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# Withdrawal-Like Symptoms in Young and Adult Rats Maternally Exposed to Methadone[1]

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ZAGON, I. S. AND P. J. McLAUGHLIN. *Withdrawal-like symptoms in young and adult rats maternally exposed to methadone*. PHARMAC. BIOCHEM. BEHAV. **15**(6)887–894, 1981.—Rats of 30, 45, 60 and 120 days of age, maternally exposed to methadone (5 mg/kg daily) during gestation and/or lactation, were evaluated on a variety of behavioral and physiological parameters related to drug withdrawal. Animals were tested before and after an acute injection of naloxone (10 mg/kg). Prior to naloxone injection, methadone-exposed rats were subnormal in body temperature at 30 days of age, hypoalgesic at 45 days, and weighed less than controls at 60 days. Additionally, and in contrast to control rats, methadone-exposed animals at most ages displayed head shake and wet-dog shake behaviors. After naloxone administration, methadone-exposed rats exhibited an increase in the mean number of head and wet-dog shakes over pre-injection levels. Although control rats injected with naloxone also demonstrated head shakes (at all ages) and wet-dog shakes (at 45 days), these behaviors were usually not of the magnitude as noted for methadone-exposed offspring receiving naloxone. Perturbations in body weight and hypothermia during development, along with head shake and wet-dog shake behaviors which were exacerbated following naloxone administration, suggest a protracted state of physical dependence/withdrawal and/or permanent damage as a result of perinatal exposure to methadone.

Methadone Perinatal exposure Withdrawal Rats Naloxone Opiates

METHADONE is a synthetic analgesic that is frequently employed in detoxification and maintenance programs for narcotic-addicted pregnant women [3]. This drug crosses the placenta and enters the fetal circulation of humans [2] and laboratory animals [28,33], and has been detected in the milk of lactating humans on methadone maintenance [2,20]. Infants born to mothers using methadone, and other narcotic analgesics, typically exhibit signs of drug withdrawal such as irritability, tremors, hyperreflexion, and disturbed sleep. The onset of appearance of these withdrawal symptoms may extend up to several weeks after birth [19]. The abstinence syndrome in infants also may be quite prolonged, and early symptoms described in the neonatal addict may continue in attenuated or "subacute" form for periods of up to 4–6 months [5, 7, 19, 21, 44]. Although the short- and long-term consequences of perinatal opiate addiction are unclear, some investigators suggest that infants maternally subjected to narcotics may be considered at risk for growth retardation and neuro-developmental dysfunction [5, 39, 43–45].

In experiments with laboratory animals, symptoms of withdrawal have been identified in neonates maternally subjected to methadone. Newborn rats delivered by mothers chronically receiving methadone experience drug withdrawal (e.g., tremors, cyanosis), and a concomitant increase in infant mortality occurs when these pups are cross-fostered at birth to drug-naive mothers [48]. The duration of drug withdrawal is unknown, although offspring perinatally exposed to methadone have tremors, retarded growth, and temporal delays in the ontogeny of spontaneous motor and sensorimotor behaviors in the preweaning period [49]. At weaning, investigations in our laboratory [52] and others [11] have shown that methadone-exposed pups are often hypoactive in comparison to control animals. Hutchings and colleagues [16] have recorded disturbances in the rest-activity cycle of 17- and 22-day old rats prenatally treated with methadone which disappear by day 30. Rats exposed to methadone in early life are also known to exhibit numerous long-term difficulties including neuroanatomical and neurochemical abnormalities, physiological dysfunction, elevated nociceptive thresholds, aberrant drug response, delayed behavioral development, and impaired learning ability [30, 34 48–53]. Some disturbances recorded in adolescent and adult animals such as hypothermia [36,37], perturbations in body weight gain [26, 36, 48, 50], and hyperactivity [52] resemble those associated with the withdrawal syndrome.

The purpose of the present study was to determine whether rats maternally exposed to methadone exhibit withdrawal-like symptoms during the post-weaning period. To explore this matter further, 30-, 45-, 60-, and 120-day old rats subjected to methadone during gestation and/or lactation were compared to control animals on a variety of behavioral and physiological parameters related to the withdrawal syndrome. These included: wet-dog shakes, head shakes, diarrhea, teeth chattering, body weight reductions, and changes

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in nociception and core temperatures. Complementing these observations on natural (abrupt) withdrawal, we were also interested in determining whether drug withdrawal could be precipitated. To this effect, we administered naloxone, an opiate antagonist, and evaluated drug response in offspring of opiate-exposed and control mothers.

