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Naloxone Suppression of Self-Stimulation Is Independent of Response Difficulty[1]

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TRUJILLO, K. A., J. D. BELLUZZI AND L. STEIN. *Naloxone suppression of self-stimulation is independent of response difficulty.* PHARMACOL BIOCHEM BEHAV **33**(1) 147–155, 1989.—The action of the opiate antagonist naloxone on relatively easy (nose-poke) and relatively difficult (lever-press) self-stimulation behaviors was compared, in order to determine if opiate antagonists suppress self-stimulation by interfering with the ability of the animal to respond, or by reducing the reinforcement value of the stimulation. Naloxone (0.2, 2.0 and 20 mg/kg) significantly suppressed both nose-poking and lever-pressing self-stimulation rates, and the degree of suppression was virtually identical for both tasks at all doses examined. If naloxone had interfered with the ability of the animal to respond, then lever-pressing—which requires more motor output than nose-poking—should have been more suppressed than nose-poking. The results suggest that opiate antagonists do not interfere with the ability of the animal to respond, and are therefore consistent with the hypothesis that these drugs reduce the reinforcement value of the stimulation.

Naloxone Self-stimulation Lever-press Nose-poke Reinforcement Endorphins Response difficulty

EVIDENCE from a variety of studies suggests that endogenous opioids are important in reinforcement function. This is supported by the observation that self-stimulation behavior is suppressed by the opiate antagonists naloxone and naltrexone (2, 4, 22, 31–33, 35, 36, 39, 40, 44, 46, 47). While failures to find suppression of self-stimulation by opiate antagonists have been reported (29, 38, 42), the specific methodology used plays a critical role; opiate antagonists do indeed suppress self-stimulation if sensitive methods are used (31, 35, 40, 46). Interpretation of the suppression of self-stimulation by these drugs remains a matter of controversy. While some investigators suggest that opiate antagonists suppress self-stimulation principally by blocking the reinforcing effects of stimulation-released endogenous opioids (2, 4, 22, 32, 36, 40), others believe that the effects of these drugs result from motor incapacitation or other nonspecific performance deficits (14, 29, 38). The latter interpretation is apparently supported by studies that demonstrate suppressive effects of opiate antagonists on locomotor behavior (1, 8, 20, 21). However, the effects of these compounds on locomotion are relatively subtle and occur at doses higher than those necessary to suppress self-stimulation [see (46)]. The present study is a further attempt to determine if opiate antagonists suppress self-stimulation by interfering with reward or motor performance.

Distinctions between reinforcement and performance deficits can be made by comparing drug effects on self-stimulation responses that differ in difficulty. Drugs that cause motor debilitation should produce greater impairment of a difficult response than of a simple one. On the other hand, drugs that primarily interfere with reinforcement function should suppress different responses equally (26). Nose-poking is a natural exploratory behavior for the rat—these animals typically explore an environment by actively poking their noses into holes and corners. In contrast, lever-pressing is a less natural and more complex act for this animal. Gerhardt and Liebman (16) have demonstrated in self-stimulation experiments that lever-pressing is more susceptible than nose-poking to suppression by drugs that affect the motor capacity of the animal, while the two responses are suppressed equally by compounds thought to act specifically on reinforcement function. Thus, while the hypnotic pentobarbitol and the muscle relaxant methocarbamol suppressed lever-pressing to a greater extent than nose-poking, the dopamine antagonist haloperidol suppressed both tasks equally.

In the present study, the effects of naloxone on self-stimulation of the nucleus accumbens was determined in the rat, using nose-poking and lever-pressing as response measures. The nucleus accumbens contains high concentrations of opioid peptides and opiate receptors, and self-stimulation of this nucleus is sensitive to suppression by opiate antagonists (3, 22, 35, 39, 40). Furthermore, several studies implicate the nucleus accumbens in the mediation of the reinforcing effects of opioids (19, 28, 34, 41, 43). If, at such an opioid-dependent site as the nucleus accumbens, response difficulty was the major determinant of naloxone's suppressant action on self-stimulation, then the motor impairment hypothesis would be supported. In contrast, a lack of involvement of response difficulty in the effects of naloxone would be consistent with the hypothesis that endogenous opioids play an important role in the reinforcing properties of self-stimulation.

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METHOD

Animals

Experimentally-naive male albino Sprague-Dawley rats (Charles River) were used. The animals weighed 305 to 335 g at the time of surgery, and were individually housed on a 12-hour light/dark cycle with food and water available at all times.

Surgery

Rats were anesthetized with sodium pentobarbitol (50 mg/kg IP) and stereotaxically implanted with bipolar electrodes (Plastic Products MS 303/8) aimed at the nucleus accumbens (coordinates, skull level with horizontal: A-P = +2.0 mm from bregma; Lat. 1.2 mm from midline; D-V −6.0 from the brain surface). Electrodes were attached to the skull using stainless steel screws and dental cement.

Apparatus

Twelve chambers (28 × 25 × 30 cm high) each containing a lever (3.8 × 1.3 × 1.5 cm) mounted on the rear wall 4 cm above the grid floor were used for lever-press experiments. A light located above the lever remained on when the stimulation was available. A second (inactive) lever was located on the door of the chamber; responses at this lever were counted, but produced no stimulation. Chambers were constructed of Plexiglas with black rear and side walls, and clear door and ceiling.The same chambers were used for nose-poke experiments except that the door was replaced with one containing an 8 × 14 cm stainless steel panel. The panel had two 1.5-cm holes located side by side (6.5 cm apart; 4 cm from the grid floor), each of which contained a photocell apparatus. A light located above the active hole remained on when the stimulation was available. Nose-pokes through the inactive hole were counted but produced no stimulation. A white Plexiglas wall blocked access to the rear lever during nose-poke experiments. Self-stimulation chambers were individually housed in sound-insulated compartments with white noise. A single lever-press (10 g force required) or nose-poke (no force required; animal needed only to break a light beam with its nose) delivered a 150 msec train of electrical brain stimulation consisting of monophasic rectangular pulses of 0.2 msec duration presented at 100 pulses per second through an isolation transformer. Electrical connection through a commutator allowed the rat free movement in the chamber at all times. Responses were automatically counted and recorded at five-minute intervals by a computer interfaced with the chambers via a BRS-LVE Interact system. In addition, cumulative recorders continuously monitored responding throughout the session.

