Pharmacology Biochemistry & Behavior, Vol. 39, pp. 329–335. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Acute Opiate Withdrawal in Rats Undernourished During Infancy: Impact of the Undernutrition Method

C. A. COHEN,[1] J. TONKISS[2] AND S. B. SPARBER[3]

Departments of Pharmacology and Psychology, University of Minnesota, Minneapolis, MN 55455

Received 1 October 1990

COHEN, C. A., J. TONKISS AND S. B. SPARBER. *Acute opiate withdrawal in rats undernourished during infancy: Impact of the undernutrition method.* PHARMACOL BIOCHEM BEHAV **39**(2) 329–335, 1991.—Acute morphine withdrawal was assessed in adult rats following early postnatal undernutrition produced by two different methods (Large Litter procedure—20 pups/litter and Modified Slob procedure—rats cross-fostered on days 2, 4, and 6 to nonlactating dams for 24-hour periods). Response rates were first stabilized on a FR16 operant schedule. A single dose of morphine (20 mg/kg) was then administered, followed 4 h later by a single injection of naloxone (2.5 mg/kg). Males reared in large litters showed little behavioral disruption after morphine, suggesting either insensitivity to the opiate or the rapid development of tolerance. After naloxone, Modified Slob males displayed milder withdrawal than those in the well-nourished control or large litter groups. Thus the method of undernutrition influenced morphine's action and expression of withdrawal. A clear sex difference was also evident, females appearing to be generally less sensitive to the opiate- and naloxone-induced withdrawal than males. Body temperature underwent a characteristic elevation following morphine and a depression following naloxone across all groups, but undernutrition did not affect these responses. Hence, behavior proved to be the more sensitive measure for revealing differences in opiate dependence and withdrawal following early life undernutrition, under the test conditions employed.

Neonatal undernutrition Acute opiate withdrawal Tolerance Dependence

RATS exposed to opiates in utero show an altered sensitivity to these compounds in adulthood; a finding which has been interpreted as evidence of a direct and specific effect of the drug (11, 17, 18, 26). Prenatal opiate administration has also been associated with stunting of the growth of the offspring, with large reductions in brain weight, protein and nucleic acid concentrations (20, 26, 29, 30), similar to changes observed after some types of neonatal undernutrition. It is therefore unclear whether findings of altered opiate sensitivity result from a direct effect of the opiate or from an indirect effect of undernutrition (and its associated stresses) arising from acute toxicity and/or withdrawal abstinence postnatally (23,25). Since the number of opiate receptors increases rapidly during the first few weeks of life (3,14), it is possible that early postnatal undernutrition, in the absence of exposure to opiates, may alter receptor development and function in later life. Alternatively, other transmitter/receptor systems involved in adaptive processes (dependence) or its expression (withdrawal) may be affected.

Sparber and Lichtblau (25) demonstrated that preventing rat pups from suckling during postnatal days 2, 4, and 6 not only retarded their growth and altered brain measures comparable to alterations produced by opiate exposure and/or withdrawal during development, but also altered their sensitivity to morphine when administered in adulthood. Low doses of morphine did not differentiate the previously malnourished and well-nourished control rats in their performance of an autoshaping task, but when higher doses were administered, undernourished rats proved more sensitive to morphine's behavior disrupting properties, manifest as reduced lever touching. Moreover, lower basal colonic temperatures were seen in the previously undernourished subjects, suggesting that early postnatal undernutrition may have lasting effects on rats' thermoregulatory mechanisms (25). Since this change in temperature was similar to that previously reported to be the direct result of exposure to opiates during development, the potential importance of undernutrition as an epiphenomenon in developmental opiate studies was clearly demonstrated. Nevertheless, it remained to be determined whether undernutrition per se altered responsiveness to opiates later in life, or if the experiential factors related to the specific undernutrition manipulations (e.g., maternal separation stress, cooling) were equally or more important. One way to address this issue is to compare different methods of inducing the same degree of early postnatal undernutrition, which engender different early experiences, and then to challenge the rehabilitated adults with an acute dose of morphine. If the results of the acute morphine challenge and/or precipitated withdrawal are dissimilar, despite evidence of a

[1]Current address: Research Building, Tufts University, 490 Boston Ave., Medford, MA 02155.
[2]Current address: Behavioral Development, M921, Boston University School of Medicine, 80 East Concord St., Boston, MA 02118.
[3]Requests for reprints should be addressed to Dr. Sheldon B. Sparber.

similar magnitude of undernutrition, then the behavioral differences could be attributed to early experience factors associated with each technique for inducing early undernutrition.

Two undernutrition methods were selected for study: the large litter procedure (13) associated with overcrowding of the pups and altered maternal behavior (4, 9, 21) and the so-called Modified Slob procedure [(22) as modified by (25)] involving rotation of pups between lactating and nonlactating females over the first few days of life. Clearly the latter is more acutely stressful owing to maternal separation and the total absence of any fluid intake over the 24-h deprivation periods. Opiate withdrawal was assessed using colonic temperature measures (16) and sensitive operant procedures (1, 5, 8, 10, 24, 27).

