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Development of Motor Activity in Young Rats Following Perinatal Methadone Exposure[1]

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ZAGON, I. S., P. J. MCLAUGHLIN AND C. I. THOMPSON. *Development of motor activity in young rats following perinatal methadone exposure*. PHARMAC. BIOCHEM. BEHAV. **10**(5)743–749, 1979.—Ambulatory behaviors of 21, 45, and 60 day old rats exposed to methadone (5 mg/kg) during gestation and/or lactation were studied by assessing locomotion in an activity cage, open field, and activity wheel, and latency times to step down from an elevated platform. Methadone-exposed rats were found to be generally less active than controls at 21 days of age and more active than saline-treated pups at the 45 and 60 day test periods. In addition, behavioral responses appeared to be dependent on the timing and duration of opioid treatment. These data suggest that prenatal and/or postnatal methadone exposure affects behavior in young rats and provide a functional correlate to our earlier observations of microscopic and neurochemical changes in the brains of methadone-treated offspring.

Methadone Motor activity Open field Activity wheel Activity cage Behavioral development Rats

METHADONE is a synthetic narcotic analgesic that is commonly utilized in detoxification and maintenance programs for narcotic-addicted pregnant women [1]. In spite of its widespread clinical use, the short-term and long-term consequences of methadone exposure on perinatal development have not been elucidated. However, in the limited number of clinical studies that have been conducted, children delivered by methadone-exposed mothers have been reported to be retarded in body growth [11,12] and to exhibit behavioral abnormalities [11].

The developing nervous system of laboratory animals appears to be particularly sensitive to methadone [4, 5, 8, 14–18]. In studies utilizing different schedules of maternal methadone treatment during gestation and/or lactation, drug-exposed offspring were found to have altered patterns of brain and cerebellar development during both the preweaning and postweaning periods [15, 16, 17]. In particular, 21-day old rats treated with methadone during either gestation or lactation had reductions in brain and cerebellar wet weights as well as concomitant decreases in DNA concentration and content; brain DNA content of animals in the gestation-lactation group was also significantly reduced from control values. Although rat pups were removed from methadone exposure at weaning (i.e, Day 21), alterations in brain and cerebellar wet weight, macroscopic dimensions, and neurochemical composition were still observed in these animals 5 1/2 weeks after cessation of drug treatment. Thus, at 60 days of age all methadone-treated animals had significant reductions in brain DNA content, and rats in the gestation and gestation-lactation groups also had marked decreases in cerebellar weight and DNA content.

Although Zagon and McLaughlin [18] found retardation in the ontogeny of spontaneous motor and sensorimotor behaviors in methadone-exposed offspring during the preweaning period, behavioral profiles of opioid-treated animals after weaning have not been ascertained. The present investigation was undertaken in order to determine the effects of perinatal methadone exposure on motor activity at three age periods: 21 (weaning), 45 (juvenile), and 60 (sexual maturity) days. Performance in an activity cage, open field, and activity wheel, as well as latency time to step-down from an elevated platform were measured in offspring subjected to methadone during gestation and/or lactation. The results of this behavioral study were correlated with our previous anatomical, neurochemical, and behavioral findings [8, 14–18].

METHOD

Animals

Female (180–200 g) and male (250–300 g) Sprague-Dawley rats (Charles River Labs, Wilmington, MA) were utilized in this study and housed under controlled conditions (15) with water and Purina Lab Chow available ad lib. All animals were allowed 6 days to acclimate to their surroundings prior to the beginning of drug injections.

