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Perinatal maternal exposure to picrotoxin: Effects on sexual behavior in female rat offspring

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Abstract

A previous study in our laboratory showed that perinatal maternal picrotoxin exposure (0.75 mg/kg) in rats improved heterosexual behavior in male offspring. In the present study, we examined the effects of this maternal treatment on sexual behavior in the female offspring. The dams received 0.75 mg/kg picrotoxin treatment (PT) once a day on the 18th and 21th day of pregnancy, 2 h after parturition and once a day during the first 4 days of lactation. The results showed that (1) at birth, the body weight and anogenital distance were not modified by treatment; (2) female sexual behavior was improved in experimental animals. These results demonstrate that perinatal picrotoxin exposure improves adult sexual behavior in female rat offspring as suggested by increase in the lordosis quotient.

Keywords: Perinatal toxicology; Sexual development; Fertility; Stress; Reproductive behavior; Dopamine; GABA

1. Introduction

In gonadally intact female rats, the sequential release of ovarian estradiol followed by progesterone maximizes the probability that a female will assume the lordosis posture when mounted by a conspecific male (Uphouse, 2000). Female rat sexual behavior includes attractivity, proceptivity and receptivity (Beach, 1976; Clemens and Weaver, 1985). Attractivity refers to those characteristics of the female that increased the probability of male engaging in copulatory activity with that female. Behaviors which bring the female reproductive status to the attention of the male, followed by the pacing of the mating interaction (McCarthy and Pfaus, 1996) are generally referred to as proceptive behaviors. These aspects of the behavioral repertoire can be viewed as reflecting a motivational component (Pfaus and Everitt, 1995). Receptivity refers to the female's consummatory response (the lordosis reflex) required for successful copulation (Beach, 1976; Uphouse, 2000).

The lordosis reflex (arching of the back, elevation of the rump, dorsoflexion of the tail, and extension of the neck) is a stereotyped posture elicited by stimulation of cutaneous mechanoreceptors in the flank, rump, tail base, and perineum and is adopted by a sexually receptive female in response to tactile stimulation from the male (Pfaff, 1970). Since Beach (1976) introduced the lordosis quotient (or lordosis to mount (L/M) ratio $\times 100$) as a measure of lordosis frequency, this has been the most commonly assessed parameter of female rat sexual behavior. In a naturally cycling female rat, both estrogen and progesterone initiate sexual receptivity. Progesterone-induced lordosis facilitation may be due to activation of dopamine D1 receptors in the ventral tegmental area (Frye et al., 2004; Petralia and Frye, 2004; Apostolakis et al., in press; Sumida et al., 2005).

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In sexual behavior studies in our laboratory, the female rat lordosis reflex has been used to study the effects of prenatal and postnatal maternal exposure to several drug treatments on sexual behavior of male and female offspring observed in adult age (Silva et al., 1998; Sant'ana et al., 2001; Teodorov et al., 2002; Baso et al., 2003). The females were previously ovariectomized and, after 21 days (time necessary for endogenous female hormones to be eliminated from the animal body), the estrous was induced by pharmacological estrogen plus progesterone doses.

It is now known that female sexual behavior is controlled by the presence or absence of appropriate hormones and various central neurotransmitters, such as estrogen, progesterone, norepinephrine, dopamine, serotonin, GABA, endogenous opiates and several peptides essential to the copulatory behavior control. Thus, this behavior is abolished by ovariectomy but was restored by estrogen–progesterone replacement. GABAergic drugs when administered during pregnancy produce both anatomic and neurologic malformations in the offspring (Manning et al., 1971; Middaugh et al., 1975) as well as sexual dysfunction in later life (Cagiano et al., 1990).

A previous study from our laboratory on sexual brain differentiation of rats showed that maternal exposure to picrotoxin (0.75 mg/kg immediately after parturition and once a day during the first 5 days of lactation) during the perinatal sexual differentiation period of the central nervous system leads to sexual behavior injuries in male offspring, suggesting occurrence of demasculinization similar to those produced by stress (Silva et al., 1998). In another study, data showed that heterosexual behavior was improved while picrotoxin treatment (2 h after birth and once a day during the first 5 days of lactation) reduced the lordotic response of homosexual behavior in inexperienced male rats (Teodorov et al., 2002). Similar results were obtained when picrotoxin was injected postnatally in dams, 2 h after birth. 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 (Silva et al., 1998; Teodorov et al., 2002; Baso et al., 2003), suggesting that different mechanisms mediated the sexual interferences produced by perinatal picrotoxin exposure.

