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The Involvement of Interoceptive Factors in the Maintenance of Heroin-Seeking Behavior

C. MADDEN, G. SINGER[1] AND T. P. S. OEI

Department of Psychology, La Trobe University, Bundoora, Victoria, 3083, Australia

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MADDEN, C., G. SINGER AND T. P. S. OEI. *The involvement of interoceptive factors in the maintenance of heroin-seeking behavior.* PHARMAC. BIOCHEM. BEHAV. 11(4) 445–448, 1979.—The role of interoceptive stimuli in conditioned heroin-seeking behavior was investigated using forty-two naive male Wistar rats. A 21-day Phase I period in which 28 animals self-injected heroin in saline solution was followed by a 5-day Phase II period in which 7 animals were allowed access to the original solution; 7 animals were allowed access to saline only; 7 animals were allowed access to distilled water; and 7 animals were allowed no access to any solution. Results showed that only the heroin and (heroin-associated) saline groups showed a significant difference in operant rate compared to animals that had been continually exposed to saline only. It was concluded that heroin-seeking behavior was maintained in the (heroin-associated) saline group as a result of physiological conditioning following repeated association of saline with heroin.

Heroin Conditioning Schedule Self-injection

EVIDENCE for the involvement of conditioned stimuli in the maintenance and relapse of opiate-seeking behavior in rats, monkeys and man is derived from two sources of research. Environmental stimuli contiguous with the withdrawal syndrome have been shown to increase the frequency of withdrawal symptoms, and to continue eliciting these symptoms for up to five months after morphine treatment has been terminated [12, 18, 19]. Furthermore, investigators [3,4] have demonstrated the production of withdrawal symptoms in opiate-dependent rhesus monkeys by a conditioned stimulus (light or tone) after several associations of the stimulus with nalorphine injections. The implicit argument in these studies is that once physical dependence has been established, withdrawal-associated symptoms lead to enhanced opiate-seeking behavior as a means of avoiding the impending withdrawal reaction. Thus perceived withdrawal onset, whether it results from physiological or environmental cues, will lead to either maintenance or relapse of opiate-seeking behavior.

A further source of evidence for the involvement of conditioned stimuli in the maintenance and relapse of opiate-seeking behavior is derived from studies in which environmental stimuli associated with opiate injections come to elicit certain physiological and behavioral effects of opiates [13,17], including opiate-seeking behavior [1,15].

In studies by both Schuster and Woods [15] and Crowder *et al.* [1] a light (or buzzer) was associated with morphine injections during the conditioning period. It was later shown that presentation of the conditioned stimuli, with saline, temporarily maintained operant (opiate-seeking) behavior. While the above-mentioned studies show that conditioned environmental stimuli combined with saline substitution for drug, maintains responding, the relative importance of these exteroceptive and interoceptive stimuli has not been demonstrated. Fluid infusion per se may be sufficient to temporarily maintain behavior.

In the present experiment, saline was continuously associated with heroin to determine whether interoceptive cues alone can maintain heroin-seeking behavior following heroin removal. The schedule-induced self injection technique [7, 10, 11] was chosen as the method of drug administration in order to maximize the occurrence of drug dependence.

METHOD

Animals

Forty-two naive male albino Wistar rats were reduced to 80% of free-feeding body weight (340–390 g) and housed individually in temperature controlled conditions (23 ± 1°C) with a 12 hr light-dark cycle. Each animal was anaesthetized with an IP injection of pentathesin, and a polythene (SP 28) catheter was surgically implanted into the right jugular vein. Catheters were held in position by leather jackets.

Apparatus

The apparatus was identical to that described in Oei *et al.* [10,11]. Briefly, an operant box contained a bar that triggered the delivery of 0.07 ml solution via a syringe infusion pump (Sage Instruments, Model 341) into a

[1]Requests for reprints to Dr. G. Singer, Department of Psychology, La Trobe University, Bundoora, Victoria, 3083, Australia.