Drug Treatment

Females were treated daily with an intraperitoneal injection of either 5.0 mg/kg *dl*-methadone hydrochloride (Dolophine, Eli Lilly Company, Indianapolis, IN) or an

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equivalent volume of saline. Rats were weighed every two days and appropriate dosage adjustments made. Five days after initiating drug treatment, females were mated (one female to one male) and the presence of sperm in vaginal smears indicated the onset of pregnancy (=day 1 of gestation). Three days prior to parturition, pregnant females were placed in solid-bottom cages to deliver their young. Litter size was maintained at 8 pups per mother, with an equal number of males and females. Within 4 hr of birth, 4 groups of animals (based on treatment schedule) were established. One group of litters delivered by methadone-treated females was cross-fostered to mothers receiving saline injections throughout gestation and lactation; these experimental pups were considered to have been subjected to methadone during "gestation alone." A second group of pups delivered by saline-injected females was transferred to mothers receiving methadone during gestation and lactation and was considered to have been subjected to methadone during "lactation alone." Another group of pups, delivered by methadone-treated mothers, was fostered by other mothers who had received methadone during gestation and lactation; these pups were considered to have been given a combined "gestational-lactational" methadone treatment. Finally, offspring delivered by saline-injected females were fostered by other mothers receiving saline throughout gestation and lactation; these pups were considered "controls." At weaning (postnatal day 21), rat pups were removed from their mothers, placed in separate cages by sex, and received no further drug or saline treatment. All offspring were weighed at birth and on Days 21, 45, and 60.

Sixty-four animals, 8 males and 8 females from each of 4 treatment groups, were tested for activity levels at 19–21 days of age. Four males and 4 females from each treatment group were retained for a repetition of these tests at 44–45 days, and again at 59–60 days; the remaining animals were used in other studies not reported here.

Apparatus and Procedures

Activity cage. A cylindrical activity cage (Lehigh Valley Electronics, Model 145–03), 60 cm in diameter and 38 cm high which contained 6 banks of infrared photobeams, was utilized to assess locomotor activity in a darkened area. Inside walls were flat black to minimize ambient light reflections. An animal's movement was measured as the total number of photobeam interruptions during a 5 min period.

Open field. The open field was constructed of masonite with a 52.5×52.5 cm surface divided by painted lines into 25 squares; the walls were 20 cm high. Illumination was provided by standard fluorescent ceiling lights and all lights were turned on throughout the test period. During testing each rat was placed in the center square of the field and allowed to explore the area for 5 min. Locomotion was scored as the total number of squares entered with all four paws.

Activity wheel. Behavior in the activity wheel (Wahmann Manufacturing Company, Model LC-34) was recorded as the number of revolutions during a 5 min period. The shuttle door from the side cage was closed throughout the period in order to confine the animals to the activity wheel.

Elevated platform. Measurements of step-down latencies from an elevated platform were conducted by placing each animal on a wooden platform (7×7×3 cm) located in the center of a 50×60 cm floor that was enclosed by 35 cm high walls. Rats were allowed 2 min to acclimate to the floor and then were placed on the platform. If an animal did not step down within 60 sec, the trial was terminated and a 60 sec latency was recorded. Testing consisted of 5 trials with a 60 sec intertrial interval on each day of examination.

Animals were tested at three age periods: Days 19–21, Days 44–45, and Days 59–60. At the onset of the first tests (i.e., Day 19), rats were removed from their home cages, marked for identification, placed in plastic holding cages (5 rats per cage), and allowed one hr to acclimate to the testing location. Each rat received all four tests; each test required approximately 5 min, with a minimum interval of 50 min between tests. Test order was randomized for all animals in order to control for effects of order of presentation. Rats were returned to their home cages at the end of each test day. This procedure was replicated on each of the next two days.

At 44–45 and 59–60 days, 4 males and 4 females from each of the original groups were re-examined on all four activity measures. The test procedure was identical to that used on days 19–21, except that the tests were administered on only two, rather than three, consecutive days.

Data Analysis

Body weights were evaluated by analysis of variance with Treatment Schedule and Sex considered as between-group variables and Age as a repeated measure. Individual group comparisons between experimental and control animals subsequent to the analysis were made using Dunnett's procedure. [3].

Performance on each of the four activity measures was evaluated by analysis of variance. Because group sizes were reduced following 19–21 day tests, data from this period were analyzed separately from data obtained at 44–45 and 59–60 days.

