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BRIEF COMMUNICATION

Effect of Maternally Administered Heroin on the Motor Activity of Rat Offspring[1]

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LASKY, D. I., I. S. ZAGON AND P. J. MCLAUGHLIN. *Effect of maternally administered heroin on the motor activity of rat offspring.* PHARMAC. BIOCHEM. BEHAV. 7(3) 281–284, 1977. – The behavior of 21-day old rats whose mothers were administered heroin (5 mg/kg daily) throughout gestation and lactation was studied utilizing an activity wheel, activity cage, open field test, and step-down latency times from an elevated platform. The total score of all behavioral tasks of offspring from heroin-injected females was statistically different from that of pups from saline-injected mothers, with heroin-treated animals appearing more active.

Heroin Behavior Development Motor

THE WITHDRAWAL syndrome in infants of heroin-addicted mothers is well-documented [2, 5, 8], but little is known about the long-term behavioral development of these children. Newborns of narcotic-treated females have been reported to be irritable and hyperactive, often exhibiting a high pitched cry, tremors, and myoclonic jerks. Studies on addicted children from birth to 34 months of age reveal that 50% of these infants demonstrate behavioral disturbances, predominantly hyperactivity, brief attention spans, and temper tantrums [13].

Although the pharmacological effects of heroin on young adult laboratory animals have been reported [11,12], behavioral studies conducted on developing animals exposed to heroin are limited. Previous investigators [9] have shown a dose-related response to locomotor activity of mice subjected to chronic intravenous or subcutaneous injections of heroin, with the duration of hyperactivity increasing with higher drug concentrations; a maximum dosage of 10 mg/kg was used in these studies. A disruption in the normal diurnal pattern of behavior was observed in adult rats chronically treated on a daily basis for two weeks with 5 mg/kg or 20 mg/kg of heroin [10]. After each heroin injection, these investigators found an initial phase of depressed behavior that was followed by a period of hyperactivity, with the latter response being more pronounced as tolerance to the depressant effects developed. A differential effect on the locomotor activity of two different inbred strains of mice has been reported by Oliverio and Castellano [6], suggesting that mechanisms responsible for individual differences in reactivity to heroin may be genetically controlled [7]. However, when the behavioral activity of heroin was potentiated by the addition of amphetamine or alcohol, previously unreactive mice had increased motor activity [3].

The present investigation was undertaken in order to determine the effects of chronic maternal heroin administration on the behavioral patterns of 21-day old rat offspring that were exposed to this drug during gestation and lactation. Utilizing several behavioral tasks, motor activity was measured in offspring subjected to heroin or saline.

METHOD

Animals and Drug Treatment

Female (180–200 g) and male (250–300 g) Sprague-Dawley rats (Charles River Labs) were housed under controlled conditions [14] with water and Wayne Laboratory Chow available ad lib. All animals were allowed 6 days to acclimate to their surroundings before the beginning of drug injections. Females were treated with an intraperitoneal injection either once daily (0800) with 5 mg/kg or twice daily (1800 and 1800) with 2.5 mg/kg/injection of diacetylmorphine (heroin); control animals were treated with an equivalent volume of saline under similar conditions. Animals were weighed every 2 days and appropriate dosage adjustments made.

Five days after the beginning of drug treatment, females

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were placed with drug-free males for breeding; the presence of sperm in vaginal smears indicated Day 0 of gestation. At birth, litter size was adjusted to 8 pups per mother, with an equal distribution of males and females. All pups were weighed on the day of birth and at weaning (postnatal Day 21). Rat pups were separated from their mothers on Day 21. Twenty-four hr elapsed from the time of the last maternal injection for the 5 mg/kg once daily group, and 14 hr elapsed from the mother's last injection for the 2.5 mg/kg twice daily group, before behavioral tests were administered.

