Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 3, pp. 625-633, 1994

0091-3057/94 $6.00 + .00

0091-3057(93)E0077-H

Differentiation of Intracranial Morphine Self-Administration Behavior Among Five Brain Regions in Mice

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Received 7 July 1993

DAVID, V. AND P. CAZALA. *Differentiation of intracranial morphine self-administration behavior among five brain regions in mice.* PHARMACOL BIOCHEM BEHAV **48**(3) 625-633, 1994. – BALB/c mice were unilaterally implanted with a guide cannula, the tip of which was positioned 1.5 mm above either the lateral hypothalamus (LH) the medial hypothalamus (MH), the mesencephalic central gray area (CG), or either the dorsal (DRF) or ventral parts (VRF) of the reticular formation. On each day of the experimental period a stainless steel injection cannula was inserted into these brain structures to compare the self-administration of two doses of morphine (5 ng or 50 ng), using a spatial discrimination task in a Y-maze. At the dose of 5 ng, LH-, MH-, CG-, and VRF-injected mice all showed a regular self-administration response. At the dose of 50 ng, a discrimination between the reinforced arm and the neutral arm of the Y-maze was observed in LH-, MH-, and VRF-injected mice. Animals of the MH group exhibited the highest level of discrimination performance. At this dose, long injection latencies (>15 min) were recorded in the CG group, which constrained us to reduce the number of daily trials from 10 to 4. In these modified conditions, CG animals clearly self-injected the dose of 50 ng of morphine. Subcutaneous injections of naloxone (4 mg/kg) reduced the number of self-administrations of morphine at each of the four responding structures. Marked signs of physical dependence (escape attempts) were observed in the four groups but with a higher frequency in CG and MH animals. When the injections of naloxone were suspended, a regular self-administration reappeared. Animals injected into DRF did not discriminate the reinforced arm from the neutral arm even when a high dose of morphine (150 ng) was applied, which suggests that the drug has no reinforcing effects at this brain site. The differential reactivity to morphine observed at the level of closely apposed brain structures such as LH and MH, or CG and DRF, suggests that opiate receptors located in the immediate vicinity to the injection site are directly involved in the expression of the observed drug-seeking behavior.

Intracranial self-administration Morphine Lateral hypothalamus Medial hypothalamus Mesencephalic central gray Reticular formation

PREVIOUS studies from our laboratory have shown that BALB/c mice self-administer morphine into the lateral hypothalamus (LH) (14) and the mesencephalic central gray (CG) (12). However, the characteristics of the dose-effect function for responding were very different in these two brain areas. Whereas self-administration could be induced in both LH and CG by a low dose of the drug (5 ng), this behavior remained regular only in the LH when a higher dose of morphine was available (50 ng). Indeed, concerning CG, we observed that, at this dose, mice seemed after only a few injections to hesitate to self-administer again; they rapidly adopted a strategy of delaying and spacing out the injections.

Our previous behavioral studies were performed in a Y-maze where the subjects were required to discriminate between a neutral arm and a reinforced arm to self-inject morphine, each daily session being composed of 10 successive trials. However, despite the low number of responses made by mice injected into the CG with the dose of 50 ng of morphine, these animals appeared to present an effective drug-seeking behavior. Therefore, one aim of the present study was to determine if CG mice injected with 50 ng of morphine could discriminate the reinforced arm from the neutral arm, using a somewhat more restricted number of daily trials.

One important theoretical problem raised by intracerebral self-administration paradigms concerns the anatomical specificity and spatial delimitation of the reinforcing effects produced by the substance injected (6,27). A precise study of the neuroanatomical substrates of positive central reinforcement

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using the present technique implies that the rewarding effects observed result from the activation of receptors located in the immediate proximity of the injection site. This is the main reason that it is necessary, particularly when using mice, to determine if it is possible to differentiate patterns of self-administration of morphine for brain structures that are closely apposed. We have attempted to provide an answer to this point by comparing the self-administration profiles of this drug in the lateral (LH) and medial (MH) parts of the hypothalamus and, on the other hand, in the CG and the adjacent mesencephalic reticular formation. The injection sites in LH and MH as well as in CG and the reticular formation were situated in the same frontal plane but separated by a distance of 0.6 mm laterally. Two regions were particularly studied in the reticular formation: the first concerning the dorsal part (DRF), the other being situated more ventrally (VRF); these two sites being separated one from another, in depth, by 1 mm.

It may be noted that, to our knowledge, no previous studies on self-administration of opiates into the MH have been reported to date. Concerning the reticular formation, only one previous study appears to have been carried out and concluded as to the absence of self-administration of Met-enkephalin into this structure (31).

