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Naloxone and Shuttlebox Self-Stimulation in the Rat

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STILWELL, D. J., R. A. LEVITT, C. A. HORN, M. D. IRVIN, K. GROSS, D. S. PARSONS, R. H. SCOTT AND E. L. BRADLEY. *Naloxone and shuttlebox self-stimulation in the rat.* PHARMAC. BIOCHEM. BEHAV. 13(5) 739–742, 1980.—Rats self timed electrical brain stimulation on and off periods in a shuttlebox. Electrodes for self-stimulation were located either in the lateral hypothalamic area (LHA) or the periaqueductal gray (PAG). Doses of the narcotic antagonist, naloxone, were administered intraperitoneally immediately prior to self-stimulation testing. Doses of 1.0, 5.0, 10.0 or 50.0 mg/kg failed to alter shuttlebox self-stimulation behavior. These results are inconsistent with one lever-press self-stimulation study employing PAG electrodes [3], but agree with other studies using LHA electrodes [9, 15, 21, 24]. Possible reasons for the discrepancy are suggested.

Naloxone Narcotics Self-stimulation

THE reinforcing properties of narcotic drugs may be mediated, either directly or indirectly, by the same neural pathways which support intracranial self-stimulation (ICSS). In the lever-press paradigm, analgesic doses of morphine (5 to 10 mg/kg) produce an initial inhibition of responding which persists for 2 to 3 hours followed by a facilitation which also lasts for several hours. Tolerance develops to the initial suppression, but studies have failed to find the development of tolerance to the delayed facilitation [4,14]. It has been suggested that the early inhibition seen in the lever-press paradigm may be due to sedative or motor inhibitory effects of the narcotics [6, 11, 12, 23]. Immediate facilitative effects, however, have been found using low doses of 1.0 to 2.5 mg/kg of morphine with the lever-press design [8, 13, 16].

The shuttlebox paradigm may be less susceptible to sedative and motor inhibitory effects [11, 12, 23]. Furthermore, it permits the rat to easily control both the onset and duration of rewarding electrical brain stimulation and requires no shaping during training. In this paradigm, doses of 5 and 10 mg/kg morphine sulfate selectively increase ON times per crossing without altering OFF times. In contrast to the biphasic effect seen in lever-press studies, this facilitation begins within ten min after injection and lasts for more than 3 hours. A high dose of morphine (20 mg/kg) produces a non-selective increase in both ON and OFF times that appears to be due to the profoundly sedating and motor inhibitory effects of this large dose [11].

In the shuttlebox, tolerance does not develop over 5 daily injections to the increases in average ON time per crossing produced by morphine or etorphine [2,12]. This is similar to the aforementioned failure to find tolerance to the increased lever-press rates seen in other ICSS studies [4, 6, 14]. Tolerance, however, does develop to the increases in average OFF time produced by large doses of morphine and etorphine [2,12]. This may be analogous to the development of tolerance to the initial behavioral suppression seen in lever-press studies. One lever press study has reported tolerance to the immediate 10 percent facilitation produced by a low (2.5 mg/kg) dose of morphine [8]. This 10 percent facilitation is however much smaller than the delayed facilitation produced by 5–10 mg/kg morphine in lever-press studies.

The immediate morphine-produced increases in shuttlebox ON times as well as their approximate duration is in agreement with the time course for the hedonic effects of opiates in humans. It has been suggested that the delayed facilitation in lever-press rates, shuttlebox ON time increases, and lowered self-stimulation threshold levels are all due to sensitization of the reinforcement pathways by narcotic drugs [5, 8, 13, 16]. However, not all investigators share this interpretation. Atrens *et al.* [1] suggest that OFF times more effectively reflect changes in stimulation reinforcement value. Furthermore, it has not been demonstrated conclusively that the increased lever-press rates or the acceptance of longer durations of shuttlebox stimulation under morphine is not due to analgesia or a reward-independent interaction with ICSS [23]. However, the failure to find tolerance to the facilitation of lever-press rates or to the increased shuttlebox ON times makes the analgesia interpretation less likely [2, 4, 5, 7, 12].

