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Prenatal Exposure to Nicotine: Effects on Prepulse Inhibition and Central Nicotinic Receptors

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POPKE, E. J., Y. TIZABI, M. A. RAHMAN, S. M. NESPOR AND N. E. GRUNBERG. *Prenatal exposure to nicotine: Effects on prepulse inhibition and central nicotinic receptors.* PHARMACOL BIOCHEM BEHAV **58**(4) 843–849, 1997.—The present experiment examined effects of prenatal nicotine exposure (6 mg/kg/day via osmotic minipump) throughout gestation on prepulse inhibition of the acoustic startle response (PPI) and on the density of nicotinic acetylcholine receptors (nAchRs) in the brains of 5-week-old Sprague–Dawley rats. A total of 117 male and 103 female offspring were used. Prenatal nicotine reduced subsequent percent PPI to a 98 dB stimulus in female but not in male offspring. There was an inverse correlation between the percent of PPI and nAchR density in the cortex of male rats and the striatum of female rats. © 1997 Elsevier Science Inc.

Prenatal nicotine Prepulse inhibition Nicotinic receptors Sensory gating Sex differences

CIGARETTE smoking by mothers during pregnancy is associated with cognitive and behavioral deficits in developing offspring. Children of mothers who smoked during pregnancy have impaired vigilance performance (14) and are at greater risk of attention deficits (8,19) than are the children of nonsmoking mothers. Animal studies suggest that some of the effects of smoking during pregnancy may result from prenatal exposure to nicotine. Sorenson et al. (27) reported that female rats that had been exposed to nicotine prenatally had impaired radial-arm maze performance when compared with female rats exposed to saline prenatally. Yanai et al. (35) reported similar effects of prenatal nicotine to impair radial-arm maze performance, and also to impair Morris water maze performance, in male, as well as in female mice. These data suggest that prenatal exposure to nicotine can impair motor performance, memory, and attention as reflected by impaired maze performance. More importantly, these data suggest that prenatal exposure to nicotine may be responsible for some of the deficits in attention and vigilance observed in children of mothers who smoked during pregnancy. The mechanisms by which nicotine may exert these effects, however, have not been identified.

Prepulse inhibition of the acoustic startle response (PPI) provides a sensitive measure of time-dependent sensory-motor gating deficits (5,6,29) and has been interpreted as reflecting processes that underlie attention (1,2). One purpose of the present experiment was to examine effects of prenatal nicotine exposure on PPI in young rats.

Additionally, it has been reported that prenatal exposure to nicotine increases the number of central nAchRs in brain (26). Yet, little is known regarding the relationship between these receptor changes and behavioral effects of prenatal exposure to nicotine. The second purpose of the present experiment, therefore, was to examine the relationship between PPI and nAchR densities in discrete regions of the brain following prenatal exposure to saline or prenatal exposure to nicotine. Based on published reports (26,27,35), it was hypothesized that prenatal exposure to nicotine would result in an increased density of nAchRs in discrete brain regions and would have effects to reduce PPI. Because sex differences ex-

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ist with respect to nicotine sensitivity (11,21,28), male and female subjects both were included in the present experiment.

METHOD

Prenatal Drug Treatment

Nicotine dihydrochloride (6 mg/kg/day nicotine base) or saline was administered to 24 pregnant Sprague–Dawley dams by osmotic minipump (Alzet model 2002). Pumps were implanted in the intrascapular space (SC) on gestational day 4 to provide nicotine or saline solution at a constant infusion rate of 0.5 μl/h for the remainder of the 22-day gestation period. Methoxyflurane (Metophane), administered by inhalation, was used to anesthetize the dams during the implantation surgery. Incisions (roughly 1.25 cm) were closed with 9 mm stainless steel wound clips (Becton Dickinson). Physiologic saline was used to prepare the nicotine solutions from nicotine dihydrochloride and also was used as the control solution. The dosage of nicotine used presently has been reported to produce behavioral (23) and biochemical (23,24) effects in prenatally exposed rats.

Subjects and Housing

The subjects were 117 male and 103 female Sprague–Dawley rat pups born to 24 pregnant dams. These pups were weaned 18 days after birth and were group housed with same-sex litter mates (two to six pups in a cage) in 35.6 × 15.2 × 20.3 cm cages with absorbent Pine-Dri, wood-chip bedding. The subjects were maintained under a 12-h light/dark cycle (lights on at 1900 h) at approximately 23°C and 50% relative humidity. Water and a standard laboratory chow (Agway Prolab 3000) were available continuously. The average weight of male subjects at the time of testing was 142 g (range = 96–189 g). The average weight of female subjects at the time of testing was 129 g (range = 95–172 g). Subjects were roughly 35 days old at the time of testing.

