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Morphine Differentially Affects Ventral Tegmental and Substantia Nigra Brain Reward Thresholds[1]

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NAZZARO, J. M., T. F. SEEGER AND E. L. GARDNER. *Morphine differentially affects ventral tegmental and substantia nigra brain reward thresholds.* PHARMAC. BIOCHEM. BEHAV. **14**(3) 325–331, 1981.—In order to differentiate the roles of the nigrostriatal and mesolimbic-mesocortical dopamine systems in the action of opiates on dopaminergically mediated intracranial self-stimulation (ICSS), the effects of chronic morphine administration and acute naloxone administration on ICSS were tested in rats with electrode placements in the substantia nigra pars compacta (A-9) and the ventral tegmentum (A-10). Acute morphine (5.0 mg/kg SC) did not affect ICSS thresholds of rats with electrodes in the A-9 nucleus when tested 1, 3, 5, and 23 hours after administration. With repeated daily administration, though, these animals showed increases in thresholds which grew progressively larger with each day of morphine treatment. This threshold elevation was not reversed by naloxone given 0.5 hour after the final morphine treatment on the fifth day. In contrast, acute morphine significantly lowered self-stimulation thresholds in rats with A-10 placements. Tolerance to this facilitatory effect was evident with chronic administration. Naloxone attentuated the lowering of threshold caused by opiate administration in these A-10 animals. The present data suggest a specificity of action of opiates on different brain systems subserving reward and reinforcement. These findings also suggest that the mesolimbic-mesocortical system may play an important role in mediating the rewarding properties of morphine.

Intracranial self-stimulation ICSS Opiates Morphine Naloxone Ventral tegmentum Substantia nigra

A SUBSTANTIAL amount of recent evidence suggests a functional and anatomical interrelationship between endogenous opioid peptide (enkephalinergic) and dopaminergic neuronal systems. Such evidence also strongly indicates that the effects of enkephalins and opiates on brain dopaminergic neurotransmission varies in different brain sites. That is, in some neurobehavioral brain loci, opiates appear to have a net DA antagonist effect [8] whereas in other sites, a net DA agonist effect is suggested [4].

One behavior in which dopamine has been strongly implicated as a primary substrate is intracranial self-stimulation (ICSS) [32]. Anatomical, biochemical, neurophysiological, and pharmacological studies have demonstrated decreased reward value by drugs which interfere with dopamine neurotransmission [34,56] while enhanced dopamine transmission facilitates ICSS [5,56].

In view of the evidence for heterogeneous functional interrelationships between enkephalinergic and dopaminergic systems in the forebrain, it might be hypothesized that specific dopamine systems play differing roles in mediating the rewarding and reinforcing properties of opiates. In order to test this hypothesis, the present study investigated the effects of opiate agonist and antagonist administration on ICSS elicited from the mesencephalic ventral tegmental nucleus and the substantia nigra, two dopaminergic nuclei giving rise, respectively, to the mesolimbic-mesocortical and nigrostriatal dopaminergic systems [33], both of which systems contain high concentrations of enkephalin nerve fibers [13, 39, 50].

The large majority of studies utilizing the ICSS paradigm have relied on changes in response rate as the dependent measure [14]. Rate, as an index of reward, may not control

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for drug-induced non-selective alterations in motor activity, arousal level or coordination [16,55]. In order to avoid the possibility of such contamination, especially in light of the extensive literature concerning the effects of morphine on motor output and performance ability [1], the present study utilized a rate-independent threshold reward titration paradigm for ICSS [48].