METHOD

Nutritional Treatment and Handling Protocol

The nutritional methods have been described in more detail elsewhere [see (28)]. Briefly, one half of the pups in a given litter (N=5, 3 males and 2 females or 2 males and 3 females) were cross-fostered to a nonlactating "aunt" for 24 h using the Modified Slob procedure (MS) on days 2, 4 and 6 of life. Two half litters were combined so that each "aunt" cared for 10 pups and litter size remained constant. The pups from the other half of each litter (Controls, C, N=5) were fostered to lactating dams at these times. In order that each of the lactating dams also cared for 10 pups, their litter sizes were adjusted by adding reserve, nonexperimental pups, of the same age. Eight litters were used for this condition. In addition, five large litters of 20 pups per litter (10 males and 10 females) were created from a pool of remaining litters. Because we were interested in carrying out this control for the large litter subjects as well, they too were fostered to lactating dams on days 2, 4, and 6 so that they would have a similar handling and fostering experience (7). On day 10, two pups were taken from each litter for brain analyses, which has been reported elsewhere (28). Also, on day 10, the remaining pups in each C/MS litter (N=8) were fostered to lactating dams. The large litters (now comprising 18 pups) were split into litters of 9 and fostered to lactating females, for nutritional rehabilitation. All rats were weighed daily from day 2 to 12, then every 3 days until weaning on day 21. At weaning, all subjects were ear punched for identification and were housed in stainless steel hanging wire mesh colony cages (50×25×18 cm) with foster litter mates of the same sex in groups of 4 or 5 per cage. C/MS "litter mates" were housed together and the split large litter subjects (9 pups in each) were further subdivided into either 4 or 5/cage. There were no deaths during any of the preweaning manipulations; however, one LL animal died shortly after weaning.

Subjects

At 9 months of age, 8 Control (C) males, 7 C females, 8 Modified Slob (MS) males, 8 MS females, 10 Large Litter (LL) males and 9 LL females were taken from the larger pool of subjects to test their sensitivity to morphine and the development of acute physical dependence. (One C female was excluded from the study because of an abdominal tumor and one LL female was excluded due to a severe ear infection.) No more than 2 males and females were from the same large litter and no more than 1 male and 1 female were from each C and MS litter. All rats had been tested previously in autoshaping and progressive fixed ratio tests.

Apparatus

Rodent operant chambers measuring 31×25×32 cm (Model 143-22, BRS/LVE, Laurel, MD) were equipped with retractable levers, which remained extended and fixed during the behavioral session (Model RRL-015, BRS/LVE), a dispenser for the delivery of 45 mg food pellets (Formula #0021; BioServ, Inc., Frenchtown, NJ) and a speaker for introduction of white masking noise. Behavioral sessions were controlled, and data collected by custom-made microcomputer interfaces for TRS-80 Color Computers (Tandy Radio Shack, Fort Worth, TX). Timing and monitoring of behavioral events were provided by 60-Hz interrupt driven machine language routines. The data were transferred to magnetic cassette tapes at the end of every behavioral session for later analysis.

Acute Morphine Withdrawal Protocol

In preparation for the present study, which included repeated determination of rectal temperature, the rats were habituated to the insertion of a lubricated temperature probe (Model 702, Yellow Springs Instrument Co., Yellow Springs, OH). The probe was inserted 6 cm into the rats' rectum and held in place by a piece of split rubber tubing fitted around their tails. They were then allowed to move freely in a plastic cage and temperature was recorded 2 min later from a telethermometer (Model 5810, United Systems Corporation, Dayton, OH). One temperature determination was made at the same time on each of 3 successive days. In addition to familiarizing the rats with the procedure, this phase of the experiment also generated data which served as baseline for the 6 groups under free-feeding conditions. The rats were then placed on a restricted feeding regime to reduce their weights to 85% of their free-feeding weight.