Startle and Prepulse Testing

Prepulse inhibition (PPI) was tested using a four-station acoustic startle system (Coulbourn Instruments, Allentown, PA) based on published reports (1–3). Specifically, animals were enclosed in 8 × 8 × 16 cm open air cages that restrict locomotion but do not restrain the animal. Cages were placed on one of four platforms in a sound-attenuating test chamber. Background noise within the sound-attenuating startle chamber was produced by a ventilating fan and was measured at 56 dB SPL (sound pressure level). Startle-eliciting acoustic stimuli consisted of 20-ms noise bursts of 98 dB SPL, 112 dB SPL, or 122 dB SPL. Prepulse stimuli consisted of a 20-ms, 1 KHz pure tone of 68 dB SPL (12 dB above background). The onset of the prepulse stimuli preceded the onset of the startle-eliciting stimuli by 100 ms. Trials with no stimuli and trials with prepulse alone were also presented. Each subject's movement in response to each stimulus was measured as voltage change by a strain gauge and was converted to grams of body weight change following analog to digital conversion. Responses were recorded by an interfaced microcomputer as the maximum response occurring within 200 ms of the onset of the startle-eliciting stimuli. A single test session included eight presentations of each stimulus intensity both with and without prepulse. The order of presentation was randomized within blocks to ensure that each stimulus type was presented within seven trials of its last presentation and that none of the stimuli occurred more than once in sequence. Intertrial intervals ranged randomly from 10–30 s. It should be noted that some rats emit ultrasounds when startled by an acoustic stimulus (17). Although these ultrasounds are unrelated to the acoustic startle response within subjects, it is possible that ultrasonic vocalizations may affect the acoustic startle response if several animals are tested simultaneously in the same apparatus. To minimize the impact of these effects, both drug groups were represented in every group of four animals run concurrently.

Procedure

Orientation to the PPI testing procedure was begun on postnatal day 30, an age when nAchRs in the brain have reached adults levels (25). This orientation consisted of a single test session designed to acclimate subjects to the acoustic startle apparatus. This acclimation procedure has been used to minimize the effect that the stress of a novel environment may have on prepulse inhibition during testing (2,3). Testing of prepulse inhibition began on postnatal day 35 and consisted of a second test session as outlined in the preceding section on startle and prepulse testing. Orientation sessions and test sessions for each animal were separated by at least 4 days to minimize effects of habituation on PPI (31). Testing was balanced such that nicotine pretreated subjects and saline pretreated subjects were equally represented in the chamber during each test session.

Startle amplitudes were determined for each animal by subtracting the response to the no-stimulus control trials from the average peak response recorded during each of the other trial types. The amount of PPI was determined by subtracting the response to the prepulse trials from the response to the trials in which the same stimulus was presented without prepulse. The amount of prepulse inhibition was divided by the response amplitude from trials using similar stimuli without prepulse to determine the percentage of the response inhibited (percent PPI). The purpose of converting these data to percentages is to minimize the effect that initial startle responses have on the measurement of subsequent PPI effects. This data handling procedure is consistent with published reports examining effects of drugs on PPI (1,2).

Subjects were selected for biochemical analysis on the basis of the average percent PPI. Specifically, percent PPI for each subject was averaged across stimuli to obtain a composite percent PPI score. Subjects with average percent PPI in the highest 25% of all scores were classified as having high PPI, whereas subjects in the lowest 25% of all scores were classified as having low PPI. Using these criteria, male subjects were classified as having high PPI if the prepulse inhibited the startle response by more than 35% ($n = 17$) and were classified as having low PPI if the prepulse inhibited the startle response by less than 11% ($n = 18$). Female subjects were classified as high in PPI if the prepulse inhibited the startle response by more than 35% ($n = 17$) and were classified as having low PPI if the prepulse inhibited the startle response by less than 9% ($n = 17$). A third group of subjects having neither high nor low PPI and representing a range of locomotor activity [see (32)] also was included in the biochemical analysis. This third group was comprised of 39 males and 24 females. Application of this classification criteria ensured a sufficient sample size to allow statistical analysis of biochemical data.

