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Site-Specific Naloxone Blockade of Brain Self-Stimulation Duration

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FREEDMAN, N. L. AND D. PANGBORN. *Site-specific naloxone blockade of brain self-stimulation duration.* PHARMACOL BIOCHEM BEHAV 20(3) 361–366, 1984.—Moderate doses of naloxone (1, 5, 10 mg/kg) did not reduce response rate measured by the initiation of intracranial self-stimulation bursts to the medial forebrain bundle or central gray, at several current intensities (30, 60, or 90 μA). Raising current intensity increased rates and duration of lever pressing at both sites. Naloxone produced a dose dependent reduction in self-selected current duration at both sites averaged over intensity. However, naloxone completely blocked the effect of intensity only at the central gray site. In contrast to research showing effects of only high naloxone doses (e.g., 40 mg/kg) on self-stimulation at opiate-rich and opiate-poor receptor sites, this study indicates that endogenous opiates and opiate receptor mechanisms interact with aversive properties to modify central gray self-stimulation.

Self stimulation Naloxone Endogenous opiates

A number of investigators have proposed that the endorphins play a role in pleasure and reward [7, 22, 24]. Naloxone antagonizes the action of endorphins by occupying opiate receptor sites [13,27] and does not appear to produce motor decrements at low doses [1]. Naloxone has been used to evaluate the role of endorphins in behavior rewarded by brain stimulation [2, 4, 24, 28, 30]. The conclusions are not unanimous. Examination of the various naloxone doses, routes of administration, and variety of stimulation parameters and sites investigated (Table 1) suggest some possible reasons for the discrepancies. A number of studies report no effect of naloxone over a wide range of dosages from 0.3 to 50 mg/kg on rate [4, 8, 10, 14] or threshold [6, 15, 17, 26] of self stimulation responding. Other studies [2, 23, 25, 28] report reduced rates of lever pressing at doses of 10 mg/kg or greater. Clear dose response effects of moderate naloxone doses are seen in one study [20] employing fixed ratio (FR 15) reinforcement at relatively high stimulation current.

Though the results of the various investigations appear equivocal several general observations can be made. First, naloxone depresses rate of lever pressing for brain stimulation when relatively high doses are used [2, 24, 28, 30]. Second, the effect is observed more frequently when the range of stimulation currents exceeds 120 microamps [20, 28, 30] or when the stimulation duration exceeds 500 milliseconds. While it is tempting to argue that naloxone blocks rewarding properties of stimulation-released endorphins, alternative explanations are possible. For example, endorphins may be released by relatively intense current spreading to non-reward structures or by aversive consequences of long forced durations.

If endorphins are activated by long stimulation durations then rats allowed to self-determine reinforcement duration should select shorter durations of brain stimulation with naloxone. If the release of endorphins depends on spread to adjacent non-reward structures then the effect of naloxone should be more marked at higher current densities regardless of site stimulated. If the endorphins acts upon receptor subtypes which are differentially sensitive to naloxone [18] then naloxone should be differentially effective in blocking stimulation at reward-relevant brain sites which are relatively rich in Type 1 ("μ-oid") receptors.

The present experiment examines the effect of several moderate naloxone doses (1, 5, 10 mg/kg) and moderate current intensities (30, 60 and 90 μA) on self-determined duration and rate of brain stimulation at rewarding central gray and medial forebrain bundle sites.

METHOD

Subjects

Fourteen male Sprague-Dawley hooded rats were maintained on ad lib food and water in individual cages. Since naloxone effects and activity vary diurnally a 12-hour on, 12-hour off light-dark cycle was programmed in the colony room with the onset of the dark cycle at 0800 hr so rats would be maximally active in daytime when they were run.