The purpose of the present study was to evaluate the effects of perinatal exposure to picrotoxin administered once a day on GD18 and GD21 only, as well as in the first 5 days of lactation (postnatal day PND1–PND5) on some reproductive parameters of female offspring rats during development and on sexual behavior at adult age.

2. Methods

2.1. Animals

Male and female Wistar adult rats from the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo were used. The animals were housed in polypropylene cages (32 × 40 × 18 cm) under controlled temperature (22 ± 2 °C), with a 12 : 12 light/dark schedule (lights on at 06:00 h) and free access to food and water during the experimental procedure. The animals used in this study were maintained in accordance with the guidelines from the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

2.2. Preparation of animals

Twenty adult female rats were randomly divided into two groups with 10 animals per group. The female rats were placed overnight with one untreated sexually experienced male rat for mating. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears on the following morning and was considered day 1 of pregnancy (GD1). Pregnant rats were housed two per cage until GD18 and then individually until the end of experiments. The picrotoxin (0.75 mg/kg sc, Sigma) or distilled water (1 ml/kg sc) administrations occurred once a day on GD18 and GD21 only as well as in the first 5 days of lactation (postnatal day PND1–PND5) in the morning (09:00 h) during the gestation and lactation periods and 2 h after parturition. Picrotoxin dose of 0.75 mg/kg was chosen after previous studies in our laboratory (Silva et al., 1998) and the dilution was in distilled water. This dose did not induce mother and fetal intoxication, and it did not cause convulsive symptoms and significantly increased plasmatic corticosterone levels in the prenatal period (Silva et al., 1998). No cross-fostering procedure was used (Chiavegatto and Bernardi, 1991). Parturition was considered PND0 and all litters were examined externally, sexed and weighed, with eight pups (four males and four females) left with each dam until weaning (PND21).

The anogenital distance and body weight were obtained at birth. A pachymeter was used to measure the anogenital distance considered as the length (millimeters) from anal opening to genital. The unit of analysis was the litter. On PND21, the littermates were separated, housed together by gender and grouped under same laboratory conditions as their parents. One animal of each litter was used for the experiment in adulthood. Female offspring aged 100 days were used to investigate the sexual behavior.

2.3. Female sexual behavior

Sexual receptivity was quantified using a previously reported system (Felicio and Nasello, 1989). All tests for evaluation of sexual behavior in female rats were performed in 12-h inverted light/dark cycle (lights on at 22:00 h). The sexual behavior was observed in a wooden cage (56 × 35 × 31 cm) provided with a moveable cover and frontal glass. A layer of sawdust served as bedding for the animals. During observation, a lamp of 40 W provided room illumination with a red filter. The minimum period

Table 1
Effects of perinatal treatment with picrotoxin (0.75 mg/kg) once a day from GD18, GD21 and PND1 to PND5 on body weight and body weight gain (g) and anogenital distance (mm) of offspring

Parameters	Groups	
	Control	Experimental
Body weight (g) — Days		
1	5.9±0.4	6.0±0.3
10	16.9±0.6	15.4±0.4
21	40.4±2.7	40.0±2.2
60	220.0±8.5	207.4±9.4
75	252.1±9.4	236.1±6.2
Body weight gain (g)		
1–10 days	11.0±0.2	9.3±0.1
11–21 days	23.6±2.1	24.6±1.8
Anogenital distance (mm)	2.4±0.2	2.1±0.3

There are no differences between groups in relation to body weight and body weight gain ($P>.05$ — two-way ANOVA followed Student's t test). There are no differences between groups in relation to anogenital distance ($P>.05$ — Student's t test). The anogenital distance was measured in the first postnatal day. Data are reported as the means±SEM. The unit of analysis was the litter ($n=10$).

considerated for animal adaptation to the inverted light/dark cycle was 15 days before the beginning of sexual behavior studies.

