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Morphine Withdrawal Produces Differential Effects on the Rate of Lever-Pressing for Brain Self-Stimulation in the Hypothalamus and Midbrain in Rats

GERALD J. SCHAEFER AND RICHARD P. MICHAEL[1]

Department of Psychiatry, Emory University School of Medicine, Atlanta, GA 30322 and Georgia Mental Health Institute, 1256 Briarcliff Road, N.E., Atlanta, GA 30306

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SCHAEFER, G. J. AND R. P. MICHAEL. *Morphine withdrawal produces differential effects on the rate of lever-pressing for brain self-stimulation in the hypothalamus and midbrain in rats.* PHARMACOL BIOCHEM BEHAV **18**(4) 571–577, 1983.—Rats were implanted with stimulating electrodes either in the medial forebrain bundle-lateral hypothalamus (MFB-LH) or the midbrain-central gray area (MID-CG), and were trained to lever-press for electrical brain self-stimulation (ICSS). The animals were made tolerant to morphine (15 mg/kg) by twice daily injections for a four-day period. Withdrawal was then induced either by substituting saline (spontaneous withdrawal) or by administering naloxone (1.0 mg/kg) (precipitated withdrawal). Changes in body weight, in the incidence of diarrhea, and in rates of lever-pressing for ICSS were recorded during the five-day withdrawal period. In both the MFB-LH implanted and the MID-CG implanted groups, the duration and magnitude of changes in lever-pressing were greater when withdrawal was precipitated than when it was spontaneous. Independently of the type of withdrawal, however, the behavioral disruption was greater for animals implanted in the MFB-LH than for animals implanted in the MID-CG. The changes in body weight were similar for both electrode sites and both types of withdrawal. Diarrhea only occurred in the precipitated withdrawal group and its incidence was similar for animals implanted in the two sites. Three additional groups of animals were implanted in the MFB-LH, made tolerant to morphine, and given naloxone as above. They were administered clonidine (10, 30 or 100 μg/kg) 30 min prior to naloxone to attenuate the effects of withdrawal. The 30 μg/kg dose of clonidine produced maximal attenuation of the disruption in lever-pressing. None of the doses of clonidine attenuated weight loss, but all three doses reduced the incidence of diarrhea. The ICSS procedure demonstrated that the behavior during withdrawal can be related to the brain area that is stimulated.

Brain self-stimulation Spontaneous morphine withdrawal Midbrain-central gray Precipitated morphine withdrawal Medial forebrain bundle-lateral hypothalamus Clonidine Morphine Naloxone

THERE are several reports describing the effects of morphine on brain self-stimulation (ICSS) in rats [4]. These studies have amply demonstrated that tolerance occurs to the rate-depressing effects observed during the first hour after treatment with moderate to high doses of morphine (5–30 mg/kg), although tolerance does not occur to the rate-increasing effects which appear three to five hours after morphine administration. When chronic morphine treatment is terminated either by withholding morphine or by administering an opioid antagonist, a pattern of behavioral and physiological events occurs that characterizes the morphine withdrawal syndrome. Morphine withdrawal after chronic treatment also modifies lever-pressing for brain self-stimulation [6, 8, 13]. In these studies, the majority of rats were implanted in the medial forebrain bundle-lateral hypothalamus (MFB-LH), although animals implanted in the zona incerta and dorsal brainstem were also used and showed effects similar to the MFB-LH implants. Morphine was administered chronically for between three to six days, and a variety of dose regimens and stimulation parameters were used. Lever-pressing was generally depressed under these conditions, but there was no consistent pattern during withdrawal. Furthermore, the time-course and magnitude of the behavioral changes during withdrawal have not been systematically investigated. The purpose of the present study was to evaluate the effects of both spontaneous and precipitated withdrawal on lever-pressing for ICSS in rats made moderately tolerant to morphine. In a previous study [21], tolerance to the rate-depressing effects of morphine on ICSS was demonstrated after multiple injections over a period of three days, and a four-day procedure has been used in the present study. Additionally, changes in body weight and in

[1]Requests for reprints should be addressed to Richard Michael at above address.

the incidence of diarrhea were monitored during the withdrawal period [1,10]. It was also of interest to compare effects obtained on the MFB-LH with those obtained in the midbrain-central gray area (MID-CG) which has been demonstrated to contain high concentrations of opiate receptors and enkephalins [11, 19, 23] and may be an important brain area for the development of morphine tolerance [14,15]. A final objective was to evaluate the effects of clonidine on lever-pressing during precipitated withdrawal since it has been reported that clonidine can attenuate the morphine withdrawal syndrome both in rats [24,25] and in man [7].

