Pharmacology Biochemistry & Behavior, Vol. 26, pp. 719–723. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Increased Sensitivity to the Stimulus Properties of Morphine in Food Deprived Rats

M. GAIARDI,[1] M. BARTOLETTI, A. BACCHI, C. GUBELLINI AND M. BABBINI

Institute of Pharmacology, University of Bologna, I-40126 Bologna

Received 4 November 1985

GAIARDI, M., M. BARTOLETTI, A. BACCHI, C. GUBELLINI AND M. BABBINI. *Increased sensitivity to the stimulus properties of morphine in food deprived rats.* PHARMACOL BIOCHEM BEHAV **26**(4) 719–723, 1987.—Recent research has shown that food deprivation increases opiate self-administration; in this line a first purpose of the present experiments was to determine whether the food deprivation effect could be replicated by the use of place conditioning, an alternative procedure for the study of drug reinforcement. It was found that the conditioned reinforcing properties of morphine (2.5 mg/kg IP) paired cues are greater in food deprived rats both after 1 and 3 conditioning sessions. A second objective of the work was to examine the possibility that food deprivation could also influence the discriminative stimulus properties of opiates. To this end rats trained to discriminate 10 mg/kg IP of morphine from saline were submitted to morphine generalization tests when food deprived or after 15 min supplemental feeding in the home cages. The ED_{50} value was significantly lower for food deprived (6.09 mg/kg) than for partially satiated (7.79 mg/kg) rats. It was concluded that food deprived rats are mores sensitive to both the reinforcing and the discriminative stimulus properties of morphine.

Food deprivation Morphine, discriminative properties of Morphine, reinforcing properties of Narcotic cue

FOOD deprivation has been shown to produce a substantial increase in opiate-maintained behaviors. This finding has been generalized across species, routes of self-administration, reinforcement schedules and has been interpreted in terms of food deprivation augmenting the opiate reinforcing efficacy as assessed by self-administration tests (see review by Carroll and Meisch) [1]. In this line a first purpose of the present experiments was to determine whether the food deprivation effect could be replicated by the use of place conditioning, an alternative procedure for the study of drug reinforcement (experiment 1).

There is very little literature dealing with the effects of food deprivation on behavioral actions of drugs other than their reinforcing effects. Since the ability to induce a discriminative stimulus complex is a peculiar feature of a rewarding drug [6], a second objective of the work was to examine the possibility that food deprivation could also influence the discriminative stimulus properties of opiates (experiment 2).

EXPERIMENT 1

METHOD

Subjects

Male Sprague Dawley NOS rats, approximately 6 months old, were housed 4 to a cage under standard laboratory conditions (light on 7:00 a.m.–7:00 p.m., temperature 22±1°C). Water was freely available; food access was unlimited or restricted as specified under Procedure.

Drugs

Morphine hydrochloride was dissolved in saline. Doses are expressed as the salt. All treatments were administered IP (2 cc/kg).

Apparatus

The testing apparatus consisted of two highly distinctive interconnected chambers; one compartment was illuminated and had a rectangular grid floor, the other was dark with a triangular mesh floor; three photocells allowed to measure the time spent by the animals in each compartment. Each box was enclosed in a sound insulated and ventilated shell.

Procedure

The experiment consisted of four phases:

(1) Habituation phase: animals acclimated to the apparatus during three sessions (days 1–3); over this time side preference developed.

(2) Deprivation phase: rats were left in their home cages

[1]Requests for reprints should be addressed to Prof. Margherita Gaiardi, Istituto di Farmacologia, Università di Bologna, Via Irnerio, 48, I-40126 Bologna, Italia.

