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Perinatal Exposure to Morphine Affects Adult Sexual Behavior of the Male Golden Hamster

HEATHER M. JOHNSTON,*[1]
ANTHONY P. PAYNE* AND DESMOND P. GILMORE†

**Department of Anatomy and †Institute of Physiology, University of Glasgow, Glasgow, Scotland G12 8QQ, UK*

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JOHNSTON, H. M., A. P. PAYNE AND D. P. GILMORE. *Perinatal exposure to morphine affects adult sexual behavior of the male golden hamster.* PHARMACOL BIOCHEM BEHAV **42**(1) 41–44, 1992.—Duromorph, a long-acting form of morphine, was administered to pregnant golden hamsters and/or their pups over the last 4 days of pregnancy and/or the first 4 days after birth. As adults, offspring were gonadectomized, primed with estrogen and progesterone, and tested for their ability to display feminine sexual behavior when placed with a stud male. They were then given testosterone over a 4-week period and tested for their ability to display masculine sexual behavior in the presence of a receptive female. Perinatal morphine exposure had little effect on the females' ability to display either feminine or masculine sexual behavior. In contrast, feminine sexual behavior was significantly enhanced in males exposed to morphine over the perinatal period. This suggests that exposure to opiates during the critical period of sexual differentiation may prevent the defeminization process in this species.

Opiates Sexual behavior Sexual differentiation

PATTERNS of adult sexual behavior in the rodent are laid down during the perinatal period. For masculinization of behavior, testicular androgens must be present during a short critical period in early postnatal life. Thus, phenotypic males deprived of androgens during this period do not respond to androgens as adults by displaying normal masculine copulatory behavior when exposed to a receptive female. However, these males will, when primed with estrogen and progesterone, show increased amounts of feminine receptive behavior (lordosis) when placed with a vigorous stud male (8,27).

Recent studies in adult male rats have shown that prenatal exposure to opiates reduces masculine copulatory behavior and increases feminine sexual behavior (26). Prenatal stress has similar effects (25). Stress is reported to trigger a release of endogenous opioids in adults (2,10,19,29), and it is possible, therefore, that fetuses may be exposed to high levels of these if mothers are stressed during pregnancy. This would suggest that the endogenous opioids might play a role in the development and establishment of adult sexual behavior patterns.

In the present investigation, the effect of morphine exposure during the perinatal period on adult sexual behavior in another laboratory species, the golden hamster, was examined.

METHOD

Subjects

Eighty female and 88 male golden hamsters were used in this study. All were derived from a closed colony established in the Glasgow University Anatomy Department in 1968. Animals were kept in an air-conditioned, light-reversal room with 9 h dull red light and 15 h bright light.

Treatments

To reduce handling of animals to a minimum, a long-acting form of morphine, Duromorph (5), was used in this investigation that allowed injections to be reduced to two per day (see Appendix). Duromorph is an aqueous suspension of morphine of which 95% is in microcrystalline form. The drug was kindly donated by Laboratories for Applied Biology Ltd., London.

Mothers of treated and control hamsters were placed with vigorous stud males for 2–3 h on the afternoon of estrus. The day of mating was taken as day 0. Gestation in hamsters lasts 16 days. After mating, females were housed individually in PVC cages measuring 26 × 24 × 10 cm and allowed access to food and water ad lib. The following treatments were administered:

[1] Requests for reprints should be addressed to H. M. Johnston, Department of Anatomy, University of Glasgow, University Avenue, Glasgow, Scotland G12 8QQ, UK.

1. *Prenatal morphine* (n = 19 females, 18 males): Duromorph (10 mg/kg) was administered IP twice daily to pregnant females from day 13 of pregnancy until parturition.
2. *Pre- and postnatal morphine* (n = 18 females, 26 males): Duromorph (10 mg/kg) was administered IP twice daily to pregnant females from day 13 of pregnancy until parturition. Pups were similarly treated from day of birth for 4 days.
3. *Postnatal morphine* (n = 28 females, 25 males): Duromorph (10 mg/kg) was administered IP to pups twice daily from day of birth for 4 days.
4. *Untreated controls* (n = 15 females, 19 males): Pups were housed and treated from birth in a manner similar to experimental animals.

