Pharmacology Biochemistry & Behavior, Vol. 15, pp. 37–41, 1981. Printed in the U.S.A.

A Within-Subject Comparison of the Effects of Morphine on Lateral Hypothalamic and Central Gray Self-Stimulation[1]

S. SCHENK, A. COUPAL, T. WILLIAMS AND P. SHIZGAL

Center for Research on Drug Dependence, Department of Psychology, Concordia University
1455 de Maisonneuve Blvd. West, Montreal, Quebec, Canada H3G 1M8

Received 16 December 1980

SCHENK, S., A. COUPAL, T. WILLIAMS AND P. SHIZGAL. *A within-subject comparison of the effects of morphine on lateral hypothalamic and central gray self-stimulation.* PHARMAC. BIOCHEM. BEHAV. **15**(1) 37–41, 1981.—The effects of chronic administration of morphine (20 mg/kg) on self-stimulation (SS) of the central gray and lateral hypothalamus were investigated in a within-subject design. The magnitude and time course of the drug-produced changes in SS at the two placements were similar within subjects but varied substantially across subjects. These results are interpreted in the light of evidence pertaining to the anatomical linkage of the substrates for the rewarding effects of central gray and lateral hypothalamic stimulation. The facilitation of SS may be due to a drug-produced sensitization of reward-related neurons. If so, morphine acts either beyond the point of convergence of the two substrates or at an earlier stage in each substrate. The across-subject variability is attributed to individual differences in sensitivity to the effects of the drug. The importance of controlling for this subject variable is stressed.

Self-stimulation Lateral hypothalamus Central gray Morphine

IT has been reported that morphine facilitates self-stimulation (SS) of various brain sites including the lateral hypothalamus [1], ventral tegmentum [3], locus coeruleus [7], medial prefrontal cortex [9], and dorsal raphé nucleus [8]. These effects have been interpreted as the result of a direct action of the drug on the neural substrate for brain stimulation reward [4]. Some investigators have also reported that the modulation of SS by opiates is site specific [7, 8, 9]. For example, electrode placements within 0.2 mm of the midline at the level of the dorsal raphé nucleus have been shown to be less sensitive to the facilitatory effects of morphine on SS than more lateral placements [8]. Conceivably, opiates act directly only on specific elements within the circuits subserving brain stimulation reward.

The neural circuitry subserving SS has not been clearly delineated. Recent evidence from our laboratory has led to the suggestion that different elements are responsible for the rewarding effects of lateral hypothalamic (LH) and periaqueductal gray stimulation [2]. In light of these findings, we were interested in determining whether morphine would produce different effects on SS at these two placements. Since we have noticed large across-subject differences in the opiate modulation of SS [11], a multiple electrode implant design was employed to control for the subject variable.

METHOD

Subjects

The subjects were six male hooded rats of the Royal Victoria strain (Canadian Breeding Farms, St. Constant, Quebec) weighing approximately 350 g at the time of surgery. They were individually housed in wire mesh cages and maintained on a 12 hr light/dark cycle. Purina rat chow and water were available in the home cage at all times.

Electrode Implantation

Electrodes were 254 μm stainless steel wire insulated with Formvar to within 0.25 mm of the rounded tip. Using standard stereotaxic procedures, we aimed the electrode tips at the LH (AP: −0.4 mm relative to bregma; lateral: +1.7 mm; 8.0 mm below the dura) and the dorsal raphé nucleus (DR) (AP: −6.0 mm; lateral: 0.00 mm; 6.0 mm below the dura). The incisor bar was set at +5.0 mm. Sodium pen-

[1]This work was supported by F.C.A.C. grant no. Eq-09 from the Ministère de l'Education de Québec.