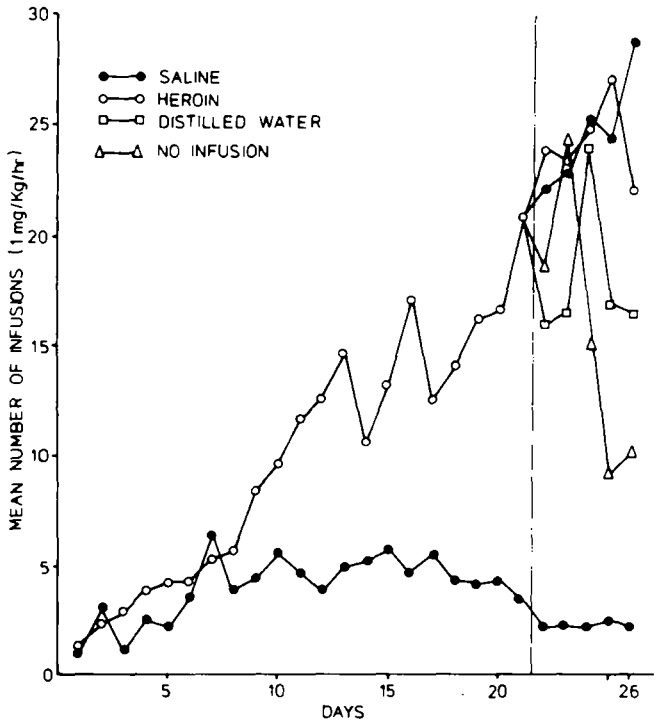

FIG. 1. Mean number of self infusions/hr (0.1 mg/kg) for the experimental and control groups during Phase 1 and Phase 2 of the experiment.

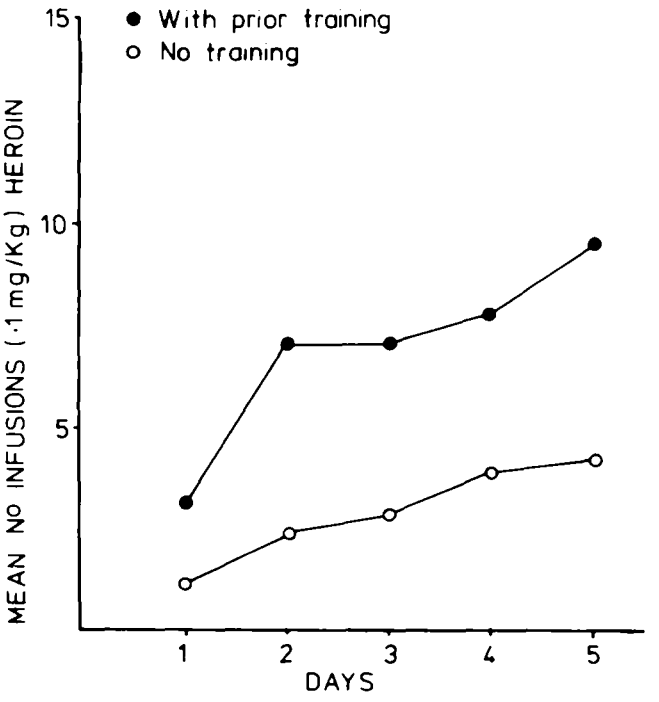

FIG. 2. Mean number of self infusions (0.1 mg/kg) for animals with (Group 6) and without prior training.

polythene catheter. The catheter was connected to a flexible swivel system which allowed the animals free movement in the operant box. The infusion system allowed only one infusion per 5 sec interval. Infusion frequency was recorded on a continuous graph recorder. A fixed time 1 min (FT 1) food delivery schedule was used in all experimental conditions.

Procedure

On recovery from surgery (2 days postoperative), animals were given a daily one hour session in the operant box for 21 days during Phase 1 and another 5 days during Phase 2. A 21-day Phase 1 period was selected on the basis of results from a previously conducted pilot study.

Freshly prepared diacetylmorphine hydrochloride (0.1 mg/kg; Victorian Health Department) in 0.9% saline solution was available to 28 animals, and 0.9% saline only was available to the remaining 14 animals during Phase 1. An initial priming dose was administered prior to each experimental session. Following Phase 1, the 28 animals that had been self-injecting heroin were randomly allocated to the following 4 groups: heroin (Group 1); saline (Group 2); distilled water (Group 3) and no infusion (Group 4). The 14 saline self-injecting animals were allocated to either saline (Group 5); or heroin (Group 6) self-injection, and a 5 day Phase 2 period followed. All animals were given the hot plate (paw lick) latency test (45°C) immediately before and after each alternate experimental session.

