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RAPID COMMUNICATION

The Effects of Prenatal Nicotine on Radial-Arm Maze Performance in Rats

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SORENSON, C. A., L. A. RASKIN AND Y. SUH. *The effects of prenatal nicotine on radial-arm maze performance in rats.* PHARMACOL BIOCHEM BEHAV **40**(4) 991–993, 1991.—Studies have revealed lasting cognitive impairments, including deficits in attention and learning, in the offspring of women who smoke. Animal models have shown that prenatal nicotine can induce behavioral impairments, including deficits in learning and memory, and one study showed that only females were impaired on a maze task. The purpose of the present experiment was two-fold: 1) to attempt to replicate the reported sex difference in maze learning and 2) to assess the ability of nicotine-treated subjects to learn a maze that placed particularly heavy demands on their attentional capabilities. Pregnant mothers were given 6.0 mg/kg/day of nicotine in their drinking water. Offspring of both sexes were tested following weaning in an 8-arm-radial maze using a confinement procedure. Results showed that prenatal nicotine treatment produced significant impairments in performance in the radial-arm maze. These impairments were seen in animals of both sexes, a finding which challenges the view that only females prenatally treated with nicotine show deficits in maze learning.

Prenatal nicotine Spatial memory and sex differences

A number of studies have reported that the offspring of women who smoked during pregnancy show a variety of behavioral and cognitive impairments. Neonatal offspring of smokers have been reported to show deficits in responsiveness to auditory stimuli (15,16), to show both an increase (16) and a decrease (8) in irritability, and to show deficits in learning of simple operant tasks (10). Children exposed to smoking prenatally may also show specific deficits in attention; prospective correlational studies of both 4-year-olds (18) and 7-year-olds (11) have reported lower scores on vigilance tasks in these children. In addition, there appears to be an increased incidence of diagnosed cases of attention deficit and hyperactivity disorder (ADHD) among the children of smokers (5, 6, 11). Such children show problems in academic performance: they perform less well in the first grade of school (6), show impaired performance on standardized intelligence tests (6,11), on tests of reading (2), and on tests of block design and vocabulary (6).

The evidence that smoking might produce some persisting cognitive impairment in humans has led to a few preliminary efforts to determine, through the use of animal models, whether prenatal nicotine exposure might be the causal agent. These studies have shown that prenatal nicotine treatment does produce a subtle cognitive impairment, which could be secondary to an attentional deficit. On simple tasks, such as shuttle avoidance, there appears to be minimal impairment (1,14). Deficits are seen, however, on more complex tasks or on tasks requiring the animal to choose from more alternatives, such as spontaneous alternation (7), bar pressing on fixed ratio or DRL schedules (9), or maze learning (14). In the maze learning study, learning deficits were seen only in female offspring, a provocative finding which might suggest that there is a sex difference in the teratogenic effect of nicotine on the performance of complex learning tasks in rats.

The purpose of the present study was to further evaluate the nature of the cognitive deficit seen in animals following prenatal nicotine exposure. Given reports suggesting that children show deficits in sustained attention after exposure to prenatal nicotine, we selected a task that would be extremely sensitive to impairments of attention: the radial-arm maze with the confinement procedure. This task requires the animal to sustain attention to extramaze cues throughout a period of confinement between each choice in an 8-arm maze (12). In light of the previous finding that nicotine-induced deficits in maze learning were seen only in females (14), animals of both sexes were used in this study.

