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Prior experience with wheel running produces cross-tolerance to the rewarding effect of morphine

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Abstract

The rewarding effect of wheel running is hypothesized to be mediated by endogenous opioids. Thus, prior experience with wheel running might be expected to affect the reward value of an opiate drug like morphine. In three similar experiments to test this idea, 10 rats (wheel-morphine group) were confined in running wheels for 2 h on each of eight consecutive days during the first phase; the 10 in the cage-morphine group were confined in small metal cages. Then, in the second phase, a distinctive place was paired with morphine (1 mg/kg) on three occasions to produce conditioned place preference (CPP). In all experiments, CPP occurred in the cage-morphine group, but not in the wheel-morphine group, implying that prior wheel running resulted in cross-tolerance to the rewarding effect of morphine. This finding supports the idea that the rewarding effect of wheel running is mediated by endogenous opioids.

Keywords: Wheel running; Endogenous opioids; Morphine; Conditioned place preference; Cross-tolerance; Cross-sensitization

1. Introduction

A substantial body of evidence indicates that wheel running has a rewarding effect in rats. Wheel running occurs spontaneously and increases with experience (Eayrs, 1954), implying that wheel running is self-reinforcing (Bolles, 1975; Gross, 1968; Sherwin, 1998). It can also act as a reinforcer for instrumental behavior. For example, rats will bar press vigorously to gain a brief period of wheel running (e.g., Belke and Heyman, 1994; Iversen, 1993). There is also evidence that pairings of the aftereffect of wheel running with a distinctive place result in Pavlovian conditioning of a preference for that place (Lett et al., 2000).

It has been hypothesized that the rewarding effect of wheel running is mediated by endogenous opioids (Epling and Pierce, 1992). In experiments to test this hypothesis (Lett et al., 2001), rats were first allowed to wheel run for 2 h; then each was injected with naloxone (0.1 or 0.5 mg/kg) and 10 min later placed in a distinctive chamber. Other rats were similarly treated except that saline was injected instead of naloxone. Consistent with the notion that the rewarding effect of wheel running is mediated by endogenous opioids, a conditioned preference for the distinctive place occurred in the rats injected with saline but not in those injected with naloxone.

If the rewarding effect of wheel running is mediated by endogenous opioids, then wheel running must activate at least some of the same systems that are activated by morphine and other opiates. Repeated exposures to morphine can increase (e.g., Lett, 1989; Schnur, 1985) or decrease (e.g., Baker and Tiffany, 1985) the sensitivity of the relevant brain systems to morphine. Thus, repeated activation of certain opioid reward systems by wheel running might also be expected to change the sensitivity of these systems to morphine. The purpose of the present three experiments was to test this idea. The procedures of these experiments were almost the same. During the first phase, the rats in the wheel-morphine group were confined in running wheels for 2 h on each of eight consecutive days, while those in the cage-morphine group were put in small metal cages. Then in the second phase, the effect of prior

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wheel running on the strength of the conditioned place preference (CPP) induced by morphine was tested.

2. Method

2.1. Subjects

In each of the three experiments, the subjects were 30 male Sprague–Dawley rats (*Rattus norvegicus*) obtained from the breeding colony at Memorial University of Newfoundland. Upon arrival, the mean weight of the rats was 184 g (S.D. = 9.3) in Experiment 1, 187 g (S.D. = 6.9) in Experiment 2, and 207 g (S.D. = 8.9) in Experiment 3. Each rat was housed in a clear plastic cage ($47 \times 24 \times 20$ cm) with wood chip bedding. All training procedures occurred in the room where the rats were housed. This room was maintained at a temperature of 22 °C. During Experiments 1 and 2, the room lights automatically turned on at 12:00 a.m. and off at 12 noon; during Experiment 3, lights went on at 3:30 a.m. and off at 3:30 p.m.

