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Dual Action Effects of Morphine on the Electrical Activity of the Dorsal Tegmentum

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GILDEN, L. *Dual action effects of morphine on the electrical activity of the dorsal tegmentum.* PHARMAC. BIOCHEM. BEHAV. 9(5) 597–602, 1978.—The midbrain tegmentum has been identified as an important locus for development of negative reinforcement with electrical stimulation of the brain. It also plays a central role in the motivational-affective component of pain, and is a site of the analgesic action of morphine. The present study reports the effects of morphine on the electrical activity of areas of the dorsal tegmentum of rats that were also tested for the aversive effects of brain stimulation. The results of spectral analysis of the EEG indicated that IP injections of 16 mg/kg of morphine significantly depressed intensity of EEG, while 8 mg/kg of morphine tended to increase intensity. The results were interpreted in terms of the dual action hypothesis of morphine action and Winters' model of drug effects on electrical activity of the brain. It was concluded that morphine may produce complementary inhibitory and excitatory effects on the negative and positive reinforcement systems of the brain respectively.

Morphine EEG Brain stimulation Hypothalamus Dorsal tegmentum

TWO LINES of research converge in studies of the motivational systems of the midbrain tegmentum. Many experiments have demonstrated that the dorsal tegmentum is one of the chief sites of the organization of negative reinforcement arising with electrical stimulation of the brain [12,15]. Other studies suggest that the affective component of pain, giving rise to the experience of the agony of aversive stimulation, also develops in that general region [9]. The research pertaining to the central control of pain includes many experiments designed to clarify the analgesic action of morphine. Liebeskind and Paul [8] have recently reviewed the literature on pain and have discussed morphine's site and mechanism of analgesic action.

A wide variety of electrophysiological techniques have been employed to determine morphine's effect on the central nervous system, including evoked potential [11,18], single unit [7,16], multiple unit [6,19] and EEG recordings [1,10]. The powerful technique of spectral analysis has not however been employed to measure the effects of morphine on the electrical activity of midbrain structures involved in motivational processes. This was the primary purpose of the present study. A second aim entailed determination of the relationships between the forms of electrical response to morphine by midbrain structures and the behavioral effects of electrical stimulation of those same structures. An attempt was made therefore to answer the question, What electrophysiological responses, as measured by spectral analysis, occur following intraperitoneal (IP) injections of morphine in tegmental structures shown to yield aversive reactions to electrical stimulation?

METHOD

Animals

Eight Long-Evans male rats weighing 210–285 g were used. Animals were maintained on ad lib food and water and housed separately.

Surgery

Unilateral stereotaxic implantation of bipolar, stainless steel electrodes, 0.01 in. dia., was carried out under sodium pentobarbital, with the dorsal tegmentum as the target site. Placement of the electrodes was made with respect to bregma, using the following coordinates: 6 mm posterior, 1 mm lateral and 5 mm below the surface of the dura. The stereotaxic instrument was adjusted, and the rat's head fixed in a position such that the interaural line was 5 mm below the level of the upper incisor bar.

Procedure

After a week of postoperative recovery, the animals were subjected to five 1 hr periods of habituation to a restraining apparatus designed to reduce movement artifact to a minimum. The apparatus consisted of an adjustable collar, through which the rat's head was inserted, attached to a plastic base fitted with leather straps which kept the trunk restrained.

Habituation to the restraining apparatus was facilitated by administering IP injections of 6 mg/kg of diazepam (Valium) 15 min before the first habituation session, and 3 mg/kg of

diazepam before the second habituation session. No drug was administered for the remaining 3 days of habituation training. By the fifth day all the animals were trained to remain quietly in restraint for the entire recording session, which lasted approximately 75 min.

Recordings were obtained from the restrained animals placed in a grounded aluminum box situated inside an Industrial Acoustic Corporation double wall, sound damped chamber. EEG signals were carried out of the chamber, amplified by a Tektronix 2A61 differential amplifier set at a gain of 2×10^4, filtered through a Krohnhite bandpass filter set to pass 0.2 to 40 Hz, and recorded on a Technical Measurements Corporation FM tape recorder with a model LC data converter. Recordings were made at a tape speed of $1^7/_8$ ips.

