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Increasing the Work Requirements Lowers the Threshold of Naloxone for Reducing Self-stimulation in the Midbrain of Rats

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WEST, C. H. K., G. J. SCHAEFER AND R. P. MICHAEL. *Increasing the work requirements lowers the threshold of naloxone for reducing self-stimulation in the midbrain of rats.* PHARMACOL BIOCHEM BEHAV **18**(5) 705–710, 1983.—Rats were trained to lever-press for intracranial self-stimulation (ICSS) with electrodes in the midbrain central gray area. The effects of naloxone (0.1–30.0 mg/kg, SC) on a continuous reinforcement (CRF) schedule were determined. Rats were then re-trained on higher fixed-ratio (FR) schedules, and naloxone was re-tested at FR: 5, 10, 15 and 20. Only moderate reductions in lever-pressing rates were obtained at the highest dose of naloxone under CRF and FR: 5 schedules. In contrast, pronounced, dose-dependent reductions in ICSS rates occurred at FR: 10, 15 and 20. The time-course for this reduction at FR: 20 was consistent with an opiate-antagonistic action of naloxone. The modest decrease in locomotor activity produced by naloxone in a matched group of control rats was not sufficient to account for the effects on ICSS. The threshold of naloxone for reducing the rate of ICSS lever-pressing was lowered by increasing the effort and/or time requirement for each reinforcement.

Brain self-stimulation Naloxone Fixed-ratio schedules Midbrain-central gray Spontaneous locomotor activity

It is well known that psychotropic drugs alter the behavior maintained by intracranial self-stimulation (ICSS) in rats. In the typical paradigm animals are trained to press a lever for ICSS on a continuous reinforcement schedule, and changes in the rate of responding are attributed to a drug effect. This procedure has been widely used to study opioid compounds [5]. As we have noted previously [23], studies with opioid antagonists, such as naloxone, have not given consistent results. With doses of naloxone up to 30 mg/kg, no significant changes [11, 13, 28], modest decreases [25], and marked decreases [26] in response rates have all been reported. The observed effects of other psychotropic drugs on the rate of ICSS have been shown to be influenced by the behavioral task itself [8, 10, 29]. Since this could contribute to the discrepancies in the literature, we have tested the effects on the rate of lever-pressing when the ICSS reinforcement was controlled by partial reinforcement schedules. With a fixed-ratio: 15 (FR: 15) schedule, it was found that both naloxone and naltrexone produced dose-dependent reductions in the rate of lever-pressing when the stimulating electrodes were implanted in either the medial forebrain bundle-lateral hypothalamus (MBF-LH) or in the midbrain-central gray (MID-CG) [23]. These effects do not appear to be peculiar to fixed-ratio schedules, since Franklin and Robertson [9] reported that naloxone produced a dose-dependent reduction in lever-pressing for ICSS when the reinforcement was delivered on a random interval 10 sec schedule.

Since the opioid antagonists represent a phenomenon in which the drug effect depends, at least in part, on the schedule of reinforcement, it seemed important to study systematically the effects of various fixed-ratio schedules on the rate of lever-pressing for ICSS. In this report we compare the effects of continuous reinforcement with FR: 5, 10, 15 and 20 schedules following graded doses of naloxone. In addition, we tested the effects of naloxone on spontaneous locomotor activity to control for any generalized behavioral change which could affect lever-pressing behavior. Finally, the time-course of the reduction in lever-pressing following 30 mg/kg naloxone was determined with the FR: 20 schedule in order to compare the duration of effects produced by naloxone in the ICSS procedure with its duration of action in other test conditions.

METHOD

Animals

The subjects were 16 adult male Sprague Dawley rats (King Animal Labs, Inc., Oregon, Wis) (weighing 310–360

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g). Rats were housed 2–4 per cage in a colony room lighted between 7:00 a.m. and 7:00 p.m. with free access to food and water.

