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BRIEF COMMUNICATION

Prenatal Morphine Administration Alters Behavioral Development in the Rat

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SOBRIAN, S. K. *Prenatal morphine administration alters behavioral development in the rat.* PHARMAC. BIOCHEM. BEHAV. 7(3) 285–288, 1977. – Female rats were administered increasing doses of morphine sulfate 5 days prior to mating and during gestation until 4–6 days before the birth of their litters. Prenatal morphine exposure altered the normal developmental pattern of spontaneous motor activity by inducing in the offspring a sustained period of hyperactivity during the 3rd and 4th postnatal weeks. This disruption in behavioral ontogeny did not coincide with changes in physical parameters. Decreased body weight and higher mortality were observed in morphine-treated offspring only during the first postnatal week. The appearance of behavioral disturbances in the absence of physical abnormalities stresses the need for follow-up studies of infants born to narcotic-dependent mothers after signs of physical withdrawal or retarded growth have disappeared.

Prenatal morphine Behavioral ontogeny Physical maturation Hyperactivity

ADMINISTRATION of morphine to pregnant mice and rats during critical periods of fetal development can induce behavioral changes in the offspring. Most of the research concerning the effects of prenatal morphine has focused on alterations in the behavior of the post weanling animal. When tested at 30 and 70 days of age, the offspring of females administered morphine on Days 5–18 of gestation show an increase in open field activity and rearing; spontaneous locomotor activity, however, is significantly increased over control levels only in 70-day-old offspring [4]. An attenuated responsiveness and long-term tolerance to the analgesic effect of a single dose of morphine have been reported in the 5–10 week old offspring of rodents given morphine pregestationally [7,8] and prenatally [5, 12, 14, 21].

Developmental observations have been limited to changes in infant mortality and growth. Increases in neonatal mortality and decreases in the postnatal growth rate occur in the offspring of female rats given morphine pregestationally or during the prenatal period [4, 6, 9]. Treatment of male rats with morphine prior to mating produces similar results in the offspring [17].

The consequences of prenatal morphine treatment on the behavior of the neonate have been ignored. The primary objective of the present experiment was to systematically study an aspect of behavioral development in rats prenatally exposed to morphine. In an effort to parallel the pattern of drug use most commonly seen in clinical studies [20], morphine was administered to female rats prior to conception and during gestation. The development of spontaneous motor activity was the major dependent variable, because of reports of hyperactivity in human infants born to narcotic addicts [20].

METHOD

Twenty-six nulliparous Sprague-Dawley adult female rats (Bio-Breeding Inc., Ottawa, Canada) were housed individually with ad lib water and Purina rat chow. They were maintained on a 12 hr reversed light-dark cycle (lights off at 0800) throughout the experiment. Nine randomly selected females were given an initial 5 day exposure to increasing doses of morphine sulfate administered subcutaneously (Day 1, 10 mg/kg; Day 2, 3, and 4, 20 mg/kg; Day 5, 30 mg/kg), followed by a 13-day drug free period. Six days into this drug-free period, females were housed with drug free males (in the ratio of 3:2) for seven days, and then removed to individual plastic maternity cages. Morphine administration was resumed at this time and continued for 14 consecutive days. The 10 mg/kg starting dose was increased every second day by 5 mg/kg to a maximum of 40 mg/kg. With a seven day mating period, births were expected 2–7 days after treatment termination. This schedule of discontinuous pregestational and gestational morphine treatment was adopted because the continuous administration of morphine to females prior to and during mating and throughout gestation resulted in 94% mortality rate in the offspring within 48 hr after birth (Sobrian, unpublished observations).

Dosages of morphine were calculated on the quantity of free base represented by the sulfate sale administered. Fifteen control females received 0.9% sodium chloride on

an injection schedule identical to that used to administer morphine. All injections were given between 1200 and 1300 hr.

