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Learning Ability in Adult Female Rats Perinatally Exposed to Methadone[1]

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ZAGON, I.S., P.J MCLAUGHLIN AND C.I. THOMPSON *Learning ability in adult female rats perinatally exposed to methadone* PHARMAC BIOCHEM BEHAV. 10(6): 889–894, 1979.—Cognitive functioning of adult female rats that were maternally exposed to methadone (5 mg/kg daily) during gestation and/or lactation was studied by assessing performance on a food-motivated light-dark discrimination learning test and on active and passive shock-avoidance tests. Methadone-exposed rats exhibited difficulties on the light-dark discrimination learning and the active avoidance tests, and behavioral deficits appeared to be related to the timing and duration of drug treatment. On the light-dark discrimination test only 33% of the rats in the gestation group and 25% of the animals in the lactation group met criterion in comparison to 87% of the control rats Thirty-three percent of the animals in either the gestation or gestation-lactation groups met criterion on the active avoidance test in contrast to 87% of the controls. These data suggest that perinatal exposure to methadone impairs cognitive abilities in the adult female rat.

Methadone Adult learning Female rats Active avoidance Passive avoidance Light-dark discrimination Perinatal Narcotics

METHADONE is a synthetic analgesic that is frequently employed in detoxification and maintenance programs for heroin-addicted pregnant women [2]. This drug crosses the placenta and enters the fetal circulation of humans [1,6] and laboratory animals [7, 12, 14], and has been detected in the milk of lactating humans on methadone maintenance [1]. Despite its widespread use, the short- and long-term consequences of perinatal methadone exposure on offspring have not been firmly established. In fact, it is known that the fetus may become passively addicted to methadone and infants delivered by narcotic-addicted mothers often experience the abstinence syndrome [1,2]. Furthermore, methadone-exposed children may have a retarded body growth [18,19] and exhibit behavioral abnormalities [9, 13, 18].

The developing nervous system of laboratory animals appears to be particularly sensitive to methadone. In our studies utilizing maternal methadone treatment during gestation and/or lactation, drug-exposed offspring have been found to have altered patterns of brain development at both 21 and 60 days of age [10, 20–23]. In particular, 21-day old rats treated with methadone during either gestation or lactation exhibited significant reductions in brain and cerebellar wet weights, as well as concomitant decreases in DNA concentration and content; brain DNA content of rats exposed to methadone during both gestation and lactation was also significantly reduced from control values. Although rat pups in these studies received no further methadone exposure after weaning (i.e., Day 21), significant alterations in macroscopic dimensions and neurochemical composition were still observed 5½ 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 exposed to the drug during either the gestation or gestation-lactation periods also had marked decreases in cerebellar weight and DNA content.

Offspring subjected to methadone *in utero* and/or during lactation also appear to have aberrant patterns of behavioral development [24,25]. Thus, methadone-treated rats examined during the preweaning period were retarded in the development of spontaneous motor and sensorimotor behaviors as compared to control animals [24]. At weaning, methadone-exposed offspring were less active than controls in a variety of test situations related to motor activity, but at 45 and 60 days of age these animals were hyperactive in comparison to controls [25].

In view of the morphological, biochemical, and behavioral alterations already known to occur in rats perinatally exposed to methadone, the present study was undertaken in order to determine whether adult learning ability is also impaired in these animals. The performance of 120-day old rats that had been maternally subjected to methadone during gestation and/or lactation was evaluated on three cognitive

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tasks. These included an active shock-avoidance test, a passive avoidance test, and a light-dark discrimination test rewarded by food.

METHOD

Animals

Male (250–300 g) and primiparous female (180–200 g) Sprague-Dawley rats (Charles River Labs, Wilmington, MA) parented the offspring that were tested in this study. These adults were housed under standard laboratory conditions that have been described in detail elsewhere [21], with water and Wayne Laboratory Chow available ad lib. All animals were allowed 6 days to acclimate to their surroundings prior to the beginning of drug or saline injections (described below).

