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Effect of Social Isolation on the Reinforcing Properties of Morphine in the Conditioned Place Preference Test

NOPPAMARS WONGWITDECHA[1] AND CHARLES ALEXANDER MARSDEN

Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK

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WONGWITDECHA, N. AND C. A. MARSDEN. *Effect of social isolation on the reinforcing properties of morphine in the conditioned place preference test.* PHARMACOL BIOCHEM BEHAV 53(3) 531-534, 1996.—It is widely accepted that early environmental influences may affect the behaviour of the adult animal and the responsivity to psychotropic drugs. Isolation rearing is an important variable in this regard. The present experiments compared the effects of isolation and social rearing of rats on the reinforcing properties of morphine. Male Lister hooded rats were raised from weaning either alone (isolation reared rats) or in groups of four rats per cage (socially reared rats). Four weeks later, the rats were tested for their sensitivity to morphine using a conditioned place preference test. Comparisons were made between socially and isolation-reared rats with respect to preconditioning and postconditioning following either saline or morphine (1 and 5 mg/kg SC) pretreatment. The results from the preconditioning phase demonstrated that rats reared either socially or in isolation had least preference for one quadrant that was then selected as the treatment quadrant. After saline conditioning, the socially reared rats still showed a significant ($p < 0.05$) less preference for the treatment quadrant relative to the opposite quadrant. Following morphine (1 and 5 mg/kg) conditioning, socially reared rats spent significantly more time ($p < 0.05$) in the treatment quadrant relative to the opposite quadrant, whereas isolation-reared rats failed to display morphine-induced place preference. These results demonstrate that social isolation decreases the reinforcing properties of morphine.

Social isolation Conditioned place preference Morphine Rat

THERE is growing evidence that social isolation at an early age has profound consequences for behaviour in later life. In particular, rearing rats in social isolation from weaning has been suggested to enhance the propensity to self-administer drugs of abuse (2,15), and hence to have important implications for the aetiology of human drug abuse. Thus, isolation rearing has been shown to increase the rate of the self-administration of some drugs of abuse, such as ethanol (26), morphine (3), heroin (8), and cocaine (25), while other studies have shown impairment of the reinforcing properties of cocaine and amphetamine (6,19,29). In contrast, isolation housing in adulthood has been demonstrated not to affect the self-administration of cocaine (8). Hence, the term "isolation rearing" in this paper implies a continuous period of social isolation, beginning at weaning. Rearing conditions have been reported to alter the sensitivity to exogenously administered opiates. Rats that are reared in isolation are less sensitive to opiate-induced analgesia (15) and show less severe opiate withdrawal syndrome (1) than rats that are socially reared. Moreover, isolation rearing impairs severely the capacity of heroin to establish a conditioned place preference (21), and impairs the establishment of a conditioned taste aversion to morphine (24). The early social environment may therefore be one of the critical factors that determines the behavioural sensitivity of a mature animal to morphine. The present study examines the effects of different environmental rearing conditions on the conditioned place-preference produced by morphine.

MATERIALS AND METHODS

Animals

Male Lister hooded rats (Nottingham University, Medical School) were obtained at weaning (21 days postnatal). They were randomly divided into two groups and were housed

[1] To whom requests for reprints should be addressed.

either in groups of four per cage (socially reared rats), or singly (isolation-reared rats). All cages were constructed of plastic and were lined with sawdust. The socially reared rats were housed in cages 52 × 32 × 20 cm high, whereas the isolation-reared rats were housed in cages 41 × 26 × 20 cm high. Both groups had food and water available ad lib and were housed within the same room for 6 weeks before use in the place-preference trials. A constant dark-light cycle (on 06.00 h, off 20.00 h) was maintained in the animal house and temperature controlled at 22 ± 1°C.

Apparatus

For the place preference experiments, each rat was conditioned and tested in a circular open field arena 83 cm in diameter by 32 cm in height, of metal construction. Black lines divided the floor into four quadrants of equal size and identical floor and wall textures (12). Surrounding the arena were two screens, attached to which were a series of visual cues so rats could orient themselves. This uses the same well-established method of visual cues as used with the Morris water maze (16) for spatial memory. The positioning of these cues in relation to each quadrant was constant for the experiment. The arena was placed beneath a video camera so trials could be recorded for later analysis.

Procedures

All experiments were performed between 09.00 and 17.00, which is the normal inactive period for the rats. Morphine was administered during the light period, as the relative inactivity of the rats made the measurement of line crossings more accurate and less subjected to error. The procedure used was essentially the same as that described by Wongwitdecha and Marsden (29), which was a modification of that reported by Hasenohrl et al. (12). Each experiment was carried out over 6 consecutive days. There were three phases of behavioural testing: Phase I, the preconditioning phase or baseline trial (Day 1); Phase II, the conditioning phase or drug treatment (Days 2-5); and Phase III, the postconditioning phase or a test trial (Day 6).

