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# Dose-Discrimination Performance of Mice for Self-Administration of Morphine Into the Lateral Hypothalamus

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CAZALA, P. AND V. DAVID. *Dose-discrimination performance of mice for self-administration of morphine into the lateral hypothalamus.* PHARMACOL BIOCHEM BEHAV **51**(1) 49-55, 1995.—Two experiments were performed in BALB/c mice implanted bilaterally with guide cannulae. In the first experiment, the tips of the guide cannulae were positioned 1.5 mm above the lateral hypothalamus (LH). On each experimental day, injection cannulae were inserted into each side of the LH. The experiment, carried out in a Y-maze, was composed of two phases. During the initial acquisition period, which lasted 4 days, animals were allowed to self-inject, successively, on alternate days, one dose of morphine into one side of the LH and a different dose in the other side. From the fifth day, the subjects were given the possibility of choosing between these two doses by entering into a given arm of the Y-maze. When the two doses available were 5 ng and 50 ng or 15 ng and 50 ng, the subjects rapidly discriminated them and preferentially triggered the injection of the higher dose (50 ng). When the two doses available were 30 ng and 50 ng, the mice triggered indifferently the two doses during the first three sessions. A discrimination between these two doses began to become apparent from the fourth session, with the subjects preferring to trigger the dose of 50 ng. In a second experiment, the tips of the guide cannulae were positioned either 1.5 mm or 2.6 mm above the LH, the bilateral injection cannulae consequently being inserted either into the LH or into the overlying ventral thalamus (TH). Experimental conditions were the same as that of Experiment 1. During a preliminary phase (4 days), animals were allowed to self-inject morphine successively into the LH or the TH, on alternate days. From the fifth day, subjects were given the possibility of choosing between the two sites. For one group, a single low dose of morphine (5 ng) was applied in both structures. In an other group, the doses used were, respectively, 5 ng for the LH and 50 ng for the TH. A marked preference for injection into the LH was observed in the two groups. These results show that mice are capable of discriminating, at the intracerebral level, the motivational or rewarding components of two different doses of morphine even when the dose levels are relatively close (30 ng vs. 50 ng). Moreover, these effects of morphine seem to remain localized to the proximity of the injection sites, suggesting strongly that opiate receptors present in the LH mediate the self-administration response for the drug in this brain region.

Intracranial self-administration  Dose discrimination  Morphine  Lateral hypothalamus  Mice

PREVIOUS studies from our laboratory showed that BALB/c mice self-administer morphine into various brain sites. Placed in a Y-maze, where they were required to discriminate between a neutral arm and a reinforced arm, the animals entered more frequently into the reinforced arm to self-inject morphine into the lateral hypothalamus (LH) and the mesencephalic central gray (4,5). However, the characteristics of the self-administration response induced by morphine varied as a function of the particular brain sites even when these were closely apposed such as LH and medial hypothalamus (6). Moreover, self-administration performance was highly influenced by the dose of morphine injected. Thus, whereas a clear self-administration response was induced by injection of either 5 ng or 50 ng of morphine into LH, paradoxically the discrimination performance of mice was somewhat better with the lower dose (4,6). This result suggests that the motivational and/or emotional effects of the drug are not identical at these two doses. These data raise the following question: is an animal able to discriminate between two different doses of morphine using the intracranial self-administration procedure? To attempt an answer

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to this question, BALB/c mice, implanted bilaterally with two guide cannulae, were placed in an experimental situation offering the possibility of choosing between different doses of morphine self-injected into the LH.

## METHOD

### *Animals and Surgery*

The present experiments used 27 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 cycle (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 bilaterally implanted with a guide cannulae (outer diameter 0.460 mm; inner diameter 0.255 mm).