Experiment 1 Procedure

Animals were trained to nose-poke for self-stimulation at 350 μA current intensity in 60-minute sessions, five days per week. After stable response rates were achieved, a descending rate-intensity function was determined for each rat to identify the lowest current that would maintain stable responding. This was achieved in a single self-stimulation session as follows: rats began responding in self-stimulation at 350 μA current intensity as normal. Current intensity was readjusted downward by 25 to 50 μA every five minutes, until responding became disrupted or intermittent. At this point, current was adjusted up and down around this intensity to establish the lowest value that would maintain stable responding. This current intensity was identified for each animal as the "baseline" current and was maintained at the new value for the remaining nose-poke sessions. Drug experiments began after response rates restabilized at the new "baseline" current intensities. Since drug effects are more pronounced at low currents than at maximal ones, use of these low baselines provides a more sensitive assessment of the reinforcement mechanisms underlying the self-stimulation behavior than use of higher current intensities (44).

During drug experiments, animals were tested seven days per week, with naloxone doses (0.2, 2.0 and 20 mg/kg) administered in a random order, and with at least 3 days between drug injections. A saline injection on the day prior to each drug injection served as the control for that drug test. If response rate changed by more than 10% during the saline session the drug test for that animal was postponed another 3 days. Naloxone HCl was dissolved in sterile saline and administered subcutaneously (SC) in a volume of 1 ml/kg immediately prior to the experimental session. After receiving all naloxone doses in nose-poke tests, animals were switched to lever-press. Current intensities were readjusted over the following days in order to equate lever-press response rates with those observed during nose-poking. After responding restabilized at the new baseline current intensity, animals again received all naloxone doses as described above.

Experiment 2 Procedure

Animals were allowed to self-stimulate at 350 μA current intensity in 60-minute sessions, five days per week. The first seven days, animals were exposed on alternate days to nose-poke and lever-press, counterbalanced for order of exposure across rats; i.e., some rats received exposure to nose-poke on the first day while others experienced lever-press during this session, followed by the alternate task the following day. Therefore, each animal experienced nose-poke and lever-press conditions for at least three days each during this period. Following this period of acquisition, half of the animals were assigned to nose-poke and half to lever-press for further training. After stable response rates were obtained at 350 μA current intensity, a descending rate-intensity function, as described above, was determined for each rat to identify the lowest current that would maintain stable responding. Current intensity remained at the new value for the remainder of the animal's history. Drug experiments began after response rates restabilized at the new "baseline" current intensities. During the course of drug experiments animals were allowed to self-stimulate five days per week. Days one, two, and five, animals received no treatment; day three served as saline control session; injections of 0.2, 2.0 or 20 mg/kg naloxone HCl occurred on day four. Doses were presented in a random order, and at least seven days separated drug injections. If response rate changed by more than 10% during the saline session, no experimental manipulation was performed that week. Naloxone was dissolved in sterile saline and administered subcutaneously (SC) in a volume of 1 ml/kg immediately prior to the experimental session. After all doses were tested in the first task, animals were switched to the alternate task (i.e., if they were lever-pressing, they were switched to nose-poking, and vice versa), maintained at the same current intensity, and all doses were again administered as described above.

Histological Analysis

Upon completion of experiments, animals were given an overdose of chloral hydrate and perfused intracardially with saline followed by 10% formalin. Brains were removed, frozen, and sectioned at 40 μ. Electrode placements were verified using the atlas of König and Klippel (23).

Data Analysis

The number of lever presses during the final 45 minutes of a

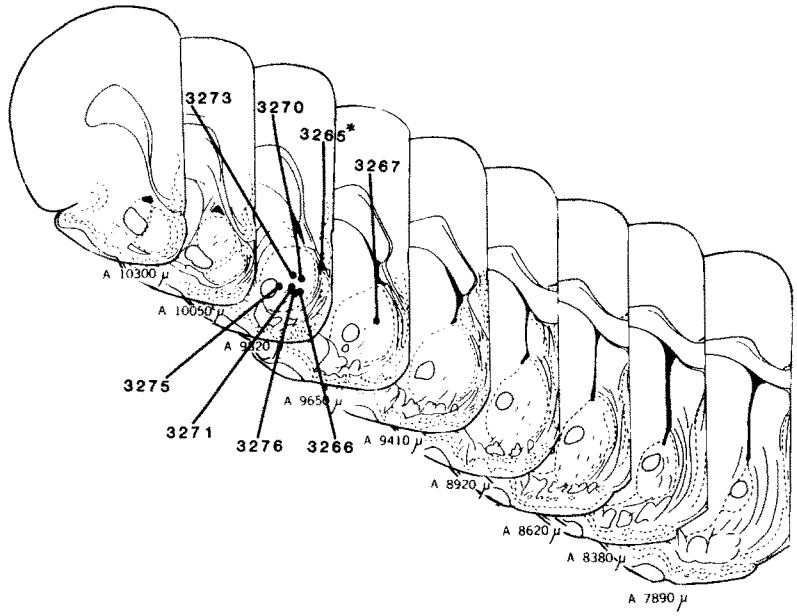

FIG. 1. Summary of diagram of electrode placements in Experiment 1. Electrode tips are indicated by filled circles on representative sections from the atlas of König and Klippel (23). Animal identification numbers are shown in bold. Seven out of eight electrodes are in the nucleus accumbens. The remaining electrode (indicated by *) is slightly medial to the accumbens.

drug session was compared to the final 45 minutes of the preceding saline control session and are expressed as mean percent of control. Paired *t*-test analysis assessed whether experimental effects were different from control, or whether nose-poke was different from lever-press.

RESULTS

Experiment 1

Of the 12 animals implanted with electrodes, eight completed drug testing on both nose-poke and lever-press tasks. Of the four that did not finish, two died of illness and two were lost due to electrode problems. Histological analysis revealed that seven out of eight of the electrode tips were located in the nucleus accumbens (Fig. 1). The electrode tip for the eighth animal was located just medial to the accumbens; since self-stimulation behavior and drug effects for this animal were similar to the nucleus accumbens rats, the data were included in the analysis. Response rates were dependent on current intensity—reduction in current resulted in an intensity-related reduction in responding, as observed in the rate-intensity session (Table 1). The difference in response difficulty between nose-poke and lever-press is indicated by the increase in current intensity necessary after the switch in task to attain the same level of responding on lever-press as seen on nose-poke (Table 2; although the current intensity was substantially higher on lever-press, the difference was not statistically significant). Naloxone dose-dependently suppressed both nose-poking and lever-pressing for self-stimulation. In addition, the effects of this drug were nearly identical for both tasks at all three doses tested (Fig. 2).

Experiment 2

Seventeen of 25 animals implanted with an electrode were used in the experiments. Of the eight that did not finish, seven lost their electrodes and one had repeated seizures during self-stimulation. Histological analysis revealed that 16 out of 17 electrode tips were located in the nucleus accumbens (Fig. 3). The seventeenth electrode was located adjacent to the accumbens in the anterior olfactory nucleus. Since the self-stimulation behavior and drug responses of this animal were no different than the remaining 16 animals, the data were included in the analysis.