Phase I. The preconditioning phase or the baseline trial was used to determine a treatment quadrant for each rat. During this phase, socially or isolation-reared rats were individually placed in the centre of the open field arena facing away from the experimenter and were allowed free access to all parts of the apparatus. A rat was determined to be in a particular quadrant when their two forepaws crossed the dividing lines. The time spent in each of the four quadrants was recorded over a 10-min period for 18 socially reared and 18 isolation-reared rats. These rats were tested in an alternating schedule (socially, isolation, socially, isolation, etc.) to maintain consistency of circadian rhythms between the two groups. By this measure the normal preference for the four quadrants was determined for each rat. The quadrant in which each individual rat spent the least time in this baseline trial was selected as the subsequent treatment quadrant. We tested the initial preference only once because from previous studies we have found that the initial preference has a stable pattern and can be reproduced on the following days.

Phase II. The conditioning phase consisted of 4 consecutive days of conditioning sessions. On each day of this phase transparent plexiglas barriers were inserted into the open field arena to restrict the animals to a particular quadrant. Rats were divided into six groups of six (three groups of socially reared and three groups of isolation-reared rats). The groups of socially and isolation-reared rats were treated with one treatment, either saline (SC) or morphine (1 and 5 mg/kg, SC) once a day. Ten minutes after injection, each rat was placed in the treatment quadrant for 15 min and then returned to its home cage. We placed animals in the apparatus 10 min after injection to allow the drug to reach the site of action. This period was established as the time of onset of the behavioural effects of morphine. The 15 min conditioning period was selected from our previous experiments; we have conditioned the animals for both 10 and 15 min and found with the longer conditioning time a higher place preference for the treatment quadrant is obtained.

Phase III. The postconditioning test was on Day 6. The plexiglas barriers were removed and neither morphine nor saline was injected. Each rat was placed into the centre of the open field arena with access to the entire arena, and the time spent in each of the four quadrants was measured over a 10-min period. The observer of the test trial was blind as to which quadrant each animal had been treated in. Previous studies have used 5- or 10-min test periods to determine place preference (12,29). In the present study a 10-min test period was used and results analysed for 5- and 10-min periods, but as the place preference results were not significantly different at 5 and 10 min, the results given are the 10-min values.

Drug

Morphine sulphate (Sigma) was dissolved in 0.9 % saline and injected in a volume of 1 ml/kg.

Data Analysis

Data are expressed as mean ± SEM. Analysis of data was performed using one-way analysis of variance (one-way ANOVA) followed by Dunnett's test to compare the time the animals spent in the four quadrants. The place preference in socially and isolation-reared rats were compared using two-factor ANOVA. The effect of different housing on the conditioned place preference response to saline and morphine administration was also analysed by two-factor ANOVA. In all statistical tests a value of $p < 0.05$ was considered to be significant.

RESULTS

The results from the baseline (preconditioning) trial (Fig. 1) demonstrate that each isolation [$F(1, 34) = 10.69$, $p < 0.01$] and socially [$F(1, 34) = 11.38$, $p < 0.01$] reared rat had least preference for one quadrant; this quadrant was later selected as the treatment quadrant. Neither socially [$F(1, 24) = 0.90$, $p > 0.1$] nor isolation [$F(1, 24) = 1.26$, $P > 0.1$] reared rats given saline showed any significant change in their quadrant preference compared to the preconditioning values (Fig. 1A and 1B).

Socially reared rats treated with morphine (1 and 5 mg/kg), however, spent more time in the treatment quadrant than in the opposite quadrant. Rats given morphine (1 mg/kg) showed a significant [$F(1, 21) = 5.35$, $p < 0.05$] increase in their selection of the treatment quadrant relative to preconditioning and to saline controls (Fig. 1A). There was no significant difference between the time spent in the treatment quadrant and the other quadrants. However, the higher dose of morphine (5 mg/kg) resulted in a trend for the rats to select the treatment quadrant that was significant [$F(1, 22) =$

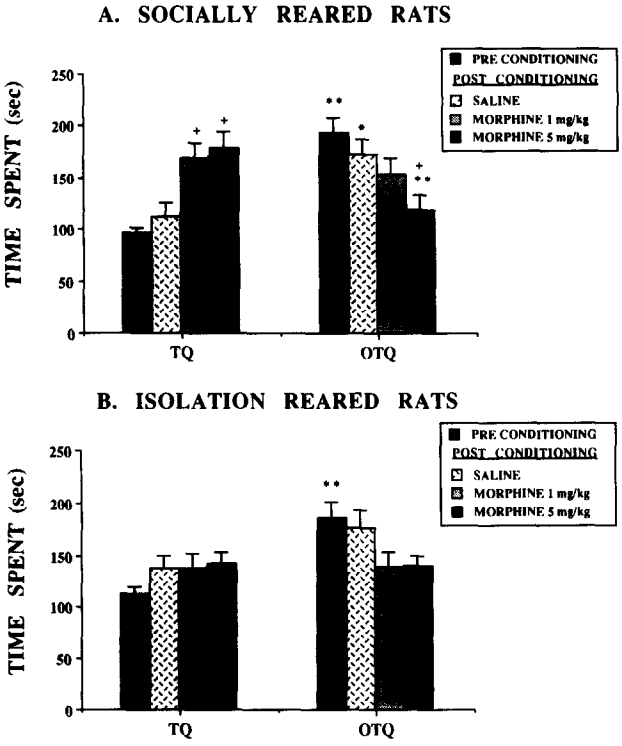

FIG. 1. Time spent on the treatment quadrant (TQ) and the quadrant opposite the treatment quadrant (OTQ) before conditioning and after conditioning with saline or morphine (1 and 5 mg/kg) in (A) socially reared rats and (B) isolation-reared rats. Data represent mean ± SEM of 18 rats/group in preconditioning test and 6 rats/group in postconditioning test. $*p < 0.05$; $**p < 0.01$, significantly different from the treatment quadrant. $+p < 0.05$, significantly different from saline treated group in the same quadrant.