Procedure

Rats were anesthetized with 65 mg/kg body weight sodium pentobarbital (Nembutal 65 mg/ml). Using standard procedures bipolar stimulating electrodes (Plastic Products Co., MS-303-0.005 in) were aimed at medial forebrain bundle (MFB) sites in four rats and central gray (CG) sites in ten rats. The coordinates were AP −3.5 mm from bregma, L 1.5 mm, DV −8.5 mm from top of skull with lambda at the same level as bregma for the MFB placement and AP −6.3 mm, L 0.4 mm, and DV −5.8 mm with the nosebar 5 mm above the

TABLE 1

REPRESENTATIVE STUDIES OF THE EFFECTS OF VARIOUS DOSES OF NALOXONE ON BRAIN STIMULATION REWARD

Investigators	Doses	Stimulation Parameters and Results
Wauquier, Niemengeers and Lal (1974)	5 mg/kg, IP	200 A, 0.5 sec, MFB. Reduced lever-pressing.
Bozarth and Reid (1977)	1 mg/kg, SC	5–26 A, 0.24 sec, LH. Failed to effect fast or slow responders.
Holtzman (1976)	0.3, 1.0, 3.0, 10, 30 mg/kg, SC	5–250 A, 0.2 sec square wave, LH. No effect on lever pressing.
Van der Kooy, Le Piane and Phillips (1977)	5, 10, 20, 40, 80, 160 mg/kg, IP	20–150 A, 0.2 sec, LH, CNU. Depressed lever press rates at 40 mg/kg for both LH and CNU even though CNU has greater conc. of opiate receptors and Enkephalin.
Belluzzi and Stein (1977)	0.1, 1.0, 5, 10 mg/kg, SC 12.5–200 mg/kg, DDC	Parameters unspecified, CG. Dose related decreases in response rates under both drugs.
Stein (1978)	10, 20, 40 mg/kg, SC	Reward Paradigm. Dose related decrease in responding. Escape Paradigm. Dose related increase in responding.
Stapleton, *et al.* (1979)	10 mg/kg, SC	85 A, 0.3 sec, to CG, SN, LH or NA. Reduced lever pressing at all sites.
Stillwell, *et al.* (1980)	1, 5, 10, 50 mg/kg, IP	20–80 A RMS, shuttlebox procedure. CG, LH. No effect on on- or off-time at either site.
Perry, Esposito and Kornetsky (1981)	16 mg/kg, IP for 5 days	Threshold procedure, 0.5 sec biphasic square. No effect on threshold VT or MFB.
Schaefer and Michael (1981)	0.1, 0.3, 1.0, 3.0, 10 and 30 mg/kg, SC	45–280 A, 0.2 sec square wave, MFB or CG. Reduced lever pressing for FR 15 at both sites. CG more sensitive to naloxone.

Abbreviations: IP=Intraperitoneal; SC=Subcutaneous; MFB Medial Forebrain Bundle; NA=Nucleus Accumbens; VT=Ventral Fegmental Area; LH=Lateral Hypothalamus; CG–Central Gray; DDC=Diethyldithiocarbamate.

interaural line for the CG placement. Following two weeks of post-surgical recovery rats were trained to lever press for self-determined trains of 60 Hz current at 30 microamperes. Rats not acquiring the lever-press response after two three-hour sessions were not tested further. Rats acquiring the response received four 70-min sessions, each separated by four days over a sixteen day period. Each session consisted of a thirty min pre-injection phase, drug injection followed by a ten min waiting period, and a thirty min post-injection phase. On any given day, 1 cc/kg of either naloxone hydrochloride (1, 5, 10 mg/cc) or isotonic saline (0.9 mg/cc) were injected by random determination such that each rat received all drugs and doses over the four sessions.

During each session rats received 60 Hz current for each lever press. As long as the lever was held down current was delivered to the brain site. In both the pre-injection and post-injection phase, rats responded for 10 min at each of 3 current intensities (30, 60 and 90 μA) in ascending order. Both number of lever-presses (rate) and total lever press duration (seconds/ten min) were recorded for each condition.