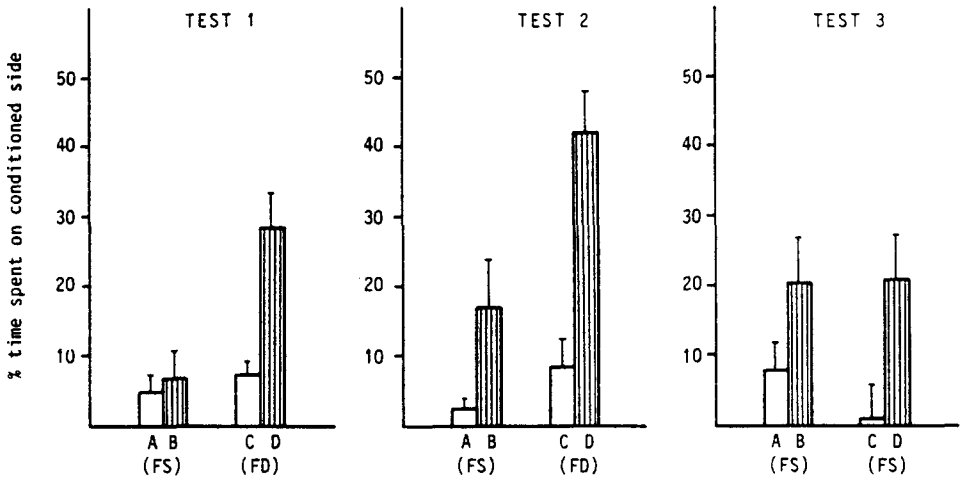

FIG. 1. Percent time spent on the initially least preferred side (as difference from the preconditioning value) after place conditioning with saline (groups A and C) or morphine (2.5 mg/kg) (groups B and D). Tests are performed in food satiated (FS) or food deprived (FD) rats, after 1 (test 1) or 3 (tests 2 and 3) conditioning sessions (see text for further details). Each column represents the mean of 7–8 values±SEM.

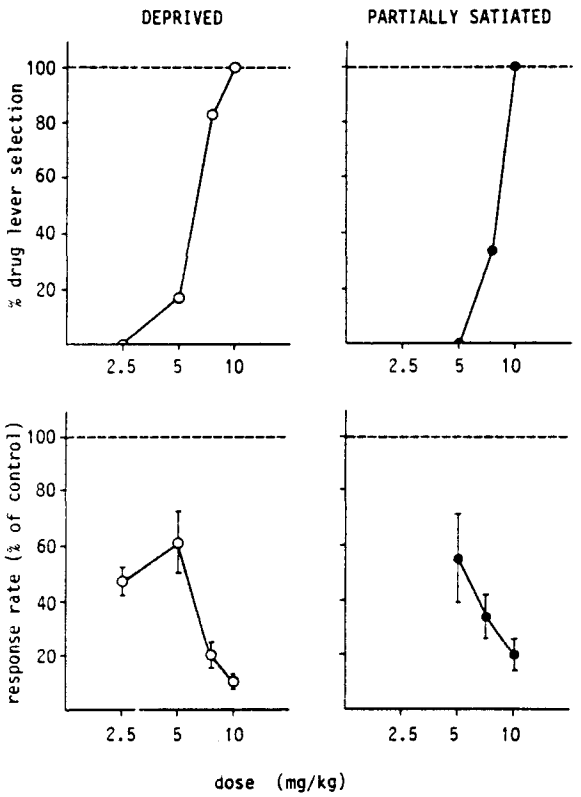

FIG. 2. Effects of various morphine doses in food deprived or partially satiated rats trained to discriminate 10 mg/kg morphine from saline. Top panels: percentage of subjects selecting the morphine lever. Bottom panels: mean response rate±SEM. Each point is based on 5–6 determinations.

under the appropriate feeding conditions (see below) (days 4–5).

(3) Conditioning phase: the animals were injected with saline (group A: n=8; group C: n=8) or morphine (2.5 mg/kg) (group B: n=7; group D: n=8) and immediately confined in the least preferred ("conditioned") side, the access to the second chamber being prevented by a removable door. This procedure was repeated for 3 days (day 6, 8 and 9).

(4) Testing phase: after the first conditioning session the rats were placed in the apparatus and allowed to explore both compartments (day 7: test 1). The same procedure was repeated after the third conditioning session (day 10: test 2) and again eight days later (day 18: test 3).

The experimental sessions lasted always 30 min and were at least 24 hr apart. Food was freely available for half the rats (groups A and B); the others (groups C and D) were: (1) fed ad lib during the habituation phase and from the end of test 2 onwards; (2) deprived for 72 hours between the last habituation session and the first conditioning session; (3) allowed to eat only for 30 min after each session for the remainder of the experiment (i.e., after the three conditioning sessions and after the first test session).

Statistical Analysis

Percent times spent on the conditioned side during the test sessions were expressed as differences from the corresponding values obtained in the last habituation session. Data relative to test 1 and 2 were analyzed together according to a three way ANOVA with repeated measures on one factor, while data from test 3 were separately submitted to a two-way ANOVA.