Response rates during acquisition for nose-poking were greater than those for lever-pressing (Fig. 4), supporting the suggestion that lever-pressing is more difficult for the rat than nose poking. Rate-intensity data revealed that response rates were dependent on current intensity—reduction in current resulted in an intensity-related reduction in responding (Table 3). Note that at each current intensity except the highest, response rates for nose-poke were greater than for lever-press.

Nine animals received their first drug treatment on nose-poke,

TABLE 1

RATE-INTENSITY DATA FOR ANIMALS IN EXPERIMENT 1

Current Intensity (μA)	Mean Number of Responses/5 min
350	121 (8)
300	100 (8)
250	88 (8)
200	89 (8)
150	61 (8)
100	62 (5)

Data are from the rate-intensity sessions for the eight animals in Experiment 1. Number of rats tested at each intensity is identified in parentheses. Note that response rate decreases as current is decreased.

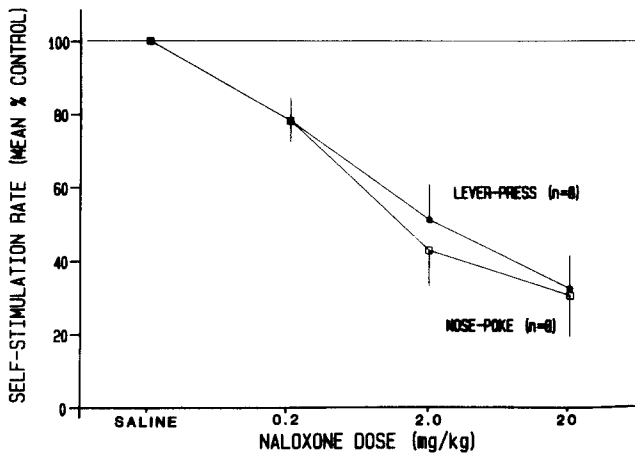

FIG. 2. Effects of naloxone on nose-poking and lever-pressing for self-stimulation: Experiment 1. Data points represent mean percent control ± standard error. Each animal (n = 8) was tested first on nose-poke at each dose of naloxone, then on lever-press. Naloxone significantly suppressed self-stimulation for each task at all doses examined (Nose-poke: 0.2 mg/kg, 78.6 ± 6.2% of saline control, $p<0.05$; 2.0 mg/kg, 43.0 ± 10.3%, $p<0.02$; 20 mg/kg, 30.4 ± 10.7%, $p<0.01$. Lever-press: 0.2 mg/kg, 78.0 ± 5.4%, $p<0.05$; 2.0 mg/kg, 50.9 ± 9.9%, $p<0.02$; 20 mg/kg, 32.2 ± 8.9%, $p<0.01$). There were no significant differences between the two tasks at any dose.

and eight on lever-press. Mean baseline current intensity and mean control response rate were very similar for these two groups (Table 4). Naloxone dose-dependently suppressed self-stimulation for both nose-poke and lever-press (Fig. 5). The effects were virtually identical for both tasks at 0.2 and 2.0 mg/kg. Although not statistically significant, naloxone at 20 mg/kg suppressed lever-press slightly more than nose-poke, perhaps reflecting motor effects of the drug at this high dose.

Thirteen of the original seventeen animals completed the second dose-response; six were switched from nose-poke to lever-press, and seven were switched from lever-press to nose-poke. Animals that were switched from lever-press to nose-poke had no apparent difficulty in responding after the switch—response rates remained stable, and were, in fact, slightly increased on the first day postswitch (Fig. 6). In contrast, animals switched from nose-poke to lever-press showed a substantial decline in response rate after the switch. When response rates restabilized after the switch, the control rates for the lever-press to

TABLE 2

BASELINE CURRENT INTENSITY AND SALINE CONTROL RESPONSE RATE FOR NOSE-POKE AND LEVER-PRESS: EXPERIMENT 1

	Baseline Current Intensity (μA)	Response Rate*
Nose-poke	147 ± 16.7	1391 ± 143
Lever-press	181 ± 30.5	1405 ± 129

*Response rate is expressed as mean number of responses ± standard error for the final 45 minutes of saline control sessions (8 animals, 3 determinations for each animal). There are no significant differences between nose-poke and lever-press for current intensity or response rate. Although current intensity does appear higher for lever-press, this difference was not significant.

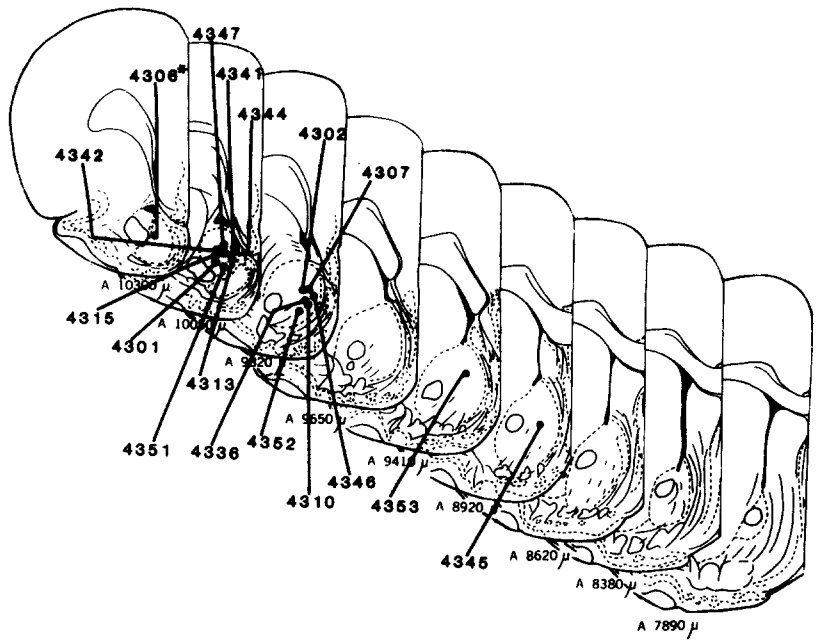

FIG. 3. Summary diagram of electrode placements in Experiment 2. Electrode tips are indicated by filled circles on representative sections from the atlas of König and Klippel (23). Animal identification numbers are shown in bold. Sixteen out of 17 electrodes are in the nucleus accumbens. The remaining electrode (indicated by *) is in the anterior olfactory nucleus.