Behavioral Tasks

Rat pups were allowed 1 hr acclimation to the testing location before the beginning of experimentation; all tests were performed on postnatal Day 21. The same animals in each group were tested for all behavioral tasks, and only one animal per group (i.e., saline, 2.5 mg/kg twice daily, 5.0 mg/kg once daily) was placed in a testing apparatus. After 10 min of observation, the rat pup was removed and returned to its home cage; 50 min elapsed before each animal was retested. Rats from each group were rotated among the behavioral tasks in order to control for possible effects of time of day and order of presentation of tests.

Open field test. The open field was constructed of masonite with a 52.5 cm × 52.5 cm surface, divided into 10 cm × 10 cm squares; walls were 20 cm high. Illumination was provided by standard fluorescent ceiling lights and a 60 W light bulb suspended over the center of the field. Each rat was placed in the center square of the field and tested for ten-min time periods. The score for each rat was the number of entries into a single square with all four paws.

Activity cage. A cylindrical activity cage (Lehigh Valley Electronics, Model 145–03), 50 cm in diameter × 31 cm high, was utilized to assess total activity in a darkened area. Inside walls were flat black to minimize ambient light reflections, and the animal's movement was measured by two parallel banks of infrared photobeams. The combined readings of the 2 banks, each connected to its own counter was used as the measure of total movement within the activity cage by each animal for a period of 10 min.

Elevated platform. Measurements of step-down latencies were conducted by placing each animal on a wooden platform, 7 cm × 7 cm × 3 cm, located in the center of a 50 cm × 60 cm grid floor that was enclosed by 35 cm high walls. Rats were allowed to acclimate to the grid floor for 2 min prior to experimentation. Testing consisted of 5 trials, the last 3 of which were averaged to obtain the score for each animal. If the animal did not respond within one min, the trial was terminated and a 60 sec latency was recorded.

Activity wheel. Behavior in the activity wheel (Wahmann Manufacturing Company, Model LC–34) was recorded as the number of revolutions over a ten-min period. The shuttle door from the side cage was closed throughout the experimentation in order to confine the animal to the wheel.

Analysis of Data

Data were normalized for all four behavioral tasks and an average score obtained on each animal by converting raw scores to standardized T-scores. Short latencies on the step-down from the elevated platform were assigned *t*-scores above the mean so that high scores would consistently reflect greater activity. The heroin and saline groups were compared by use of the *t*-test for independent samples for total performance as well as performance on each of the four behavioral tasks. Because no significant differences were found between the two heroin groups on the behavioral tasks, these groups were combined and compared to the saline animals for each of the *t*-tests.

Pearson product-moment correlation coefficients (r) were calculated to determine whether behavioral tasks were measuring similar behavioral activity.

In addition to comparisons between the experimental and control groups, habituation was studied for the open field test, activity cage, and activity wheel by comparing the first five min with the second five min for each of the tasks.

RESULTS

Mean birth weights of rat pups in the control, 5.0 mg/kg heroin daily, and 2.5 mg/kg heroin twice daily groups were 6.19 g, 5.43 g and 5.13 g, respectively. Birth weights for both experimental groups were significantly less ($p<0.01$) than controls, but the mean birth weights of the two heroin-treated groups did not differ significantly from each other. At weaning (Day 21), the body weight of the heroin-subjected rats in the 5.0 mg/kg daily group (40.7 g) and the 2.5 mg/kg twice daily group (41.0 g) were significantly less ($p<0.05$) than controls (46.6 g).

The total score of all behavioral tasks were statistically different ($t = 2.79$; $p<0.01$) between offspring of saline- and heroin-subjected mothers (Table 1); no statistical differences existed between males and females, thus results were combined for both sexes in each group. Although drug-treated pups recorded slightly more counts in the activity cage than the control group (394.4 and 360.2, respectively), they had a mean of 53.5 revolutions in the activity wheel for 10 min in comparison to the control value of 30.0 revolutions. In the open field, heroin pups entered twice as many squares as did control rats (81.2 and 40.0, respectively). The only significant differences ($t = 1.98$, $p<0.05$) between experimental and control animals for an individual behavioral task was the latency response on the step-down from an elevated platform; heroin-treated pups had a decreased latency time of 6.8 sec in comparison to the mean latency time of 15.0 sec for saline-treated pups.