At the 19–21 day age period, three-factor analyses of variance were used to evaluate: (a) the number of squares entered in the open field, (b) number of revolutions in the activity wheel, (c) number of photobeam interruptions in the darkened activity cage, and (d) latency to leave the elevated platform (totaled for the five trials given each day). Sex and Treatment Schedule (gestation, lactation, gestation-lactation, or control) were treated as between-group variables, and performance on each of the three consecutive test Days was treated as a within-group variable.

Data obtained at 44–45 and 59–60 days on each of the four tests were evaluated using a four-factor analysis of variance. In each of these analyses there were two between-group variables: Age (44–45 and 59–60 days) and Days (two days at each age).

All subsequent comparisons involving groups in the four Treatment Schedules were made using Dunnett's procedure [3]; that is, each of the three methadone-exposed groups was compared with the appropriate control group. Subsequent tests that did not involve Treatment Schedules were made using the Newman-Keuls procedure [13].

RESULTS

Body Weights

Rats that were perinatally exposed to methadone tended to weigh less than controls (Table 1), but this difference was reliable only at certain ages for males. Statistical significance was obtained for the Treatment Schedule ×Age×Sex interaction, $F(6,48)=4.48$, $p<0.01$, and these data are presented

TABLE 1

THE EFFECT OF DIFFERENT SCHEDULES OF MATERNALLY ADMINISTERED METHADONE ON BODY GROWTH OF THE YOUNG RAT

Treatment	Age in days:	Males 21	Males 45	Males 60	Females 21	Females 45	Females 60
Control		47.2*	180.2	239.2	46.8	139.0	174.0
Gestation		39.2	170.7	209.8†	37.5	139.0	161.5
Lactation		41.7	151.2†	196.0‡	41.0	118.8	156.8
Gestation-Lactation		44.0	189.0	216.0	42.0	116.8	193.8

*Values are mean weights (g), N=4.
†Significantly different from controls at $p<0.05$.
‡Significantly different from controls at $p<0.01$.

TABLE 2

THE EFFECT OF PERINATAL METHADONE EXPOSURE ON PERFORMANCE OF YOUNG RATS IN THE ACTIVITY CAGE

Treatment	Age in Days:	Males 19	Males 20	Males 21	Males 44/59	Males 45/60	Females 19	Females 20	Females 21	Females 44/59	Females 45/60
Control		257.2*	263.7	237.9	256.8	233.4	267.1	285.1	268.8	176.8	148.0
Gestation		183.1†	184.1†	163.6†	286.4	177.1	192.8†	213.1†	252.8	200.6	212.4
Lactation		163.8†	205.6‡	179.4‡	384.1†	369.5†	214.0‡	275.5	246.8	348.0†	196.2
Gestation-Lactation		168.1†	240.5	315.5†	331.2‡	160.5‡	190.2†	250.0	220.5	271.5†	266.6†

*Mean number of photobeam interruptions in 5 min. Values at 44/59 and 45/60 are presented as half the mean value of the two days; N=8 on Days 19–21, N=4 on Days 44–60.
†Significantly different from controls ($p<0.01$).
‡Significantly different from controls ($p<0.05$).