Apparatus

Intracranial self-stimulation. The animals were tested in an operant chamber with inside dimensions of 31×30×29 cm high [23]. A model G6312 lever (Ralph Gerbrands) was positioned on one wall 10 cm above the grid floor. The chamber was placed inside a sound attenuating box. Electrical pulses were produced by two Model S44 Grass stimulators and the output from each stimulator was passed through a constant current unit and a stimulus isolation unit. Stimuli of alternating polarity were generated by a switching device which triggered first one stimulator, programmed for a positive pulse train, and then the other stimulator, programmed for a negative pulse train for each successive reinforcement. The stimuli consisted of 200 msec trains of square-wave pulses at 100 Hz with a pulse duration of 1 msec. The stimuli were delivered to the brain through a two channel commutator [22] connected to the rat's skull by a length of spring-shielded hearing aid wire. A permanent record of lever-pressing activity was obtained on a cumulative recorder together with a digital readout for each session.

Locomotor activity monitor. Locomotor activity was measured using an Omnitech Digiscan Model RXY activity monitor which measured horizontal locomotor activity by counting the total number of infrared light beam interruptions. During monitoring, the animal was placed inside a clear acrylic cage (39.4×39.4×30.5 cm high, inside dimensions), positioned within the "main frame" of the Digiscan monitor, itself within a sound attenuating box. In addition to the number of beam interruptions being displayed on a digital electronic counter, an analogue measure of animal running speed was produced by feeding the output of the monitor into a Beckman strip-chart recorder.

Surgery and Histology

Rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and were given atropine sulfate (0.25 mg, SC) to reduce respiratory distress. After placement in a stereotaxic instrument, the skull was exposed and drilled, and the dura was incised. A bipolar platinum electrode (tip diameter = 0.125 mm, Plastic Products) was angled 10° toward the mid-sagittal plane and was lowered into the exposed brain toward the midbrain-central gray area using the following coordinates: AP 0.0, L −0.5, H −2.5 [16]. The electrode was secured with cranioplastic cement and stainless steel screws. When the experiment was completed, animals were decapitated and the heads immersed in 10% formalin. Frozen sections were cut at 50 μ, and stained either with Weil's strain or cresyl violet to locate accurately the electrode tips.

Procedures

Intracranial self-stimulation. After recovery, animals were trained for 15 min per day. Animals that responded reliably were trained on a CRF schedule until response rates stabilized. For each CRF and FR study, a current-intensity/response rate curve was determined for each animal, and the final current intensity was selected on that part of the curve where a high, stable rate was produced [19]. Once the appropriate current intensity was selected, it was kept constant for the duration of the study.

In each study, rats were tested twice per week with naloxone (0.1–30 mg/kg, as free base; courtesy Endo Laboratories, Garden City, NY) in a random sequence and with vehicle (0.9% saline) on the preceding days. All injections were given SC 15 min before a 20-min test session. At the beginning of each session, up to ten noncontingent "priming" reinforcements were given if the animal did not begin to lever-press spontaneously. When each study was completed, the animals were re-trained on the next higher schedule of reinforcement, and current intensities were adjusted as described above. The effects of naloxone were determined using this procedure for CRF, FR: 5, FR: 10, FR: 15 and FR: 20. Two of the seven rats could not be trained to lever-press reliably for FR: 15, and after this experiment, three drug-naive rats were added to the group for the FR: 20 study. After the animals completed the FR: 20 experiment, the time-course of the effects of naloxone was determined. Animals received 30 mg/kg naloxone either 15, 60, 120 or 180 min prior to the 20-min test session.

Spontaneous locomotor activity. Five rats that did not reliably lever-press for ICSS on the CRF schedule were used to measure the effects of naloxone on locomotor activity. After three days of habituation to the activity monitor and saline injections, animals were administered either saline or naloxone 15 min before the activity session which always occurred between 2:00 and 3:45 p.m. The total number of infrared beam interruptions during the last 10 min of the 12-min activity session provided the locomotor activity score. The doses of naloxone were administered in random sequence and were given twice for each animal on different days.

Data Analysis

Intracranial self-stimulation. The total number of lever presses made during the 20-min test session provided the data for all the ICSS experiments. The response rate for a given dose of drug is presented as a percentage of the rate of responding on the preceding saline day. By using this transformation, direct comparisons between each FR experiment can readily be made, as well as comparisons between animals having different baseline rates of responding. Analyses of variance, using a randomized block design, were used to assess the significance of differences between response rates [14]. Dunnett's test (two-tailed) was used to compare differences between response rates after saline administration and different doses of naloxone.