## METHOD

### *Animals and Drug Treatment*

Female (180–200 g) and male (400–500 g) Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used in this study. All animals were housed under controlled laboratory conditions [48,50], with Purina Laboratory Chow and water available ad lib. Animals were allowed at least 6 days to acclimate to their surroundings prior to the beginning of experimentation. Females were randomly divided into groups of 5 rats per cage and treated daily with an intraperitoneal (IP) injection of either 5.0 mg/kg *dl*-methadone hydrochloride (Dolophine, Eli Lilly Company, Indianapolis, IN) or an equivalent volume of saline. Animals were weighed every 2 days and appropriate dosage adjustments made.

Five days after the beginning of drug treatment, the nulliparous females were placed with drug-free males for breeding; the presence of sperm in vaginal smears indicated the first day of pregnancy. Within 4 hr of birth, all litters were cross-fostered and four groups of animals were established as described elsewhere [48–50]. One group of animals was exposed to methadone only during gestation (i.e., the G or gestation group), another group received methadone only during lactation (i.e., the L or lactation group), and a third group of pups was continuously subjected to methadone throughout gestation and lactation (i.e., the GL or gestation-lactation group); litters from saline-injected mothers served as controls (i.e., the C or control group). Litters were maintained at 8 pups per mother with an equal distribution of males and females. Animals were weighed on days 30, 45, 60, and 120.

### *Apparatus and Procedures*

*Core temperature.* Core temperatures were determined using a Yellow Springs Telethermometer (Model 47). The thermistor probe was inserted 5–6 cm into the rectum and readings were made after 1 min had elapsed. Rats were placed in plastic restrainers of appropriate size for the time required to obtain a core temperature reading.

*Hot-plate.* The hot-plate technique of Woolfe and MacDonald [44] was used in this study. Animals were placed on an Analgesia Meter (Technilabs Instrument, Pequannock, NJ) maintained at 55±0.5°C and the latency (±0.1 sec) of their reflex response to heat was recorded. Animal responses employed as endpoints included the licking of a paw or withdrawal of one of the hindlimbs from the plate; any subject not responding within 30 sec was removed from the hot-plate.

*Behavior.* Observations of withdrawal were made by individually placing rats in Plexiglas cages (30×19.5×25 cm) that contained a layer of bedding material. All movements were recorded by 2 observers who were "blind" to the animals' treatment (interobserver reliability exceeded 0.90); head shakes, wet-dog shakes, teeth chattering, and number of boli were recorded for 15 min periods. "Wet-dog shakes" were considered to be brief episodes of rapid shaking of the entire body, while "head shakes" consisted of side to side movements of the head only.

Animals were tested on days 30, 45, 60, and 120. Four to six rats of each sex were randomly chosen from every group and injected IP with 10 mg/kg naloxone hydrochloride (Endo Laboratories, Garden City, NY). Immediately prior to the injection of naloxone, rats were weighed and rectal temperatures, hot-plate latencies, and behavior within a 15 min observation period (=pre-naloxone period) were recorded. Animals then were placed in separate cages containing bedding material and allowed to acclimate for 20 min before injection of naloxone. Following naloxone administration (=post-naloxone period), the behavior of drug-injected rats was recorded for the first 15 min; core temperatures and hot-plate latencies were determined at 1 hr post-injection. An animal was tested only at one age period and then discarded.

### *Data Analysis*

Body weights were analyzed at each age using a two-way analysis of variance, with Treatment Schedule and Sex as between group variables. Rectal temperatures, hot-plate scores, number of head shakes, number of wet-dog shakes, as well as the number of boli, were each analyzed at every age using analysis of variance with Treatment Schedule and Sex as between group variables, and Time (pre-naloxone, post-naloxone) as a repeated measure. Subsequent tests between controls and experimental groups were made using the Newman-Keuls test. The proportion of rats that demonstrated a given behavior (i.e., head shakes and wet-dog shakes) were compared separately using a chi square test; within-group differences across the two observation periods, as well as differences within each observation period between groups, were noted [46].