2.2. Apparatus and materials

Each of the 15 running wheels had a circumference of 113.1 cm and a floor width of 11.5 cm. Each wheel had a rotating and a stationary wall made of solid metal and a floor made of wire mesh. There was a sliding door on the stationary wall. Water was available from a spout that protruded into the wheel through a small opening in the door. Wheel turns were electronically counted and recorded by a computer. The small metal cages ($18.0 \times 25.0 \times 18.0$ cm) were suspended in a metal rack. Each cage had a wire mesh floor and front wall; the ceiling and the other three walls were solid metal. Water was available from a spout inserted into the front wall of the cage.

Place conditioning was conducted in one of eight CPP apparatuses ($80 \times 25 \times 38$ cm) consisting of two joined chambers that were each 40 cm long. In one chamber, the walls had black and white horizontal stripes (3 cm wide) and the floor was a metal lattice of diamond-shaped openings. In the adjoining chamber, the walls had vertical stripes and the floor was a grid of metal rods. The rods were parallel to the midline where the two chambers were joined. The lid of each chamber was a metal grid. During CPP training, opaque white dividers were inserted to confine a rat to a particular chamber. The dividers, one in each chamber, were placed 7 cm from the midline of the CPP apparatus. The placement of the dividers reduced the length of each chamber and resulted in a separation, 14 cm wide, between the two chambers. Such a separation seemed desirable because both chambers were occupied simultaneously during CPP training. The dividers were removed before CPP testing began.

In all experiments, morphine sulfate was injected at a dose of 1 mg/kg. The drug was dissolved in isotonic saline at a concentration of 1 mg/ml so that the injection volume was 1 ml/kg.

2.3. Procedure

The rats were treated the same way in each of the three experiments except when specified otherwise. Several days after arrival in the laboratory, the rats were divided into two equal-sized groups with the same mean body weight. One group was assigned to the wheel condition; the other to the cage condition. On each of the next 8 days, a rat in the wheel condition spent 2 h in a running wheel while each in the cage condition was confined in a small cage. The rats were put into the wheels and cages 30 min before the dark period began. Wheel access was given at this time because rats run more at night (Eikelboom and Mills, 1988). The room lights were turned on briefly to permit the removal of the rats from the wheels and cages. Throughout the experiment, food and water were continuously available in the home cage. Water, but no food, was available in the wheels and small cages.

After the eighth period of wheel running was completed, the rats were assigned to one of three groups for the CPP training that began on the next day. Ten of the 15 rats in the wheel condition were assigned to the wheel-morphine group; 10 of the 15 rats in the cage condition were assigned to the cage-morphine group; the remaining 5 rats from each condition were assigned to the saline group. In Experiments 1 and 2, the 10 rats with the highest scores on the eighth day of wheel running were assigned to the wheel-morphine group. This was done to increase the likelihood of detecting the effect of wheel running. The five less active rats were assigned to the saline group. In Experiment 3, the rats in the wheel condition were assigned to the wheel-morphine and saline groups so that the mean scores on the last day of wheel running were similar in the two groups. In all experiments, the rats in the cage condition were assigned to the cage-morphine and saline groups so that the mean weights of the groups were similar.

To produce place conditioning, a biased CPP procedure was used (Bardo et al., 1995; Schechter and Calcagnetti, 1993). In all three experiments, the rewarding effect of morphine was paired with the horizontal chamber, which previous findings indicated was less preferred than the vertical chamber (Lett et al., 2001). In the wheel-morphine and cage-morphine groups, the rats were given three trials during which each rat was injected with morphine and then confined in the horizontal chamber for 30 min. On these occasions, the rats in the saline group were each injected with saline before placement in the horizontal chamber. All rats were also given three trials during which the rat was simply removed from its home cage and confined in the vertical chamber for 30 min. In Experiments 1 and 2, the morphine and saline were injected subcutaneously; in Experiment 3, intraperitoneally.

The six training trials were given one per day on consecutive days. Half the rats in each group were put in

the horizontal chamber on odd-numbered days and the vertical chamber on even-numbered days; vice versa for the remainder. This training began each day at 11 a.m. (Experiments 1 and 2) or 2:30 p.m. (Experiment 3) and ended about 90 min later. The room lights were left on until the training session ended.