12.25, $p < 0.01$] relative to the opposite quadrant and the left quadrant. In contrast the morphine conditioning in the isolation-reared rats did not cause an increase in selection of the treatment quadrant relative to saline controls [morphine 1 mg/kg $F(1, 21) = 0.75, p > 0.1$; morphine 5 mg/kg $F(1, 22) = 1.04, p > 0.1$] as shown in Fig. 1B.

DISCUSSION

The present studies show differential effects of morphine in the conditioned place preference test as a function of the initial preference in different rearing conditions. Animals reared in isolation from weaning were less sensitive to morphine than were those reared in social groups, as demonstrated by their failure to exhibit place preference to morphine. Our results add to existing reports on the effects of early social environment on sensitivity to opiates and the possibility that early social contact plays an important role in the subsequent behavioural expression of the effects of opiates. If the social contact is restricted, such as in the case of isolation immediately postweaning, the sensitivity to opiates is decreased. This suggests that the early social environment may influence specific neurochemical systems in the developing nervous system, thus differentially affecting the mature rat's sensitivity to drugs of abuse.

We have previously provided evidence that conditioned place preference produced by psychostimulant drugs such as amphetamine and cocaine is reduced in isolation-reared rats (6,29). The present data show that isolation rearing also reduces sensitivity to the reinforcing properties of morphine. In our apparatus, we observed that both socially and isolation-reared rats initially spent least time to one quadrant with preference for the opposite quadrant. Before conditioning, the time spent in the treatment quadrant was significantly less than the opposite quadrant and the right and the left quadrants.

Socially reared rats treated with morphine (1 and 5 mg/kg) showed a significant place preference for the treatment quadrant relative to saline controls with respect to the same quadrant as well as the opposite quadrant. These data add to the growing number of studies employing conditioned placed preference as a procedure for identifying the rewarding properties of psychoactive drugs (5,9,11,28). Rats housed in isolation immediately postweaning, however, failed to show place preference to morphine. This finding is consistent with other studies using cocaine (6,23), amphetamine (29), and heroin (22). These results suggest that the sensitivity of the mature rat to morphine may, in part, be a reflection of the extent of development of the endogenous opioid system (which may be inhibited by social isolation in the young rat), as previous neurochemical studies show the number of opiate binding sites reduced by isolation rearing (20). The development of the opiate receptor system, in turn, may determine the sensitivity of the rats to the behavioural effects of opiates as indicated by the present results and previous studies showing reduced sensitivity to opiate-produced analgesia (15) and conditioned place preference (21,22).

Several studies have shown that isolation rearing produces long-term effects on various neurotransmitter systems including dopamine (13,14), for which there is extensive evidence for an involvement in the rewarding properties of opiates. For example a number of studies suggest that the behavioural effects of opioids may involve an action in the ventral tegmental area (7,18), site of origin of mesolimbic dopamine neurones. Reinforcing effects have been shown when morphine was injected into the ventral tegmental area (18). Lesions of this area disrupt the acquisition of intravenous heroin self-administration (7). Moreover, dopamine antagonists reportedly block heroin-induced place preference conditioning (27). Studies with isolation-reared rats have shown increased presynaptic dopamine function, increased release in response to K^+ and amphetamine stimulation in the nucleus accumbens (13,14), but reduced D_1 receptor responsiveness (14). In contrast, electrophysiological studies have shown an increased responsiveness to dopamine neurones in the prefrontal cortex (17), an area innervated by mesocortical dopamine neurones that are involved in the reciprocal inhibition of the dopamine pathways to the nucleus accumbens. Thus, the failure of morphine to demonstrate place preference in isolation-reared rats in the present study may reflect not only changes in opiate receptor mechanisms in these rats, but also dopaminergic function associated with reward behaviour. Isolation-reared rats also show decreased presynaptic serotonergic function (4) and supersensitivity of presynaptic α_2 adrenoceptors, resulting in decreased noradrenaline release (10). Both these neurotransmitters have been implicated in behaviours associated in reward and the actions of opiates.

In summary, isolation-reared rats demonstrate a dysfunction in opiate reward mechanisms in common with those of cocaine and other rewarding drugs. This dysfunction may reflect alterations not only in brain opiate mechanisms but also

other neurotransmitters such as dopamine, serotonin, and noradrenaline. The isolation-reared rats may be a model to investigate developmental factors involved in predisposition to drug abuse in later life.

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