Effect of exposure to morphine throughout gestation on feminine and masculine adult sexual behaviour in golden hamsters

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Adult female hamsters were treated with a long-acting form of morphine before mating and throughout pregnancy; the drug was gradually withdrawn during lactation. The offspring produced were gonadectomized as adults and tested for their ability to display feminine and masculine sexual behaviour after appropriate hormonal priming. Chronic exposure to morphine during development resulted in males that showed an increase in both feminine and masculine sexual behaviour when compared with controls.

Introduction

Exposure to opiates during prenatal or early postnatal development may result in changes in a variety of behaviours including passive avoidance learning (Rech *et al.*, 1980; Casilli and Freeman, 1983), social dominance (Thompson and Zagon, 1983) and open field locomotion (Grove *et al.*, 1979; Lall and Sahoo, 1990).

Other behavioural patterns that might be expected to show alterations following opiate administration include sexually dimorphic patterns (such as sexual behaviour itself) that become dimorphic as a result of the presence or absence of androgen during a short perinatal 'critical period' of central nervous system (CNS) development (Carter *et al.*, 1972; Hayisha and Gorski, 1974; Johnson, 1975; Gray *et al.*, 1976). Opiates might be expected to affect the differentiation process either by suppressing LH secretion (Purohit *et al.*, 1979; Akabori and Barraclough, 1986) or by altering the speed of CNS development (Kornblum *et al.*, 1987; Davila-Garcia and Azmitia, 1989). Indeed, Johnston *et al.* (1992) showed that exposure to opiates for 4 days before or after birth (or over both periods) significantly increased the capacity for feminine sexual behaviour (lordosis) in adult male golden hamsters, but had little effect on their masculine behaviour. This finding suggests that opiate treatment reduces the defeminization process in these males.

However, a difficulty of interpretation was proposed by Lichtblau and Sparber (1984), who suggested that changes in behaviour observed in animals exposed to opiates for a relatively short period during development might not be mediated by opiates themselves, but be caused by their withdrawal. This study was therefore undertaken to investigate whether any behavioural changes occurred in animals exposed to opiates throughout gestation and the early postnatal period and whether these changes were different from those recorded after short-term exposure.

Materials and Methods

Animals and treatments

Chronic morphine-treated animals. Adult female hamsters were housed singly and treated once a day with a long-acting form of morphine, Duromorph (10 mg kg⁻¹ i.p.), an aqueous suspension of morphine of which 95% is in microcrystalline form (The Laboratories for Applied Biology Ltd, London). Treatment was given for 4 days before mating and then throughout pregnancy. Morphine was withdrawn during lactation, the dose being reduced by 10% day⁻¹; using this method there were no obvious withdrawal symptoms. The mothers were killed after the pups had been weaned. The pups ($n = 10$ males, $n = 12$ females) were not treated separately with morphine as there is evidence that morphine passes to the pups through the milk (Ghodse, 1990).

Control animals. Animals whose mothers were untreated were used as controls ($n = 11$ males, $n = 20$ females).

Saline-treated animals. In this group, mothers were injected once a day with 0.1 ml physiological saline i.p. during pregnancy. Pups ($n = 19$ males; $n = 15$ females) were given 0.02 ml saline i.p. for 4 days after birth, since there is no evidence that saline given to mothers reaches the pups.

Hormone treatments and tests of feminine and masculine sexual behaviour. Pups were weaned at 21 days after birth and housed with littermates of the same sex, two to three per cage under reversed lighting conditions (9 h red light:15 h bright light). At 3 months of age, they were bilaterally gonadectomized under sodium pentobarbitone anaesthesia (Sagatal, 90 mg kg⁻¹; May and Baker, Dagenham); 2 weeks later, each animal was given a s.c. injection of oestradiol benzoate (5 µg in 0.1 ml oil of arachis; Sigma, Poole) followed 24 h later by a s.c. injection of progesterone (500 µg in 0.1 ml oil of arachis;

Table 1. Feminine sexual behaviour as displayed by adult hamsters treated with morphine, or saline, or untreated during gestation

