Pharmacology Biochemistry & Behavior, Vol. 18, pp. 171–178, 1983. Printed in the U.S.A.

Shuttlebox Self-Stimulation in the Rat: An Anatomical Analysis and the Effect of Morphine with Two Current Levels[1]

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Received 16 May 1981

POPOV, A. E., D. S. PARSONS AND R. A. LEVITT. *Shuttlebox self-stimulation in the rat: An anatomical analysis and the effect of morphine with two current levels.* PHARMACOL BIOCHEM BEHAV **18**(2) 171–178, 1983.—A shuttlebox paradigm was used to train rats to turn electrical stimulation ON and OFF by crossing back and forth in a stabilimeter cage. Two experiments are presented. In the first experiment a threshold current level was used in testing four electrode sites: the lateral hypothalamic area (LHA), lateral septal nucleus (LSN), periaqueductal grey (PAG), and the mesencephalic reticular formation (MRF). In the second experiment, a suprathreshold current level was used to explore two electrode sites: the PAG and the MRF. Stimulation with electrodes in the MRF produced an aversive behavioral response; animals shuttled mainly to turn electrical stimulation OFF. At the other electrode sites, both rewarding and aversive properties were apparent: animals shuttled to turn the stimulation ON as well as OFF. Systemic morphine (10 mg/kg) injections nonselectively increased both average ON and OFF times for the three rewarding sites (minimum $p<0.05$) at the threshold current level. Systemic morphine injections (10 mg/kg) in animals stimulated at a suprathreshold current level in the PAG selectively increased time spent with stimulation ON ($p<0.05$) as opposed to time spent with stimulation OFF. No significant behavioral change due to morphine was seen in the aversive MRF at either current level. Animal behavior also was found to vary as a function of site of stimulation ($p<0.05$). The use of suprathreshold currents appears necessary to produce selective reward facilitation effects of morphine such as those found in the PAG or LHA.

Morphine Narcotics Reward Self-stimulation Shuttlebox

NUMEROUS experiments have assessed the reinforcing properties of electrical stimulation of the brain [4, 32–34, 36, 39–40]. For the most part the lever-press self-stimulation paradigm has been the dominant methodological approach and the rat the most frequent experimental subject. The limitations of the lever-press approach have been pointed out [4,49] and several alternative rate-independent methods have been used. One such approach has been the shuttlebox self-stimulation technique [2, 8, 12–13, 15].

In the dominant lever-press paradigm, self-stimulation behavior can be produced with a variety of electrode sites and with lever-press rates that differ as a function of anatomical site. Such characteristics as rate of lever-pressing, current threshold, alteration of rate with increasing current, and alteration of rate with chronic stimulation have been studied [4, 32, 33, 36]. The structure most frequently employed in studies of rewarding self-stimulation in the rat has been the lateral hypothalamic area (LHA).

Lever-press self-stimulation studies using electrodes in the septal area also have shown bar press rates indicative of rewarding stimulation. These rates are slower than those for LHA placements and show lever-pressing trends that decline (satiate) more rapidly with chronic stimulation. The septal area has been implicated in sex-related reward [27,49] and relief from intractable pain or anxiety in humans has been reported from stimulation of some rhinencephalic sites [9]. In rats, it has been shown that septal stimulation suppresses aversive effects elicited from tegmental stimulation [35, 47, 48].

The periaqueductal grey (PAG), another rewarding self-stimulation site [5, 8, 12], has been implicated in stimulation produced analgesia (SPA) [29]. In as much as the PAG has a high density of opiate binding sites, it is thought that SPA is mediated through activation of the paleospinothalamic pain inhibitory neuronal pool which utilizes an endogenous opioid as a neurotransmitter neuromodulator [24,30].

[1]A portion of this research was conducted as a doctoral dissertation at Southern Illinois University by A. E. Popov. Requests for reprints should be addressed to R. A. Levitt at the above address.

Electrophysiological studies of cells in the mesencephalic reticular formation (MRF) as well as the medullary reticular formation have shown them to be responsive to nociceptive stimuli [6, 7, 10] as well as stimulation produced antinoceptive effects [18–19, 44]. Thus, this site classically associated with sensory arousal, has been suggested as one of the possible locations of action for the antinociceptive effects of morphine [18]. Primarily, aversive effects result from electrical stimulation of this area [46].