RESULTS

The data are summarized in Fig. 1. Results relative to test 1 and 2 indicate that more time was spent on the conditioned side by morphine treated rats; the effect was significantly greater in food deprived rats ("treatment × deprivation" interaction: $F(1,27)=9.69$, $p<0.01$) and after 3 conditioning sessions ("treatment × test" interaction: $F(1,27)=4.56$,

TABLE 1

RAT BODY WEIGHTS (g) AT THE TIME OF DIFFERENT TEST SESSIONS

Groups	Test 1	Test 2	Test 3
A + B (food satiated)	+4.667 ± 1.297	+6.533 ± 1.912	+33.533 ± 3.504
C + D (food deprived)	−63.500 ± 1.767	−86.500 ± 2.438	+8.438 ± 3.392

Groups have been pooled according to the feeding conditions. The data represent mean differences from the last habituation session ± SEM.

$p<0.05$); no "treatment × deprivation × test" interaction was found ($F<1$). In the third test the morphine effect was still present ($F(1,27)=8.84$, $p<0.01$), but similar in previously deprived and non deprived animals ($F<1$).

BRIEF DISCUSSION

Morphine conditioning caused a significant increase in the amount of time spent by rats on their least preferred side. The effect increased with the number of conditioning trials as already found by Mucha and Iversen [10]. Moreover a larger preference shift was obtained in food deprived than in food satiated animals; in this regard it is worth noting that the analysis revealed: (1) a significantly higher difference between treatments in food deprived than in food satiated rats (see the "treatment × deprivation" interaction), indicating that the increased percent of time spent in the conditioned side had been the result of morphine conditioning and not deprivation alone, and (2) no difference in food deprivation effect between test 1 and 2 (see the "treatment × deprivation × test" interaction), suggesting that pairing of environmental cues with drug effects had not been influenced by deprivation. Thus the present results demonstrate that the effect of food deprivation is not limited to the primary reinforcing properties of morphine (as assessed by self administration experiments) [1], but extends to the conditioned reinforcing properties of morphine paired cues.

EXPERIMENT 2

METHOD

Subjects

Male Sprague Dawley NOS rats, approximately 18 months old, were housed 3 to a cage under standard laboratory conditions (light on 7:00 a.m.–7:00 p.m., temperature 22±1°C). Water was freely available; food access was restricted as specified under Procedure.

Drugs

Morphine hydrochloryde was dissolved in saline. Doses are expressed as the salt. All treatments were administered IP (2 cc/kg).

Apparatus

The experimental chambers were six Skinner boxes equipped with a food tray (for a 70 mg food pellet as reinforcer), two levers (left and right) and a 3 W bulb light to provide a low level illumination during the trials. Each box was enclosed in a sound insulated and ventilated shell; experimental events and contingencies were programmed in an adjacent room by electronic circuits; responses were registered on pen recorders and/or digital counters.

Procedure

Six rats with varying experiences in drug discrimination studies served as subjects. They were trained to discriminate morphine from saline in a two lever food reinforced operant task (tandem VI 60 FR 10). The experimental sessions took place between 10:00 and 12:00 hr and food was available in the home cages only between 12:30 and 14:00 hr. Treatments (10 mg/kg morphine or 2 cc/kg saline) were administered according to the following two sequences, which were presented alternatively: M, S, S, M, M and S, M, M, S, S. The subjects were placed in the operant chambers 30 min after the treatment and were allowed to respond for 30 min. Two types of data were recorded following each session: (1) the number of responses the animal made on either of both levers before obtaining the first reinforcer (FRF) (and, thus, before having made 10 responses on the injection-appropriate lever); (2) the total number of responses (TR) made on both levers together during the entire session. Stimulus generalization tests began when a subject reached the training criterion consisting of FRF≤12 on at least 8 out of 9 consecutive daily training sessions (errors defined as FRF>12). The training procedure was continued during the test period and testing was postponed or omitted if the FRF exceeded 15 on either of the two most recent training days (errors defined as FRF>15). The rats were submitted to morphine generalization tests when food deprived (training conditions) or partially food satiated (15 min supplemental feeding in the home cages just before the administration of the test dose); the sequence of testing was randomized for each rat. On generalization tests it was noted on which lever the animal totalized 10 responses first (selected lever); then the rat was given its first food pellet and was reinforced throughout the trial upon pressing (tandem VI 60 FR 10) that lever. The total number of responses the animal made on both levers together was also measured.

Statistical Analysis

ED_{50} values and potency ratios for generalization gradients were calculated according to the method suggested by Waud [11] for a parallel line assay involving quantal responses.

The TRs were expressed as a percent of the TRs found in the most recently preceding saline session; dose effect curves were fitted using the method of the orthogonal polynomials.