RESULTS

Infusions

The data from Phase 1 and Phase 2 were analysed separately. The mean infusions/hr for Phase 1 are shown in Fig. 1. A two-way ANOVA, with repeated measures on days, was applied to the data for Phase 1 (Days 1–21). Significant main effects for drug treatment, $F(1,40)=13.921$, $p<0.001$; for days, $F(20,800)=5.871$, $p<0.001$; and for the interaction of drugs and days, $F(20,800)=3.637$, $p<0.001$; indicate that the rate of self-infusion for heroin was significantly higher than that of saline.

One-way ANOVA's were applied to the data in Phase 2 using the first 5 groups (Groups 1–5) on each of Days 25 and 26. Significant differences were found for Day 25, $F(4,30)=4.093$, $p<0.01$; and for Day 26, $F(4,30)=4.162$, $p<0.01$. Figure 1 indicates a pattern of responding resembling partial extinction for the no infusion group. Post hoc Scheffé analysis for Days 25 and 26, respectively, showed that only Group 1 (heroin) and Group 2 (saline) maintained behavior at a level significantly higher than Group 5 (the saline control group).

The animals in Group 6, having self-injected saline for 21 days during Phase 1, self-administered heroin during Phase 2. In order to determine whether prior exposure to the self-injection procedure effects heroin intake, the data from group 6 was compared to the data from the first 5 days of heroin infusion for Phase 1 (see Fig. 2). A two-way ANOVA with repeated measures showed no significant main effects

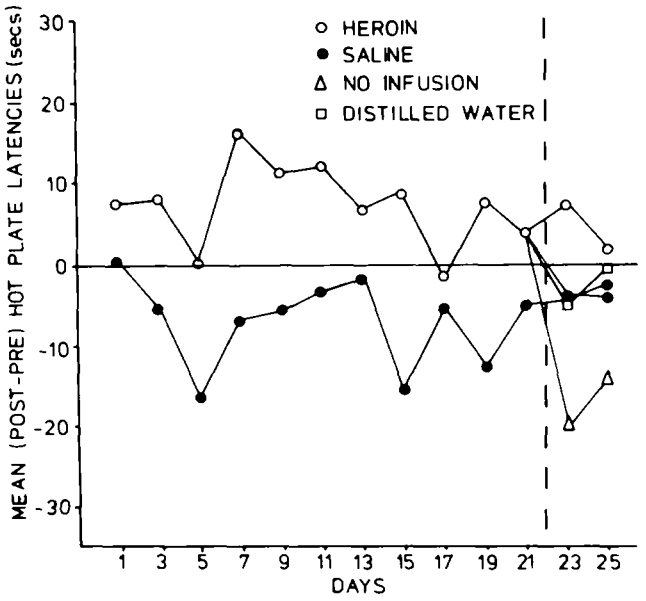

FIG. 3. Mean (Post-Pre) hot plate latencies in seconds for the experimental and control groups during Phase 1 and Phase 2 of the experiment.

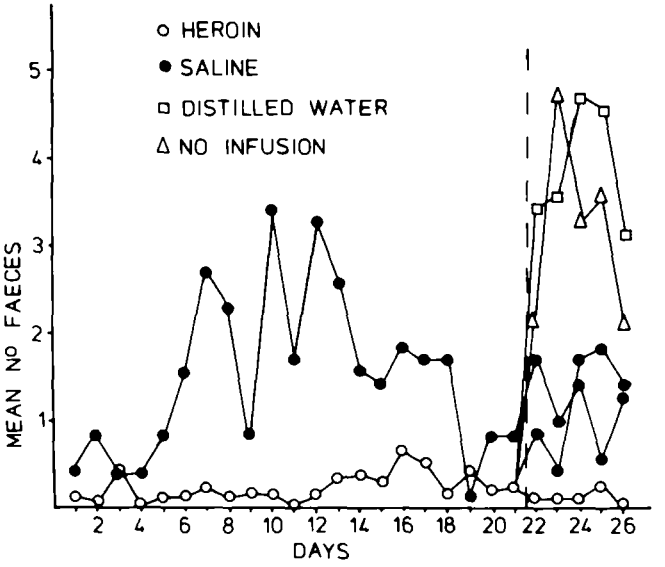

FIG. 4. Mean faeces count for the experimental and control animals during Phase 1 and Phase 2 of the experiment.

suggesting that prior exposure to the infusion process did not enhance heroin-seeking behavior.

