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Prenatal Nicotine Exposure Modifies Behavior of Mice Through Early Development

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AJAREM, J. S. AND M. AHMAD. *Prenatal nicotine exposure modifies behavior of mice through early development.* PHARMACOL BIOCHEM BEHAV **59**(2) 313–318, 1998.—Studies in humans and animal models (including rodents) have revealed lasting behavioral and cognitive impairments in offspring prenatally exposed to nicotine. Offspring of pregnant mouse dams prenatally subjected to 9–10 daily subcutaneous injections into the nape of the neck during pregnancy have been postnatally subjected to several developmental and behavioral tests to investigate the effects of prenatal nicotine exposure on those offspring at various stages of their life. The prenatal exposure to nicotine has resulted in significant reduced postnatal body weight gain, as well as in significant delay in eye opening, in the appearance of body hairs, and in sensory motor reflexes. However, motor activity was significantly stimulated in early adulthood of mouse pups prenatally exposed to nicotine, and such exposure proved to have long-lasting hyperactive effects on mice. Thus, exposure to nicotine during a critical prenatal period of brain development deduced from the present study in mice raises the alarm of the possible hazard of prenatal exposure to nicotine in humans. Hence, smoking by pregnant women might constitute a serious hazard to their in utero developing children. © 1998 Elsevier Science Inc.

Anxiety Mice Behavior Nicotine Offspring Prenatal

A number of studies have revealed the deliterious effects of smoking by pregnant women on their offspring (7,9,15,24,28, 34,37,44,45). On the other hand, animal models have confirmed that nicotine can induce behavioral impairments in offspring of nicotine-treated dams (3,4,12–14,20,27,38,42,43). Such impairments in rodents included deficits in learning and memory (5,16,22,32,33,41). Moreover, ample evidence that of prenatal nicotine effects on the offspring and knowledge that nicotine can penetrate the preimplantation blastocyte and cross the placental barrier, does exist (10,26); however, such studies have failed to evaluate the behavioral changes produced by the prenatal exposure of nicotine in newly born mouse pups. Hence, in the present study an attempt has been made to assess the effects of nicotine on the early development of reflexes in developing mouse pups. Furthermore, the effects of nicotine on the anxiety and on the locomotor activity of weaned mouse pups have also been evaluated.

METHOD

Mice

Male and Female Swiss–Webster strain mice, 8–9 weeks old were used throughout the experiments. They were housed in opaque plastic cages (three females to a single male in each cage) measuring 30 × 12 × 11 cm, in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia. They were kept under reversed lighting conditions with white lights on from 2230 to 1030 h (local time). The ambient temperature was regulated between 18 and 22°C. After pregnancy (the date of finding the vaginal plug was considered as day 1 of pregnancy), the males were removed from the cages. Females were left undisturbed for further 9 days and then subjected to experimental conditions. Food (Pilsbury's Diet) and water were available ad lib.

Treatments

Twenty pregnant mice were randomly separated into two categories of 10 each and were given daily subcutaneous injections (constant injectable volume of 0.1 ml per mouse) in the nape of the neck with either vehicle (normal saline, 0.9% saline) or 0.5 mg/kg body weight, of pure nicotine (Eastman Organic Chemicals, U.S.A.) dissolved in normal saline.

Tests of Developing Pups

The litters from dams that had received 9–10 daily injections of nicotine were chosen to ensure adequate exposure of the foetus to the drug. On the day of birth (postnatal day 0,

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PD0), the pups were culled to only eight per dam and left with their mothers until PD22. During this weaning period, three pups of each litter were colour marked from others and were subjected to various behavioral tests under dim lighting (ca 8 lx). In all, 21 pups belonging to seven litters from each treatment category were considered. All tests were begun on PD1 and repeated every other day until PD21 in the same three pups of each litter. These tests were used to measure the early development of sensory motor coordination reflexes together with morphological development in the pups. For statistical analysis, the mean of all three color marked pups per litter was considered as a single score. Thus, seven replicates from each treatment category were considered in these tests.

Weight

Weight is a useful indicator of physical development. Thus, the pups were weighed every other day using a Mettler digital balance (Mettler, Model PL 3000, Switzerland).

Righting Reflex

The time taken for a pup placed on its back to turn over and place all four paws on the substrate was recorded. An upper limit of 2 min being set for this test.