One of the uses to which the self-stimulation paradigm has been put is the assessment of the reinforcing properties of drugs. The effects of narcotic drugs, including the prototype morphine and related exogenous and endogenous opioids, have been assessed using lever-press [1, 25–26, 37], threshold [13,15] and shuttlebox [2, 5, 21] methodologies. These studies have used primarily LHA electrode sites.

In the lever-press paradigm using LHA sites, moderate doses of morphine (5–10 mg/kg) depress behavior for about two hours, followed by a period of several hours of increased lever-pressing [1, 25–26, 37]. Lever-press threshold studies using similar doses of morphine produce immediate decreases in reinforcement thresholds [13,14]. Similarly, in the shuttlebox paradigm, moderate doses of morphine produce immediate selective increases in shuttlebox "ON" times per cross without affecting "OFF" times [5,21].

The present two experiments further investigate the effects of opioids on shuttlebox self-stimulation. Both the site of stimulation and the current intensity employed are varied. The sites explored in this study were chosen because of their possible involvement in the antinociceptive effects of opioids.

EXPERIMENT 1

The first experiment examined the effects of morphine on shuttlebox self-stimulation behavior with threshold current levels. Four electrode sites were explored: three rewarding self-stimulation sites (LHA, LSN, PAG) and one aversive site (MRF). The purpose was to investigate differences in animal behavior as a function of the site stimulated and of morphine injection.

METHOD

Subjects and Surgery

Male and female naive adult Long-Evans strain rats served as subjects. Animals weighed between 250 and 375 g at time of surgery. All subjects were housed in individual cages with food and water available ad lib throughout the experiment, except during training and testing in the shuttleboxes. A 12 hr light-dark cycle (lights on between 08:00 and 20:00 hr) was maintained during the course of the study. All experimental procedures were carried out during the light phase.

Under sodium pentobarbital anesthesia (50 mg/kg) with atropine (0.2 mg/kg, Lilly) administered to minimize respiratory congestion, each subject was stereotaxically implanted unilaterally with a stainless steel bipolar electrode. All injections were given intraperitoneally. Implants were aimed at four sites: the lateral hypothalamic area (LHA), the lateral septal nucleus (LSN), the periaqueductal grey (PAG), and the midbrain reticular formation (MRF). Surgeries were performed with the incisor bar set at 5 degrees (5 mm) above the interaural plane. The stereotaxic coordinates for these sites, with reference to bregma, are shown in Table 1.

TABLE 1
SURGERY COORDINATES BY SITE

	Anterior/Posterior	Lateral	Below Skull Surface
LHA	−0.40 mm	1.75 mm	9.5 mm
LSN	2.00	0.75	6.5
PAG*	−5.00	2.00	7.5
MRF	−5.60	2.00	6.0

*Inclined at 12° from vertical towards the midline at the skull surface. Actual lateral electrode placement was 0.75 mm.

Experimental Apparatus

Electrical stimulation was provided by a Grass (Model BPS-1) brief pulse square-wave stimulator. The pulse duration was set at 0.01 sec with a stimulus interval of 0.1 sec. The stimulation frequency was set at 60 Hz. Pulses were bi-directional. The current intensity was varied as described below under "Procedure" and monitored on an oscilloscope (Tektronix 5102N) which registered the voltage drop across a 100 ohm resister by means of a differential amplifier.

The electrical stimulation was delivered to the subjects through flexible cords (electrodes, cords, and connectors were obtained from Plastic Products Co., Roanoke, VA) connected to the stimulator by means of a mercury commutator (Scientific Prototype Co., NY). This system allowed the subjects to move freely about in the stabilimeter cages. The stabilimeter cages (35 cm l × 20 cm w × 20 cm h) were set on central fulcra. The subjects movement caused the cages to tilt which closed a microswitch, turning the stimulation ON or OFF. The sides designated ON and OFF automatically reversed every 2 min by means of a timer and relays. Thus, subjects were exposed to both ON and OFF conditions at least every 2 min.

Several modes of data collection were employed. A cumulative timer was used to monitor three 30-min sequential periods of a one-day session for each cage. A session timer also provided the total ON time for each 30-min period. A Sodeco counter, wired to each stabilimeter cage indicated the number of crosses per 30-minute period for each subject.