Upon termination of morphine treatment, maternity cages were inspected daily at 1000, 1500 and 2000 hr for live births. Day 1 was defined as the day of birth and all subsequent ages were calculated on this basis. Pups tested on Day 1 were a maximum of 14 hr old. On Day 1, original litter size and the condition of the pups were recorded; those found dead at this time were recorded as stillborn. Litters were then culled to 10. Pups were weighed every 5 days and litters were inspected for deaths daily. At 30 days of age litters were weaned.

In order to insure that observed behavioral changes resulted from prenatal exposure to morphine and not morphine-induced changes in postnatal maternal behavior, the offspring of morphine treated females were cross-fostered to saline-injected controls on Day 1. The litters of saline controls were fostered to mothers within this treatment group, thus holding the postnatal manipulations involved in introducing pups to a non-biological mother constant for all litters. Pups tested for Day 1 activity were allowed to nurse the foster mother at least once before testing.

The spontaneous motor activity of 5 male and 5 female pups from both of the treatment groups was measured on postnatal Days 1, 5, 10, 15, 20, 25 and 30. All animals were tested at each age and each testing group consisted of offspring from 5 litters. The apparatus and procedure used for activity testing have been described in detail elsewhere [18]. Briefly, activity was recorded for 30 min with a field capacitance movement sensing device. Pups were individually placed in a cardboard box containing wood shavings which rested on the sensing coil. Activity counts were automatically recorded by a digital logic array. Pups were tested during the 12 hr dark cycle.

RESULTS

All births occurred 4–6 days after drug administration had been discontinued. Six of the nine (66.7%) morphine-treated females and 14 of the 15 (93.3%) saline-treated females gave birth to viable litters.

The perinatal effects of prenatal morphine administration are listed in Table 1. No differences in litter size or male/female ratio of pups were found between morphine and saline groups. However, significantly greater neonatal mortality occurred among morphine offspring at birth and during the first postnatal week ($x^2 = 9.71$, $p<0.01$). In subsequent weeks until weaning, no further deaths were recorded in either group.

The body weights of prenatally treated male and female morphine pups were significantly lower than like-sex controls at birth and remained so during the first postnatal week, $F(1,18) = 23.66$, $p<0.01$. Weight differences were not evident in pups 10–40 days of age (Table 2).

The developmental changes in spontaneous motor activity are illustrated in Fig. 1. Analysis of variance and simple main effects indicated that, in agreement with previous reports [2, 15, 18, 19], activity in the control offspring increased sharply during the first two weeks of life, peaked at 15 days of age and then declined to adult levels between 25–30 days of age. Rat pups prenatally exposed to morphine sulfate showed normal motor activity between birth and 10 days of age. After this time, however, they became significantly more active than controls and their activity remained elevated until 30 days of age (Treatment × Days interaction: $F(6,108) = 3.703$, $p<0.05$).

DISCUSSION

Prenatal exposure to morphine altered both the physical and behavioral development of the offspring. However, these effects were manifested at different ages. Lowered body weights and higher infant mortality were observed in morphine-treated pups only during the first postnatal week. Changes in the development of spontaneous motor activity were not evident until after 10 days of age. Morphine-treated pups failed to show the normal decrease in activity at 20 days of age and exhibited a sustained period of

TABLE 1

PERINATAL EFFECTS OF PRENATAL MORPHINE ADMINISTRATION TO THE RAT

	Morphine	Saline
Percent females delivering offspring	66.67†	93.33
Mean number of offspring per litter	11.33 ± 1.6	12.14 ± 0.8
Total number of live births	66	169
Male/Female Ratio	1.06/1	1.24/1
Mean birthweight in grams		
Males	5.2 ± 0.32*	6.2 ± 31
Females	5.1 ± 0.28*	6.1 ± 0.35
Percent infant mortality		
Stillborn	1.5*	0.5
Day 2–7	13.64*	2.9
Day 8–28	0.0	0.0

†Significantly different from saline-treated females, $p<0.05$.
*Significantly different from offspring of saline-treated females, $p<0.05$.

