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Perinatal Methadone Exposure and its Influence on the Behavioral Ontogeny of Rats[1]

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ZAGON, I. S. AND P. J. McLAUGHLIN. *Perinatal methadone exposure and its influence on the behavioral ontogeny of rats.* PHARMAC. BIOCHEM. BEHAV. 9(5) 665–672, 1978.—The ontogeny of spontaneous motor and sensorimotor behaviors were evaluated daily from postnatal days 2 to 19 in rats maternally exposed to methadone (5 mg/kg) throughout gestation and/or lactation. In the methadone-treated groups, the age at which a specific behavior initially appeared for any group member and the ages at which 50% and a maximal (usually 100%) number of animals demonstrated a particular behavior was often delayed several days in comparison to controls. In addition, the time interval between the age of initial appearance and maximal achievement of a positive response was protracted. Rats subjected to methadone during either gestation or lactation exhibited the most retarded behavioral development. This study demonstrates that the timetable of behavioral maturation is altered in preweaning rats perinatally exposed to methadone, with the degree of response dependent on the timing and duration of opioid treatment. In addition, these results provide a functional correlate to our earlier observations of macroscopic and neurochemical changes in the brains of methadone-treated offspring.

Methadone Behavioral development Sensorimotor reflexes Spontaneous movement

METHADONE is a synthetic narcotic analgesic that is often used in the therapeutic treatment of heroin addicts, many of whom are females of childbearing age [7]. Clinical studies reveal that methadone-exposed children are lighter in weight and smaller in stature than control children [30,33], have behavioral profiles characterized by hyperactivity and a high intensity of response in the first two years of life [30], and often have subnormal head circumference measurements [33].

The developing nervous system of laboratory animals appears to be particularly sensitive to methadone [14, 15, 18, 20, 35–38]. In studies utilizing different schedules of methadone during gestation and/or lactation, drug-exposed offspring were found to have altered patterns of brain and cerebellar development during the preweaning period. In particular, 21-day old rats treated during either gestation or lactation had reductions in brain and cerebellar wet weights and macroscopic dimensions, 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.

In view of the above observations and the need for obtaining further information on the physical and behavioral development of children born to methadone-treated mothers, the present investigation was undertaken in order to determine the effects of perinatal methadone exposure on the behavioral ontogeny of laboratory animals. From postnatal days 2 to 19, physical characteristics and the maturation of spontaneous motor activity and sensorimotor reflexes were evaluated in rat pups subjected to methadone during gestation and/or lactation. The results of this behavioral study will be correlated with our previous anatomical and neurochemical findings [20, 35–38]

METHOD

Animals and Drug Treatment

Female (180–200 g) and male (250–300 g) Sprague-Dawley rats (Charles River Labs, Wilmington, MA) were housed under controlled conditions [36], 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 experimentation. Females were treated daily (0800) with an intraperitoneal injection of either 5 mg/kg *dl*-methadone hydrochloride (Dolophine, Eli Lilly Company, Indianapolis, IN) or an equivalent volume of physiologic saline. Animals were weighed every 2 days and appropriate dosage adjustments made.

Five days after the beginning of drug treatment, females were placed with drug free males for breeding; the presence of sperm in vaginal smears indicated Day 0 of gestation. Within 4 hr of birth, litters of methadone-treated mothers were cross-fostered either to saline-injected control mothers or to other methadone-injected mothers; these two groups of pups were considered to have been subjected to methadone

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TABLE 1
CATEGORIES OF BEHAVIORAL DEVELOPMENT

A. Spontaneous Motor Behavior
1. Unilateral head turn and no return.
2. Unilateral head turn to right and return.
3. Unilateral head turn to left and return.
4. Head raise:vertical lifting of the head.
5. Pivoting less than 360° with the hindlimbs remaining stationary.
6. Pivoting greater than 360° with the hindlimbs remaining stationary.
7. Simultaneous movement of head and forelegs.
8. Simultaneous movement of forelegs and hindlegs.
9. Simultaneous movement of head, forelegs, and hindlegs.
10. Crawling: forward progression for the distance of the animal's body.
11. Walking: forward progression for the distance of the animal's body with the abdomen lifted from testing surface.