In 19 animals, the tips of the guide cannulae were positioned bilaterally 1.5 mm above the lateral hypothalamus (LH). The stereotaxic coordinates used were the following: antero-posterior distance (AP) referring the 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. In the eight other animals, the tips of the guide cannulae were positioned in one hemisphere 1.5 mm above the LH and in the other 2.6 mm above the LH. The stereotaxic coordinates used in this group were the following: AP +2.10 mm; L ±1.10 mm; V +3.90 and +2.80 mm, respectively. The incisor bar was level with the interaural line. Mice were allowed to recover from the operation for 1 week.

### *Materials and Experimental Protocol*

*Self-injection procedure.* On each experimental day, two stainless-steel injection cannulae (o.d. 0.229 mm; i.d. 0.127 mm) were inserted into the injection sites and were held fixed in position by means of a small connector. Injection cannulae were connected by flexible polyethylene tubing to two independant microinjection systems, each of which housed a 5 μl Hamilton syringe. The tip of the injection cannulae projected beyond the guide cannulae by 1.5 mm. Consequently, in 19 animals injection cannulae were bilaterally inserted into LH, whereas in 8 animals they were inserted into the LH in one hemisphere and into the thalamus (TH) in the other hemisphere. By interrupting photocell beams in the Y-maze (see the Behavioral Procedure section), mice could obtain the reinforcement (injection of morphine sulfate dissolved in ringer solution). Each self-injection (50 nl) lasted 4 s (normal drug flow was controlled visually 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 two injectors in the same direction as the animal's movement. This process avoided the rolling up of the two flexible tubings; consequently, self-administration could be studied in freely moving mice (5).

*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. Two experiments were performed.

*Experiment 1.* After the first habituation session to the Y-maze during which no injection was delivered, the 19 animals with the injection cannulae bilaterally inserted into the LH were submitted to two successive experimental phases. During a preliminary phase, to begin a trial, a mouse was placed in the starting box, and after 1 min the door to the stem was opened. During the first day, in each group (see below) a certain number of animals learned to trigger injection of morphine by interrupting the photocell beam situated in the right arm (the left arm being closed), whereas the others had to go to the left arm (the right arm being closed). 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. This phase lasted 4 days. An animal could obtain delivery of one dose of morphine (d1) during the first and the third day (in the right arm for example: only one of the two cannulae being active) and delivery of another dose (d2) during the second and the fourth day (in the left arm for example: the other cannula now only being active). For another subject, d1 could be delivered in the left arm and d2 in the right arm. A given dose was always delivered in the same cerebral hemisphere, which was different from one animal to another. Each daily session was composed of 10 trials.

Three groups of subjects were constituted. In the first group, the experiment was performed with the 5 ng and 50 ng doses of morphine ($n = 7$), in the second group using the 15 ng and 50 ng doses ($n = 6$), and in the third group using the 30 ng and 50 ng doses ($n = 6$). In each group, the order of presentation of the doses used differed from one subject to another (for example, in the group 5 ng vs. 50 ng, the order was, during 4 successive days either: 5 ng, 50 ng, 5 ng, 50 ng or 50 ng, 5 ng, 50 ng, 5 ng).

The second phase of Experiment 1 began on the fifth day. During this phase, the two arms of the Y-maze were opened and the animals could choose freely between the two different doses of morphine (the two cannulae being active). Each session was composed of 10 trials. This phase lasted 6 days in the groups 5 ng vs. 50 ng and 15 ng vs. 50 ng and 7 days in the group 30 ng vs. 50 ng. Automatic equipment, triggered by opening the door of the start box to begin a trial, recorded the latency to trigger each injection during the two phases.