All EEG recordings were screened, and artifact-free samples 40.96 sec in length were analysed. After being digitized at a rate of 100 samples/sec, the data were subjected to a Fast Fourier Transform (FFT) procedure on a Xerox Sigma 7 computer. From the output of the FFT a power spectrum was computed. For each 1 Hz band of the power spectrum 40 data points were averaged in overlapping segments of 20 data points giving a resolution of 0.5 Hz.

For purposes of statistical analysis the frequency span (0.2 to 25 Hz) was partitioned into the commonly used EEG frequency bands: delta (0.2–3.8); theta (4.0–7.8); alpha (8.0–12.2); sigma (12.4–14.6); and beta, divided into 2 bands referred to as $beta_1$ (14.8–20.0) and $beta_2$ (20.2–25.0). The units of measurement of spectral analysis were $\mu V^2/Hz$, as recommended by Walter [20], and referred to as intensity or power. In addition, the percent of the total spectral intensity in each frequency band was computed for each 40.96 sec sample.

The design of the experiment involved 3 treatments for each animal administered in randomly selected order. The treatments were IP injections of 0.5 cc physiological saline, 8 mg/kg morphine or 16 mg/kg morphine. Five to 7 days were allowed to elapse between each drug treatment.

Sets of ten 40.96 sec samples occurring in close temporal contiguity during the periods 1–12 min, 31–43 min, and 62–75 min into the recording session were compared to identify the period of peak drug effect. To accomplish this, matched sample t tests were calculated, comparing all 3 conditions with each other. Differences in intensity and percent of total spectral intensity were identified, and the period in which the greatest number of significant values of t occurred relative to the control condition, across all frequency bands, was thereby defined as the period of peak effect for each animal. All results reported below refer to data obtained during the peak effect period. Statistical statements refer to comparisons made across experimental conditions based on matched sample t tests.

After completion of the recording sessions each rat was tested to determine the aversive effects of electrical stimulation of the brain at the site of the implanted electrode. Avoidance conditioning training was carried out in a shuttle box 7 1/2 in. wide and 19 1/2 in. long. The floor consisted of 3/16 in. dia. metal rods and across the center of the shuttle box was a hurdle 3 in. high. Simple avoidance conditioning was conducted by placing the animal in one compartment of the box facing away from the hurdle, timing out 10 sec and then delivering to the brain 0.5 sec trains of 60 Hz sine wave pulses every 1 sec until the animal crossed the hurdle or until 90 sec elapsed. Stimulation current was controlled by measuring the voltage across a constant resistance in series with the electrodes on a Tektronix oscilloscope, and adjusted between trials by gradually raising intensity from 10 μamp rms to a level at which the animals displayed definite responses to the stimulation, or until a maximum ot 50 μamp rms was reached.

After each trial the animal was permitted to remain in the safe, nonstimulation compartment for 30–45 sec then returned to the other compartment for the next trial. If an animal crossed the hurdle during the time stimulation was being delivered, the behavior was considered to be an escape response. If it crossed during the 10 sec prestimulation period, the response was counted as an avoidance response. A criterion of 8 out of 10 consecutive trials was used as an index of both escape learning and avoidance learning. Thirty training trials were given each day until criterion for avoidance learning was reached or for a maximum of 4 days.

After the experiment was completed, electrode placements were verified from frontal histological sections 50 μ thick and stained with cresyl violet.

RESULTS

Histology

Histological examination revealed that the tips of the electrodes were located in the dorsal tegmentum but distributed over a relatively wide area in the dorsal-ventral and medial-lateral dimensions between planes 5.6 and 6.2, according to Pellegrino and Cushman [13] (Fig. 1). Since different brain sites subserve different functions and are therefore likely to generate different electrical activity, it was deemed inappropriate to average the EEG data derived from diverse sites. Instead, the data of each animal will be reported separately and comparisons made among individual animals.