Procedure

After at least 7 days to recover from surgery, all subjects were run 90 min per day on a consecutive 5 day schedule. Thirty animals completed the experiment; 7 or 8 with electrodes (histologically verified) implanted in each of the 4 different brain sites. Of these, 6 (1 or 2 with electrodes implanted in each of the four sites) served as non-stimulated controls.

On the first day (training), subjects were placed in the shuttlebox and a threshold current level established. The current initially was set at 200 μA for 15 min. The current was increased by 40 μA for each additional 15 min period until the subject's behavior demonstrated a change in either total ON time or in crossings from control baseline (random exploratory behavior without stimulation) in a 15 min period or until a limit of 400 μA was attained. Total ON times assumed to reflect threshold were those that exceeded 10 min for rewarding stimulation or less than 5 min for aversive stimulation (during a 15 min period). The threshold for stimu-

TABLE 2
DATA SUMMARY FOR EXPERIMENT 1*

		Site of Stimulation				
		Control	LHA	LSN	PAG	MRF
Current Levels (μA; square waves)		None	233 (33)	208 (8)	208 (8)	242 (27)
	Day					
Total	2	58 (10)	678 (314)	193 (52)	1000 (287)	62 (2)
Crosses	5	44 (5)	486 (254)	135 (31)	1380 (518)	64 (8)
	Vehicle	38 (3)	588 (342)	181 (64)	1258 (478)	61 (7)
	Morphine	32 (4)	514 (355)	71 (25)	546 (198)	55 (3)
Total	2	45 (0.4)	52 (9)	36 (3)	37 (9)	3 (1)
ON	5	45 (0.6)	58 (8)	31 (6)	35 (8)	3 (1)
Time	Vehicle	43 (1)	52 (8)	28 (2)	36 (10)	3 (1)
(min)	Morphine	44 (0.7)	56 (8)	41 (2)	51 (7)	3 (1)
Mean	2	56 (13)	13 (5)	15 (3)	3 (0.5)	3 (1)
ON	5	64 (6)	17 (5)	22 (10)	2 (0.7)	2 (0.8)
Time/Cross	Vehicle	69 (5)	16 (5)	14 (4)	2 (0.6)	2 (0.8)
(sec)	Morphine	88 (11)	40 (18)	57 (15)	28 (14)	4 (1)
Mean	2	58 (14)	11 (6)	23 (6)	10 (5)	84 (3)
OFF	5	65 (6)	11 (6)	36 (10)	6 (3)	88 (11)
Time	Vehicle	76 (5)	15 (8)	31 (7)	9 (5)	92 (10)
(sec)	Morphine	92 (11)	36 (21)	63 (14)	26 (15)	96 (6)

*Standard errors are in parentheses.

lation effectiveness was also assumed to be reached when subjects exceeded 20 crossings in a 15 min period. Thus nonrandom shuttling behavior produced by electrical stimulation was reflected in a change from baseline in either total ON times ($\leq$5 min or $\geq$10 min during a 15 min period; a mean of 7.5 min was expected) or by increased crosses (>20 during a 15 min period, even with no increase in total ON time; a mean of 8 crosses was expected). See the control 90 min data in Table 2 for comparisons. Following a 15 min period in which the subject's behavior met one of these threshold criteria, subjects spent their remaining time on the first day and the full ninety minutes on each subsequent day at this amperage.

On each of days two through five, data were recorded for three consecutive 30 min periods (a total of 90 min per day). Morphine injections (10 mg/kg) were administered on day three in a counterbalanced manner for half of the subjects at each site with the other half receiving distilled water. On day four those animals which received morphine on day three received the vehicle and vice versa. All injections were given intraperitoneally. No injections were administered on days two and five which served as control days.

In the process of behavioral testing, it was found that a few subjects would meet the behavioral criteria for shuttling behavior on day one, but then not show shuttling on subsequent days. Consequently, an additional criterion was that each subject would also have to meet the original shuttling criteria on both of the control days to be included in the study. These criteria, an extension of those for a 15 min training period, were greater than 120 crossings in a 90 min session, less than 30 min total ON time per session for aversive stimulation, or greater than 60 min total ON time per session for rewarding stimulation during each of the 90 min control day test sessions. Four subjects were thus excluded: one LHA, two LSN, and one MRF.