Thirty-one female progeny were utilized in the present study. Male offspring were used in another experiment not described here. Animals were 120 days old when testing began.

Drug Treatment

Adult 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 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 day of pregnancy (=day 1 of gestation). Three days prior to parturition pregnant females were placed in solid-bottom cages containing Easi-Litter (Westminster Scientific Co., Westminster MD) to deliver their young.

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 to other mothers receiving saline throughout gestation and lactation: these pups were considered "controls." Litter size was maintained at 8 pups per mother, with an equal number of males and females.

At weaning (Postnatal Day 21) rat pups were removed from their mothers, placed in separate cages according to sex (5 or 6 animals per cage), and received no further drug or saline treatment. Prior to the onset of testing (i.e., 120 days of age), all offspring were weighed.

The present study tested 6–9 females from each of the four drug treatment schedules. Nine animals had been exposed to methadone only during gestation, 8 had been exposed to methadone only during lactation, 6 had received methadone exposure throughout gestation and lactation, and 8 were controls that had been exposed to saline during both gestation and lactation.

Apparatus

All training procedures were conducted in a test room that was maintained at an ambient temperature of 21±0.5°C and a humidity of 50±10%. Three test apparatuses were used, one for each of the three learning paradigms, and these are described below.

Active avoidance Active avoidance tests were conducted in a standard 2-way shuttle box (Lafayette Instrument Co.): each compartment was 30.5 X 19.0 X 20.0 cm. The floor consisted of stainless steel rods, 2.3 mm in dia., through which shock could be delivered. The two compartments were separated by a 9.5 X 9.5 cm opening flush with the floor. A 6 W light bulb was suspended from the ceiling in both compartments; these lights provided illumination and were used as the conditioned stimulus. The unconditioned stimulus was a 1 mA shock passed through a Lehigh Valley model 1531 scrambler.

Discrimination learning. Animals were tested in a 4-unit discrimination box [8]. The apparatus consisted of a start box, 4 two-choice discrimination units and a goal box. Total length of the unit was 2.6 m and the width was 25 cm. Guillotine doors between compartments were opened prior to starting each trial and closed after the animal passed through the area. Each discrimination unit contained two 9.5 cm^2 alley entrances which were flush to the floor. A moveable barrier was positioned 11.4 cm beyond the entrance to either of the alleys in each unit in order to prevent the rat from entering the next discrimination compartment before a correct choice was made. Black cloth curtains were hung 10 cm beyond the alley entrances to prevent rats from being able to see the barrier. Either of the alleys could be illuminated by a 15 W incandescent bulb located immediately beyond the alley entrance. Overhead lights in the room were off during the experiment.

Passive avoidance. A wooden platform (7×7×3 cm), located in the center of a 50×60 cm grid floor which was enclosed by 35 cm high walls, comprised the apparatus for testing passive avoidance. The grid floor was connected to a shock source similar to that used in the active avoidance tests. A Plexiglas cage that surrounded, but could be lifted from, the wooden platform restrained the rats prior to each trial.

Procedure

The order of administration for the three learning tasks was kept constant for all groups of animals and consisted of the following: active avoidance, discrimination learning, and passive avoidance. Within each of these three paradigms the order of animal testing was randomly predetermined; this test order remained constant until testing had been completed for each task. On any given day testing always began at 8:00 A.M. and was generally completed by noon; the only exception was the light-dark discrimination task, which sometimes required until midafternoon to complete.

