Pharmacology Biochemistry & Behavior, Vol. 13, pp. 919–924. Printed in the U.S.A.

Facilitation of Self-Stimulation in Rats by Methadone[1]

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Received 6 June 1979

STUTZ, R. M., A. N. MAROLI, W. K. TSANG AND P. A. HARVAN. *Facilitation of self-stimulation in rats by methadone*. PHARMAC. BIOCHEM. BEHAV. 13(6) 919–924, 1980.—The effects of morphine and its derivatives on self-stimulation behavior have been widely studied. In those experiments which have used multiple injections (over days) and multiple post-injection tests (within days), the typical findings includes a depression of responding after the initial injections followed by a facilitation of responding on subsequent days. There have been only a few reports which have tested the effects of methadone in this paradigm. Some investigators have observed only depression of self-stimulation while others have reported both the transient depression and the subsequent facilitation generally obtained with morphine. In the present experiment we administered either 5 mg/kg or 10 mg/kg methadone IP over a five day period and tested MFB-LH self-stimulation at 2, 4, 6, 8, 10 and 23 hours post-injection. Compared to saline controls, the 10 mg/kg dose produced the typical opiate-induced changes in self-stimulation, i.e., an initial depression which lasted for two hours on the first two days but was replaced by significant facilitation by hour 4 of day 3. This facilitation persisted for at least 10 hours on all 5 days of the experiment. Except for a transient (days 2–3) depression of self-stimulation, 5 mg/kg was without effect. The present experiment demonstrates that methadone does facilitate self-stimulation but that its ability to do so is highly dose-dependent.

Self-stimulation Opiates Methadone

IN the past several years a number of reports have appeared concerning the effects of opioids on self-stimulation (SS) in rats [1, 2, 5, 7, 8, 10–16]. The effects of opioids on intracranial reinforcement are complex, being dependent upon electrode locus, drug dosage, prior drug history, route of administration, and topography of the response used. Esposito and Kornetsky [6] have provided a comprehensive review of this literature.

In general, two behavioral effects have been widely reported in those experiments which studied the effects of morphine on self-stimulation over several days and at several post-injection intervals within each day. Following the first one or two daily injections, self-stimulation is profoundly depressed for 1–4 hours with responding returning to control levels shortly thereafter. On subsequent days, the degree of suppression of self-stimulation is diminished in both magnitude and duration and is followed by a facilitation of responding. Response rates generally return to control levels by 5–6 hours post-injection. Usually, by the third of fourth day of treatment, no drug induced suppression is observed and the facilitation of responding occurs within one hour of the injection and persists for about four hours. The magnitude of this facilitation may reach 200–300% over control levels and persists without diminution throughout experiments which have lasted up to 20 days [6]. While there are occasional studies which found only a suppression of self-stimulation following morphine injections (e.g. [13,19]), the reason for this discrepancy has not been well documented. Minor differences in electrode locus [13,19], drug regimen [19], or stimulation parameters [19] may have been contributing factors.

Methadone, a synthetic opiate, has not been studied extensively using the self-stimulation paradigm. Pert and Hulsebus [18] reported that methadone affected self-stimulation in much the same way that morphine does (see above). However, the results of their study were published only in the form of an abstract and the procedural details are therefore unavailable. This absence of details is unfortunate since, in a later report, Pert [17] reported that methadone (0.1, 1.0, 5.0 mg/kg) failed to produce reliable increases in posterior-lateral hypothalamic self-stimulation. This report leaves unclear the prior drug history of the rats which were tested only at three hours following administration of the drug. Furthermore, at the lowest dose used (0.1 mg/kg) there was a slight inhibition of response rate. Schaefer and Holtzman [19] reported that methadone (0.3 and 3.0 mg/kg, IP) reliably inhibited medial forebrain bundle-lateral hypothalamic (MFB-LH) self-stimulation rates without producing any statistically significant facilitation of responding. Inhibition occurred 1 and 3 hours post-injection when 0.3 and 3.0 mg/kg were used but not when 0.1 or 1.0 mg/kg was administered.

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There is no apparent reason why methadone should not affect self-stimulation as other opioids do (i.e., a dose dependent depression followed by a facilitation of responding). Therefore, we decided to examine the effect of methadone on self-stimulation using doses which partially overlap and extend those previously used [17,19] and at a greater number of post-injection intervals. Specifically, rats with MFB-LH electrodes were tested for self-stimulation with either 5.0 or 10.0 mg/kg methadone HCl at 2, 4, 6, 8, 10, and 23 hours following injection.