Cliff Avoidance

Pups were placed on the edge of a table top with the forepaws and face over the edge. The time taken for the pup to back away and turn from the "cliff" was recorded. Again, an upper limit of 2 min was chosen. A latency of 2 min was attributed when the animal fell from the "cliff."

Rotating Reflex

The surface that was used to measure the rotating reflex was the same as that used for righting reflex, except that it was inclined at an angle of 30°. The pups were placed on this surface with their heads pointed downwards. The time taken by a pup to rotate its body through 180° geonegatively and face its head upwards, was recorded as the "rotating time." The upper limit of this test was also set at 2 min.

Eye Opening and Hair Appearance

The days at which the body hair fuzz appeared and the eyes opened were also recorded because these two parameters are also useful indicators of development.

Tests of Young Adults

The offspring were weaned on PD21 and thereafter, the males were isolated and kept in groups of two or three, for 14 days. Subsequently, 10 mice from each treatment group (including representatives from each 10 litters) were subjected to anxiety tests and locomotor activity tests.

Anxiety Test

Anxiety in weaned mouse pups was measured using an elevated plus-maze apparatus consisting of two open (30 × 5 cm) and two closed arms (30 × 5 × 15 cm) extending from a central square (5 × 5 cm), arranged so that the arms of the same type were opposite each other. The floor of the entire maze (plus-maze) was made of black Plexiglas and the walls of the enclosed arms were made of clear Plexiglas. The apparatus was elevated to a height of 38.5 cm above the floor. Testing was conducted in a dimly illuminated (ca 8 lx) room.

Each mouse was placed in the center of the plus-maze facing one of the open arms, and allowed to move freely for 5 min. During the 5-min test the number of entries into each type of arms and the time spent in each arm were recorded by an observer seated 50 cm away from the centre of the maze. A mouse was taken to have entered an arm when all four legs were on the arm. At the end of each trial, the number of entries into the open arms was expressed as a percentage of the total number of arm entries. The time spent on the open arms was also expressed as a percentage of the time spent on both the open and the closed arms. The maze was cleaned after each trial with a 5% Dettol solution (Reckitt and Coleman, Ltd., Hull) to minimize potential effects of odors from previous occupants.

Locomotory Tests

Young adult males were placed in an experimental wooden arena measuring 80 × 80 × 30 cm and the floor was divided into 64 equal-sized squares. Various behavioral "elements" were observed as described by Ajarem (2). Elements of locomotor activity included the numbers of squares crossed, numbers of wall rears, wash, squats, as well as durations of locomotion and immobility. The observations in the arena lasted 300 s for each animal.

Statistical Analysis

The data of body weights, dates of morphological developments, data of sensory motor reflexes, and plus-maze tasks were compared within the experimental groups by the analysis of variance (ANOVA) using a Minitab computer programme, and were subsequently analysed by Student's *t*-test (46). Data of locomotory tests were compared within the experimental groups by the analysis of variance (ANOVA) (39).

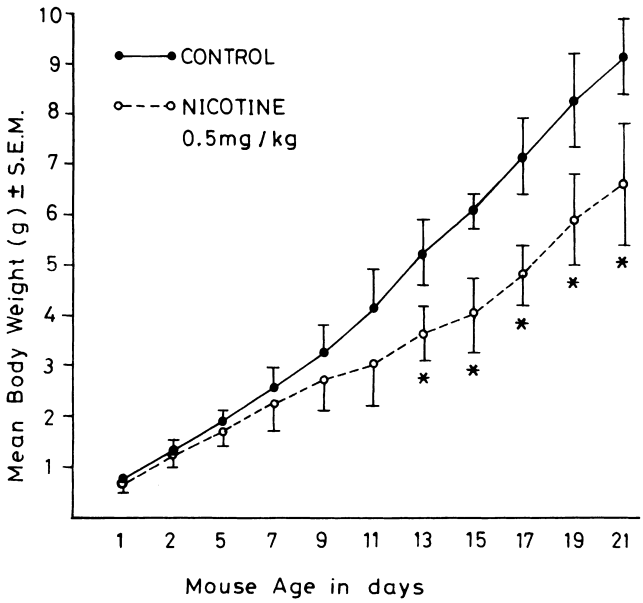

FIG. 1. Effect of prenatal nicotine exposure on the body weight gain of mouse pups. *Siginficantly different ($p < 0.001$) from controls. S.E.M. = Standard error of the mean.

