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BRIEF COMMUNICATION

Morphine and Shuttlebox Self-Stimulation in the Rat: Tolerance Studies

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LEVITT, R. A., D. J. STILWELL AND T. M. EVERS. *Morphine and shuttlebox self-stimulation in the rat: Tolerance studies.* PHARMAC. BIOCHEM. BEHAV. 9(4) 567–569, 1978.—Rats were trained to turn lateral hypothalamic electrical brain stimulation on and off by crossing back and forth in a shuttlebox. Injections of 10 mg/kg morphine doubled the amount of time animals left the stimulation ON without altering OFF times. Tolerance did not develop to this action during 5 daily trials. Injections of 20 mg/kg produced a 4- to 5-fold increase in average ON times together with a large increase in average OFF times. Tolerance did not develop to the ON time increase over 5 daily injections, but did partially develop to the OFF time increase. The ON time increase appears to be based on a mechanism separate from the analgesic action of morphine and from the OFF time increase. Differentiable neurological structures and receptor systems may mediate these actions.

Morphine Narcotics Self-stimulation

THE SELF-STIMULATION paradigm has been used as a means of studying the reinforcing properties of narcotic drugs. Narcotic drugs when administered in analgesic doses have been found in the rat to produce an initial suppression of self-stimulation lever pressing rates lasting for several hours, followed by a facilitation also lasting several hours [9,11]. Tolerance developed over several daily injections to the early inhibitory effect, but tolerance did not develop to the delayed facilitative action [2,9].

Recently, doses of morphine have been found to reduce the self-stimulation threshold for electrical stimulation shortly after drug injection, and tolerance has also been found not to develop to this action [4]. Recently, also, a rate-free technique employing a shuttlebox has been used to evaluate the effects of narcotic drugs on the reinforcing properties of electrical stimulation of the brain in the rat. Doses of 5 to 10 mg/kg of morphine or 10 to 20 μg/kg of etorphine have been found to increase the amount of time rats leave the stimulation ON in a shuttlebox at each crossing, without altering average OFF times. These actions began within 10 min of drug injection and lasted at least 90 min [1,12]. Moreover, tolerance did not develop to this action of etorphine over 5 consecutive days of injection [1].

It is tempting to suggest that electrical self-stimulation is related to reward processes and mood enhancement or euphoria in humans. It is also tempting to suggest that the alterations in self-stimulation behavior produced by narcotics reveal something about the action of narcotics on mood and their ability to produce a euphoric state, and several investigators including us have made this suggestion [1, 2, 4, 8, 9]. In fact, it is this notion which is responsible for much of the interest in the effect of narcotics on self-stimulation. It should, however, be stated that there is as yet no direct evidence to support this claim.

The current experiment investigated the development of tolerance in the shuttlebox to the actions of 2 doses of morphine, one that selectively increases ON times (10 mg/kg) and a second dose that increases both ON and OFF times (20 mg/kg).

METHOD

Animals and Surgery

Adult Long-Evans strain rats of both sexes (weighing 250–350 g) were used. Animals were housed individually with free access to food and water. Under sodium pentobarbital anesthesia, each animal was stereotaxically implanted with a stainless steel bipolar electrode insulated except at the tip (Plastic Products Co.). The electrodes were aimed for the medial forebrain bundle as it passes through the lateral hypothalamic area, a site commonly used in self-stimulation research [4,9]. Implantation coordinates were 0.4 mm posterior to Bregma, 1.75 mm lateral to the midsaggital suture, and 9.5 mm below the surface of the skull ([12]; in this system the incisor bar is placed 5.0 mm above the ear bars).

Apparatus

Testing occurred in wire mesh cages (shuttleboxes) measuring 35×20×20 cm, set on a fulcrum at the center, and with a microswitch under one end of the cage. The shuttleboxes were adjusted so that the animal's weight on

one side of the cage would close the microswitch, which would open when the animal moved to the other side of the cage.

Electrical stimulation to the brain was provided by a Grass square wave stimulator (BPS-1; 60 Hz, 300 to 400 μA). Current was monitored on a Tektronix oscilloscope (5103N). A bipolar flexible cord covered by stainless steel wire (Plastic Products Co.) connected the implanted electrode to the stimulator via a mercury commutator (Scientific Prototype Co.). Standard electromechanical devices (Lehigh Valley Co.) were used to program and time the experiment.