Spontaneous locomotor activity. Since each animal received each dose of naloxone twice, the two scores at each dose were averaged and the mean value was used for statistical analysis. All scores for saline days were averaged, and the activity scores for each drug dose are presented as a percentage of the mean saline scores. An analysis of variance followed by Dunnett's test was then performed on these data as described above for the ICSS data.

RESULTS

Spontaneous Locomotor Activity

The effects of graded doses of naloxone on locomotor activity are shown in Fig. 1A, and it appears that changes in locomotor activity do not account for the changes in ICSS responding described below. The analysis of variance was significant, $F(4,16)=3.2$, $p<0.05$, but Dunnett's test indicated that only the 1.0 mg/kg dose was significantly different from

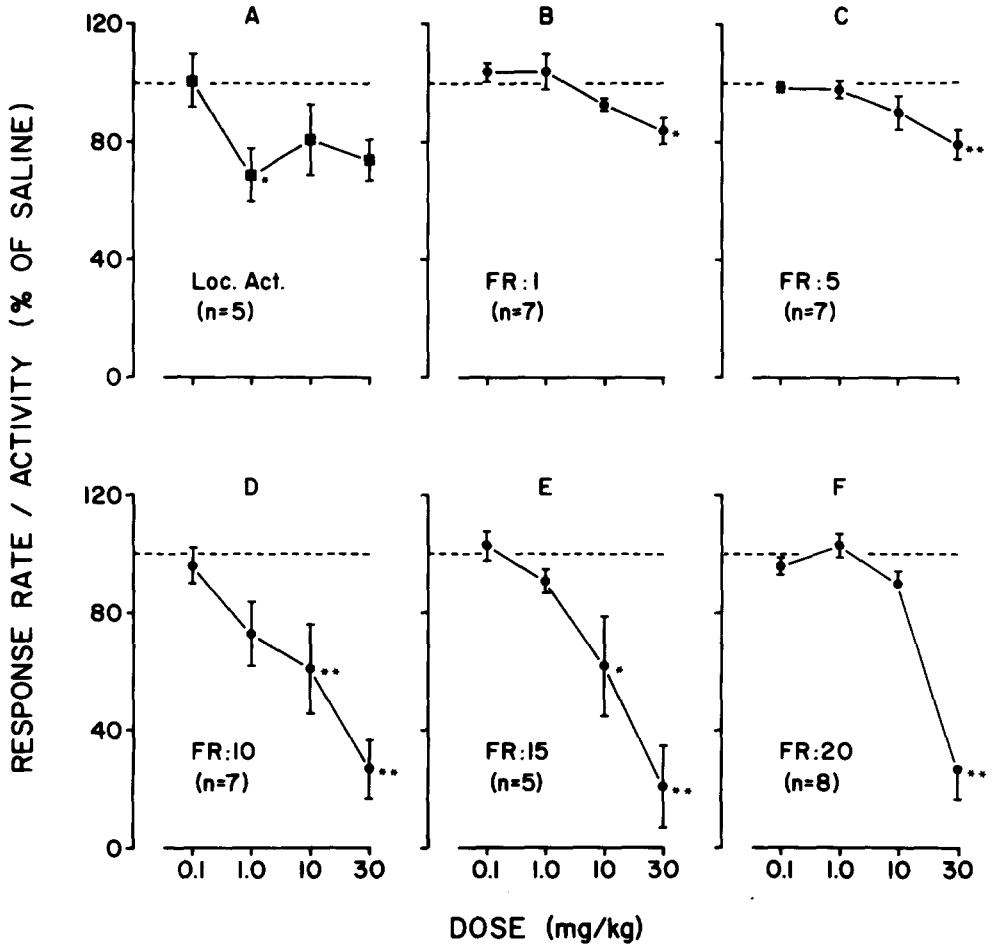

FIG. 1. Effects of graded doses of naloxone on spontaneous locomotor activity (A) and on lever-pressing for intracranial self-stimulation (B–F) using different schedules of reinforcement. Horizontal interrupted lines in this and the subsequent figure give saline values during control periods. Vertical bars give standard errors of means. n=number of animals per group. Significantly less than saline control at $p<0.05$*, and at $p<0.01$**.

saline. The average number of beam interruptions following saline administration was 927±129 (±SE) during the 10 min activity sessions (range: 701–1246). Clearly, the modest decreases in locomotor activity at 10 and 30 mg/kg naloxone cannot be responsible for the decrements in lever-pressing at these doses.