METHOD

Animals

Forty male rats (325 to 445 g) were used that were the first generation offspring of CFE rats obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Between test sessions, animals were housed in groups (3–4 per cage) in a colony room where they were allowed free access to food and water. The lights were on in the colony room between 07:00–19:00 hr.

Apparatus

Rats were tested in an operant chamber (31 cm by 30 cm by 29 cm high) constructed in our laboratory. The back, front and top were made of Plexiglas, and the sides were of aluminum. The grid floor was constructed of steel rods positioned 1.3 cm apart. On one side, a lever (Model G6312, Ralph Gerbrands Co., Arlington, MA) was positioned 10 cm above the grid floor. The operant chamber was placed inside a ventilated box that was sound-attenuated and lightproof. Electrical pulses were produced by a model S44 square wave stimulator (Grass Instruments, Quincy, MA), and were passed through a model CCU-1 constant current unit (Grass Instruments). Biphasic pulses were generated by a switching device which alternated the position of ground with each lever-press and produced a 200-msec train of 100 pulses/sec with a pulse duration of 2 msec. The animals used in this study were tested at current intensities which varied from 40 to 270 μA for the MFB-LH and from 70 to 220 μA for the MID-CG area. A two-channel commutator, constructed in our laboratory, was used to deliver electrical stimuli to the animal's brain [20] without inhibiting the animal's freedom of movement in any way. Spring-shielded standard hearing-aid wire (Plastic Products Co., Roanoke, VA) was connected from the base of the commutator to the electrode assembly on the head of the animal. The operant chamber was interfaced with a Digital PDP-12 computer (Digital Equipment Corp., Maynard, MA) that controlled the session clock and recorded the number of lever presses. In addition, the cumulative number of presses was displayed on an Esterline Angus X-Y Recorder (Esterline Corp., Indianapolis, IN) interfaced with the computer.

Surgery and Histology

Rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and given atropine sulfate (2.5 mg/kg, SC) to reduce respiratory distress. After positioning the animals in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA), a small burr hole was drilled in the exposed skull and the dura was incised. Four stainless steel screws were fixed to the skull to anchor the bipolar platinum electrode (Plastic Products Co.). Using the atlas of Pellegrino and Cushman [17], for one group of rats, electrodes (tip diameter = 0.25 mm) were aimed vertically at the MFB-LH using coordinates AP 5.2, L 1.7, H −2.2. A second group of animals was implanted in the MID-CG area with electrodes aimed at an angle of 10° towards the mid-sagittal plane using coordinates AP 0.0, L −0.5, H −2.5. Cranio-plastic cement was then applied to the anchor screws and electrode base to secure the electrode firmly in place. The animals were administered 100,000 units of benzathine penicillin G and procaine penicillin G IM post-operatively.

After the experiments were completed, animals were killed with an overdose of sodium pentobarbital and perfused via the heart with 10% formol-saline. Brains were placed on a microtome stage, frozen and sections were cut at 50 μm; alternate sections were stained with cresyl violet and Weil's stain. The histological material was then examined to determine the precise locations of the electrode tips.

TABLE 1

SUMMARY OF PROCEDURE FOR ESTABLISHING TOLERANCE AND EVALUATING WITHDRAWAL FROM MORPHINE ON BRAIN SELF-STIMULATION

		Baseline	Tolerance Development	Withdrawal
	Days	M T W	T F S S	M T W T F
Groups		1 2 3	4 5 6 7	8 9 10 11 12
1, 5		Tested Saline	No Testing Saline, twice/day	Tested Saline
2, 6		Tested Saline	No Testing Saline, twice/day	Tested Naloxone (1 mg/kg)
3, 7		Tested Saline	No Testing Morphine, twice/day (15 mg/kg)	Tested Saline
4, 8		Tested Saline	No Testing Morphine, twice/day (15 mg/kg)	Tested Naloxone (1 mg/kg)

