rats. Science 1980;207:205–7.

Lauder JM, Han VKM, Henderson P, Verdoorn T, Towle AC. Prenatal ontogeny of the GABAergic system in the rat brain: an immunocytochemical study. Neuroscience 1986;19:465–93.

Laurie DJ, Seeburg PH, Wisden W. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain: II. Olfactory bulb and cerebellum. J Neurosci 1992;12:1063–76.

Lloyd KG, Worms P, Zivkovic O, Sactton B, Bartholini G. Interaction of GABA mimetics with nigro striatal dopamine neurons. Brain Res Bull 1980;5:439–45.

Lyubimov BI, Smolmikova NM, Strekolova SN. Effects of diazepam on development of the offspring. Bull Exp Biol Med 1975;78:1156–8.

Ma W, Behar T, Barke JL. Transient expression of GABA immunoreactivity in the developing rat spinal cord. J Comp Neurol 1992;235:271–90.

Ma W, Saunders PA, Somogyl R, Poulter MO, Barker JL. Ontogeny of $GABA_A$ receptor subunit mRNAs in rat spinal cord and dorsal rat ganglia. J Comp Neurol 1993;338:337–59.

Malmnãs CO. Monoaminergic influence on testosterone-activated copulatory behavior in castrated male rat. Acta Physiol Scand Suppl 1973; 395:1–128.

Malmnãs CO. The significance of dopamine, versus other catecholamines, for L-DOPA induced facilitation of sexual behavior in the castrated male rat. Pharmacol Biochem Behav 1976;4:521–6.

Manning DE, Stoud AG, Jemp JW. Effects of maternal phenobarbital administration on same aspects of neonatal brain development. Fed Proc Fed Am Soc Exp Biol 1971;30:495.

Mas M, Del Castillo AR, Guerra M, Davidson JM, Battaner E. Neurochemical correlates of male sexual behavior. Physiol Behav 1987;41: 341–5.

Meyerson BJ, Palis A, Sietnieks A. Hormone–monoamine interactions and sexual behavior. In: Beyer C, editor. Endocrine control of sexual behavior. New York: Raven Press; 1979 (389 pp.).

Middaugh LD, Santos III CA, Zemp JW. Effects of phenobarbital given to pregnant mice on behavior of mature offspring. Dev Psychobiol 1975; 8:305.

Paredes RG, Agmo A. GABA and behavior: the role of receptor subtypes. Neurosci Biobehav Rev 1992;16:145–70.

Paysan J, Bolz J, Mohler H, Fritschy JM. The $GABA_A$-receptor α1-subunit: an early marker of cortical parcellation. J Comp Neurol 1994;350: 133–49.

Perez de La Mora M, Fuxe K, Hökfelt T, Ljungdahl A. Effect of apomorphine on the GABA turnover in the DA cell rich area of the mesencephalon. Evidence of the involvement of an inhibitory GABAergic feedback control of the ascending DA neurons. Neurosci Lett 1975; 1:104–14.

Poulter MO, Barker JL, O'Carrol AM, Lolait SJ, Mahan LC. Co-existence expression of GABAA receptor β2, β3, and γ2 subunit messenger RNAs during embryogenesis and early postnatal development on the rat central nervous system. Neuroscience 1993;53:1019–33.

Silva MRP, Oliveira CA, Felicio LF, Nasello AG, Bernardi MM. Perinatal

treatment with picrotoxin induces sexual, behavioral and neuroendocrine changes in male rats. Pharmacol Biochem Behav 1998;60:203–8.
Smart JL, Dobbing J. Vulnerability of developing brain: II. Effects of early nutritional deprivation on reflex ontogeny and development of behaviour on the rat. Brain Res 1971;28:85–95.
Soderstein P, Eneroth P. Effects of exposure to pups on maternal behaviour, sexual behaviour and serum prolactin concentrations in male rats. J Endocrinol 1984;102:115–9.
Teodorov E, Salzgeber SA, Felício LF, Varolli FM, Bernardi MM. Effects of perinatal picrotoxin and sexual experience on heterosexual and homosexual behavior in male rats. Neurotoxicol Teratol 2002;24:235–45.