METHOD

Nicotine was administered to adult female rats following the method of Peters and Tang (13), which reportedly produced reliable behavioral and neuroanatomical changes. Twelve female Sprague-Dawley rats received nicotine in their drinking water beginning 15 days prior to mating and continuing throughout the gestation period. The concentration of nicotine was approximately 6.0 mg/kg/day. Twenty-four control females received

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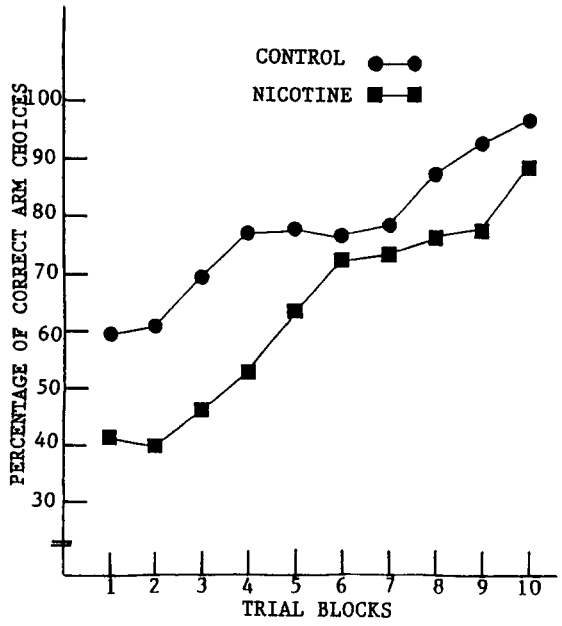

FIG. 1. Percentage of correct arm choices plotted as a function of trial blocks for control and nicotine-treated females.

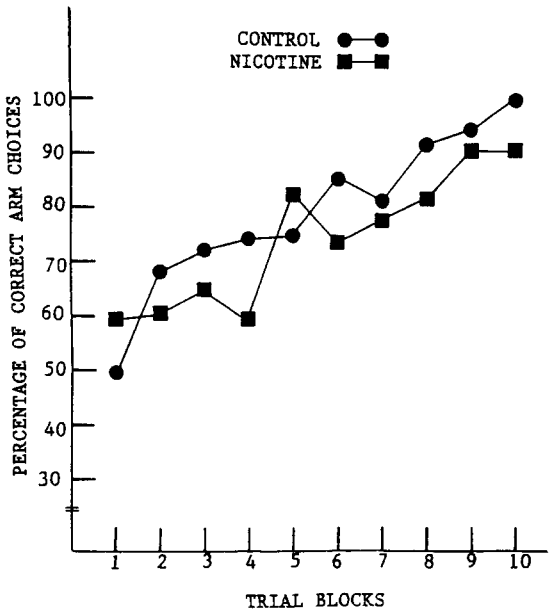

FIG. 2. Percentage of correct arm choices plotted as a function of trial blocks for control and nicotine-treated males.

plain tap water. Twenty-four hours after birth, all litters (nicotine-treated and controls) were cross-fostered to other control dams; five days later, litters were culled to 10 pups (5 males and 5 female). All pups were weaned on postnatal day 30 and housed in a colony room maintained on a reverse light-dark cycle (12:12) at 25°C.

Radial-arm maze testing was carried out on 4 groups of 6 animals each by taking 4 offspring (2 males and 2 females) at random from each of 3 separate nicotine and control litters. Maze performance was tested in an 8-arm maze, constructed of transparent Plexiglas. The center platform was 16-sided and measured 51 cm across. Each of the 8 arms were 12 cm wide × 18 cm high and extended 46 cm symmetrically from the center platform. A rubber food cup was placed 3 cm from the distal end of each arm. The maze was elevated 47 cm above the floor on a wooden table covered with black opaque paper. The confinement apparatus, made of transparent Plexiglas, fit snugly into the central platform, and was raised and lowered into the central platform by hand.

Training and testing were carried out in a small (2.5 × 3 × 2.2 m) dimly lit room. Animals were trained for 10 days, beginning at 35 days of age. For the first 5 days of training, each animal was placed in the center of the maze with the confinement apparatus lowered and food cups empty. After 30 seconds the confinement apparatus was raised and the animal was allowed to explore the maze for 10 minutes. Between the fifth and sixth days of training animals were deprived of food beginning 13–14 hours prior to the next trial. Beginning on day 6 of training and continuing throughout testing, each food cup was baited with one 45 mg Dustless Precision pellet (Bioserve). For each training trial the animal was confined to the central platform for 30 seconds and then allowed to run through the maze and consume all 8 pellets.