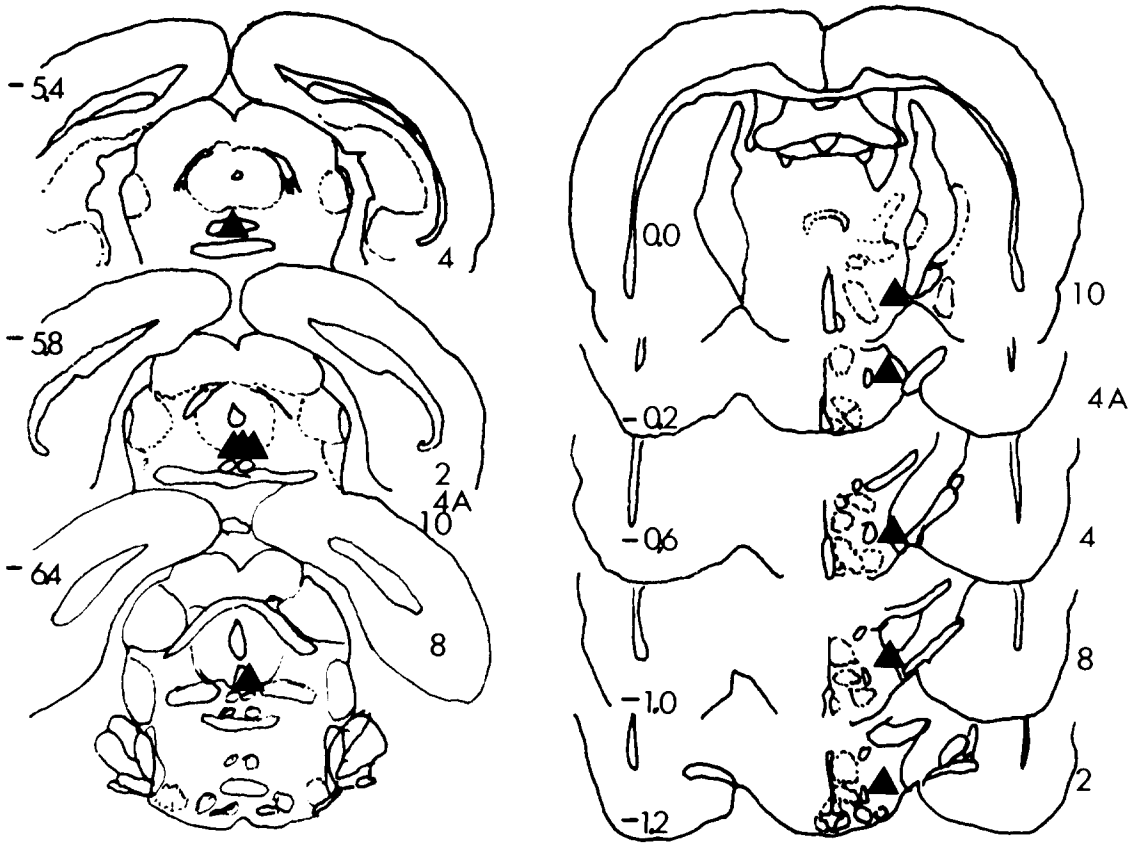

FIG. 1. Reconstructions of electrode placements for 5 of the 6 subjects. No histology was available for rat no. 5. Pertinent sections are from the Pellegrino *et al.* [10] atlas.

tobarbital (60 mg/kg) was used as the anesthetic. A flexible stainless steel wire wrapped around four stainless steel skull screws served as the current return. The electrodes were fixed to the skull with dental acrylic.

Apparatus

Testing was carried out in a wooden box with a Plexiglas front (25×25×82 cm). Depression of a lever (Lehigh Valley Electronics 121-05) mounted in one corner of the chamber triggered a 500 msec train of rectangular, cathodal pulses, 0.1 msec in duration. Current intensity and frequency were manipulated for each electrode placement in each animal so that a maximal rate of responding was attained. These intensities were similar for each placement, ranging from 250–500 μa, and were fixed for the remainder of the experiment.

Required frequencies were determined by decreasing the pulse frequency every 2.5 minutes in 0.1 $\log_{10}$ steps from the value that produced maximal response rates to a value for which the animal failed to respond (<10 responses/2.5 minutes). The stimulation frequency that supported a half maximal rate of responding was defined as the required frequency and was determined by interpolation. These required frequencies ranged from 60–90 Hz.

Procedure

Roughly 1 week after surgery animals were trained by successive approximation to self-stimulate at both electrode placements. Performance was stabilized over a period of about 7 days by determining required frequencies at up to 7 hourly intervals during each daily session. After stabilization a vehicle test was conducted. Ringer's solution was injected (1 ml/kg), and required frequencies were determined at hourly intervals for 7 hours. The difference between any 2 required frequencies was not greater than 0.1 $\log_{10}$ units on this control day, indicating that SS performance was stable over this entire testing session of 7 hours. Within 4 days of the vehicle test, drug testing began. At hour 1 on each drug day, a pre-injection baseline test was run; morphine was administered after the first required frequency determination. Drug effects were then assessed for 6 hours. The LH placement was tested on the hour and the central gray (CG) placement on the half hour. Drug effects were determined by comparing the post injection to the pre-injection required frequency for that test day.

Drug solution. Morphine HCl was dissolved in injectable Ringer's solution (20 mg/ml, as measured by weight of the salt). The drug was injected daily (20 mg/kg, IP) for 5 days, and animals were tested on days 1, 3, and 5.