Tissue Collection

Twenty-four hours after the last acoustic startle test, pups were sacrificed by decapitation. The brains were rapidly frozen in powdered dry ice. The brains were stored at −80°C for

several weeks before being dissected on an ice-cold plate under a magnifying lens. Cerebral cortex (left hemisphere), striatum, hippocampus, thalamus, and colliculi (superior and inferior) were separated and stored frozen at −80°C until assay. These brain regions were selected for assay on the basis of their established role in cognitive, behavioral, or sensory and motor functions, and on the abundance of their nAchRs (7,20,34).

Nicotinic Receptor Binding Assay

nAchR binding was assessed based on the procedures of Pabreza et al. (20). Tissue was homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.0 at room temperature) and then centrifuged at 38,000 × *g* for 12 min at 4°C. The pellet was washed twice by suspension in fresh buffer and centrifuged again. Aliquots of homogenate equivalent to approximately 10–20 mg tissue were used in triplicate for total binding and in duplicate for the nonspecific binding. For total binding, 4 nM [^{3}H]cytisine (39.6 Ci/nmol, NEN, Boston, MA) was incubated in a final volume of 0.25 ml at 2°C for 75 min. Nonspecific binding was obtained in the presence of 100 μM (−)-nicotine bitartrate. Membrane-bound [^{3}H]cytisine was separated from free ligand by filtration using Brandel GF/B filter paper and a Brandel cell harvester. The binding affinity was determined in cortical tissue, using six concentrations (0.25–8 nM) of [^{3}H]cytisine. Scatchard plots to calculate B_{max} and K_d were obtained using nonlinear least-squares regression LIGAND analysis (18). Protein concentration in the final homogenate was determined by the Bradford method (4). The findings are reported as nAchR binding, generally, but most likely reflect the α4β2 subtype of nAchR because cytisine has been shown to be specific for this type of nAchR (9).

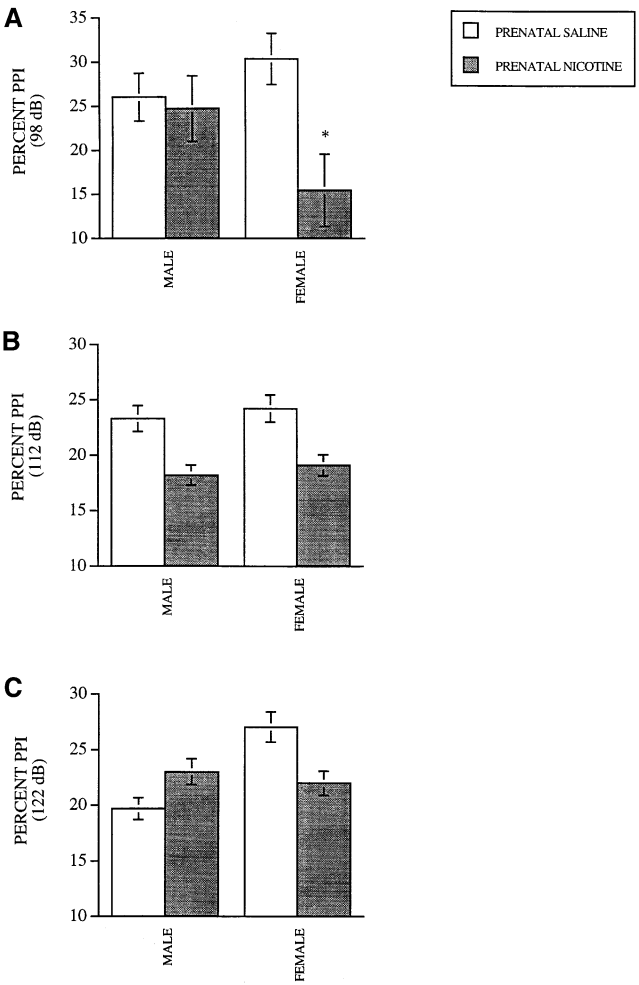

FIG. 1. Percent PPI in response to each of the three acoustic startle stimulus intensities (mean ± SEM). Among males, $n = 64$ saline and 54 nicotine. Among females, $n = 47$ saline and 56 nicotine. *Denotes groups that differ from prenatal saline ($p < 0.05$).

Treatment of Data and Statistical Analysis

Percent PPI data were analyzed for each stimulus intensity using a two-way ANOVA with sex and prenatal exposure entered as between-subjects factors. Because initial analyses revealed significant interactions of prenatal treatment and sex, effects of prenatal treatment on males and females were examined separately using one-way ANOVA.