B. Reflex Tests
1. Startle response: a jumping reaction to a loud noise.
2. Righting reflex: the ability of an animal to turn itself over instantaneously with all four feet on the ground after being placed on its back.
3. Falling reflex: rats were held in a supine position 30 cm above a bed of litter and dropped; turning in midair and landing with all four limbs under the body was considered a positive response.
4. Visual orientation: a visual stimulus was passed through both visual fields at a distance of 10 cm from each eye. Five passes through each field in both naso-temporal and temporal-nasal directions were performed. Tracking (turning head) toward the stimulus as it passed through the field was a positive response.
5. Auditory reflex: a stimulus was presented 3 times at 1 sec intervals, 20 cm from head at ear level; turning the head to the side of the stimulus sound was considered a positive response.
6. Olfactory reflex: olfactory stimuli (warm chocolate or cedarwood oil) were placed on cotton swabs and held 5–10 cm from the nose. A positive response consisted of movement toward the stimulus (in the case of chocolate) or movement toward the odorant tip (in the case of cedarwood oil) and withdrawal (approach-avoidance).
7. Vibrisseal stroking: vibrissae on alternate sides of the head were stroked at 10 sec intervals with a cotton swab; turning the head and attempting to grasp the stimulus was a positive response.
8. Edge aversion: the rat was placed on the edge of the table with forepaws and head extending over the edge; a positive response was considered to be turning, withdrawing, and walking away from the edge of the table.
9. Pain: the hindpaw was quickly grasped, squeezed with surgical forceps, and the rat was then observed for 10 sec. The procedure was repeated on both hindpaws, alternating the side of the initial stimulation every day. Turning the head or withdrawing the paw was considered a positive response for pain.
10. Cross extensor reflex: pinching the dorsal surface of the hindpaw causes flexion of the stimulated limb and simultaneous extension of the contralateral hindlimb.
11. Bar grasping: a wooden dowel was simultaneously placed against the ventral surface of both forepaws as the animal was held in a vertical position. Curvature of both paws around the dowel and the ability to hang from the dowel for 5 sec was considered a positive response.
12. Tail hanging: pups were held by the tail (head down) for 15 sec. Twisting and flexing of the torso to produce head elevation was considered a positive response.

during "gestation alone" or given a combined "gestational-lactational" treatment, respectively. A third group of pups, delivered by control females, was placed with methadone-injected mothers and considered to be exposed to methadone during "lactation alone." Finally, pups delivered by saline-injected mothers were transferred to other females receiving saline injections throughout gestation and lactation; these offspring were considered "controls." Litters were randomly culled to 8 pups per mothers, with an equal distribution of males and females. All pups were weighed at birth and at weaning.

Apparatus

Spontaneous movement (Table 1A) and reflexive (Table 1B) tests were performed on a flat table (60×70 cm) covered by a laboratory cloth. Illumination was provided by ceiling lights and a 60 W bulb suspended directly over the center of the table; this lamp, as well as the laboratory cloth, provided added heat for the pups during testing. A complete description of the spontaneous motor and reflexive tests utilized in this study can be found in Table 1; additional information in regard to the simple and complex reflexive parameters (Table 1B) is as follows. Odorant substances used as olfactory stimuli included cedarwood oil and solid chocolate bars (Hershey). Startle and sound stimuli for auditory tests were produced by the snapping of a ball-point pen and toy-clicker, respectively. Painful stimuli were administered by surgical forceps. The stimulus for visual orientation was measured with a white cardboard square (1×1×1 cm) with 5 black-inked vertical lines spaced at 1 mm intervals; the cardboard was glued to a wooden dowel. Somatosensory orientation was examined by vibrisseal stroking with cotton Q-tips, while bar grasping was measured with a wooden dowel of 1 cm thickness.

Procedure

Every rat was observed between 0800 and 1400 daily on postnatal days 2 through 19 on the following 3 behavioral categories:

Spontaneous motor behavior. Methodology for investigating spontaneous motor behavior (Table 1A) was adapted from Blanck *et al.* [4] and Gard *et al.* [13]. Each animal was placed in the center of the examination area and all movements were recorded for 2 min/day.

Reflex tests. After completion of the above 2 min observation period, pups were subjected to an entire battery of

sensorimotor tests adapted from Fox [12] and Almli and Fisher [1]; each animal was examined for 5 to 10 min/day, depending on the level of performance. These sensory and motor tests were designed to determine the presence (positive response) or absence (negative response) of a given response to a specific stimulus. The frequency, coordination, or velocity of behaviors was not, in general, included in the operational definition of positive responses.

Physical characteristics. The time of appearance of 4 physical features was recorded as follows: (1) eye opening—visible break in eye lid and membrane covering eye; (2) ear opening—opening of external auditory meatus; (3) incisor eruption—eruption of upper incisors; and (4) hair covering—appearance of a complete coat of white hair.