Procedure

Beginning one week after surgery, rats were placed in the operant chamber and trained to press the lever to receive brain stimulation on a continuous reinforcement schedule. The current intensity was adjusted over a 2–3 week period until the daily variability in the rate of responding was less than 20% of the mean rate. At this time the range of response rates for all animals was between 2000–4000 per hour. The testing phase was then begun. Animals were weighed at the same time each day before administration of either saline or naloxone which was injected 15 min before each 1 hr test session. The procedure for establishing tolerance and evaluating the effects of morphine withdrawal is summarized in Table 1. For each electrode site, four groups of animals were tested. All groups were initially tested for three days (M-T-W) with saline injections (1 ml/kg). These three daily sessions served as a baseline against which to compare operant behavior during withdrawal. For the next four days (T-F-S-S) the animals were not tested, but received two injec-

tions per day at approximately 8:00 a.m. and 6:00 p.m. Groups 1, 2, 5 and 6 received saline (1 ml/kg, SC) while Groups 3, 4, 7 and 8 received morphine sulfate (15 mg/kg, SC). For the remaining five days (withdrawal phase) animals were tested for lever-pressing behavior using the same parameters as during the baseline phase with saline. Drug treatments were as follows: Groups 1, 3, 5 and 7 received saline (1 ml/kg) 15 min before testing; Groups 2, 4, 6 and 8 received naloxone hydrochloride (1. 0 mg/kg) 15 min before testing. In all cases, test sessions were of 1 hr duration. Rats made tolerant to morphine were never re-used. Different groups of animals were used to evaluate the effects of clonidine. Animals were implanted in the MFB-LH and trained to lever-press as previously described. One group of rats was used to assess the effects of clonidine on lever-pressing prior to morphine administration. These animals were injected SC with either saline or clonidine (10–100 μg/kg) 45 min before being tested for 1 hr. Other groups were made tolerant to morphine as previously described. During the withdrawal phase, the animals were administered clonidine (10–100 μg/kg) 30 min prior to receiving naloxone (1.0 mg/kg), and after 15 min were placed in the test chamber for 1 hr. Animals were observed for diarrhea for 90 min after the injection of saline or naloxone during each day of withdrawal, and the number of animals in each group that showed diarrhea was recorded. The lever-pressing behavior and changes in body weight were recorded as described previously.

Drugs

The drugs used were morphine sulfate, (Merck and Company, Inc., Rahway, NJ), naloxone hydrochloride (courtesy of Endo Laboratories, Garden City, NJ) and clonidine hydrochloride (courtesy of Boehringer Ingelheim Ltd., Ridgefield, CT). The vehicle for all drugs was 0.9% saline, and they were injected SC in a volume of 1.0 ml/kg body weight. Doses were expressed as the free base.

Data Analysis

The total number of lever-presses during a one hour test (response rate) was used for statistical analysis. For each animal, response rates during the three-day baseline period were averaged and the mean response rate served as the control for comparison with behavior during withdrawal. Response rates during withdrawal are presented as percentages of the mean rate during the baseline period. The response rate data for the four groups of animals implanted in the MFB-LH were first analyzed by a factorial analysis of variance with repeated measures on one factor [16]. Specific comparisons between means in the MFB-LH groups as well as comparisons between MFB-LH and MID-CG groups were made by *t*-tests (two-tailed). The response rate data for the animals in the MID-CG were also analyzed with a factorial analysis of variance followed by *t*-tests for specific comparisons. These procedures were also used to analyze body weight changes. Body weight during the withdrawal period was compared with the mean body weight during the baseline period. To analyze the clonidine dose-response curve, a randomized block design [16] was used, followed by Dunnett's test [3] to determine which doses were significantly different from vehicle. Finally, when clonidine was administered prior to nalonone during withdrawal, the response rate and weight data were treated as described above for the MFB-LH results.