## RESULTS

The body weights of male and female rats in the methadone-exposed groups did not differ from their counterparts in the control group at 30 and 45 days. At 60 days, animals in the G, L, and GL groups were subnormal in body weight; male rats were reduced 31%, 24%, and 33%, respectively, and females were reduced 25%, 20%, and 23%, respectively, from control weights. Although the mean body weights of rats perinatally exposed to methadone also tended to be subnormal at 120 days of age, only male rats in the GL group were significantly reduced (13%) from control values. No changes in body weight were noted within the 1 hr observation period following naloxone administration.

### *Core Temperature*

At 30, 45, and 120 days of age, the Treatment Schedule×Sex×Time interactions were significant, $F(3,24)=6.26$, $p<0.05$; $F(3,24)=82.54$, $p<0.01$; and $F(3,24)=8.00$, $p<0.01$, respectively, whereas at 60 days of age the Treatment Schedule×Sex interaction was reliable, $F(3,24)=40.00$, $p<0.01$; these values are presented in Table 1. Prior to an injection of naloxone, 30-day old male rats in the G and L groups and female rats in the GL group were significantly colder than their respective controls. After naloxone administration, 30-day old male rats in the L group and females in the GL group had significant increases in core temperature with regard to pre-injection levels, and their temperatures no longer were lower than those of controls. In contrast, females in the L group demonstrated a marked reduction in body temperature after naloxone injection, and these rats were now colder than controls.

TABLE 1

THERMOREGULATION BEFORE AND AFTER ACUTE NALOXONE INJECTION IN YOUNG AND ADULT RATS PERINATALLY EXPOSED TO METHADONE

| | | Male Core Temperatures | | | Female Core Temperatures | | |
|---|---|---|---|---|---|---|---|
| Age | Group | Pre-Naloxone | Post-Naloxone | $p$ values | Pre-Naloxone | Post-Naloxone | $p$ values |
| 30 | C | 37.60 ± 0.14 | 37.10 ± 0.11 | NS | 37.30 ± 0.15 | 37.12 ± 0.11 | NS |
| | G | 36.67 ± 0.37† | 37.00 ± 0.37 | NS | 37.02 ± 0.06 | 36.70 ± 0.08 | NS |
| | L | 36.20 ± 0.13† | 36.72 ± 0.12 | <0.01 | 36.72 ± 0.08 | 35.67 ± 0.31† | <0.01 |
| | GL | 36.90 ± 0.09 | 37.07 ± 0.09 | NS | 36.15 ± 0.50† | 36.85 ± 0.13 | <0.05 |
| 45 | C | 37.80 ± 0.25 | 37.00 ± 0.08 | <0.05 | 37.37 ± 0.00 | 36.65 ± 0.15 | <0.05 |
| | G | 37.20 ± 0.11 | 36.70 ± 0.12 | NS | 36.80 ± 0.40 | 36.82 ± 0.15 | NS |
| | L | 37.30 ± 0.42 | 37.40 ± 0.41 | NS | 36.90 ± 0.15 | 37.20 ± 0.15 | NS |
| | GL | 37.40 ± 0.19 | 37.40 ± 0.19 | NS | 37.05 ± 0.09 | 37.32 ± 0.08 | NS |
| 60 | C | 37.38 ± 0.19 | | | 37.62 ± 0.17 | | |
| | G | 37.51 ± 0.13 | | | 37.30 ± 0.08† | | |
| | L | 37.47 ± 0.15 | | | 37.12 ± 0.24† | | |
| | GL | 37.50 ± 0.11 | | | 37.66 ± 0.11 | | |
| 120 | C | 37.42 ± 0.04 | 36.80 ± 0.11 | <0.01 | 37.85 ± 0.06 | 37.17 ± 0.11 | <0.05 |
| | G | 36.92 ± 0.13* | 36.55 ± 0.09 | NS | 37.92 ± 0.06 | 37.77 ± 0.08* | NS |
| | L | 38.05 ± 0.18* | 37.47 ± 0.13* | <0.05 | 37.57 ± 0.11 | 37.82 ± 0.08* | NS |
| | GL | 37.57 ± 0.14 | 37.15 ± 0.09 | NS | 38.00 ± 0.08 | 38.77 ± 0.13† | <0.01 |

Values represent means (°C) ± SE for 4–6 animals per sex per Treatment Schedule. Significantly different from corresponding control values at $p<0.05$ (*) and $p<0.01$ (†). $p$ values reflect differences between pre-naloxone and post-naloxone core temperatures; NS=not significant. At 60 days of age, only the Treatment Schedule × Sex interaction was significant; thus, the combined values for pre-naloxone and post-naloxone periods are presented for each sex.