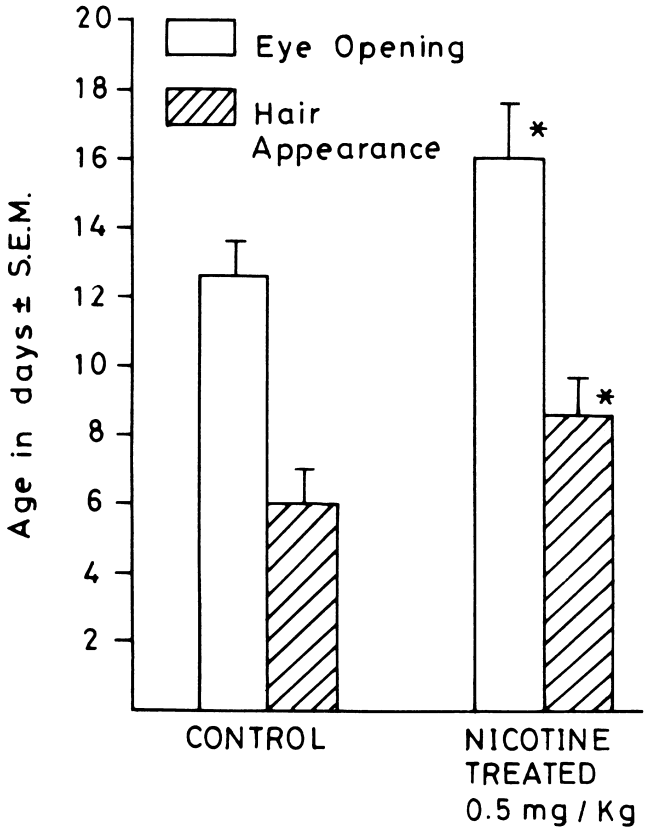

FIG. 2. Effect of prenatal nicotine exposure on the hair appearance and eye opening in mouse pups. *Significant ($p < 0.001$) compared to controls.

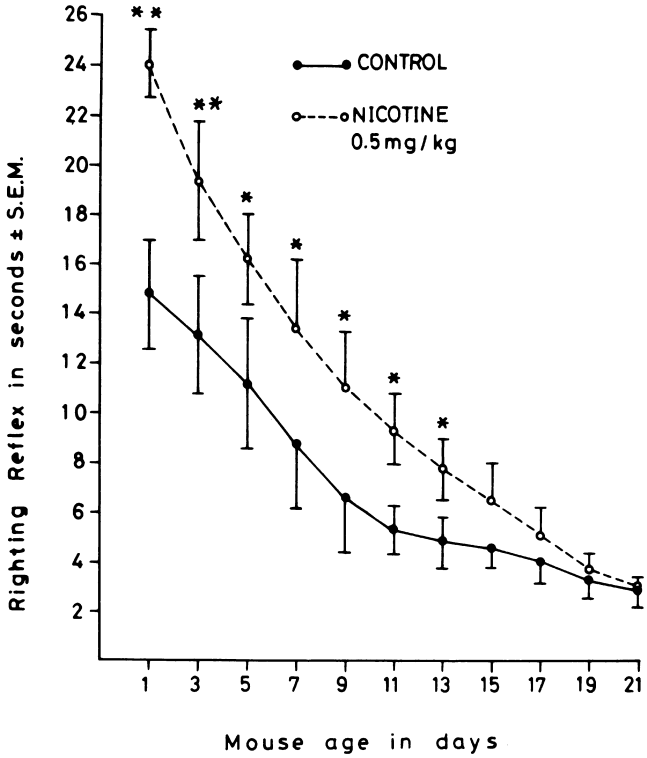

FIG. 3. Effect of prenatal nicotine exposure on the mean righting reflex of mouse pups. *Significant ($p < 0.05$) compared to controls. *Significant ($p < 0.001$) compared to controls.

RESULTS

The body weights of pups born to nicotine-treated and control mice were similar at birth and showed no significant differences until PD11. While the weights of nicotine-exposed pups lagged behind those of controls significantly on PD13, 15, 17, 19, and 21, $F(1) = 17.75, 21.90, 28.60, 19.52$, and 13.89, respectively, $p < 0.001$ (Fig. 1).