METHOD

Animals and Surgery

The present experiments used 76 male mice of the BALB/c By JICO strain (Iffa-Credo). At 9 weeks of age, they were housed individually with ad lib access to food and water in a temperature-controlled room (23°C) with a 12 L : 12 D (lights on at 0800 h). The animals were aged 11–12 weeks (body weight 27–30 g) at the beginning of the experiments. Under deep sodium thiopental anesthesia (90 mg/kg) the animals were unilaterally implanted with a guide cannula (outer diameter 0.460 mm; inner diameter 0.255 mm) the tip of which was positioned 1.5 mm above either the lateral hypothalamus (LH $n = 16$), the medial hypothalamus (MH $n = 15$), the mesencephalic central gray area (CG $n = 15$), the dorsal reticular formation (DRF $n = 18$), and the ventral reticular formation (VRF $n = 12$). The stereotaxic coordinates used were the following: LH: antero-posterior (AP) referring to interaural line: +2.10 mm; lateral (L) referring to sagittal line ±1.10 mm; vertical (V) from the surface of the skull: +3.90 mm. MH: AP +2.10 mm, L ±0.50 mm; V +3.90 mm. CG: AP −0.40 mm; L ±0.30 mm; V +1.20 mm. DRF: AP −0.40 mm; L ±0.90 mm; V +1.20 mm. VRF: AP −0.40 mm; L ±0.90 mm, V +2.20 mm. The incisor bar was level with the interaural line. Mice were allowed to recover from operation for 1 week.

Materials and Experimental Protocol

Self-injection procedure. On each day of the experimental period, a stainless steel injection cannula (o.d. 0.229 mm; i.d. 0.127 mm) was inserted into the injection site and was held fixed in position by means of a small connector. The injection cannula was connected by a flexible polyethylene tubing to the microinjection system, which housed a 5 μl Hamilton syringe. The tip of the injection cannula projected beyond the guide cannula by 1.5 mm. By interrupting one photocell beam in the Y-maze (see the Behavioral Procedure section) mice could obtain the reinforcement (injection of morphine sulfate dissolved in Ringer's solution); each self-injection (50 nl) lasted 4 s (normal drug flow was controlled visually both before and after injection of each animal). The least movement of the animal in the Y-maze was detected by an optical system. This information was transmitted to a microcomputer that commanded the rotation of the injector in the same direction as the animal's movement. This process avoided the rolling up of the flexible tubing; consequently, self-administration could be studied in freely moving mice (14).

Behavioral procedure. Self-administration behavior was studied in an opaque Plexiglas Y-maze, the two arms of which were separated by an angle of 90°. The stem and arms were 31 cm long, 8 cm wide, and 12 cm high. The starting box (14 × 8 cm) was separated from the stem by a horizontal sliding door. Horizontal sliding doors were also located at the entrance of each arm. By interrupting the photocell beam in one of the two arms, mice could obtain the reinforcement. The other arm was neutral (no injection).

To begin a trial, a mouse was placed in the starting box and after 1 min the door to the stem was opened. In each group, a certain number of animals had to learn to trigger the injection by interrupting the photocell beam situated in the right arm, whereas the others had to go to the left arm. Each daily session was composed of either 10 trials or 4 trials, as the case may be. During the first 4 trials only of the first 10 trials session, if the animals made an error (in choosing the neutral arm) they were allowed the opportunity of entering the correct arm and receiving injection. From the fifth trial on, when the animal made an error, the chosen arm was closed off. After a 10-s confinement the mouse was removed and replaced directly into the starting box for the following trial. During the acquisition phase, the number of self-administrations was recorded. Automatic equipment, triggered by opening the door to the start box to begin a trial, recorded the latency to enter the correct goal box and trigger the injection. When the injection was terminated, the mouse was replaced into the starting box where it was retained for 1 min, after which the door was reopened to begin a new trial.

The effects of three doses of morphine were studied: 5 ng (6.5 pmol) and 50 ng (65 pmol) for all brain sites and 150 ng (195 pmol) only for DRF. The experiment with the dose of 50 ng comprised the following successive periods: a) acquisition of the self-administration response (6 days in the case of LH, MH, DRF, and VRF, and 10 days in the case of CG); b) evolution of the acquired and stabilized self-administration response rate following a subcutaneous injection of naloxone HCl (4 mg/kg) 10 min before each self-injection session (5 days); c) during the last period, which lasted 3 days, naloxone was replaced by vehicle (NaCl 0.9%). For the doses of 5 ng and 150 ng, only the acquisition phase was studied. The doses of morphine and naloxone used were expressed in terms of the salt. A control group composed of 8 mice (LH $n = 3$; MH $n = 2$; CG $n = 3$) was given access only to vehicle (Ringer).