On PND100, one rat of each litter was used for the mating tests. To investigate sexual behavior, female rats of each litter were ovariectomized and sexually activated with exogenous estradiol (50 μg/kg sc, 54 h before the tests) and progesterone (2.0 mg/kg sc, 6 h before the tests) and mated with a sexually experienced stud male rat (male rats with previous sexual experience which had not been used in another experimental procedure), after a recovery period following ovariectomy for 15 days. The time and dose of progesterone and estradiol established before sexual tests were used in our laboratory because they were appropriate to induce sexual receptivity in females. Briefly, the female rat sexual behavior is characterized by a series of mount, with or without vaginal insertion by the male, and the female responds to each mount with a lordosis response: a dorsoflexion of the spine and deflection of the tail to one side allowing vaginal access to the male.

The following parameters of female sexual behavior were recorded: first mount and lordosis latencies, full time for 10 mounts, number of lordosis in ten mounts and lordosis quotient (LQ: number of lordosis/number of mounts × 100). All sexual behavior tests were held 4–8 h after the beginning of the dark period.

2.4. Statistical analysis

Data of pups' body weights were analyzed prior to a two-way (days × treatment) analysis of variance (ANOVA). The Student's t test was employed for anogenital distance as well as post hoc test when no interaction was observed between factors. For the sexual behavior (nonparametric data), the Mann–Whitney U test was used. In all cases, results were considered significant for $P<.05$.

3. Results

The two-way ANOVA was applied between body weight and body weight gain data of female offspring exposed or not to picrotoxin showed that no interaction occurred between groups. Thus, the Student t test revealed that there are no significant differences between groups, as well as in relation to anogenital distance (Table 1).

Female rats perinatally exposed to picrotoxin exhibited a more intense reproductive behavior than control animals. A significant increase in the lordosis quocient (LQ) was observed in experimental females compared to control animals (Fig. 1), whereas the other parameters were not altered (Table 2).

4. Discussion

The present study indicates that maternal perinatal picrotoxin exposure did not induce maternal toxicity, revealed by the lack of effects on dam body weight or food and water consumption (data not shown). The 0.75 mg/kg picrotoxin dose was chosen after previous studies in our laboratory (Silva et al., 1998). This dose did not induce mother and fetal intoxication, nor did it not cause convulsive symptoms. However, it significantly increased plasmatic corticosterone levels in the prenatal period (Silva et al., 1998).

A previous study in our laboratory on sexual brain differentiation of rats showed that maternal exposure to picrotoxin (0.75 mg/kg immediately after parturition and once a day during the first 5 days of lactation) during the perinatal sexual differentiation period of the CNS led to sexual behavior injuries in male offspring, suggesting

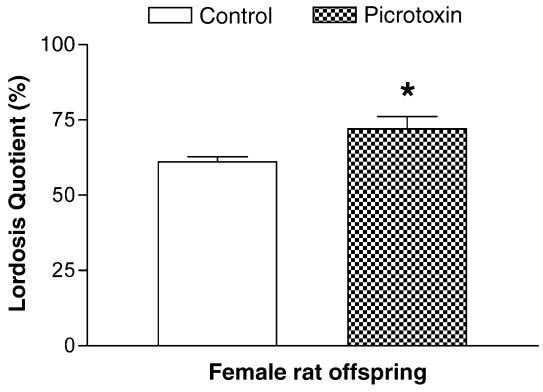

Fig. 1. Effects of maternal administration of picrotoxin (0.75 mg/kg) once a day from GD18, GD21 and PND1 to PND5 in lordosis quotient (LQ) of adult female offspring. Data are reported as the means±SEM ($n=10$/group). *$P<.05$ compared to the control group (Mann–Whitney U test). (LQ=number of lordosis/number of mounts × 100).