Histology. At the completion of the experiment, animals were administered an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline followed by 10% Formalin. The brains were then removed and stored in 10% Formalin solution for at least 1 week. They were then frozen and sliced into 40 μm sections.

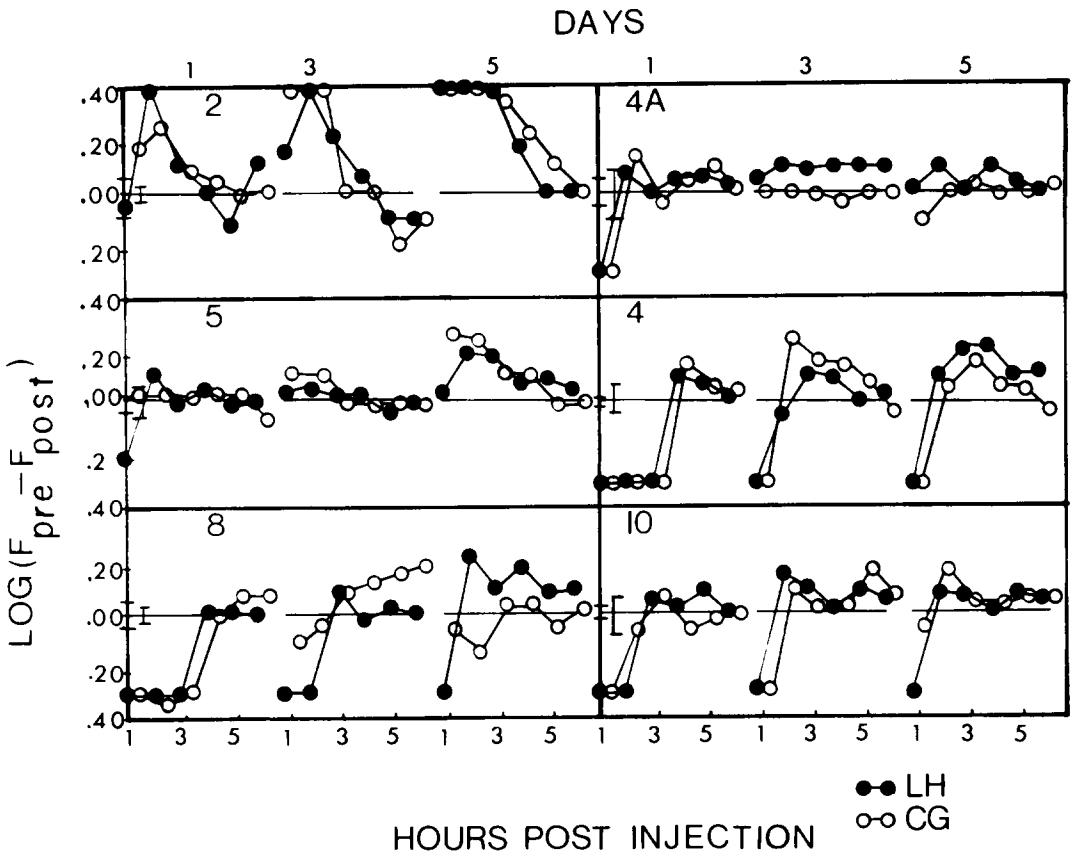

FIG. 2. Changes in required frequency of both sites in each subject as a function of hours post injection and days of treatment. Open circles refer to the posterior placement (CG). Close circles refer to the anterior placement (LH). The statistic, log ($f_{pre}-f_{post}$) reflects the proportional changes in the number of pulses required to maintain a half maximal rate of responding following drug treatment as compared to the pre-injection baseline.

RESULTS

Figure 1 shows reconstructions of electrode placements for 5 of the 6 animals. Sections that include the tip of the electrode track are not available for animal no. 5. The tips of all anterior electrodes were located within the LH. The placements for rats no. 4 and no. 8 bordered the internal capsule, while the placement for rat no. 2 was located just below the zona incerta. For the posterior electrodes, two placements (no. 2 and no. 10) were in the DR, one (no. 4A) was just ventral to the DR, another (no. 4) bordered the medial longitudinal fasciculus at the level of the third ventricle, and the final electrode tip (no. 8) was adjacent to the DR in the periaqueductal gray.

Figure 2 shows the changes in SS of both sites in each subject as a function of hours post injection and days of treatment. The ordinate is expressed as the function, log ($f_{pre}-f_{post}$), where f_{post} refers to the post injection required frequency and f_{pre} to the pre-injection required frequency. This statistic allows facilitation of SS to be expressed as a positive change from the pre-injection baseline. At the extreme left of each set of curves 95% confidence intervals are shown. The left-most confidence interval is for the LH site and the confidence interval to its right is for the CG site. These intervals are derived from the 7 hourly threshold determinations per electrode obtained on the Ringer's treatment day.