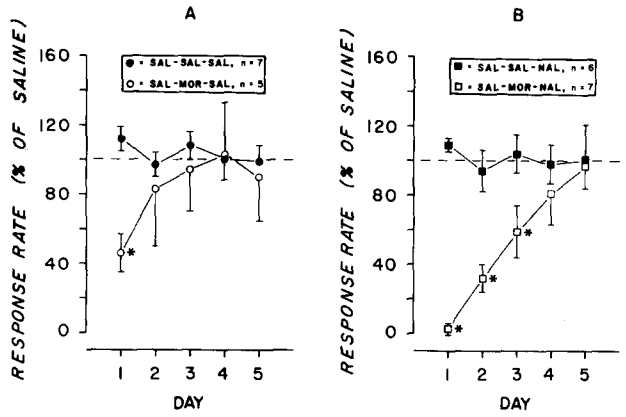

FIG. 1. The effects of withdrawal from morphine on the rate of lever-pressing for ICSS with electrodes implanted in the MFB-LH. Panel A shows the effects of spontaneous withdrawal over a five-day period (○) compared with the control group that received saline during the development of tolerance (●). Panel B shows the effects of withdrawal precipitated by naloxone (1.0 mg/kg) over a five-day period (□) compared with a control group that received saline during the development of tolerance (■). Horizontal interrupted lines in this and subsequent figures give saline values during the Baseline period. Vertical bars give standard errors of means (SEM). n=number of animals per group. The mean control rates (±SEM) of responding for the groups were: SAL-SAL-SAL = 3828±231; SAL-MOR-SAL = 3463±359; SAL-SAL-NAL = 3714±615; SAL-MOR-NAL = 3518±396. *Significantly different from corresponding control group ($p<0.05$–0.01).

RESULTS

Medial Forebrain Bundle-Lateral Hypothalamus

The factorial analysis of variance used here generated three F-ratios. The first ratio (withdrawal conditions) evaluated the significance of changes in the rate of lever-pressing between the four groups during withdrawal irrespective of the individual daily changes. The second ratio (days) evaluated changes in the rate of lever-pressing between the five days of withdrawal irrespective of whether withdrawal was spontaneous or precipitated. The third ratio (withdrawal conditions × days interaction) simultaneously compared the rate of lever-pressing between the four groups during each day of withdrawal. The interaction effect, therefore, evaluated whether or not there was a significant difference in the pattern of recovery of lever-pressing during withdrawal. Comparable ratios were produced for the weight data in the MFB-LH groups, as well as for response rate and weight data in the MID-CG groups.

Figure 1A shows the mean response rates of rats with electrodes in the MFB-LH during the five days of spontaneous withdrawal from morphine (open circles) (Group 3) compared with its control group (closed circles) (Group 1). Figure 1B shows the data for rats with electrodes also in the MFB-LH during precipitated withdrawal from morphine (open rectangles) (Group 4) compared with its control group (closed rectangles) (Group 2). The analysis of variance indicated no significant effect of withdrawal conditions. However, both the days, $F(4,84)=89.02$, $p<0.001$, and the withdrawal conditions × days interaction effect, $F(12,84)=61.79$, $p<0.001$, were highly significant. Therefore, changes in the rates of lever-pressing during the five days of withdrawal

TABLE 2

CHANGES IN BODY WEIGHT DURING THE WITHDRAWAL PERIOD†

		Day 1	Day 2	Day 3	Day 4	Day 5
			A. MFB-LH			
Group						
1	(Sal-Sal-Sal)	102 ± 0.56	102 ± 0.51	103 ± 0.53	103 ± 0.62	103 ± 0.53
2	(Sal-Sal-Nal)	102 ± 0.31	101 ± 0.31	101 ± 0.31	102 ± 0.40	102 ± 0.37
3	(Sal-Mor-Sal)	94 ± 1.57*	90 ± 2.21*	88 ± 1.96*	92 ± 1.98*	94 ± 1.75*
4	(Sal-Mor-Nal)	96 ± 0.89*	91 ± 1.02*	90 ± 1.10*	93 ± 1.13*	95 ± 1.38*
			B. MID-CG			
Group						
5	(Sal-Sal-Sal)	103 ± 0.71	103 ± 0.48	104 ± 0.50	104 ± 0.65	104 ± 0.63
6	(Sal-Sal-Nal)	101 ± 0.75	100 ± 0.65	99 ± 0.58	99 ± 1.03	99 ± 1.20
7	(Sal-Mor-Sal)	95 ± 0.81*	89 ± 0.73*.	88 ± 1.12*	90 ± 1.07*	92 ± 0.80*
8	(Sal-Mor-Nal)	92 ± 3.76	91 ± 1.89*	90 ± 1.68*	90 ± 1.97*	92 ± 1.50*
			C. Clonidine Pre-Treatment (MFB-LH)‡			
Clonidine dose (μg/kg)						
10		92 ± 1.12*	89 ± 1.89*	89 ± 1.32*	90 ± 1.25*	92 ± 1.63*
30		92 ± 1.21*	90 ± 0.68*	90 ± 0.58*	89 ± 0.89*	90 ± 0.93*
100		94 ± 2.01*	89 ± 1.39*	88 ± 1.55*	89 ± 1.69*	90 ± 2.28*

*Significantly different from corresponding control group $p<0.05$–0.01 (See Results).