Table 2
Effects of perinatal treatment with picrotoxin on sexual behavior in female rat offspring

Parameters	Groups	
	Control	Picrotoxin
Latency to first mount (s)	11.9±3.5	13.7±3.9
Latency to first lordosis (s)	21.1±0.1	17.1±0.2
Full time for 10 mounts (s)	167.7±5.0	160.4±4.7
Lordosis quotient (%)	61.0±1.8	72.0±4.2*

The parameters are presented as means±SEM for ten animals per group.
* $P<0.05$ in relation to a control group (Mann–Whitney U test).

occurrence of demasculinization (Silva et al., 1998) similar to those produced by stress. In another study, data showed that heterosexual behavior was improved while picrotoxin treatment (2 h after birth and once a day during the first 5 days of lactation) reduced the lordotic response of homosexual behavior in inexperienced male rats (Teodorov et al., 2002). Similar results were obtained when picrotoxin was injected postnatally in dams, 2 h after birth. 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, suggesting that different mechanism mediated the sexual interferences produced by perinatal picrotoxin exposure (Silva et al., 1998; Teodorov et al., 2002; Baso et al., 2003).

There is a correlation between the anogenital distance in infancy and the brain sexually dimorphic areas size in adult age (Faber and Hughes, 1992). These changes should be an indication of interference with brain sexual differentiation (Ixart et al., 1983). In this study, no differences in anogenital distance were detected between female control and experimental pups, suggesting no interference with female sexual organization.

The receptivity of female sexual behavior consists of the lordosis reflex: a characteristic arching of the back, elevating of the head and rump, and deflection of the tail. The reflexive nature of this response is generated by the requirement of somatosensory stimulation of the flank and perineal region of the female by the male and it is required for the male to gain intromissions and for the female to receive vaginocervical stimulation (McCarthy and Pfaus, 1996; Kow et al., 1985). In order to better understand the natural expression of female sexual behavior, it is important to distinguish the variables that modulate the proactive motivational components of sexual behavior and the lordosis reflex.

It is possible that picrotoxin induces moderate stress at fetal levels since subconvulsive dose of this drug showed an anxiogenic effect (Silva et al., 1995). Thus, the interference with female sexual behavior described in the present study can result from picrotoxin-induced moderate levels of anxiety associated with the presence of sexually receptive male (Domjan et al., 1992). This would lead the female rat to display a major performance of sexual behavior, i.e., an increase in the lordosis quotient. This fact could be considered an improvement of sexual behavior in rats.

The effects of prenatal or perinatal stress on the organization of the developing CNS and brain sexual differentiation of female rats are controversial. Beckhard and Ward (1983) studied the behavior and physiology of female offspring in rats stressed during pregnancy. Mothers were restrained and placed under bright, hot lights from day 14 through day 21 of gestation. This treatment, which is known to disrupt the sexual behavior of male offspring, did not alter the reproductive function of female offspring. The females showed evidence of normal cyclicity, sexual behavior, pregnancy, parturition, pup survival, and maternal behavior when tested beginning at 70 or at 140 days of age.

On the other hand, Herrenkhol (1979) showed that female rats subjected to prenatal stress later experienced fewer pregnancies, and fewer viable young than nonstressed rats. The offspring of the prenatally stressed rats were lighter in weight and less likely to survive the neonatal period. The author proposed that prenatal stress may influence the balance of adrenal and gonadal hormones during the critical stage of fetal hypothalamic differentiation, thereby producing a variety of reproductive dysfunctions in adulthood.

While the objectives of this study were primarily descriptive, the nature of the findings presented here deserves some thought about potential mechanisms involved in the perinatal action of picrotoxin, an antagonist drug that acts in $GABA_A$ receptors, in female sexual behavior.

In a naturally cycling female rat, both estrogen and progesterone initiate sexual receptivity. Estrogen prepares the female to show the reflex, while progesterone triggers the reflex (Knobil and Neill, 1984). In relation to neurotransmitters, central monoaminergic neurotransmission is very important to the control of lordosis reflex in female rats (Ahlenius, 1993), as well as the role of noradrenergic neurotransmission in ovulation LH-induced in these animals. These neurotransmitter systems are important to the control of lordosis and serves as a major link between endocrine status and this effect on sexual behavior.