TABLE 2

NEONATAL BODY WEIGHT (IN GRAMS) OF OFFSPRING FROM MORPHINE AND SALINE TREATED FEMALES. VALUES LISTED ARE COMBINED MEANS ± SEM FOR MALE AND FEMALE PUPS

Age (Days)	Morphine	Saline
1	5.1 ± 0.20*	6.1 ± 0.22
5	9.1 ± 0.18*	11.2 ± 0.41
10	18.1 ± 0.48	19.0 ± 0.35
15	26.7 ± 1.5	29.3 ± 0.78
20	36.6 ± 0.89	39.3 ± 1.3
25	56.5 ± 1.6	57.8 ± 1.9
30	80.7 ± 1.1	79.4 ± 61
40	123.6 ± 6.6	127.3 ± 5.1

*Significantly different from offspring of saline-treated females, $p<0.05$.

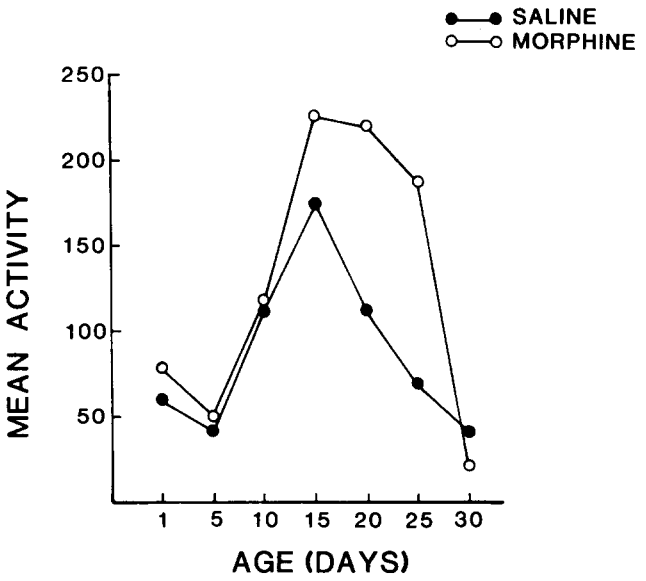

FIG. 1. The development of spontaneous motor activity in the offspring of females treated with morphine (○) or saline (●) during gestation. Each point represents the mean activity levels of 10 pups recorded during a 30 min test session. Activity levels of saline-treated offspring increased steadily from birth, peaked at 15 days of age and then decline. Pups exposed prenatally to morphine showed a similar development of locomotor activity until 10 days of age; between 15 and 25 days of age they exhibited a sustained period of hyperactivity. At 30 days of age, activity levels in saline and morphine offspring were indistinguishable.

hyperactivity during the third and fourth postnatal weeks. In agreement with a previous report [4], activity levels of saline and morphine pups did not differ during the fifth postnatal week.

Following prenatal morphine exposure, increased levels of motor activity were first evident during the third postnatal week. This finding parallels clinical reports of the emergence of hyperkinesis between 1 and 2 years of age in human infants born to narcotic dependent mothers [20]. The delayed appearance suggests that behavioral alterations are probably not the direct consequence of drug withdrawal but may reflect temporary or chronic disruptions in the developing nervous system [20].

The sustained hyperactivity seen in the offspring of morphine-treated females suggests altered CNS maturation. The normal ontogenetic changes in activity have been correlated with the maturation of adrenergic hindbrain and cholinergic forebrain structures [2,3]. Moreover, depletion of brain catecholamines produces hyperactivity in rat pups 2–4 weeks old [16].

It is difficult to postulate a mechanism whereby prenatal morphine exerts an effect on neonatal behavioral development. Although teratogenic effects of prenatal morphine have been reported in rodents [1, 10, 12], the behavioral changes reported here occurred in absence of gross malformations. The effect is apparently of prenatal origin as morphine-treated offspring were cross-fostered at birth to non-drugged mothers and not subsequently exposed to the drug as neonates. Reduced maternal food intake and intrauterine nutritional factors have been implicated in weight differences [11] and may be responsible for the subsequent behavioral alterations. However, prenatal morphine exposure can also directly effect fetal growth by reducing the number of cells in several organs [13].

