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Prenatal Exposure to Morphine Affects Juvenile Play Behavior and Adult Social Behavior in Rats

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HOL, T., R. J. M. NIESINK, J. M. VAN REE AND B. M. SPRUIJT. *Prenatal exposure to morphine affects juvenile play behavior and adult social behavior in rats.* PHARMACOL BIOCHEM BEHAV **55**(4) 615–618, 1996.—The effects of morphine exposure *in utero* on play behavior and social behavior were investigated in a longitudinal study. Wistar rat dams were SC injected daily with saline (control) or 10 mg/kg morphine from day 8 to day 21 of gestation. Play behavior of the offspring was measured at 3 and 4 weeks of age and social behavior at 3 months of age. Pinning, a measure for play behavior and social grooming of the morphine-treated offspring were significantly elevated compared to saline controls, especially on day 21. The onset-latency of pinning behavior was not changed. Furthermore, prenatal morphine treatment resulted in more social approach and less social avoidance behavior in adulthood, whereas changes in general locomotor activity were not observed. The results are discussed in relation to the effects of in utero exposure of morphine on the development of incentive aspects of play and social behavior. **Copyright © 1996 Elsevier Science Inc.**

Prenatal morphine Play behavior Social behavior

DURING development endogenous opioids and their receptors are in balance: it has been suggested that the development of this balance is particulary sensitive to prenatal administration of opioid agonists and antagonists (10,27). Physiological and behavioral effects of in utero treatment with opiates have been the subject of a number of studies. Changes in pain sensitivity (7), sexual behavior (31), responsiveness to stress and stimulants (2,32) and play behavior (13,14) have been reported. The motivational and rewarding aspects of play behavior and adult social behavior are thought to be regulated by endogenous opioids systems in a comparable but dose-dependent fashion: low doses of opioids stimulated both behaviors, whereas administration of high doses inhibited play and social behavior (12,17,18,30). Scott (1962) already suggested that social behavior of young animals, such as play, is of functional significance for the development of adult social behavior (23). Since then, several studies have analysed the impact of social interactions during the juvenile period on the development of appropriate social behavior in later life (3,4,21).

The present study examines the effects of morphine given during the prenatal development of the endogenous opioid systems on the maturation of social behavior: play behavior in juvenile rats and social behavior of the same animals were assessed in adulthood.

METHOD

Subjects

Twenty pregnant Wistar rats from the laboratory stock (RMI, Utrecht, The Netherlands) were housed individually in standard macrolon cages and under controlled temperature (25 ± 1°C) and light conditions (lights on 0500, lights off 1900). Daily injections with morphine (Morphine HCl, O.P.G., Utrecht, The Netherlands) 10 mg/kg (s.c.) or saline (control group) started on day 8 of gestation and were continued until parturition. Directly after birth, male pups were placed with foster mothers (8 pups per foster mother) to avoid possible behavioral disturbances caused by the behavior of the treated mother (9). At day 21 the pups were weaned; this was also the day when their play behavior was scored for the first time.

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TABLE 1

PRENATAL MORPHINE TREATMENT INCREASES PLAY BEHAVIOR, EXPRESSED AS PINNING, AND SOCIAL GROOMING IN THE JUVENILE MALE RAT

	Pinning			Social Grooming	
	Frequency	Duration	Latency	Frequency	Duration
Day 21					
Saline	9.5 ± 2.1	35.0 ± 10.8	117.4	73.5 ± 4.0	134.0 ± 10.3
Morphine	24.0 ± 4.3†	70.5 ± 11.9*	115.3	88.2 ± 4.7*	186.3 ± 17.6*
Day 28					
Saline	9.6 ± 2.1	34.8 ± 10.3	191.6	60.6 ± 7.7	92.8 ± 10.8
Morphine	21.7 ± 3.9*	62.2 ± 11.0	171.4	64.5 ± 5.1	93.3 ± 9.6

Animals were observed for 15 min. Data are expressed as means ± SEM. Student's *t*-test: *$p < 0.05$, †$p < 0.01$.

For the present study, two pups per litter were randomly chosen resulting in twenty animals per treatment group; the other pups were used for other experiments. From day 21 till the end of the experiments rats from both, morhine- and saline-treated mothers were housed together in groups of four animals per cage. Commercial rat chow and water were available ad libitum.