Procedure

One week was allowed for surgical recovery. Animals were then tested in the shuttlebox for 90 min a day. On the first day of testing each animal was placed in the shuttlebox and connected to the stimulator set at 350 μA. Mean ON and OFF times and number of crossings were recorded for each of 3 consecutive 30 min periods. Current was adjusted, if necessary, after the first and second 30 min periods to obtain shuttling behavior with mean ON times between 4 and 25 sec. Only animals that displayed such stable shuttling behavior by the third 30 min period were kept in the experiment. About 50% of the animals met this criterion. No shaping of behavior was required and stable shuttling behavior began within the first 30 min in the shuttlebox. The shuttlebox was also programmed so that the ON and OFF sides automatically switched every 2 min in order to help counteract the behavioral inhibitory effects of morphine.

There were 3 groups of 8 animals. Each animal was run in the shuttlebox on 10 consecutive days. The first day was a training day and its data were discarded. Beginning with the next day the current for each animal was not varied. This next day (Day 1) was a predrug control day. Immediately prior to being placed in the shuttlebox on the next 5 consecutive days (Days 2 through 6) the 3 groups of animals received an intraperitoneal (IP) injection. The injections consisted of either morphine sulfate at a dose of 10 mg/kg or 20 mg/kg, or of the isotonic sodium chloride (saline) vehicle used to prepare the morphine. The 5 injection days were then followed by 3 postdrug control days (Days 7 through 9).

Histology

After the completion of the experiment, animals were overdosed with pentobarbital. They were then perfused intracardially with formal saline and their brains were removed. Histological analysis was then made of thionin-stained 40 micra frozen sections to locate the electrode tips. Histological verification was obtained for 21 of the 24 animals. All electrode stimulation sites were located within the medial forebrain bundle-lateral hypothalamic area. Stimulation sites ranged between 0.4 and 1.2 mm posterior to Bregma, 1.3 to 2.4 mm lateral to the midsaggital suture, and 8.0 to 9.5 mm below the dura [12].

RESULTS

Since no differences were apparent in shuttlebox behavior or in drug actions between male and female animals (there were 4 animals of each gender in each group), the data have been pooled across gender. The data are shown in Figs. 1, 2 and 3 (each figure shows the means for the group of 8 animals). The data were analyzed by means of 2 analyses of variance with appropriate post-ANOVA comparisons: one

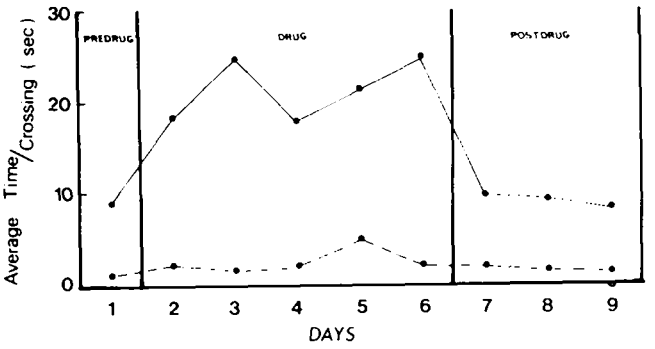

FIG. 1. The effect of 10 mg/kg morphine on shuttlebox self-stimulation (solid line=ON times, dashed line=OFF times).

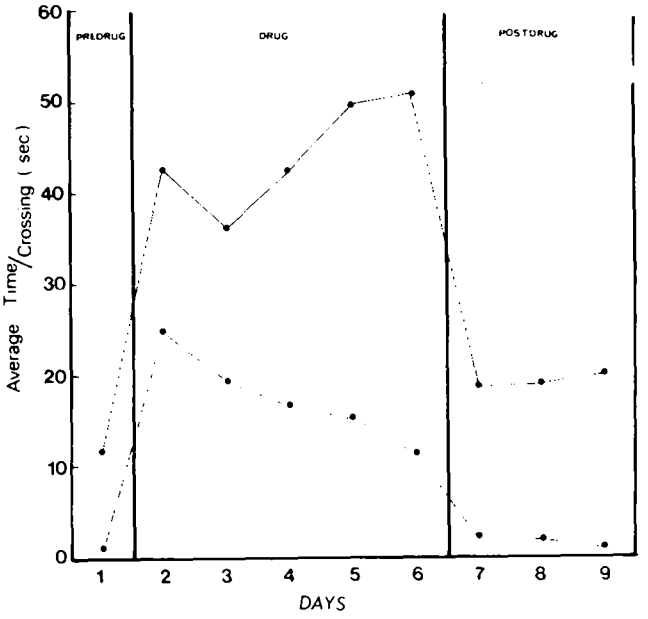

FIG. 2. The effect of 20 mg/kg morphine on shuttlebox self-stimulation (solid line=ON times, dashed line=OFF times).

