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Prenatal stress produces deficits in socio-sexual behavior of cycling, but not hormone-primed, Long–Evans rats

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Abstract

Prenatal stress (PNS) alters behavior of adult offspring in novel environments or in social interactions; variable effects of PNS on female reproductive behavior have been reported. Effects of exposure to restraint and lights for 45 min/day on Gestational Days 14–20 were examined on the motor and socio-sexual behavior of adult female offspring. In a novel arena, proestrous PNS rats displayed greater behavioral inhibition as indicated by significantly fewer beam breaks made in the horizontal crossing task compared to that of proestrous non-PNS rats. In a standard mating test, in which females are exposed to males in a relatively small space for a restricted time or number of sexual contacts, PNS females in proestrus were found to have significant decreases in the intensity of lordosis and in the number of solicitation behaviors that they directed towards the male compared to non-PNS rats. In a seminatural mating test, in which females can control the timing of the sexual contacts from the male, PNS females in proestrus engaged in significantly less pacing of their sexual contacts compared to that of the non-PNS females. When additional PNS and non-PNS rats were ovariectomized (ovx) and tested following hormone priming, behavioral differences were abrogated. PNS decreased motor behavior in a novel arena, lordosis intensity, and solicitation behavior in a standard mating paradigm, as well as adaptive, approach–avoidance behavior in a seminatural mating situation of endogenously cycling proestrous rats but not ovx, hormone-primed rats. Thus, hormone priming may override or mask effects of PNS on some aspects of socio-sexual behavior.

Keywords: Lordosis; Receptivity; Progestin; Prenatal stress; Hypothalamic–pituitary–adrenal axis; Pacing; Long–Evans rats

1. Introduction

The offspring of pregnant animals that are exposed to gestational stress can display long-term physiological and behavioral changes. Varied means have been used to induce gestational stress (e.g. exposure to heat, shock, restraint, bright light, loud sound, social crowding or disruption, handling, or saline injection). Consistent between stressors, and across species, are the profound and relatively uniform effects produced in offspring born of stressed mothers (Morgan et al., 1999).

Prenatal stress (PNS) alters physiological parameters, such as endocrine responses. Infant rats exposed to PNS show disruption of the hypothalamic–pituitary–adrenal axis (Weinstock, 1997). Animals exposed to PNS typically have higher basal levels of plasma cortisol or corticosterone (Clarke et al., 1994; Fride et al., 1986) and a greater and/or prolonged elevation of corticosterone in response to environmental challenge compared to animals not exposed to PNS (Fride et al., 1986; Henry et al., 1994; McCormick et al., 1995; Takahashi et al., 1990, 1992b; Weinstock et al., 1992).

Concomitant with endocrine changes, which suggest heightened sensitivity to disturbance, PNS animals show more pronounced behavioral reactivity to external stimuli (Ader and Plaut, 1968; Clarke and Schneider, 1993; Fride et al., 1986; Hockman, 1961; Polytrev et al., 1996; Takahashi et al., 1992a; Thompson, 1957). For example, PNS rats demonstrate behavioral changes that are typically inter-

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preted as indicating increased fearfulness/anxiety (Becker and Kowall, 1977; Masterpasqua et al., 1976; Vallee et al., 1997). PNS rats display reduced motor behavior when placed in a novel open field compared to non-PNS controls (Fride et al., 1986; Thompson, 1957). Similarly, the behavior of PNS rats in the elevated plus-maze is characterized by reduced exploration, as indicated by fewer open-arm entries and less time spent in the open arms, compared to non-PNS control rats (Polytrev et al., 1996; Schmitt and Hiemke, 1998; Wakshlak and Weinstock, 1990).

In addition to behavioral inhibition in response to novel or environmental challenges, PNS animals also show behavioral disturbances in social situations. Juvenile rhesus monkeys, whose mothers have been exposed to unpredictable noise stress during gestation, are socially inhibited and show increased rates of abnormal behavior, such as self-clasping, when placed in a novel group situation (Clarke and Schneider, 1996). Exposure to PNS also affects social behavior of rodents. Decreased rates of social play are seen in juvenile rats exposed to PNS compared to non-PNS controls (Takahashi et al., 1992a). As well, female mice that were exposed to PNS show increased postpartum aggression when presented with a social challenge (Kinsley and Svare, 1988). Male PNS mice also show increased aggression in a resident–intruder paradigm than do control mice (Kinsley and Svare, 1986). Together, these studies suggest that PNS animals are hyperreactive (both physiologically and behaviorally) to stressful stimuli, including novel social stimulation.