At least 7 males and 7 females from each group, arbitrarily chosen from 3 litters, were coded and examined. The same animals were tested each day for all behavioral parameters. Each pup was removed from its foster-mother's cage, examined for development of spontaneous motor and sensorimotor behavior, and then returned to the home cage. Group order varied daily and the pups within a given group were also rotated daily in terms of the order of tesing. During the observation period, all animals were allowed to move freely. Inasmuch as possible, excessive handling and undue stress of both mothers and offspring were avoided. The same individual performed all examinations throughout the experiment and was "blind" to the group of animals tested.

The age at which a specific movement initially appeared for any group member (=the age of initial appearance), the age at which 50% of the animals displayed a particular behavior (=the median age), and the age at which a maximal number (usually 100%) of the animals demonstrated a particular behavior (=the age of maximal response) were recorded. In addition, the range of maturational performance (i.e., number of days between the age at which a positive response was initially observed and the age at which a maximum number of rats displayed a positive response) was also noted.

Data Analyses

Overall group differences in the age of appearance of a given behavior were analyzed using the chi square test [25]. The point at which these comparisons were made was the age at which 50% of the animals in the combined groups first exhibited a given behavior or positive response. If the results of the chi square test revealed significant overall group differences, subsequent comparisons were then made between each experimental group and the controls using the Fisher exact probability test [25]. This analysis thus provided a test of differences in the proportion of animals within each group that had exhibited the behavior by the time of its median age of appearance.

Preliminary analyses indicated that there were no differences between males and females on any of the behavioral responses. Therefore, animals of both sexes were combined within each group for purposes of the above statistical analyses.

RESULTS

No difficulties in mating, gestation, or parturition could be observed in either the 8 methadone-treated or 8 control females utilized in this study, nor were differences in gestation time noted. The body weights of methadone-exposed mothers were lower than controls throughout pregnancy and lactation, and a full account of these changes, including an analysis of food and water consumption, can be found in White *et al.* [32]. The mean litter size and the number of stillborn pups of females receiving methadone were comparable to controls. The mean birthweights of pups subjected to methadone *in utero* were slightly lower than control values (5.93 g and 6.33 g, respectively), but the difference was not statistically significant, $t(31)=1.40$, $p>0.05$. By weaning, the mean body weights of control animals (47.00 g) was significantly different from pups in the gestation, 38.37 g, $t(26)=12.68$, $p<0.01$, lactation, 41.37 g, $t(26)=9.44$, $p<0.01$, and gestation-lactation, 43.00 g, $t(25)=4.51$, $p<0.01$, groups. These results are consonant with those reported in earlier studies [20, 35–38] in which a systematic exploration of these parameters was conducted.

Spontaneous motor behavior. The ontogeny of gross motor development in methadone-exposed and control rat pups is presented in Table 2. Neonates of the control group were usually immobile in an open field situation. When movement did occur in the first postnatal week, the characteristic behaviors were head turns (with and without return) and a swimming-like forelimb movement. These movements often resulted in a pivoting or circling motion from a fixed axis consisting of the rump and/or rear limbs (which remained stationary). In the second week, rats were able to fully support their heads, the hindlimbs began paddling movements, and a relatively uncoordinated crawling pattern of locomotion was observed. Between 11 and 13 days of age, the crawling behaviors became more coordinated and the rats were able to fully support their body weight with all 4 limbs.

Overall, the development of gross motor abilities in rats subjected to methadone during gestation and/or lactation was delayed in comparison to controls. In 5 of the 11 behavioral categories, all methadone-treated offspring showed delays of 1–5 days in both the age of initial appearance and median age of response. In a few cases, the initial age (e.g., pivoting less than 360° in the gestation-lactation group) or median age (e.g., pivoting more than 360° in all drug-treated groups) of appearance of a behavioral activity in methadone groups occurred 1–2 days earlier than controls. In 6 of the 11 behavioral categories, all methadone-treated offspring also showed delays of 1–6 days in the age at which a maximal number of animals exhibited an activity. For some behaviors, the age of maximal response in drug-treated groups was achieved 1–2 days earlier than controls (e.g., pivoting more than 360°). In addition, the range of maturational performance was often protracted by 1 to 6 days in the drug-treated groups, however, some exceptions were noted (e.g., pivoting more than 360° and crawling).

Statistical evaluation of each spontaneous motor parameter was performed (see Method) and those behaviors with responses most notably different from controls are presented in Table 4. The greatest number of delays for the median age of appearance of a behavioral parameter, when pups from the methadone-treated and control groups were combined, occurred in the gestation group [5], followed by the lactation [2], and gestation-lactation [1] groups. Walking, often considered a milestone of behavioral achievement, was particularly retarded in animals of the gestation and lactation groups, with 35% and 31%, respectively, of those animals walking by Day 13 in comparison to 100% of the controls.