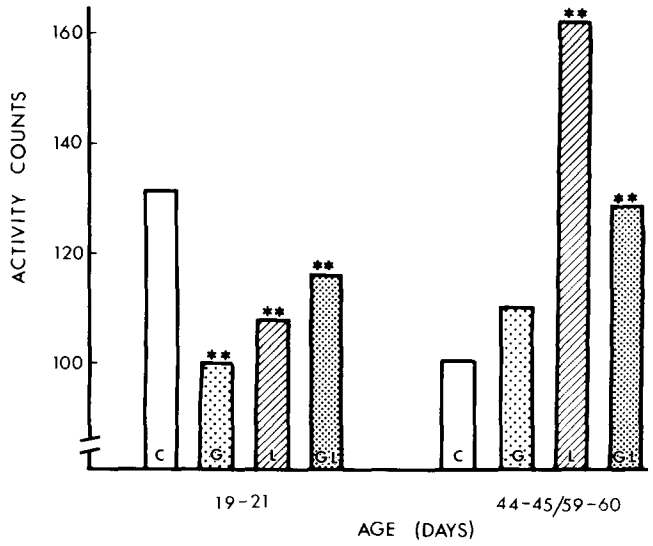

FIG. 1. The effect of perinatal methadone exposure on performance in a darkened activity cage. Each bar represents mean number of photobeam interruptions during 5 min for 8 male and 8 female rats at the 19–21 day period and 4 male and 4 female animals at the 44–45 and 59–60 day periods. Rats were subjected to methadone during gestation (G), lactation (L), or gestation-lactation (GL); C=saline-treated controls. Significantly different from controls at $p<0.01$ (**).

in Table 1. Males exposed to methadone only during the lactation period weighed significantly less than controls at both 45 and 60 days of age, while males exposed only during gestation weighed less than control rats at 60 days. The body weights of animals exposed to methadone during both gestation and lactation did not differ from control values in this study.

19–21 DAY TEST PERIOD

Methadone-treated animals were generally less active than controls at the 19–21 day period (Figs. 1, 2, 3). Pups in the gestation group were significantly less active than controls in all parameters except the activity wheel. Rats exposed to methadone during lactation or both gestation and lactation exhibited marked differences from controls in the activity cage and open field tests.

Activity cage. The interaction between Treatment Schedule, Sex, and Days was statistically reliable in the analysis of activity cage data at 19–21 days, $F(6,112)=2.25$, $p<0.05$, and these data are presented in Table 2. On Day 19, rats of both sexes in all methadone-exposed groups made fewer photobeam interruptions than control rats. Males and females in the gestation-lactation group and females in the lactation group were the only animals observed to be significantly more active on Day 20 than Day 19, and the subnormal activity levels recorded for these animals on Day 19

were not apparent on Day 20. All other methadone-treated animals were significantly less active than controls in activity cage performance on Day 20. On Day 21, males in the gestation and lactation groups were less active than controls in the activity cage, while the activity of females in the methadone groups was not significantly different from controls. The activity of males in the gestation-lactation group was even higher on Day 21 than on Day 20, and rats in this drug-treated group actually made more photobeam interruptions than control males on Day 21.

In general, female rats were more active than males from the same group (Table 2), however, this sex difference was reversed for animals in the gestation-lactation group on Day 21.

Open field. The overall Treatment Schedule effect was statistically reliable in the open field test, $F(3,56)=6.90$, $p<0.01$. Methadone-exposed rats in all groups entered significantly fewer squares on Days 19–21 than control offspring (Fig. 2).

The overall Days effect was statistically significant, $F(2,112)=13.21$, $p<0.01$, reflecting the fact that animals in all groups entered an increasing number of squares on successive days. Rats entered an average of 39.2 squares on Day 19, this increased to 50.3 squares on Day 20 ($p<0.05$) and to 70.5 squares on Day 21 ($p<0.01$, relative to Day 20).

Elevated platform. The overall Treatment Schedule effect was statistically reliable in the analysis of latencies to step down from an elevated platform, $F(3,56)=4.20$, $p<0.05$, and these data are presented in Fig. 3. All methadone-treated groups tended to remain on the platform for a longer period than controls; however, this difference was statistically reliable only for rats in the gestation group.

The overall Days effect was statistically reliable $F(2,112)=82.67$, $p<0.01$, indicating that animals in all groups took less time to step down on each successive day. Average latencies to leave the platform were 191.1 sec on Day 19, 80.4 sec on Day 20 ($p<0.01$), and 48.7 sec on Day 21 ($p<0.01$, relative to Day 20).

Activity wheel. There were no significant differences between methadone-exposed rats and controls in the number of activity wheel revolutions made during the 19–21 day test period. The overall Days effect was significant, $F(2,112)=24.79$, $p<0.01$, which reflected a tendency for animals to become more active as the test period progressed. Rats averaged 31.1 revolutions during the 5 min period on Day 21, which was significantly more than the 17.4 revolutions recorded on Day 20 ($p<0.01$) and the 13.8 revolutions on Day 19 ($p<0.01$); the number of revolutions on Day 19 did not differ from that measured on Day 20.