Intracranial Self-stimulation

Rats that were tested with graded doses of naloxone on a CRF schedule had a mean saline baseline response rate of 1083±122 (±S.E.) presses per 20 min (range: 653–1539). These response rates were produced at a mean current intensity of 141±23 μA (range: 60–240). Fig. 1B shows the effects of 0.1–30 mg/kg doses on response rates with CRF. A significant analysis of variance in this group, $F(4,24)=5.6$, $p<0.005$, was the result of a reduction in lever-pressing to 84±4% of baseline at the 30 mg/kg dose level. A similar effect occurred with FR: 5, $F(4,24)=2.8$, $p<0.05$, where the 30 mg/kg injection produced a decrease to 79±5% of baseline (Fig. 1C). For this experiment the mean baseline response rate was 2133±227 presses per 20 min (range: 1383–2981) which occurred at a mean current intensity of 176±25 μA (range: 110–300). A steep dose-dependent decrease in response rates occurred with FR: 10 (Fig. 1D). A significant analysis of variance, $F(4,24)=12.8$, $p<0.001$ resulted from decreases to 61±15% and 27±10% at the 10 and 30 mg/kg dose levels, respectively. The mean rate during saline sessions was 1827±138 presses per 20 min (range: 1413–2335), while the mean current intensity was 194±27 μA (range: 110–320). A similar dose-dependent decrease in the rate of lever-pressing occurred with FR: 15 (Fig. 1E), which also produced a significant analysis of variance, $F(4,16)=16.9$, $p<0.001$. At the 10 mg/kg level, the rate of responding was 62±17% of baseline and after the 30 mg/kg dose it was 21±14%. The mean baseline rate was 2059±125 presses per 20 min (range: 1599–2294) at a mean current intensity of 242±34 μA (range: 170–350). The FR: 20 study is shown in Fig. 1F. Three animals were added to this experiment in which the mean response rate was 1924±84 presses per 20 min (range: 1430–2195) and mean current intensity was 296±32 μA (range: 190–445). There was a highly significant analysis of variance, $F(4,28)=38.0$, $p<0.001$, due principally to the decrease to 27±10% of baseline rates after the 30

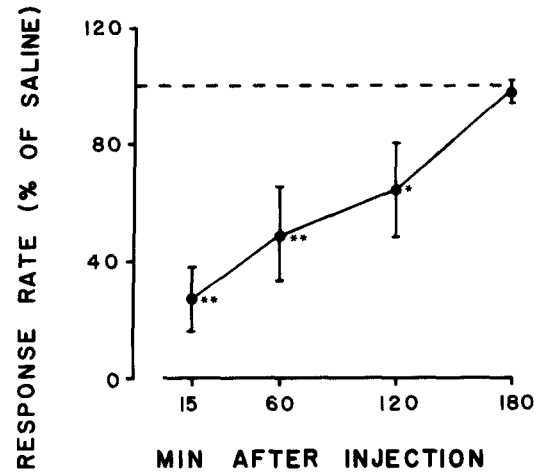

FIG. 2. The effects of 30 mg/kg naloxone on lever-pressing for ICSS reinforced on a FR: 20 schedule as a function of the interval between drug injection and the beginning of the test session (n=7). Other symbols as in Fig. 1.

mg/kg dose. Thus, the CRF and FR: 5 studies produced relatively modest decreases in the rates of lever-pressing and more marked decreases occurred when the fixed-ratio requirement was 10 or greater.

As can be seen from the time-course of the 30 mg/kg dose (Fig. 2), responding on FR: 20 had returned to baseline at 3 hr after injection, and the greatest effect occurred 15 min after injection. The analysis of variance was significant, $F(4,24)=11.7$, $p<0.001$, and there was still a significant decrease in the mean response rate when the animals were tested 2 hr after injection.