Studies have demonstrated that estrogen potentiates the action of norepinephrine in sites of hypothalamus and that the norepinephrine is important in the natural expression of lordosis (Etgen and Petitti, 1986). In cycling female rodents, estrogen and progesterone are elevated when lordosis is displayed. Because of this, the relevance dopamine has in normal female sexual behavior is not clear. In female rats, dopamine release in the nucleus accumbens (Nacc) increases when the female is allowed to control (i.e., pace) the timing of the pair mating (Mermelstein and Becker, 1995; Pfaus et al., 1995). The timing of the copulatory behavior, however, is just one factor regulating the amount of dopamine released in the Nacc and striatum, but this function is believed to affect rewarding aspects of mating (Becker et al., 2001; Jenkins and Becker, 2003a,b). Estrogen and progesterone replacement to ovariectomized rats

increases dopamine content in the Nacc, whereas progesterone alone only increased dopamine in the ventral tegmental area (VTA) (Russo et al., 2003).

The role of serotonin in the regulation of lordosis has been extensively reviewed (Mendelson, 1992). It was concluded that serotonin can either inhibit or facilitate lordosis, depending on which subtypes of central serotonin receptors become activated. A lack of clear steroid modulation of the serotonin system led to the speculation that the effects of pharmacological manipulations of serotoninergic activity on lordosis would reflect a physiological role for this neurotransmitter (Mendelson, 1992; McCarthy and Pfaus, 1996).

Dopamine exerts its effects via at least five dopamine receptor (DAR) isoforms that are divided into two families: D1-like (D1 and D5) receptors and D2-like (D2, D3, and D4) receptors (Jackson and Westlind-Danielsson, 1994; Missale et al., 1998). Within the D1-like family, D1 and D5 dopamine receptors share 78% sequence homology; yet, they have different distributions in the brain, suggesting distinct functional roles (Sunahara et al., 1991). For example, neuropharmacological and molecular studies suggest a role for a D1-like DAR, particularly the D5, in controlling female rat sex behavior (Fienberg and Greengard, 2000; Mani, 2001). Dopamine can act via cyclic AMP (cAMP) that activates cAMP-dependent protein kinase, which stimulates phosphorylation of several neuronal phosphoproteins including dopamine- and cAMP-regulated phosphoprotein-32 (DARPP-32). DARPP-32 in turn may phosphorylate the progesterone receptor (PR) and enhance expression of female sexual behavior (Mani et al., 2000; Auger et al., 2001; Apostolakis et al., 2004; Kudwa et al., 2005).

Dopamine appears to facilitate lordosis by activation of the progesterone receptor, as well as progesterone-induced lordosis facilitation may be due to activation of dopamine D1 receptors in the VTA (Frye et al., 2004; Petralia and Frye, 2004; Apostolakis et al., in press; Sumida et al., 2005). In fact, there is well-established literature on the ability of estrogen to stimulate dopamine release (Becker, 1992). Thus, it appears that brain dopamine system can be primed by estrogens and may, therefore, play a role in female sexual behavior (McCarthy and Pfaus, 1996). Behaviorally effective doses of estradiol increase the phosphorylation of the DARPP-32 protein within the female hypothalamus, specifically the medial preoptica area (mPOA) (Auger et al., 2001). Vaginal cervical stimulation received during mating also induces the phosphorylation of DARPP-32 in these same areas (Meredith et al., 1998).

The dopamine appears to facilitate lordosis by activation of the progesterone receptor (Becker, 1992). The brain dopamine systems can be primed by estrogens and may, therefore, play a role in female sexual behavior (McCarthy and Pfaus, 1996). In male rats the GABAergic drugs affect sexual behavior only indirectly, via an impairment of motor execution (Agmo et al., 1987), but in female this parameter was not observed. The reduction of sexual behavior was always associated with reduced social interactions and exploratory behaviors (Agmo and Paredes, 1985).

It is now known that central dopaminergic systems are neural substrates for motivational aspects of male and female rat sexual behavior and that dopamine is intimately associated with GABAergic system. Pharmacological stimulation of dopamine receptors leads to an enhancement of sexual desire and arousal in human males and activates copulatory behavior in male and female rats (Sapolsky and Mcarrey, 1986). Inhibition of dopaminergic transmission has the opposite effect on rat sexual behavior (Felicio and Nasello, 1989). In male rats, blocking the access of dopamine to receptors in preoptic area slowed the rate of copulation, decreased ex copula erections and decreased specifically sexual motivation (Pehek et al., 1988). Thus, endogenous dopamine facilitates copulation and enhances genital reflexes and sexual motivation in male and female rats.