*Experiment 2.* After a first habituation session during which no injection was delivered, the eight animals with the injection cannulae inserted respectively into LH and TH were submitted to two experimental phases. During a preliminary phase, to begin a trial, a mouse was placed in the starting box, and after 1 min the door to the stem was opened. During the first day in each group (see below) a certain number of animals learned to trigger injection of morphine by interrupting the

TABLE 1

MEAN VALUES (SECONDS) OF THE LATENCY (± SEM) TO TRIGGER THE INJECTION OF EACH DOSE OF MORPHINE RECORDED DURING THE PRELIMINARY PHASE IN THE THREE GROUPS

| Group | | Latencies |
|---|---|---|
| 5 ng vs. 50 ng | 5 ng | 49.8 ± 4.9 |
| | 50 ng | 30.2 ± 2.4 |
| 15 ng vs. 50 ng | 15 ng | 36.5 ± 2.9 |
| | 50 ng | 23.8 ± 2.8 |
| 30 ng vs. 50 ng | 30 ng | 27.5 ± 3.4 |
| | 50 ng | 24.9 ± 3.6 |

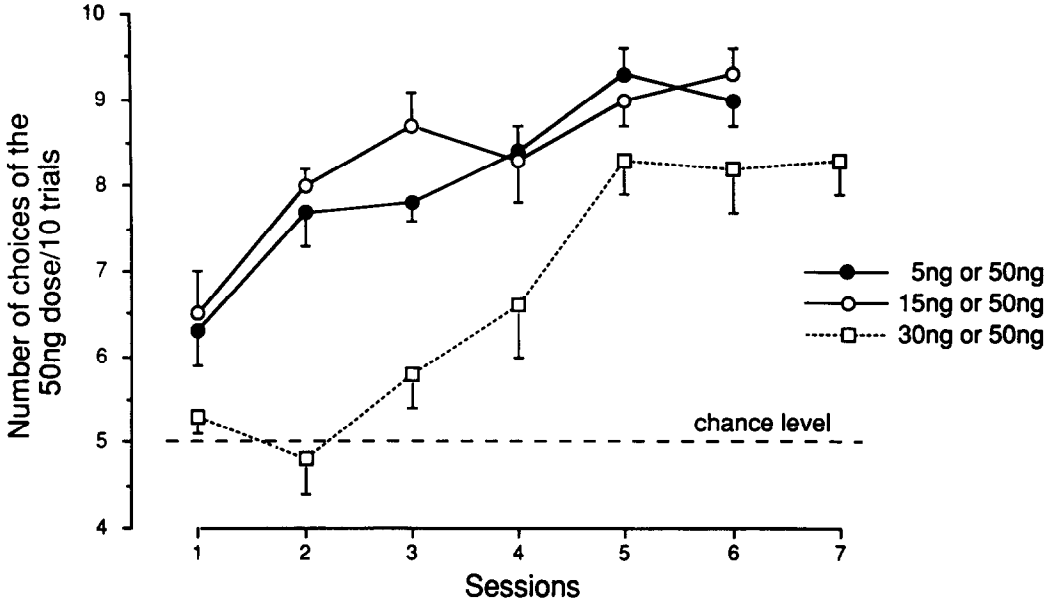



FIG. 1. Mean number of self-injections of the 50 ng dose of morphine (± SEM) recorded in the 5 ng vs. 50 ng, 15 ng vs. 50 ng, and 30 ng vs. 50 ng groups, during the choice phase of the spatial discrimination task in the Y-maze.

photocell beam situated in the right arm (the left arm being closed), whereas the others had to go to the left arm (the right arm being closed). 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. This phase lasted 4 days. An animal could obtain delivery of morphine into LH during the first and the third day (in the right arm, for example; only one of the two cannulae being active) and delivery of morphine into TH during the second and the fourth day (in the left arm, for example; the other cannulae now only being active). For another subject, morphine could be delivered into LH by entering in the left arm and into TH by entering in the right arm). The cerebral hemisphere chosen for injection into LH or TH was different from one animal to another. The order of presentation of injections into LH or TH was also different from one subject to another (for example during the 4 successive days the order of injection for a given animal was either; LH, TH, LH, TH or TH, LH, TH, LH). Each daily session was composed of 10 trials.

Two groups of subjects were constituted. In the first group ($n$ = 4) the experiment was performed using the 5 ng dose of morphine for the two structures. In the second group ($n$ = 4), the doses delivered were, respectively, 5 ng into LH and 50 ng into TH.