The rats responded for 2 days under an FR4 schedule, in which every fourth lever press produced food (6). Behavioral sessions lasted for 20 min. They were then trained for 12 consecutive days under an FR16 schedule, until their response rates had stabilized (when the mean of each groups' response rate did not differ by more than 10% for 3 consecutive days). Once a stable rate of responding was achieved, the rats received three 10-min FR16 sessions/day for the next 2 days. The rectal probe was inserted immediately prior to all three sessions and saline injections (1 ml/kg, IP) were given immediately following the first 2 sessions. Session 2 began 3 h 50 min after completing Session 1. Session 3 began 1 h 30 min after completing Session 2. This procedure served to familiarize the rats with performing during multiple sessions within the same day, to habituate them to IP injections, and to refamiliarize them with the insertion of the temperature probe. The following day served as a control for the morphine treatment day and was also used to verify that the naloxone dose chosen (2.5 mg/kg) did not affect the behavioral baseline being studied. The procedure for both the Control and the Drug day is given in Table 1. Briefly, five 10-min sessions were given throughout the day. The first session occurred prior to administering saline (Control day) or morphine (Drug day). Sessions 2 and 3 began 2 h 50 min and 3 h 50 min following the injection, respectively. These sessions were run to measure long-term behavioral effects of the morphine, if present, and to allow behavior to return toward control rates sufficiently to enable response rate reductions to be witnessed as a result of subsequent naloxone-precipitated withdrawal. Upon termination of Session 3, the rats were given an injection of naloxone on both the Control and Drug days. Thirty minutes and 1 h 36 min after naloxone administration, Sessions 4 and 5 were run, respectively. These sessions were instituted to measure the independent effect of naloxone administration (Control day) and its ability to precipitate withdrawal (Drug day). Rectal temperatures were monitored immediately prior to each session. The behavioral sessions occurred at the same time of day on the Control and Drug days.

TABLE 1

PROTOCOL FOR THE ACUTE MORPHINE WITHDRAWAL EXPERIMENT

Time (min)	Event
0	Weigh rat and insert rectal probe
2	Record temperature; remove probe; place rat in operant chamber; start behavioral session
12	Remove rat from operant chamber; give injection (Control day = saline; Drug day = morphine 20 mg/kg, IP) and return rat to home cage
180	Insert temperature probe
182	Record temperature; remove probe; place rat in operant chamber; start behavioral session
192	Remove rat from operant chamber and return rat to home cage
240	Insert temperature probe
242	Record temperature; remove probe; place rat in operant chamber; start behavioral session
252	Remove rat from operant chamber; give injection (naloxone 2.5 mg/kg, IP) and return rat to home cage
280	Insert temperature probe
282	Record temperature; remove probe; place rat in operant chamber; start behavioral session
292	Remove rat from operant chamber and return rat to home cage
346	Insert temperature probe
348	Record temperature; remove probe; place rat in operant chamber; start behavioral session
358	Remove rat from operant chamber and return rat to home cage

Drugs

Morphine SO_4 (S. B. Penick and Co., New York, NY) and naloxone HCl (generously supplied by DuPont Pharmaceuticals, Wilmington, DE) were used. Both drugs were dissolved in 0.9% NaCl and were administered IP in a volume of 1 ml/kg at a dose of 20 mg/kg for morphine and 2.5 mg/kg for naloxone, as the salts.

Data Analysis

Male and female body weights were analyzed by separate one-way ANOVA with Nutrition as the main factor. Rectal temperatures over the three habituation days and the five 10-min sessions on the Control day were analyzed by 3-way ANOVAs [Nutrition × Sex × Day (or Session)] with Day (or Session) as a repeated measure. Since there was a difference in baseline temperature between the nutritional groups, Control day values were subtracted from Morphine day values and a 3-way ANOVA (Nutrition × Sex × Session), with Session as a repeated measure, was applied. Rate of lever pressing on the Control day, and on the Drug day (% of Control day), was analyzed by 2-way (Nutrition × Session) ANOVA, with Sessions as a repeated measure. These analyses were applied to the two sexes separately. Significant main effects were followed by planned comparisons using a Duncan's Multiple Range test. Repeated measure ANOVAS were applied to both rate of lever pressing (% of Control day) and body temperature (difference from Control day) on the Drug day. These analyses compared the session prior to morphine with each subsequent session, and the second session after morphine with the two sessions after naloxone, for each group individually.

RESULTS

One male control animal was excluded from all analyses because its behavior after morphine was greater than 3 standard deviations above the group mean, indicating a high probability that a poor injection was received.

At 90 days of age, male rats of both previously undernourished groups weighed less than the controls (mean body weight ± SE; C = 474 ± 10, LL = 437 ± 8, MS = 431 ± 10; Duncan's Multiple Range test, $p<0.05$). In contrast, MS females were significantly lighter than both C and LL females (C = 285 ± 8, LL = 266 ± 7, MS = 247 ± 8; Duncan's Multiple Range test, $p<0.05$).