Two days after CPP training ended, the testing for CPP began. Half the rats were tested on the first day and half on the next day at about the same time of day that training occurred. During the test, each rat was given free access to both chambers for 10 min. At the start of the test, the rat was placed along the midline of the CPP apparatus. The amount of time spent in each chamber was measured. A rat was considered to be in a chamber only when all four paws were in that chamber.

The experimental protocol was approved by the local institutional committee on animal care.

2.4. Data analysis

A score of place preference was calculated for each rat as the percentage of time spent in the horizontal chamber during the CPP test relative to total time in both chambers. The difference between the groups on this measure was initially evaluated with a one-way analysis of variance (ANOVA); when appropriate, pairwise comparisons were evaluated by a *t* test. An analysis for a linear trend (Winer, 1962) in the number of wheel turns made by the 15 rats in the wheel condition was used to assess whether the amount of wheel running changed with experience.

3. Results

3.1. CPP test

Fig. 1 shows the mean score of place preference for each group during the CPP test in Experiments 1–3. The pattern of results was similar in these experiments. There was a reliable difference between groups in these preference scores, $F(2,27) = 14.6$, $P < .001$ in Experiment 1, $F(2,27) = 10.8$, $P < .001$ in Experiment 2, and $F(2,27) = 5.2$, $P < .05$ in Experiment 3.

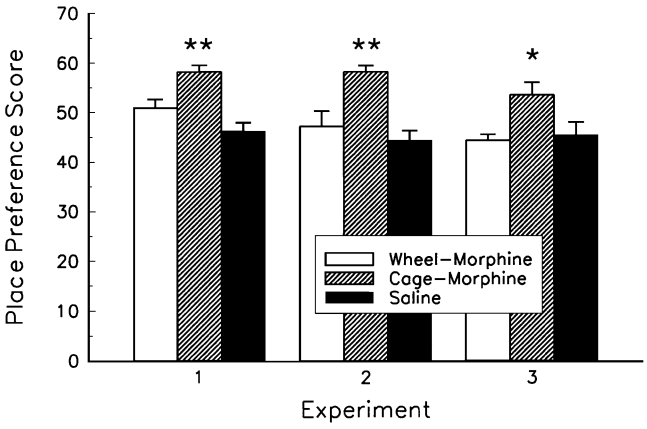

Fig. 1. Mean score of place preference (±S.E.) for each group during the CPP test in Experiments 1–3. $*P < .05$; $**P < .0001$ compared with saline controls.

Table 1
Wheel turns (mean and S.E.) made by all 10 rats in the wheel-morphine group and 5 rats in the saline group during each 2-h session before the start of CPP training in each experiment

	Wheel running sessions							
Groups	1	2	3	4	5	6	7	8
Experiment 1								
Morphine								
M	253	358	501	708	899	1211	1395	1442
S.E.	48	62	90	91	107	127	154	164
Saline								
M	129	262	346	455	499	519	703	682
S.E.	15	28	91	55	49	60	48	33
Experiment 2								
Morphine								
M	304	590	752	884	1036	1177	1255	1300
S.E.	48	61	54	83	91	90	76	81
Saline								
M	268	359	524	598	698	615	572	667
S.E.	33	99	95	83	85	53	50	82
Experiment 3								
Morphine								
M	342	538	654	737	914	965	1314	1267
S.E.	41	36	60	68	97	97	167	156
Saline								
M	326	642	616	931	942	933	1169	1404
S.E.	87	172	122	191	152	172	226	355

In each experiment, the mean preference score of the cage-morphine group was greater than that of the saline group. This difference was reliable, P's $< .0001$ in Experiments 1 and 2 and $P < .05$ in Experiment 3, indicating that CPP occurred in the cage-morphine group in all three experiments. In contrast, reliable CPP was not obtained in the wheel-morphine group in any of these experiments. Although in Experiments 1 and 2 the wheel-morphine group did have a higher mean preference score than the saline group, this difference was not reliable, $.05 < P < .10$ in Experiment 1 and $t < 1$ in Experiment 2. Moreover, in Experiment 3 the mean preference score of the wheel-morphine group was slightly lower than that of the saline group, $t < 1$. Also, in each of the three experiments the mean preference score of the wheel-morphine group was reliably lower than that of the cage-morphine group, P's $< .01$. This pattern of findings indicates that the eight periods of wheel running that occurred before CPP training attenuated the effectiveness of morphine in producing CPP.