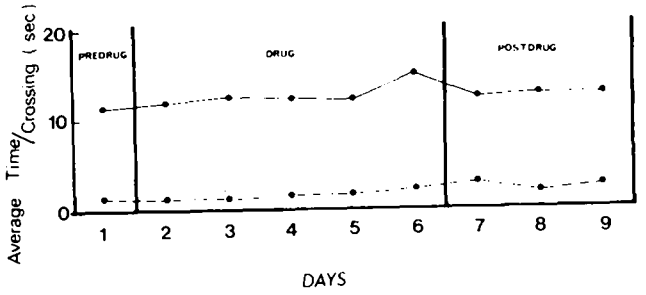

FIG. 3. The effect of isotonic saline on shuttlebox self-stimulation (solid line=ON times, dashed line=OFF times).

analysis was applied to mean ON time scores and the other to mean OFF time scores (all p's are based on two-tailed tests of significance). For each analysis there were 2 factors: dose of drug (10 mg/kg, 20 mg/kg, saline) and days (1 through 9).

For the 10 mg/kg group mean ON times on the predrug day (Day 1) and mean ON times on the 3 postdrug days (Days 7–9) did not differ from each other. Mean ON times on the 5 drug days (Days 2–6) also did not differ from each other, but were significantly higher ($p<0.01$) than on the predrug and postdrug days. Mean OFF times for the 10 mg/kg group did not differ between the 9 days of the experiment.

For the 20 mg/kg group mean ON times on the 3 postdrug days averaged about 7 sec above predrug ON times, but this difference was not statistically significant. Mean ON times on the 5 drug days also did not differ from each other, but were significantly higher than on both the predrug day ($p<0.01$) and the three postdrug days ($p<0.01$). Mean OFF times on the predrug day and on the postdrug days did not differ from each other. Mean OFF times on the 5 drug days, including the last drug day (Day 6) were greater than on the predrug and postdrug days (all p's<0.01). However, mean OFF times on the last drug day (Day 6) were less than on the first drug day (Day 2; $p<0.05$).

There were no significant differences between mean ON times or mean OFF times between days for the saline group. Comparing between groups, mean ON times for the 20 mg/kg group on the 5 drug days were higher than for the 10 mg/kg and saline groups (both p's<0.01), and mean ON times for the 10 mg/kg group were higher than for the saline group ($p<0.01$). Mean OFF times on the 5 drug days were higher for the 20 mg/kg group than for the 10 mg/kg and saline groups (both p's<0.01), which did not differ from each other in OFF times.

DISCUSSION

Injections of 10 mg/kg of morphine selectively increased mean ON times in a shuttlebox self-stimulation paradigm, confirming previous experiments with morphine [8] and etorphine [1]. The ON time increase also did not show tolerance, confirming the previous finding for etorphine [1]. Injections of 20 mg/kg produced an even greater increase in ON times together with a large increase in OFF times, also confirming the previous finding [8]. The OFF time increase, however, did show some tolerance over 5 daily injections.

The increase in average ON times produced by morphine confirms the reinforcement enhancement action noted in lever-press self-stimulation studies and in threshold determination experiments [5,9]. The increase in average OFF times produced by higher doses of morphine confirms the behavioral depressant action of morphine also revealed in lever-press self-stimulation studies [9].

In previous papers we have suggested that the selective increases in ON times produced by analgesic doses of morphine or etorphine were due to an inhibition of an aversive system in the brain and a related delay in the development of aversive effects from prolonged electrical stimulation of the brain [1,8]. Our failure to find tolerance to the increase in average ON times is similar to the lack of tolerance to the increased lever-pressing found in other self-stimulation studies [2,9]. The tolerance to the increase in average OFF times is also similar to the tolerance to decreased lever-pressing found in other self-stimulation studies [2,9].

Since the analgesic and sedative actions of these doses of morphine (10 to 20 mg/kg) do show considerable tolerance over 5 daily injections [3,5], it may be that the increase in average ON times found in shuttlebox self-stimulation experiments is separable from these actions. If this were the case, our earlier suggestion that the ON time increase is based on the inhibition of the development of an aversive effect, an action also responsible for analgesia, would be incorrect. The separation of self-stimulation enhancement from analgesia and sedation may be based on the involvement of different neuroanatomical structures [6,13] as well as on differentiable receptor systems [7,10].

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