†Values obtained by expressing individual animals weights as a percentage of the mean weight during the 3-day Baseline period and then calculating the group mean and SEM.

‡Animals received 1.0 mg/kg naloxone 30 min after receiving clonidine.

differed in the four groups. Subsequent t-tests indicated that Group 3 was significantly less than Group 1, $t(10)=5.45$, $p<0.001$, on the first day of withdrawal only, while Group 4 was significantly less than Group 2 on the first three days of withdrawal, $t(11)=34.09$, $p<0.001$; $t(11)=4.5$, $p<0.001$; $t(11)=2.35, p<0.05$, respectively. Furthermore, Group 4 was significantly less than Group 3 on the first day of withdrawal, $t(10)=4.57, p<0.01$.

Changes in the body weights of these animals are shown in Table 2A. The control values indicated that the animals tended to gain weight over the 12-day procedure used here. The analysis of variance for the weight data produced both significant effects of withdrawal conditions, $F(3,21)=34.16$, $p<0.001$, and days, $F(4,84)=38.00, p<0.001$, as well as the withdrawal conditions × days effect, $F(12,84)=10.24$, $p<0.001$. Using t-tests to further examine these effects, it was found that Group 3 significantly differed from Group 1 on all five days of withdrawal, while Group 4 significantly differed from Group 2 during the entire 5-day period. However, Group 3 did not differ from Group 4 on any withdrawal day. Therefore, both spontaneous and precipitated withdrawal produced similar reductions in body weight. The greatest weight loss occurred on the third day of withdrawal for both groups, although the most severe disruption of lever-pressing occurred on the first day of withdrawal. Seven out of seven animals, and five of seven animals showed diarrhea on the first and second days, respectively, of precipitated withdrawal, and none showed diarrhea thereafter. Diarrhea was not observed in any animals during spontaneous withdrawal.

Midbrain-Central Gray Area

Figure 2A shows the mean response rates of rats with electrodes in the MID-CG area during the five days of spontaneous withdrawal from morphine (open circles) (Group 7) compared with its control group (closed circles) (Group 5). Figure 2B shows the data for rats with electrodes also in the MID-CG area during precipitated withdrawal from morphine

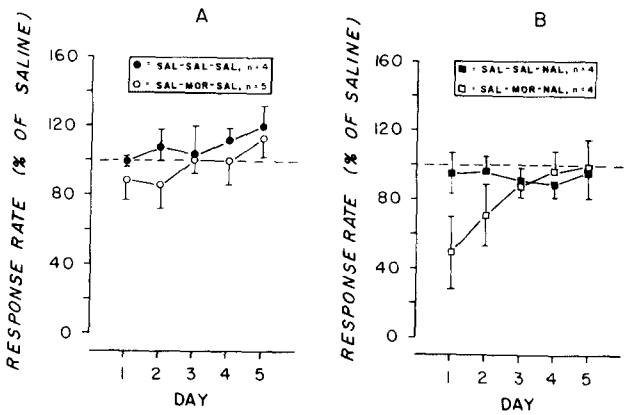

FIG. 2. The effects of withdrawal from morphine on the rate of lever-pressing for ICSS with electrodes in the MID-CG area. Panel A shows the effects of spontaneous withdrawal over a five-day period (○) compared with the control group that received saline during the development of tolerance (●). Panel B shows the effects of withdrawal precipitated by naloxone (1.0 mg/kg) over a five-day period (□) compared with a control group that received saline during the development of tolerance (■). The mean control rates (±SEM) of responding for the groups were: SAL-SAL-SAL = 3239±494; SAL-MOR-SAL = 2936±376; SAL-SAL-NAL = 2294±467; SAL-MOR-NAL = 2221±414. Other abbreviations as in Fig. 1.