METHOD

The experimental subjects were male, albino, Sprague-Dawley rats (n=10), weighing approximately 250 g at the time of surgery and individually housed in a 12 hour on-off light cycle colony with free access to food and water at all times. The animals were anesthetized with sodium pentobarbital and implanted with chronic bipolar stainless steel electrodes (Plastic Products type MS303/1; 0.25 mm tip diameter) using standard surgical and stereotaxic technique. In one group of animals, the electrodes were aimed at the ventral tegmental nucleus (A-10) at the following coordinates: AP +2.6, L 1.2, H −3.5, according to the atlas of Pellegrino and Cushman [38]. In the other group, electrodes were aimed at the substantia nigra (A-9) at the coordinates: AP +2.7, L 2.0, H −2.7. ICSS training and testing was conducted in a clear plexiglass experimental cage (enclosed in a lighted, ventilated, sound proof chamber) equipped with two side-by-side levers on one of the cage walls and a rotating commutator on top for connection of an electrical cable to the animal. Stimulation was produced by a Nuclear-Chicago model 7150 constant current stimulator modified by the addition of a precision resistor network and logic circuitry to produce decremental brain stimulation. Each depression of the primary lever in the rat cage resulted in a 250 msec stimulus train consisting of 60 Hz bipolar rectangular pulses of 0.3 msec duration, with a 0.15 msec delay between the pulses of each bipolar pair. Maximum stimulation current was 250 μA, decremented by 15 μA with each succeeding lever press. The animal could reset the current to maximum at any time by pressing the second lever, which did not itself deliver stimulation. All stimulation parameters were constantly monitored by oscilloscope. The current step at which the animal chose to reset was automatically recorded and the mean of the resultant frequency distribution was taken as the ICSS current threshold for that test period.

ICSS training was begun 10 days after surgery, initially with shaping to a single ICSS lever until reliable responding was elicited, followed by introduction of the second lever and shaping to the two-lever decremental titrating ICSS threshold paradigm. After each animal had reached asymptotic performance levels in the threshold paradigm, baseline data were collected for 6 consecutive days 1, 3, and 5 hours following sham or intraperitoneal injection of 0.9% saline. Test sessions were 10 minutes each preceded by a 3 minute stabilization period during which data were not recorded.

On the next day (drug day 1) animals were injected with naloxone (2 mg/kg SC, dissolved in 0.9% saline) and tested 1, 3, and 5 hours post injection. Morphine sulfate (5.0 mg/kg SC, dissolved in 0.9% saline) was administered on days 2 through 5 and the animals were tested 1, 3, 5, and 23 hours post morphine injection. On drug day 6, both morphine and naloxone were administered and the animals were tested at 1, 3, 5, and 23 hours.

After completion of the experiment, verification of stimulation site was performed on all animals by standard histological procedure.

RESULTS

The ventral tegmental and substantia nigra electrode placements are shown in Fig. 1, and ICSS performance is shown in Fig. 2.

Analysis of variance of ICSS thresholds of both the substantia nigra and ventral tegmental animals for the four days of chronic morphine administration revealed a significant overall difference between morphine's effects on ICSS in these two brain loci, $F(1,8)=7.87$, $p<0.025$. In addition, a statistically significant overall effect for days, $F(3,24)=3.81$, $p<0.025$, and a significant locus × hours interaction, $F(3,24)=6.61$, $p<0.005$, were revealed. Thus, morphine's effect on ICSS was significantly different for the two ICSS loci.

For the ventral tegmental animals (see Fig. 2), analysis of variance revealed a significant hours effect, $F(3,12)=8.61$, $p<0.005$, and a significant hours × days interaction, $F(9,36)=2.19$, $p<0.05$. Tests of individual differences for these animals showed that ICSS thresholds were not affected by acute injection of naloxone alone, but that the first administration of morphine caused a significant lowering of threshold at 1 hour ($t=5.90$, $p<0.01$) and 3 hours ($t=3.08$, $p<0.05$) post-injection. The average percent decrease in ICSS threshold caused by the acute morphine (drug day 2) was 32% at 1 hour and 18% at 3 hours. At 5 and 23 hours after this first morphine injection (drug day 2), ICSS values had returned to near baseline. On the second day of morphine administration (drug day 3) threshold values were again significantly lowered 1 hour post-injection ($t=6.22$, $p<0.01$) but not at 3, 5, or 23 hours. On the following two days of chronic morphine, all threshold tests following drug were not significantly different from baseline. Thus, both by analysis of variance and by tests for individual differences, morphine produced an acute facilitation of ICSS in the ventral tegmental area with tolerance developing to the facilitatory effect of morphine on ventral tegmental ICSS threshold.