The socio-sexual behavior of rodents may be directly influenced by changes in endocrine parameters that are produced by exposure to PNS. For example, PNS male rats can show disrupted sexual behavior. Exposure to PNS demasculinizes and feminizes sexual behavior of male rats (Dahlof et al., 1977; Dunlap et al., 1978; Gotz and Dorner, 1980; Meisel et al., 1979; Rhees and Fleming, 1981; Velazquez-Moctezuma et al., 1993; Ward, 1972, 1977; Ward and Reed, 1985; Whitney and Herrenkohl, 1977), suppresses testosterone secretion (Ward and Weisz, 1980; Ward et al., 1994), and attenuates sexual dimorphisms in enzymes that metabolize testosterone, as well as sexual dimorphisms in catecholamine levels (Reznikov et al., 2001).

The effect of PNS on socio-sexual behavior and physiology of female rats is less clear. Some reports indicate that the estrous cycle (Herrenkohl and Politch, 1978), sexual receptivity (Gutierrez et al., 1989; Rojo et al., 1986), and pregnancy (Herrenkohl and Scott, 1984) are disrupted, fertility and fecundity are reduced (Herrenkohl, 1979), and adrenal, ovarian, and uterine weights are decreased at autopsy (Herrenkohl and Scott, 1984) in PNS female rats as compared to non-PNS controls. However, there are other reports that estrous cyclicity (Beckhardt and Ward, 1983), sexual behavior (Beckhardt and Ward, 1983; Herrenkohl and Scott, 1984), pregnancy (Beckhardt and Ward, 1983), parturition (Beckhardt and Ward, 1983), pup survival (Beckhardt and Ward, 1983), maternal behavior (Beckhardt and Ward, 1983), and adrenal and ovarian weights (Rojo et al., 1986) are not different in PNS compared to non-PNS control female rats. It has been proposed that some of the discrepant findings between laboratories may reflect some investigators' examination of PNS effects on reproductive parameters of endogenously cycling rats, while other investigators have utilized ovariectomized (ovx), hormone-primed rats (Beckhardt and Ward, 1983).

The present experiments were conducted to further examine effects of PNS on socio-sexual behavior of female rats. Some laboratory paradigms used to assess reproductive behavior of female rats vary considerably in the manner in which sexual behavior is investigated. Previous investigations of PNS effects on lordosis have utilized a standard mating paradigm in which males typically control sexual contacts (due to the small size of the testing arena). Because the response to a novel environment or a social challenge has been demonstrated to be different in PNS compared to control rats, we compared the socio-sexual behavior of PNS and non-PNS rats in a standard mating paradigm, in which the female has less control of sexual contacts, and in a seminatural mating paradigm, in which the female has more control over the sexual contacts. If PNS alters endocrine and reproductive responses in female rats, we predicted that standard measures of sexual behavior would be disrupted. If PNS effects on reproductive behavior are a sole consequence of dysregulation of social behavior, then we would predict that only approach–avoidance behavior, which is quantifiable in the seminatural mating paradigm, would be disrupted. To address effects of PNS on reproductive behavior of naturally cycling (Experiment 1) and ovx, hormone-primed (Experiment 2) rats, experiments were conducted using both groups of subjects.

2. Method

These methods were preapproved by the Institutional Animal Care and Use Committee.

2.1. Animals and housing

Long–Evans rats, approximately 55 days of age, were bred in our laboratory from stock previously obtained from Taconic Farms (Germantown, NY). Rats were group housed (four per cage) in polycarbonate cages ($45 \times 24 \times 21$ cm) until breeding and thereafter were housed individually in a temperature-controlled room (21 ± 1 °C) in the laboratory animal care facility. The rats were maintained on a 12/12-h reversed light cycle (lights off 08:00 h) with access to Purina Rat Chow and tap water in their home cages.