TABLE 2

HOT-PLATE RESPONSE BEFORE AND AFTER ACUTE NALOXONE INJECTION IN YOUNG AND ADULT RATS PERINATALLY EXPOSED TO METHADONE

| | | Male Latency Scores | | | Female Latency Scores | | |
|---|---|---|---|---|---|---|---|
| Age | Group | Pre-Naloxone | Post-Naloxone | $p$ values | Pre-Naloxone | Post-Naloxone | $p$ values |
| 30 | C | 7.92 ± 0.64 | 8.12 ± 2.04 | NS | 5.20 ± 0.39 | 6.55 ± 0.58 | NS |
| | G | 6.42 ± 0.15 | 9.37 ± 1.43 | <0.01 | 10.32 ± 1.54 | 6.60 ± 0.61 | <0.01 |
| | L | 6.62 ± 0.45 | 5.55 ± 0.83* | NS | 6.55 ± 0.66 | 6.32 ± 0.54 | NS |
| | GL | 13.87 ± 1.47† | 11.87 ± 1.19* | NS | 11.90 ± 1.71 | 11.32 ± 1.22 | NS |
| 60 | C | 8.80 ± 0.70 | 6.30 ± 0.60 | NS | 4.62 ± 0.59 | 5.42 ± 0.23 | NS |
| | G | 8.97 ± 0.32 | 9.77 ± 0.98 | NS | 9.12 ± 1.06 | 5.35 ± 1.02 | NS |
| | L | 9.12 ± 0.44 | 14.12 ± 0.78† | <0.01 | 6.67 ± 0.68 | 9.45 ± 0.79 | NS |
| | GL | 8.00 ± 0.15 | 8.07 ± 0.57 | NS | 7.22 ± 0.20 | 8.17 ± 1.34 | NS |

Values represent means (sec) ± SE for 4–6 animals per sex per Treatment Schedule. Significantly different from corresponding control values at $p<0.05$ (*) and $p<0.01$ (†). $p$ values reflect differences between pre-naloxone and post-naloxone hot-plate latencies; NS=not significant.

At 45 days of age, no differences in core temperature were recorded between control and methadone-exposed animals either prior to or after naloxone injection. In addition, no differences were noted between the pre- and post-naloxone periods for methadone-treated rats. However, control rats of both sexes had significant reductions in core temperatures after naloxone administration with respect to their pre-injection levels.

At 60 days of age, body temperatures of male rats in the control and methadone-exposed groups did not differ; however, female rats in the G and L groups had markedly lower core temperatures than control females.

Prior to an injection of naloxone, 120-day old male rats in the G group were significantly colder than male control rats, whereas males in the L group were warmer than their control counterparts. Following naloxone administration, male rats in the L group had significantly lower body temperatures in regard to their pre-injection levels, but these animals remained markedly warmer than controls. Comparison of pre-injection and post-injection values revealed a notable increase in body temperature for female rats in the GL group, and this group of animals along with females in the G and L groups were significantly warmer than controls in the post-naloxone period. After naloxone injection, both male and