In addition, but not shown in Fig. 2, a separate group of five animals with ventral tegmental electrode placements was acutely administered 5 mg/kg morphine sulfate and followed 1/2 hour later by an injection of 2 mg/kg naloxone and tested according to the above schedule. In contrast to the first group, these animals showed thresholds which were very close to, and statistically not significantly different from, baseline. Thus, naloxone reversed the threshold lowering effect of acute morphine in these ventral tegmental animals.

For the substantia nigra animals (see Fig. 2), analysis of variance revealed a statistically significant hours effect, $F(3,12)=4.30$, $p<0.05$. Tests of individual differences for these animals revealed that they were essentially identical to the ventral tegmental animals in their lack of response to acute injections of naloxone. However, in contrast to the ventral tegmental animals, ICSS thresholds in the substantia nigra animals were not altered by morphine at 1, 3, 5, and 23 hours post-injection on the first morphine day (drug day 2). On the second morphine day (drug day 3), ICSS threshold was significantly elevated (+17%) 1 hour after administration ($t=2.73$, $p<0.05$) while tests 3, 5, and 23 hours post-injection were not significantly different from baseline. On the third day of chronic morphine (drug day 4) threshold values were elevated an average of 23% and 16% at 1 hour ($t=5.20$, $p<0.01$) and 3 hours ($t=2.6$, $p<0.05$), respectively, post-drug while on the fourth morphine day (drug day 5) significant increases in threshold values were observed at 1 hour