Subjects with percent PPI in the top or bottom 25% of all scores were further examined using chi-square analyses to determine the relative proportion of nicotine-exposed subjects and saline-exposed subjects that fell in the extreme tails of the overall distribution. The purpose of this analysis was to determine whether prenatal exposure to nicotine increased the incidence of low PPI or decreased the incidence of high PPI relative to saline.

Results of the nAchR binding assay were analyzed using three-way ANOVA with prenatal treatment, sex, and PPI status entered as between-subjects factors. Because initial analyses revealed significant sex by PPI status interactions, effects of prenatal treatment in males and females were examined separately using two-way ANOVA.

Correlation analyses were performed to examine the relationships between nAchR changes in specific brain regions and percent PPI. All statistical tests were two tailed and used an alpha level of 0.05 or less to determine significant statistical significance.

RESULTS

Figure 1a presents percent PPI using 98 dB stimulus. Two-way ANOVA revealed a significant main effect of prenatal drug treatment, $F(2, 215) = 5.05$, $p < 0.05$, and a significant drug by sex interaction, $F(2, 215) = 3.94$, $p < 0.05$. Subsequent one-way analyses, examining effects of prenatal nicotine on percent PPI in males and in females separately, revealed a significant effect of prenatal drug treatment to reduce percent PPI in females, $F(1, 101) = 8.18$, $p < 0.05$, but not in males. There were no effects on prenatal nicotine on the startle amplitudes of either sex in the absence of the prepulse. Figure 1b presents percent PPI using the 112 dB stimulus. There were no statistically significant effects in either male or female subjects considered separately. There were no effects on prenatal nicotine on the startle amplitudes of either sex in the absence of prepulse. Figure 1c presents percent PPI using the 122 dB stimulus. Two-way ANOVA revealed a significant drug by sex interaction, $F(2, 215) = 3.92$, $p < 0.05$. There were no effects of prenatal nicotine on the startle amplitudes of either sex in the absence of the prepulse.

Female subjects that received nicotine prenatally were significantly more likely to have low PPI, and significantly less

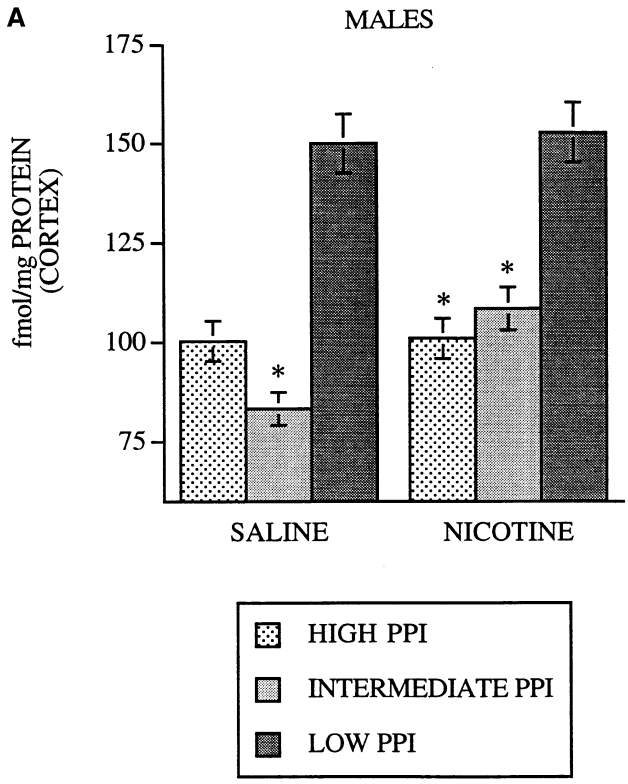

FIG. 2. nAchR binding in cortex as a function of sex, PPI status, and prenatal drug treatment (means ± SEM). Among males, $n = 8$ saline-high PPI; 17 saline-intermediate PPI, 6 saline-low PPI, 8 nicotine-high PPI, 19 nicotine-intermediate PPI, and 7 nicotine-low PPI. Among females, $n = 7$ saline-high PPI; 11 saline-intermediate PPI, 6 saline-low PPI, 7 nicotine-high PPI, 12 nicotine-intermediate PPI, and 9 nicotine-low PPI. *Denotes groups that differ from the low PPI group of the same drug treatment ($p < 0.05$).