TABLE 3

HEAD-SHAKE BEHAVIOR BEFORE AND AFTER ACUTE NALOXONE INJECTION IN YOUNG AND ADULT RATS PERINATALLY EXPOSED TO METHADONE

| | | Mean number head-shakes | | | Percent rats with head-shakes | | |
|---|---|---|---|---|---|---|---|
| Age | Group | Pre-Naloxone | Post-Naloxone | $p$ values | Pre-Naloxone | Post-Naloxone | $p$ values |
| 30 | C | 0 | 6.50 | <0.01 | 0 | 100 | <0.01 |
| | G | 2.00† | 3.75 | <0.01 | 88* | 88 | NS |
| | L | 4.12† | 5.38 | <0.01 | 100† | 100 | NS |
| | GL | 0.25 | 0.62 | NS | 25 | 12† | NS |
| 45 | C | 0.50 | 6.50 | <0.01 | 25 | 100 | <0.01 |
| | G | 0.38 | 4.12† | <0.01 | 38 | 88 | <0.05 |
| | L | 1.75† | 9.75† | <0.01 | 100† | 100 | NS |
| | GL | 2.62† | 14.50† | <0.01 | 100† | 100 | NS |
| 60 | C | 0 | 1.50 | <0.01 | 0 | 75 | <0.05 |
| | G | 0.87* | 0.25† | <0.05 | 67* | 33 | NS |
| | L | 2.25† | 7.25† | <0.01 | 88† | 75 | NS |
| | GL | 3.12† | 7.87† | <0.01 | 100† | 100 | NS |
| 120 | C | 0.50 | 2.00 | <0.01 | 25 | 50 | NS |
| | G | 1.62† | 3.25† | <0.01 | 75 | 75 | NS |
| | L | 3.25† | 3.50† | NS | 92* | 100 | NS |
| | GL | 2.87† | 7.25† | NS | 82* | 73 | NS |

N=8–12 animals/treatment schedule (equal number of males and females). Pre-naloxone and post-naloxone observation periods were 15 min in length. Significantly different from corresponding control values at $p<0.05$ (*) and $p<0.01$ (†). $p$ values reflect the differences between pre-naloxone and post-naloxone observation periods; NS=not significant. At 120 days of age, a Treatment Schedule × Sex × Time interaction was significant (see details in text), but the combined data for males and females are presented in this table.

female rats in the control group were significantly colder than before drug administration.

*Nociception*

At 30 and 60 days of age, the Treatment Schedule×Sex×Time interactions were significant, $F(3,24)=4.37$, $p<0.05$ and $F(3,24)=23.84$, $p<0.01$, respectively, and these data are presented in Table 2. Prior to naloxone administration, 30-day old male rats in the GL group were slower to respond to the hot-plate than control males. In contrast to pre-injection levels, 30-day old males in the G group and 60-day old males in the L group were significantly slower to react to the hot-plate after receiving naloxone, but female rats of 30 days in age had shorter latencies after naloxone injection in comparison to their pre-injection levels. In comparison to controls, 30-day old males in the GL group and 60-day old males in the L group were significantly slower to react to the hot-plate during the post-naloxone period, but 30-day old males in the L group were faster to step off the hot-plate in this period.

At 45 days of age, only the Treatment Schedule×Time interaction was significant, $F(3,24)=27.59$, $p<0.01$, with both sexes of animals in each group having similar responses to the hot-plate test. Prior to naloxone administration, animals in the G, L, and GL groups had latencies of 13.03, 10.63, and 12.13 sec, respectively, in comparison to a latency of 4.31 sec for control rats; the latencies for all methadone-treated groups were significantly ($p<0.01$) longer than that of controls. After naloxone administration, only rats in the control group exhibited changes relative to their pre-injection latencies; these rats were significantly ($p<0.01$) slower to respond to the hot-plate after naloxone injection (10.37 sec) than before drug administration (4.31 sec).

At 120 days of age, no significant differences were observed between any group of methadone-treated animals and controls at any time period (pre- or post-naloxone) investigated.

*Head Shakes*

The mean number of head shakes per group, before and after naloxone injection, are presented in Table 3. The interaction of Treatment Schedule×Time was significant for the number of head shakes recorded at 30, 45, and 60 days of age, $F(3,24)=3.62$, $p<0.05$; $F(3,24)=6.61$, $p<0.01$; $F(3,24)=5.75$, $p<0.01$, and the Treatment Schedule×Sex×Time interaction was statistically reliable at 120 days of age, $F(6,76)=5.62$, $p<0.01$. Prior to administration of naloxone, few head shakes were recorded for rats in the control group; however, this behavior was commonly observed in rats perinatally subjected to methadone. G and L animals at 30 days, L and GL rats at 45 days, and all methadone-exposed rats at days 60 and 120 (both sexes) had significantly more head shakes than control rats. Following naloxone injection, control rats at 30, 45, and 60 days of age, as well as female control rats at 120 days of age, had significant increases in head shake behavior in contrast to their pre-injection values, with head shake behavior being especially prominent at 30 and 45 days. Except for GL rats at 30 days and G rats at 60 days, 30-, 45-, and 60-day old rats perinatally subjected to methadone had more head shakes