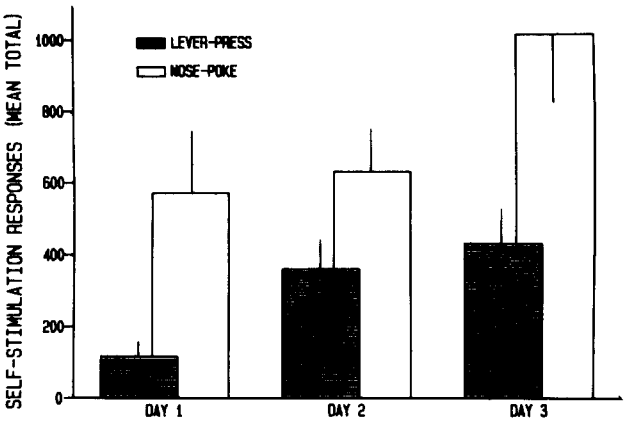

FIG. 4. Initial three days of acquisition for nose-poke and lever-press: Experiment 2. Each animal received exposure to nose-poke and lever-press on alternate days during the first days of self-stimulation training. Data are expressed as the mean total number of responses for all animals (n = 24) for the first, second and third exposure to each task (Day 1, Day 2 and Day 3, respectively). Note that for each exposure, the responding in nose-poke is greater than in lever-press (Day 1: lever-press = 117 ± 66, nose-poke = 575 ± 174, $p<0.005$; Day 2: lever-press = 360 ± 127, nose-poke = 632 ± 171, $p<0.05$; Day 3: lever-press = 432 ± 95, nose-poke = 1019 ± 190, $p<0.01$).

nose-poke group were substantially higher than before the switch (Table 5; Fig. 7). On the other hand, in the animals that were switched from nose-poke to lever-press, the stabilized response rates were only slightly higher than before the switch (Table 5; Fig. 7).

The effects of naloxone were also dependent on the direction of the switch. In the animals that were switched from nose-poke to lever-press, the effects of naloxone were identical for both tasks at all doses (Fig. 8). In contrast, in the animals that were switched from lever-press to nose-poke, naloxone was less effective (although not significantly) on nose-poke at all doses (Fig. 9).

DISCUSSION

The present experiments compared the effects of naloxone on two self-stimulation responses that differ in difficulty, lever-press and nose-poke, in order to determine if opiate antagonists suppress self-stimulation by interfering with the ability of the animal to respond. If opiate antagonists suppressed self-stimulation by interfering with performance, naloxone would have affected the more difficult response (lever-pressing) more strongly than the simpler response (nose-poking) (16,26). Our results show that naloxone affects nose-poke and lever-press similarly, suggesting that opiate antagonists do not interfere with the ability of the animal to perform the self-stimulation response.

Several observations support the suggestion that lever-pressing is indeed a more difficult task than nose-poking. First, nose-poking is a species-specific behavior requiring little movement and no force, while lever-pressing is a less natural response requiring more complex motor output and 10 grams of force. Second, increased current intensity was necessary to maintain response rates after switching the animals from nose-poke to lever-press in Experiment 1. Third, consistently higher response rates were

TABLE 3

RATE-INTENSITY DATA FOR ANIMALS IN EXPERIMENT 2

Current Intensity (μA)	Mean Number of Responses/5 min Nose-Poke Animals	Lever-Press Animals
350	134 (9)	135 (8)
300	130 (9)	119 (8)
250	116 (9)	108 (8)
200	114 (9)	80 (8)
150	92 (8)	35 (7)
100	56 (8)	—*

Data are from the rate-intensity sessions for the 17 animals in Experiment 2. Number of animals tested at each intensity is identified in parentheses. *Only two out of eight lever-press animals were responding at this current intensity making for an invalid comparison (one had 27 responses and the other 93 responses for the 5-minute period). Note that response-rate decreases as current is decreased, and that lever-press animals respond less than nose-poke animals for each current intensity except the highest.

TABLE 4

BASELINE CURRENT INTENSITY AND SALINE CONTROL RESPONSE RATE FOR NOSE-POKE AND LEVER-PRESS ANIMALS PRIOR TO THE SWITCH IN TASKS: EXPERIMENT 2

	Baseline Current Intensity (μA)	Response Rate*
Lever-press	181 ± 9.1	808 ± 62
Nose-poke	172 ± 11.4	792 ± 74

*Response rate is expressed as the mean number of responses ± standard error for the final 45 minutes of saline control sessions (n = 8 animals, 3 determinations for each animal for lever-press; n = 9 animals, 3 determinations for each animal for nose-poke). There are no statistical differences between groups on either measure as determined by an independent *t*-test analysis.

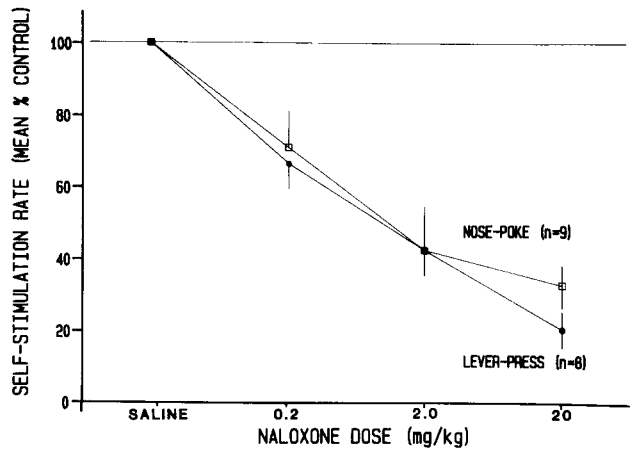

FIG. 5. Effects of naloxone on nose-poking and lever-pressing for nucleus accumbens self-stimulation: Experiment 2. Data points represent mean percent control ± standard error. Naloxone significantly suppressed self-stimulation for each task at all doses examined [Nose-poke (n = 9): 0.2 mg/kg, 71.3 ± 10.1% of saline control, $p<0.01$ different from saline control; 2.0 mg/kg, 42.9 ± 11.9%, $p<0.001$; 20 mg/kg, 32.7 ± 5.9%, $p<0.001$. Lever-press (n = 8): 0.2 mg/kg, 66.7 ± 7.1% $p<0.01$; 2.0 mg/kg, 42.4 ± 7.1%, $p<0.01$; 20 mg/kg, 20.8 ± 5.2%, $p<0.01$]. There were no significant differences between the two tasks at any dose.

TABLE 5

EFFECTS OF SWITCH IN TASK ON CONTROL RESPONSE RATES IN SELF-STIMULATION

Response Rate			% Change in Response Rate
Lever-press	------>	Nose-poke	
796 ± 70		1152 ± 114	144 ± 5.4*
Nose-poke	------>	Lever-press	
864 ± 104		1016 ± 153	113 ± 6.9†

Response rates for the final 45 minutes of all saline control sessions (mean ± standard error), and % change resulting from the switch in tasks are shown for animals examined on both nose-poke and lever-press. Arrows represent direction of the switch in tasks.