Reflex tests. A general retardation in the achievement of a battery of sensory and motor reflexive tests was observed in

TABLE 2

THE MEDIAN AGE OF APPEARANCE OF SPONTANEOUS MOTOR ACTIVITIES IN PREWEANING RATS MATERNALLY SUBJECTED TO METHADONE

Behavior	Treatment			
	Control	Gestation	Lactation	Gestation-Lactation
Unilateral head turn with no return	2 (2–3)*	2 (2–8)	3 (2–5)	3 (2–5)
Unilateral head turn to right and return	5 (4–6)	6 (5–12)	4 (4–11)	5 (4–10)
Unilateral head turn to left and return	6 (5–8)	10 (6–12)	8 (5–11)	6 (4–11)
Head raise	8 (5–8)	11 (7–12)	10 (3–11)	7 (4–8)
Pivoting<360°	7 (4–11)	9 (4–10)	9 (4–13)	7 (3–11)
Pivoting>360°	12 (5–12)	11 (8–11)	10 (7–10)	11 (7–10)
Head and foreleg movement	2 (2–6)	6 (2–12)	3 (2–9)	5 (2–5)
Foreleg and hindleg movement	9 (7–11)	10 (9–12)	11 (9–14)	10 (9–13)
Head, foreleg, hindleg movement	9 (7–12)	12 (10–12)	11 (9–16)	11 (9–13)
Crawling	11 (7–12)	12 (12–14)	13 (10–13)	12 (9–13)
Walking	12 (11–13)	14 (12–18)	14 (13–17)	13 (12–15)

*Values represent age (days) at which a positive response was recorded for at least 50% of the offspring in each group. Numbers in parentheses indicate the range of maturational performance (in days), and includes the age at which a maximum number (usually 100%) of the animals displayed a positive response (N=at least 14 animals per group).

methadone-treated offspring (Tables 3 and 4). In all groups of drug-treated rats, delays of 1 to 6 days were noted in the initial age of appearance for four activities, while delays of 1 to 6 days were recorded in the median age of response for seven activities and in the maximal age of response for eight activities. In three reflexive tests: falling reflex, vibrisseal stroking, and tail hanging, the ages for all dependent variables were delayed 1 to 6 days in all opiate-subjected animals. In a pattern similar to that for gross motor development, the range of maturational performance was often prolonged in the methadone-exposed groups, however, a shorter interval was recorded for the auditory reflex of all drug-treated rats.

The median age for appearance of sensorimotor behavior, for which drug-treated and control groups were combined, is presented in Table 4. The gestation and lactation groups exhibited some of the most marked delays in achievement of response, but all methadone-treated rats were significantly different from controls in tail hanging, startle, and visual orientation reflexes.

Physical characteristics. Delays in the appearance of mature physical characteristics were often noted in methadone-subjected pups (Tables 3 and 4). Retardation in the initial age and median age of appearance of these physical characteristics were recorded for some methadone-treated groups, with all drug-exposed groups requiring 1 to 3 days longer for the appearance of mature physical features. Results from statistical studies show that animals in the gestation group were significantly different from controls in every parameter (Table 4), while ear opening was the only category in which all groups were significantly altered from controls.

In addition to the behaviors analyzed, several subjective observations were recorded throughout the period of experimentation. Pups in all methadone-treated groups were found to have extensive hindlimb splaying (i.e., the hind-limbs did not rest under the body as in the normal crouching position) during the first two postnatal weeks. Moreover, offspring of the lactation group were observed to experience tremors (i.e., generalized body shaking while the rat stood still) from postnatal days 2 to 8, while less severe and more infrequently occurring tremors were noted during the same

TABLE 3

THE MEDIAN AGE OF APPEARANCE OF SENSORIMOTOR REFLEXES AND PHYSICAL CHARACTERISTICS IN PREWEANING RATS MATERNALLY SUBJECTED TO METHADONE