Pups remained with their natural mothers until weaned at day 21. They were then housed with litter mates of the same sex (three to four per cage) under reversed lighting conditions. At 3 months of age, they were gonadectomized under sodium pentabarbitone (Sagatal) anesthesia. Testes and epididymides were removed from males and ovaries from females. After a recovery period of 3 weeks, each animal received a subcutaneous injection of estradiol benzoate (EB) (5 μg) dissolved in peanut oil, followed 24 h later by 500 μg progesterone (SC, in oil). Four to 6 h after the second injection, each animal was tested for its ability to display feminine sexual behavior when placed with a vigorous stud male.

Sexual behavior of a receptive female hamster consists of a depression of the back with the head and tail raised (lordosis). A normal female will remain in this position between successive mounts and dismounts by the males and may remain so throughout the test period. Each test period lasted for 10 min.

Tests were carried out in a Plexiglas cage measuring 38 × 24 × 16 cm in a room with normal lighting. Previous studies have shown that, although the test is carried out during the dark period, animals are undisturbed by the bright conditions. Each test session was recorded in video using an overhead camera and the information stored for later analysis.

The first test was used to acclimatize animals to the test conditions and the data obtained were not used in subsequent analysis. A second test was carried out 1 week later following a second priming with EB and progesterone. The data collected during this test are reported below. Those aspects of behavior used in analysis were:

1. latency to lordosis (in seconds)
2. total time spent in lordosis during the test session (in seconds)
3. longest single episode of lordosis (in seconds).

Statistics

Data were analyzed by one-way analysis of variance (ANOVA) (F) followed by intergroup comparisons using least significant difference computations. Tests were carried out using a Statgraphics program (Statistical Graphics Corp.).

RESULTS

Sexual Behavior

Females. All control females (Table 1) showed lordosis, adopting the lordotic stance within 60 s of being placed with a stud male and remaining in that position for most of the test period. Exposure to morphine had little effect on the females' ability to display typical receptive behavior. However, exposure to morphine prenatally significantly increased the latency to lordosis; while it reduced both the total and maximum length of lordosis, these reductions did not reach significance.

Males. In comparison to females, normal male hamsters (Table 2) show little feminine sexual behavior; only 53% of controls displayed any lordosis and even then duration was relatively short. However, exposure to morphine significantly increased the amount of feminine behavior displayed by males. Of those treated postnatally, 88% showed feminine copulatory behavior and 92% of those treated both pre- and postnatally did so. Pre- and postnatal morphine exposure significantly decreased latency and increased the total duration of lordosis displayed by males. Postnatal treatment alone also decreased lordosis latency but did not increase the total amount of lordosis, while prenatal morphine exposure alone was ineffective.

DISCUSSION

In the present investigation, it has been shown that perinatal exposure to opiates has little effect on adult sexual behavior of female golden hamsters. Females exposed prenatally to morphine showed a significant increase in lordosis latency (possibly suggesting that opiate exposure has interfered with mechanisms involved in sexual response), but this was small.

TABLE 1

COMPONENTS OF FEMININE SEXUAL BEHAVIOUR AS SHOWN BY ADULT FEMALE HAMSTERS TREATED PERINATALLY WITH MORPHINE

Treatment	Prenatal Morphine (n = 19)	Pre- and Postnatal Morphine (n = 18)	Postnatal Morphine (n = 28)	Control (n = 15)	F (3, 76)
Lordosis latency	145 ± 48*	13 ± 3	73 ± 26	25 ± 4	3.79 ($p < 0.01$)
Total lordosis	365 ± 46	498 ± 15	445 ± 35	431 ± 44	1.62 (NS)
Maximum lordosis	264 ± 44	256 ± 27	298 ± 38	322 ± 60	0.45 (NS)
% Showing behavior	89%	100%	96%	100%	

See text for details. All figures are mean times (seconds) ± SEM. NS, not significant.
*Differs from controls $p < 0.01$.