Active avoidance. These tests were designed to measure the ability of an animal to respond to the onset of a 6 W cue light by changing compartments within 5 sec in order to avoid receiving footshock. Each animal was tested for six consecutive days. On the first day (habituation), the rat was allowed to explore both compartments in the shuttle box for a 15 min period during which no stimulus was presented and no footshock was administered. Avoidance training occurred on Days 2–6 On each of these 5 days, 20 training trials with a

45 sec intertrial interval were conducted. Thus, each rat received a total of 100 trials. Illumination of the test compartment signalled that shock would be administered in 5 sec. If the rat crossed to the alternate compartment within this interval no footshock occurred and an avoidance response was recorded. If an avoidance was not made, a 1 mA footshock was administered continuously until the animal crossed to the other compartment (escape): if the rat did not successfully cross within 10 sec, the footshock was terminated and the trial ended. Dependent variables were the number of animals meeting a criterion of 5 consecutive avoidances, and the number of trials required to meet this criterion. The total number of avoidances, number of times footshock was allowed to continue for 10 sec, and number of crossings from one compartment to the other (both during and between trials) were also noted.

Discrimination learning. During these tests, rats were maintained on a one hour per day feeding schedule, with food available for one hour immediately following each test session.

Rats were habituated to the discrimination apparatus by allowing them to explore the apparatus for 10-min sessions on each of 5 days. No barriers were present during habituation and all compartments were lighted. A palatable wet mash was present in the goal box.

During actual training, one randomly chosen alleyway was illuminated and a barrier was positioned across the darkened exit on the opposite side. The rat was placed in the start box and allowed to find its way to the goal box. The guillotine doors of each compartment were lowered after the animal passed into the next compartment in order to prevent the rat from returning to the start box. A one minute interval in the goal box was allowed between runs, during which time the animals had access to the wet mash. Each rat made 5 runs (20 discriminations) per day for 5 days. A wrong choice occurred if the rat's head and forefeet passed through the opening of a blocked alley. For half of the rats in each treatment schedule the lighted side of the alley was correct: for the remaining rats the dark side was correct. The sequence of lighted and darkened choicepoints was randomized for each of the 25 runs. Dependent variables were the number of errorless trials out of 100, the number of trials required to reach a criterion of 9 errorless trials within a series of any 10 consecutive discriminations, and the proportion of animals in each group that met this criterion.

Passive avoidance. In this test rats were trained to remain on a wooden platform in order to avoid a footshock that was administered when they stepped onto the grid floor. This test required 5 days to complete.

On the first 2 days rats were habituated to the apparatus by individually placing them on the platform inside the Plexiglas enclosure. After 15 sec the Plexiglas frame was lifted and the rats were allowed to step down and explore the grid floor for 2 min. Training occurred on Day 3 and involved the same procedure as described for habituation except that immediately after the animals stepped down a 1.5 mA footshock was continuously administered for 2 sec. One-half of the animals received the training procedure (i.e., shock), whereas the other half received no footshock and served as a nonshocked control group. All rats were immediately removed from the grid floor after stepping down from the platform. On Day 4 (testing), all animals were allowed to step off the platform, but no footshock was administered; the trial was terminated if 180 sec elapsed before the rat stepped down. On Day 5, extinction was measured by placing the rats on the platform and monitoring the latency to step down. No footshock was administered and animals were allowed to remain on the platform for a maximum of 180 sec.

Learning was assessed as an increased latency to leave the platform on Day 4 relative to Day 3 for animals that had received footshock; rats that did not receive footsock on Day 3 served as controls for the effects of time. Group differences in recovery of step-down performance were measured on Day 5.

RESULTS

Body Weight

The body weights of methadone-treated females were not significantly different from control females at 120 days of age. The mean body weight (±SE) of control females was 305.75 (±14.13 g), while females in the gestation, lactation, and gestation-lactation groups weighed 287.00 (±17.31 g), 312.40 (±20.86 g), and 319.00 (±13.45 g), respectively.