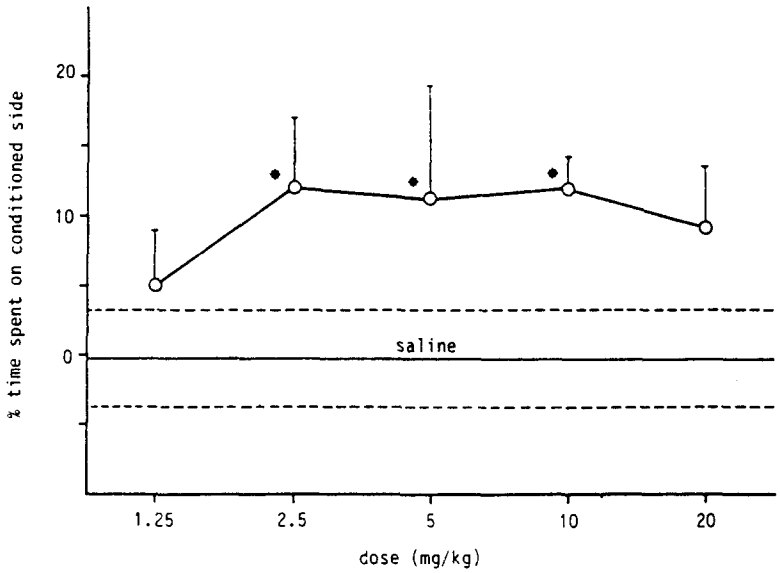

FIG. 3. Percent time spent on the initially least preferred side (as difference from the preconditioning value) after place conditioning with saline (n=17) or morphine (n=8 per dose). Tests are performed in food satiated rats after 3 conditioning sessions. The data are presented as means±SEM. The asterisk denotes a significant difference from 0 ($p<0.05$).

RESULTS

The panels of Fig. 2 show the effects of a range of morphine doses on the percentage of rats selecting the morphine lever. A Waud [11] analysis of these data indicates that gradients do not differ in slope, t(36)=0.76, p=NS; the ED_{50} value (i.e., the dose that had discriminative stimulus effects similar to those of 10 mg/kg in 50% of the animals tested) is significantly lower for food deprived (6.09 mg/kg) than for partially satiated (7.79 mg/kg) rats (potency ratio: 0.78; f.l. 95%: 0.62–0.99).

From the bottom panels of Fig. 2 it can be noted that morphine induced a linear dose related decrease in response rate both in food deprived, tb(28)=3.77, $p<0.01$, and in partially satiated, tb(28)=3.11, $p<0.01$, rats, the differences between the two regression coefficients and the two intercepts being far from significance.

BRIEF DISCUSSION

Partially food satiated rats were less sensitive to the discriminative stimulus properties of morphine. The shift in ED_{50} values was small; however, if we consider that the rats were fed only for a period so short (15 min, half an hour before the experimental session) that neither the baseline response rate under the tandem VI 60 FR 10 schedule (not shown in the figure) nor the rate decreasing effects of morphine (see Fig. 2) were significantly altered, we can conclude that the narcotic cue is substantially affected by changes in the feeding regimen.

GENERAL DISCUSSION

A first purpose of the present study was to determine whether the food deprivation effect could be replicated by the use of place conditioning. It was found that the conditioned reinforcing properties of morphine paired cues are greater in food deprived rats both after 1 and 3 conditioning sessions.

Since the deprivation effect disappeared in test 3, when animals were food satiated (even if still underweight) (see Table 1), deprivation during testing seems to have played a critical role. Possibly both interoceptive stimuli related to food deprivation and environmental cues have become paired to the morphine effect so that environmental cues alone are not enough to cause the high preference shift observed in test 2. It could be argued that in this case the test did not directly follow a conditioning session; however the same was true for all groups. Whatever the explanation, the data are fairly reminiscent of Carroll and Boe results (reported in [1]): in fact they find that rats with a previous history of both cocaine self administration and food deprivation exhibit a high rate of saline-maintained responding only when food deprived.

With regard to the magnitude of the deprivation effect, "the basic finding that the rate of drug-maintained behavior nearly doubles in food-deprived animals" has been reported [1] to hold "across routes of administration, species, and type of drug"; on the basis of the present data we can now tentatively suggest that this finding holds across tests too (i.e., self-administration and place conditioning). Furthermore an ancillary experiment we performed in control rats demonstrated that the large preference shift exhibited by food deprived animals is not attainable with any morphine dose (up to 20 mg/kg IP) in food satiated animals (see Fig. 3); therefore food deprivation does not simply enhance the reinforcing action of 2.5 mg/kg of morphine, but just increases the drug reinforcing efficacy as assessed by place conditioning. It is well known that not all drugs are abused to the same degree and the relative reinforcing efficacy of drugs is the presumed basis of their relative abuse potential [9]; conversely factors changing the reinforcing efficacy of a given

drug are expected to change its abuse potential. Thus the present results suggest that the opiate abuse potential is increased in food deprived rats.