Female rats ($n = 30$) were screened daily between 07:00 and 09:00 h to determine the day of the estrous cycle. Rats were cycled through two normal estrous cycles (4–5-day cycle), and then 17 were successfully mated on behavioral estrus. Fourteen days following mating, pregnant rats were

randomly assigned to receive 45 min of restraint stress, under bright lights, in a Plexiglas restrainer ($n=10$, PNS condition) everyday between Gestational Days 14 and 20. Rats in the control condition ($n=7$) remained undisturbed in their home cages. NB: We were unable to prevent litter effects by taking one pup to represent each litter (as utilization of 84 litters was not possible). However, we did attempt to counterbalance the assignments of female offspring from each litter to Experiments 1 and 2 by including one to three pups from each litter to each group. Cursory analyses of the maternal origin revealed no effects of cohort or litter on the behavioral outcomes investigated.

2.2. Behavioral testing

For Experiment 1, the female offspring of the dams ($N=58$; $n=31$ were from dams that were stressed and $n=27$ were from dams that were not stressed) were cycled when they were over 60 days of age. Rats in proestrus (also known as behavioral estrous) were tested in the behavioral tasks described below. For Experiment 2, adult female offspring ($N=26$; $n=13$ were from dams that were stressed and $n=13$ were from dams that were not stressed) were anesthetized with 60 mg/kg ip rhompun and 80 mg/kg ip ketaset so that the ovaries could be removed and rats could receive a subcutaneous silastic implant (0.062 mm i.d., 0.125 mm o.d.) containing estradiol-17 benzoate (EB; Sigma, St. Louis, MO, BETA-E_2 3-Benzoate, 30 mg/mm; 10 mm/100 g body weight). Four hours prior to behavioral testing, rats were injected with 500 μg progesterone sc.

All of the rats were exposed to the tasks consecutively and without any rest periods in the order indicated.

2.2.1. Horizontal crossings

Rats were placed in a brightly lit $39 \times 39 \times 30$ cm Digiscan Optical Animal Activity Monitor (Accuscan Instruments, Columbus, OH). The number of beam breaks that occurred in a 5-min test was mechanically recorded.

2.2.2. Standard test for sexual behavior

To assess sexual receptivity, experimental females were placed in a glass aquarium ($25 \times 48.5 \times 30$ cm) with a sexually experienced male. The male was allowed to mount the female 10 times or for a total interaction time of 10 min, whichever occurred first. Receptivity of rats was quantified by rating dorsiflexion during lordosis (0–3) (Hardy and DeBold, 1971, 1972, 1973). Lordosis quotients [LQ=(no. of lordosis responses/no. of contacts) × 100], lordosis ratings [LR=(summation of lordosis rating for all contacts/no. of contacts)], and proceptivity quotients [PQ=(no. incidents of solicitation behaviors (hopping, darting, ear wiggling)/ no. of contacts) × 100] were calculated and used for statistical analyses (Frye et al., 1996a,b). In our laboratory, interrater reliability for these indices of female sexual behavior has a concordance rate of greater than 95%.

2.2.3. Pacing test for sexual behavior

Paced mating tests were carried out using previously reported procedures (Erskine, 1985; Frye and Erskine, 1990). Paced mating tests occurred in a large chamber ($37.5 \times 75 \times 30$ cm), which was equally divided by a partition that had a small (5 cm in diameter) hole in the bottom center, to allow the female free access to the male's side of the cage. A pacing test was initiated when a receptive female was placed in the side of the chamber opposite the male rat. During the test, the female freely entered and left the chamber containing the male. The male was unable to follow the female to the other side of the test chamber because he was too big to fit through the hole and he was conditioned to stay away from the hole (by tapping the male on the nose with a marking pen each time he was within one body length of the hole). Rats were behaviorally tested for an entire ejaculatory series. Behaviors recorded were the frequency of mounts and intromissions that preceded an ejaculation. Pacing measures included the percentage of times that the female left the compartment containing the male after receiving a particular copulatory stimuli (%