Upon completion of testing brains of all rats were per-

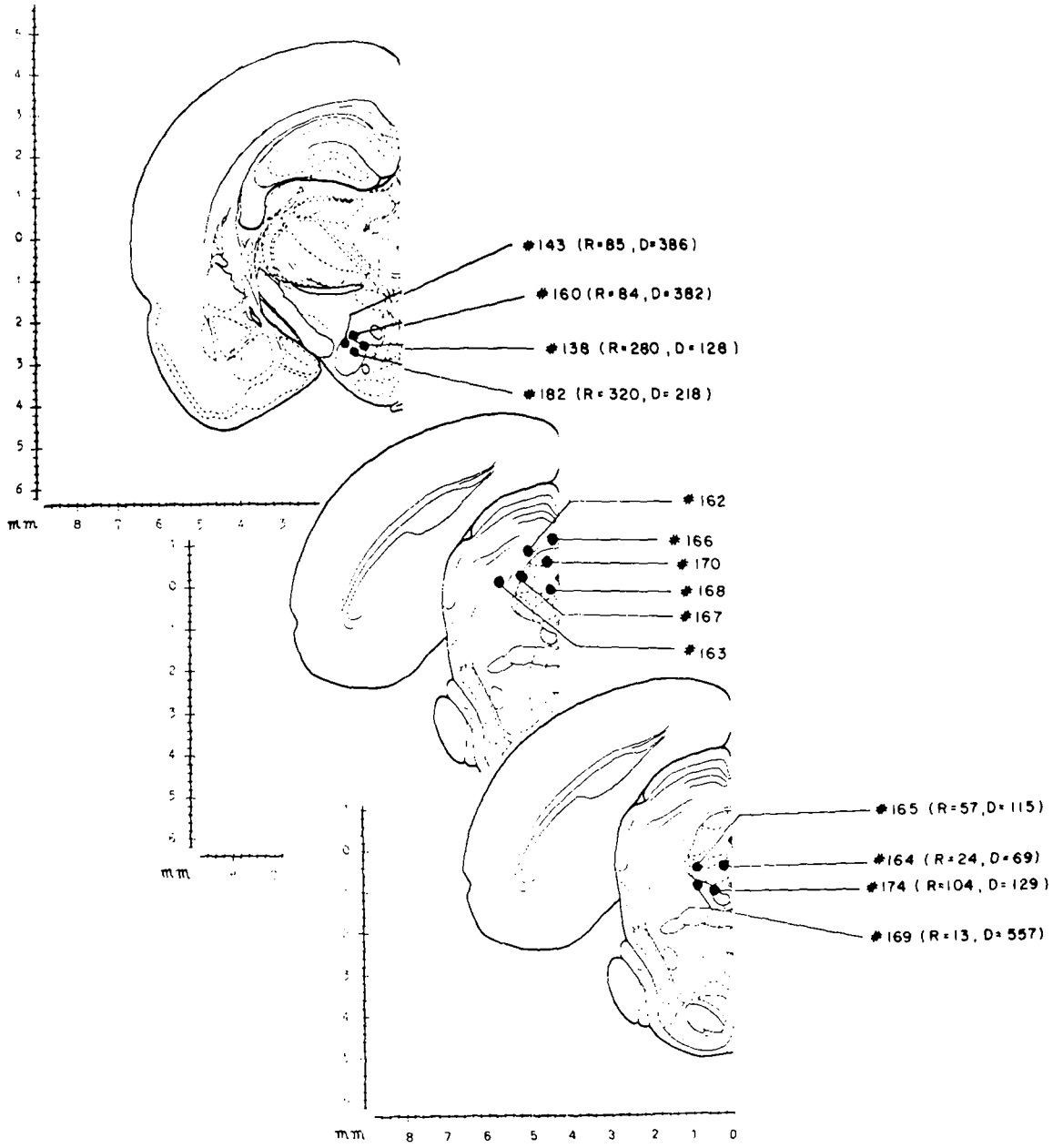

FIG. 1. Distribution of electrode tip locations for MFB rats (Top), non-responsive dorsal CGR rats (Middle) and responsive CGR rats (Bottom).

fused with isotonic saline, then formol saline and removed from the skull. Brains sectioned at 40 microns were mounted and counterstained with metachromatic thionin and electrode placements confirmed for responders and non-responders.

RESULTS

Histology

Figure 1 summarizes the electrode tip locations. All MFB electrode tips shown at the top of Fig. 1 were located in or just lateral to the MFB at the level of the lateral hypothalamus. Electrode tips of the six non-responding CGR rats, shown in the middle brain slice in Fig. 1, were located outside or in the dorsal CGR. Electrode tips for CGR responders in the bottom brain slice in Fig. 1 were located ventrally or just lateral to CGR relative to non-responders.