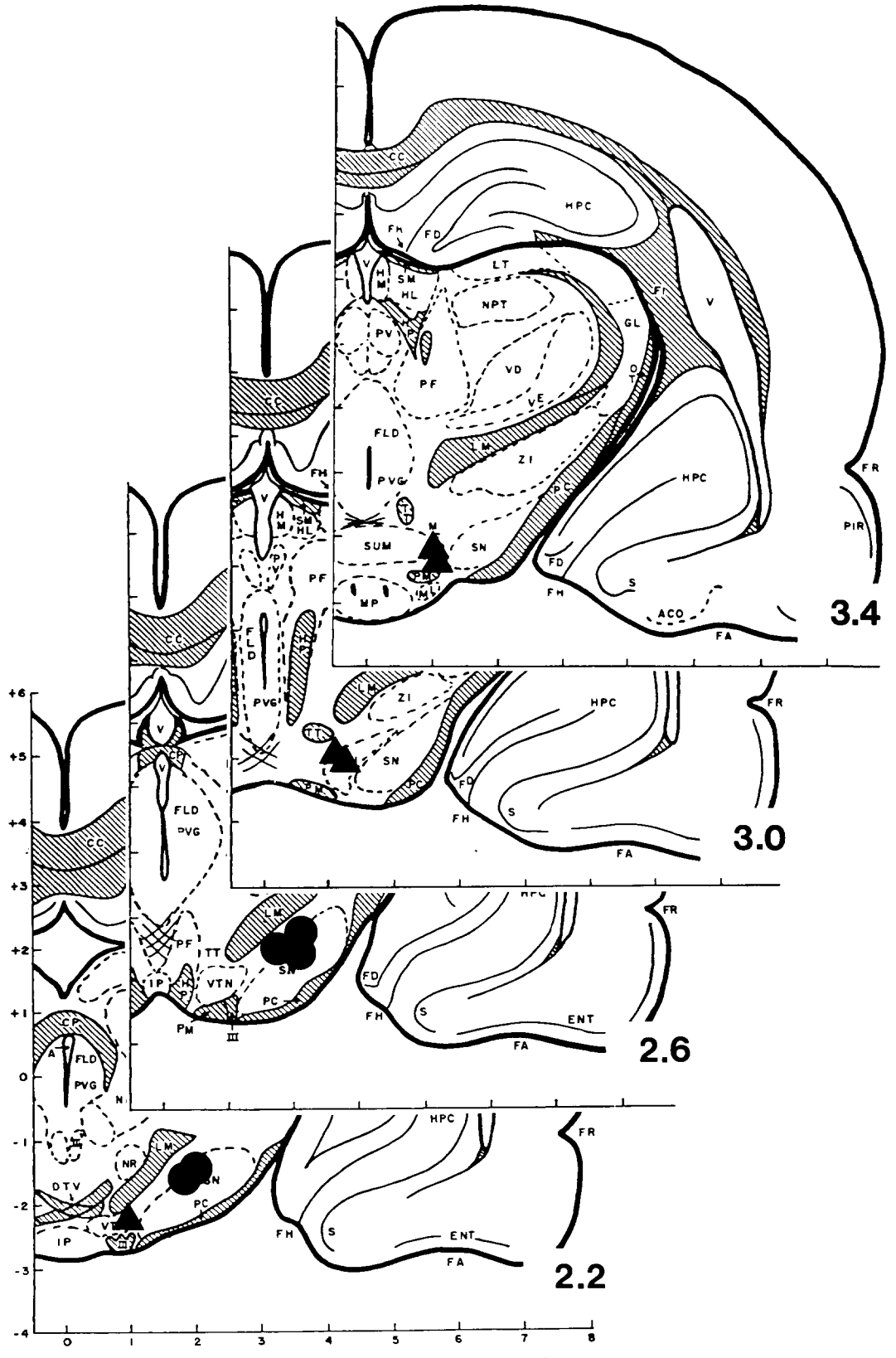

FIG. 1. ICSS electrode placements. The medial (ventral tegmental) placements are indicated by triangles (▲); the lateral (substantia nigra) placements are indicated by circles (●). For the nigral placements, all electrode tips were within 0.3 mm of the dorsal border of the substantia nigra. Vertical plane coordinates are in mm anterior to the interaural line.