Histological Verification

All subjects on which data were collected were sacrificed within ten days. The animals were perfused with Formalin and then the brains were stored in Formalin. Frozen sections were taken through the location of the electrode track. Sections were then stained using a formal-thionin staining technique which uses thionin to stain for cell bodies. Electrode placements were identified according to Pellegrino and Cushman [41]. A projection microscope was used with a viewing screen which had been dimensionally calibrated. Only those subjects (n=24) for which the stimulation electrodes were confirmed histologically to be in the appropriate structure were included in the study. The electrodes of the six non-stimulated control subjects were similarly verified to be in the following sites: one LHA, two LSN, two PAG, and one MRF.

RESULTS

The data summary for Experiment 1 (Table 2) lists current levels, Total Crosses, Total ON Time, Mean ON Time per Cross, and Mean OFF Time per Cross for each group of six animals. Standard errors are provided following each measure. These data are based on the full 90 min test sessions on control days 2 and 5, on morphine injection days, and on the water vehicle injection days for the five groups (control, LHA, LSN, PAG and MRF).

TABLE 3
DUNCAN'S RANGE TESTS

Total ON Times	MRF (3 ± 1 min)	LSN (28 ± 2 min)	PAG (36 ± 10 min)	Control (43 ± 1 min)	LHA (52 ± 8 min)
Mean ON Times per Cross	MRF (2 ± 0.8 sec)	PAG (2 ± 0.6 sec)	LSN (14 ± 4 sec)	LHA (16 ± 5 sec)	Control (69 ± 5 sec)
Mean OFF Times per Cross	PAG (9 ± 5 sec)	LHA (15 ± 8 sec)	LSN (31 ± 7 sec)	Control (76 ± 5 sec)	MRF (92 ± 10 sec)

Shuttlebox Behavior as a Function of Anatomical Site Stimulated

The data were first analyzed using a treatment by subjects analysis of variance on each of the following variables: Total ON Time, Mean ON Time per Crossing, and Mean OFF Time per Crossing. Total Crosses and Total OFF Time were not analyzed since they are a function of Total ON Times and Mean ON and OFF Times. The control days 2 and 5, and the water vehicle day were included in this analysis, but not the morphine day. The purpose of this analysis was to verify differences in shuttlebox behavior as a function of the anatomical site of electrical stimulation (irrespective of drug injections).

There was a significant group (site of stimulation) effect for all three variables analyzed: Total ON Time $p<0.001$, both Mean ON and OFF Times $p<0.0001$. The day (control days 2 and 5, and water vehicle day) and the interaction factors were not significant for any of the three analyses. These results demonstrate that behavioral performance differs as a function of electrode site for all three measures, but that there are not significant changes in shuttling behavior over days (i.e., the two control days and the vehicle day). This analysis also shows that injection of the vehicle did not influence shuttling behavior.

Duncan's range tests were performed to elucidate further the statistically significant treatment (site) effects found in each of the analyses of variance. All possible pair-wise comparisons were made on all three measurements. The data are cast in ranges (below) and thus are distinguishable in a quantitatively increasing order (critical ranges are a $p<0.05$; continuous underlining indicates non-statistically distinct groups; means are averages for the three control days: 2, 5, and water vehicle days).

The results of the Duncan's range tests show the aversive MRF site's total ON time (3 min average ±1 over the three control days) was significantly different from total ON times for all other sites. This is also true for the rewarding LHA site (52 min ±8). Although the total ON time for PAG stimulation (36 min ±10) did not differ statistically from LSN or Control, LSN (28 min ±2) was significantly different from Control (44 min ±1).

In terms of Mean ON times per cross, the Control group (69 sec) was higher than all four stimulated sites. The Mean ON times per cross for animals receiving stimulation fell into two statistically distinct groups: the MRF and PAG (both averaging 2 sec per cross) in one group, and the LSN and LHA (14 and 16 sec, respectively) in the other group.

The mean OFF times per cross for the three control days further differentiates the shuttling behavior for the five groups. The MRF group (92 sec) was significantly higher than all other groups, confirming the aversive nature of stimulation of this site; animals in this group shuttled mainly to keep the current off. The lowest Mean OFF times were recorded for the PAG and LHA sites (9 and 15 sec, respectively) which together are statistically distinct from the other groups. Although animals shuttling with LHA or PAG electrode sites did not differ statistically in Mean OFF times, they did differ significantly in Mean ON times. These data justify the inference that shuttlebox behavior is markedly different as a function of stimulation site.