Other morphological developments such as the opening of the eyes and appearance of body hair have also significantly, $F(1) = 82.54$, and $F(1) = 32.35$, $p < 0.001$, delayed in the nicotine-treated pups compared to the controls (Fig. 2).

The righting reflex of the nicotine-treated pups was significantly suppressed from the day they were born (PD1) until PD13 [PD1, $F(1) = 84.52$; PD2, $F(1) = 16.80$, $p < 0.001$; PD5, $F(1) = 6.20$; PD7, $F(1) = 6.30$; PD9, $F(1) = 7.37$; PD11, $F(1) = 29.29$; PD13, $F(1) = 19.55$, $p < 0.05$ (Fig. 3). On the other hand, the prenatal nicotine exposure had no effect on the development of rotating reflexes of the pups except on the day of birth (PD1), when their rotating reflexes were significantly suppressed, $F(1, 13) = 63.37$, $p < 0.001$ (Fig. 4), while cliff avoidance was not significantly affected by early nicotine exposure.

In the plus-maze test, it was found that nicotine has significantly increased, both of the percentages of entries made into the open arms and the time spent into those arms, $F(1) = 39.45$ and $F(1) = 230.62$, $p < 0.001$. Locomotor activity in the plus-maze, as measured by the total number of arm entries, was also significantly increased, $F(1, 19) = 35.75$, $p < 0.001$, in the nicotine-treated mice compared to that of the controls (Fig. 5).

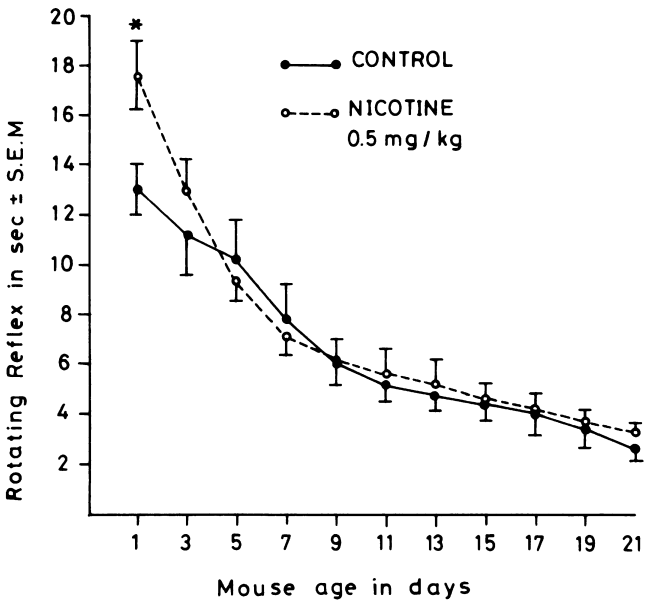

FIG. 4. Effect of prenatal nicotine exposure on the mean rotating reflex of mouse pups. *Significant ($p < 0.001$) compared to controls.

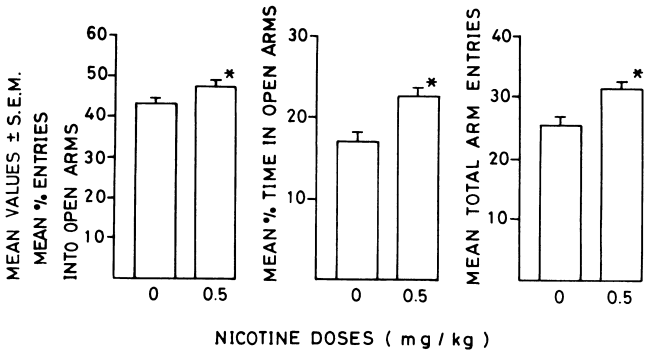

FIG. 5. Effect of prenatal nicotine exposure on the mean percentages of entries into open arms and time spent in those arms, as well as the total number of arm entries by male offspring. *Significantly different ($p < 0.001$) from controls.

The locomotor activity test (Table 1), has shown that prenatal nicotine exposure has a significant stimulatory effect on the numbers of squares crossed, as well as in wall rears and in locomotion duration in weaned animals, $F(1) = 5.36$, $F(1) = 5.71$, and $F(1) = 6.25$, respectively, $p < 0.05$. On the other hand, the numbers of wash and squats, and the duration of immobility has significantly decreased, $F(1) = 5.39$, $F(1) = 5.50$, and $F(1) = 5.73$, respectively, $p < 0.05$, compared to that in the controls.