During habituation to the insertion of the rectal probe (under free-feeding conditions), no differences in average rectal temperatures were observed between the different nutritional groups [overall means (°C): C = 38.26, MS = 38.41, LL = 38.54]. Likewise, there was no Sex × Nutrition interaction. However, the females had higher rectal temperatures than the males; males = 38.05, females = 38.75, $F(1,45) = 29.1$, $p<0.001$. On the Control day when the rats were at 85% of their free-feeding weight, females continued to display higher temperatures than the males, $F(1,43) = 3.97$, $p = 0.05$. However, unlike the nondeprived condition, a difference emerged between the nutritional groups, $F(2,43) = 3.30$, $p<0.05$, due to the LL animals having higher temperatures than either the C or MS rats (LL = 38.48, C = 38.21, MS = 38.19; Duncan's Multiple Range test, $p<0.05$). Thus food deprivation appeared to unmask a difference in body temperature regulation between the different treatment groups. Body temperature rose slightly during the Control day, $F(4,172) = 50.50$, $p<0.001$, but was found to be stable over Sessions 3 to 5, indicating that naloxone had no independent effect on temperature regulation. Nevertheless, since temperature was subject to change, and there was a baseline difference between the nutritional groups, Control day values were subtracted from Drug day values to facilitate analysis of drug effects. ANOVA applied to these temperature differences revealed a significant main effect of Session, $F(4,172) = 66.08$, $p<0.001$ (Fig. 1). Body temperature was elevated relative to the first measurement taken on the drug day for the two postmorphine sessions in both the males and females, $F(2,44) = 19.011$, $p<0.001$ and $F(2,42) = 15.881$, $p = 0.001$, respectively. After naloxone administration there was a significant decrease in body temperature for both sexes, indicating the animals were experiencing a withdrawal induced hypothermia, $F(2,44) = 6.998$, $p = 0.002$ and $F(2,42) = 6.94$, $p = 0.003$, respectively.

Table 2 shows the mean number of lever press responses emitted during each of the five 10-min sessions using an FR16 schedule of reinforcement for the control day. In the males, the response rates were unaffected by the saline and naloxone injections throughout the five behavioral sessions. In the females, response rates were significantly lower in the first session of the day (i.e., baseline, preinjection) than in the following four sessions, $F(4,84) = 8.06$, $p<0.001$. There was no significant difference between the nutritional groups for either sex, and the interaction of Nutrition × Session was also not significant. When individual behavioral sessions were analyzed for main effects of Nutrition and Sex, a sex difference emerged in the pretreatment session, females making fewer responses than the males, $F(1,43) = 4.781$, $p = 0.03$. Figure 2 (upper) shows the responses/10-min session for the 5 sessions on the Drug day, expressed as a percentage of the responses on the Control day for the male rats. ANOVA showed significant effects of Nutrition, $F(2,22) = 7.70$, $p<0.01$, and of Session, $F(4,88) = 29.91$, $p<0.001$, and a significant Nutrition × Session interaction, $F(8,88) = 2.66$, $p<0.05$. In both the C and MS males, there was a significant decrease in lever pressing during the two postmorphine sessions [M1: C males, $F(1,6) = 22.5$, $p<0.01$, MS males, $F(1,7) = 10.9$, $p<0.05$; M2: C males, $F(1,6) = 7.8$, $p<0.05$, MS males, $F(1,7) = 8.1$, $p<0.05$], while no decrease in responding

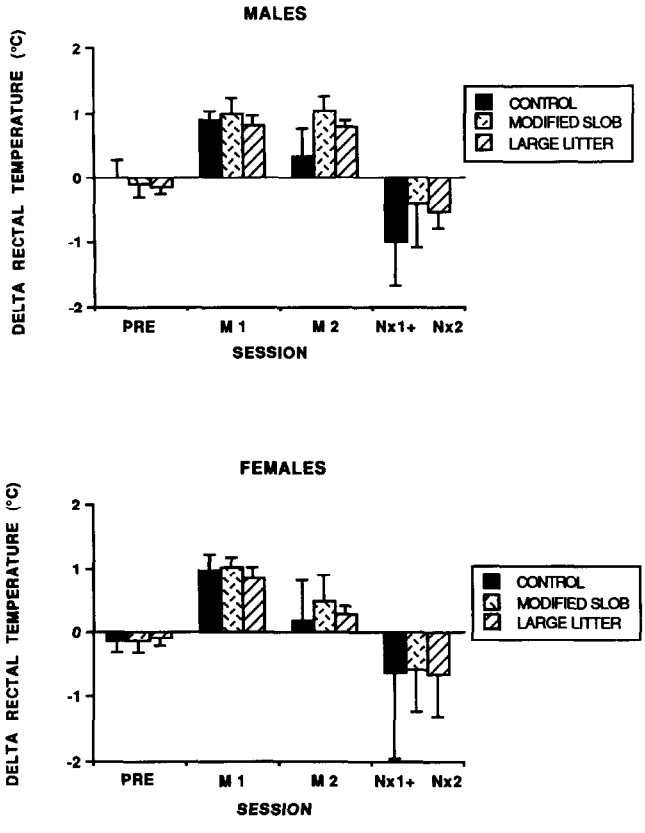

FIG. 1. Difference in rectal temperature between the Drug day and the Control day for male and female rats of the three nutritional treatment groups. PRE = Initial session (preinjections), M1 = 2 h 50 min after morphine injection, M2 = 3 h 50 min after morphine injection, Nx1 = 30 min after naloxone injection, Nx2 = 1 h 36 min after naloxone injection. Since there were no significant differences between the two sessions, Nx1 and Nx2 were combined for ease of display. Error bars are standard errors of the mean.