*Significant difference in response rates on the two tasks, n = 7, $p<0.001$; †significant difference in rates, n = 6, $p<0.05$; as determined by paired t-test analysis.

obtained on the nose-poke task during response acquisition in Experiment 2. Similar differences in acquisition between these tasks were also noted by Gerhardt and Liebman (16). Fourth, in rate-intensity sessions, nose-poke rates were typically higher for a given current intensity than lever-press rates. Finally, in Experiment 2, rats switched from nose-poke to lever-press initially decreased responding, whereas rats switched from lever-press to nose-poke increased responding.

In Experiment 1 careful attention was paid to equalizing the response rates obtained on nose-poke and lever-press trials; therefore, drug trials were performed on rats responding for different current intensities on the two tasks. Nevertheless, naloxone suppressed nose-poke and lever-press responses equally.

In Experiment 2, in the initial between-group analysis of nose-poke and lever-press, baseline current intensity and response rate were unexpectedly equivalent. Although there was a slight tendency in the nose-poke group toward a lower baseline current intensity, this was not significant. The fact that baseline current intensity and response rate did not differ between the two groups of animals aids the interpretability of the comparison; the importance of having these factors equivalent when comparing different tasks in self-stimulation has been previously stressed (13, 16, 26). The actual differences between the tasks may have been masked by the variability in the two groups of rats. This is supported by the differences in response rate observed when the animals were switched to the opposite task.

As was seen in Experiment 1, the effects of naloxone in the between-group analysis in Experiment 2 were very similar for animals nose-poking and lever-pressing. Interestingly, in this comparison, while the two tasks were suppressed equally by naloxone at the lower doses (0.2 and 2.0 mg/kg), at the highest dose (20 mg/kg) lever-pressing was slightly more suppressed than

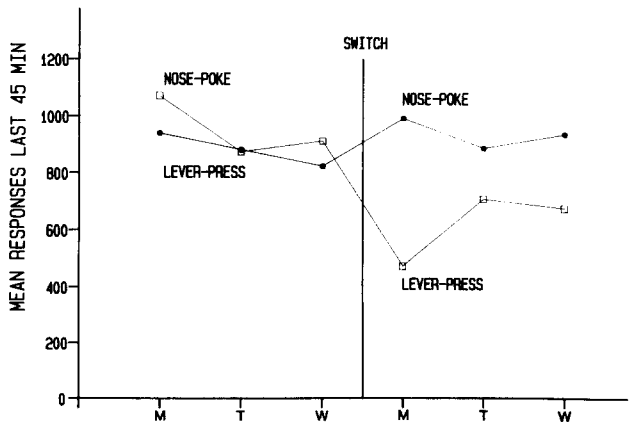

FIG. 6. Immediate effects of switch in task on response rates for self-stimulation: Experiment 2. The mean number of responses during the final 45 minutes of the session on Monday (M), Tuesday (T) and Wednesday (W) prior to the switch, and M, T and W after the switch in task are shown. Note that responding decreased for the group switched from nose-poke to lever-press, while responding slightly increased for the group switched from lever-press to nose-poke.

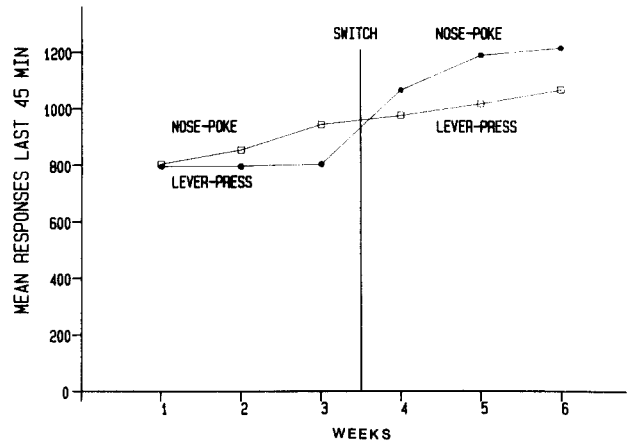

FIG. 7. Effects of switch in task on response rates for self-stimulation. The mean number of responses during the final 45 minutes of the session for each consecutive saline control day is shown before the switch and after the switch in task. Data shows the large increase in control rate for the animals switched from lever-press to nose-poke, as well as the change in rate over time that is typically observed in nucleus accumbens self-stimulation.

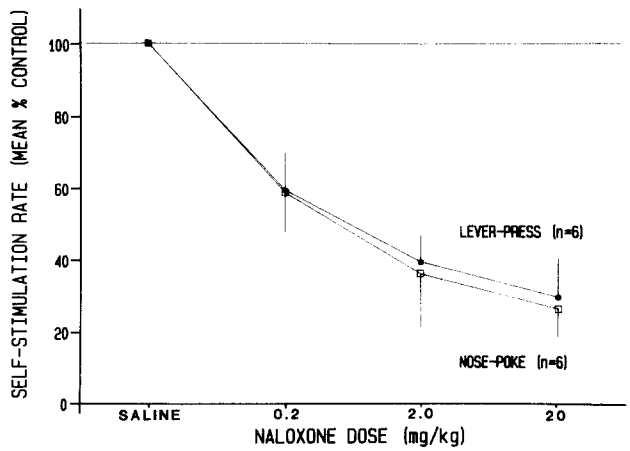

FIG. 8. Effects of naloxone on nose-poking and lever-pressing for nucleus accumbens self-stimulation for animals tested on nose-poke first, then switched to lever-press. Data points represent mean percent control ± standard error (n = 6). Naloxone significantly suppressed self-stimulation for each task at all doses examined (Nose-poke: 0.2 mg/kg, 58.8 ± 11.0% of saline control, $p<0.001$ different from saline control; 2.0 mg/kg, 36.4 ± 15.2%, $p<0.01$; 20 mg/kg, 26.2 ± 7.4%, $p<0.01$. Lever-press: 0.2 mg/kg, 59.6 ± 10.6%, $p<0.02$; 2.0 mg/kg, 39.4 ± 8.1%, $p<0.05$; 20 mg/kg 29.9 ± 11.0%, $p<0.05$). There were no significant differences between the two tasks at any dose.