TABLE 4

WET-DOG SHAKE BEHAVIOR BEFORE AND AFTER ACUTE NALOXONE INJECTION IN YOUNG AND ADULT RATS PERINATALLY EXPOSED TO METHADONE

| | | Mean number wet-dog shakes | | Percent rats with wet-dog shakes | | |
|---|---|---|---|---|---|---|
| Age | Group | Pre-Naloxone | Post-Naloxone | Pre-Naloxone | Post-Naloxone | $p$ values |
| 30 | C | 0 | 1.00 | 0 | 50 | NS |
| | G | 1.00 | 2.25 | 50 | 62 | NS |
| | L | 0.75 | 0.62 | 37 | 37 | NS |
| | GL | 0 | 0.87 | 0 | 37 | NS |
| 45 | C | 0 | 4.00 | 0 | 100 | <0.01 |
| | G | 0.12 | 0.62 | 12 | 50 | NS |
| | L | 1.50 | 5.37 | 87† | 100 | NS |
| | GL | 0.62 | 6.12 | 37 | 100 | <0.05 |
| 60 | C | 0 | 1.25 | 0 | 37 | NS |
| | G | 0 | 0.87 | 0 | 33 | NS |
| | L | 0.25 | 5.62 | 25 | 100* | <0.05 |
| | GL | 2.00 | 4.50 | 87† | 100* | NS |
| 120 | C | 0 | 0 | 0 | 0 | NS |
| | G | 0 | 0.87 | 0 | 37 | NS |
| | L | 0.25 | 0.25 | 12 | 25 | NS |
| | GL | 0 | 1.12 | 0 | 37 | NS |

N=8–12 animals (equal number of males and females). Pre-naloxone and post-naloxone observation periods were 15 min in length. Since the statistical analysis of mean number of wet-dog shakes revealed different levels of interaction at all ages, values of significance were not noted in the Table; see Results for further details. Data of the percentages of rats with wet-dog shakes were analyzed using a chi square test. Significantly different from corresponding control values at $p<0.05$ (*) and $p<0.01$ (†). $p$ values reflected the difference between pre-naloxone and post-naloxone observation periods; NS=not significant.

after naloxone administration than before drug injection. At 120 days of age, male rats in the G and GL groups also demonstrated marked increases in head shake behavior after naloxone administration in relation to their pre-injection values. In comparison to controls during the post-naloxone period, 45- and 60-day old L and GL rats, as well as 120-day old male rats in the G, L, and GL groups had fewer head shakes; 45- and 60-day old rats in G groups had fewer head shakes at this time.

In regard to the percentage of rats in a group exhibiting head shakes (Table 3), this was rarely recorded in the control group prior to administration of naloxone. However, head shakes were frequently observed in rats maternally exposed to methadone, being most prominent in animals of the L group. After naloxone administration, more rats in the 30-, 45-, and 60-day old control group, as well as the 45-day old G group, exhibited head shakes in respect to their pre-injection levels. Comparison of the percentage of rats with head shakes during the post-naloxone period revealed few differences between control and methadone-exposed groups.

*Wet-Dog Shakes*

Statistical analysis of the mean number of wet-dog shakes revealed different levels of interaction at all four ages of animals examined and details of these interactions, as well as the levels of significance, are stated below. Values for the mean number of wet-dog shakes, derived from the Treatment Schedule×Time interaction, are presented in Table 4; data are presented in this manner in order to provide a sense of comparison to the results regarding the percentage of rats displaying wet-dog shakes.

At 30 days of age, the Treatment Schedule×Sex×Time interaction was statistically significant, $F(6,46)=28.14$, $p<0.01$. Prior to an injection of naloxone, male rats in the G group and female rats in the G and L groups displayed significantly more wet-dog shakes than control animals, whereas after naloxone administration, only female rats in the G group had more wet-dog shakes than control animals.