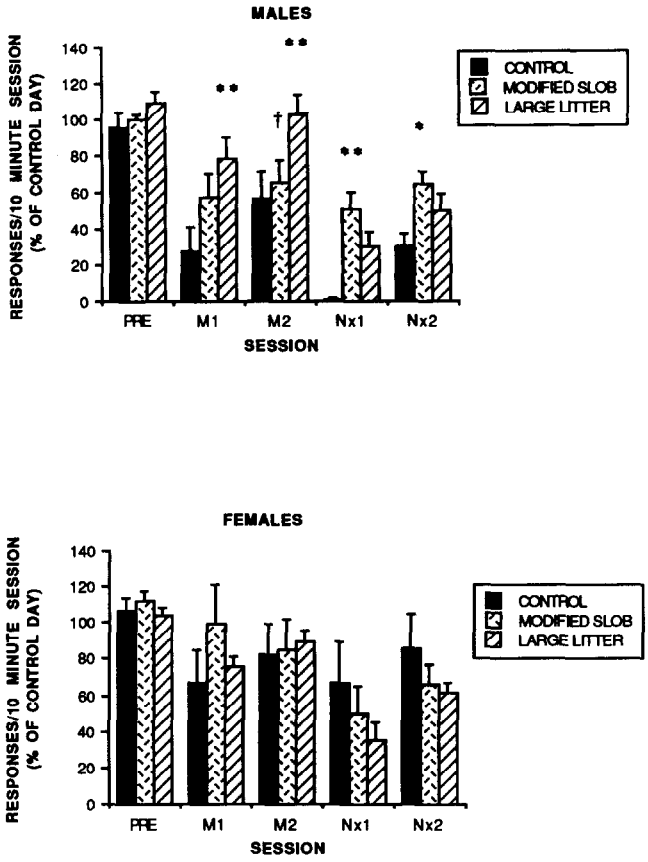

FIG. 2. Responses per 10-min session expressed as a percent of the control day for male and female rats of the three nutritional treatment groups. PRE = Initial session (preinjections), M1 = 2 h 50 min after morphine injection, M2 = 3 h 50 min after morphine injection, Nx1 = 30 min after naloxone injection, Nx2 = 1 h 36 min after naloxone injection. Differences from Control rats are indicated by $*p<0.05$ and $**p<0.01$ as measured by Duncan's Multiple Range test. Differences from Large Litter rats are indicated by † $p<0.05$. A clear sex difference existed in the expression of acute opiate withdrawal. The males, in general, appeared more sensitive to the naloxone-induced withdrawal than the females.

was seen in the LL males. Planned comparisons revealed that the LL rats were responding at a significantly higher rate than the C rats during the first session after morphine (M1) and at a higher rate than both the C and MS rats during the second postmorphine session (M2).

The administration of naloxone after morphine could result in one of two effects on fixed ratio performance. It could either reverse the morphine-induced decrement in responding by displacing the opiate at its receptor(s) in the nondependent animal, or further decrease response rates in the morphine-dependent animal (recall that this dose of naloxone alone had no effect on FR16 behavior). Relative to the second postmorphine session (M2), C males showed a decrease in responding during the first session after naloxone (Nx1), indicating that they were dependent on morphine, $F(1,6) = 14.1$, $p<0.05$. This effect was no longer present during the second postnaloxone session (Nx2). However, responding was still significantly lower than the first behavioral session, suggesting that the rats were still in withdrawal, $F(1,6) = 156.0$, $p<0.001$. Contrast these effects of naloxone postmorphine with those for male rats of both previously undernourished groups. The MS group did not show any statistical decrease in responding from M2 to Nx1 and Nx2. Since this group was still significantly below baseline, $F(1,7) = 22.1$, $p<0.01$, and naloxone administration did not result in a return to premorphine response rates, there was apparently some degree of physical dependence (and withdrawal). During the two postnaloxone sessions, the MS rats had higher rates of responding than the C rats, but were no different from the LL rats, again confirming that there was a difference in the degree of physical dependence observed across the undernutrition groups in the males. When naloxone was given to the LL rats, their response rates were significantly decreased during both postnaloxone sessions, as compared to the premorphine and prenaloxone behavioral sessions, $F(1,9) = 36.3$, $p<0.001$ and $F(1,9) = 18.6$, $p<0.01$. This confirms that the LL animals had indeed become acutely tolerant and dependent.