The second phase of Experiment 2 began on the fifth day. During this phase, the two arms of the Y-maze were opened and the animals were able to choose freely the brain structure into which they could self-administer morphine (the two injection cannulae being active). This phase lasted 7 days.

Automatic equipment triggered by opening the door of the start box to begin a trial recorded the latency to trigger each injection during the two phases.

TABLE 2

MEAN NUMBER OF ENTRIES (± SEM) INTO THE ARM PREVIOUSLY REINFORCED BY THE 50 ng DOSE OF MORPHINE, RECORDED DURING THE EXTINCTION PHASE IN THE 15 ng VS. 50 ng AND 30 ng VS. 50 ng GROUPS

| | Sessions | | | |
|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 |
| 15 ng vs. 50 ng | 8.3 ± 0.3 | 8.0 ± 0.2 | 6.0 ± 0.2 | 5.6 ± 0.3 |
| 30 ng vs. 50 ng | 8.5 ± 0.5 | 7.8 ± 0.3 | 6.6 ± 0.6 | 5.6 ± 0.4 |

*Histology*

At the end of the experiments, the animals were sacrificed by an overdose of thiopental. The head, with the guide cannulae attached, was placed in 10% formol for a period of 72 h. The guide cannulae were 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

*Experiment 1*

During the preliminary (acquisition) phase we observed, in the two first groups (5 ng vs. 50 ng and 15 ng vs. 50 ng), that the latency to trigger the injection of the 50 ng dose of morphine was significantly shorter than that to trigger the injection of the 5 ng dose, $F(1, 12) = 12.42, p < 0.01$ (12), or the 15 ng dose, $F(1, 10) = 9.76, p = 0.01$. In the group 30 ng vs. 50 ng, on the other hand, the latency to trigger the two doses was similar, $F(1, 10) = 0.26$; NS (Table 1). It may be noted that the differences observed between the latencies for self-injection of the two doses in a given group are greater when the difference between the doses is large, $F(2, 10) = 7.05$, $p = 0.02$.