The Treatment Schedule×Time interaction at 45 days of age was statistically reliable, $F(6,48)=3.79$, $p<0.05$. Prior to an administration of naloxone, rats in the L and GL groups exhibited significantly more wet-dog shakes than controls. In comparison to their pre-injection levels, rats in the C, L, and GL groups demonstrated more wet-dog shakes after naloxone administration. During the post-naloxone period, L and GL rats had more wet-dog shakes than controls, but G rats had fewer wet-dog shakes.

At 60 days of age, the Treatment Schedule×Sex interaction was significant, $F(3,22)=5.36$, $p<0.01$. Male and female rats in the GL group had more wet-dog shakes than controls.

At 120 days of age, no interactions were reliable. The overall Treatment Schedule, however, was statistically significant and indicated that the GL animals had more wet-dog shakes than control rats, regardless of sex and time.

Prior to administration of naloxone, no control animals

were observed to have wet-dog shakes, but a marked number of rats in the L group at 45 days and the GL group at 60 days exhibited this behavior (Table 4). After naloxone injection, a greater percentage of animals in the control and GL groups at 45 days, as well as the L group at 60 days, exhibited wet-dog shakes relative to their pre-injection levels. Comparison of the percentage of rats with wet-dog shakes during the post-naloxone period revealed few differences between control and methadone-exposed groups.

*Other Behavioral Parameters*

With the exception of the parameters mentioned above, the control and methadone-exposed rats were similar in behavior prior to injection with naloxone. After naloxone administration, G, L, and GL rats often exhibited increased motor activity and prolonged periods of grooming; teeth chattering, hiccupping, repetitive sneezing, and tremors occurred less frequently in these animals. No differences in the number of boli or the proportion of rats with diarrhea were noted between control and methadone-exposed groups in the pre-naloxone and post-naloxone periods.

## DISCUSSION

The results of the present study confirm and extend our earlier observations showing abnormalities in core temperature [36,37] and nociception [51] in post-weaning rats perinatally subjected to methadone. Our findings of hypothermia, and head shake and wet-dog shake behaviors in young and adult rats maternally subjected to methadone, along with results obtained in earlier investigations showing hyperactivity [52] in 45- and 60-day old methadone-treated animals, bear a striking similarity to the signs described for the withdrawal syndrome in rodents [1, 6, 25, 38, 40, 41]. In at least one parameter, wet-dog shakes, a relationship between this behavior and the schedule of drug treatment could be discerned. The greatest number of wet-dog shakes and the highest incidence of this behavior for any drug-exposed group were noted to occur at 30 days for rats in the gestation group, at 45 days for animals in the lactation group, and at 60 days for rats subjected to methadone during both gestation and lactation. These results could suggest that the expression of this withdrawal syndrome appears dependent on the timing and duration of drug exposure, with animals given cumulative exposure (i.e., gestation-lactation group) having the longest interval between cessation of drug exposure and the period of most marked behavioral alteration.

A prolongation of the symptoms related to narcotic withdrawal is known to occur in infants of narcotic-addicted mothers [5, 7, 19, 21, 44], as well as in adult organisms exposed to opiates [15, 24, 25, 42]. In the case of neonates passively addicted to methadone or heroin, these infants undergo overt withdrawal within several weeks after birth, but experience symptoms of drug withdrawal in an attenuated or "subacute" form for 4 to 6 months [5, 7, 19, 21, 44]. Within the context of evidence gathered in the present study, and given differences in routes of drug administration and/or pharmacokinetics between methadone exposure in humans and laboratory animals, it appears that a protracted state of drug withdrawal may be a common phenomena in offspring perinatally exposed to narcotics.

One observation recorded in this and another study [51] that is not entirely consistent with the hypothesis of a prolonged period of withdrawal is our findings of analgesia in rats maternally exposed to methadone. The withdrawal syndrome is usually associated with an increase in nervous system irritability, and hyperalgesia has been reported in animals undergoing opiate withdrawal [38]. The presence of analgesia and withdrawal, simultaneously, is a paradox that needs further study. Our observations could suggest that not all alterations associated with withdrawal are necessarily manifested at one time, and that the period of readjustment after cessation of methadone exposure could include a spectrum of drug persistence (e.g., analgesia) and drug withdrawal (e.g., hypothermia). Alternatively, the effects of methadone exposure in early life as regards pain perception may differ entirely from those related to the regulation and control of body temperature and behavior.