Figure 2 (lower) shows the responses/10-min session for the 5 sessions on the Drug day, expressed as a percentage of the responses on the Control day, for the female rats. A somewhat different pattern emerged than that seen in the males. In both the C and MS females, there were no significant decreases in responding during the two postmorphine sessions, compared to the baseline session. However, when morphine was administered to the LL females, a significant decrease in responding was observed during both postmorphine sessions [M1: $F(1,8) = 23.9$,

TABLE 2

RESPONSES/10-MIN SESSION MEAN ± SE

Group	PRE	SAL 1	SAL 2	Nx 1	Nx 2
Males					
C	1194.4 ± 144.1	1222.0 ± 147.2	1199.7 ± 151.0	1160.1 ± 156.1	1176.0 ± 145.3
MS	1141.0 ± 105.1	1220.5 ± 140.1	1251.5 ± 143.5	1202.8 ± 113.7	1244.5 ± 131.5
LL	1162.5 ± 122.5	1214.1 ± 108.3	1253.4 ± 118.5	1264.4 ± 111.6	1295.1 ± 104.8
Females					
C	842.1 ± 185.4	981.3 ± 218.1	970.7 ± 210.3	951.1 ± 178.9	935.0 ± 166.8
MS	887.8 ± 188.2	921.9 ± 178.0	981.4 ± 180.8	995.4 ± 177.2	963.5 ± 163.8
LL	1043.0 ± 109.3	1153.8 ± 125.6	1159.6 ± 133.2	1198.1 ± 125.7	1201.6 ± 129.8

C = Control; MS = Modified Slob; LL = Large Litter.
PRE = Baseline; SAL 1 = First session after saline; SAL 2 = Second session after saline; Nx 1 = First session after naloxone; Nx 2 = Second session after naloxone.

$p<0.01$; M2: $F(1,8)=5.1$, $p=0.05$]. After the administration of naloxone, the behavior of the C females did not change significantly, compared to the baseline and prenaloxone behavioral sessions. Thus normal females appear to be resistant to both the rate-suppressing and dependence-inducing effects of morphine. While the MS females did not show a significant decrease in responding after naloxone, compared to the prenaloxone session (M2), they were significantly lower than baseline, indicating a withdrawal response, $F(1,7)=16.0$, $p<0.05$. The LL females were also acutely dependent on morphine because their response rates were significantly lower after naloxone administration, as compared to baseline [Nx1: $F(1,8)=55.2$, $p<0.001$; Nx2: $F(1,8)=51.3$, $p<0.001$] and the prenaloxone session (M2) [Nx1: $F(1,8)=49.9$, $p<0.001$; Nx2: $F(1,8)=22.4$, $p<0.01$].

DISCUSSION

There were four main findings in the present study: (A) later behavioral sensitivity to morphine and to subsequent naloxone-precipitated withdrawal following early postnatal undernutrition was dependent upon the method used to impose the nutritional restriction, (B) the sex of the rat interacted with the undernutrition method in determining opiate sensitivity and withdrawal, (C) female rats were generally more resistant than males to the behavioral disruption caused by acute opiate administration and withdrawal, and (D) under the test conditions employed, behavior was a more sensitive measure than colonic temperature in revealing differences in opiate dependence and withdrawal following early life undernutrition.

Consider the first two findings. The LL males did not show the same degree of behavioral disruption after morphine as that seen in C or MS groups. This finding may be interpreted as the LL males being either less sensitive to morphine, better able to adapt to the disrupting effects of the drug (i.e., were more tolerant) or differed in morphine pharmacokinetics from the other groups. Clearly, monitoring of the plasma levels of the drug would be necessary to investigate the latter possibility. During naloxone-precipitated withdrawal, MS rats of both sexes displayed less suppression of their operant responding than either the LL or C animals. However, it was evident from their lower than baseline response rates that they were in mild withdrawal. These data suggest that the MS procedure results in rats which are more resistant to opiate withdrawal-abstinence. Nonetheless, the incomplete behavioral recovery in the various groups prior to naloxone administration must be recognized as a complicating factor.

Since the drug effects were not equivalent in the two undernutrition procedures, it suggests that altered opiate sensitivity and withdrawal may indeed be a consequence of experimental factors accompanying the undernutrition, in addition to (or rather than) the undernutrition itself. For instance, crowding of the pups and competition for access to the nipples could contribute to the decreased sensitivity of the LL males to morphine. Likewise, the more acutely stressful MS procedure may allow subjects to adapt to the stress of opiate withdrawal more readily than the LL and C animals. However, such conclusions must be approached cautiously. One of the requirements of the study was that there should be equivalence of the severity of undernutrition imposed by the two procedures. Although growth was retarded to the same extent during the nutritional deprivation, it was found that MS rats had greater deficits than the LL rats in brain weight (21.3% and 10.3%, respectively), protein and nucleic acid concentrations (28) in a sample of rats taken from each population at the end of the undernutrition period (day 10). Furthermore, MS females clearly demonstrated a more severe body weight deficit than the LL females in the present experiment. Thus the possibility remains that the differential effects of the two undernutrition procedures on opiate sensitivity and withdrawal may be a consequence of dissimilarly altered neurochemistry, receptor number or distribution in response to an unequal severity of undernutrition.