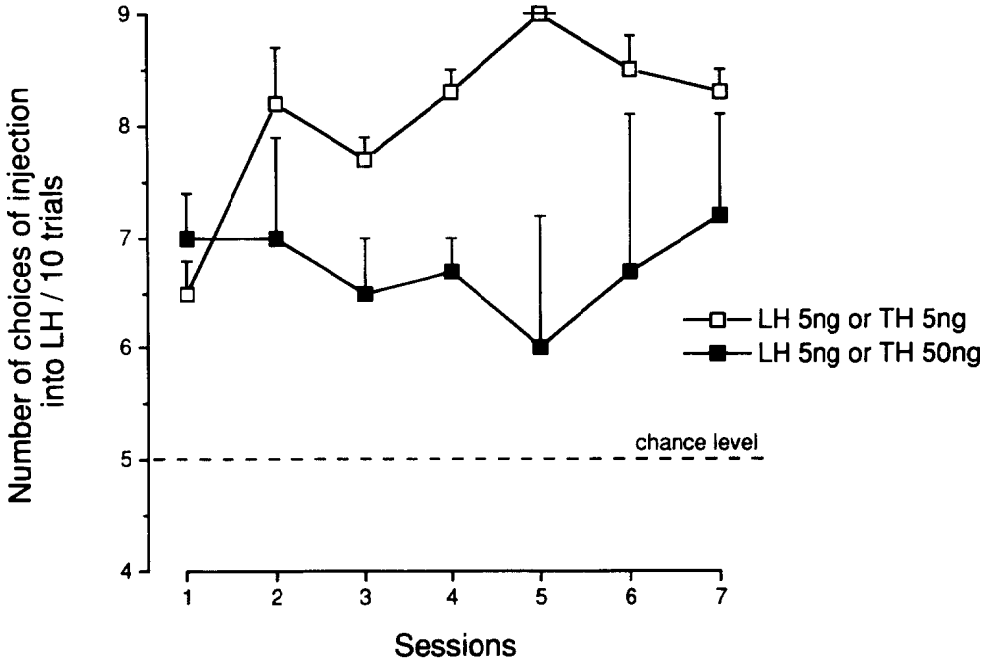
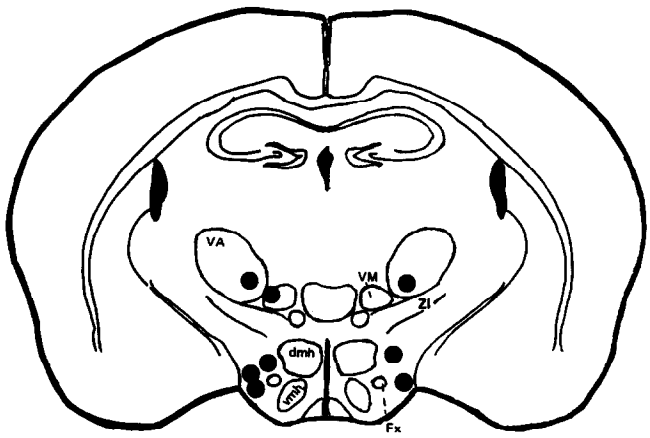
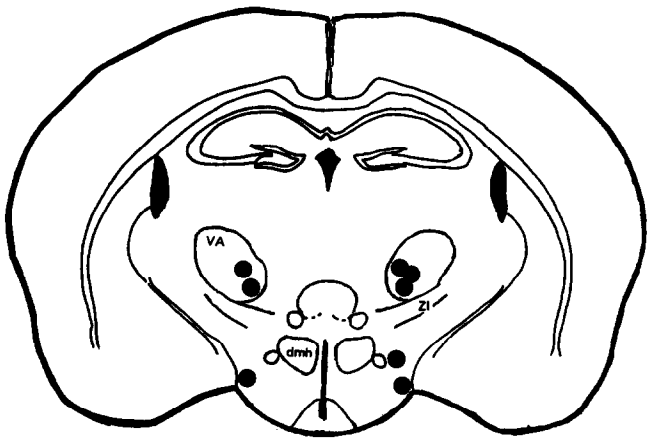



FIG. 2. Mean number of self-injections of morphine (± SEM) into the LH, recorded in the LH 5 ng vs. TH 5 ng and LH 5 ng vs. TH 50 ng groups, during the choice phase of the spatial discrimination task in the Y-maze.

FIG. 3. Photomicrograph of thionin stained section (60 $\mu$m) through guide cannulae (a) and injection cannulae (b) tracks at the level of lateral hypothalamus.




2900 μ




2750 μ

FIG. 4. The placements of the injection sites in the lateral hypothalamus and in the thalamus (Experiment 2) are plotted on frontal section diagrams. The values in microns (μ) indicate the distance of the section from the frontal plane zero situated 1 mm posterior to the interaural line (atlas by A. Lehman CNRS, 1974). dmh: nucleus dorsomedialis hypothalami; Fx: fornix; VA: nucleus ventralis thalami pars anterior; VM: nucleus ventralis thalami pars medialis; vmh: nucleus ventromedialis hypothalami; ZI: zona incerta.

During the choice phase, the 5 ng vs. 50 ng and 15 ng vs. 50 ng groups rapidly differentiated between the two doses of morphine.

This discrimination appeared from the beginning of the first session in these two groups [by comparison with chance level: 5 ng vs. 50 ng group: $t(6) = 3.25, p < 0.02$; 15 ng vs. 50 ng group: $t(5) = 3.75, p < 0.02$]. During the following sessions the discrimination performance further increased and the subjects of the two groups entered more and more frequently into the arm reinforced by the injection of the higher dose of morphine (50 ng) [5 ng vs. 50 ng group: $F(5, 30) = 11.53, p < 0.001$; 15 ng vs. 50 ng group: $F(5, 25) = 8.06, p < 0.001$].