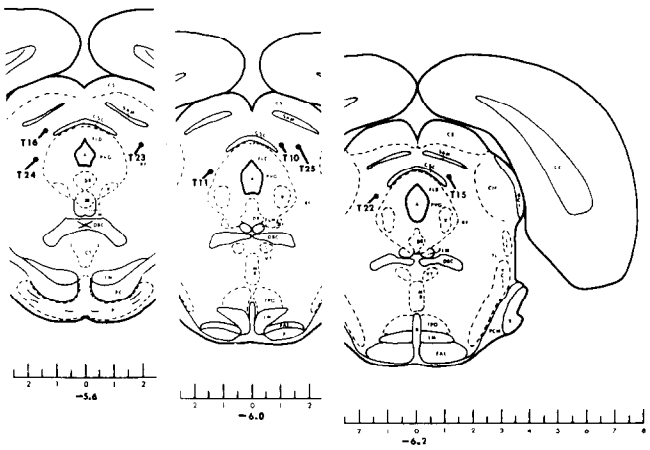

FIG. 1. Loci of electrode tips of animals shown in schematic representations of coronal sections of the rat midbrain after Pellegrino and Cushman [13].

Control Profiles

The spectral profiles of all the animals under the control conditions had the characteristic features of EEG spectra, progressive decrease in power as frequency increased from the delta to the $beta_2$ band (Fig. 2). The variances of the intensities of the different spectral bands tended to be consis-