Belluzzi and Stein [3,19] recently suggested that endogenous opioid peptides may serve as reward transmitters in at

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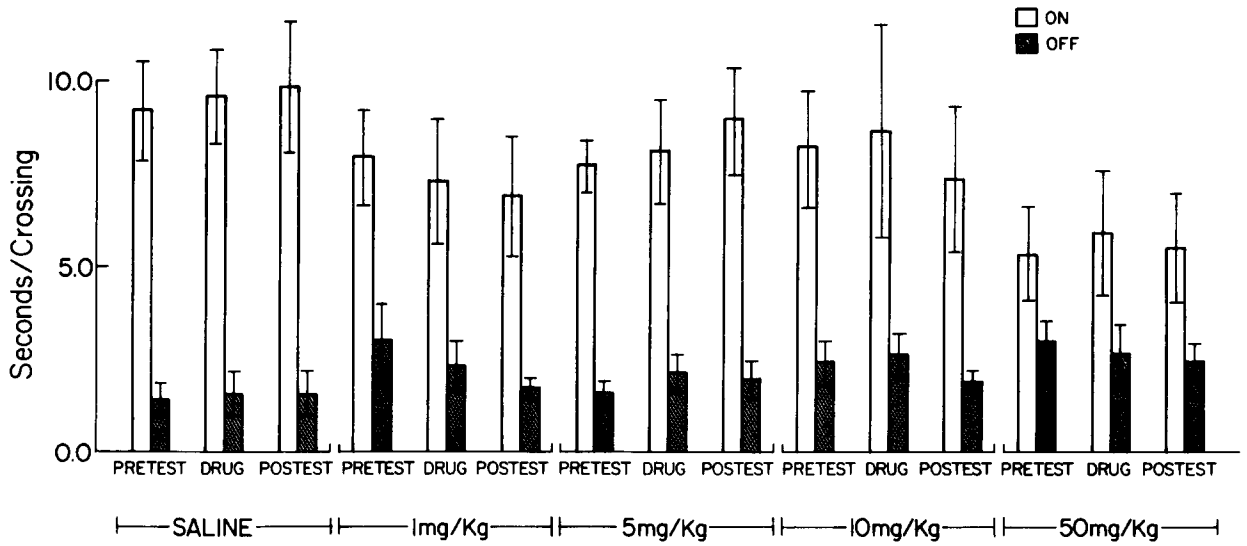

FIG. 1. The effect of naloxone on mean self-stimulation ON and OFF times in the shuttlebox with LHA electrodes (standard errors are shown by the vertical lines).

least some subset of those neural pathways which support intracranial self-stimulation. Neural loci which support high rate self-stimulation often overlap precisely with regions of high enkephalin-like immunoreactivity. If their hypothesis is correct, then self-stimulation of at least some of these regions should be attenuated following the administration of the specific opiate receptor antagonist naloxone. In a series of experiments Stein and Belluzzi demonstrated dramatic dose-dependent suppressions of self-stimulation in the periaqueductal gray (PAG), septum, lateral hypothalamic area (LHA), locus coeruleus, substantia nigra, and nucleus paratenialis [19]. In a duel component brain stimulation study, rats were trained to manipulate one lever to initiate a train of rewarding brain stimulation and, in alternate 5 min periods, another lever to escape bursts of forced stimulation that were automatically delivered each second. Naloxone substantially decreased PAG self-stimulation while increasing escape responding [19]. In light of these results, it might be expected that the administration of naloxone to an animal that is shuttling for rewarding brain stimulation would decrease ON and increase OFF times per crossing.

In this paper, we report our attempt to find naloxone induced changes in shuttling behavior in animals with either periaqueductal or lateral hypothalamic implants.

METHOD

Subjects and Surgery

Sixty Long-Evans strain rats of both sexes and weighing 250–300 g at the time of surgery served as subjects. The animals were housed individually in a controlled environment colony room, and had free access to food and water throughout the experiment.

Under sodium pentobarbital anesthesia (50 mg/kg) and according to standard stereotaxic procedure, each subject was chronically implanted with a single stainless steel bipolar electrode (Plastic Products Co.). Electrodes were aimed at either the periaqueductal gray (PAG) (n=30) or the medial forebrain bundle as it passes through the lateral hypothalamic area (n=30). Implant coordinates were: *LHA:* 0.4 mm posterior to bregma, 1.6 mm lateral to the midline, and 9.5 mm below the surface of the skull, and *PAG:* 5.0 mm posterior to bregma, 1.7 mm lateral to the midsaggital suture, and 7.0 mm below the surface of the skull, angled toward the midline at 12° from the vertical [17].