The behavior of the mice of the 30 ng vs. 50 ng group was somewhat different from that of the two preceeding groups, $F(2, 16) = 15.78, p < 0.001$. Indeed, during the first three sessions the discrimination performance recorded was not different from the chance level. However, beginning from the fourth session, $t(5) = 2.66, p < 0.05$, the subjects of this group triggered more frequently the 50 ng rather than the 30 ng dose of morphine, $F(5, 25) = 16.09, p < 0.001$ (Fig. 1).

When discrimination performance had stabilized, the subjects of the 15 ng vs. 50 ng and 30 ng vs. 50 ng groups were submitted to an extinction phase in which only vehicle (ringer) was available. During the two first sessions, the two groups continued to enter, preferentially, into the arm previously reinforced by the 50 ng dose of morphine. Beginning on the third day, the mice tended to alternate their entries into each of the two arms. The performance recorded was identical in the two groups, $F(1, 10) = 0.2$, NS (Table 2).

Following the end of the extinction period, three subjects of the 15 ng vs. 50 ng group and three subjects of the 30 ng vs. 50 ng group were resubmitted to the preceeding experimental protocol (preliminary phase and choice phase) but using doses of 40 ng and 50 ng. No significant discrimination was observed, performance remaining at chance level during seven successive sessions (mean number of choices of the 50 ng dose/10 trials (± SEM) recorded during the seven sessions: 5.1 ± 0.4, 5.6 ± 0.5, 5.1 ± 0.4, 4.8 ± 0.7, 4.3 ± 0.6, 5.5 ± 0.7, 4.8 ± 0.6).

*Experiment 2*

During the preliminary (acquisition) phase, we observed that the latency to trigger the injection of morphine into LH was shorter than that to trigger the injection into TH; this tendency being identical whatever the dose of the drug delivered into TH. Consequently, the data recorded in the two groups (LH 5 ng vs. TH 5 ng; LH 5 ng vs. TH 50 ng) were pooled, which shows clearly a significant difference between the two brain structures [mean values (s) of the latency to trigger the injection into LH: 35.6 (SEM ±4.6); into TH: 63.9 (SEM ±6.6), $t(7) = 5.61, p < 0.001$].

During the choice phase, the LH 5 ng vs. TH 5 ng group rapidly differentiated between the two brain structures. This discrimination appeared from the beginning of the first session [by comparison with chance level: $t(3) = 5, p < 0.02$]. During the following sessions, the discrimination performance increased further and the subjects of this group entered more and more frequently into the arm reinforced by the injection into LH, $F(6, 18) = 11.14, p < 0.001$ (Fig. 2).

In the LH 5 ng vs. TH 50 ng group, no subject chose to self-inject morphine into TH. Two subjects showed a clear preference for the injection of the 5 ng dose into LH, whereas the two others performed at random, which accounts for the fact that no significant progression of the mean level of the discrimination performance was observed during the successive sessions, $F(6, 18) = 0.23$, NS.

*Histology*

Injection sites were precisely located by using the tracks of injection cannulae. Concerning the LH sites, they were all located in a frontal plane corresponding most frequently to the medial or posterior part of the ventromedialis nucleus of the hypothalamus. Scatter of these injection sites was similar for subjects of the different groups studied. Injection sites into TH were located in the nucleus ventralis thalami (pars anterior or medialis) just above the zona incerta (Figs. 3 and 4).

## DISCUSSION

The results of Experiment 1 show that BALB/c mice are clearly able to differentiate between two different doses of morphine during self-administration into the LH. In spite of the fact that the role of LH in internal reward mechanisms for opiates is contested by certain authors (3), it should be noted, however, that the self-administration of morphine into this region has also been observed in the rat (10,11). In our experiment, the discrimination performance was rapid when the concentration difference between the two doses available was marked (5 ng vs. 50 ng or 15 ng vs. 50 ng) with the animals preferentially choosing to self-inject the higher dose. This preference was already statistically significant beginning from the first session of the choice phase. These data suggest that the discrimination had already begun during the preliminary phase in which the subjects alternatively triggered either low or high doses of morphine. This hypothesis seems to be confirmed by the fact that, during this phase, the latency for triggering the doses of 50 ng was shorter than that to trigger the doses of 5 ng or 15 ng and which clearly indicates the stronger motivational effect of the higher dose.