The pattern of drug effects is quite similar across placements within each subject. In rats no. 4, no. 4A, and no. 10 there is an initial suppression of SS lasting from 1–3 hours post-injection. In subjects no. 4 and no. 10, the suppression is followed by facilitation. During chronic treatment the suppression undergoes tolerance and the facilitation occurs earlier. Rat no. 2 shows no suppression on Day 1 of treatment but shows some stereotypic responding (gnawing on the lever) as indicated by a high level of non-reinforced responses. This stereotypic responding is observed on all three test days, increasing in duration with continued treatment. On Day 5 clear facilitation is seen at hours 3 and 4 post injection. Rat no. 5 shows very little effect of the drug on Day 1, but there is some facilitation on Day 3, which increases in magnitude and duration on Day 5. For all of these rats, the pattern and magnitude of the morphine-produced changes in SS are consistent across placements. In contrast, the across subject variability in both the pattern of the drug-produced changes in SS and the magnitude of the facilitation is quite large. The size of the maximum facilitation observed on Day 5 ranges from 0.01–3.5 $\log_{10}$ steps and occurs anywhere from 1–4 hours post injection. The only marked across placements differences are found in rat no. 8. While the performance of all other animals returns to the pre-injection baseline threshold by 6 hours post injection, this subject shows a large enhancement of responding for CG stimulation on Day 3 that seems to increase over the test

session. On Day 5 the discrepancy between the drug-produced effects of the two placements appears to be the greatest.

DISCUSSION

Both the time course and magnitude of the drug-produced changes in SS at the two placements are similar within subjects. The one exception is rat no. 8. This rat's behavior was different from the typical behavior reported in the literature [1,4] and generally observed in our laboratory. The performance of subjects that show changes in SS following morphine administration typically returns to the pre-injection level within 6 hours of the injection. In contrast on Day 3 of morphine treatment, the performance for central gray stimulation in rat no. 8 was elevated following morphine injection and remained so over the entire testing session. On Day 5 the pre-injection baseline was still elevated relative to this rat's past performance. One cannot confidently attribute the apparent discrepancy between the drug-produced changes in SS at the two placements to a site-specific action of morphine on reward-related neurons. These data may reflect changes in the effects of the CG stimulation that are unrelated to the action of the drug.

The consistency of the morphine-produced changes in SS across placements for the other 5 subjects stands in contrast to the differences in the excitability characteristics of the reward relevant neurons excited by LH and CG stimulation [2]. The simplest explanation of these differences is that the rewarding effects of stimulation at the two sites are due to the activation of different neural populations. The observation that there is strong summation of the rewarding effects of LH and CG stimulation [2] implies that the directly stimulated cells converge at some point. Our findings may be interpreted within this framework. Either the drug works beyond this proposed point of convergence or the drug sensitizes both substrates prior to this point with a similar time course. Electrophysiological studies may help choose between these two possibilities [6].

Nonetheless, one cannot rule out the possibility that the drug-produced changes in SS are due to factors other than an increase in the rewarding effect of the stimulation. Although we have shown that the facilitation of SS by morphine is not due to a generalized enhancement of operant performance [11], it is possible, for example, that the drug acts on the substrate for the priming effect [5].

In this study there is little evidence for a site specific modulation of SS by morphine. There are large across subject differences both in the magnitude and time course of the drug-produced effects (see Fig. 2). It is possible that the across subject variable represents differences in the subjects' sensitivity to the drug's modulatory effect rather than differences in electrode placement. Otherwise it is difficult to account for the similarity within subjects of the drug's effect at the two placements.

The value of the within subject design, which makes it possible to separate placement from subject effects, is dramatically illustrated in Fig. 3. The data from Fig. 2 are rearranged to show how misleading results could have been obtained had we not done within subject comparisons.