It was surprising to find that female rats were more resistant than the males to the behaviorally disruptive effects of opiate exposure and withdrawal. Moreover, in contrast to the males, nutritional history had no significant influence upon fixed ratio performance following the drug challenges. These sex differences could be due to dissimilar pharmacokinetics. However, the appropriate temperature changes were observed in response to morphine and naloxone when assessed immediately prior to the behavioral sessions. A recent paper by Kellogg et al. (12) reported sex differences in adult rats that had been prenatally exposed to diazepam. Rather profound effects were found in males in both behavioral and biochemical measures, while little if any effect was seen in the females. Thus it is not uncommon to find sex differences following an early environmental manipulation.

The finding that fixed ratio performance was the more sensitive index of acute opiate dependence and withdrawal contrasts with an earlier report of alterations in both behavior and rectal temperature in rats undernourished using the MS procedure (25). This apparent inconsistency is unlikely to be due to differences in the undernutrition procedure, since the same protocol was employed in both cases. The key may lie in the dose of morphine selected for use and the time at which rectal temperatures were monitored. Sparber and Lichtblau (25) reported differential effects upon body temperature in MS and C rats which emerged between 1 and 3 h after the acute administration of 30 mg morphine/kg. Lower morphine doses did not produce these effects. Thus, either the opportunity to observe a difference in thermal response was missed, since we did not measure body temperature until approximately 3 h after morphine, and/or the dose of morphine used in this study was too low to reveal such a difference.

Some baseline rectal temperature differences were observed as a consequence of early undernutrition. Basal body tempera-

tures were very similar in all groups when free feeding. It was only upon reduction of body weight to 85% of their free-feeding weight that the LL animals proved to have higher body temperatures compared with both the C and MS subjects. Thus food deprivation unmasked a difference in body temperature regulation between this group and the others. The finding that the body temperature of the MS and C rats did not differ at any time during the experiment is in contrast to the consistently lower temperatures in one-year-old MS rats (of both sexes) reported by Sparber and Lichtblau (25). This difference could be due to our use of younger animals or dissimilarities in pretest experiences in the two studies. Similar to the findings of Sparber and Lichtblau (25) and Neal et al. (15), the females had higher basal body temperatures than males. However, there were no sex differences in the degree of hyper- and hypothermia induced by morphine exposure and naloxone-precipitated withdrawal. Neither were there differences in the thermic response to morphine and/or precipitated withdrawal associated with undernutrition.

These data show that neonatal undernutrition and altered early experience can influence later sensitivity to opiates similar to that observed in rats prenatally exposed to such compounds. Toxicity and/or withdrawal (distress) associated with neonatal abstinence could lead to impaired suckling, lower activity, and disrupted mother-infant interactions, thus producing indirect as well as direct effects of the drug. The data also demonstrate the importance of considering and including both sexes in such studies, bearing in mind the possibility that sex differences in responsiveness to drugs may be enhanced or masked (12,19) by one or another manipulation during development. Since severe undernutrition and/or neonatal withdrawal are controllable in dependent neonates born to women in methadone maintenance programs, it is premature to conclude from animal research, in which such variables are not usually controlled, that exposure to therapeutically (maintenance) relevant protocols are deleterious to the developing human.

ACKNOWLEDGEMENT

Supported in part by USPHS grants DA01880, HD20111 and T32DA07097.