Testing began on day 11 and lasted for 20 days (subject age 45–65 days). As in training, animals were placed in the center of the maze and confined for 30 seconds. The confinement apparatus was lifted and latency to enter the first arm with all four paws was recorded. When the subject returned to the central platform, it was confined for 15 seconds before the next choice. This confinement occurred after each arm choice. Test sessions lasted 10 minutes or until all 8 food pellets were eaten.

RESULTS

The first 3 nicotine-treated litters born were cannibalized by the foster mothers when the litters were cross-fostered on the day of birth. Subsequent cross-fostering 24 h after birth prevented such cannibalism. Analysis of the birth weights of prenatally nicotine treated offspring vs. controls using a two-tailed *t*-test showed that nicotine treated subjects had slightly but significantly ($p<0.05$) reduced birth weights. The mean weight for nicotine subjects was 5.8 grams, and for control subjects was 6.3 grams. These findings are consistent with those of other animal studies showing significant depression of birth weight following prenatal nicotine administration (11,16). Group weight differences were no longer present at day 35 when radial-arm-maze training began. There was no significant difference in body weight at parturition between nicotine and control dams.

The results of the radial-arm maze testing were tabulated in terms of a percentage of correct arm choices and latency to enter the first arm. For each of these variables the 20 observations for each animal (one for each of 20 days of testing) were collapsed into 10 blocks of 2 trials each.

The percentage of correct arm choices was calculated by dividing the total number of correct arm choices (entrance into a previously unentered arm and eating the pellet) by the total number of arms entered, and then multiplying this fraction by 100.

Given the importance of litter effects in data such as these, an overall analysis was run using litter means as the unit of analysis. Despite the small number of litters used, this ANOVA revealed a highly significant effect of nicotine treatment, $F(1,8)=12.28$, $p<0.01$, a highly significant effect of trials, $F(1,9)=33.42$, $p<0.01$, and an effect of sex, $F(1,8)=4.83$, $p=0.05$. The treatment × sex interaction was not statistically reliable. The treatment × trials and the treatment × sex × trials inter-

actions were also not statistically significant.

Although there was no treatment by trials interaction, a comparison of Figs. 1 and 2 appears to show that the magnitude of the detrimental effect of nicotine on maze learning is greater for females than for males.

The latencies to enter the first arm during the testing period were uniformly short in all animals, with mean latencies ranging from 0.8 to 1.6 s. Statistical analysis revealed no significant main effect or interaction effect.

DISCUSSION

The results of this study show that rats of both sexes exposed prenatally to 6.0 mg/kg/day of nicotine were impaired in their ability to learn an 8-arm maze. These findings agree with those of other studies (7, 9, 14), which have shown impairments in performance on complex tasks after fetal exposure to nicotine. The performance decrements observed in nicotine-treated animals cannot be attributed to diminished levels of motivation, because there were no differences between nicotine-treated animals and controls in runway speed (latency to enter the first arm). The findings of the present study are, therefore, consistent with the view that nicotine exposure impairs learning of complex mazes. These results also suggest that attentional mechanisms may be disrupted by prenatal nicotine exposure since the radial-arm maze, and particularly the confinement procedure, places substantial demands on attentional processes.

Our findings appear to differ from those of Peters and Ngan (14), who reported that females, but not male nicotine animals, were impaired in maze learning. The difference between these studies might be attributable to the fact that the maze used in the present study was intentionally more complex: the animal had more choices to make, and choices had to be delayed because of the confinement procedure. Our findings do not preclude the possibility that females are more severely impaired than males by nicotine pretreatment, as is suggested by a comparison of Figs. 1 and 2. Future research is necessary to determine whether, with more subjects, a sex difference might emerge.