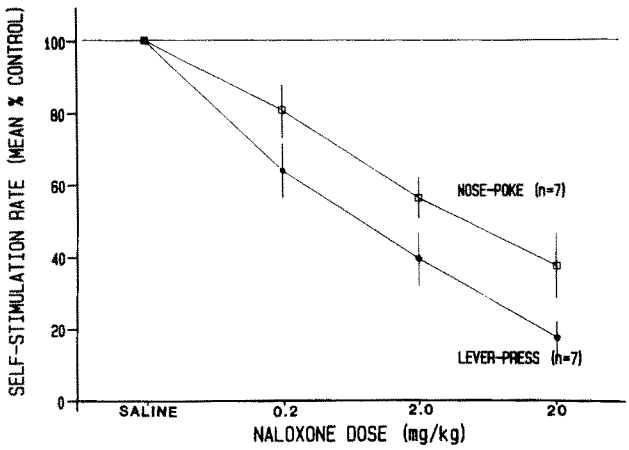

FIG. 9. Effects of naloxone on nose-poking and lever-pressing for nucleus accumbens self-stimulation for animals tested on lever-press first, then switched to nose-poke. Data points represent mean percent control ± standard error. Naloxone significantly suppressed responding at all doses except the 0.2 mg/kg dose for nose-poking (Nose-poke: 0.2 mg/kg, 80.6 ± 7.4% of saline control, n.s. different from saline control; 2.0 mg/kg, 56.4 ± 5.8%, $p<0.01$; 20 mg/kg, 37.7 ± 8.9%, $p<0.001$. Lever-press: 0.2 mg/kg, 64.0 ± 7.6%, $p<0.01$; 2.0 mg/kg, 39.4 ± 7.4%, $p<0.01$; 20 mg/kg 17.5 ± 4.6%, $p<0.01$). There were no significant differences betwen the two tasks at any dose.

nose-poking. It appears at the lower doses, that the effects of naloxone are not the result of a performance deficit. The difference between the tasks observed at the highest dose, however, suggests that in addition to suppressing reinforcement, at this dose naloxone may also have effects on motor capacity. These findings are consistent with studies demonstrating that suppression of locomotor activity by naloxone occurs only at doses of 10 mg/kg or greater (1, 8, 20, 21).

In the within-group comparisons in Experiment 2, differences in control response rate were dependent upon the direction animals were switched. Despite the fact that current intensities were not changed, animals that were switched from lever-press to nose-poke showed a large increase in control rate after the switch, while those that were switched from nose-poke to lever-press showed only a slight increase. These differences in response rate were reflected in the effects of naloxone on the two responses. When animals were switched from nose-poke to lever-press, the effects of naloxone on self-stimulation were virtually identical for the two tasks at all doses examined. However, when animals were switched in the opposite direction, from lever-press to nose-poke, naloxone was slightly less effective in suppressing nose-poke. The differences in sensitivity to naloxone in the lever-press to nose-poke group may have therefore resulted form the large difference in response rates between the two tasks for this group. This suggestion is supported by the observation that the group of animals that had only a small change in rate (those switched from nose-poke to lever-press) showed equal effects of naloxone on both tasks. As noted above, it is important to have equivalent baseline responses rates when comparing different tasks in self-stimulation. Reasons for the increase in rate observed in the lever-press to nose-poke group are probably two-fold: 1) the decreased motor output required by these animals on the nose-poke task allowed for greater response rates (16), and 2) a normal increase in response rate over time is typically observed in animals working for nucleus accumbens self-stimulation [(9,35); Trujillo, unpublished observations]. In the nose-poke to lever-press group, the greater difficulty of the lever-press task apparently countered the normal increase in rate over time, resulting in only slightly higher response rates.

It is important to note that methodological factors may play a role in the ability of lever-press and nose-poke to distinguish between reward and performance effects in self-stimulation. While Gerhardt and Liebman (16) observed that the dopamine antagonist haloperidol suppressed nose-poking and lever-pressing for self-stimulation equally, Ettenberg, Koob and Bloom (11) observed that lever-pressing was more suppressed than nose-poking by the dopamine blocker alpha-flupenthixol. In the Ettenberg *et al.* study, which has been the subject of controversy (7), the authors tested the animals first on nose-poke, then on lever-press, using rate-intensity functions. In contrast, Gerhardt and Liebman (16) tested both operants in a single session, in a counterbalanced manner, examining response rates at a fixed current intensity. In the present experiments, we used a procedure similar to that of Ettenberg *et al.* (Experiment 1: testing animals on nose-poke first, then lever-press) as well as one similar to Gerhardt and Liebman (Experiment 2: counterbalanced testing, although we tested animals on only one operant per session). While we did not take into account all methodological factors (i.e., using fixed currents as opposed to rate-intensity function as Ettenberg *et al.*, and testing nose-poke and lever-press on different days as opposed to within a single session by Gerhardt and Liebman), the fact that we obtained similar results with both procedures suggests that the similar effects of naloxone on nose-poke and lever-press do generalize to different experimental situations.

The results of the present experiments, demonstrating that naloxone suppresses nose-poking and lever-pressing equally, are at odds with the conclusion drawn by West, Schaefer and Michael (46), that increasing the work requirements increases the ability of naloxone to suppress self-stimulation. In their study, West *et al.* utilized fixed ratio schedules in order to increase the work required by the animal to obtain a reinforcement, and observed that the higher ratio schedules were more suppressed by naloxone than lower schedules or continuous reinforcement. However, confounding the interpretation of West *et al.* is the fact that changes in the schedule of reinforcement also alter the density of reinforcement. With decreased reinforcing stimulations per unit time, there would be a concomitant decrease in he amount of endogenous opioids released, and less naloxone would be necessary to antagonize the behavior. Therefore, as alluded to by these investigators, the richness or density of reinforcement rather than the increased work, was more likely responsible for their effects. In the present studies, the three comparisons where the density of reinforcement (response rate) was closely matched, the effects of naloxone were equivalent for the two tasks. On the other hand, consistent with the study of West *et al.*, the one comparison in which the density of reinforcement was increased (increased response rate), the effects of naloxone were decreased. Thus, while our results are not inconsistent with those of West *et al.*, our experiments lead us to quite different conclusions about the role of work requirements in the ability of naloxone to suppress self-stimulation.