METHOD

Subjects

The subjects were 21 male rats of the Long-Evans strain obtained from the animal colony maintained in the Department of Psychology at the University of Cincinnati. At the time of surgery the animals weighed between 350–400 g. All animals were individually caged with food (Purina Lab Chow) and water continuously available.

Surgery

A single bipolar stainless steel electrode (Plastic Products Company, MS-303-.018-.312-.010) was stereotactically aimed in the medial forebrain bundle of each rat. Surgery was performed under sodium pentobarbital (Nembutal) anesthesia (42 mg/kg, IP) with atropine sulfate (0.2 mg, IP) administered to reduce respiratory congestion. With the skull horizontal between bregma and lambda, the coordinates were: 4.5 mm posterior to bregma, 1.5 mm lateral to the mid-line, and 8.5 mm below the surface of the skull. This corresponds to the deGroot [4] coordinates: AP 5.0, V −2.75, L 1.75.

Self-Stimulation Testing

Ten days following surgery, the animals were trained to press a lever in order to receive reinforcing electrical stimulation of the brain [21]. Each depression of the lever resulted in the delivery of a 300 msec train of 60 Hz current through a constant current circuit. The initial training phase consisted of daily 30 minute sessions during which the animals were shaped to self-stimulate. On each of these days the animals were exposed to several different current levels in order to determine the lowest intensity which would support the criterion performance of 600 lever pressing responses per 30 minutes on a continuous reinforcement schedule. These current levels ranged between 10–70 μA. The current thus selected for an individual animal was used for all subsequent testing. The animals then were assigned to one of three groups (n=7) which were statistically equated for initial lever pressing rates. The groups received either saline, 5 mg/kg methadone, or 10 mg/kg methadone. A given rat received only one type of injection: 5 mg/kg methadone, 10 mg/kg methadone or saline.

Drug Tests

At 9 a.m. on each of the next 5 days the animals received a single IP injection of either 5 mg/kg methadone HCl, 10 mg/kg methadone HCl, or the vehicle (0.9% saline). The drug was prepared by dissolving methadone HCl (Lilly) in 0.9% saline at appropriate concentrations such that each animal received 1 ml of the solution per kg of body weight.

During the five days of the experiment, the effects of methadone on SS behavior were determined by allowing the animals to self-stimulate during 15 minute test sessions given 2, 4, 6, 8, 10, and 23 hours post-injection. Self-stimulation rates were collected only during the last 10 minutes of a session. It an animal failed to self-stimulate immediately, priming stimulation was administered by the experimenter during the five minute warm-up period.

Histology

Following completion of the behavioral tests, the animals were killed with an overdose of sodium pentobarbital and perfused intracardially with physiological saline followed by 10% Formalin. After removal from the skull, the brains were embedded in celloidin and 40 μm sections were taken throughout the electrode tract. The cresylviolet stained brain sections were then mounted on slides and microscopically examined to verify the electrode placements.

RESULTS

A one-between (drug) two-within (days and hours) analysis of variance was performed on the 10 minute self-stimulation rates. The analysis yielded a significant main effect for days, $F(4,68)=6.29$, $p<0.001$, a significant Drug × Hour interaction, $F(10,85)=2.16$, $p<0.03$, and a significant Drug × Day × Hour interaction, $F(40,340)=1.50$, $p<0.03$. These effects are displayed separately for the 5 mg/kg and the 10 mg/kg groups in Figs. 1 and 2, respectively. The drug effects are compared to the same saline control group in both figures.

In Fig. 1 it can be seen that 5 mg/kg of methadone failed to consistently alter self-stimulation during any of the testing periods. While the depression seen at Hours 2 and 4 of Day 2 was statistically significant, the episodes of apparent facilitation seen in Fig. 1 were not statistically different from the saline control group. Analysis of the individual protocols revealed that a single subject receiving 5 mg/kg showed a substantial rate increase contributing to the differences in the means. Figure 2 shows a pattern of responding following 10 mg/kg of methadone which is similar to that most commonly produced by daily injections of morphine (i.e., initial depression followed by facilitation of responding).

Two hours following the first two injections, responding was suppressed although tolerance to this effect occurred by the third day and a statistically significant facilitation was evident on Day 5. The suppression produced by 10 mg/kg of methadone dissipated by Hour 4 and facilitation occurred by Hour 6 of the first day. Statistically reliable potentiation of responding occurred by Hour 4 of Day 3 and persisted through Hours 6, 8, and 10 on this and all successive days. Twenty-three hours after injection there was still a slight elevation of rates for animals receiving 10 mg/kg of methadone although the magnitude of this effect was not statistically significant. Obviously, the facilitatory effect dissipates at some point between 10–23 hours post-injection.