Brain Histology

The sites of the electrode tips for all animals used in these studies are shown in Fig. 3. The filled triangles refer to animals used in the ICSS experiments and the filled squares refer to animals used in the locomotor activity experiment. The tips were located in the ventral part of the mid-brain-central gray area in AP planes 1.4 to −0.4 [16]. These sites are similar to those observed in our previous experiment [23].

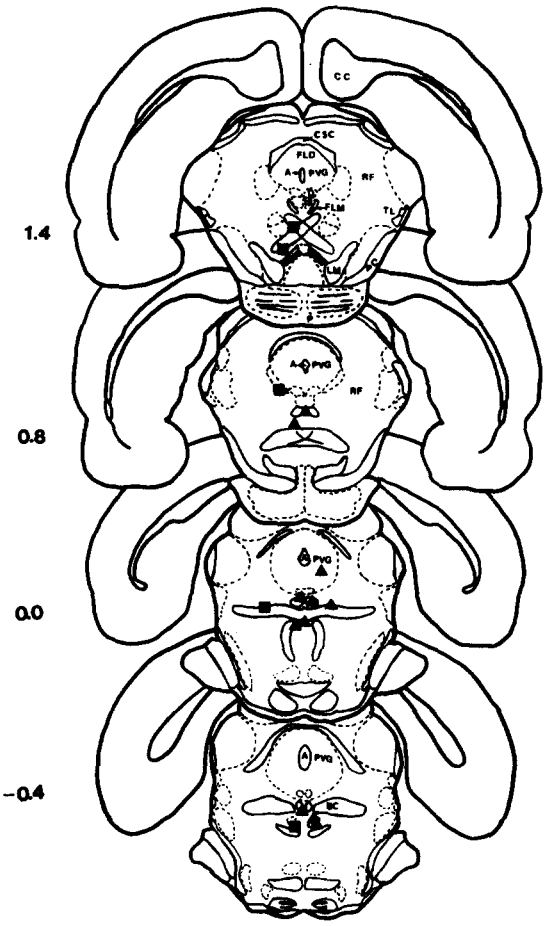

FIG. 3. Maps of the placements of the electrode tips for animals used in the locomotor activity (■) and ICSS (▲) experiments (Atlas of Pellegrino, Pellegrino and Cushman). Numbers to the left of the sections give anterior-posterior co-ordinates. Abbreviations: A, aqueduct of Sylvius; BC, brachium conjunctivum; CC, corpus callosum; CSC, commissure of superior colliculus; D, nucleus of Darkschewitz; FLD, dorsal longitudinal bundle; FLM, medial longitudinal bundle; LM, medial lemniscus; P, pons; PC, cerebral peduncle; PVG, central gray substance; TL, lateral tegmental nucleus; III, nucleus of oculomotor nerve.

DISCUSSION

In all of the intracranial self-stimulation experiments reported here, naloxone produced a decrease in the rate of lever-pressing; the extent to which this occurred depended upon the amount of effort required to obtain the reinforcement. When each lever-press was reinforced (CRF) or when five lever-presses (FR: 5) were required for one reinforcement, only the highest dose of naloxone (30 mg/kg) significantly reduced the rate of responding, and then only to a modest degree. When the fixed-ratio requirement was increased to 10 or more, the rate-decreasing effects of naloxone were much more pronounced. Monitoring spontaneous locomotor activity suggested that the effects of naloxone were not due to any generalized suppression of behavior; although naloxone reduced locomotor activity, this effect was modest and not dose-dependent. Previous studies have shown either no significant depressant effects on activity [1,12] or significant decreases [18,20]. It was for this reason that we were careful to test for spontaneous locomotor activity in a group of animals matched to those studied during the ICSS procedure. While fatigue or behavioral disruption may contribute somewhat to the ability of naloxone to reduce lever-pressing for ICSS, it does not appear to be a critical or major factor. The time-course of these effects on lever-pressing for ICSS in the FR: 20 schedule is similar to that reported for reversing opiate agonist effects [2]. Other studies have described a similar time-course for the action of naloxone in reducing food intake in food deprived rats [21] and in increasing plasma corticosterone levels [4]. The agreement between the present results and these other studies suggests that the effects of naloxone on ICSS are a direct, rather than a blocking, action of the drug.