In the VTA, the site brain most studied for female rat sexual behavior, formation of progesterone metabolite 5α-pregnan-3α-ol-20-one (3α,5α-THP) is important for facilitating lordosis (Petralia and Frye, 2005). These authors observed that picrotoxin infusions blocked FGIN 1-27-mediated (mitochondrial benzodiazepine receptors — MBRs) increases in lordosis of rats and hamsters, proceptivity of rats, and sexual responsiveness of hamsters.

When inhibitors of progesterone biosynthesis and/or metabolism are applied to the VTA, 3α,5α-THP production and lordosis decline. 3α,5α-THP facilitates lordosis, in part, through its actions at γ-aminobutyric acid ($GABA_A$)/benzodiazepine receptor complexes, denominated GBRs. Activation of GBRs leads to membrane hyperpolarization. 3α,5α-THP is highly effective at increasing GBR-mediated inhibitory post-synaptic potentials and prolonging chloride channel opening duration (Majewska et al., 1986; Fancsik et al., 2000). As well, when applied to the VTA, 3α,5α-THP is one of the most effective progestins at enhancing GBR binding and at facilitating lordosis (Frye and Vongher, 1999). Thus, since that picrotoxin is an antagonist drug for $GABA_A$ receptors, probably it makes the same effects for promoting the facilitation in parameters of sexual behavior.

Notably, in the VTA, inhibiting GBRs attenuates, and activating GBRs, enhances progestin-facilitated lordosis of rodents (Frye and DeBold, 1992; Frye and Gardiner, 1996; Frye, 2001a,b,c). In rats and hamsters, picrotoxin infusions produced modest increases in general activity, probably related with dopaminergic system (Petralia and Frye, 2005). In the same study results provide information demonstrating that, in VTA, synthesis of 3α,5α-THP is important for facilitating proceptive and receptive behaviors of rodents and may facilitate an array of prosocial behaviors, in addition to lordosis, of female rodents. Thus, some other hypotheses can be raised to explain the role of GABAergic system on modulation of dopaminergic system to promote the results described here for female sexual behavior.

First, early postnatal exposure of the offspring to picrotoxin increased the excitability of the CNS and then facilitated female 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., 1986). This blockade of GABA receptors produced neuronal 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; Fritschy and Mohler, 1995; Hornung and Fritschy, 1996). 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 α1-subunit (Poulter et al., 1993; Fritschy et al., 1994). The α1-subunit, one the most abundant subunits in the adult brain (Benke et al., 1991; 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 in the present study. 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 (Fernández-Guasti et al., 1986).

Central dopaminergic systems are neural substrates for male and female sexual motivation aspects and behavior (Ahlenius et al., 1987; Hull et al., 1999). There are several evidences showing that the increase in GABAergic system activity induces central dopamine system inhibition (Fuxe et al., 1975; Lloyd et al., 1980; Perez de La Mora et al., 1975). Then, it is possible that the perinatal treatment with picrotoxin had altered dopaminergic system activity through the GABAergic system. Picrotoxin administered in dams would reach offspring through placenta and would block their GABAergic transmission during early development, promoting changes in $GABA_A$ receptors conformation. In adult animals, the GABA receptors might have become subsensitive and less responsive to endogenous GABAergic neurotransmission, permitting the dopaminergic system to be freely active. This may have led to the improvement of some female sexual behavior described here, eliminating the hypothesis of polycystic ovarian occurrence. On the other hand it is possible that perinatal exposure to picrotoxin interfered with the switch of $GABA_A$ receptors, which remained excitatory (Teodorov et al., 2002; Baso et al., 2003). This also explains the improvement of sexual behavior observed here, because the GABAergic system normally inhibits sexual behavior (Fernández-Guasti et al., 1986).

However, the central nervous system is complex and interactions between neurotransmitter systems are poorly understood. Thus, the interpretation of these data contributes to a better understanding of the effect of perinatal exposure to drugs that change neurotransmitter systems. The interference of perinatal picrotoxin exposure on female sexual behavior observed here needs further investigations.

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