Histology

Immediately after testing, all subjects were perfused under deep sodium pentobarbital anesthesia, and their brains were removed and stored in 10 percent Formalin. The brains were later frozen, sectioned at 80 μm, and thionin-stained. Verified stimulation sites were distributed in the LHA or PAG within about 1.0 mm of the loci specified by the surgical coordinates.

Apparatus

Rats were tested in wire mesh stabilimeter shuttleboxes which measured 35×20×20 cm and were set on individual central fulcra. Crossing from one side of the cage to the other caused the cage to tip, thereby operating a microswitch under one end of the cage. In shuttlebox self-stimulation, the subject receives electrical brain stimulation while on one side of the box and no stimulation while on the other. The ON and OFF sides of each shuttlebox were programmed to switch automatically every 2 min.

Electrical stimulation to the brain was provided by independent Lafayette #82408 constant current sine wave stimulators (60 Hz). Standard electromechanical programming equipment (BRS/LVE) was used to record cumulative ON time and crosses for each rat per 20 min test period. From these data, mean ON and OFF times per crossing were calculated for each subject.

Procedure

At least one week was allowed for recovery following surgery. Each subject was tested in the shuttlebox for 80 min on 4 consecutive days. On the first day of testing, individual current levels were adjusted between 20 and 80 μA rms in an

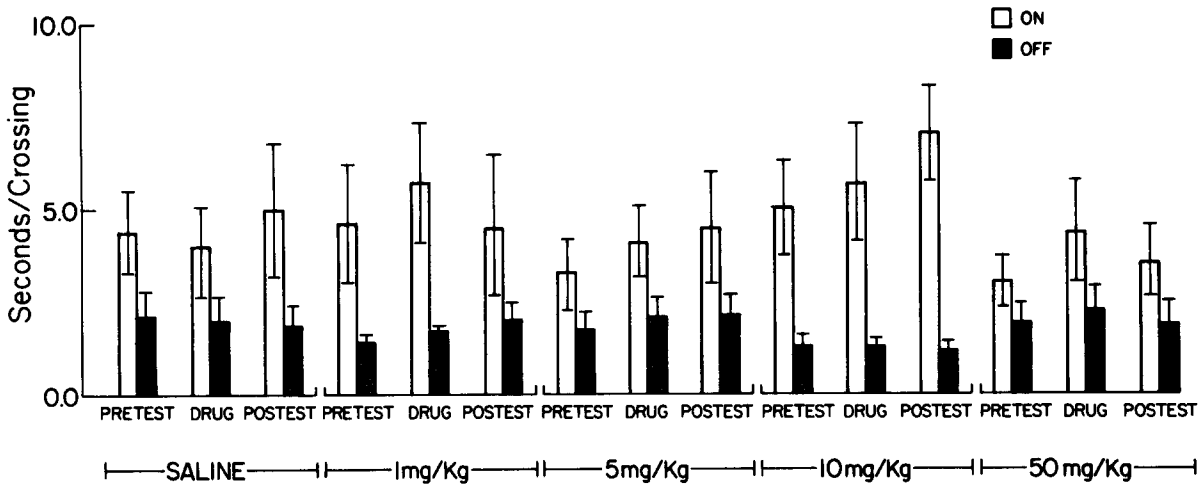

FIG. 2. The effect of naloxone on mean self-stimulation ON and OFF times in the shuttlebox with PAG electrodes (standard errors are shown by the vertical lines).

attempt to produce stable shuttling behavior. On the following three days (predrug, drug, postdrug) current levels were not varied. On the drug day, the animals received equivalent-volume intraperitoneal injections of naloxone hydrochloride (Endo) in doses of 1, 5, 10, or 50 mg/kg, or the isotonic sodium chloride vehicle. Days 2 and 4 served as predrug and postdrug no treatment control days.