Morphine Action

The second set of statistical analyses were directed to discern the effects of morphine injections on shuttlebox behavior (Table 2). This was a three factor repeated measures analysis of variance for each site and measure, across the four days. These analyses were followed by planned comparisons comparing all three control days to morphine (in the first analysis, above, it was found that the data did not differ significantly across the three control days). The morphine injections increased all three measures (Total ON time, Mean ON and OFF times) in the LSN and PAG indicating a general depressant effect of morphine on shuttling activity (LSN=Total ON time: 28 vs 41 min, $p<0.05$; Mean ON time: 14 vs 57 sec, $p<0.01$; Mean OFF time: 31 vs 63 sec, $p<0.01$; PAG=Total ON time: 36 vs 51 min, $p<0.01$; Mean ON time: 2 vs 28 sec, $p<0.01$; Mean OFF time: 8 vs 26 sec, $p<0.05$). However, since Total ON time was increased by morphine with LSN or PAG electrodes, there is also the suggestion of a selective facilitation of reward, in addition to the general depression.

The morphine injections increased both Mean ON and OFF times in the LHA and control groups but did not significantly alter Total ON times (Control—Mean ON times: 69 vs 88 sec, $p<0.05$; Mean OFF times: 76 vs 92 sec, $p<0.05$; LHA—Mean ON times: 16 vs 40 sec, $p<0.05$; Mean OFF times: 15 vs 36 sec, $p<0.05$). For the MRF group, none of the three measures were significantly altered by the morphine injections.

DISCUSSION

This experiment demonstrated the differences in shuttlebox self-stimulation behavior among groups of animals with electrodes in four anatomical sites. The 4 groups differed significantly from a non-stimulated control group, as well as from each other, in their shuttling behavior [2, 5, 21].

Morphine injections (10 mg/kg) produced a non-selective sedative or depressant effect on shuttlebox S-S behavior. This depressant effect is revealed by the increases in both Mean ON and OFF times per crossing for the three groups with electrodes in rewarding sites (LHA, LSN, PAG), as well as for the non-stimulated control group. Some selective facilitation of reinforcement is revealed by the statistically significant increases in Total ON time found for the LSN and PAG electrodes, and may indicate a morphine-induced change in the reward value of stimulation at these sites. Morphine had no effect on the behavioral measures among animals with electrodes in the aversive MRF.

The failure to find a selective facilitation of reinforcement in Total ON time for the LHA site as opposed to the other two rewarding sites is interesting in that a previous study from this laboratory has demonstrated a selective increase in Mean and Total ON times with an identical dose of morphine (10 mg/kg). In the earlier study a suprathreshold current level was employed, and this may account for the differences observed [21]. To investigate further the effects of current level and morphine on shuttlebox S-S behavior, a second experiment was performed utilizing suprathreshold current levels. Two sites were examined: the rewarding PAG, which has high densities of opiate binding sites, and the aversive MRF.

TABLE 4

DATA SUMMARY FOR EXPERIMENT 2*

		Site of Stimulation	
		PAG	MRF
Current Levels (μA, sine waves)		43 (6.8)	34 (8.7)
	Day		
Total Crosses	Control (Averaged)	625 (130.3)	63 (11.6)
	Vehicle	521 (163.2)	65 (16.9)
	Morphine	266 (11.1)	69 (8.7)
Total ON Time (min)	Control (Averaged)	58.1 (5.9)	1.2 (0.3)
	Vehicle	59.7 (6.5)	1.3 (0.4)
	Morphine	68.9 (1.2)	1.4 (0.2)
Mean ON Time (sec)	Control (Averaged)	5.6 (1.1)	1.2 (0.2)
	Vehicle	6.9 (1.8)	1.2 (0.2)
	Morphine	15.6 (2.9)	1.3 (0.1)
Mean OFF Time (sec)	Control (Averaged)	2.1 (0.2)	75 (7.9)
	Vehicle	2.3 (0.2)	72.7 (8.6)
	Morphine	2.5 (0.4)	67.8 (6.7)

*Standard errors are in parenthesis.

EXPERIMENT 2

Hypotheses concerning the effect of morphine on self-stimulation emphasize the importance of the enkephalinergic neuronal systems. It has been shown that morphine and the enkephalins inhibit spontaneous or pain-induced activation of single neurons in certain regions, suggesting an inhibitory mechanism of neuronal action [17,18].