The electrode placements for all rats are displayed in Fig. 3. The electrode tips encroached upon or were located directly in the medial forebrain bundle at the level of the lateral hypothalamus. Examination of individual protocols revealed that none of these placements was associated with persistent depression of self-stimulation following methadone injections.

DISCUSSION

The pattern of changes observed in self-stimulation following administration of 10 mg/kg methadone HCl is com-

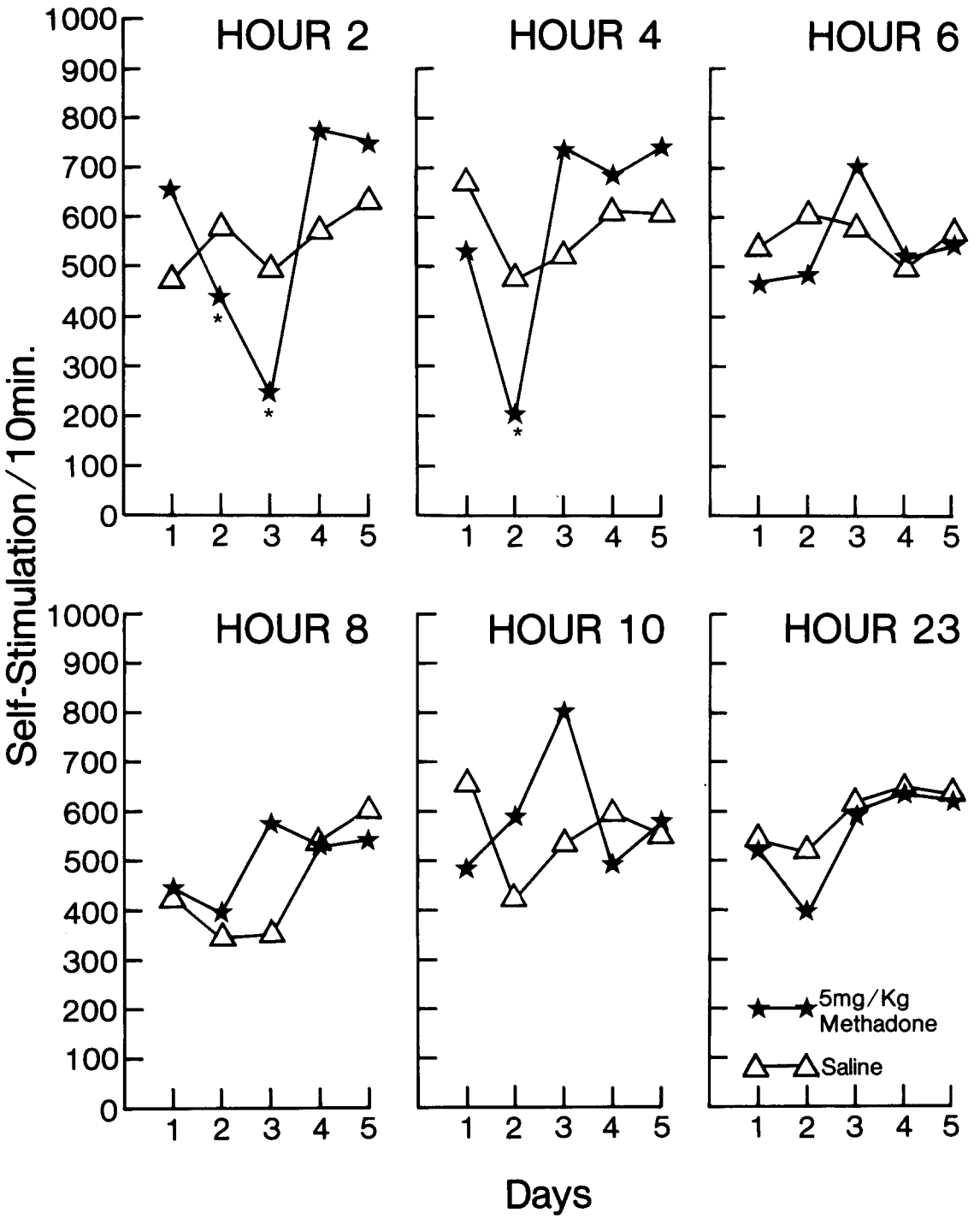

FIG. 1. Effect of 5 mg/kg methadone on self-stimulation rates. Asterisks indicate the points at which the drug group differed significantly from the saline control group (Scheffé tests, $p<0.05$). The drug was administered on each of the five days represented in the figure.