Both physical and behavioral development is altered in rat pups prenatally exposed to morphine. Behavioral change is not immediately discernible and occurs in the absence of retarded physical development. These data indicate the need of follow-up studies of infants born to narcotic dependent-mothers after physical symptoms of withdrawal or retarded growth have disappeared.

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REFERENCES

1. Arcuri, P. A. and R. F. Gautieri. Morphine-induced fetal malformations III: Possible mechanisms of action. *J. Pharmac. Sci.* **62:** 1626–1634, 1973.
2. Campbell, B. A., L. D. Lytle and H. C. Fibiger. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* **166:** 637–638, 1969.
3. Campbell, B. A. and P. D. Mabry. The role of catecholamines in behavioral arousal during ontogenesis. *J. comp. physiol. Psychol.* **81:** 371–379, 1973.
4. Davis, W. M. and C. H. Lin. Prenatal morphine effects on survival and behavior of rat offspring. *Res. communs. chem. pathol. Pharmac.* **3:** 205–214, 1972.
5. Friedler, G. The role of immune factors in the development of tolerance to morphine. Unpublished Ph.D. thesis, Boston University Graduate School, 1968.
6. Friedler, G. Long-term effects on growth of mice offspring following morphine treatment of mothers. *Pharmacologist* **13:** 262, 1971.

7. Friedler, G. Long-term effects of opiates. In: *Perinatal Pharmacology: Problem and Priorities,* edited by J. C. Huang. New York: Raven Press, 1974, pp. 207–219.
8. Friedler, G. Effect of pregestational morphine administration to mice on behavior of their offspring. *Pharmacologist* **16:** 203, 1974.
9. Friedler, G. and J. Cochin. Growth retardation in offspring of female rats treated with morphine prior to conception. *Science* **175:** 645–656, 1972.
10. Harpel, H. S. and R. F. Gautieri. Morphine-induced fetal malformations. I. Exencephaly and axial skeletal fusion. *J. Pharmac. Sci.* **57:** 1590–1597, 1968.
11. Hutchings, D., 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.
12. Johannesson, T. and B. A. Becker. The effect of maternally administered morphine on rat fetal development and resultant tolerance to the analgesic effect of morphine. *Acta pharmac. tox.* **31:** 305–313, 1972.
13. Naeye, R. L., W. Blanc, W. Leblanc and M. A. Khatamee. Fetal complications of maternal heroine addiction: Abnormal growth, infection, and episodes of stress. *J. Pediat.* **83:** 1055–1061, 1973.
14. O'Callaghan, J. P. and S. G. Holtzman. Prenatal administration of morphine to the rat: Tolerance to the analgesic effect of morphine in the offspring. *J. Pharmac. exp. Ther.* **197:** 533–544, 1976.
15. Pappas, B. A., D. A. V. Peters, S. K. Sobrian, A. Blouin and B. Drew. Early behavioral and catecholaminergic effects of 6-hydroxydopamine and guanethedine in the neonatal rat. *Pharmac. Biochem. Behav.* **3:** 681–685, 1975.
16. Shaywitz, B. A., R. D. Yager and J. H. Klopper. Selective brain dopamine depletion in developing rats: An experimental model of minimal brain dysfunction. *Science* **191:** 305–308, 1976.
17. Smith, D. J. and J. M. Joffe. Increased neonatal mortality in offspring of male rats treated with methadone or morphine before mating. *Nature* **253:** 202–203, 1975.
18. Sobrian, S. K. Aversive prenatal stimulation: Effects on behavioral, biochemical, and somatic ontogeny in the rat. *Devl Psychobiol.* **10:** 41–51, 1977.
19. Sobrian, S. K., M. Weltman and B. A. Pappas. Neonatal locomotor and long-term behavioral effects of d-amphetamine in the rat. *Devl Psychobiol.* **8:** 241–250, 1975.
20. Wilson, G. S., M. M. Desmond and W. M. Verniaud. Early development of infants of heroin-addicted mothers. *Am. J. dis. Child.* **126:** 457–462, 1973.
21. Zimmerberg, B., A. D. Charap and S. D. Glick. Behavioral effects of in utero administration of morphine. *Nature* **247:** 376–377, 1974.