Stimulus generalization experiments demonstrated that feeding conditions do not exclusively interact with the reinforcing actions of drugs; in fact the animals were less sensitive to the discriminative stimulus properties of morphine when partially food satiated.

A question could be raised about the specificity of this deprivation effect. In this regard it is worth noting that Jarbe *et al.* [8] found that a stimulus (light or complete darkness) common to both drug and saline trials (as is our case) apparently exerts no control over responding in a T-maze discrimination, since tests conducted under light/dark conditions equal to or different from that occurring during training give similar generalization gradients. Thus their findings lend some support to the view that the narcotic cue is specifically affected by feeding conditions. It has been repeatedly hypothesized that food deprivation lowers the organism's threshold of responsiveness to relevant environmental stimuli [2,5]; therefore the increased sensitivity to the discriminative stimulus properties of morphine we had observed in deprived animals could be a subset of this more general phenomenon.

Iversen and Fray [7] maintain that motivation has to be viewed as a non specific final common response to activation by a wide range of innate and learned stimuli; according to this theory the most salient stimulus condition at a particular moment in time dominantes the motivational state and results in the emergence of responses relevant to that stimulus. Thus, if motivation is flexible rather than rigid in nature, we can think that food deprived rats with no food available but exposed to morphine would behave like morphine deprived animals; in this regard it is worth noting that, consistent with the present results, post-dependent (i.e., morphine deprived) rats have been found more sensitive to both the reinforcing [3] and the discriminative [4] stimulus properties of morphine.

ACKNOWLEDGEMENTS

The work was supported by CNR on the "Progetto Finalizzato Medicina Preventiva e Riabilitativa, SP7" (Contract No. 85.00435.56).

REFERENCES

1. Carroll, M. E. and R. A. Meisch. Increased drug-reinforced behavior due to food deprivation. In: *Advances in Behavioral Pharmacology*, Vol 4, edited by T. Thompson, P. B. Dews and J. E. Barrett. Orlando: Academic Press, 1984, pp. 47–88.
2. Franklin, J. C., B. C. Schiele, J. Brozek and A. Keys. Observations on human behavior in experimental semistarvation and rehabilitation. *J Clin Psychol* **4**: 28–45, 1948.
3. Gaiardi, M., M. Bartoletti, A. Bacchi, C. Gubellini and M. Babbini. Previous morphine experience as a factor in determining the reinforcing efficacy of the drug. Proc. XXII Congress of the Italian Pharmacological Society, abstract No. 143, 1984.
4. Gaiardi, M., M. Bartoletti, C. Gubellini, A. Bacchi and M. Babbini. Sensitivity to the narcotic cue in non-dependent, morphine-dependent and post-dependent rats. *Neuropharmacology* **25**: 119–123, 1986.
5. Hughes, R. N. and K. M. Swanberg. Effects of food deprivation on exploration in deprivationally naive rats. *Am J Psychol* **22**: 79–83, 1970.
6. Iversen, S. D. Brain endorphins and reward function: some thoughts and speculations. In: *The Neurobiology of Opiate Reward Processes*, edited by J. E. Smith and J. D. Lane. Amsterdam: Elsevier Biomedical Press, 1983, pp. 439–468.
7. Iversen, S. D. and P. Fray. Brain catecholamines in relation to affect. In: *The Neural Basis of Behavior*, edited by A. Beckman. New York: Spectrum Publications Inc., 1982, pp. 229–269.
8. Järbe, T. U. C., T. Laaksonen and R. Svensson. Influence of exteroceptive contextual conditions upon internal drug stimulus control. *Psychopharmacology (Berlin)* **80**: 31–34, 1983.
9. Johanson, C. E. and C. R. Schuster. Animal models of drug self-administration. In: *Advances in Substance Abuse: Behavioral and Biological Research*, Vol 2, edited by N. K. Mello. Greenwich, CT: JAI Press, 1981, pp. 219–297.
10. Mucha, R. F. and S. D. Iversen. Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. *Psychopharmacology (Berlin)* **82**: 241–247, 1984.
11. Waud, D. R. On biological assays involving quantal responses. *J Pharmacol Exp Ther* **183**: 577–607, 1972.