Treatment	Number of animals	Lordosis latency	Total lordosis	Maximum lordosis	Percentage showing lordosis
Males					
Untreated	11	482 ± 63	50 ± 36	19 ± 11	27
Chronic morphine-treated	10	118 ± 55**	163 ± 46*	43 ± 14	90
Saline-treated	19	360 ± 60	76 ± 23	28 ± 8	53
F value		6.85	2.65	1.01	
P value		< 0.003	< 0.08	< 0.37	
Females					
Untreated	20	60 ± 18	417 ± 32	265 ± 39	100
Chronic morphine-treated	12	76 ± 48	411 ± 60	285 ± 58	92
Saline-treated	15	25 ± 4	431 ± 44	322 ± 60	100
F value		1.07	0.10	0.39	
P value		< 0.35	< 0.91	< 0.68	

Values are group means ± SEM.

*Significantly different from untreated animals ($P < 0.05$).

**Significantly different from untreated animals ($P < 0.01$).

Sigma). Four to six hours after progesterone administration, each animal was tested for its ability to display feminine sexual behaviour when placed with a vigorous stud male. This first test was used to acclimatize the animals to the test conditions and the procedure was repeated 1 week later. The test lasted for 10 min. Only the data collected during the second test were analysed. The following aspects of behaviour were used in the analysis: (1) the time elapsing between the introduction of the male and the test animal assuming the lordotic position (latency); (2) the total time spent in lordosis during the test session; (3) the longest single episode of lordosis.

After the second test for feminine behaviour, each animal was given a s.c. injection of testosterone propionate (1.5 mg three times a week; Sigma) for 4 weeks and then tested on three consecutive days for the ability to display masculine sexual behaviour towards a receptive female. The test period was again 10 min on each day. The specific aspects of behaviour used for analysis were: (1) the time between introduction of the treated animal to the test cage and it mounting the receptive female (average over three tests); (2) the total number of mounts (regardless of orientation) from the three test periods; (3) the total number of intromissions from the three tests; and (4) the total number of genital self-grooms from the three tests.

Statistical analysis

Data were analysed by one-way analysis of variance (F). After this, intergroup differences were obtained using least significant differences. Tests were carried out using a STATGRAPHICS program (Statistical Graphics Corporation).

Results

Feminine sexual behaviour

Normal female hamsters responded to the introduction of a vigorous male by adopting a lordotic posture within 60 s and

remained in that position during most of the test period (417 s out of a possible 600 s), and the longest single episode of lordosis lasted an average of 265 s. Neither the saline-treated nor the chronic morphine-treated females differed significantly from this (Table 1).

When given oestrogen and progesterone, normal control males did show limited feminine behaviour, but they were much less responsive than were females. Control males took 482 s to adopt the lordotic posture and spent only 50 s of a possible 600 s in lordosis; the longest single episode lasted 19 s. By contrast, males exposed chronically to morphine throughout gestation showed enhanced feminine sexual behaviour. They adopted the lordotic posture within 118 s of the start of the test and spent a total of 163 s in lordosis. Although all male groups showed significantly less lordosis than any of the female groups, the time taken by the morphine-treated group to adopt a lordotic stance, and the total time spent in lordosis, was significantly different from that of the controls (Table 1). Overall, only 27% of control males showed any feminine behaviour, in comparison with 100% of control female and 90% of morphine-treated males.

Masculine sexual behaviour

None of the female groups displayed significant amounts of masculine sexual behaviour (Table 2). Males, however, showed variable levels of mounting with the males treated with morphine showing the greatest amount of masculine sexual behaviour. This group displayed significantly more mounts, genital groomings and intromissions than did the other two male groups.