Offspring subjected to heroin recorded significant differences between the first 5 and second 5 min of response (Table 2) in the activity cage ($t = 7.59$; $p<0.01$), open field test ($t = 3.89$; $p<0.01$), and activity wheel ($t = 3.77$; $p<0.01$). Evidence of habituation was not as marked for the saline-subjected pups, however, activity during the second five-min period was significantly reduced in the activity cage ($t = 8.18$; $p<0.01$) and open field test ($t = 2.86$; $p<0.05$).

The Pearson product-moment correlation was significant between the activity cage and activity wheel ($r = .61$; $p<0.01$); other comparisons of activities were not significant ($r = .30$ to $.10$).

DISCUSSION

The results of the present study demonstrate that 21-day

TABLE 1

BEHAVIORAL TASKS OF 21-DAY OLD RATS MATERNALLY SUBJECTED TO HEROIN

	Treatment	
	Saline (n = 6)	Heroin (n = 12)
Total scores (mean T-scores)	44.96	52.44‡
Activity cage* (no. photocell interruptions)	360.2	394.4
Elevated step-down (Response latency, sec)	15.0	6.8†
Open field* (No. squares entered)	40.0	81.2
Activity wheel* (No. revolutions)	30.0	53.5

*Mean values for 10 min. trials.
†Significantly different from controls at $p<0.05$.
‡Significantly different from controls at $p<0.01$.

TABLE 2

COMPARISON OF BEHAVIORAL PERFORMANCE DURING THE FIRST 5 MIN AND SECOND 5 MIN IN 21-DAY OLD RATS

Behavioral Tasks	Time Periods	Treatment*	
		Saline (n = 6)	Heroin (n = 12)
Activity cage (No. photocell interruptions)	First 5 min	233.0	248.0
	Second 5 min	123.3‡	146.8†
Open field (No. squares entered)	First 5 min	31.3	59.4
	Second 5 min	8.7†	21.8‡
Activity wheel (No. revolutions)	First 5 min	17.7	35.2
	Second 5 min	9.2	18.2‡

*All animals were maternally subjected to heroin or saline throughout gestation and lactation.
†Significantly different from first 5 min at $p<0.05$.
‡Significantly different from first 5 min at $p<0.05$.

old rats maternally treated with heroin throughout gestation and lactation were more active than offspring of saline-injected mothers. These findings are consistent with other investigations that have examined the behavioral effects of perinatal exposure to opioids. In this regard, Davis and Lin [4] have reported high motility scores (i.e., ambulation, rearing) for 30- and 70-day old rats that were prenatally subjected to morphine.

Although evaluation of all behavioral tasks in the present study indicated that the heroin-treated rats were more active than controls, only two tests (activity cage and wheel) were significantly correlated, demonstrating similar locomotor activities involved with these tasks. A lack of correlation between other behavioral measurements suggests that although these tests have been used to study motor activity, they may in fact be measuring ambulation or emotional reactivity [1].

In order to determine the pattern of behavioral movements in the open field tests, the number of inner and outer squares traversed were compared. Both control and experimental animals showed a strong preference for occupying outer squares, suggesting higher emotionality among both groups [4]. Comparison of activity levels during the first and second 5 min periods of each 10 min trial for activity cage, wheel, and open field indicated habituation. Although heroin-subjected offspring showed significantly higher levels of total activity for the behavioral tasks, these pups seemed to habituate to the tests as readily as control animals.

Although the results of the present investigation are of a preliminary nature, the increased activity level of offspring maternally treated with heroin is similar to that reported in clinical studies [13] for children of heroin-addicted mothers. These findings suggest that drug abuse of heroin during pregnancy may have important implications in terms of the behavioral development of drug-exposed children.

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