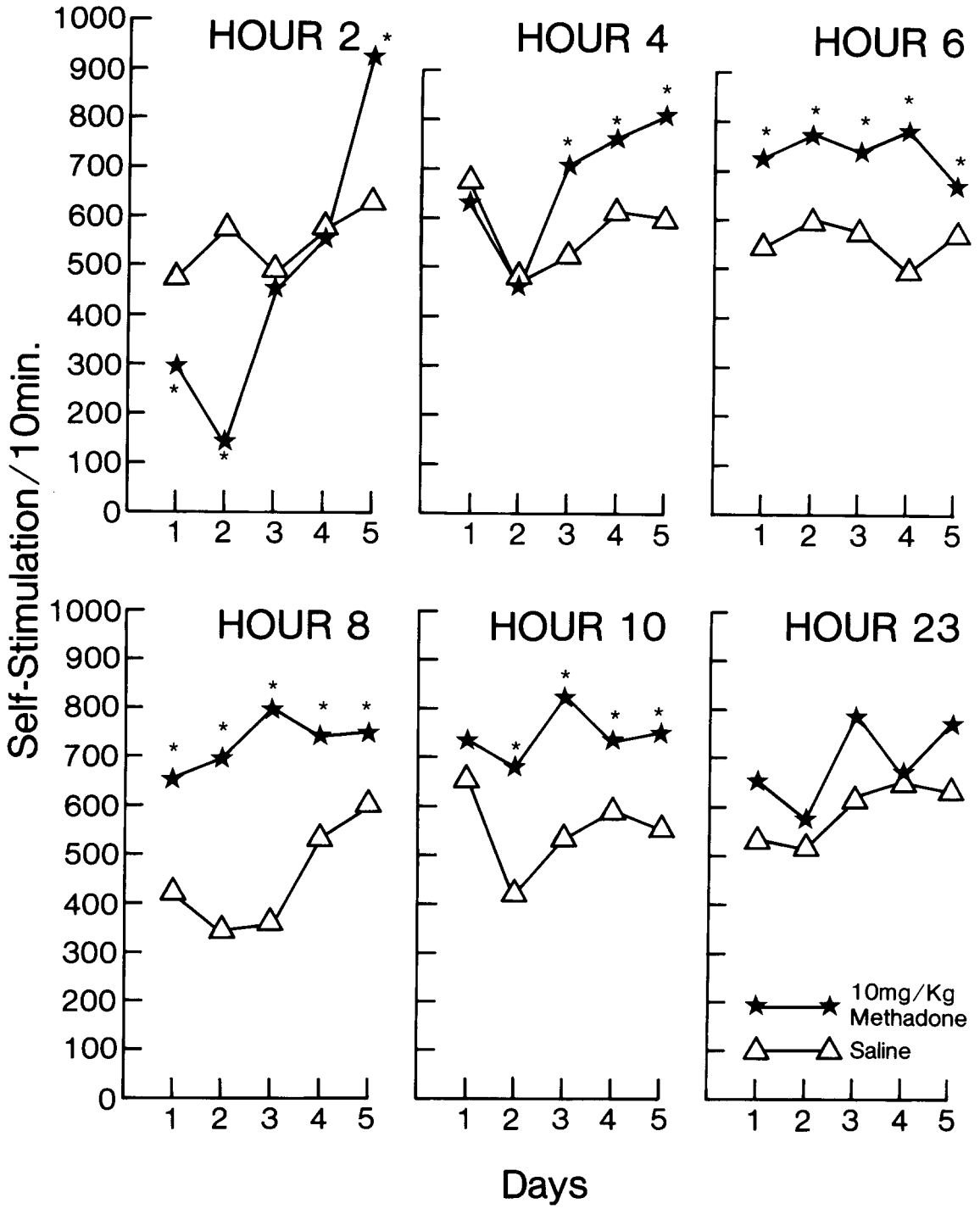

FIG. 2. Effect of 10 mg/kg methadone on self-stimulation rates. Asterisks indicate the points at which the drug group differed significantly from the saline control group (Scheffé tests, $p<0.05$). The drug was administered on each of the five days represented in the figure.