As described earlier, half the rats in the saline group had prior wheel running experience and half had not. This difference in experience was not expected to have, and did not have, an effect on the place preference scores of these rats, $P > .25$ in Experiment 1, t's < 1 in Experiments 2

and 3. When combined across the three experiments, the rats in the saline group that were given prior wheel experience had a mean preference score of 46.2% (S.E. = 1.9) while those placed in small cages had a mean score of 44.5% (S.E. = 1.4), $t < 1$.

3.2. Wheel running

Table 1 shows the mean number of wheel turns made by the 10 rats in the wheel-morphine group and the 5 rats in the saline group in each experiment. In Experiments 1 and 2, as described earlier, the rats assigned to the wheel-morphine group made many more wheel turns than did those assigned to the saline group. In Experiment 3, the assignment of rats ensured that these groups were similar in mean wheel turns. In each of the three experiments, wheel running increased with experience, P's < .0001.

4. Discussion

In each of the three experiments, morphine produced CPP in the cage-morphine group but not in the wheel-morphine group. This finding indicates that the eight sessions of wheel running that occurred before CPP training made morphine less effective in producing CPP. It implies that the repeated activation of opioid reward system(s) by wheel running decreased the rewarding impact produced by morphine. This type of effect is often called cross-tolerance.

If wheel running produced cross-tolerance to the rewarding effect of morphine, tolerance to the rewarding effect of wheel running should also have occurred. Thus, wheel running might have been expected to decrease with repeated experience. However, wheel running did not decrease; instead, as noted earlier, it increased. Various factors may have led to this increase. One factor might be that the physical fitness of the animals increased with experience in the wheel (Mueller et al., 1999). A second could be that practice led to better coordination of the rat's movements. Both would make wheel running easier and, therefore, more likely to occur. Another possibility is that instrumental learning occurred because the motor behavior of wheel running was repeatedly followed by the rewarding effect that it produced. Such instrumental learning would increase the probability of wheel running. Also, it seems possible that the increases in wheel running occurred in order to compensate for a decrease in the reward value of wheel running. Thus, the reward value of wheel running may have decreased with experience even though the probability of its occurrence increased.

In contrast to the effect produced by repeated experience with wheel running, prior exposures to morphine have been found to increase the effectiveness of morphine in producing CPP (Gaiardi et al., 1991; Lett, 1989; Shippenberg et al., 1996, 1998). Presumably, repeated activation of opioid reward system(s) by morphine increased the sensitivity of these systems thereby increasing the drug's reward value. This effect is an example of sensitization.

On the hypothesis that the rewarding effect of wheel running is mediated by an opioid system, repeated experience with wheel running was expected to increase, rather than decrease, the reward value of morphine. Thus, the present findings suggest that this hypothesis may be incorrect. However, as noted earlier, there is other evidence that supports the hypothesis. During CPP training in which the rewarding aftereffect of wheel running was paired with a distinctive chamber, administration of the opiate antagonist, naloxone, attenuated CPP (Lett et al., 2001). Indeed, the present finding of an interaction between wheel running and the reward value of morphine in itself provides support for the hypothesis that the rewarding effect of wheel running is mediated by an opioid system.

It has been suggested that the opioid system susceptible to sensitization involves the dopaminergic neurons that project to the nucleus accumbens (Shippenberg et al., 1996). The present findings suggest that the rewarding effect of wheel running is not mediated by this opioid system. However, it should be noted that there is evidence for more than one opioid reward system (Vaccarino et al., 1989; Wise, 1989). It seems possible that such systems have different properties. If so, the present findings suggest that tolerance occurs in the opioid system that mediates the rewarding effect of wheel running, as it does in the system that underlies the analgesic effects of opiates (e.g., Baker and Tiffany, 1985). Presumably, any tolerance produced in this reward system by repeated exposures to systemic morphine or other opiates would usually be masked by sensitization occurring elsewhere.

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