Table 1
Summary of data (mean ± S.E.M.) for Experiments 1 and 2

	Spontaneous cycling (Experiment 1)		Hormone primed (Experiment 2)	
	Non-PNS	PNS	Non-PNS	PNS
Beam breaks (s)	1447.6 ± 75.3	1240 ± 61.6 *	1189.0 ± 62.6	1153.2 ± 66.2
Lordosis quotient (%)	99.3 ± 0.5	95.5 ± 4.1	90.8 ± 3.1	91.5 ± 4.2
Lordosis rating	2.26 ± 0.06	1.97 ± 0.09 *	1.64 ± 0.13	1.67 ± 0.14
Proceptivity quotient (%)	29.6 ± 3.8	16.5 ± 2.6 *	25.4 ± 7.0	26.9 ± 5.8
Percent exits following mounts (%)	25.5 ± 5.5	10.0 ± 3.5 *	29.2 ± 6.9	11.3 ± 3.8 *
Percent exits following intromissions (%)	43.2 ± 7.0	29.2 ± 4.7 **	8.6 ± 1.5	9.1 ± 1.0
Percent exits following ejaculations (%)	88.9 ± 6.2	67.7 ± 8.5 *	66.7 ± 16.7	75.0 ± 16.4
Return latency following mounts (s)	47.4 ± 13.0	25.1 ± 3.8 **	32.6 ± 10.4	39.0 ± 9.0
Return latency following intromissions (s)	32.0 ± 4.8	27.6 ± 2.7	74.6 ± 11.0	111.8 ± 19.3
Return latency following ejaculations (s)	351.3 ± 20.3	390.8 ± 26.8	280.7 ± 54.9	322.2 ± 35.2

* Indicates a significant difference ($P<.05$) between non-PNS and PNS groups for the indicated measure for the particular experiment.

** Indicates a tendency ($P<.10$) to be different between groups for the indicated measure for the particular experiment.

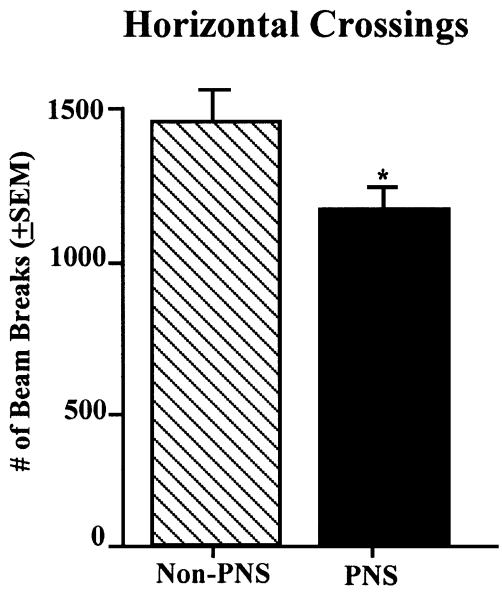

Fig. 1. The mean (±S.E.M.) number of beam breaks made during 5 min in the horizontal crossing apparatus of nonprenatally stressed female rats in behavioral estrus (non-PNS; striped bars) and prenatally stressed female rats in behavioral estrus (PNS; black bars). Asterisk (*) indicates a significant difference between groups ($P < .05$).

exits after mounts, intromissions, and ejaculations) and latencies in seconds to return to the male compartment after these stimuli (mounts, intromissions, and ejaculations).

2.3. Statistical analyses

Multiple one-way analyses of variances (ANOVAs) were used to examine effects of PNS condition on motor behavior (no. of horizontal crossings) and sexual behavior in the standard (lordosis quotients, lordosis rating, proceptivity quotient, and number of contacts) and pacing tests (percent exits and return latencies following mounts, intromissions, and ejaculations). Where appropriate, ANOVAs were followed by Fisher's *post hoc* tests and least-squares mean comparisons between groups.

3. Results

All results are summarized in Table 1.

3.1. Experiment 1: effects of PNS on behavior of rats in behavioral estrus

3.1.1. Horizontal crossings

Female rats in behavioral estrus that were prenatally stressed made significantly fewer beam breaks in the horizontal crossing task than did proestrous females that had not been prenatally stressed [$F(1,56) = 4.642$, $P \leq .03$; see Fig. 1 and Table 1].