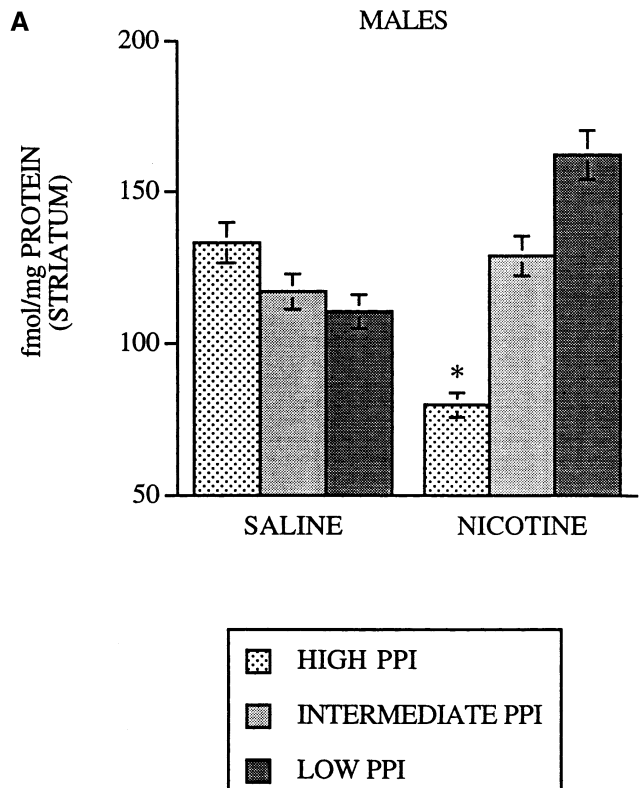

FIG. 3. nAchR binding in striatum as a function of sex, PPI status, and prenatal drug treatment (mean ± SEM). Among males, $n = 7$ saline-high PPI; 17 saline-intermediate PPI, 9 saline-low PPI, 8 nicotine-high PPI, 18 nicotine-intermediate PPI, and 7 nicotine-low PPI. Among females, $n = 7$ saline-high PPI; 11 saline-intermediate PPI, 7 saline-low PPI, 6 nicotine-high PPI, 10 nicotine-intermediate PPI, and 7 nicotine-low PPI. *Denotes groups that differ from the low PPI group of the same drug treatment ($p < 0.05$).

likely to have high PPI, than were female subjects that received saline prenatally ($\chi^2 = 5.1, p < 0.05$). There was a similar trend in male subjects that did not achieve statistical significance.

Figure 2 presents nAchR binding in cortex as a function of sex, PPI status, and prenatal drug treatment. Three-way ANOVA revealed a significant sex by PPI status interaction, $F(2, 103) = 6.21, p < 0.01$. Subsequent two-way analyses, examining nAchR binding in the cortex of males (Fig. 2a) and females (Fig. 2b) separately, revealed a significant effect of PPI status in males, $F(2, 58) = 6.03, p < 0.01$, but no effects in females. Specifically, male rats with low PPI had more nicotinic binding sites in cortex than did male rats with high PPI or with intermediate PPI, regardless of prenatal drug condition.

Figure 3 presents nAchR binding in striatum as a function of sex, PPI status, and prenatal drug treatment. Three-way ANOVA revealed a significant drug treatment by PPI status interaction, $F(2, 101) = 5.121, p < 0.05$. Subsequent two-way analyses, examining nAchR binding in the striata of males (Fig. 3a) and females (Fig. 3b) separately, indicated that this significant interaction was a result of a drug treatment by PPI status interaction among males, $F(2, 59) = 4.158, p < 0.05$, but not among females. Among males that received nicotine prenatally, subjects with high levels of PPI had fewer nicotine binding sites than did subjects with either low PPI or intermediate PPI ($p < 0.05$).

There were no significant effects involving sex, PPI status, or prenatal drug treatment on nAchR binding in hippocampus, thalamus, or colliculi. Table 1 presents correlation coefficients to describe the relationships between nAchR binding and PPI, for male and female pups. For males (Table 1 a), there was a significant negative correlation between nAchR density in the cortex, and percent PPI when the 98 dB stimulus was used ($p < 0.01$) but there were no significant effects when the other two stimulus intensities were used. There also was a significant negative correlation between nAchR density in cortex and average percent PPI. For females (Table 1 b), there was a significant negative correlation between nAchR density in the striatum and percent PPI when the 98 dB stimulus was used ($p < 0.05$). There also was a significant negative correlation between nAchR density in striatum and average percent PPI. There were no effects of prenatal nicotine on binding affinity (K_d values ranged from 0.56 nM to 0.68 nM; $n = 9$ subjects per group) and no relationship between binding affinity and PPI.