Histology. At the end of the experiment, the animals were sacrificed under deep chloroform anesthesia. The head, with the guide cannula attached, was placed in 10% formol for a period of 72 h. The guide cannula was then withdrawn, the brain dissected, and placed in a solution of formol containing 30% sucrose for a further week. Frozen brains were then cut in a microtome to provide 60 μm sections which were stained using 0.1% thionin to identify the injection site.

RESULTS

In the eight control animals having only vehicle (Ringer) available we observed an absence of discrimination between the two arms whatever the brain region considered (LH, MH,

or CG). Therefore, the results obtained for the three structures were pooled; an analysis of variance (35) confirmed the absence of any self-administration response in control mice, $F(3, 15) = 0.66$, NS.

In mice that had access to morphine, we observed, particularly at the dose of 50 ng in MH and CG groups, a marked increase in the latency of injection. As previously observed (12) subjects do not appear to show any willingness to retrigger injection and remain in the starting box. Consequently, the session was terminated each day in these animals when the interinjection delay exceeded 15 min. Therefore, for all experiments, self-injection performance was expressed as the percentage of reinforced trials in comparison with the total number of trials made. Angular transformation of these values (35) has permitted the use of analysis of variance for statistical comparisons.

Dose of Morphine (5 ng)

At this dose, a slight tendency towards injection latencies in excess of 15 min was observed in CG and MH animals during the first three sessions of acquisition, but this tendency disappeared during the following sessions. Consequently, all groups could be submitted to the 10 daily trials. The results obtained are summarized in Fig. 1. At the dose of 5 ng, a progressive discrimination between the reinforced arm and the neutral arm was observed in LH ($n = 6$), $F(5, 25) = 6.72, p < 0.001$, MH ($n = 6$), $F(5, 25) = 11.33, p < 0.001$, CG ($n = 6$), $F(5, 25) = 4.18, p < 0.01$, and VRF ($n = 6$), $F(5, 25) = 14.17, p < 0.001$, groups. However, self-administration performance appeared to be highly dependent upon the brain structure injected, $F(3, 20) = 5.08, p < 0.01$. In the DRF group ($n = 6$), no discrimination between the two arms of the Y-maze was detected, $F(5, 25) = 0.52$, NS.

Globally, the mean value of the latency to trigger the injection was similar in the four responding structures (Table 1), $F(3, 20) = 0.75$, NS. However, if we compared the latencies recorded during the first five trials and the last five trials of each session we observed a differential evolution of this parameter in the four brain structures. Whereas the latency remained stable in VRF or tended to decrease in LH group, its value increased significantly in CG, $F(1, 5) = 11.03, p < 0.05$, and MH, $F(1, 5) = 13.06, p < 0.02$, groups [latency × structure interaction: $F(3, 20) = 8.67, p < 0.001$; Table 2].

Dose of Morphine (50 ng)

At this higher dose, all six mice injected in the CG showed a marked increase of injection latency (>15 min) after only a few injections. Consequently, after four sessions, this group was dissociated from the others. In the MH group ($n = 7$) the same phenomenon was observed during the first two sessions of the acquisition phase (four and five animals, respectively). However, the value of injection latency rapidly decreased during the next sessions (only one animal during the sixth session). Thus, the 10 daily trials were maintained in the MH group. Such behaviors were not observed in the LH, DRF, and VRF groups.

At this dose, discrimination between the reinforced arm and the neutral arm of the Y-maze was observed in LH ($n = 7$), $F(5, 30) = 14.57, p < 0.001$, MH ($n = 7$), $F(5, 30) = 3.99, p < 0.01$, and VRF ($n = 6$), $F(5, 25) = 13.58, p < 0.001$, groups (Fig. 2). Concerning LH, it may be noted that performance recorded at the dose of 5 ng was better than that recorded at 50 ng during the second and the third acquisition sessions ($p < 0.001$ in the two cases).

Despite the long injection delay observed in the MH group, these animals exhibited better performance than the two other groups during the first two sessions of the acquisition phase, $F(2, 17) = 5.04, p < 0.02$. The mean value of the latency to trigger the injection was similar in the three groups $F(2, 17) = 0.98$, NS (Table 3). No discrimination between the two arms of the Y-maze was recorded in the DRF group at this dose, $F(5, 25) = 0.88$, NS.