3.1.2. Standard mating test

There was no significant difference in the lordosis quotients of PNS and non-PNS female rats [$F(1,56) = 0.741$, $P \geq .39$; see Fig. 2, top and Table 1] that were in behavioral estrus. There was a significant decrease in the lordosis rating of the proestrous rats that had been prenatally stressed compared to those that were not prenatally stressed

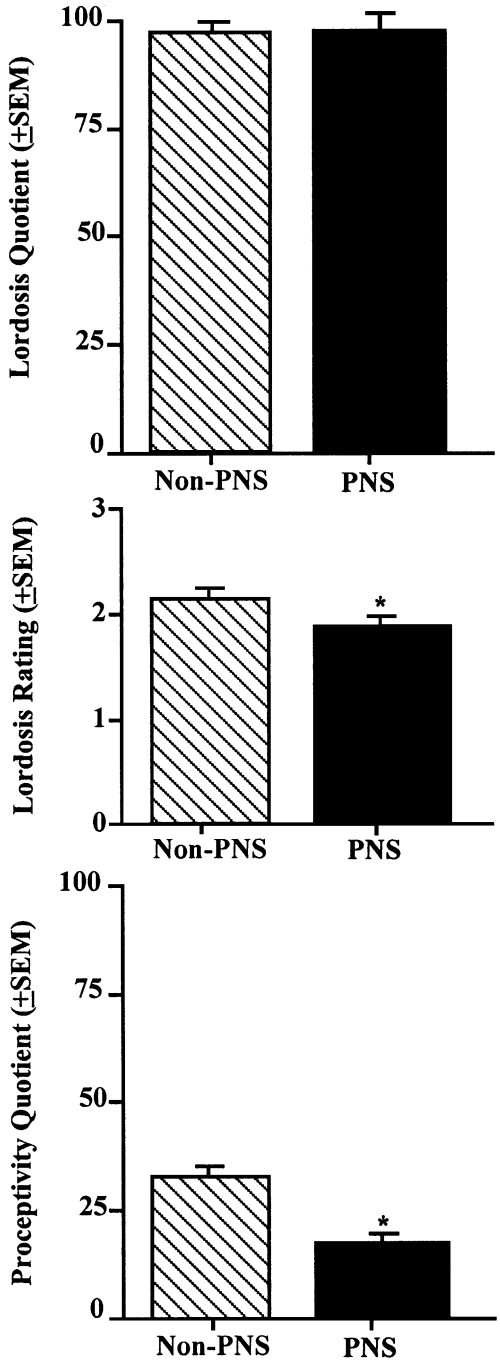

Fig. 2. The mean (±S.E.M.) lordosis quotients (top panel), lordosis ratings (middle panel), and proceptivity quotients (bottom panel) of nonprenatally stressed (non-PNS; striped bars) and prenatally stressed (PNS; black bars) female rats in behavioral estrus that were tested in the standard mating test. Asterisk (*) indicates a significant difference between groups ($P < .05$).

[$F(1,56) = 5.822$, $P \leq .01$; see Fig. 2, middle and Table 1]. Proceptivity quotients were also significantly lower among the behavioral estrus PNS rats compared to the rats that were not prenatally stressed [$F(1,56) = 8.768$, $P \leq .005$; see Fig. 2, bottom and Table 1].

3.1.3. Paced mating test

Prenatally stressed rats in behavioral estrus were significantly less likely to leave the male side of the pacing chamber following a mount compared to the non-PNS female rats; this is demonstrated by the significant decrease in the percentage of exits following a mount [$F(1,56) = 5.955$, $P \leq .01$; see Fig. 3, top left and Table 1]. Proestrous rats that had been prenatally stressed also tended to make fewer exits from the male side of the chamber following an intromission, compared to the percentage of exits that the non-PNS females demonstrated [$F(1,56) = 2.884$, $P \leq .09$; see Fig. 3, top middle and Table 1]. The percentage of exits from the male side of the chamber after ejaculation was also significantly lower in the proestrous PNS rats as compared to the non-PNS rats [$F(1,56) = 3.828$, $P \leq .05$; see Fig. 3, top right and Table 1.