As noted above, drugs that interfere with the ability of the animal to respond should suppress lever-pressing for self-stimulation more strongly than nose-poking, while drugs that interfere with reinforcement should affect both responses equally. The present observation that naloxone suppresses nose-poking and lever-pressing equally is therefore consistent with the suggestion that opiate antagonists interfere with the reinforcement value of the brain stimulation reward rather than with the ability of the animal to respond. There are, however, other possible explanations for the similar effects of naloxone on nose-poke and lever-press. For example, naloxone may interfere with a portion of the response that is common to both tasks. Alternatively, this drug may produce sickness or aversion, thereby causing a generalized decrease in

responding. Thus, the present results do not prove that a motor deficit does not exist, nor do they provide direct support for a reinforcement interpretation. However, while the present studies do not completely rule out alternative explanations, a variety of studies support our suggestion that the suppression of self-stimulation is due to decreased reinforcement and not motor impairment, sickness, or other general debilitation. First, although naloxone has been observed to suppress locomotor activity, this effect requires doses of 10 mg/kg or greater (1, 8, 20, 21). In contrast, the observation in the present study, as well as in previous studies (2, 14, 32, 33, 39), that suppression of self-stimulation occurs at much lower doses suggests that motor effects are not responsible for the actions of naloxone on brain-stimulation reward. In a direct comparison of these behaviors, West, Schaefer and Michael (46) concluded that the modest effects of naloxone on locomotor behavior could not account for the suppression of self-stimulation. Second, if motor impairment, sickness or other nonspecific action were responsible for the suppressant effects of opiate antagonists, one would expect that all operant responding would be suppressed by these drugs. However, a variety of studies have demonstrated that naloxone can, in some experiments, facilitate responding (12, 18, 37, 45). Third, the observation that opiate antagonists have different effects at different self-stimulation brain sites [(15, 17, 24, 25, 27, 32, 37), but see also (14)] suggests that the actions of these drugs are site-specific, and not the result of a general suppression of behavior. Fourth, recent studies using threshold measures of self-stimulation (25, 44, 47) support our suggestion that naloxone interferes with the reinforcing value of the stimulation, rather than with the ability of the animal to respond. Finally, if the effects of opiate antagonists on self-stimulation were caused by sickness, aversion, motor-impairment or other nonspecific actions, then suppression of responding should be seen throughout the experimental session. However, our observation that opiate antagonists produce an extinction-like response decrement pattern in self-stimulation, with initial normal rates of response followed later by suppression (40), suggests that these compounds are indeed suppressing reinforcement rather than causing sickness, aversion or motor debilitation.

Despite the number of studies demonstrating significant suppression of self-stimulation by opiate antagonists, these effects remain controversial. Why do some studies observe effects of these drugs while others do not? Why do opiate antagonists typically only suppress self-stimulation rather than completely blocking this behavior? First, as noted above, careful examination of the studies that have used opiate antagonists in self-stimulation experiments reveals that methodology plays an important role in whether or not suppression is observed with these drugs—particularly important variables include electrode implant site and length of test session [see (40) for explanations of why these variables may be important]. Opiate antagonists do indeed suppress self-stimulation if appropriate methods are used. Second, although complete blockade of self-stimulation behavior by opiate antagonists has been observed (4), self-stimulation of most electrode sites is merely suppressed by these drugs. The most parsimonious explanation for these partial effects of opiate antagonists is that endogenous opioids do not play an exclusive role in self-stimulation. Endogenous opioids may be one of several neurotransmitters involved in this behavior—at some sites endogenous opioids may be of primary importance to the behavior; at some sites catecholamines may be of primary importance; and at other sites both endogenous opioids and catecholamines (as well as perhaps other transmitters) may contribute. Therefore, as would be expected, self-stimulation of some sites is completely blocked by opiate antagonists, self-stimulation of other sites is unaffected by these drugs, and self-stimulation of a third group of sites is suppressed, but not completely blocked.

In conclusion, the present study demonstrating that nose-poking and lever-pressing for self-stimulation are equally suppressed by the opiate receptor antagonist naloxone adds further evidence that motor debilitation is not responsible for the effects of opiate antagonists on self-stimulation. These results are consistent with the suggestion that opiate antagonists suppress self-stimulation by specifically blocking the reinforcing actions of stimulation-released endogenous opioids, and add to the increasing evidence that endogenous opioids may play an important role in reinforcement function (2, 5, 6, 10, 30, 36, 37).

REFERENCES

1. Amir, S.; Solomon, M.; Amit, Z. The effect of acute and chronic naloxone administration on motor activation in the rat. Neuropharmacology 18:171–173; 1979.
2. Belluzzi, J. D.; Stein, L. Enkephalin may mediate euphoria and drive-reduction reward. Nature 266:556–558; 1977.
3. Childress, A. R. Naloxone suppression of brain self-stimulation: Evidence for endorphin-mediated reward. Doctoral dissertation, Bryn Mawr College; 1979.
4. Collier, T. J.; Routtenberg, A. Electrical self-stimulation of dentate gyrus granule cells. Behav. Neural Biol. 42:85–90; 1984.
5. Cooper, S. J. Benzodiazepine-opiate antagonist interactions and reward processes: Implications for drug dependency. Neuropharmacology 22:535–538; 1983.
6. Cooper, S. J. Sweetness, reward and analgesia. Trends Pharmacol. Sci. 5:322–323; 1984.
7. Corbett, D.; Stellar, J. R.; Stinus, L.; Kelley, A.; Fouriezos, G.; Bielajew, C.; Wise, R. A.; Ettenberg, A.; Koob, G. F.; Bloom, F. E. Time course of alpha-flupenthixol action explains "response artifacts" of neuroleptic action on brain stimulation reward. Science 222:1251–1254; 1983.
8. DeRossett, S. E.; Holtzman, S. G. Effects of naloxone and diprenorphine on spontaneous activity in rats and mice. Pharmacol. Biochem. Behav. 17:347–351; 1982.
9. Douglin, D. C.; Glassman, R. B. Gradual increase in self-stimulation response rates: effect of electrode loci. Physiol. Psychol. 7:135–138; 1979.
10. Dum, J.; Herz, A. Endorphinergic modulation of neural reward systems indicated by behavioral changes. Pharmacol. Biochem. Behav. 21:259–266; 1984.
11. Ettenberg, A.; Koob, G. F.; Bloom, F. E. Response artifact measurement of neuroleptic-induced anhedonia. Science 213:357–358; 1981.
12. Ettenberg, A.; Pettit, H. O.; Bloom, F. E.; Koob, G. F. Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology (Berlin) 78:204–209; 1982.
13. Fibiger, H. C. Drugs and reinforcement mechanisms: A critical review of the catecholamine theory. Annu. Rev. Pharmacol. Toxicol. 18:37–56; 1978.
14. Franklin, K. B. J.; Robertson, A. Effects and interactions of naloxone and amphetamine on self-stimulation of the prefrontal cortex and dorsal tegmentum. Pharmacol. Biochem. Behav. 16:433–436; 1982.
15. Freedman, N. L.; Pangborn, D. Site-specific naloxone blockade of brain self-stimulation duration. Pharmacol. Biochem. Behav. 20: 361–366; 1984.
16. Gerhardt, S.; Liebman, J. M. Differential effects of drug treatments on nose-poke and bar-press self-stimulation. Pharmacol. Biochem. Behav. 15:767–771; 1981.
17. Gimino, F.; Farrell, R.; Tempel, A.; Steiner, S. S.; Ellman, S. J. The site specific effects of naloxone on intracranial self-stimulation rates in the rat. Soc. Neurosci. Abstr. 5:648; 1979.
18. Glick, S. D.; Weaver, L. M.; Meibach, R. C. Asymmetrical effects of morphine and naloxone on reward mechanisms. Psychopharmacology (Berlin) 78:219–224; 1982.