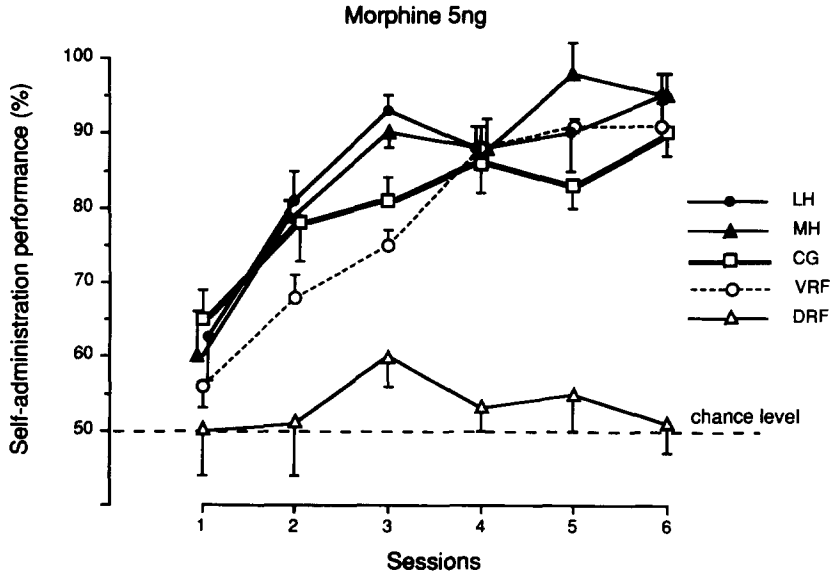

FIG. 1. Self-administration performance (mean ± SEM expressed as the percentage of reinforced trials/total number of trials made) recorded in animals injected into LH, MH, CG, DRF, or VRF with the 5 ng dose of morphine.

TABLE 1

MEAN VALUES (SECONDS) OF THE LATENCY (±SEM) TO TRIGGER THE INJECTION OF THE 5 ng DOSE OF MORPHINE IN ANIMALS INJECTED INTO LH, MH, CG, OR VRF

	Sessions					
Group	1	2	3	4	5	6
LH	55 ± 12	51 ± 12	41 ± 8	30 ± 9	24 ± 5	21 ± 4
MH	47 ± 7	81 ± 20	52 ± 15	36 ± 10	34 ± 12	42 ± 20
CG	58 ± 23	51 ± 16	78 ± 23	59 ± 12	40 ± 12	34 ± 6
VRF	80 ± 13	70 ± 7	70 ± 11	38 ± 6	34 ± 6	34 ± 5

When naloxone (4 mg/kg) was injected subcutaneously 10 min before each session, the number of self-administrations was progressively reduced in the three brain areas (Fig. 2). This decrease tended to be more marked in the MH group. It may be noted that during this phase, injection latencies tended to increase again in the MH group (one animal during the first session under naloxone and three during the fifth session). In each of the three groups, mice appeared to be very disoriented. They increased the number of visits to the neutral arm; some of them even jumped out of the maze. These behavioral disturbances were more frequently observed in MH animals than in the two other groups [mean number of escapes observed: LH 1.3 ± 0.2; MH 2.8 ± 0.3; VRF 0.7 ± 0.1; $F(2, 15) = 14.31$, $p < 0.001$]. In the three groups, the mean number of escapes increased during the five successive sessions under naloxone, $F(4, 60) = 2.68$, $p < 0.05$. During this phase, the latency to trigger the injection increased in the three groups: LH, $F(4, 24) = 5.07$, $p < 0.01$; MH, $F(4, 16) = 5.08$, $p < 0.01$; VRF: $F(4,20) = 8.79$, $p < 0.001$. When naloxone was replaced by vehicle (NaCl 0.9%) a regular self-administration response reappeared in the three brain structures (Fig. 2). It may be noted that despite the absence of discrimination observed in mice injected into the DRF, these animals were also injected with naloxone, as were the three other groups. No modification of the behavior of DRF animals was observed, and particularly no escape attempt was recorded.

Concerning the CG, due to the high proportion of subjects exhibiting injection latencies in excess of 15 min, and which were then removed from the Y-maze, no significant progression in the overall measures of discrimination performance was observable for this group for the four consecutive sessions of 10 trials, $F(3, 15) = 1.15$, NS. This phenomenon was observed for a given subject from the first session and remained constant throughout successive periods of testing. Consequently, the number of trials per daily session was reduced to four (Fig. 3). With this revised protocol, CG mice were effectively observed to progressively discriminate the reinforced arm from the neutral arm to self-inject the dose of 50 ng of morphine, $F(9, 45) = 4.89$, $p < 0.001$. The number of self-administrations was greatly reduced by subcutaneous injection of naloxone (4 mg/kg). Escapes out of the Y-maze were observed; their number progressively increased during the five sessions of this phase. When naloxone was replaced by vehicle, a regular self-administration response reappeared in CG mice. During each of these three phases, no significant modification of injection latencies was recorded.