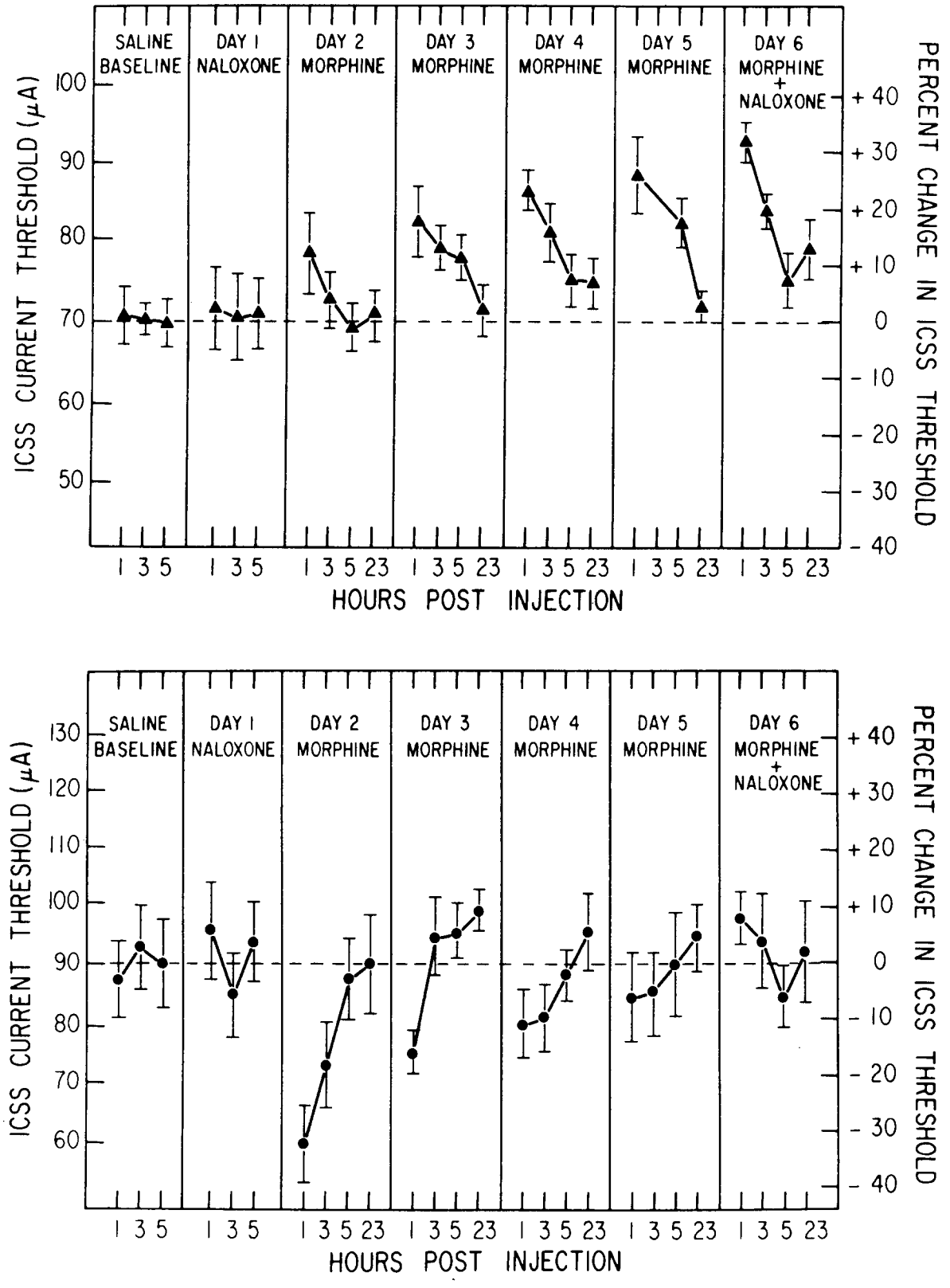

FIG. 2. Effects of morphine and naloxone on intracranial self-stimulation (ICSS) thresholds in rats (n=5) with electrodes in the substantia nigra (top) and in rats (n=5) with electrodes in the ventral tegmental area (bottom). The data points plotted are means ± S.E.M.

($t=3.58$, $p<0.02$) and 5 hours ($t=3.78$, $p<0.02$). When naloxone and morphine were administered on drug day 6, ICSS threshold was again elevated an average of 32% ($t=3.74$, $p<0.02$) 1 hour after morphine injection. The altered thresholds observed following the morphine-naloxone injections did not differ significantly from thresholds on drug days 4 and 5, when morphine was given alone. Thus, a progressive increase in ICSS threshold was observed in the substantia nigra animals over four days of repeated morphine injections, and naloxone did not alter this increase in ICSS threshold induced by chronic morphine in animals with substantia nigra electrode placements.

DISCUSSION

These findings support previous suggestions for a specificity of action of opiates on different brain systems subserving reward and reinforcement [14] and further suggest that the mesolimbic-mesocortical dopaminergic system may, at some level, play an important role in mediating the rewarding properties of morphine. It is noteworthy that recent evidence has implicated this system in mediating the reinforcing properties of other drugs of abuse [31]. Although some of the fibers of the mesolimbic-mesocortical and nigrostriatal systems pass close to each other [33] and recent evidence suggests the possibility of a dopaminergic interfacing between the two systems [2], our post-drug data, coupled with the differential basal ICSS threshold values (in terms of microamperes) of the two groups of animals used in the present study (see Fig. 2), tend, in our view, to suggest that our results reflect the behavioral output of anatomically distinct systems. In this regard, we also feel that evidence for the discreteness of the anatomical area stimulated by implanted electrodes in the self-stimulation paradigm should be noted [49].