Active avoidance

Group differences in the numbers of animals successfully meeting the criterion of 5 consecutive avoidances within 100 test trials were analyzed using the Fisher Exact Probability test [15], and these data are presented in Table 1. When data from the three experimental groups were combined and compared with controls, a significantly smaller proportion of methadone-treated rats met criterion (43.5% methadone and 87.5% control, $p=0.037$). Further comparisons between control animals and rats from the individual methadone treatment schedules revealed that significantly fewer animals treated with drug either *in utero* or during both gestation and lactation met the criterion of 5 consecutive avoidances within 100 test trials: in each of these two groups only 33% of the animals met this criterion, in comparison to 87% of the controls. Sixty-two percent of the rats in the lactation group met the criterion within 100 trials, and this proportion did not differ significantly from control levels.

Testing was continued an additional four days for all animals that did not make five consecutive avoidances within the first 100 trials: these animals thus received a total of 180 trials. All control animals met the criterion within this period, but one animal each in the lactation and gestation-lactation groups, and four animals in the gestation group, failed to meet criterion within 180 trials. The mean number of trials required to meet criterion for rats in each of the four treatment groups (with a score of 180 assigned to animals that did not meet criterion by 180 trials) is presented in Table 1. Animals in the three methadone-treated groups tended to require a greater number of trials to meet the learning criterion than did controls, but these differences were not statistically reliable.

The total number of avoidances, 10-sec footshocks, and crossings from one compartment to the other (both during and between trials) during the first 100 trials were each analyzed by a one-way analysis of variance. No significant differences between groups were found on any of these measures. When all four groups were combined, rats averaged 21.8 avoidances, 1.1 ten-sec footshocks, and 153.2 compartment changes during the first 100 trials of testing.

Discrimination learning

Group differences in the proportion of animals successfully meeting the criterion of 9 errorless trials within 10

TABLE 1

ACTIVE AVOIDANCE PERFORMANCE IN ADULT FEMALE RATS THAT WERE PERINATALLY EXPOSED TO METHADONE

	Treatment Schedule			
	Control	Gestation	Lactation	Gestation-Lactation
Proportion of rats meeting criterion within 100 trials	7/8*	3/9†	5/8	2/6†
Mean number (± S.E.) of trials to reach criterion within 180 trials‡	87.8 (± 12.1)	126.9 (± 19.2)	111 0 (± 13.2)	110.2 (± 21.8)

*Significantly different from the three methadone-exposed groups combined, $p = 0.037$
†Significantly different from controls, $p = 0.031$
‡If criterion was not met within 180 trials, a score of 180 was assigned to that animal

TABLE 2

DISCRIMINATION LEARNING PERFORMANCE IN ADULT FEMALE RATS THAT WERE PERINATALLY EXPOSED TO METHADONE

	Treatment Schedule			
	Control	Gestation	Lactation	Gestation-Lactation
Proportion of rats meeting criterion within 100 trials	7/8*	3/9†	2/8‡	3/5
Mean number (± S.E.) of trials to reach criterion§	62 75 (± 10.6)	82 5 (± 11 4)	83 2 (± 11.0)	77 6 (± 10 0)

*Significantly different from the three methadone-exposed groups combined, $p = 0$ 011
†Significantly different from controls, $p = 0.047$
‡Significantly different from controls, $p = 0.01$
§If criterion was not met within 100 trials, a score of 100 was assigned to that animal

consecutive discriminations were analyzed using the Fisher Exact Probability test, and these data are presented in Table 2. When all three methadone groups were combined, 36% of the drug-exposed animals met the learning criterion: this percentage was significantly lower than the 87% of control rats that met the criterion ($p=0.011$). Further tests between controls and the individual methadone-treated groups were then conducted. These tests revealed that a significantly lower percentage of animals in both the gestation (33%) and the lactation (25%) groups met criterion than did the controls ($p = 0.047$ and $p = 0.01$, respectively). The percentage of animals in the gestation-lactation groups that met criterion (60%) was also somewhat reduced in relation to control levels, but this difference was not statistically reliable.