TABLE 2

MEAN VALUES (SECONDS) OF THE LATENCY (±SEM) TO TRIGGER THE INJECTION OF THE 5 ng DOSE OF MORPHINE DURING THE FIRST FIVE AND THE LAST FIVE TRIALS OF ACQUISITION SESSIONS, RECORDED IN ANIMALS INJECTED INTO LH, MH, CG, OR VRF

Group	Trials 1-5	Trials 6-10
LH	44 ± 6	32 ± 3
MH	27 ± 4	65 ± 11
CG	27 ± 3	62 ± 12
VRF	55 ± 6	55 ± 12

Dose of Morphine (150 ng)

The effect of this dose was only studied in the DRF group that did not show any drug-seeking behavior at 5 ng and 50 ng doses. However, as for the two other doses, no discrimination between the two arms of the Y-maze was observed in animals injected into DRF with 150 ng of morphine [percentage of self-injection responses recorded during the six successive sessions: mean ± SEM 56 ± 4; 51 ± 4; 56 ± 3; 55 ± 5; 53 ± 3; 53 ± 5; $F(5, 25) = 0.40$, NS]. A comparison of the effects of the three doses of morphine confirmed the total absence of discrimination in the DRF group, $F(2, 15) = 2.33$, NS, and revealed that the latency of entering in the two arms decreased at the dose of 150 ng, $F(2, 15) = 27.30$, $p < 0.001$.

Histology

Injection sites were precisely located by using the track of injection cannula. Figure 4 summarizes these placements into LH, MH, CG, DRF, and VRF. In the five brain regions, the scattering of cannula tracks was identical for animals receiving the dose of 5 ng as for those receiving the dose of 50 ng.

DISCUSSION

The major results of our study confirm the existence of self-administration of morphine into CG (12) and, furthermore, reveal that this behavior is also observable at the level of two other brain structures: the medial hypothalamus (MH) and the ventral reticular formation (VRF).

Although intracerebral self-administration of drugs is "perhaps the most powerful method to demonstrate that a drug has its rewarding action at a particular receptor field" (6), a certain number of methodological problems have to be taken into account to confirm the validity of the results. These precautionary measures as outlined by Bozarth (6) and by Wise and Hoffman (36) are of three main categories: behavioral

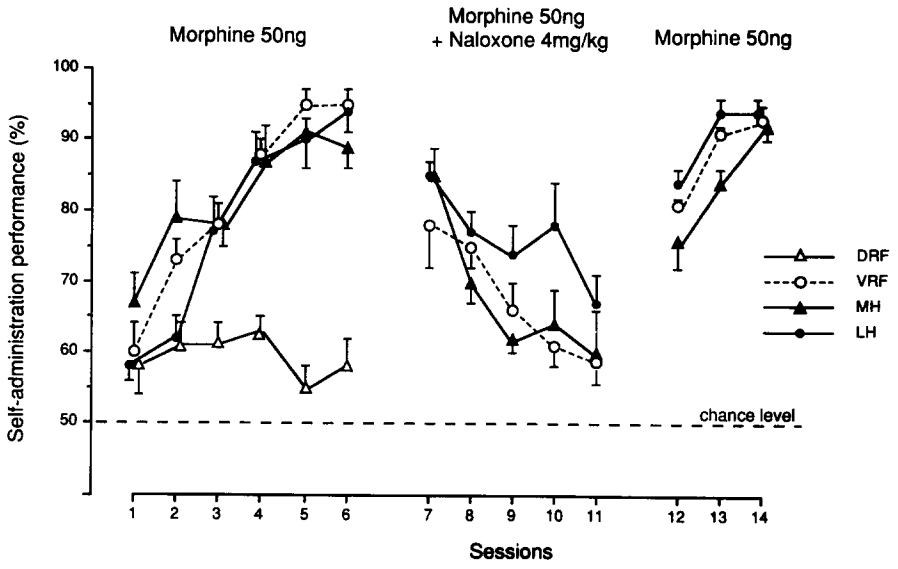

FIG. 2. Self-administration performance (mean ± SEM expressed as the percentage of reinforced trials/total number of trials made) recorded in animals injected into LH, MH, DRF, or VRF with the 50 ng dose of morphine [sessions 1–6: morphine alone; sessions 7–11: morphine and naloxone (SC, 4 mg/kg), sessions 12–14: morphine and vehicle (SC, Nacl 0.9%)].

specificity, pharmacological specificity, and anatomical specificity.

Behavioral Specificity

Concerning behavioral specificity, the first condition required to affirm the existence of a real self-administration behavior is to demonstrate the progressive acquisition of the response to the new drug (5) and not simply the perseveration of a previously learned response to a different drug.

At the dose of 5 ng, naive BALB/c mice learned to self-administer morphine into the LH, MH, CG, and VRF. In spite of the fact that the role of LH in internal reward mechanisms generated by opiates is contested by certain authors (10), it should be noted, however, that the self-administration of morphine into this region has also been observed in the rat (26,30).