Behavior	Treatment			
	Control	Gestation	Lactation	Gestation-Lactation
Startle	3* (2–3)	4 (2–5)	3 (2–5)	3 (2–6)
Righting reflex	2 (2–5)	3 (2–9)	5 (3–8)	2 (2–4)
Falling reflex	12 (11–14)	15 (12–15)	14 (12–17)	13 (13–16)
Visual orientation	14 (14–15)	17 (16–17)	19 (10–19)	15 (13–16)
Auditory reflex	12 (12–16)	17 (17–19)	16 (13–16)	13 (12–13)
Olfactory reflex				
Chocolate	7 (5–13)	8 (2–12)	13 (12–13)	13 (4–13)
Cedarwood oil	5 (2–8)	3 (2–10)	6 (2–10)	5 (2–5)
Vibrisseal stroking	9 (9–14)	15 (10–18)	11 (10–16)	10 (10–16)
Edge aversion	8 (8–13)	14 (9–15)	11 (8–14)	10 (8–14)
Pain				
Turn head	2 (2–9)	2 (2–10)	4 (2–4)	3 (2–4)
Withdraw paw	5 (4–5)	7 (5–10)	4 (4–9)	3 (3–9)
Cross extensor reflex	4 (2–4)	2 (2–10)	4 (2–7)	3 (2–5)
Bar grasping	9 (7–14)	10 (8–17)	15 (8–16)	9 (8–14)
Tail hanging	10 (9–12)	14 (11–17)	14 (11–15)	11 (10–18)
Ear opening	13 (13–13)	16 (15–16)	13 (13–14)	14 (13–14)
Eye opening	13 (10–14)	16 (13–17)	13 (13–16)	13 (11–15)
Incisor eruption	8 (8–9)	11 (9–12)	10 (10–10)	9 (9–11)
Hair covering	10 (10–11)	12 (12–12)	13 (10–14)	11 (8–12)

*Values represent age (days) at which a positive response was recorded for at least 50% of the offspring in each group (N=at least 14 animals per group). Numbers in parentheses indicate the maturational performance range (in days) and includes the age at which a positive response was initially observed for any rat and the age at which the maximum number (usually 100%) of animals displayed a positive response.

TABLE 4

STATISTICAL EVALUATION OF SENSORIMOTOR AND PHYSICAL PARAMETERS IN RATS PERINATALLY EXPOSED TO METHADONE

Parameter	Age (days)	Treatment			
		Control	Gestation	Lactation	Gestation-Lactation
A. Spontaneous Motor					
Unilateral head turn with no return	2	0.85*†	0.61	0.38‡	0.40‡
Unilateral head turn to left and return	5	0.90	0.20‡§	0.50	0.82
Head raise	8	0.70	0.09‡	0.38	0.41
Pivoting<360°	8	0.80	0.30‡	0.38‡	0.38
Foreleg and hindleg movement	11	0.90	0.46‡	0.64	0.69
Head, foreleg, hindleg movement	12	0.90	0.93	0.21‡	0.70
Walking	13	1.00†	0.35‡§	0.31‡§	0.78
B. Reflex Tests					
Startle	3	1.00†	0.38‡	0.50‡	0.53‡
Righting reflex	3	0.80	0.54	0.19‡	0.63
Pain-paw withdrawal	5	1.00†	0.30‡	0.73	0.64
Bar grasping	11	0.80	0.31‡	0.45	0.85
Tail hanging	12	1.00†	0.28‡	0.50‡	0.38‡
Edge aversion	12	0.80	0.43	0.28‡	0.44
Visual orientation	15	1.00†	0.23‡	0.57‡	0.61‡
Auditory reflex	16	1.00†	0.71	0.57	0.44‡
C. Physical Development					
Incisor eruption	9	1.00†	0.43‡	0.00‡	0.73
Hair covering	11	1.00	0.00‡	0.48‡	0.61
Ear opening	13	1.00†	0.12‡	0.44‡	0.55‡
Eye opening	14	1.00†	0.15‡	0.46	0.83

*Values represent the proportion of positive response by each treatment group (N=at least 14 animals per group) at the median age of all animals (i.e., includes pups from both experimental and control groups).

†Significantly different from total of the three experimental groups at $p<0.05$.

‡Significantly different from controls at $p<0.05$.

§Significantly different from the gestation-lactation group at $p<0.05$.

period in animals subjected to methadone *in utero* (i.e., gestation group).

DISCUSSION

The treatment schedules, drug concentration, and route of administration utilized in this study were similar to those reported by Zagon and McLaughlin [35–38] and McLaughlin *et al.* [20] and have been shown to evoke morphological and biochemical changes in offspring exposed to methadone during gestation and/or lactation. The maternal dosage of methadone (5 mg/kg) was above the intraperitoneal threshold dose for analgesic action in rats (1 mg/kg; 9), but well below the LD_{50} toxicity concentration of 23 mg/kg [3]. Since this synthetic opioid is known to cross the placenta and enter the fetal circulation of humans [6,16], mice [24], and rats [17, 21, 22], and has been detected in the milk of lactating humans on methadone maintenance [5], fetuses and pups of drug-treated females in the present investigation were considered to have been directly exposed to methadone. Although the amount of drug reaching these animals was not ascertained, maternal

dosages were sufficiently high to precipitate the abstinence syndrome in neonates removed from drug exposure at birth.