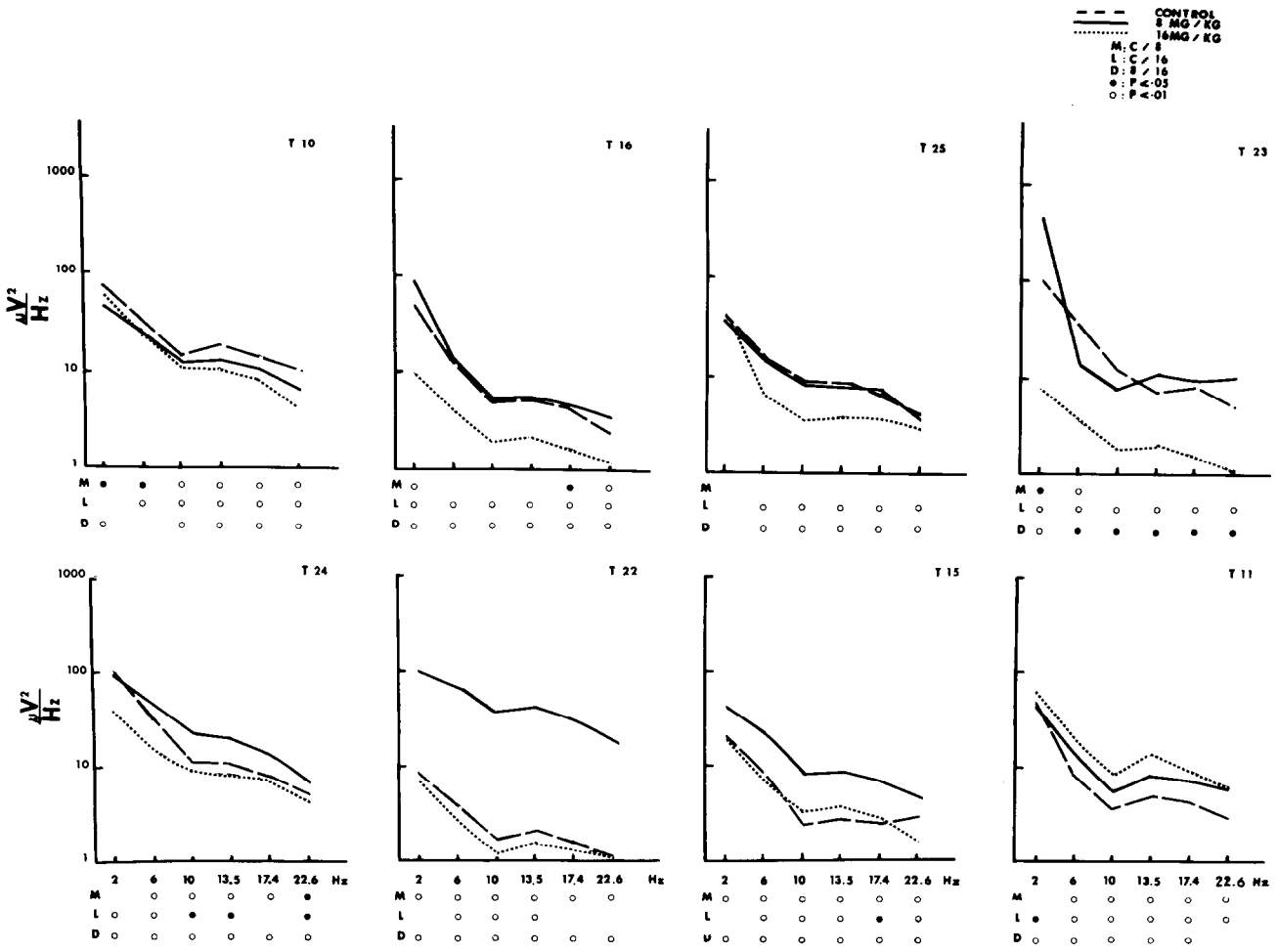

FIG. 2. Power spectra for 8 animals obtained after physiological saline (control), 8 mg/kg and 16 mg/kg morphine treatments. Data are plotted at the center frequency of the 6 frequency bands between 0.3 and 25 Hz. The symbols beneath each graph indicate the value of p for comparisons between control and 8 mg/kg (M), control and 16 mg/kg (L) and the 2 drug (D) conditions. – – – – Control; ———— 8 mg/kg; ..... 16 mg/kg. M: c/8; L: c/16; D: 8/16; ●=$p<.05$; ○=$p<0.01$.

tent throughout the control condition, due to the initial habituation to the experimental situation and the resulting passive response of the animals. Three animals (T10, T11 and T22) had discernible peaks in the sigma band. In most respects the spectral profiles of the dorsal tegmentum resembled those of the hypothalamus obtained in a previous study by Gilden and Kozakiewicz [3], although there was a stronger tendency in hypothalamic recordings for prominent alpha peaks to be present.

Intensity Changes with 16 mg/kg of Morphine

The larger dose of morphine significantly suppressed power production relative to the control condition in most or all frequency bands in 6 of the 8 animals (Fig. 2). Animal T10, for example, displayed significant ($p<0.01$) reductions in every band but delta. T16 and T23 developed significant ($p<0.01$) reductions in all frequency bands. The extent of total power reduction was measured by computing the sum of intensities in all frequency bands and comparing the results across conditions. It was thus determined that the range of total power reduction extended from 8% of control intensity in T23 (strong depression) to 78% of control intensity in T22 (moderate depression).

One animal, T15, responded to the higher dose of the drug by developing significantly less theta and beta$_2$ intensity, but significantly more alpha, sigma and beta$_1$ intensity. Another animal, T11, significantly increased power in all frequency bands with 16 mg/kg of morphine. The total power increase in this animal was 61% greater than the control condition.

Some significant changes also appeared in the spectral profiles with 16 mg/kg. Relative intensity of delta increased significantly compared to control in 3 animals (T10, T24 and T25) and decreased significantly in 3 others (T11, T16 and T23). Relative intensity of theta activity decreased in 3 animals (T15, T22 and T25). In the other animals there was no significant difference in theta relative to control. Percent of alpha intensity increased in 5 animals (T11, T15, T16, T23 and T24) and decreased in 2 animals (T22 and T25). The most consistent change in relative intensity with 16 mg/kg occurred in the beta$_1$ band in which 6 animals (T11, T15, T16, T22, T23 and T25) developed significant increases in power.

In general, with 16 mg/kg of morphine the spectral profiles tended to lose the steep slope of the delta-theta limb,

and, by increasing the intensity of the alpha and beta bands, became somewhat flattened in appearance.

Intensity Changes with 8 mg/kg of Morphine

The direction of changes in the frequency spectra with 8 mg/kg of morphine were the same as with 16 mg/kg in only 2 animals, T10 and T11. In the case of T10 a smaller, but still significant, reduction in intensity occurred in all frequency bands, whereas, with T11, a smaller, but significant increase in intensity occurred in all frequency bands but delta. Another animal, T25, displayed no difference in electrical activity relative to control with 8 mg/kg.

The smaller dose of morphine, however, paradoxically induced the opposite effect of the higher dose in some or all of the frequency bands of 5 of the 8 animals. This effect is seen most clearly in T24, which developed significantly more intensity at 8 mg/kg than in the control condition in bands theta through $beta_2$, while the intensity was significantly suppressed across the spectrum with 16 mg/kg. Similar paradoxical effects were found, but to a lesser degree, in T16, T22, T23 and T15.