TABLE 2

COMPONENTS OF FEMININE SEXUAL BEHAVIOUR AS SHOWN BY ADULT MALE HAMSTERS TREATED PERINATALLY WITH MORPHINE

Treatment	Prenatal Morphine (n = 18)	Pre- and Postnatal Morphine (n = 26)	Postnatal Morphine (n = 25)	Control (n = 19)	F (3, 84)
Lordosis latency	360 ± 61	123 ± 33*	187 ± 43*	360 ± 60	6.45 ($p < 0.01$)
Total lordosis	76 ± 27	165 ± 25*	126 ± 22	76 ± 23	3.36 ($p < 0.02$)
Maximum lordosis	26 ± 9	47 ± 8	42 ± 7	28 ± 8	1.64 (NS)
% Showing lordosis	50%	92%	88%	53%	

See text for details. All figures are mean times (seconds) ± SEM. NS, not significant.
*Differs from controls $p < 0.01$.

Vathy et al. (24) reported a reduction in soliciting behavior and a decrease in lordosis responses in female rats exposed prenatally to morphine. The lack of change in feminine sexual behavior in females exposed both pre- and postnatally or postnatally only might suggest that, while mechanisms involved in sexual response may be established prenatally, short-term exposure to opiates during this period is more deleterious than long-term exposure.

Unlike females, male hamsters exposed to opiates during the perinatal period retained their capacity to display feminine sexual behavior. In contrast with females, prenatal opiate treatment was not effective, but postnatally and pre- and postnatally treated males showed significantly more lordosis than did controls; the latter treatment regimen was the more effective. However, levels of lordosis did not reach those seen in normal females. In addition, since feminine behavior in prenatally treated males did not differ from that of control males, defeminization must occur primarily in the early postnatal period. This would account for the more marked effects of pre- and postnatal treatment.

There are many possible explanations as to how opiates might affect the process of defeminization. Defeminization in rodents appears to be dependent on the presence of androgens during the perinatal period (7,16). Opiates are known to reduce androgen production in adults by suppressing gonadotrophin release (1,4,17) and the same phenomenon has also been reported in the neonatal rat (21). Therefore, in this experiment opiate treatment carried into the postnatal period may have caused a sufficient reduction in androgens during the critical period to impair the defeminization process in these males.

Alternatively, opiates have been shown to delay neural development by inhibiting neural outgrowth and synaptogenesis both in vitro and in vivo (6,11,18,20). Indeed, endogenous opiates are thought to play a regulatory role in neural development (12,13,30). Thus, exposure to exogenous opiates during a period when the CNS is undergoing considerable growth and development may prevent neurons involved in the defeminization process from being at the right stage of development to respond to androgens. Treatment with opiates during the perinatal period has also been shown to alter opiate receptor density and distribution (3,11,22,23); this in turn could affect development by permitting incorrect neuronal connections to be made and delaying neuronal outgrowth.

A third possibility for the observed changes may be an alteration in neurotransmitter levels. During early development of the CNS, neurotransmitters are thought to act as signals for growth and synaptogenesis (14,15,28). Opiates have been reported to alter transmitter concentrations (9). Therefore, alterations in levels or distributions of these may interfere with the mechanisms involved in the establishment of behavior patterns.

It may be that no individual mechanism is responsible for the observed changes, but rather that several mechanisms or an interdependent sequence is involved. Nevertheless, the present experiment suggests that exposure to exogenous opiates during the perinatal period has an inhibitory effect on the defeminization mechanism in the male golden hamster. How this effect is mediated remains unclear and is the subject of further investigation.

APPENDIX

The dosage of 10 mg/kg Duromorph was chosen for this investigation as this concentration of the drug was shown to give up to 12 h of analgesia in hot-plate tests. It was, however, necessary to determine that fetuses were being exposed to morphine. To this end, samples of plasma and brain from the mothers plus pooled samples of fetal brains were processed and analyzed for morphine content by high-performance liquid chromatography in the Department of Medicine and Therapeutics in the Western Infirmary, Glasgow. Animals were sacrificed within 2 h of injection on day 15 of pregnancy and the maternal and fetal concentrations of morphine were:

	Adult Plasma	Adult Brain	Fetal Brain
Morphine concentration (μg/ml)	0.18 ± 0.2	1.03 ± 3.8	3.7 ± 2.2
n	5	5	5

Although these results do not quantify the dosage that each individual pup receives during the prenatal period, they do show that morphine readily passes the placental barrier and accumulates in the fetal CNS.

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