44–45 and 59–60 DAY TEST PERIODS

In contrast to the reduced activity levels measured at weaning (19–21 day test period), methadone-treated animals at the 44–45 and 59–60 day test periods were generally more active than controls (Figs. 1, 2, 3). Animals subjected to methadone only *in utero* were more active than controls in the open field (Fig. 2) and had shorter latency times in the elevated platform test (Fig. 3). In comparison to controls, rats in the lactation group were more active in activity cage (Fig. 1) and open field tests (Fig. 2), while animals in the gestation-lactation group were more active in the activity cage (Fig. 1) and had shorter latency times in the elevated platform test (Fig. 3).

Activity cage. The Treatment Schedule×Sex×Days interaction was statistically reliable in this analysis,

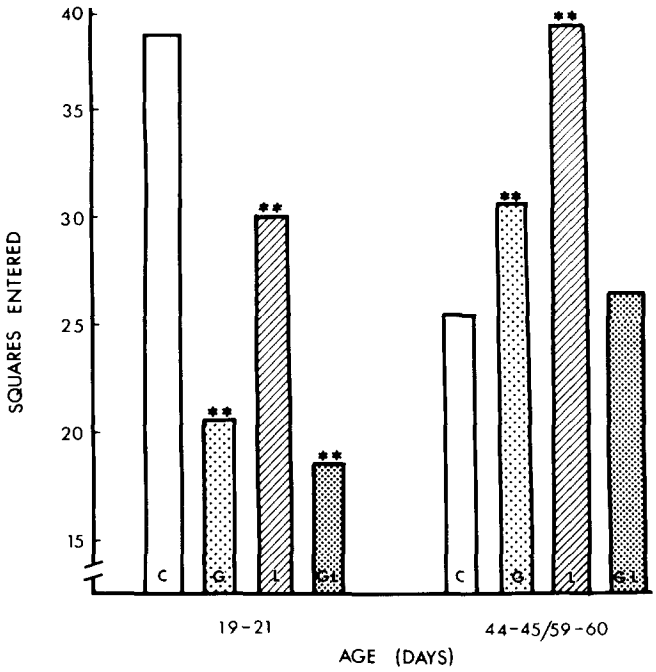

FIG. 2. The effect of perinatal methadone exposure on motor activity in an open field. Each bar represents mean number of squares entered during 5 min for 8 male and 8 female rats at the 19–21 day period and 4 male and 4 female animals at the 44–45 and 59–60 day periods. Rats were subjected to methadone during gestation (G), lactation (L), or gestation-lactation (GL); C = saline-treated controls. Significantly different from controls at $p<0.01$ (**).

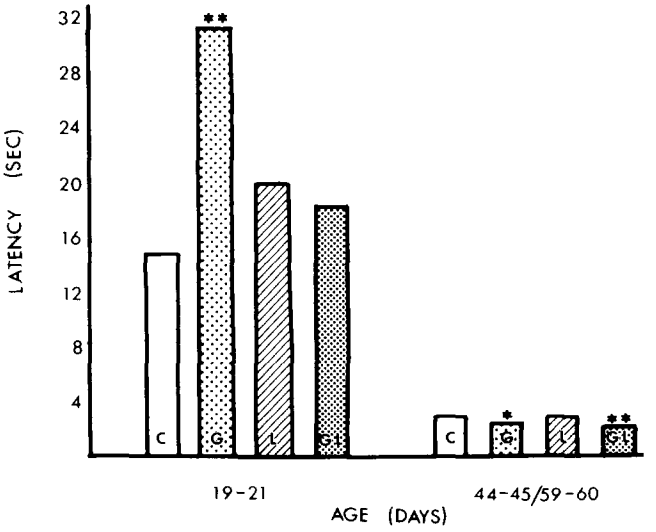

FIG. 3. The effect of perinatal methadone exposure on latency (sec) to step down from an elevated platform. Each bar represents mean latencies for 8 male and 8 female rats at the 19–21 day period and 4 male and 4 female animals at the 44–45 and 59–60 day periods. Rats were subjected to methadone during gestation (G), lactation (L), or gestation-lactation (GL); C=saline-treated controls. Significantly different from controls at $p<0.05$ (*) and $p<0.01$ (**).