The most clear-cut changes in spectral profile with 8 mg/kg of morphine occurred once again in the alpha and beta bands. Percent of total power increased in the alpha and $beta_1$ bands, relative to measurement under control conditions, in 5 animals after the lower dose of morphine. A dramatic development occurred in Animal T23, which produced a four-fold increase in delta intensity with 8 mg/kg—from 108 μV^2. The percent of total intensity occurring in the band increased from 55–64%. This effect was correlated with the development of high amplitude, slow wave and spike-dome activity.

Effects of Brain Stimulation on Behavior

Electrical stimulation of the brain, using the same electrodes from which the recordings were obtained, yielded information concerning the involvement of particular tegmental structures in aversive motivational processes. Six animals (T10, T15, T16, T22, T24, T25) learned to perform escape responses to brain stimulation, reliably crossing the partition shortly after onset of brain stimulation. In all of these cases the animals learned within 28 trials to turn around and move to the other side of the shuttle box with shorter and shorter latencies, often in as little as 2 sec subsequent to onset of stimulation. Four of these animals (T10, T15, T22 and T25) learned to avoid brain stimulation, meeting the criterion of 8 out of 10 crossings before the 10 sec prestimulation period elapsed. The mean was 55 trials to criterion for the 4 animals, with a range between 39 and 76 trials to criterion. Two of the escape animals (T16 and T24) showed no signs of learning to avoid, and training was terminated after four 30-trial training sessions.

Two other animals, T23 and T11, did not have any apparent aversive responses to brain stimulation. In both cases stimulation induced arousal behavior such as rearing, sniffing and other exploratory activity. But there were no signs of the quick, darting behavior followed by running to the opposite end of the shuttle box that characterized the escape behavior of the other animals. The training procedure was also terminated with these animals after 4 sessions.

DISCUSSION

Three aspects of the results call for some explanation: (1) the significant depression of intensity of electrical activity in the dorsal tegmentum with 16 mg/kg of morphine in 6 of the 8 animals, (2) the seemingly paradoxical increase in intensity with 8 mg/kg of morphine in 5 animals, and (3) the relationships between electrographic data and behavioral responses to brain stimulation.

The depressant and paradoxical effects of morphine appear to be manifestations of the well-known dual action of morphine hypothesized by Seevers and Deneau [17], and can be understood more fully in terms of the multidirectional modes of action of various drugs on the electrical activity of the CNS, as described by Winters [22].

Seevers and Deneau postulated, on the basis of analysis of the broad spectrum of the drug's actions, that morphine induces both excitatory effects, involving widespread increases in synaptic excitability throughout the nervous system, and specific, well-localized depressant effects. Insofar as behavioral changes are concerned, the excitatory and depressant effects appear to be dose related. Babbini and Davis [2] have found that single doses of 1.25, 2.5 and 5 mg/kg of morphine IP had an excitatory effect on locomotor activity of non-tolerant rats, whereas, 10, 20 and 40 mg/kg induced initial depression followed by a delayed excitatory effect.

Studies of the effects of morphine on the electrical activity of the CNS also support the dual action hypothesis, but have yielded diverse and sometimes contradictory results. Ignatov [4], for example, found that even within circumscribed areas different neurons may be excited, depressed or unaffected by constant doses of the drug. One multiple unit recording study has reported that IP doses of morphine from 5 mg/kg to 25 mg/kg produced a pronounced depression of spontaneous multiple unit activity in widespread areas of the brain, particularly the cingulate cortex and hippocampus, but not to a noteworthy degree in the central periaqueductal gray [6]. On the other hand, Urca *et al.* [19] found that systemic injection of 10 mg/kg of morphine led to large increments of spontaneous multiple unit activity in the periaqueductal gray matter of the awake rat. Those researchers also observed, consistent with the findings of others [1, 5, 21], that morphine produced abundant cortical synchrony. Recordings from the hypothalamus also indicate that morphine induces high voltage, slow wave activity, including spike-wave complexes in subcortical sites [3]. In that study significant increases in power were observed across the frequency spectrum, as well as, significant increases in the percent of total power of theta, alpha and sigma activity.