DISCUSSION

The present experiment examined effects of prenatal nicotine exposure on prepulse inhibition in 5-week-old male and in female offspring. Changes in prepulse inhibition have been interpreted as reflecting changes in sensory-motor gating (5,6,29,30) and possibly attention (1,2). Results indicate significant effects of prenatal nicotine to reduce percent PPI in females to a 98 dB stimulus. It is possible that the effects of prenatal nicotine to impair PPI may reflect nicotine withdrawal rather than a direct effect of nicotine administration on the developing fetus. However, previous reports have indicated that nicotine withdrawal can increase the amplitude of the acoustic startle response measured in the absence of a prepulse tone (12,13). Because there were no such effects revealed presently, it seems more likely that the deleterious effects of prenatal nicotine result from a deleterious effect of nicotine on the developing fetus as suggested by previous reports (16,26). The fact that the significant findings only occurred at the lowest intensity suggests that even lower intensity stimuli might be more sensitive to these prenatal nicotine effects.

Biochemical analysis revealed an inverse correlation between the percent of PPI and nAchR density in the cortex of male rats, regardless of prenatal drug condition (see Table 1), and an inverse relation between the amount of PPI and nAchR density in the striatum of male rats that had received nicotine (see Fig. 3a). Although females had nAchR densities in the striatum that were negatively associated with percent PPI (see Table 1), they had few biochemical changes associated with prenatal nicotine (see Figs. 2b and 3b). This biologic sex difference is in contrast to the behavioral sex difference, where impairments were manifest primarily in females. These results suggest that prenatal nicotine exposure can impair PPI without nAchR changes in females. In contrast, nAchR densities in the striatum of males varied with PPI in animals that

TABLE 1

CORRELATION COEFFICIENTS DESCRIBING THE RELATIONSHIP BETWEEN NICOTINIC RECEPTOR BINDING IN SPECIFIC BRAIN REGIONS AND PERCENT PPI USING EACH STIMULUS INTENSITY

	Hippocampus			Colliculi			Striatum			Cortex			Thalamus		
dB	Saline	Nicotine	Both	Saline	Nicotine	Both	Saline	Nicotine	Both	Saline	Nicotine	Both	Saline	Nicotine	Both
Table 1 a (Males)															
98	0.20	0.13	0.17	−0.17	−0.22	−0.21	0.00	−0.26	−0.17	−0.56†	−0.24	−0.38†	−0.02	0.11	0.04
112	0.16	0.20	0.19	0.20	−0.04	−0.13	0.02	−0.22	−0.13	0.08	−0.13	−0.23	0.15	−0.02	−0.06
122	0.24	0.10	0.15	−0.04	−0.09	−0.07	0.09	−0.24	−0.13	−0.10	−0.02	−0.01	−0.05	−0.02	−0.01
AVG	0.29	0.19	0.24	−0.02	−0.17	−0.13	0.05	−0.33	−0.20	−0.41*	−0.19	−0.29*	−0.06	−0.06	−0.01
Table 1 b (Females)															
98	−0.20	−0.07	−0.08	−0.04	−0.14	−0.17	−0.34	−0.29	−0.31†	−0.08	−0.16	−0.12	−0.05	−0.26	0.03
112	−0.06	0.03	0.05	0.11	0.03	0.00	−0.42*	−0.08	−0.23	0.22	−0.06	0.09	0.25	−0.09	0.10
122	0.13	0.00	0.10	0.11	−0.11	−0.02	−0.32	−0.14	−0.22	−0.03	0.15	0.04	0.02	0.14	0.05
AVG	0.01	−0.03	0.00	0.10	−0.13	−0.10	−0.46*	−0.26	−0.34*	0.12	0.12	0.12	0.12	0.14	0.01

Data are presented for saline and nicotine subjects seperately, as well as for drug groups when collapsed. Data for males are presented in Table 1 a. Data for females are presented in Table 1 b.

*$p < 0.05$.