Our data, though, are only partially in agreement with the work from other laboratories. While it appears that acute morphine administered either peripherally [15] or intracerebrally [3] facilitates ventral tegmental ICSS, Esposito *et al.* [15] have recently reported a lack of tolerance to the ICSS threshold lowering effect of morphine in ventral tegmental animals. This discrepency may reflect experimental procedural differences (such as threshold techniques utilized, doses of drug administered, and schedule of ICSS testing) between the present study and that of Esposito *et al.* It is also worthy of note that most other investigators, using both rate-dependent and threshold techniques different from that utilized in the present study, have not found tolerance to the facilitatory effects of morphine on medial forebrain bundle (i.e., mixed aminergic) ICSS [14,21].

The present study, though, suggests that the important question of tolerance be further closely examined, particularly with respect to possible differences in the development of tolerance in the different specific catecholaminergic nuclei of origin implicated in reward processes [18,33].

With respect to morphine's acute effects on substantia nigra ICSS, other investigators (using rate-dependent ICSS measures) have reported facilitation, inhibition, and no-effect [27, 28, 36]. Although the specific sites of stimulation in some of these studies were not clearly delineated, the differences in results appear to stem from small variations in electrode placements [28], and in this regard our results support, overall, the findings of Liebman and Segal [28].

In recent years, considerable evidence has accumulated for a dopaminergic substrate of ICSS behavior [6, 9, 32, 40, 42, 46]. A substantial amount of evidence has also accumulated which indicates a functional interrelationship between forebrain dopaminergic and enkephalinergic systems [7, 12, 25, 26, 52, 54]. Other evidence suggests that the nature of this opiate-dopaminergic interaction may vary from one neurobehavioral system to another, since in some systems the effects of opiates appear to be dopamine antagonistic (e.g., [7]), while in other systems the effects of opiates appear to be dopamine agonistic. In this latter regard, for example, locomotor activity, which appears to be linked to dopamine activity in mesolimbic dopamine neurons [22], is increased by microinjection of dopamine into the nucleus accumbens [10] and also by direct intracerebral injection of D-Ala-enkephalin into the ventral tegmentum [4].

While the precise functional relationship between enkephalinergic and dopaminergic neurons in the systems studied remains to be fully elucidated, the findings of the present study suggest a dopamine-system specific effect of morphine. That is, in the mesolimbic-mesocortical system, our results suggest a net dopamine agonist effect of morphine to which tolerance developed. In the nigro-striatal dopamine system, though, acute morphine may have activated both dopamine agonist and antagonist interactions. Recent evidence of both pre- and post-synaptic innervation of DA neurons by enkephalinergic interneurons in the nigro-striatal pathway [11] supports such an interaction. As in the mesolimbic-mesocortical system, tolerance may have developed only to the dopamine agonist effect, resulting in the final expression of elevated ICSS thresholds. In this regard, it is noteworthy that striatal [^{14}C]-dopamine formed from [^{14}C]-tyrosine significantly increases with chronic morphine treatment [8]. These suggestions, though, in no way negate the possible roles of other neurotransmitters in mediating the observed morphine-induced effects.

Our finding that the opiate antagonist naloxone failed to affect basal ICSS threshold in both brain loci is in agreement with previous work from this laboratory [35] and also several other published reports [19, 24, 30, 44, 51, 53]. However, our finding that naloxone failed to affect short-term chronic morphine induced inhibition of substantia nigra ICSS is both unique and problematic. While naloxone has been demonstrated to block the enhancing effects of several classes of drugs of abuse on medial forebrain bundle ICSS, including morphine [14], heroin [23], amphetamine [19], alcohol [30], and also most recently both the inhibitory and enhancing effects of acute phencyclidine elicited from ventral tegmentum electrode placements (Nazzaro, Seeger & Gardner, in preparation), our data suggest that some of the effects of morphine on specific reward mechanisms are not naloxone sensitive. In this regard, evidence has accumulated which suggests the existence of distinct classes of opiate receptors in nervous tissue, with variation in regional brain distribution and to which significant differences in the binding ability of naloxone have been demonstrated [20, 37, 47]. An effect of morphine on brain opiate receptors in the nigro-striatal system that are relatively naloxone insensitive might yield behavioral results similar to those observed in the present experiment. Such hypotheses, of course, are speculative at this juncture, and a fuller explanation of these findings must await further investigation.

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