Behavioral Testing

Play behavior. On postnatal day 21 play behavior of the male rats—ten pairs consisting of morphine-treated versus morphine-treated ($n = 2 \times 10$) and ten pairs of saline-treated versus saline-treated ($n = 2 \times 10$)—have been observed and analyzed. All tests were performed in a double-blind design under low light conditions. As play behavior is the result of the behavior of two animals the number of subjects is statistically the number of pairs (i.e., ten), although, per group, twenty animals are involved. The testing procedure has been described earlier by Niesink and Van Ree (12). To enhance play behavior, rats were kept singly 3 hours before the observation. The test arena was a transparant acrylic cage (35 × 35 × 50 cm with approximately 2 cm of wood shavings on the floor), located in a sound-proof chamber. Two rats, which had not been housed together were placed in the test arena for 15 min. The body weights of the two animals did not differ more than five grams. Following behaviors were registered through an on-line video display in an adjacent chamber: pinning (i.e., one of the animals is lying with its dorsal surface on the floor of the test cage with the other animal standing over it: frequency, duration, onset-latency) and social grooming (frequency, duration). On day 28 this procedure was repeated with the same animals, but with a different partner than on day 21.

Social behavior. At the age of 3 months a randomly selected part of the offspring (ten out of twenty animals per treatment group) was tested in the social interaction test. This test assesses the relative differences between the two interacting animals by measuring approach and avoidance of each individually recognized animal. This can only be measured by using two different animals, since similar animals will either approach each other and then have contact or avoid each other and have no contact. Thus, the situation that one animal is following (approach) or avoiding the other can be demonstrated best by an interaction consisting of differently behaving animals. Therefore, the partners of morphine-treated animals ($n = 10$) were saline-treated animal ($n = 10$). The body weights of the pairs did not differ more than 15 grams. The two rats were placed into a large open field (125 cm dia.) under low light conditions in a sound- proof room and observed for 15 min by a fully automated observation system (EthoVision, Noldus Information Technology b.v., Wageningen, The Netherlands) described elsewhere (26). Briefly, general locomotor activity (distance walked in cm) and approach/avoidance (movements of animals towards/from the partner—in cm) were registered. The latter have been shown to be representative for social activity and social interest.

Statistical Analysis

For play behavior, the means of groups ± SEM (10 pairs per group) were calculated over 15 min; a two-tailed Student's *t*-test was used for statistical evaluation. For social behavior, the means of groups ± SEM ($n = 10$ rats per group) of three blocks of 5 min (15 min observation) were assessed. The algorithm used in the automated system (Etho-Vision) estimates every two seconds approach and avoidance distinctively for each animal in an interaction (26). Thus, per animal the total amount of approach and avoidance is calculated and expressed in cm. The outcome is a positive value, if the animal was more approaching than avoiding—or a negative value, if the animal was more avoiding than approaching. Therefore, the data from the morphine animals can be treated as independent from the saline-treated animals. Data were subjected to an ANOVA with repeated measurements followed by a post-hoc Tukey test on the five min periods. The statistical package SYSTAT (Wilkinson, Leland, SYSTAT: The system for statistics. Evanston, IL: SYSTAT, Inc., 1990) was used to perform all statistical calculations.

RESULTS

Play Behavior

Rats treated with morphine *in utero* showed significantly more play behavior (Table 1). On day 21, pinning was significantly increased in frequency ($p < 0.01$) and duration ($p > 0.05$). Pinning frequency was still increased ($p < 0.05$) on day 28 with the duration of this behavior just missing statistical significance ($p=0.06$). On both days, the onset-latency for pinning was comparable between prenatally morphine- and saline-treated animals. In morphine-treated animals, social grooming was significantly enhanced ($p < 0.05$) in frequency and duration on day 21 but not on day 28.

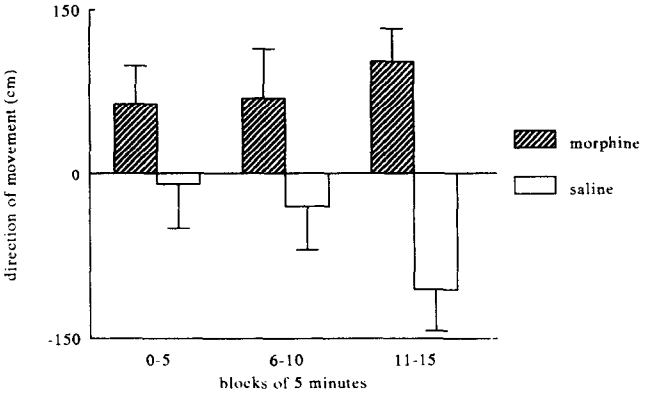

FIG. 1. Directed activity in cm (approach = upwards; avoidance = downwards; mean ± SEM) during a social interaction of young adult rats (15 min divided in blocks of five min). An interacting pair consisted of one prenatally morphine- and one saline-treated rat. *In utero* exposure to morphine resulted in higher social activity compared to their saline-treated partners.