TABLE 3

THE EFFECT OF PERINATAL METHADONE EXPOSURE ON ACTIVITY WHEEL PERFORMANCE OF RATS AT 44–45 AND 59–60 DAYS OF AGE

Treatment	Age			
	44	45	59	60
Control	9.2*	26.7	41.4	58.1
Gestation	30.6	22.5	94.4†	49.0
Lactation	40.9‡	35.2	74.1‡	95.1†
Gestation-	15.6	17.0	79.5†	65.1

*Mean number of revolutions in 5 min (n=8).
†Significantly different from controls ($p<0.01$).
‡Significantly different from ($p<0.05$).

$F(3,24)=3.65$, $p<0.05$, and these data are presented in Table 2. On the first day of each test period, Days 44 and 59, rats in the lactation and gestation-lactation groups made significantly more photobeam interruptions than controls; these differences existed for both sexes. On the second day of each test period, Days 45 and 60, only male rats in the lactation group and female rats in the gestation-lactation group were more active than controls. Males in the gestation-lactation group no longer exhibited the increased activity levels relative to controls as recorded on Days 44 and 59, and even were significantly less active than controls on the second day (Days 45 and 60). Neither males nor females in the gestation group differed significantly from controls in this apparatus.

In addition to the change in relative activity levels of methadone-exposed and control animals between the 19–21 day period and the 44–45 and 59–60 day test periods, there was a reversal in direction of the Sex and Days effects in the two analyses. In the analysis of ambulatory activity at the 44–45 and 59–60 day test periods, males generally made more photobeam interruptions than females. The only exception to these findings were males in the gestation and gestation-lactation groups who recorded fewer photobeam interruptions than their female counterparts on Days 45 and 60.

A general decrease in activity levels was observed on successive test days at both the 44–45 and 59–60 day test periods for control and methadone-exposed groups; this trend was in contrast to the increased activity levels recorded on successive days in the activity cage at the 19–21 day test period.

The overall Age factor was statistically significant, $F(1,24)=10.08$, $p<0.01$, reflecting increased mean photobeam interruptions for all animals between the juvenile test period (Days 44–45; $\bar{x}=442.6$ for 10 min) and sexual maturity (Days 59–60; $\bar{x}=560.8$ for 10 min).

Open field. The overall Treatment Schedule effect was statistically significant in the analysis of locomotor behavior in the open field, $F(3,24)=4.55$, $p<0.05$, and the mean number of squares entered by rats in each group is presented in Fig. 2. Methadone-exposed animals in both gestation and lactation groups entered significantly more squares than controls. Animals in the gestation-lactation group also appeared somewhat more active than controls, but this difference was not statistically reliable.

The interaction between Sex and Age was statistically reliable, $F(1,24)=5.66$, $p<0.05$. Males entered significantly more squares at 59–60 days of age than at 44–45 days (30.1 and 15.4 squares entered, respectively), whereas females entered approximately the same number of squares at each age (26.4 squares entered at 59–60 and 25.2 squares at 44–45 days). The sex difference was reliable at 44–45 days, with females entering more squares than males ($p<0.01$); however, by 59–60 days of age male and female rats had comparable ambulatory scores.

Elevated platforms. The overall Treatment Schedule effect was marginally significant in the analysis of latency times to leave the elevated platform at 44–45 and 59–60 days, $F(3,24)=2.41$, $p<0.10$. Subsequent planned comparisons between methadone-exposed and control animals revealed that some group differences were statistically reliable, and these data are presented in Fig. 3. Methadone-exposed rats generally required less time to leave the platform than controls, with animals in the gestation and gestation-lactation groups stepping down significantly faster than controls.