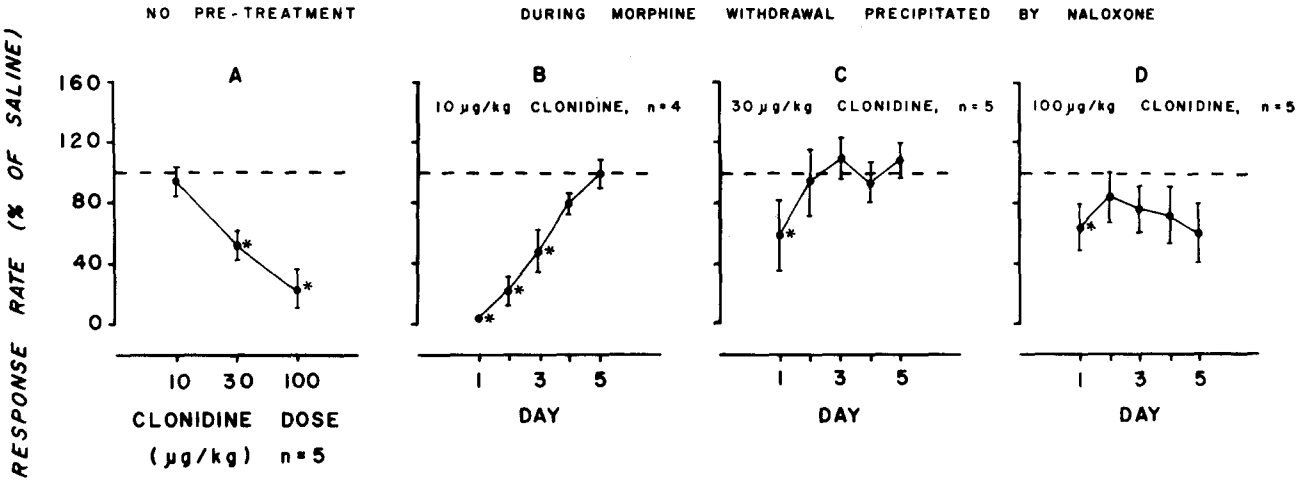

FIG. 3. Panel A: The effects of graded doses of clonidine alone on the rate of lever-pressing for ICSS with electrodes in the MFB-LH. Panels B-C-D: The effects of graded doses of clonidine on the disruption of lever-pressing for ICSS during withdrawal from morphine precipitated by naloxone (1.0 mg/kg). Clonidine was administered 30 min before naloxone, and after an additional 15 min the rats were tested for 1 hr. The 30 µg/kg dose of clonidine produced the greatest attenuation of naloxone's disruptive effects on lever-pressing. The mean control rates (±SEM) of responding for the groups were: Clonidine dose-response curve (A) = 3581±1033; 10 µg/kg clonidine (B) = 3244±1394; 30 µg/kg clonidine (C) = 3909±1340; 100 µg/kg clonidine (D) = 4942±1638. Other abbreviations as in Fig. 1.

(open rectangles) (Group 8) compared with its control group (closed rectangles) (Group 6). The analysis of variance for the MID-CG data produced only a significant days effect, $F(4,52)=6.39$, $p<0.001$. The withdrawal conditions and its interaction with the days effects was not significant as confirmed by subsequent t-tests. Throughout the 5-day withdrawal period Group 5 did not differ from Group 7, and Group 6 did not differ from Group 8. Although the response rate for Group 8 was less than that for Group 7 on the first day of withdrawal (49 vs 89%), this difference was not significant. It is also apparent from Fig. 2 that the disruption of lever-pressing was much less severe for the MID-CG animals than for the MFB-LH animals, and this was also apparent from a direct comparison of the groups using t-tests. Group 3 was significantly different from Group 7 on the first day of withdrawal, $t(8)=2.98$, $p<0.02$, while Group 4 was significantly different from Group 8 on the first two days of withdrawal, $t(9)=2.98$, $p<0.02$; $t(9)=2.30$, $p<0.05$, respectively.

Changes in body weight for the MID-CG animals are shown in Table 2B, and were generally similar to those of the MFB-LH group. During both spontaneous and precipitated withdrawal, significant reductions in body weight occurred; this resulted in a significant withdrawal conditions effect, $F(3,13)=30.38$, $p<0.001$; days effect, $F(4,52)=12.63$, $p<0.001$, and withdrawal conditions × days interaction effect, $F(12,52)=3.08$, $p<0.005$. Group 7 animals weighed significantly less than Group 5 animals during all five days of withdrawal, while Group 8 animals were significantly lighter than Group 6 animals during the last four days of withdrawal. There were no significant differences between Groups 7 and 8, between Groups 3 and 7 or between Groups 4 and 8 during the withdrawal period. Four out of four animals showed diarrhea on both the first and second days of precipitated withdrawal, and one animal had diarrhea on the third day. There was no diarrhea during spontaneous withdrawal.