Previous reports on the effects of naloxone on lever-pressing for ICSS have not described consistent results. Using a CRF schedule with electrodes in the MID-CG area,

Stapleton *et al.* [25] described effects quite similar in magnitude to the present results with CRF and FR: 5 schedules. Contrary to this, Stein and Belluzzi [26] reported dramatic decreases in this same brain area on a CRF schedule, perhaps because of certain critical procedural differences. First, their animals were injected immediately before being placed in the test chamber in contrast to the 15 min delay before testing employed here. Second, Stein and Belluzzi used 75 min test sessions, and our previous work [23] has shown that the effects of naloxone are greater with 1 hr test sessions than with the 20 min sessions used here.

As the present results confirm, opiate antagonists, such as naloxone, can alter responding for ICSS in a dose- and time-dependent manner, but several factors appear to be involved. Among them are: (1) length of test session—long test sessions (1 hr or more) show greater effects than short sessions [9, 23, 25, 26]; (2) schedule of reinforcement—partial reinforcement schedules are more sensitive than CRF, and the more stringent the work requirements, the greater the drug effects [9, 23, 24]; (3) electrode implant site—the areas in and around the central gray appear to be the most sensitive [23,26], although other investigators do not agree [9,25]; (4) stimulation parameters—more marked effects occurred when monophasic stimulation of alternating polarity was used rather than biphasic stimulation [23,24]. This phenomenon may be related to the fact that animals with bipolar electrodes often press faster for one current polarity over the other. Perhaps such animals are primarily reinforced with every other stimulus during alternating polarity stimulation which, in fact, effectively increases their fixed-ratio requirement.

In addition to the variables discussed above, the affinity of the antagonist for receptors appears to affect behavior. It has been shown that the effects of naltrexone were similar to those of naloxone [23]. Both these drugs appear to bind with relatively greater affinity to the mu-opiate receptor subtype than to the delta-receptor subtype [3,15]. In contrast, dipprenorphine, the potent oripavine-derived opiate antagonist which binds with equally high affinity to both the mu- and delta-receptor subtypes, was considerably less potent than naloxone or naltrexone in reducing lever-pressing for ICSS [24].

The effects of naloxone on ICSS have also been studied with other operant procedures. Stilwell *et al.* [27] observed that doses of up to 50 mg/kg did not alter responding for ICSS when the rats were required to move alternately from one side of a shuttlebox to the other. Similarly, Perry *et al.* [17] reported that naloxone did not alter the reinforcement thresholds for brain stimulation. However, these same investigators found that naloxone blocked the effect of *d*-amphetamine, which was to lower reinforcement thresholds, and that it potentiated the effect of chlorpromazine, which was to raise reinforcement thresholds [6,7]. On balance, then, these reports would suggest that naloxone does modify brain stimulation behavior and that procedural differences are crucial in determining the extent of this modification.

The present finding that higher fixed-ratio requirements increase the ICSS depressing action of naloxone raises the question of what physiological changes might be responsible for this phenomenon. The total motor output of the animals should not have changed significantly since the rate of lever-pressing was approximately the same at all fixed-ratios. However, the amount of reinforcement received per amount of motor output or per unit time was reduced at the higher fixed-ratios, which might make this procedure more sensitive to the actions of opiate antagonists. Support for this suggestion comes from the study by Franklin and Robertson [9] in which naloxone was effective in reducing the rate of lever-pressing for ICSS near the central gray area on a random interval 10 sec schedule, producing a rate of reinforcement nearly equivalent to that in our study with the FR: 10 schedule. This limiting of the rate of reinforcement may activate a process that normally tends to support or facilitate lever-pressing for ICSS but that only becomes necessary to maintain lever-pressing under partial reinforcement conditions (e.g., ACTH secretion or endogenous opiate analgesic activity). This facilitating process is perhaps blocked by naloxone.

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