The mean number of trials required to reach the 9 out of 10 learning criterion for rats in each treatment group is presented in Table 2. These data were analyzed by analysis of variance with a score of 100 assigned to those animals that did not reach criterion within 100 trials. Methadone-treated rats tended to require a greater number of trials to meet criterion than did controls, but these differences were not statistically significant.

The total number of errorless trials was evaluated by analysis of variance, and this measure did not reliably discriminate between groups. Animals in the control group averaged 55 errorless trials out of 100, whereas rats in the gestation, lactation, and gestation-lactation groups averaged 50, 53 and 58 errorless trials, respectively.

In the absence of any learning, animals would be expected to make a correct initial response on 50% of the trials on the light-dark discrimination task. Differences between the actual number of correct initial responses and the expected levels were analyzed for each of the four groups using the t-test for single means. On the last 50 discriminations, the average percentage of correct responses for the rats in each group was as follows: gestation, 53%; lactaton, 54%; gestation-lactation, 64%; and control, 62%. These percentages differed from chance levels only for animals in the control and the gestation-lactation groups (both p's < 0.05). In order to determine whether learning might have appeared at a later time for rats in the gestation and lactation groups,

the same analyses were performed on the last 20 trials for these animals. The gestation group averaged 55% correct responses over the last 20 trials, and the lactation group also averaged 55% correct: once again, these performance levels did not differ significantly from those expected by chance. Thus, only the control rats and animals exposed to methadone during both gestation and lactation demonstrated any evidence of learning on the light-dark discrimination test.

Passive avoidance

Prior to administration of footshock there were no between-groups differences in latency to leave the elevated platform. An analysis of variance performed on latency scores of the training day (Day 3) indicated that animals in the four Treatment Schedules remained on the platform for similar periods of time prior to stepping down (overall $\bar{X}$ = 4.6 sec).

Latencies to leave the elevated platform during retention testing (Day 4) were analyzed using a two-way analysis of covariance which included Treatment Schedule and Footshock/No Footshock as between-group variables, with latencies on the training day (Day 3) as the covariate. Rats that had received footshock on Day 3 took an average of 67.9 sec to leave the platform on Day 4, compared to an average of 20.6 sec for animals that had not been subjected to footshock ($F(1,21) = 4.73$, $p<0.05$): thus, there was evidence that learning had occurred on this test. There were not, however, any significant differences among animals from the various Treatment Schedules on passive avoidance performance.

Latencies on the extinction day (Day 5) were analyzed by an analysis of covariance similar to that described above. Overall differences due to administration of footshock were no longer evident on Day 5 and differences in passive avoidance performance related to Treatment Schedule were not statistically significant.

DISCUSSION

The results of the present study suggest that female rats maternally exposed to methadone during gestation and/or lactation have an impairment in learning ability as adults. On the discrimination learning test these decrements in cognition were manifested as a reduction in the number of rats from the gestation and the lactation exposure groups that were able to meet a learning citerion within 100 trials, as well as by a failure to exceed chance levels of performance for these two groups. On the active avoidance test there was a reduction in the number of rats from the gestation and gestation-lactation groups that were able to meet a learning criterion within 100 trials. Even when the period of active avoidance testing was extended to 180 trials, by which time all of the controls had met criterion, 12–44% of the animals in each of the methadone-exposed groups were still unable to meet this performance standard.

On both the aversive (active avoidance) and the appetitive (discrimination learning) tests, behavioral responses appeared to be related to the timing and duration of opioid treatment. In general, rats subjected to methadone during the prenatal period (gestation group) were the most affected, with only 33% of these animals meeting criterion on either the active avoidance or the discrimintion tests; in contrast, 87% of the control rats met criterion on both these tests. Rats in the other two methadone groups were significantly different from controls on only one of the behavioral tests, with 25% of the lactation animals meeting criterion on the discrimination learning task and 33% of the gestation-lactation rats meeting criterion on the active avoidance test.