The neuronal loci which support high rates of self-stimulation often are found to overlap with regions of high enkephalin binding [43]. Although high doses of morphine (20 mg/kg) produce nonselective increases in both ON and OFF times due to sedation, selective excitation has been reported with low doses [3].

Results from this laboratory have demonstrated selective increases in ON times without affecting OFF time measures using moderate (5–10 mg/kg) doses of morphine [21]. Morphine also has been found to produce a decrease in self-stimulation current thresholds [14]. The above findings suggest that morphine acts to facilitate reward processes due to a sensitization by narcotic drugs of the reinforcement pathways.

This experiment examined the effects of morphine on shuttlebox self-stimulation at supra-threshold current levels. The increase in current level was intended to overcome the generally depressant effects of morphine.

METHOD

Subjects and Apparatus

Sixteen male and female Long-Evans rats weighing 250–400 g prior to surgery served as subjects. Their experimental histories were similar to the subjects in the previous experiment.

The apparatus used in this experiment was similar to the previous experiment except that constant sine wave stimulators (Lafayette #82408, Lafayette Instrument Co.) were used.

Procedure

After subjects were allowed to recover from surgery, they were run 80 min per day on a consecutive five day schedule similar to that of experiment one.

On the first day (training), subjects were placed in the shuttlebox and a threshold current level established. The current was initially set at 5 μA (60 Hz full sine wave) for 10 min. The current was increased 10 μA for each additional 10 min period until the subject's behavior demonstrated a change in either total ON time or crosses from control baseline non-stimulated behavior. Criteria assumed to reflect threshold were: Total ON $\geq$6.6 min for rewarding stimulation, $\leq$3.4 min for aversive stimulation; crosses $>$14, all measures expressed within a ten min period.

Following a period where the subject's behavior met one of these threshold criteria, the current was then increased 10 μA (up to 90 μA) every ten min until the animal's crosses began to decrease in number or attain an average of less than 2 sec per cross on the ON side of the box for a 10 min period. Subjects then remained at that current intensity for their remaining time on the first day and the 80 min on each subsequent day.

Days two through five each consisted of four consecutive 20 min periods during which data were recorded. Morphine

(10 mg/kg) or saline (0.9%) injections were given in a counter-balanced, consecutive day manner, as described in the previous experiment. Morphine sulfate was dissolved in the vehicle, 0.9% saline solution, at a concentration of 10 mg/ml (as calculated by the salt). Testing began immediately following injections.

Only those subjects (n=16) in which the stimulation electrodes were confirmed histologically to be in the appropriate structure and that met the behavioral criteria for stimulation effectiveness (described above) were included in the study. As in previous studies, it was found that a few subjects met the behavioral criteria for shuttling on day one, but then failed to show shuttling on subsequent days. In order to be included in this study, animals had to show ON and OFF times for their post-drug control day within 50% of their own pre-drug control day behavior. Five subjects were thus excluded: two MRF and three PAG animals. The procedure for histology was the same as in experiment one.

RESULTS

The data summary for Experiment 2 is presented in Table 4. These data are based on the full eighty minute test session on control days 2 and 5 (averaged together), the morphine days, and saline vehicle injection days for the two groups (PAG and MRF). The two control days were combined for each site (n=8 per site) since no significant difference was found between them at either site: PAG=Mean ON time, $p>0.425$, Mean OFF time, $p>0.831$; MRF=Mean ON time $p>0.317$, Mean OFF time, $p>0.582$.

A 3×2×2 factorial design was employed which analyzed the two stimulation variables (Mean ON or OFF) for the two sites (PAG and MRF) over the three drug conditions (Control, saline and morphine). Total ON times were not included in this analysis but analyzed later under Planned Comparisons (below). Total crosses and Total OFF times were not analyzed since they are a function of Total ON and Mean OFF times.

The ANOVA showed a significant difference between stimulation modes (ON vs OFF) ($p<0.0001$) and a significant difference between sites ($p<0.0001$). These results support the conclusion that significant performance differences exist across both sites for the two stimulation modes. There was no overall drug condition effect. Three significant interactions were found: drug-stimulation ($p<0.05$), drug-site ($p<0.04$) and site-stimulation ($p<0.0001$). These interactions suggest at least one drug condition had a differential effect on shuttle time depending on the stimulation mode at each site. No three-way interaction (drug-stimulation-site) was found ($p>0.87$).