Activity wheel. The Treatment Schedule × Days × Age interaction was statistically reliable in the analysis of activity wheel revolutions, $F(3,24)=6.78$, $p<0.01$, and these data are presented in Table 3. In accord with the other measures of ambulatory behavior at these ages, methadone-exposed animals tended to make more revolutions than controls. On Day 59 all methadone-treated groups were significantly more active than controls, while on Days 44 and 60 rats treated only during lactation made significantly more revolutions than controls.

DISCUSSION

In an earlier study [18], we examined the effects of perinatal methadone exposure on the ontogeny of gross motor development, the appearance of certain physical characteristics, and the maturation of both simple and complex sensory and motor behaviors during the preweaning period (i.e., Days 2–19). The results of this previous investigation revealed that the age at which a specific behavior initially appeared for any group member and the ages at which 50% and a maximal number of animals demonstrated a particular behavior were often delayed several days for methadone-treated offspring in comparison to controls. Moreover, the time interval between the age of initial appearance and maximal achievement of a positive response was also found to be protracted. However, every drug-exposed rat eventually expressed all of the behavioral responses by the conclusion of the observation period. Although methadone-exposed rats had only a transient retardation in preweaning behavioral development and achieved a full complement of spontaneous motor and sensorimotor behaviors by postnatal Day 19, the present findings indicate that perinatal methadone treatment also has a deleterious effect on behavior in the postweaning period. Our results demonstrate that rats treated with methadone *in utero* and/or during lactation, and examined on a battery of tests generally related to ambulation, were less active than controls at weaning, but more active by postnatal Day 45; these increased activity levels were still present at sexual maturity (Day 60). Thus, methadone exposure during gestation and/or lactation has a profound effect on the activity of young rats, with the direction of the effect dependent upon age. Furthermore, our data reveal that the magnitude of behavioral alterations is related to the schedule of opioid treatment at weaning, with animals in the gestation group exhibiting the most marked changes at the 19–21 day period. However, the magnitude of behavioral alterations for all 3 schedules of opioid treatments were comparable at both the 44–45 and 59–60 day test periods.

It should be mentioned, however, that a number of behavioral patterns of drug-treated rats closely resembled those found for controls. Females of both control and methadone groups generally had greater mean activity levels in the darkened activity cage than males at the 19–21 day period, but males were more active than females in this test at the 44–45 and 59–60 day periods. Moreover, all control and methadone animals were generally more active in every behavioral parameter on the last day (Day 21) of the 19–21 day test period than on the first day (Day 19). Finally, both drug-exposed and control rats had overall heightened activity responses in the activity wheel and activity cage tests at 59–60 days of age in comparison to the 44–45 day period. Thus, perinatal methadone exposure does not totally disrupt normal behavior but rather exerts a selective influence upon certain aspects of behavioral ontogeny.

The present results show that not only did all three of the methadone groups differ from the controls in terms of behavior but, even more importantly, they differed from one another. These differences not only pertained to the degree of functional loss, but different measures of activity were altered by different drug schedules, suggesting that the behavioral abnormalities associated with perinatal methadone exposure may depend on the specific central and/or peripheral nervous system structures damaged. Since the ontogeny of the nervous system proceeds on a precise timetable, with neuronal cells arising in a discrete chronological order, it may be expected that changes in behavior within each group are directly related to the timing of the insult introduced by methadone. Therefore prenatal drug exposure would reflect abnormalities in the neural elements originating during this period. However, the results of this study and others [14–18] reveal that predictability as to the origin of these neural abnormalities on this basis is complicated by certain pharmacologic properties of methadone. For example, continuous methadone administration during gestation and lactation does not necessarily produce a cumulative neurobiological effect. In fact, animals subjected to this drug either *in utero* or during lactation often demonstrate the most marked alterations. In addition, methadone disturbs developmental events long after cessation of drug exposure. Thus, methadone's ability to produce tolerance and its persistent effects following drug removal make the task of deciphering the brain loci and mechanisms underlying methadone's actions extremely difficult.