19. Goeders, N. E.; Lane, J. D.; Smith, J. E. Self-administration of methionine enkephalin into the nucleus accumbens. Pharmacol. Biochem. Behav. 20:451–455; 1984.
20. Haber, S.; Hatsukami, T.; Berger, P. A.; Barchas, J. D.; Akil, H. Naloxone blocks amphetamine-induced rearing: potential interaction between catecholamines and endorphins. Prog. Neuropsychopharmacol. 2:425–430; 1978.
21. Holtzman, S. G. Behavioral effects of separate and combined administration of naloxone and d-amphetamine. J. Pharmacol. Exp. Ther. 180:51–60; 1974.
22. Katz, R. J. Identification of a novel class of central reward sites showing a delayed and cumulative response to opiate blockade. Pharmacol. Biochem. Behav. 15:131–134; 1981.
23. König, J.; Klippel, R. The rat brain. A stereotaxic atlas. New York: Kreiger; 1963.
24. Lewis, M. J. Naloxone suppresses brain stimulation reward in the VNB but not MFB. Soc. Neurosci. Abstr. 6:367; 1980.
25. Lewis, M. J. Effects of naloxone on brain stimulation reward threshold in the VNB but not MFB. Soc. Neurosci. Abstr. 7:165; 1981.
26. Liebman, J. M. Discriminating between reward and performance: A critical review of intracranial self-stimulation strategy. Neurosci. Biobehav. Rev. 7:45–72; 1983.
27. Loughlin, S. E.; Leslie, F. M.; Belluzzi, J. D.; Stein, L. Self-stimulation in the region of the locus coeruleus: Opioid or catecholaminergic mechanisms? Soc. Neurosci. Abstr. 9:277; 1983.
28. Olds, M. E. Reinforcing effects of morphine in the nucleus accumbens. Brain Res. 237:429–440; 1982.
29. Perry, W.; Esposito, R. U.; Kornetsky, C. Effects of chronic naloxone treatment on brain-stimulation reward. Pharmacol. Biochem. Behav. 14:247–249; 1981.
30. Reid, L.; Siviy, S. M. Administration of opiate antagonists reveal endorphinergic involvement in reinforcement processes. In: Smith, J. E.; Lane, J. D., eds. The neurobiology of opiate reward processes. New York: Elsevier Biomedical Press; 1983:257–279.
31. Schaefer, G. J. Opiate antagonists and rewarding brain stimulation. Neurosci. Biobehav. Rev. 12:1–17; 1988.
32. Schaefer, G. J.; Michael, R. P. Threshold differences for naloxone and naltrexone in the hypothalamus and midbrain using fixed ratio brain self-stimulation in rats. Psychopharmacology (Berlin) 74:17–22; 1981.
33. Schaefer, G. J.; Michael, R. P. The effects of naloxone, naltrexone and diprenorphine on fixed ratio responding for intracranial self-stimulation in rats. Fed. Proc. 41:1301; 1982.
34. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Attenuation of heroin reward by disruption of the mesolimbic dopamine system. Psychopharmacology (Berlin) 79:278–283; 1983.
35. Stapleton, J. M.; Merriman, V. J.; Coogle, C. L.; Gelbard, S. D.; Reid, L. D. Naloxone reduces pressing for intracranial stimulation of sites in the periaqueductal gray area, accumbens nucleus, substantia nigra, and lateral hypothalamus. Physiol. Psychol. 7:427–436; 1979.
36. Stein, E. A. Effects of intracranial self-stimulation on brain opioid peptides. Peptides 6:67–73; 1985.
37. Stein, L.; Belluzzi, J. D. Brain endorphins: Possible mediators of pleasurable states. In: Usdin, E.; Bunney, W. E.; Kline, N. S., eds. Endorphins in mental health research. London: Macmillan: 1979: 375–389.
38. Stillwell, D. J.; Levitt, R. A.; Horn, C. A.; Irvin, M. D.; Gross, K.; Parsons, D. S.; Scott, R. H.; Bradley, E. L. Naloxone and shuttlebox self-stimulation in the rat. Pharmacol. Biochem. Behav. 13:739–742; 1980.
39. Trujillo, K. A.; Belluzzi, J. D.; Stein, L. Endorphin-catecholamine interactions in nucleus accumbens self-stimulation. Soc. Neurosci. Abstr. 9:277; 1983.
40. Trujillo, K. A.; Belluzzi, J. D.; Stein, L. Opiate antagonists and self-stimulation: Extinction-like response patterns suggest selective reward deficit. Brain Res.; in press.
41. Vaccarino, F. J.; Bloom, F. E.; Koob, G. F. Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. Psychopharmacology (Berlin) 86:37–42; 1985.
42. van der Kooy, D.; LePiane, F. G.; Phillips, A. G. Apparent independence of opiate reinforcement and electrical self-stimulation systems in rat brain. Life Sci. 20:981–986; 1977.
43. van der Kooy, D.; Mucha, R. F.; O'Shaughnessy, M.; Bucenieks, P. Reinforcing effects of morphine revealed by conditioned place preference. Brain Res. 243:107–117; 1982.
44. van Wolfswinkel, L.; van Ree, J. M. Effects of morphine and naloxone on ventral tegmental electrical self-stimulation. In: Colpaert, F. C.; Slangen, J. L., eds. Drug discrimination: Applications in CNS pharmacology. Amsterdam: Elsevier Biomedical Press; 1982:391–397.
45. Weeks, J. R.; Collins, R. J. Changes in morphine self-administration in rats induced by prostaglandin E and naloxone. Prostaglandins 12:11–19; 1976.
46. West, C. H. K.; Schaefer, G. J.; Michael, R. P. Increasing the work requirements lowers the threshold of naloxone for reducing self-stimulation in the midbrain of rats. Pharmacol. Biochem. Behav. 18:705–710; 1983.
47. West, T. E. G.; Wise, R. A. Effects of naltrexone on nucleus accumbens, lateral hypothalamic and ventral tegmental self-stimulation rate-frequency functions. Brain Res. 462:126–133; 1988.