These analyses were followed by Planned Comparisons to further investigate the significant interactions. First, Total ON times were used to compare the morphine drug condition to each other drug condition by sites. The process was then repeated using Mean ON followed by Mean OFF measures. These tests demonstrated that morphine produced significant increases in PAG Total ON times ($p<0.05$) and PAG Mean ON times ($p<0.006$). All other comparisons were non-significant.

DISCUSSION

Experiment 2 demonstrated a morphine-induced increase in total and mean ON times without significant changes in mean OFF times for the PAG. The findings are similar to results seen in this laboratory using LHA electrodes with similar suprathreshold current levels [21]. The selective increases in ON times suggest a reward enhancement action of morphine. The lack of an effect in the aversive MRF suggests that morphine's primary mode of action on reinforcement pathways does not involve this neuronal loci.

GENERAL DISCUSSION

The shuttlebox paradigm provides a sensitive method for exploring both rewarding and aversive properties of electrical stimulation of the brain. This paradigm is more resistant than the lever-press paradigm to the depressant effects of narcotic analgesics and in some instances more revealing of behavioral differences resulting from reinforcing stimulation at different sites. The increased sensitivity is due in large part to the increased data available for analysis (mean ON and OFF times per shuttle, as well as total ON and OFF times per period) than in single lever studies.

Morphine injections (5–10 mg/kg), using LHA electrodes, produce an initial decrease in lever press rates lasting about two hours followed by a period, lasting several hours, of increased lever-pressing. With the shuttlebox S-S paradigm, at suprathreshold current levels, morphine produces a rapid, selective increase in stimulation ON times [1, 5, 13, 21, 25–26, 37]. The shuttlebox data, however, correlate well with lever-press data. A high lever press rate generally corresponds to a large number of crossings in the shuttlebox. This, in turn, produces a low mean ON time. The shuttlebox subjects are also allowed control over the duration of stimulation ON time.

The shuttlebox paradigm is also valuable in that it may be used to study aversive sites as well. Lever press studies have relied upon escape and avoidance techniques for studies of aversive sites. The shuttlebox offers the advantage of training within a single day, thus eliminating the time that is needed for shaping and stabilization of animal behavior as seen in lever press methodologies. The depressant effect of morphine in the shuttlebox is revealed by increases in both ON and OFF times with decreased number of crossings. Morphine, although interacting with rewarding stimulation, did not effect shuttling behavior resulting from electrical stimulation of the aversive MRF site.

The effects of morphine on shuttling behavior with rewarding stimulation occurred at a similar dose and time course as that found for the analgesic action of morphine in this species [20]. The results seen in experiment two of a selective increase in ON times for the PAG suggest morphine enhances rewarding stimulation mediated by reward pathway(s) within the CNS. The above conclusion is corroborated by previous findings of morphine-induced selective facilitation in shuttlebox "ON" times using LHA electrode placements together with findings of immediate decreases in reinforcement thresholds after morphine administration [13,21].

The differences in results between the two experiments presented are most likely due to the current levels employed. The use of suprathreshold current levels in Experiment 2 produced longer total and mean ON times and less crosses for the PAG site. Suprathreshold current levels in the MRF in Experiment 2 produced no differences when compared to the threshold currents of Experiment 1 for this aversive site.

The lack of a morphine effect in the MRF contrasts with other findings of attenuation of aversive stimulation and increases in threshold for escape reported in some lever-press

escape paradigms [28,42]. These findings may not be inconsistent with a reward facilitation hypothesis. If the analgesic action of morphine in the CNS is through attenuating the perception of aversive consequences of stimulation as well as blocking the transmission of aversive stimulation itself, then this would imply that morphine is acting differentially on neural systems underlying both rewarding and aversive properties of stimulation. Thus, the reward facilitating action of morphine may not involve attenuation in transmission of aversive stimulation but may be a manifestation of its effect on higher order sensory processing of information [23]. Further support is provided by patients' reports of pain without discomfort or affective arousal under opiate administration [11].

The reward enhancements reported here for the PAG and elsewhere for the LHA [21] suggest that analgesia and reward enhancement are interrelated. However, recent studies of tolerance development [5, 13, 22] and those using synthetic opiate analogs suggest that reward enhancement and analgesia may be separable and mediated by separate opiate receptor-type substrates [16,23]. It is possible that the results reported in this study may be a function of morphine's nonspecificity in binding as well as the differential current levels employed.

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