In our experiments, the spatial discrimination task reveals that the reactivity of each of the four responding structures to the same dose of drug was not identical. Indeed, differences concerning both the self-administration performance and the evolution of the latency of injection during each session were observed. The self-administration response, thus, corresponds, effectively, to a real drug-seeking behavior and is not simply an avoidance of the neutral arm in which subjects are briefly enclosed when they make an error, because control animals that are treated similarly and having only vehicle available do not discriminate the reinforced arm from the neutral arm of the Y-maze. At the dose of 50 ng, self-administration behavior remained regular only in LH and VRF. Concerning LH, the performance scores recorded during the second and third acquisition sessions were inferior to those obtained with the dose of 5 ng, which confirms our previous results (12,14). It may be noted that injection of morphine in certain brain areas produced hyperactivity (6,21). This phenomenon, which was also induced from the LH with doses of morphine equal or superior to 50 ng (14), can temporarily disturb the spatial discrimination and, consequently, may explain the better performance recorded in mice injected with the dose of 5 ng.

TABLE 3

MEAN VALUES (SECONDS) OF THE LATENCY (±SEM) TO TRIGGER THE INJECTION OF THE 50 ng DOSE OF MORPHINE BEFORE (SESSIONS 1-6) OR AFTER (SESSIONS 7-11) SUBCUTANEOUS INJECTION OF NALOXONE, RECORDED IN ANIMALS INJECTED INTO LH, MH, OR VRF

	Sessions										
	Morphine 50 ng						Morphine 50 ng + Naloxone				
Group	1	2	3	4	5	6	7	8	9	10	11
LH	25 ± 4	47 ± 15	40 ± 6	41 ± 9	29 ± 3	30 ± 7	28 ± 5	48 ± 7	38 ± 7	31 ± 7	68 ± 10
MH	14 ± 1	45 ± 8	64 ± 13	31 ± 8	48 ± 8	32 ± 6	38 ± 8	95 ± 12	56 ± 9	124 ± 25	65 ± 13
VRF	31 ± 8	31 ± 2	30 ± 4	27 ± 7	26 ± 6	30 ± 4	54 ± 12	78 ± 13	156 ± 29	131 ± 15	155 ± 10

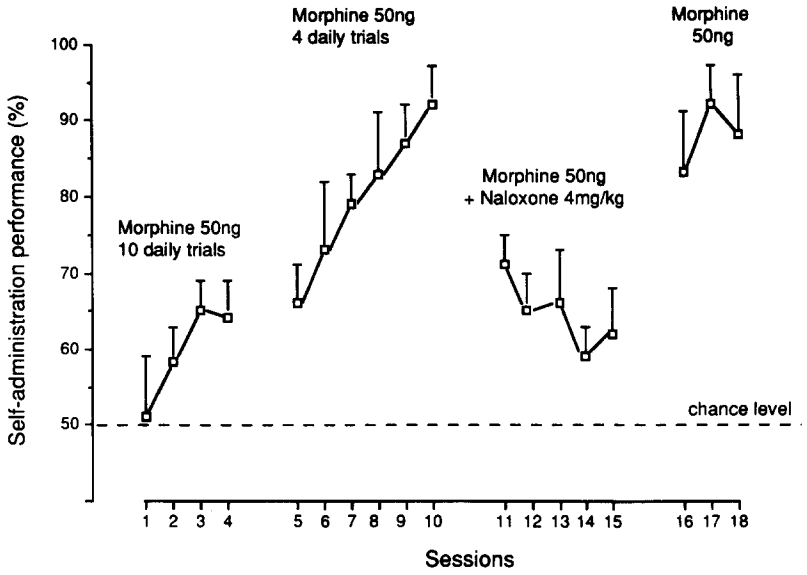

FIG. 3. Self-administration performance (mean ± SEM expressed as the percentage of reinforced trials/total number of trials made) recorded in animals injected into CG with the 50 ng dose of morphine (sessions 1–4: 10 daily trials; sessions 5–18: four daily trials).

The behavior of mice injected into MH and CG was different from that of animals injected into LH, and VRF particularly as concerns the long interinjection delay observed in the two former groups. Whereas MH and CG mice maintained a normal locomotor activity, they seemed, only after few injections, to hesitate to self-administer again.