Prenatally stressed rats in behavioral estrus tended to spend less time away from the male side of the pacing chamber following a mount compared to the non-PNS female rats [$F(1,56) = 3.053$, $P \leq .08$; see Fig. 3, bottom left and Table 1]. There were no significant differences in the interintromission interval [$F(1,56) = 0.673$, $P \geq .41$; see Fig. 3, bottom middle and Table 1] or the postejaculatory interval [$F(1,56) = 1.316$, $P \geq .25$; see Fig. 3, bottom right and Table 1] between PNS and non-PNS females that were tested on proestrus.

3.2. Experiment 2: effects of PNS on behavior of hormone-primed rats

3.2.1. Horizontal crossings

There were no significant differences [$F(1,24) = 0.154$, $P \geq .69$] in the number of beam breaks in the horizontal

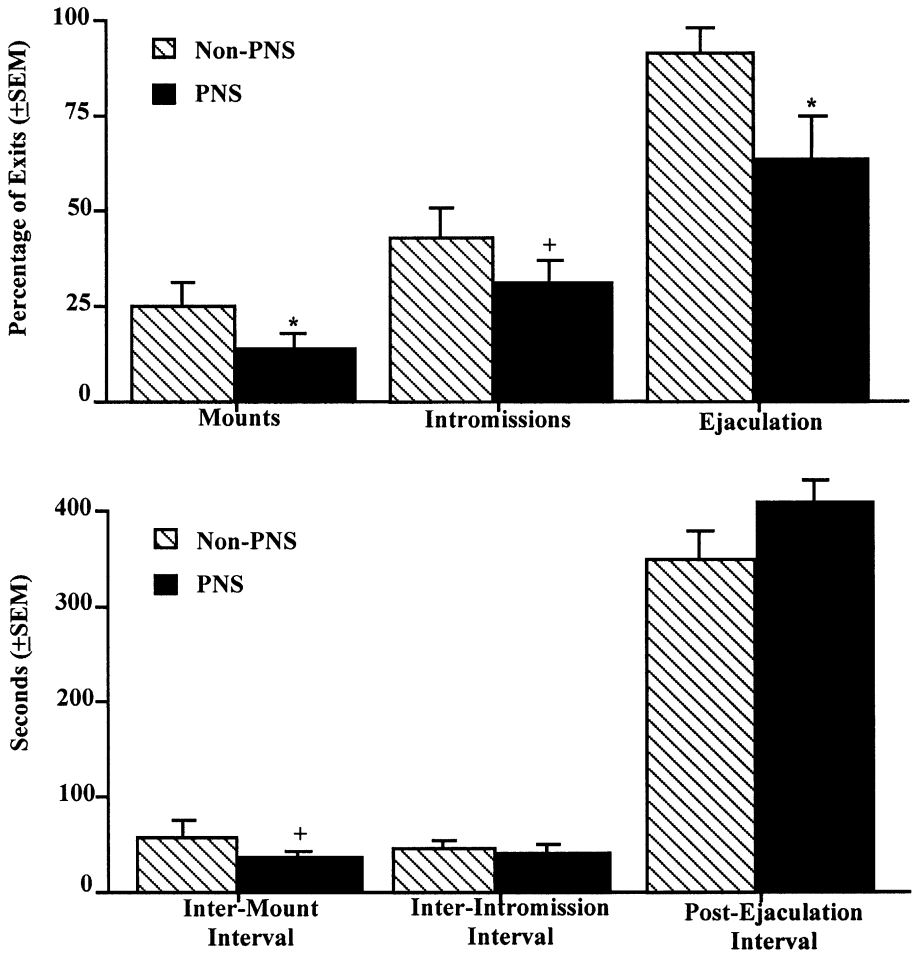

Fig. 3. The mean (±S.E.M.) percentage of exits (top panel) and time in between contacts by the male (bottom panel) of nonprenatally stressed (non-PNS; striped bars) and prenatally stressed (PNS; black bars) female rats in behavioral estrus that were tested in the paced mating test. Asterisk (*) indicates a significant difference between groups ($P < .05$). Plus sign (+) indicates a tendency to be different between groups ($P < .10$).

crossing task of hormone-primed females that had been prenatally stressed compared to those that had not been stressed (see Table 1).