†$p < 0.01$.

had received nicotine. These results suggest that a qualitative difference may exist with respect to behavioral effects and behavioral × biochemical interactions of prenatal nicotine in male and in female rats. This suggestion is supported by previous reports of sex differences in behavioral and neurochemical effects of prenatal nicotine administration observed using different behavioral and biochemical assay procedures (10,15, 16,21,22). It is worthy to note that the present experiment did not reveal general, nicotine-induced increases in the number of nAchRs in brain as previously reported (26,33). There are several possible explanations for this apparent discrepancy. Slotkin et al. (26) reported greater effects of prenatal nicotine administered by repeated acute injections than of the same dosage of prenatal nicotine administered by osmotic minipump, as in the present experiment. Second, and perhaps more notably, previous reports have not revealed nicotine-induced increases in nAchRs measured more than 30 days after birth. Because the present data were collected 35 days after birth, the fact that the present experiment did not reveal general effects of prenatal nicotine administration on nAchRs is, in fact, consistent with previous reports (26).

In summary, the present experiment examined effects of prenatal nicotine exposure on prepulse inhibition of the acoustic startle response in young rats and on the density of nAchRs in specific brain regions of young rats. Prenatal nicotine reduced subsequent percent PPI in female offspring but not in male offspring.

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REFERENCES

1. Acri, J. B.: Nicotine modulates effects of stress on acoustic startle reflexes in rats: Dependence on dose, stressor, and initial reactivity. Psychopharmacology (Berlin): 116:255–265; 1994.
2. Acri, J. B.; Morse, D. E.; Popke, E. J.; Grunberg, N. E.: Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. Psychopharmacology (Berlin) 114:369–374; 1994.
3. Acri, J. B.; Grunberg, N. E.; Morse, D. E.: Effects of nicotine on the acoustic startle reflex amplitude in rats. Psychopharmacology (Berlin) 104:244–248; 1991.
4. Bradford, M. M.: A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
5. Caine, S. B.; Geyer, M. A.; Swerdlow, N. R.: Carbachol infusion into the dentate gyrus disrupts sensorimotor gating of startle in the rat. Psychopharmacology (Berlin) 105:347–354; 1991.
6. Caine, S. B.; Geyer, M. A.; Swerdlow, N. R.: Hippocampal modulation of acoustic startle and pre-pulse inhibition in the rat. Pharmacol. Biochem. Behav. 43:1201–1208; 1992.
7. Clarke, P. B. S.; Schwartz, R. D.; Paul, S. M.; Pert, C. B.; Pert, A.: Nicotinic binding in rat brain: Autoradiographic comparison of ^{3}H-nicotine and ^{125}I-alpha-bungarotoxin. J. Neurosci. 5:1307–1315; 1985.
8. Denson, R.; Nanson, J. T.; McWatters, M. A.: Hyperkinesis and maternal smoking. Can. Psychiatr. Assoc. J. 20:183–187; 1975.
9. Flores, C. M.; Rogers, S. W.; Pabreza, L. A.; Wolfe, B. B.; Kellar, K. J.: A subtype of nicotinic cholinergic receptor in rat brain is composed of α4 and β2 subunits and is up-regulated by chronic nicotine treatment. Mol. J. Pharmacol. 41:31–37; 1992.
10. Fung, Y. K.; Lau, Y. S.: Effects of prenatal nicotine exposure on rat striatal dopaminergic and nicotinic systems. Pharmacol. Biochem. Behav. 33:1–6; 1989.
11. Grunberg, N. E.; Winders, S. E.; Popp, K. A.: Sex differences in nicotine's effects on consummatory behavior and body weight in rats. Psychopharmacology (Berlin) 91:221–225; 1987.
12. Helton, D. R.; Modlin, D. L.; Tizzano, J. P.; Rasmussen, K.: Nicotine withdrawal: a behavioral assessment using schedule-controlled responding, locomotor activity, and sensorimotor reactivity. Psychopharmacology (Berlin) 113:205–210; 1993.
13. Helton, D. R.; Rasmussen, K.; Kallman, M. J.: Clonidine attenuates increases in sensorimotor reactivity resulting from withdrawal of chronic nicotine. Poster presented at the Second Annual Conference of The Society for Research on Nicotine and Tobacco, Washington, DC; 1996.