The present experiments were conducted in order to secure a profile of preweaning behavioral ontogeny in rats perinatally exposed to methadone. The measures of behavioral development employed in this study were adapted from previous investigations [1, 4, 12, 13], and were designed to determine when, during preweaning development, the ability to perform a given behavior was attained, irrespective of the velocity and/or coordination of the response. In this regard, it should also be recognized that permanent behavioral deficits were not encountered in animals of either the methadone-treated or control groups but, in fact, every animal was observed to eventually express all of the behavioral responses by the conclusion of the observation period (postnatal day 19).

Evaluation of the present results indicates that the temporal pattern of behavioral ontogeny in our control rats is in agreement with that reported by other investigators [1, 2, 4, 13, 27]. However, a number of environmental (endogenous and exogenous) and pharmacological variables are known to influence the effects of drugs upon behavior [31]. Nevertheless, we believe that our data provide valid information about the sequence of sensory and motor development in infant rats subjected to methadone *in utero* and/or during lactation.

The results of this investigation indicate that animals perinatally exposed to methadone had alterations in the timetable of behavioral maturation during the preweaning period. However, each schedule of drug treatment is manifested by different syndromes of behavioral abnormalities, with methadone exposure during either gestation or lactation having a more detrimental effect on behavioral ontogeny than continuous opioid treatment during both gestation and lactation. In general, methadone-treated offspring exhibited temporal delays in expressing all modalities evaluated: gross motor development, the appearance of certain physical characteristics, and the maturation of both simple and complex sensory and motor behaviors. Furthermore, these delays included those involving the age of initial appearance of a particular behavior as well as the ages at which 50% and 100% of the animals expressed a specific behavioral parameter. Moreover, the time difference between the age at which a behavioral characteristic was first observed and the age at which a maximal number of rats displayed a specific activity (i.e., maturational range) was often prolonged in methadone-treated groups.

Previous investigations in our laboratory indicate that prenatal and/or postnatal methadone treatment impairs somatic and neurobiological development [20, 35–38]. Body, brain, and cerebellar weights of methadone-exposed rats often were found to be significantly lower than controls throughout the preweaning period. At weaning (Day 21), the mean body weights of all opioid-treated groups were at least 20% below control values, and brain and cerebellar weights of animals in the gestation and lactation groups were reduced by 12% to 30%. Deficits of 47%, 37% and 4% in brain DNA content were recorded for rats in the gestation, lactation, and gestation-lactation groups, respectively, while reductions of 9% to 41% in brain protein content were also observed. In addition, there was an increase in the amount of RNA per unit of DNA in the brains of all methadone-exposed offspring. Cerebellar DNA content was only noted to be significantly reduced in animals of the gestation and lactation groups, with reductions of at least 27% being recorded. If DNA reflects (with certain limitations) cell numbers [11,34], these decreases in brain and cerebellar DNA suggest deficits in the total number of neural cells. Since the rat brain develops on a precise timetable that consists of prenatal and postnatal phases, with certain regions of the brain (e.g., cerebellum, hippocampus) undergoing extensive neuronal proliferation until weaning, the DNA deficits encountered in methadone-treated animals may reflect decreases in the neuronal population. Furthermore, other abnormalities in neural ontogeny (e.g., synaptogenesis, myelination) may occur concomitantly. Although at this time it is unclear whether structural and neurochemical alterations in brain development are causally related to the disturbances in behavioral ontogeny of opioid-treated offspring, it is interesting to note that those methadone-exposed animals with the most severe deficits in brain and cerebellar weights and neurochemical content (i.e., gestation and lactation groups) also demonstrated the greatest number of behavioral changes. In addition, because each schedule of methadone treatment affected different sensory and motor behaviors to differing degrees of severity, it appears that this opioid may exert a variety of effects on one or more areas of the developing central and peripheral nervous systems.