Clonidine Pre-Treatment During Withdrawal

The effects of clonidine alone on lever-pressing in untreated animals are shown in Fig. 3A. Over the dose-range 10–100 µg/kg, clonidine produced a significant dose-dependent decrease in response rates. The effects of clonidine pre-treatment during precipitated withdrawal (1.0 mg/kg naloxone) are shown in Fig. 3B, C, D. An analysis of variance was used to compare the effects of Group 2 with the three groups administered 10, 30 and 100 µg/kg of clonidine, respectively, during precipitated withdrawal. The withdrawal conditions effect was not significant. The days effect, $F(4,64)=10.69$, $p<0.001$, and withdrawal conditions × days interaction, $F(12,64)=9.28$, $p<0.001$, were both significant. Subsequent t-tests revealed that the 10 µg/kg clonidine group was significantly different from Group 2 on days 1, $t(8)=27.80$, $p<0.001$; 2, $t(8)=4.40$, $p<0.01$; and 3, $t(8)=3.18$, $p<0.02$. The 30 µg/kg clonidine group and the 100 µg/kg clonidine groups were both significantly different from Group 2 on the first day of withdrawal, $t(9)=2.32$, $p<0.05$; $t(9)=3.06$, $p<0.02$, respectively. The animals receiving clonidine were also compared with Group 4. The 10 µg/kg group was not significantly different from Group 4 on any withdrawal day. The 30 µg/kg group was significantly different from Group 4 on day 1, $t(10)=2.85$, $p<0.02$; 2, $t(10)=3.15$, $p<0.02$; and 3, $t(10)=2.41$, $p<0.05$, while the 100 µg/kg group was significantly different from Group 4 on day 1, $t(10)=4.62$; $p<0.001$ and 2, $t(10)=3.15$, $p<0.02$, of withdrawal. An additional analysis was used to compare weight changes in Group 2 with the three clondine pre-treatment groups. The withdrawal conditions, $F(3,16)=28.90$, $p<0.001$; days, $F(4,64)=23.80$, $p<0.001$, and withdrawal conditions × days effect, $F(12,64)=4.0$, $p<0.001$, were all significant. Subsequent t-tests indicated that each of the three clonidine groups differed from Group 2 on all five days of withdrawal. Thus, pre-treatment with clonidine had no

detectable effect on the weight changes induced by precipitated withdrawal (Table 2C), but all three doses reduced the occurrence of diarrhea. There was no diarrhea with 100 μg/kg clonidine pre-treatment.

Brain Histology

The distribution of the sites of electrode tips in brains was closely similar to that already published [22], and they are not re-illustrated here. The electrode sites in the MFB-LH group were found in the lateral hypothalamic portion of the medial forebrain bundle. In the MID-CG group, the electrode tips were more widely distributed in the midbrain and central gray area.

DISCUSSION

These experiments provide clear information about the time-course of both spontaneous and precipitated withdrawal in rats made moderately tolerant to morphine using operant behavior in a brain self-stimulation procedure. It is evident that the effects on behavior depend upon the type of withdrawal that occurs. Thus, in both the MFB-LH and the MID-CG area the behavioral disruption seen on the first and subsequent withdrawal days was more pronounced in the naloxone-precipitated withdrawal group than in the spontaneous withdrawal group. This observation is consistent with the effects on operant responding for food reward during morphine withdrawal [5].