Hot Plate

The mean post-pre hot plate latencies for Phase 1 are presented in Fig. 3. Again, two-way ANOVA with repeated measures was applied to the data and only a significant between-group effect was found, $F(1,40)=13.759$, $p<0.001$, indicating a longer hot plate response latency for the heroin condition than for the saline condition. A one-way ANOVA applied to the Phase 2 data (Days 23, 25) was found to be insignificant.

Faeces Output

The mean count of faeces left in operant boxes during the experimental sessions are shown in Fig. 4. A two-way ANOVA with repeated measures was applied to the data for Days 1–21 (Phase 1). Significant main effects for drugs, $F(4,30)=5.502$, $p<0.01$; and for the interaction of drugs and days, $F(80,600)=1.388$, $p<0.05$; indicated that the saline group produced more faecol boli during the Phase 1 period than did the heroin group.

Figure 4 shows a marked increase in faeces output for Groups 3 (distilled water) and 4 (no infusion) during the Phase 2 period. A two-way ANOVA with repeated measures was applied to the data for Days 22–26 (Phase 2). Significant main effects for drugs, $F(4,30)=10.159$, $p<0.001$; and for the interaction of drugs and days, $F(16,120)=1.870$ were found. However post hoc Scheffé analysis revealed no significant differences between groups.

DISCUSSION

The linear pattern of heroin intake during the 21-day Phase 1 period concurs with that of previous studies of opiate self-injection [10, 14, 16].

The findings from Phase 2 show that (heroin-associated) saline infusion alone was sufficient to maintain responding for up to 5 days after heroin removal. It is suggested that this effect is due to interoceptive conditioning as a consequence of continuous pairing of saline with heroin for 21 days. This argument is strengthened by the finding that substitution of distilled water, and/or infusion removal disrupted the pattern of responding previously obtained under heroin infusion. Apparently, continuous and contiguous association of saline with the positive reinforcing effect of heroin rendered saline a secondary reinforcer.

It has previously been reported that the injection procedure per se may come to act as an important factor in heroin-seeking behavior [6]. The present experiment indicates that while infusion alone (see distilled water group, Fig. 1) may maintain a higher level of heroin-seeking behavior than no infusion, and control animals, this difference is not significant, suggesting that infusion per se is not of paramount importance. The critical factor was found to be the substance coupled with heroin, as shown by the significant difference between the (heroin-associated) saline group and the control group.

While prior exposure for 21 days to the self-infusion apparatus was not found to be a significant factor in subsequent heroin self-infusion, this finding should be viewed with caution as previous results have shown that heroin self-administration in the first few days may have a decreasing effect on motor activity [8] and thereby limit heroin-seeking behavior. Thus, a lower dose (<0.1 mg/kg) may be necessary to control for the motor impairment effects of heroin.

In the no infusion condition, a pattern of responding resembling an extinction curve was observed (see Fig. 1).

However, no-infusion did not completely extinguish heroin-seeking behavior; mean response rate on Day 26 was still equivalent to that observed on the 10th day of Phase 1 heroin infusion. This persistence of heroin-seeking behavior is consistent with previous studies in which opiate extinction has been investigated [19, 20, 21].

While the hot plate data show a significant analgesic effect for the heroin condition during Phase 1, lack of significance during Phase 2 most likely reflects tolerance to the analgesic effects of heroin [2] as well as reduced response latency due to heroin removal for Group 2 (saline) Group 3 (distilled water) and Group 4 (no infusion).

The significant difference in faeces output between heroin and saline conditions during Phase 1 (Fig. 4) supports previous reports of the constipatory effects of opiates [9]. Heroin removal resulted in a marked increase in faeces output for distilled water and no infusion groups. While post hoc Scheffé tests revaled no significant differences between group means during Phase 2, it is to be noted that diarrhea, which was frequently observed during this period, resulted in conservative faeces counts. These observations indicate that dependence had been established prior to heroin removal, since defecation and diarrhea are early withdrawal signs in the rat [9].

It is concluded, therefore, that (heroin-associated) saline infusion can maintain heroin-seeking behavior after dependence has been established. This implicates substances associated with heroin-infusion as factors which may increase the likelihood of continued heroin-seeking behavior or relapse. Moreover, since osmoreceptors have been localized in the CNS [5] it is likely that saline may act as a secondary reinforcer due to continued association of heroin effects with stimulation of these receptors. In this way a process of physiological conditioning may be responsible for continued heroin-seeking behavior elicited by saline infusion.

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