This phenomenon is, however, not too surprising: it is well known that a direct relation exists between the dose of drug injected and the length of the interval between successive injections (22). At the dose of 50 ng, this interval progressively decreased in the MH group but remained constant in the CG group that has constrained us to reduce the number of daily

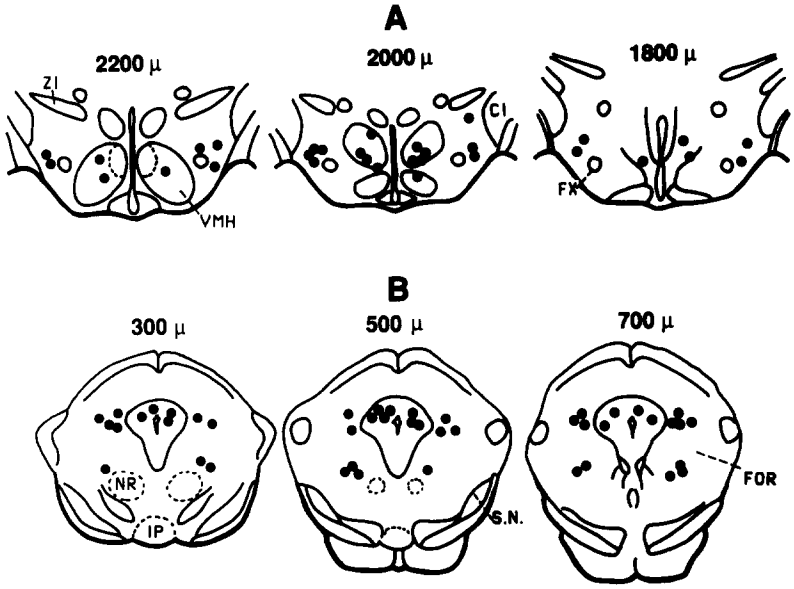

FIG. 4. Histological verification. The placements of the injection sites in the LH and MH (A) or in the CG, DRF, and VRF (B) are plotted on frontal section diagrams. The values in microns (µ) indicate the distance of the section from the interaural line. CI, capsula interna; Fx, fornix; FOR, formatio reticularis; IP, nucleus interpeduncularis; NR, nucleus ruber; SN, substantia nigra; VMH, nucleus ventromedialis hypothalami; ZI, zona incerta.

trials to four in this latter group. Then mice injected into CG showed a clear acquisition of the self-administration response. These results suggest that a rapid summation of the effects of each injection is responsible for the long interinjection delays observed during the 10 daily trials protocol.

The results obtained with the dose of 5 ng seem to corroborate this hypothesis. Indeed, although the CG group tolerates the 10 daily trials, we observed a progressive increase of the latency for the triggering of the injection during each session.

Nevertheless, another explanation is also possible. Despite the evidence that morphine has positive reinforcing properties, analysis of the consequences of its administration suggests that this drug may also have aversive effects (18,24). Moreover, we have previously shown that electrical stimulation of MH and CG has an appetitive effect inducing self-stimulation behavior which is observed immediately but which is, however, quickly followed by an intense aversive effect (11,13). It is important to note in this respect that both of the medial structures (MH and CG) have dense and bidirectional anatomical interconnections (15,28).

Pharmacological Specificity

"In the case of opiate reward, the pharmacological specificity can be established by challenging intracranial self-administration with narcotic antagonists such as naloxone" (6). Subcutaneous injection of naloxone reduced self-administration performance to a level near to chance in the four responding groups. The animals behave in the same manner as those undergoing an extinction phase (14). They appeared to be very disoriented; they increased the frequency of visits to the neutral arm or jumped out of the Y-maze. These behavioral disturbances result from the disappearance of the reinforcing properties of morphine by blockade of opiate receptors, because when the injections of naloxone were suspended, a regular self-injection response reappeared in the four brain structures. We may conclude that the self-administration response observed is not due to nonspecific factors, such as, for example, calcium chelation (6).

Escape attempts from the apparatus during withdrawal of morphine is considered to be one of the classical signs of physical dependence on this drug. Vigorous jumping is particularly exhibited when a precipitated withdrawal response is induced by naloxone (20,33). Of all the various signs, escape attempts seem to be the most suitable index for evaluation of dependence because they can be easily quantified (4,9). In our experiments, escape attempts were more frequently observed in CG and MH groups than in LH and VRF, which suggests that physical dependence was more marked in CG and MH injected animals. It may be noted that other indices of dependence were sometimes observed; in particular, diarrhea was frequently associated with the precipitated withdrawal of morphine induced by naloxone in the CG group.

Different studies have assumed that physical dependence was a necessary condition for observing the rewarding effects of opiates. The primary reinforcer for the continued use to morphine was suggested to be the avoidance of withdrawal (32,34). On the other hand, several workers have reported that morphine can be rewarding in subjects showing no obvious signs of withdrawal when the reinforcement was absent (9,37). Our results seem to agree more with this second hypothesis. Indeed, whereas all the mice injected into MH or CG showed escape attempts when opiate receptors activity was blocked by naloxone, only some of the animals injected into LH or VRF exhibited such behavioral disturbances.