Previous studies have demonstrated that withdrawal from chronic morphine treatment alters operant responding for ICSS reinforcement with stimulating electrodes in the MFB-LH, and the change is typically a reduction in response rates. In the present study, it was demonstrated that the behavioral changes are dependent upon the site of stimulation. Independently of how withdrawal was produced, animals implanted in the MFB-LH showed a greater disruption of lever-pressing than animals implanted in the MID-CG area. It is tempting to speculate that stimulation of the MID-CG area produced a greater release of enkephalins than stimulation in the MFB-LH since the former area contains higher concentrations of opiate receptors and enkephalins. Thus, the release of enkephalins would have the same functional effect as the administration of exogenous opioids during withdrawal from drugs. In support of this notion, it has recently been demonstrated that morphine will attenuate the disruption of lever-pressing for water presentation when administered after the onset of withdrawal from morphine [2]. Also consistent with this idea is a recent observation [22] that rats are more sensitive to the effects of naloxone and naltrexone in a fixed-ratio schedule of reinforcement when electrodes are implanted in the MID-CG than in the MFB-LH. However, in this study there were no differences between the MFB-LH and MID-CG groups in other signs of withdrawal, such as weight loss and diarrhea, perhaps because there was only one hour of ICSS available during each day of withdrawal. Speculation about these differences must be limited because only two sites have been investigated, but the data certainly warrant further investigation of how the release of enkephalins might influence withdrawal from chronic morphine treatment.

Akera and Brody [1] reported that weight loss is a useful index of morphine withdrawal. In the present study, the percentage of weight loss in animals receiving 30 mg/kg/day was similar to that observed by Akera and Brody in animals receiving the same dosage. They found, as we did, that the maximal loss of body weight occurred on the third day of withdrawal. Diarrhea is also a prominent feature of naloxone-precipitated withdrawal in rats [10]. In both the MFB-LH and MID-CG groups, all animals showed diarrhea on the first day of precipitated withdrawal, at a time when operant responding was maximally disrupted. Diarrhea was no longer observed by the fourth day at which time response rates had returned to baseline values. Thus, diarrhea and operant behavior showed a similar time-course during withdrawal, which was somewhat different from the time-course of weight loss. The changes in weight and bowel function were similar in both the MFB-LH and MID-CG groups, but the degree of behavioral disruption was clearly greater for animals in the MFB-LH group.

In a previous experiment [22] we reported that naloxone produced a graded decrease in the rate of lever-pressing for ICSS on a fixed-ratio: 15 schedule. Under that partial reinforcement schedule 1.0 mg/kg of naloxone produced a 17% and a 55% reduction in lever-pressing with electrodes in the MFB-LH and MID-CG, respectively. In the present experiments, which employed a continuous reinforcement schedule, naloxone administered to control groups did not produce a significant decrease in responding in animals implanted in either brain site. Thus, this differential effect of naloxone may depend upon the amount of work required to obtain a reward, namely, the schedule of reinforcement. We are currently examining the interaction between naloxone and the schedule of ICSS reinforcement in more detail (West, Schaefer and Michael, in press).

We wished to ascertain if the disruption of lever-pressing and associated changes occurring during naloxone-precipitated withdrawal could be attenuated by clonidine, an alpha-adrenergic agonist used clinically to treat hypertension [12,18]. If the ICSS procedure represents a reasonable model for the study of morphine withdrawal, then one might expect clonidine to attenuate the behavioral and autonomic changes seen during withdrawal. The present results confirmed and extended previous observations that clonidine indeed attenuates the morphine withdrawal syndrome [7, 24, 25]. When administered alone to previously untreated rats, clonidine reduced the rate of responding in a dose-dependent fashion. This was consistent with the results of Sparber and Meyer [24] using food reinforcement and with those of Herberg, Stephens and Franklin [9] using ICSS. An intermediate dose of clonidine (30 μg/kg) which itself significantly reduced the response rate in untreated rats, markedly attenuated the behavioral disruption of the animals during precipitated withdrawal. The highest dose of clonidine (100 μg/kg) attenuated the disruption of lever-pressing on the first and second day, but its own disruptive effects became apparent as the morphine-withdrawal syndrome subsided, producing a biphasic effect. All three doses of clonidine markedly reduced the incidence of diarrhea without affecting weight loss. We have previously shown that, with twice daily injections of morphine for three days, tolerance to the initial disruption of operant responding for ICSS will develop [21]. The present experiments have demonstrated that when saline is substituted for morphine after the development of tolerance, disruption of operant responding again occurs and its duration is approximately three days. When a narcotic antagonist such as naloxone is substituted for morphine, a more severe disruption of operant behavior occurs.

When tolerance to morphine has developed, the brain self-stimulation techniques employed here show that the

magnitude and duration of the behavioral changes occurring during detoxification depend on the location of the neuronal systems being stimulated.

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