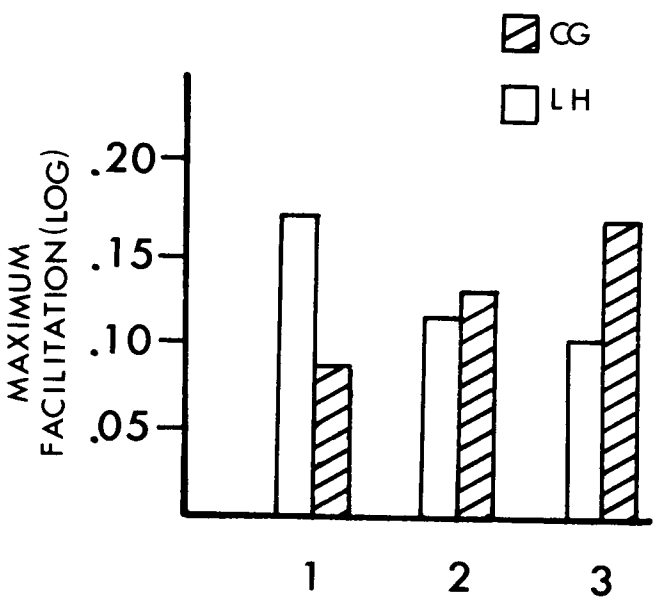

FIG. 3. Rearrangement of data from Fig. 2. Maximum facilitatory effects of morphine on SS at the two electrode placements. See text for details.

Maximum facilitatory effects of morphine on SS at the two electrode placements are plotted. Sampling without replacement, the subjects from this study are arbitrarily subdivided into three sets of 2 groups. Each group contains 3 subjects. One group of each set (termed LH) was selected on the basis of the results obtained with the anterior placement. The other group of each set (termed CG) was selected on the basis of the results obtained with the posterior placement. In each panel of Fig. 3, the LH results from three rats are compared to the CG results from three other rats. Thus, the data appear as if they were collected in an across-subject design.

In the first set (left-hand panel) subjects were chosen so that the morphine produced facilitation in the LH group is greater than that in the CG group. The converse is true for the third set (right-hand panel). In the final set (center panel) subjects were chosen so as to minimize the differences between the two groups. It is apparent from this figure that had an across subject design (one electrode per subject) been used any of the outcomes depicted in Fig. 3 could have been obtained, i.e., either no differences across the two placements or differences in either direction.

In order to reduce the likelihood of obtaining such spurious results, one would have to run a large number of subjects in order to randomize the effect of the subject variable. The time-consuming nature of threshold determination procedures recommended for use in studies of drug effects on SS [4] make this difficult to achieve. In studies with small numbers of subjects, the within subject design must be used if the variability due to placements is to be distinguished from the variability due to subject.

REFERENCES

1. Adams, W., S. Lorens and C. Mitchell. Morphine enhances lateral hypothalamic self-stimulation in the rat. *Proc. Soc. exp. Biol. Med.* **140:** 770–771, 1972.

2. Bielajew, C., C. Jordan, J. Ferme-Enright and P. Shizgal. Refractory periods and anatomical linkage of the substrates for lateral hypothalamic and periaqueductal gray self-stimulation. *Physiol. Behav.*, in press, 1981.

3. Broekamp, C. L., J. H. Van den Bogaard, H. J. Heijen, R. H. Rops, A. R. Cools and J. M. Van Rossum. Separation of inhibiting and stimulating effects of morphine on self-stimulation behavior by intracerebral micro-injections. *Eur. J. Pharmac.* **36:** 443–446, 1976.
4. Esposito, R. U. and C. Kornetsky. Opioids and rewarding brain stimulation. *Neurosci. Biobehav. Rev.* **2:** 115–122, 1978.
5. Gallistel, C. R. Self-stimulation: The neurophysiology of reward and motivation. In: *The Physiological Basis of Memory*, edited by J. A. Deutsch. New York: Academic Press, 1973.
6. Gallistel, C. R., P. Shizgal and J. S. Yeomans. A portrait of the substrate for self-stimulation. *Psychol. Rev.*, in press.
7. Jackler, F., S. S. Steiner, R. J. Bodnar, R. F. Ackerman, W. T. Nelson and S. J. Ellman. Morphine and intracranial self-stimulation in the hypothalamus and dorsal brainstem: Differential effects of dose, time and site. *Int. J. Neurosci.* **9:** 21–35, 1979.
8. Liebman, J. and D. S. Segal. Differential effects of morphine and D-amphetamine and self-stimulation from closely adjacent regions in rat midbrain. *Brain Res.* **136:** 103–117, 1977.
9. Lorens, S. A. Comparison of the effects of morphine on hypothalamic and medial frontal cortex self-stimulation in the rat. *Psychopharmacology* **48:** 217–224, 1976.
10. Pellegrino, L. J., A. S. Pellegrino and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Plenum Press, 1979.
11. Schenk, S., T. Williams, A. Coupal and P. Shizgal. A comparison between the effects of morphine on the rewarding and aversive properties of lateral hypothalamic and central gray stimulation. *Physiol. Psychol.* **8:** 372–378, 1980.