3.2.2. Standard mating test

There were no significant differences in the lordosis quotients [$F(1,24) = 0.022$, $P \geq .88$], lordosis ratings [$F(1, 24) = 0.025$, $P \geq .87$], or proceptivity quotients [$F(1,24) = 0.028$, $P \geq .86$] of hormone-primed PNS and non-PNS female rats (see Table 1).

3.2.3. Paced mating test

Hormone-primed, PNS rats were significantly less likely to leave the male side of the pacing chamber following a mount compared to the non-PNS rats; this is demonstrated by the significant decrease [$F(1,24) = 5.145$, $P \leq .03$] in the percentage of exits of the PNS compared to the control rats following a mount. Hormone-primed PNS rats made no fewer exits from the male side of the chamber following an intromission [$F(1,24) = 0.465$, $P \geq .50$] or an ejaculation [$F(1,24) = 0.084$, $P \geq .77$] as compared to the percentage of exits that the non-PNS females demonstrated (see Table 1).

There were no significant differences in the time away from the male side of the pacing chamber of the hormone-primed PNS rats following a mount [$F(1,24) = 0.208$, $P \leq .65$], intromission [$F(1,24) = 2.772$, $P \geq .11$], or ejaculation [$F(1,24) = 0.379$, $P \geq .54$] compared to the non-PNS rats (see Table 1).

4. Discussion

Proestrous PNS rats showed increased anxiety behavior, reductions in reproductive parameters, and were less likely to pace their sexual contacts compared to control, proestrous rats. In support, proestrous PNS rats made significantly fewer beam breaks when placed in a novel arena compared to that of proestrous non-PNS rats, suggesting that PNS alters anxiety behavior of cycling rats. PNS rats in proestrus showed significantly decreased lordosis ratings and manifest fewer proceptive behaviors compared to that of proestrous non-PNS rats, indicating that PNS reduces reproductive behaviors of cycling, receptive rats. The cycling PNS rats also showed some evidence for decreased pacing of their sexual contacts compared to that of non-PNS rats. This suggests that endogenously receptive PNS rats may engage in less approach–avoidance in a seminatural mating situation compared to non-PNS controls. Notably, there was little evidence for differences in motor behavior, reproductive, or pacing behavior between hormone-primed PNS and control rats. There were no differences in the number of beam breaks, lordosis, or proceptive behaviors of hormone-primed PNS vs. control rats. As well, the only difference in pacing behavior of hormone-primed rats was a reduction in the percentage of exits following mounts of PNS compared to control rats. Together, these data suggest that the behavioral inhibition of endogenously cycling PNS rats in response to novel reproductively relevant or neutral situations may be attenuated with extirpation and hormone replacement.

The present findings, suggesting that PNS rats show behavioral inhibition in response to novel environments (i.e. decreased beam breaks and pacing behavior), is consistent with previous research. Excessive fearfulness, emotional reactivity, anxiety, and physiological disruption have been observed in PNS animals (Ader and Plaut, 1968; Clarke and Schneider, 1996; Fride et al., 1986; Hockman, 1961; Polytrev et al., 1996; Takahashi et al., 1992a; Thompson, 1957; Wakshlak and Weinstock, 1990; Weinstock et al., 1992). Although moderate arousal activates behavior and extreme arousal produces behavioral inhibition in non-PNS rats, PNS rats are hyperreactive to stimulation and can respond to novel, low to moderate stress situations with behavioral inhibition.