Although the specific areas of the nervous system as well as the nature of substrates (e.g., anatomical, biochemical) involved in methadone's effect on certain aspects of behavior remain undefined, previous investigations [8, 14–18] in our laboratory have demonstrated that offspring perinatally subjected to methadone have impaired somatic and neurobiological development, with the most deleterious effects occurring in rats of both the gestation and the lactation groups. At 21 days of age, these two groups of animals had brain and cerebellar weights that were significantly reduced from control levels, as well as marked decreases in DNA content of the brain (37% and 47%) and cerebellum (11 to 19%). Although brain weights of all methadone-treated pups were comparable to controls at 60 days of age, animals in the gestation and lactation groups had brain DNA contents that were 56% and 72%, respectively, of control values and the cerebellar DNA contents of rats in the gestation and gestation-lactation groups were decreased by 9–11%. In addition, RNA and protein contents were often found to be abnormal in the brain and cerebella of methadone-treated animals, with the specific changes governed by the schedule of drug treatment. Although at this time it is unclear whether these structural and neurochemical alterations in brain and cerebellar maturation are causally related to the disturbances in behavioral ontogeny of opioid-treated offspring, it is interesting to note that those groups of methadone-exposed animals (i.e., gestation and lactation groups) with the most severe deficits in cell number (as suggested by the DNA content), cell size (reflecting protein/DNA ratios) and RNA content, often demonstrated the greatest number of behavioral changes. However, it should also be recognized that other abnormalities in neuro-ontogeny (e.g., synaptogenesis, myelination) may have occurred concomitantly and be of important consequence in accounting for methadone's deleterious effects on behavior.

Relatively few studies [2, 6, 10, 18] have examined the effects of perinatal opioid exposure on the behavior of young rats. Davis and Lin [2], investigating offspring that were maternally exposed to morphine during gestation (Days 5–18), focused on the behavior of rat pups at 30 and 70 days of age. These animals exhibited an increased activity in open field tests and in rearing at both time periods, but only 70-day old pups were found to have an increased response in activity cage tests. Sobrian [10] has explored the influence of maternal morphine treatment on the behavioral development of rat pups from 1 to 30 days of age. In her study, female rats were administered morphine 5 days prior to mating and during gestation (up to 4–6 days before birth). Sobrian found that spontaneous activity levels were similar to controls until 10 days of age, but between Days 15 and 25 these animals showed a sustained period of hyperactivity; by 30 days of age activity levels of morphine-treated and control animals were similar. An increase in motor activity was also found in 21-day old rats whose mothers were administered heroin throughout gestation and lactation [6].

The results of the present study show a biphasic effect in regard to the behavior of rat offspring exposed to methadone. Our findings of a reduction in activity levels of methadone-treated rats at the 21-day period are consistent with earlier reports [18] showing a retardation in the preweaning development of spontaneous motor and sensorimotor behaviors in these animals, but differs from the behavioral observations with morphine-exposed [2] and heroin-exposed [6] animals. It must be recognized that comparisons between studies using different narcotic drugs may be tenuous because results are dependent on the opioids utilized and experimental procedures employed (e.g., strain of rats, housing conditions, route and schedule of drug administration). Thus, it appears that preweaning behavioral response to perinatal opioid exposure is governed by the drug utilized, but that subsequent behavior (at least up to 2 months of age) is characterized by an increased level of activity.

The results of the present investigation may be correlated with clinical observations of children delivered by mothers subjected to methadone. These children tend to have electroencephalographic and behavioral changes consistent with increased central nervous system irritability and lowered overall alertness [7,9]. Behavioral profiles of these drug-exposed children during the first 2 years of life are also characterized by hyperactivity and a high intensity of response [11]. In view of these clinical findings, it appears that further research is needed to define the short- and long-term behavioral changes associated with perinatal methadone exposure.

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