DISCUSSION

The present results demonstrate that female mice treated with nicotine during pregnancy produce pups that markedly differ from their controls in the rate of physical maturation, motor development, anxiety responses, and locomotory behavior. Moreover, the postnatal suppression of body weight gain and the delay in opening of the eyes, as well as the appearance of body hairs in the nicotine treated pups, might indicate a lasting effect of the prenatal nicotine exposure in mice. Such significant nicotine-induced reduction in the postnatal body weight of the pups has previously been reported not only in the rodents (40,47) but also in humans (23,25, 35,36). Moreover, a strong evidence exists linking both active smoking (35), as well as passive smoking (23,25,35) during pregnancy with reduced birth weight of offspring.

Prenatal nicotine administration has also affected the preweaning reflexes in the mice pups. Both of the righting and rotating reflexes were significantly delayed compared to those of the controls. This clearly suggests a direct nicotine intervention with developing pups in utero because it is known to penetrate the preimplantation blastocyte (10) and cross the placental barrier (26). However, the possibility that these changes resulted also from indirect actions of nicotine treatment, such as hypoxia or ischemia cannot be ruled out (1). In this regard, the present data, particularly, the delays in eye opening and hair appearance seem to be equivalent to deficits in weight gain; suggesting that these effects might indeed be related to general growth retardation. Moreover, the postnatal retardation of development, together with the delayed righting reflex have been reported in offspring of mice prenatally treated with benzodiazepine drugs (19,29–31). Thus, nicotine exposure during fetal life does retard motor development and physical maturation, as have been suggested for other drugs (6).

The elevated plus-maze task in young adult mice might indicate that nicotine significantly increases the percentage of both time spent in, as well as the entries into, the open arms, which is apparently indicative of a possible anxiolytic effect (21) of nicotine in the offspring. However, the total number of arm entries in the plus-maze task is also significantly greater in nicotine-treated young adults than in the controls, suggesting that they are hyperactive or impulsive due to early nicotine exposure. Moreover, the observation on total arm entries (general activity) in the elevated plus-maze task indicated that the possible anxiolytic effect, as well as the stimulant (hyperactive) effect of nicotine may be dissociated from one another in the elevated plus-maze task, as suggested by Hale et al. (11). Furthermore, the locomotory test in early adulthood might also suggest hyperactivity brought about by the prenatal nicotine exposure in the offspring at a later stage in life. A stimulatory effect have also been reported in rats exposed to daily periods of sustained nicotine exposure produced by schedule-induction conditioning (20). It is, however, important to mention here that prenatal nicotine exposure in rats have also been reported to increase neuronal death in the brain stem (18), and the presence of nicotinic receptors in rat brain has already been confirmed (8) and the prenatal nicotine exposure was found to have a direct influence on the developing neurons (47).

Hence, the present results agree with those of Kandel et al. (17), who concluded that nicotine released by maternal smoking can affect the fetus, thus raising similar concerns that during a critical prenatal period of brain development nicotine exposure might modify the properties of the dopaminergic system and thus change the threshold of that system or other related systems. This might further be supported by the present

TABLE 1

EFFECT OF PRENATAL NICOTINE ADMINISTRATION ON THE LOCOMOTOR ACTIVITY OF MALE MOUSE OFFSPRING AT ADOLESCENT AGE

	Median (With Ranges) Number of Acts and Postures					
GROUP	Number of Squares Crossed	Wall Rears	Wash	Squats	Locomotion Duration (s)	Immobility Duration (s)
Controls	166 (70–232)	43.5 (9–76)	10.5 (1–23)	6.5 (2–12)	143.5 (99–125)	158 (105–199)
Nicotine Treated (0.5 mg/kg)	273* (92–245)	83* (57–101)	7* (0–14)	4.5* (1–8)	164* (104–198)	138* (94–171)

*Significantly different from controls ($p < 0.05$).

findings in the behavioral parameters of both neonates and adults prenatally exposed to nicotine. These studies provide evidence that nicotine exposure during pregnancy has potential hazards to the in utero developing child.

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