RESULTS

Preliminary analysis revealed that the shuttlebox data did not differ on the basis of the gender of the animal. Results also did not differ as a function of the four 20 min periods of each day's testing, therefore results have been collapsed for males and females and to show only means for the full 80 min of testing on each day. Figures 1 and 2 illustrate the daily mean ON and OFF times per crossing (80 min periods) for the predrug, drug and postdrug days. Figure 1 depicts the data for LHA electrodes and Fig. 2 shows the data for PAG sites. For each site there were six animals in each dosage group (saline control, 1, 5, 10, 50 mg/kg of naloxone).

Correlated t-tests designed to test for differences between predrug and postdrug ON and OFF times separately for the LHA and PAG subjects failed to reach significance, even in the absence of a conservative adjustment for multiple t-tests. Therefore the scores have been averaged to obtain one control score. The ON and OFF scores were then analyzed using a repeated measures analysis of variance with the experimental conditions (Control and Drug) nested across areas (LHA and PAG) and dose (0, 1, 5, 10, 50 mg/kg). LHA ON times were significantly larger than those of the PAG animals, $F(1,50)=12.23$, $p<0.001$, but the groups did not differ in OFF times by electrode site, $F(1,50)=1.30$, $p<0.25$. Naloxone failed to influence mean ON or OFF times at any dose with either LHA or PAG electrodes and there were no significant interactions (all F's < 1).

DISCUSSION

In the present study naloxone at doses ranging from 1.0 to 50.0 mg/kg failed to alter self-stimulation shuttling behavior in rats with either PAG or LHA electrode placements. In four recent studies [9, 15, 21, 24], doses of naloxone less than 40 mg/kg have also been found not to alter lever-press self-stimulation rates in the LHA or caudate nucleus. Another study found 10 μgm of naltrexone administered intraventricularly not to alter LHA lever-press self-stimulation [25]. These narcotic antagonists have been found quite effective, however, in low doses at inhibiting the behavioral actions produced by narcotic analgesics, including those on self-stimulation as well as analgesia [14,16]. These results suggest that naloxone-blockable enkephalin release is not the basis of ICSS at these sites, even though naloxone-blockable changes in ICSS can be produced by narcotic agonists.

In contrast to the results just reviewed is the work of Belluzzi and Stein [3,19]. These investigators found a naloxone dose of 1.0 mg/kg to suppress PAG lever-press self-stimulation by 60 percent. They have also found a similar effect with septal and substantia nigra electrodes [19]. In partial agreement with the work of Belluzzi and Stein, Stapleton *et al.* [18] report small reductions of lever-press rates using 10 mg/kg naloxone with PAG, accumbens nucleus, substantia nigra and LHA electrode placements. The dose, though ten times greater than that used by Belluzzi and Stein, resulted, however, in only a 9% reduction in barpressing for PAG placements. The small reductions obtained by Stapleton *et al.* are reliable, but may reflect non-specific behavioral inhibition. For example, naloxone has been shown to produce a conditioned taste aversion [10, 20, 22], and a similar mechanism may be responsible for the reduced performance. The shuttlebox paradigm, which results in an immediate facilitative effect with no preceding inhibition, is possibly a better test for an inhibitory effect produced by naloxone [11,12]. Our failure to find naloxone induced changes in self-stimulation behavior with PAG or LHA electrodes, as well as the similar lever-press results with LHA and caudate placements from five other laboratories [9, 15, 21, 24, 25], and the less dramatic PAG results of Stapleton *et al.* lead us to the conclusion that the work of Belluzzi and Stein should be accepted only with great caution. Their use of near threshold levels of current intensity may be creating self-stimulation that is highly sensitive to non-specific behavioral effects of a drug such as naloxone. No other laboratory has been able to obtain the dramatic (60%) decrease in self-stimulation with low doses that Belluzzi and Stein report.

The suggestion that brain stimulation by itself releases an endorphin which acts on the opiate receptor to produce a naloxone blockable effect is at the present time untenable.

In the present study, control shuttlebox behavior was found to differ as a function of electrode site (LHA electrode ON times were greater than those for PAG electrodes; about 7 sec vs 4 sec). These data suggest that anatomical studies of shuttlebox behavior and narcotic actions as a function of electrode site may be of some value.

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