REFERENCES

1. Babbini, M.; Gaiardi, M.; Bartoletti, M. Changes in operant behavior as an index of a withdrawal state from morphine in rats. Psychon. Sci. 29:142–144; 1972.
2. Bickel, W. K.; Stitzer, M. L.; Liebson, I. A.; Bigelow, G. E. Acute physical dependence in man: Effects of naloxone after brief morphine exposure. J. Pharmacol. Exp. Ther. 244:126–132; 1988.
3. Coyle, J. T.; Pert, C. B. Ontogenic development of opiate receptors in rat brain. Neuropharmacology 15:555–560; 1976.
4. Crnic, L. S. Models of infantile malnutrition in rats: Effects on maternal behavior. Dev. Psychobiol. 13:615–628; 1980.
5. Dwoskin, L. P.; Neal, B. S.; Sparber, S. B. Yohimbine exacerbates and clonidine attenuates acute morphine withdrawal in rats. Eur. J. Pharmacol. 90:269–273; 1983.
6. Ferster, C. B.; Skinner, B. F. Schedules of reinforcement. New York: Appleton-Century-Crofts; 1957.
7. Fleischer, S. F.; Turkewitz, G. Behavioral effects of rotation between lactating and nonlactating females. Dev. Psychobiol. 12:245–254; 1979.
8. Gellert, V. F.; Sparber, S. B. A comparison of the effects of naloxone upon body weight loss and suppression of fixed ratio operant behavior in morphine dependent rats. J. Pharmacol. Exp. Ther. 201:44–54; 1977.
9. Grota, L. J.; Ader, R. Continuous recording of maternal behavior in *Rattus norvegicus*. Anim. Behav. 17:722–729; 1969.
10. Holtzman, S. G.; Villarreal, J. E. Operant behavior in the morphine-dependent Rhesus monkey. J. Pharmacol. Exp. Ther. 184: 528–541; 1973.
11. Johannesson, T.; Becker, B. The effects of maternally administered morphine on rat foetal development and resultant tolerance to the analgesic effect of morphine. Acta Pharmacol. Toxicol. 31:305–313; 1972.
12. Kellogg, C. K.; Primus, R. J.; Bitran, D. Sexually dimorphic influence of prenatal exposure to diazepam on behavioral responses to environmental challenge and on γ-aminobutyric acid (GABA)-stimulated chloride uptake in the brain. J. Pharmacol. Exp. Ther. 256: 259–265; 1991.
13. Kennedy, G. C. The development with age of hypothalamic restraint upon the appetite of the rat. J. Endocrinol. 16:9–17; 1957.
14. Kirby, M. L. Development of opiate receptor binding in rat spinal cord. Brain Res. 205:400–404; 1981.
15. Neal, B. S.; Messing, R. B.; Sparber, S. B. Long term effects of neonatal exposure to isobutylmethylxanthine: II Attenuation of acute morphine withdrawal in mature rats. Psychopharmacology (Berlin); in press.
16. Nielson, J. A.; Sparber, S. B. Indomethacin facilitates acute tolerance to and dependence upon morphine as measured by changes in fixed ratio behavior and rectal temperatures in rats. Pharmacol. Biochem. Behav. 22:921–931; 1985.
17. O'Callaghan, J.; Holtzman, S. Prenatal administration of morphine to the rat: Tolerance to the analgesic effect of morphine in the offspring. J. Pharmacol. Exp. Ther. 197:533–544; 1976.
18. O'Callaghan, J.; Holtzman, S. Prenatal administration of levorphanol or dextrorphan to the rat: Analgesic effects of morphine in the offspring. J. Pharmacol. Exp. Ther. 200:255–262; 1977.
19. Perry, M. L. S.; Carrasco, M. A.; Dias, R. D.; Izquierdo, I. β-Endorphin like immunoreactivity of brain, pituitary gland and plasma of rats submitted to postnatal protein malnutrition: Effect of behavioral training. Peptides 5:15–20; 1984.
20. Peters, M. The effect of maternally administered methadone on brain development in the offspring. J. Pharmacol. Exp. Ther. 203: 340–346; 1977.
21. Seitz, P. F. D. The effects of infantile experiences upon adult behavior in animal subjects: I. Effects of litter size during infancy upon adult behavior in the rat. Am. J. Psychiatry 110:916–927; 1954.
22. Slob, A.; Snow, C.; de Natris-Mathot, E. Absence of behavioral deficits following neonatal undernutrition in the rat. Dev. Psychobiol. 6:177–186; 1973.
23. Sparber, S. B. Preclinical perinatal and developmental effects of methadone: Behavioral and biochemical aspects. In: Cooper, J.; Altman, F.; Brown, B.; Czechowicz, D., eds. Research on the treatment of narcotic addiction. Rockville, MD: National Institute on Drug Abuse; 1983:318–346. (Treatment Research Monograph Series.)
24. Sparber, S. B.; Gellert, V. F.; Lichtblau, L.; Eisenberg, R. The use of operant behavior methods to study aggression and effects of acute and chronic morphine administration in rats. In: Adler, M. W.; Manara, L.; Samanin, R., eds. Factors affecting the action of narcotics. New York: Raven Press; 1978:63–91.
25. Sparber, S. B.; Lichtblau, L. Neonatal undernutrition alters responsiveness to morphine in mature rats: A possible source of epiphenomena in developmental drug studies. J. Pharmacol. Exp. Ther. 225:1–7; 1983.
26. Steele, W.; Johannesson, T. Effects of prenatally administered morphine on brain development and resultant tolerance to the analgesic effect of morphine in offspring of morphine treated rats. Acta Phar-

macol. Toxicol. 36:243–256; 1975.
27. Thompson, T.; Schuster, C. R. Morphine self administration, food reinforced and avoidance behaviors in rhesus monkeys. Psychopharmacologia 5:87–94; 1964.
28. Tonkiss, J.; Cohen, C. A.; Sparber, S. B. Different methods for producing neonatal undernutrition in rats cause different brain changes in the face of equivalent somatic growth parameters. Dev. Neurosci. 10:141–151; 1988.
29. Zagon, I.; McLaughlin, P. The effects of different schedules of methadone treatment on rat brain development. Exp. Neurol. 56: 538–552; 1977.
30. Zagon, I.; McLaughlin, P. Perinatal methadone exposure and brain development: A biochemical study. J. Neurochem. 31:49–54; 1978.