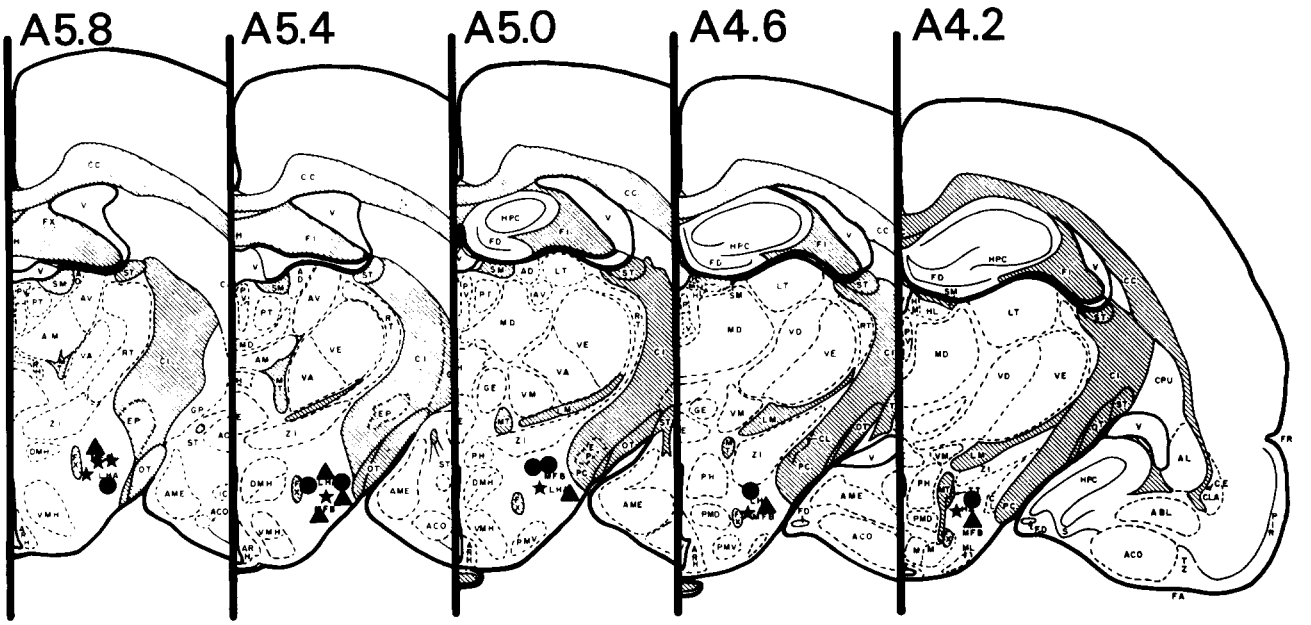

FIG. 3. Electrode placements for the animals receiving 5 mg/kg methadone (circles), 10 mg/kg methadone (stars), and saline (triangles). Figures are taken from DeGroot [4].

parable in direction and magnitude to the pattern seen following injections of 5–10 mg/kg of morphine (e.g., [1, 7, 10, 12, 14, 16]). That is, tolerance rapidly developed to an initial depressive effect and was followed (over days) by a potentiation of responding. As in the previous morphine experiments which lasted between 5–20 days (see [6]), no tolerance occurred to the facilitatory effect, at least during the five days of testing employed in the present study. In previous experiments with morphine [1, 5, 16], responding returned to base or control levels within 4–6 hours while in the present experiment 10 mg/kg methadone maintained significantly elevated rates for at least 10 hours post-injection. In Fig. 2 there is some evidence that the methadone treated rats were still responding at slightly (although not significantly) higher rates than were control Ss even at 23 hours post-injection, thereby suggesting that the effects of 10 mg/kg methadone may persist beyond 10 hours.

Since morphine and methadone have been shown to be equianalgesic [3, 9, 20] and methadone has been reported to be more potent than morphine in some ways (e.g., straub tail, hyperglycemia, catalepsy, LD 50) [3, 9, 20], it is somewhat surprising that 5 mg/kg methadone failed to significantly potentiate self-stimulation since previous experiments with 5 mg/kg morphine did so [14]. Our results—considered together with those of Schaefer and Holtzman [19] who reported that 0.3 and 3.0 mg/kg methadone depressed SS, and Pert [17] who found that 0.1–5.0 mg/kg had no significant effect—suggest the existence of an orderly dose-response relationship between methadone and self-stimulation. Lower doses (0.1–3.0 mg/kg) would appear to inhibit the reward system with the intermediate dose of 5 mg/kg failing to have a significant effect. However, 10 mg/kg produces the transient depression and the subsequent facilitation most often reported in the literature dealing with opioids and self-stimulation [6]. This bi-directional dose response relationship requires additional experimental attention. While the finding that doses of methadone below 5 mg/kg may produce inhibition but not facilitation of self-stimulation warrants further study, our results do provide evidence that methadone is not qualitatively different from other opiates in its ability to potentiate responding for reinforcing MFB-LH stimulation.

ACKNOWLEDGEMENTS

This research was supported by grants from the Biomedical Research Support Program (NIH) and the University of Cincinnati Research Council. We thank the Eli Lilly Company for generously supplying the methadone.

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