Winters [22] has formulated a schema describing the effects of drugs on the electrical activity of the brain, which is useful in explaining some of the above observations and accounting for aspects of the results of the present experiment. While his formulation is focused on the effects of anesthetics on the CNS, Winters describes processes that appear to be similar to those occurring with morphine. Different drugs are seen to induce a variety of multidirectional progressions of states of CNS excitation and depression. Briefly, the schema states that anesthetics and CNS excitants bring about an initial excitation (Stage I), which is followed either by a further state of excitation (Stage II) then myoclonus and seizures, or depressant stages (Stage III—anesthesia—and Stage IV—medullary paralysis). Furthermore, the sequence of progressions differs with different drugs. The sequence for diethyl ether, for example, is $I \rightleftarrows II \rightleftarrows III \rightleftarrows IV$, whereas, the sequence for barbiturates is $I \rightleftarrows III \rightleftarrows IV$.

The data of the present experiment, obtained from several sites in the dorsal tegmentum, indicate that morphine may

act upon many of those sites in such a way that the dose-response relationships follow aspects of Winters' schema quite closely. In the case of Animals T16, T22, T23, T24 and, to some extent, T15, 8 mg/kg of morphine increased the power of electrical activity as much as sevenfold, while 16 mg/kg severely depressed electrical activity. The dual action of morphine is, therefore, quite clear in these animals. It appears that in some areas of the dorsal tegmentum the narcotic effects of smaller doses of morphine, e.g., 8 mg/kg, are excitatory, corresponding to Winters' Stage I and Stage II of electrical activity; whereas, with larger doses, e.g., 16 mg/kg, Stage III activity is observed. Presumably, larger doses of morphine would ultimately induce Stage IV activity.

The progression of drug stages was found to be different in other areas, however. Animal T10 responded to increasing doses of morphine with progressive decrements in power. This suggests that the area in which the electrode of that animal was implanted (just dorsal to the commissure of the superior colliculus) responds to morphine by by-passing the excitatory stage (Stage II) and entering Stage III directly. On the other hand, T11 developed progressively greater intensity, and more excitatory activity, with increments in dosage of morphine. The region from which those recordings were obtained, the reticular formation dorsolateral to the nucleus of the trigeminal nerve, therefore, appears to respond to morphine by proceeding through the various excitatory stages (Stage I $\rightleftharpoons$ Stage II $\rightleftharpoons$ myoclonus and seizures) without shifting to depressant activity.

It may be concluded that it is necessary to carry out careful regional and quantitative dose-response measures of the effects of morphine on the electrical activity of the brain before an accurate account of the drug's actions may be formulated. The effects are complex, and appear to involve multidirectional progressions, depending upon the site of action and the dose of morphine injected.

The fact that 6 of the 8 animals displayed either escape or avoidance responses with electrical stimulation indicates that the sites of the recording electrodes in those animals were located in areas of the tegmentum subserving aversive reinforcement. Furthermore, all of those animals exhibited depression of electrical activity with 16 mg/kg of morphine. While the size of the sample is too small to draw definitive conclusions about correlations between the forms of EEG responses to morphine and behavioral responses, it may be conjectured that structures in the dorsal tegmentum generating negative reinforcement with electrical stimulation are reliably depressed by 16 mg/kg of morphine. Some areas, such as the most dorsal region of the tegmentum, may be highly sensitive to morphine and inhibited even by 8 mg/kg.

In 3 instances (T16, T23 and T24) the development of the dual action responses of morphine was associated with aversive reinforcement with brain stimulation, and in one instance (T15) yielded only arousal. In the one case in which excitation developed with 8 mg/kg and 16 mg/kg of morphine (T11) only arousal occurred with brain stimulation.

Since it was demonstrated in a previous study by Gilden and Kozakiewicz [3] that excitatory responses to morphine by certain structures in the hypothalamus tended to be correlated with self-stimulation behavior, it is possible that structures developing the dual action response may participate in both positive and negative reinforcement processes. Anatomical information supports this inference, for it is known that the positive reinforcement system of the tegmentum is a direct extension of the hypothalamic reinforcement system [12]. Furthermore, the fact that certain brain areas yield bivalent responses to brain stimulation has been known for some time [14]. It has also been reported that rats with electrodes in some of the areas tested in the present experiment, particularly in the dorsolateral tegmentum, engage in both self-stimulation and escape behavior [12].

The hypothesis may then be considered that the electrophysiological responses of the positive and negative reinforcement systems to morphine are complementary. If it is the case that morphine induces concomitant excitatory effects in the positive reinforcement system and depressant effects in the negative reinforcement system, the very powerful positive reinforcing properties of morphine might be explained.

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