At this time, it is difficult to distinguish between a direct effect of methadone in regard to the alterations in preweaning behavioral maturation and a secondary effect related to other factors (e.g., undernutrition, stress, hormonal unbalance, abnormal placentation, poor maternal care). A number of observations in this investigation as well as previous reports [20, 32, 35–38] suggest that inadequate nutrition may not be the primary factor involved with these changes. The significant decreases in litter size and birthweight that are often associated with pregnant rats on restricted diets [10] were not found in our studies [35,36] and preliminary observations of female rats subjected to methadone also did not reveal any abnormalities in maternal care (e.g., efficiency in retrieving pups, daily rhythms of nest occupation and desertion, frequency of licking offspring) as reported for underfed mothers [28]. In this regard, methadone-exposed offspring were noted to suckle frequently and to have milk in their stomachs [35,36]. Although the mean body weight of opiate-treated females has been found to be lower than controls throughout pregnancy and lactation, suggestive of maternal undernutrition, food and water consumption of these mothers was similar to controls [32]. Finally, there appears to be a different constellation of somatic, neurochemical, and structural effects accompanying perinatal methadone exposure in comparison to those associated with undernutrition [20, 32, 35–38]. The present results concerning alterations in preweaning behavioral development are consistent with these findings. For example, the results from behavioral studies on rats delivered by mothers subjected to protein deficient diets [29] or to a 50% dietary restriction [26] reveal that the magnitude of effects on neuromotor development is greater in animals deprived during gestation and lactation than in rats subjected to undernutrition or malnutrition during either gestation or lactation. In contrast to these findings, the present investigation shows that animals receiving a cumulative exposure to methadone (i.e., gestation-lactation group) exhibited the fewest changes in behavioral ontogeny, with offspring in the gestation or lactation groups demonstrating the most marked disturbances.

Although the results of this laboratory investigation demonstrate that chronic maternal methadone treatment interferes with preweaning maturation, the significance of

these findings on subsequent stages of behavioral growth, as well as its implications for the adult, are unclear. In this regard, some investigators have used adaptive behaviors of the infant to predict the later intelligence of the adult, but this correlation may be tenuous [8]. Nevertheless, the effect of perinatal methadone exposure on future development and maturation (e.g., learning, memory, emotionality) needs to be clarified. Our laboratory findings do suggest a parallel with data obtained from clinical reports. Children delivered by methadone-treated mothers tend to have electroencephalographic and behavioral changes consistent with increased central nervous system irritability and lowered overall alertness and attentiveness (particularly to visual stimuli) [19,23]. Behavioral profiles of these drug-exposed children during the first two years of life are also characterized by hyperactivity and a high intensity of response [30]. It appears that further clinical and laboratory investigations are needed to define the short- and long-term behavioral changes that may be associated with perinatal methadone exposure.