These data confirm and extend previous research that shows that PNS alters reproductive behavior. The significant decreases that were presently seen in lordosis ratings and proceptivity quotients are consistent with previous reports that sexual receptivity (Gutierrez et al., 1989; Rojo et al., 1986) is disrupted in rats exposed to PNS. As well, the normal pattern of female rats to pace their sexual contacts with males by being more likely to leave the presence of the male after more intense stimulation (ejaculation > intromisions>mounts) (Erskine, 1985), which enhances their fertility and fecundity (Frye and Erskine, 1990), was attenuated in PNS compared to control rats. There was no evidence from our studies to support an increase in PNS females' sexual responses unless one interprets PNS females remaining with the male rather than pacing her sexual contacts as increased responsiveness. Notably, sexual responses of PNS rats have also been found to be the same as that of non-PNS rats (Beckhardt and Ward, 1983; Herrenkohl and Scott, 1984). The similar lordosis quotients of cycling PNS and control rats and the lack of differences in almost every reproductive parameter of ovx, hormone-primed PNS vs. control rats is congruous with these previous findings. The present studies extend this past research in an important way to show that when reproductive behavior is considered in its social context, differences can be seen in naturally cycling PNS rats as compared to controls.

The mediator of PNS effects on social and reproductive behavior is unknown. However, the salient behavioral differences observed between naturally cycling and hormone-primed rats suggest that endocrine responsiveness may mediate some of the observed differences in behavior. It is well established that PNS alters the hypothalamic–pituitary–adrenal axis (Weinstock, 1997) with PNS animals typically showing a greater adrenal hormone response to stress (Fride et al., 1986; Henry et al., 1994; McCormick et al., 1995; Takahashi et al., 1992b; Weinstock et al., 1992) and adrenal hypertrophy (Herrenkohl and Scott, 1984). There are indirect measures that suggest that the hypothala-

mic–pituitary–gonadal axis may also be disrupted in PNS rats. Disruption of estrous cyclicity (Herrenkohl and Politch, 1978), pregnancy (Herrenkohl and Scott, 1984), fertility (Herrenkohl, 1979), fecundity (Herrenkohl, 1979), and reductions in ovarian and uterine weight (Herrenkohl and Scott, 1984) have been reported in PNS female rats as compared to non-PNS controls. Evidence from the present study that PNS effects on the hypothalamic–pituitary–gonadal axis may have produced changes in behaviors observed include that when feedback from gonads was eliminated by ovx, behavioral differences were abrogated.

Although it is an intriguing possibility that adrenal or gonadal dysregulation or differences in maternal care may have produced the behavioral differences observed in the PNS rats, there are a number of reasons to be cautious in this interpretation. First, gestational stress affects maternal behavior (Moore and Power, 1985; Power and Moore, 1986), and it may be that postnatal differences in maternal care are the cause of the differences observed in this study. Because of documented effects of crossfostering on maternal behavior (Maccari et al., 1995), we did not control for maternal stress-induced alterations in parental care by crossfostering pups. It remains to be determined how best to separate prenatal from postnatal influences on rat behavior. Second, it is also possible that maternal care could be responsible for adrenal or gonadal regulation. Third, there is evidence that PNS affects development (Kinsley and Svare, 1986, 1988; Lay et al., 1997). Indeed, in support of this, we observed that the average age of sexual maturation, as indicated by the first day of vaginal opening, was 4 days earlier in PNS compared to the non-PNS rats. Finally, our study has limitations insofar as subjects were from a limited number of dams. We attempted to address this limitation by counterbalancing subjects from each litter across the various experimental groups so that there were no more than a few experimental subjects from each litter in each group. However, it would be necessary to only have one subject from each litter per experimental group in order to rule out any possible cohort effects. A comprehensive examination of how these or other variables contributed to the behavioral differences observed is beyond the scope of this study, but future work in our lab will aim to ascertain the many possible contributors.

In summary, these data demonstrate that proestrous rats exposed to PNS show decreased reproductive behavior (lower lordosis intensity) and increased behavioral inhibition in a neutral open field (fewer beam breaks), in a standard mating paradigm (less solicitation behavior), or in a situation in which they can control their sexual contacts (reduced pacing behaviors) compared to non-PNS, endogenously cycling proestrous rats. Hormone-primed PNS and control rats did not differ significantly in their reproductive responses or behavior in an open field or a standard or enriched mating environment. The extent to which PNS alters the responsiveness of the hypothalamic–pituitary–gonadal axis, which may, in turn, mitigate reproductive responses and/or behavior inhibition in adulthood, will be the subject of ongoing investigation in our laboratory.

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