Discussion

In the study reported here, no changes in either feminine or masculine sexual behaviour were found in female hamsters exposed to morphine throughout gestation; this result supports some previous findings in rats (Ward *et al.*, 1983), although

Table 2. Masculine sexual behaviour as displayed by adult hamsters treated with morphine, or saline, or untreated during gestation

Treatment	Number of animals	Mount latency	Total mounts	Total intromission	Total grooms	Percentage showing mounting
Males						
Untreated	11	428 ± 38	20 ± 6	8 ± 4	17 ± 5	82
Chronic morphine-treated	10	266 ± 45*	45 ± 10**	27 ± 7**	44 ± 10**	100
Saline-treated	16	371 ± 27	14 ± 2	2 ± 1	9 ± 2	100
F value		4.42	8.04	10.3	10.13	
P value		< 0.02	< 0.001	< 0.001	< 0.001	
Females						
Untreated	12	564 ± 24	0.25	0	1	17
Chronic morphine-treated	13	572 ± 12	1	0	1	38
Saline-treated	15	520 ± 29	3 ± 1	0	2	40
F value		1.48	2.58	0.43	0.53	
P value		< 0.24	< 0.89	< 0.65	< 0.59	

Values are group means ± SEM.

*Significantly different from untreated animals ($P < 0.05$).

**Significantly different from untreated animals ($P < 0.01$).

Vathy *et al.* (1983) did note a reduction in feminine sexual behaviour in female rats exposed to morphine on days 5–12 of gestation. By contrast, exposing male hamsters to morphine throughout gestation resulted in a very significant increase in the number of animals showing feminine sexual behaviour, together with an increase in the amount of feminine behaviour shown. Thus there was a considerable reduction in lordosis latency and an increase in total lordosis, a similar trend to that reported in male rats after prenatal morphine treatment (Ward *et al.*, 1983). These findings suggest that the process of defeminization may have been compromised as a result of morphine treatment. In a study on hamsters (Johnston *et al.*, 1992), males treated with morphine for 4 days after birth or over 8 days, covering both pre- and postnatal exposure, showed an increase in feminine behaviour. As an increase in feminine sexual behaviour also occurs in males whose androgen concentrations are suppressed (Whalen and Edwards, 1967), it is possible that circulating androgen concentrations in the males in the experiment reported here may have been reduced as a result of opiate exposure. However, it must be remembered that the masculine sexual behaviour in these males was significantly higher than that observed in either of the two control groups. Since the capacity for masculine sexual behaviour also depends on androgen concentrations being relatively high during the critical period for masculinization, this could rule out the possibility that the changes in feminine behaviour are caused by an opiate-induced suppression of androgens and alternative mechanisms must be considered.

Opioid receptors are first detectable within the rat CNS on embryonic day 14 (Kent *et al.*, 1982). Their pattern of distribution changes during fetal development (Petrillo *et al.*, 1987) and opiate exposure during this period has been shown to alter the rate and distribution of receptor ontogeny (Tempel *et al.*, 1988). During development, many neurotransmitters (including the endogenous opioids) are considered to be involved in the control and direction of neurogenesis and synaptogenesis (Hauser *et al.*, 1989). In particular, exposure to exogenous opiates has been shown to delay and inhibit neuronal growth

and synaptogenesis both *in vivo* and *in vitro* (Seidler *et al.*, 1982; Hammer *et al.*, 1989). Changes in receptor distribution and ontogeny brought about by morphine administration might therefore interfere with these processes. In the experiment reported here, pups were exposed to morphine throughout development, as opiates are known to cross the placental barrier and accumulate in the fetal plasma and brain (Johnston *et al.*, 1992). By contrast, exposure of pups to the drug after birth could only be through the milk, and drug administration was phased out completely over the first few days of lactation. Defeminization occurs postnatally in hamsters (De Bold and Whalen, 1975). Thus, if neural development was delayed as a result of opiate exposure, the systems involved in defeminization might reach maturity too late in the critical period for the process to be completed. This would result in males that showed higher than normal levels of lordotic behaviour. It is not clear at present why the same males show higher than normal levels of masculine sexual behaviour.

In conclusion, we have shown that chronic exposure to morphine has the same effect on the feminine sexual behaviour of male hamsters as does acute exposure and we therefore suggest that these alterations in behaviour are not due to withdrawal of the drug. In addition it would appear that, in this species at least, the increase in feminine sexual behaviour is not due to any opiate-induced reduction in androgens since masculine behaviour was not reduced.

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