REFERENCES

1. Bertolini, A.; Bernardi, M.; Genedani, S. Effects of prenatal exposure to cigarette smoke and nicotine on pregnancy, offspring development and avoidance behavior in rats. Neurobehav. Toxicol. Teratol. 4:545–548; 1982.
2. Butler, N. R.; Goldstein, H. Smoking in pregnancy and subsequent child development. Br. Med. J. 4:573; 1973.
3. Davis, M. Neurochemical modulation of sensory-motor reactivity: Acoustic and tactile startle reflexes. Neurosci. Biobehav. Rev. 4:241–263; 1980.
4. Davis, M.; Redmond, D. E., Jr.; Baraban, J. M. Noradrenergic agonists and antagonists: effects on conditioned fear as measured by the potentiated startle paradigm. Psychopharmacology (Berlin) 65: 111–118; 1979.
5. Denson, R.; Nanson, J. L.; McWatters, M. A. Hyperkinesis and maternal smoking. Can. Psychiatry Assoc. J. 20:183–187; 1975.
6. Dunn, H. G.; McBurney, A. K.; Ingram, S.; Hunter, C. M. Maternal cigarette smoking during pregnancy and the child's subsequent development: II. Neurological and intellectual maturation to the age of 6½ years. Can. J. Pub. Health 68:43–49; 1977.
7. Johns, J. M.; Louis, T. M.; Becker, R. F.; Means, L. W. Behavioral effects of prenatal exposure to nicotine in guinea pigs. Neurobehav. Toxicol. Teratol. 4:365:369; 1982.
8. Jacobson, S. W.; Fein, G. G.; Jacobson, J. L.; Schwartz, P. M.; Donler, J. K. Neonatal correlates of prenatal exposure to smoking, caffeine and alcohol. Infant Behav. Dev. 7:253–267; 1984.
9. Martin, J. C.; Becker, R. F. The effects of maternal nicotine absorption or hypoxic episodes upon appetitive behavior of rat offspring. Dev. Psychobiol. 4:133–147; 1971.
10. Martin, J. C.; Martin, D. C.; Lund, C. A.; Streissguth, A. P. Maternal alcohol ingestion and cigarette smoking and their effects on newborn conditioning. Alcohol.: Clin. Exp. Res. 1:243–247; 1977.
11. Naeye, R. L.; Peters, E. C. Mental development of children whose mothers smoked during pregnancy. Obstet. Gynecol. 64:601–607; 1984.
12. Olton, D. S. The use of animal models to evaluate the effects of neurotoxins on cognitive processes. Neurobehav. Toxicol. Teratol. 5:635–640; 1983.
13. Peters, D. A. V.; Tang, S. Sex-dependent biological changes following prenatal nicotine exposure in the rat. Pharmacol. Biochem. Behav. 17:1077–1082; 1982.
14. Peters, M. A.; Ngan, L. L. E. The effects of totigestational exposure to nicotine on pre- and postnatal development in the rat. Arch. Int. Pharmacodyn. 257:155–167; 1982.
15. Picone, T. A.; Allen, L. H.; Olsen, P. N.; Ferris, M. E. Pregnancy outcome in North American women. II. Effects of diet, cigarette smoking, stress, and weight gain on placentas, and on neonatal physical and behavioral characteristics. Am. J. Clin. Nutr. 36:1214–1224; 1982.
16. Saxton, D. W. The behavior of infants whose mothers smoke in pregnancy. Early Hum. Dev. 2:363–369; 1978.
17. Schlumpf, M.; Gahwiler, M.; Ribary, A.; Lichtensteiger, W. A new device for monitoring early motor development: Prenatal nicotine-induced changes. Pharmacol. Biochem. Behav. 30:199–203; 1988.
18. Streissguth, A. P.; Martin, D. C.; Barr, H. M.; Sandman, B. M.; Kirchner, G. L.; Darby, B. L. Intrauterine alcohol and nicotine exposure: attention and reaction time in 4-year-old children. Dev. Psychol. 20:533–541; 1984.
19. Wertelecki, W.; Hoff, C.; Zansky, S. Maternal smoking: greater effect on males, fetal tobacco syndrome? Teratology 35:317–320; 1987.