The effects of nicotine administration in utero upon activity in the rat

JOAN C. MARTIN and R. F. BECKER,
Duke University Medical School, Durham,
N.C. 27706

Holtzman male rat offspring whose mothers were injected twice daily throughout gestation with 3.0 mg/kg nicotine differed little from saline-injected control offspring on activity measures in the wheel. Offspring whose mothers were injected with the same dose of nicotine throughout gestation and the nursing period and offspring whose mothers received a 4% hypoxic episode throughout gestation were significantly more active than the control saline group. Differences were more extreme during the second week of testing than during the first.

A number of studies have demonstrated behavioral changes in the offspring as a result of stress during the fetal period. Various agents capable of crossing the placental barrier have been utilized, including alcohol (Vincent, 1958), irradiation (Furchtgott & Echols, 1958; Martin, 1968; Werboff et al, 1961), oxygen deprivation (Meier & Menzel, 1955; Becker & Donnell, 1952), and sodium bromide (Hamilton & Harned, 1944). Additional experiments have applied environmental stressors to gravid females, such as shock (Denenberg, 1959; Thompson, Watson, & Charlesworth, 1962), handling (Denenberg & Karas, 1959), and stimuli designed to elicit audiogenic seizures (Thompson & Sontag, 1956).

The results of these experiments have not always been consistent since the animal chosen, the strain, the amount and duration of the stressor, and the gestational period during which it is applied all have an effect upon the outcome.

However, at least two investigators have suggested that the behavioral effects of different types of fetal stress appear to be nonspecific for the stressor involved, but specific for the amount of the stressor and gestation period during which it is applied (Thompson et al, 1962; Arshovsky, 1968).

Nicotine crosses the blood-brain barrier to affect the central nervous system, possibly through the release of acetylcholine (Armitage & Hall, 1967). Since it is a CNS activator that results in a change in heart rate, blood pressure, and respiration (Domino, 1967), it is possible that behavioral activation might be a consequence of the drug. Since the CNS is most easily affected during the fetal period

when cellular differentiation is taking place and neural patterns are being formed (Hicks & D'Amato, 1966), the animals in the present study underwent treatment during this period.

The following experiment was an attempt to determine the effects of nicotine administered to the gravid rat upon the activity of the offspring.

METHOD

Pregnancies

Sprague-Dawley females bred in our laboratory from a colony originally obtained from Holtzman were mated overnight at 120 days of age. Ss were assigned to one of four groups, and treatment was begun on the day sperm was found in the vaginal lavage.

Treatment

Gravid nicotine rats received 3.0 mg/kg, with weight determined on Day 1 of pregnancy, subcutaneously, twice daily, of Eastman Kodak reagent-quality pure nicotine in a saline buffer throughout gestation.

The nicotine-nursing group received the same treatment as the comparable nicotine group, with the drug continued through Postnatal Day 21. A 48-h postnatal recovery period was allowed before injections were begun after delivery.

The saline animals received 3.0 mg/kg of an 0.9% solution of isotonic saline twice daily.

It had been observed in our laboratory that nicotine administration to the pregnant guinea pig caused blanching and constriction of the uterine blood vessels. In an attempt to control for the possibility that the results of nicotine injections might be attributable to the decreased availability of oxygen to the fetus, a 4% oxygen control group (hypoxia) was added. Gravid rats were placed in an air-tight Lucite chamber connected to a nitrogen and an oxygen supply, dropped to 4% O₂ over a period of 37-41 min, and held at this level for an additional 5 min before being removed from the chamber. This procedure was repeated once a day through Day 21 of gestation.

All animals were allowed to deliver normally.

Offspring

These were weaned at 21 days of age and females were discarded. Males were placed four to a cage and were allowed free access to food and water. When the animals were 52-57 days of age, sessions in the wheel were begun.

Apparatus

Six Wahmann activity wheels with attached cages were kept in a separate room that was uniformly lighted from above with fluorescent strips. Each animal was assigned to one cage and used it throughout the study, with each wheel being chosen an equal number of times for all groups.

Procedure

The Ss were placed in the wheel and removed 1 h later. The temporal order was varied daily so that all groups were run equally often in the morning and in the afternoon. During the first five sessions, the door from the wheel to the outer cage was open and during the succeeding five sessions it was closed. All Ss were placed by hand in the wheel itself on Day 1 to insure that all had some experience with the apparatus.

RESULTS

Kruskal-Wallis H tests were performed on the weights for Day 1 of each experimental condition. An $H = 9.26$, $df = 3$, $p = .05$ and an $H = 4.81$, $df = 3$, $p = .25$ were obtained on the data for the open and closed wheels, respectively. Although weights were significantly different on Day 1 of test, the differences were no longer apparent by Week 2. The N-N and H animals were lighter at the beginning of the experiment.

Single-factor analyses of variance (Winer, 1962) were performed on the number of turns made in the open and closed wheels. An $F = 3.27$, $df = 3$, $p = .05$ and an $F = 5.18$, $df = 3$, $p = .01$ were obtained for Weeks 1 and 2, respectively. Duncan's multiple-range test (Edwards, 1962) was performed on the saline vs each other group to determine which groups were

Fig. 1. The mean number of turns in the activity wheel under open and closed wheel conditions.

significantly different from controls. During Week 1, only the H animals differed from the S group, with $p = .10$. During Week 2, both H and N-N groups differed from the S group with $p = .005$. The N animals did not differ significantly from controls. See Fig. 1.

DISCUSSION

The two lightest groups were also the most active during the first week. However, weight cannot be the entire explanation since differences in activity were even more extreme during Week 2 when weight differences were no longer significant. The failure of the N-N animals to gain as rapidly as the other groups up to 60 days of age was, in all probability, due to the postnatal injection trauma suffered by the nursing mothers. Whether the low body weights were primarily due to a lack of adequate nursing care or to the effects of the nicotine upon the neonate is not known. Nicotine is excreted in the milk (Perlman & Dannenberg, 1942) and may also be correlated with a lessened milk supply.

The higher activity rate exhibited by the N-N animals may have been due to physiological or biochemical changes wrought by the nicotine during the fetal and neonatal periods or indirectly through the effects of continuing stress to the mother. Neurological or chemical changes, if present during the fetal period, might have conceivably been maintained by the drug administration throughout the nursing period. Nicotine, as a CNS activator, might then have been manifested in the higher activity level that this group displayed.

The high activity level exhibited by the hypoxic group is consistent with the findings of Vierck, King, & Felm (1966) in a shock-avoidance paradigm, although under different experimental conditions and with one exposure to oxygen deprivation rather than the chronic episodes in the present study.

Since there were no differences between N and S groups as measured in the present study, it can be concluded that fetal administration alone does not result in measurable activity change when animals are tested at 60 days of age. The question

of whether or not nicotine has an effect indirectly through hypoxia can also be answered negatively since it was not this group, but the N-N group, whose activity patterns resembled those of the hypoxic animals. If it can be assumed that different types of fetal stressors result in similar behaviors in the offspring, then it might be hypothesized that nicotine in the doses used in these experiments must be administered throughout the nursing period to obtain the activity changes that result from hypoxic episodes during the fetal period only.

The greater differences between controls and the H and N-N groups during Week 2 as compared with Week 1 could have been due either to group differences in response to a more familiar environment or to the fact that there were fewer response options in the closed wheel during the second week and, hence, less chance of variability obscuring the results. Experiments are currently in progress to enable us to make a choice between these alternatives.

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3. Now at Michigan College of Osteopathic Medicine, Pontiac, Michigan 48057.