When more similar doses of morphine were used (i.e., 30 ng vs. 50 ng), the discrimination task was more difficult. However, after an initial period during which the animals triggered the two doses in an equivalent manner, a marked preference for injection of the 50 ng dose gradually appeared and persisted up to the end of the experiment. This result particularly reveals the high discrimination capacity of these subjects. Finally, when very similar doses of morphine were used (i.e., 40 ng vs. 50 ng) no discrimination was observed; the animals tending to alternate the triggering of the two doses. The fact that the subjects perform at chance level when the two doses used were 40 ng and 50 ng shows that the discrimination behavior observed when the dose contrast is greater (i.e., between a low and a high dose of morphine) in the three other groups is not artefactual: this dose discrimination seems effectively to correspond to both a qualitative and quantitative central appreciation of the motivational and/or emotional effects induced by each of the different doses of morphine.

When the morphine reinforcement was suppressed, vehicle only being available, a strong resistance to extinction of the self-administration response was observed during the first 2 days. Subjects of the two groups studied (15 ng vs. 50 ng and 30 ng vs. 50 ng) continued to prefer the arm previously reinforced by the injection of 50 ng of morphine. This result confirms that even in the 30 ng vs. 50 ng situation, a preference for the highest dose was clearly established during the choice phase.

Given the distance separating the two bilateral injection sites (2.2 mm), there is little or no risk of contamination between these two LH sites because Lomax (8) has shown that the injection of ($^{14}$C) morphine does not diffuse beyond a sphere of 0.6 mm radius around the injection cannula tip. The results of Experiment 2 clearly demonstrate that when the subjects could choose freely between infusions of morphine into the LH or into the TH (these two sites being situated in the same frontal plane and separated only by a vertical distance of 1.1 mm), no marked preference for injection into the TH was recorded, even when the dose applied in this structure was 10 times greater than that delivered into the LH. These data on the one hand confirm that a low dose of morphine (5 ng) has rewarding effects when applied into the LH (4,6), and on the other hand, demonstrate that the ventral thalamus is not a major site for self-administration of opiates as is also the case for the lateral TH (11). These results suggest, moreover, that, in our experimental conditions, the effects of each dose of morphine remain localized to the proximity of the injection sites and consequently that the self-adminitration of this drug into the LH is produced by activation of local opiate receptors present in this structure (7,9). In this respect, we have recently shown that with an experimental protocol and injection parameters identical to that used in the present study it is possible to clearly differentiate the reactivity to morphine for brain injection sites separated only by 0.6 mm in laterality (6) and by 0.8 mm in verticality (unpublished data). These data, thus, provide strong evidence for a limitation of dorsal and lateral efflux from the tip of the injection cannula.

In conclusion, the present study demonstrates that motivational and/or rewarding effects of two different doses of morphine can be easily differentiated using intracerebral injection procedures. Self-administration of morphine has previously been observed in various brain structures in rats (2,10,11) and mice (4–6). However, if the role of the ventral tegmental area appears to be important in this drug-seeking behavior (1), that of other structures remains, in contrast, less well understood. The utility of the present two-choice task, which enables simultaneous study of two independant intracerebral injections, will certainly render possible a classification of different brain structures along a sensitivity continuum. Furthermore, many studies have already demonstrated the capacity of animals to discriminate between different drugs when injected systemically (13). With our present novel technique, a similar approach can now be envisioned at the intracerebral level. In particular, by using drugs that are selective for the different subtypes of opiate receptor (type $\mu$ or $\delta$), it may even be possible to further describe and define the respective roles of these receptor subtypes in the mechanisms of central reinforcement.

## ACKNOWLEDGEMENTS

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