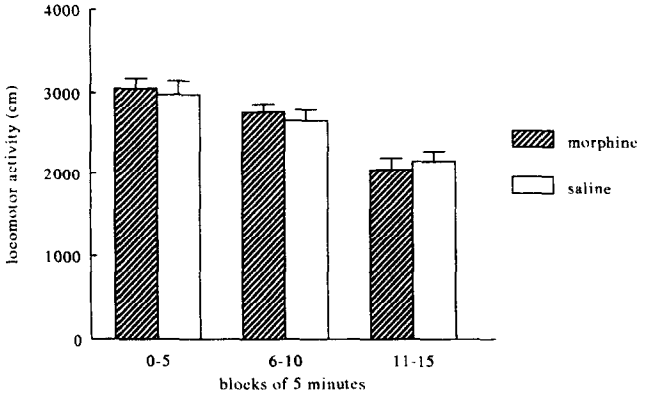

FIG. 2. Locomotor activity (mean ± SEM in cm) during a social interaction of 15 min divided in blocks of five min). The general activity of morphine-treated rats is comparable with their saline-treated partners and decreases similarly in time.

Social Behavior

Prenatal exposure to morphine significantly enhanced social behavior of young adult rats (Figure 1). These animals displayed more approach and less avoidance behavior than their saline-treated partners ($F(1,18) = 28.9$, $p < 0.001$) as it is shown by the positive direction of movement. This effect was particulary present during the last five min of the observation ($p < 0.05$). In contrast, the general locomotor activity of the morphine-treated rats during the social interaction test was comparable with their saline-treated partners ($F(1,18) = 0.4$, ns; Figure 2).

DISCUSSION

The results of the present study demonstrated that administration of morphine during prenatal development had a distinct effect on different aspects of juvenile play and adult social behavior. Not only were the levels of pinning and social grooming of young rats elevated, but also their adult social activity was increased as compared to that of prenatally saline-treated controls. This higher activity was specific for social behaviors, since the general locomotor activity was comparable between the groups.

It is reasonable to assume that brain opioid systems, which are known to be involved in the rewarding and incentive aspects of play (18) were affected by prenatal morphine treatment. Interesting parallels appear with respect to social isolation and its effects on social activity. For example, short-term social isolation in adulthood increased social activity comparable to prenatally morphine-treated animals: both groups show more approach and less avoidance behavior than their respective controls. Furthermore, the elevated social activity of these isolated animals was counteracted by morphine (6). Play behavior itself is known for its highly rewarding value (1,15). The role of the opioid system in juvenile play and adult social behavior is further substantiated by the finding that the amount of brain opioid receptors and binding was changed in juveniles related to play (28) and isolated—compared to group-housed animals (19,22,29). Surprisingly, after one social interaction this difference disappeared suggesting the release of endogenous opioids during social interaction (29).

Increased motivation for playing is expected to be expressed in shorter onset-latencies for pinning as has been shown in short-term isolated rats (12). However, in the present study the onset-latency for pinning was not affected by prenatal morphine treatment. It was the increased frequency and duration of pinning and social grooming, which is indicative for changes of the incentive value of play. A permanent alteration of the balance between opioids and their receptors is underlined by the persistence of the effect: the same animals, tested as young adults in a social interaction showed an increased interest in their social partner. It might be concluded that alterations of juvenile play behavior are predictive for social behavior in later life. This is also in line with the proposed function of social play—acquisition of communicative skills (25). It is suggested that these prenatally morphine-treated rats possess a sensitized endogenous opioid system responsible for the enhanced susceptibility for the incentive aspects of social behavior (16). This is supported by the facilitation of acquisition and self-administration of heroine in similarly treated animals (20). The sensitivity of opioid receptors as well as their number could have been affected, whereby the amygdala is a likely candidate due to its involvement in motivational as well as incentive aspects of play and adult social behavior (5,8,11,24).

Taken together, prenatal exposure to morphine increased social activity in the juvenile and young adult rat most likely by an enhancement of the rewarding aspects of play.

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