Opiate receptors of the μ type, for which morphine and naloxone have high affinity (29), have been reported to be present in higher density in CG than in RF (17,25) and, similarly, in MH than in LH (16).

Thus, although it is not possible to dismiss the hypothesis of an aversive component mediated by a different type of opiate receptor, we may propose, from our present data, that the initially observed positive reinforcing properties of morphine, as well as the subsequent increase in injection latencies at higher doses, appear to be related to μ type receptor activation. This interpretation is in agreement with previous studies by other authors who used fentanyl, a selective μ agonist (22).

Anatomical Specificity

Concerning anatomical specificity, our results demonstrate that the characteristics of the self-administration of morphine varied greatly even at the level of closely apposed brain areas. Similar data were obtained by Bozarth (8) in a study of the rewarding effects of morphine injected into the ventral tegmental area using place conditioning. In our experiment, whereas injection sites into LH and MH were separated only by 0.6 mm, the self-administration behavior in these regions was different both as regards the performance exhibited, the evolution of the injection latency, as well as by the production of behavioral disturbances (escape attempts) associated with the injection of morphine. This differential reactivity appeared even more evident at the level of mesencephalic structures. Whereas a clear drug-seeking behavior was observed in CG, injection sites located in DRF and distant only by 0.6 mm from CG did not induce any self-injection response. Indeed, mice injected into DRF did not discriminate the reinforced arm from the neutral arm of the Y-maze, whatever the dose of morphine. This result suggests that morphine had no positive reinforcing effects at the level of DRF. Consequently, the decrease of entry latency observed equally for the two arms at the dose of 150 ng, thus, probably corresponds to an aspecific behavioral activation induced by the drug. On the other hand, 1 mm below this site, a self-administration response with characteristics similar to those of self-injection into LH was recorded into VRF.

Although we have not studied the actual extent of the spread of morphine from its site of injection, various data lead us to minimise the eventual influence of an effect via distal diffusion in our study. On the one hand, according to Lomax (23), an injection of 1.0 μl of (^{14}C) morphine into the hypothalamus occupies only a sphere of 0.6 mm radius from the injection cannula. Moreover, the substance injected can also flow back up the injection cannula and disperse into the ventricles (19). This phenomenon may be considered, particularly in the case of MH injections (proximity of the third ventricle) and CG injections (proximity of the sylvian aqueduct). However, Albert and Madryga (1) reported that the functionally effective spread of 4 μl of lidocaine slowly infused was estimated to be only 0.25–0.60 mm from the injection cannula, which suggests a rapid accomodation of the tissue to the injection. Now, it may be recalled that in our study, the injections the volume of which was limited to 50 nl, were spaced by more than 1 min, which will limit even further any diffusional spread (6).

Moreover certain of our results also strongly suggest that the behavioral effects we observed are due to an action of the drug principally around the site of injection. On the one hand, concerning LH and CG, if self-administration in these two structures was induced by diffusion of morphine to a distal site, the self-administration performance would be better with the dose of 50 ng than with the dose of 5 ng (7). However our

results show the opposite, because the lower dose applied seems to represent the better condition for obtaining a more regular self-administration response. Regarding dorsal diffusion, a problem raised by intracranial injections (36), we can point out two crucial factors. First, VRF and DRF animals, in which injection sites were separated only by 1 mm, show opposite behavior because DRF animals do not choose the reinforced arm. Second, both DRF and CG are situated just below the superior colliculus, where numerous opiate receptors are located (2,25). Consequently, in the case of an effect due to dorsal efflux from the tip of injection cannula, we would obtain similar responses in CG and DRF, especially with a higher dose of morphine (50 ng).

If we consider now lateral diffusion, which seems to be larger than dorsal spread for certain authors (3), we can see that CG animals self-administer morphine, whereas DRF animals (0.6 mm lateral) do not. Moreover, concerning DRF, no increase of the entry latency in the two arms was observed when the dose of 150 ng was applied in this structure, despite the proximity of CG. On the contrary, we observed a decrease of entry latency.

In conclusion, the high specificity concerning the reactivity of each of the brain structures studied to morphine revealed by our experiments suggests that, despite an eventual diffusion of the drug, opiate receptors located in the close vicinity to the injection site are directly involved in the drug-seeking behavior observed. Consequently, these data enable us to a) envisage the mapping of the brain regions responding in vivo to morphine, and b) determine the respective roles of the opiate receptor types by studying self-administration of more selective molecules than morphine.

ACKNOWLEDGEMENTS

We would like to thank Dr. T. Durkin for correcting the English text and Mrs. J. Ducout, A. M. Perret, A. Zielinski, and M. T. Roy for technical assistance. This investigation was supported by the CNRS URA 339.

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