14. Kristjansson, E. A.; Fried, P. A.; Watkinson, B.: Maternal smoking affects children's vigilance performance. Drug Alcohol Depend. 24:11–19; 1989.
15. Levin, E. D.; Briggs, S. J.; Channelle, C. N.: Prenatal nicotine exposure and cognitive performance in rats. Neurotoxicol. Teratol. 15:251–260; 1993.
16. Lichtensteiger, W.; Schlumpf, M.: Prenatal nicotine exposure: Biochemical and neuroendocrine bases of behavioral dysfunction. Dev. Brain Dysfunct. 6:279–304; 1993.
17. Miczek, K. A.; Vivian, J. A.; Tornatzky, W.; Farrell, W. J.; Sapperstein, S. B.: Withdrawal from diazepam in rats: Ultrasonic vocalization sand acoustic startle reflex. J. Psychopharmacol. Abstr. A47; 1992.
18. Munson, P. J.; Rodbard, D.: A versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107:220–239; 1980.
19. Naeye, R. L.: Cognitive and behavioral abnormalities in children whose mothers smoked cigarettes during pregnancy. J. Dev. Behav. Pediatr. 13:425–428; 1984.
20. Pabreza, L. A.; Dhawan, S.; Kellar, K. J.: [^{3}H]cytisine binding to nicotinic cholinergic receptors in brain. Mol. Pharmacol. 39:9–12; 1991.
21. Peters, D. A. V.; Tang, S.: Sex-dependent biological changes following prenatal nicotine exposure in the rat. Pharmacol. Biochem. Behav. 17:1077–1082; 1982.
22. Ribary, U.; Lichtensteiger, W.: Effects of acute and chronic prenatal nicotine treatment on central catecholamine systems of male and female rat fetuses and offspring. J. Pharmacol. Exp. Ther. 248:786–792; 1989.
23. Richardson, S. A.; Tizabi, Y.: Hyperactivity in the offspring of nicotine-treated rats: Role of the mesolimbic and nigrostriatal dopaminergic pathways. Pharmacol. Biochem. Behav. 47:331–337; 1994.
24. Roy, T. S.; Sabherwal, U.: Effects of prenatal nicotine exposure on the morphogenesis of somatosensory cortex. Neurotoxicol. Teratol. 16:411–421; 1994.
25. Sershen, H.; Reith, M. E.; Banay-Schwartz, M.; Lajtha, A.: Effects of prenatal administration of nicotine on amino acid pools, protein metabolism, and nicotine binding in brain. Neurochem. Res. 7:1515–1522; 1982.
26. Slotkin, T. A.; Orband-Miller, L.; Queen, K. L.: Development of [^{3}H] nicotine binding sites in brain regions of rats exposed to nicotine prenatally via maternal injections or infusions. J. Pharmacol. Exp. Ther. 242:232–237; 1987.
27. Sorenson, C. A.; Raskin, L. A.; Yongsook, S.: The effects of prenatal nicotine on radial-arm maze performance in rats. Pharmacol. Biochem. Behav. 40:991–993; 1991.
28. Stone, S. V.; Dembroski, T. M.; Costa, P. T.; MacDougall, J. M.: Gender differences in cardiovascular reactivity. J. Behav. Med. 13:137–156; 1991.
29. Swerdlow, N. R.; Braff, D. L.; Geyer, M. A.; Koob, G. F.: Central dopamine hyperreactivity in rats mimics abnormal acoustic startle response in schizophrenics. Biol. Psychiatry. 21:23–33; 1986.
30. Swerdlow, N. R.; Caine, S. B.; Braff, D. L.; Geyer, M. A.: The neural substrates of sensorimotor gating of the startle reflex: A

review of recent findings and their implications. J. Psychopharmacol. 6:176–190; 1992.
31. Thompson, R. F.; Spencer, W. A.: Habituation: A model phenomenon for the study of neuronal substrates of behavior. Psychol. Rev. 73:16–43; 1966.
32. Tizabi, Y.; Popke, E. J.; Rahman, M. A.; Nespor, S. M.; Grunberg, N. E.: Hyperreactivity induced by prenatal nicotine exposure is associated with an increase in cortical nicotinic receptors. Pharmacol. Biochem. Behav. 58:1–6; 1997.
33. VanDeKamp, J. L.; Collins, A. C.: Prenatal nicotine alters nicotine receptor development in the mouse brain. Pharmacol. Biochem. Behav. 47:889–900; 1994.
34. Wonnacott, S.; Drasdo, A.; Sanderson, E.; Rowell, P.: Presynaptic nicotinic receptors and the modulation of transmitter release. In: The biology of nicotine dependence. Ciba Foundation Symposium 152:87–101; 1990.
35. Yanai, J.; Pick, C. G.; Yael, R. F.; Zahalka, E. A.: Alterations in hippocampal cholinergic receptors and hippocampal behaviors after early exposure to nicotine. Brain Res. Bull. 29:363–368; 1992.