REFERENCES

1. Almli, C. R. and R. S. Fisher. Infant rats: sensorimotor ontogeny and effects of substantia nigra destruction. *Brain Res. Bull.* **2:** 425–450, 1977.
2. Altman, J. and K. Sudarshan. Postnatal development of locomotion in the laboratory rat. *Anim. Behav.* **23:** 896–920, 1975.
3. Barnes, C. D. and L. G. Eltherington. *Drug Dosage in Laboratory Animals. A Handbook.* Los Angeles, California: University of California Press, 1966, p. 302.
4. Blanck, A., E. Hard and K. Larsson. Ontogenetic development of orienting behavior in the rat. *J. comp. physiol. Psychol.* **63:** 327–328, 1967.
5. Blinick, G., E. Jerez and R. C. Wallach. Methadone maintenance, pregnancy, and progeny. *J. Am. med. Assoc.* **225:** 477–479, 1973.
6. Blinick, G., C. E. Inturrisi, E. Jerez and R. C. Wallach. Methadone assays in pregnant women and progeny. *Am. J. Obstet. Gynec.* **121:** 617–621, 1975.
7. Blinick, G., R. C. Wallach, E. Jerez and B. D. Ackerman. Drug addiction in pregnancy and the neonate. *Am. J. Obstet. Gynec.* **125:** 135–142, 1976.
8. Bloom, B. *Stability and Change in Human Characteristics.* New York: Wiley Press, 1964, p. 237.
9. Chen, K. K. Pharmacology of methadone and related compounds. *Ann. N.Y. Acad. Sci.* **51:** 83–97, 1951.
10. Chow, B. F. and C. J. Lee. Effect of dietary restriction of pregnant rats on body weight gain of the offspring. *J. Nutr.* **82:** 10–18, 1964.
11. Dobbing, J. and J. Sands. Timing of neuroblast multiplication in developing human brain. *Nature, Lond.* **226:** 639–640, 1970.
12. Fox, M. W. Reflex-ontogeny and behavioral development in the mouse. *Anim. Behav.* **13:** 234–244, 1964.
13. Gard, C., E. Hard, K. Larsson and V. A. Petersson. The relationship between sensory stimulation and gross motor behavior during the postnatal development in the rat. *Anim. Behav.* **15:** 563–567, 1967.
14. Geber, W. F. and L. C. Schramm. Postpartum weight alteration in hamster offspring from females injected during pregnancy with either heroin, methadone, a composite drug mixture, or mescaline. *Am. J. Obstet. Gynec.* **10:** 1105–1111, 1974.
15. Geber, W. F. and L. C. Schramm. Congential malformations of the central nervous system produced by narcotic analgesics in the hamster. *Am. J. Obstet. Gynec.* **123:** 705–713, 1975.
16. Harper, R. G., G. Solish, E. Feingold, N. B. Gersten-Woolf and M. M. Sokal. Maternal ingested methadone, body fluid methadone, and the neonatal withdrawal syndrome. *Am. J. Obstet. Gynec.* **129:** 417–424, 1977.
17. Hutchings, D. E., H. F. Hunt, J. P. Towey, T. S. Rosen and H. S. Gorinson. Methadone during pregnancy in the rat: dose level effects on maternal and perinatal mortality and growth in the offspring. *J. Pharmac. exp. Ther.* **197:** 171–179, 1976.
18. Jurand, A. Teratogenic activity of methadone hydrochloride in mouse and chick embryos. *J. Embryol. exp. Morph.* **30:** 449–458, 1973.
19. Lodge, A., M. M. Marcus and C. M. Ramer. Behavioral and electrophysiological characteristics of the addicted neonate. *Addict. Dis.* **2:** 235–255, 1975.
20. McLaughlin, P. J., I. S. Zagon and W. J. White. Perinatal methadone exposure in rats: effects on body and organ development. *Biol. Neonate* **34:** 48–54, 1978.
21. Peters, M. A. Development of a blood-brain barrier to methadone in the newborn rat. *J. Pharmac. exp. Ther.* **192:** 513–520, 1975.
22. Peters, M. A., M. Turnbow and D. Buchenauer. The distribution of methadone in the nonpregnant, pregnant, and fetal rat after acute methadone treatment. *J. Pharmac. exp. Ther.* **181:** 273–278, 1972.
23. Ramer, C. M. and A. Lodge. Clinical and developmental characteristics of infants of mothers on methadone maintenance. *Addict. Dis.* **2:** 227–234, 1975.
24. Shah, N., A. G. Donald, J. A. Bertolatus and B. Hixon. Tissue distribution of levo-methadone in nonpregnant and pregnant female and male mice: effect of SKF 525-A. *J. Pharmac. exp. Ther.* **199:** 103–116, 1976.
25. Siegel, S. *Nonparametric Statistics.* New York: McGraw-Hill, 1956, p. 312.
26. Simonson, M., R. Sherwin, J. Anilane, W. Yu and B. Chow. Neuromotor development in progeny of underfed mother rats. *J. Nutr.* **98:** 18–24, 1969.
27. Small, W. S. Notes on the psychic development of the young white rat. *Am. J. Psychol.* **11:** 80–100, 1899.
28. Smart, J. L. and J. Preece. Maternal behavior of undernourished mother rats. *Anim. Behav.* **21:** 613–619, 1973.
29. Sykes, S. E. and J. A. Cheyne. The effects of prenatal and postnatal protein malnutrition on physical and motor development of the rat. *Dev. Psychobiol.* **9:** 285–295, 1976.
30. Ting, R. Y., A. Keller and L. P. Finnegan. Physical, neurological, and developmental assessment of infants born to methadone dependent mothers. *Proc. Second Natn. Drug Abuse Conf., New Orleans,* 1975.
31. Vesell, E. S., C. M. Lang, W. J. White, G. T. Passananti, R. N. Hill, T. L. Clemens, D. K. Liu and W. D. Johnson. Environmental and genetic factors affecting the response of laboratory animals to drugs. *Fedn Proc.* **35:** 1125–1132, 1976.
32. White, W. J., P. J. McLaughlin and I. S. Zagon. The effects of chronic methadone treatment on maternal body weight and food and water consumption in rats. *Pharmacology* **17:** 227–232, 1978.
33. Wilson, G. S. Somatic growth effects of perinatal addiction. *Addict. Dis.* **2:** 333–345, 1975.
34. Winick, M. and A. Noble. Cellular response in rats during malnutrition at various ages. *J. Nutr.* **89:** 300–306, 1966.
35. Zagon, I. S. and P. J. McLaughlin. Effect of chronic maternal methadone exposure on perinatal development. *Biol. Neonate* **31:** 271–282, 1977.
36. Zagon, I. S. and P. J. McLaughlin. The effects of different schedules of methadone treatment on rat brain development. *Exp. Neurol.* **56:** 538–552, 1977.
37. Zagon, I. S. and P. J. McLaughlin. Methadone and brain development. *Experientia* **33:** 1486–1487, 1977.
38. Zagon, I. S. and P. J. McLaughlin. Perinatal methadone exposure and brain development: a biochemical study. *J. Neurochem.* **31:** 49–54, 1978.