Complementing our observations on the occurrence of a natural withdrawal in offspring of mothers perinatally exposed to methadone, we also examined whether abstinence could be precipitated in these animals. Naloxone was chosen as the opiate antagonist because it is known to produce a precipitated abstinence syndrome in opiate-dependent and tolerant rats [6, 38, 40]. Since our animals were evaluated 30 to 120 days after cessation of methadone exposure, and the intensity of withdrawal is known to be related to the dose of naloxone injected [1,38], a relatively high dose of naloxone was utilized in this study to elicit a maximal response. Our results show that at least in regard to two criteria of abstinence: wet-dog and head shake behaviors, naloxone administration revealed an even deeper level of physical dependence than that observed in rats naturally withdrawing from drug exposure and suggests the presence of a state of physical dependence 1 to 4 months after termination of methadone exposure.

Naloxone administration to control animals was not without effect as demonstrated in the present study. This drug has long been regarded as a "pure" antagonist to the many biological actions of opioid substances and as devoid of significant intrinsic activity [4, 18, 31], presumably exerting its effects by blockade of opiate receptors [23]. A number of recent reports, however, indicate that naloxone may have pharmacological actions unrelated to opiate receptor blockade [31]. Naloxone's effects in drug-naive animals are unclear, with the evidence often being contradictory. Administration of naloxone to rodents has been related to a subsequent hypothermia [12] and to a reduction in exploratory activity [9]. In regard to nociception, naloxone has been reported to elevate [29], reduce [10,17], or to have no effect [13,38] on pain threshold. The results of our experiments show that the administration of 10 mg/kg naloxone to young and adult control rats often has profound effects, especially on head shake behavior and thermoregulation. The array of behavioral and physiological alterations elicited in control animals given naloxone resembles those symptoms reported for the abstinence syndrome in opiate-addicted rats. Further work is clearly needed to explain this "abstinence-like" syndrome in control animals injected with an opiate antagonist. However, it certainly could be envisioned that naloxone, with its extremely high affinity for opiate receptors, could be interfering with the interaction between opiate receptors and endogenous opioids, with consequent alterations produced in systems normally influenced by the endogenous opioids.

In summary, hypothermia and perturbations in body weight throughout weaning and young adulthood, as well as head shake and wet-dog shake behaviors which are exacerbated by naloxone administration, are shown in the present study to be characteristics of young and adult rats perinatally exposed to methadone. The mechanisms underlying these

long-term behavioral and physiological abnormalities resulting from exposure to methadone in early life are unknown at the present time, and we can only speculate about several plausible possibilities. First, it is possible that tissue-bound methadone may contribute to a prolonged state of at least partial physical dependence, with the slow egression of methadone resulting in a protracted withdrawal. Support for this suggestion comes from several investigations. Although the half-life of methadone in plasma is only a few hours in rodents [27], plasma concentrations of methadone can not be correlated with the rate of metabolism or brain concentrations of this drug [22]. Methadone is known to bind firmly to tissue proteins [35] and accumulates in the body tissues [8,27], and methadone has been reported to persist in several organs including the brain for up to 10 weeks [14]. Immature animals accumulate much larger amounts of methadone than adults [28, 32, 33]. In particular, brains of fetal [28] and preweaning [32] rats have high concentrations of methadone following acute injections, presumably because developing animals have an increased permeability due to the gradual formation of the blood-brain barrier postnatally. Immature organisms also may not be able to cope with drugs as well as adults, and fetal storage and delayed metabolism and excretion could produce pharmacological effects for longer periods than in adults (e.g., [19]). On the other hand, permanent structural and/or biochemical changes that might mediate the effects recorded in this study cannot be ruled out. Methadone exposure in our rat offspring occurs during crucial stages of cell, tissue, and organ development, and the disturbances encountered could be the results of defects in ontogeny. In fact, reductions in the concentration and content of brain DNA, as well as in macroscopic dimensions of the brain, have been reported [48,49]. Of course the concepts of protracted physical dependence/withdrawal and permanent damage are not mutually exclusive and, to a certain extent, both may be in operation to produce the effects observed in this study and perhaps the constellation of neurobiological abnormalities reported elsewhere [16, 30, 34, 48–53].

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