The tests employed in this study were designed to define learning capabilities, but it must be recognized that a number of other factors, such as alterations in emotionality, motivation, or ability to detect and respond to sensory cues, may have influenced our results. However, it does not appear that any group of drug-treated rats was undernourished, physically abnormal, or had noticeable disturbances in motor abilities that might have accounted for its impaired performance. In addition, since rats perinatally subjected to methadone exhibited learning problems on tests involving two different motivations (shock avoidance and food approach), differences in response to shock or food cannot by themselves account for the observed deficits.

The data presented in this report are consistent with the preliminary observations of Peters [11]. Utilizing 6-week old female offspring that had been maternally exposed to methadone (5 mg/kg IP) during both gestation and lactation, Peters found that drug-exposed rats, in comparison to controls, exhibited a significant decrement in responding to a conditioning stimulus in order to avoid an electric shock. The present results extend these observations into the adult period and also show that rats subjected to methadone during either gestation or lactation alone, as well as continuously throughout both periods, have impaired cognitive abilities. Neither the present study nor that of Peters examined learning abilities in males that had been perinatally exposed to methadone; further studies are needed to determine whether any sex differences might exist in this regard.

Previous evidence gathered in a series of morphological, biochemical, physiological, and behavioral investigations [10, 16, 20–25] has revealed that offspring maternally subjected to methadone during gestation and/or lactation exhibit alterations in somatic and neurobiological maturation. The specific nature of these changes appears to be dependent upon the schedule of drug treatment; however, at 21 days of age (weaning) as well as at 60 days of age (sexual maturity), both the whole brain and cerebellum of narcotic-exposed rats often have had marked deficits in DNA content, abnormalities in RNA and protein content (as well as in the ratios of RNA and protein per unit DNA), and reductions in macroscopic dimensions. Moreover, offspring maternally exposed to methadone often exhibit a variety of behavioral disturbances, including transient delays in the development of spontaneous and sensorimotor behaviors during the preweaning period, subnormal motor activity levels at weaning, and hyperactivity at 45 and 60 days of age. The present results suggest that adult female rats that have sustained perinatal exposure to methadone also are deficient in the acquisition, retention, and/or performance of learned responses. The specific area(s) of the nervous system and the nature of the substrates (e.g., anatomical, biochemical) involved in methadone's effects on the storage and retrieval of information remained undefined. However, studies assessing the effects of numerous types of local brain damage upon performance in the active avoidance paradigm [17] suggest that a wide variety of brain loci may be affected by methadone.

Methadone detoxification and maintenance programs are legally sanctioned and in widespread use for the treatment of heroin addicts. Many narcotic addicts are females of childbearing age and a question that has been frequently raised in

the last few years is to what extent the progeny of these mothers are affected by perinatal opioid exposure [2–5, 19]. Clinical studies reveal that methadone-exposed children are lighter in weight and smaller in stature than control children [18,19] and often have subnormal head circumference measurements [19]. In addition, children delivered by methadone-treated mothers tend to have electroencephalographic and behavioral features consistent with increased central nervous system irritability and lowered overall alertness and attentiveness, particularly to visual stimuli [9,13]. Furthermore, the behavioral profiles of these drug-exposed children during the first 2 years of life are characterized by hyperactivity and a high intensity of response [18].

It should be recognized that generalization of our laboratory findings to a clinical situation is attended by a number of difficulties. For example, our studies have employed a daily methadone dosage that exceeds by three times (on a per kilogram basis) even a high dosage (e.g., 120 mg) recommended for human consumption. Furthermore, we have utilized the parenteral route of drug delivery, in contrast to the oral route of administration which is used clinically. However, despite these and possibly other differences, the methadone-exposed offspring in our laboratory studies have been shown to exhibit several neurodevelopmental dysfunctions that appear to parallel those reported in children. Any extrapolation of our learning data to the clinical situation would obviously be tenuous at this time, but the possible implications are serious enough to suggest that further investigations of the intellectual abilities of humans exposed to this opioid during early life are needed.

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