Effects on Response Rate

Mean response rates (responses per 10 min) for MFB and CGR reinforcing brain stimulation are shown in Fig. 2 as a

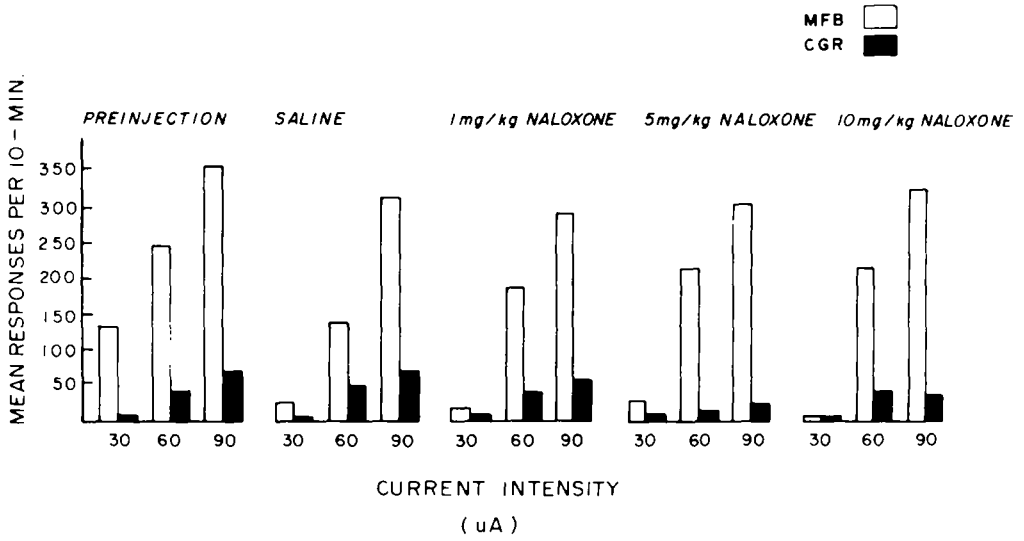

FIG. 2. Responses per 10 min in MFB and CGR implanted rats as a function of current intensity under various non-drug and drug treatment.

function of current and drug treatment. Overall response rates were greater at MFB than CGR sites, $F(1,6)=18.44$, $p<0.01$. Also increasing current at both sites enhanced response rate, $F(2,12)=10.80$, $p<0.01$, though the effect was more pronounced at the MFB site. The apparent effect of saline injection on MFB responding, $F(1,12)=7.80$, $p<0.01$, but not CGR may have been due to methodology since there was a contrast between the last pre-injection current (90 μA) and the first post-injection (30 μA). Naloxone produced no significant dose related effect on CGR (Fig. 2) response rates compared to saline across current intensity, $F(3,18)=1.01$, $p>0.41$. Thus, increasing current intensity enhances the number of times the stimulation is initiated at both sites but naloxone has no effect on response initiation. However, naloxone effects on response duration described below are more pronounced.

Effects on Response Duration

To determine the effects of naloxone on total selected current duration, the time in seconds that the lever was held down was cumulated over each 10 min session. Therefore the maximum current duration was 600 sec. Mean total self-selected stimulation durations for MFB and CGR stimulation are shown in Fig. 3 as a function of current and drug treatment. During the pre-injection baseline and saline control conditions as current was increased, rats selected longer durations of MFB and CGR stimulation, $F(2,12)=9.33$, $p<0.01$. Prior to naloxone treatment there were no significant differences between sites. Naloxone produced a dose-dependent reduction of the total stimulation duration to both sites averaged across current intensity compared to the pre-injection baseline, $F(3,18)=4.37$, $p<0.02$. A significant interaction, $F(8,48)=2.50$, $p<0.03$, confirmed the effect was more pronounced in the CGR. However, as Fig. 3 shows, a complete dose-dependent blockade of the effects of current by naloxone were seen only at the CGR site, $F_{CGR}(8,24)=3.36$, $p<0.02$; $F_{MFB}(8,24)=1.24$, $p>0.31$.

DISCUSSION

Response contingent stimulation of MFB electrode sites produced greater response rates than CGR sites; however, total response duration for stimulation was not significantly different at the two sites. Thus, response contingent MFB stimulation resulted in more frequent short duration current trains compared to CGR stimulation. Increasing current monotonically enhanced both rates and durations of lever-pressing. Furthermore, increases in rates of responding at higher current were more pronounced for MFB than CGR sites, though no site-specific effect of current was found on duration.

In a similar study using shuttle box stimulation procedures the investigators [26] report no effect of naloxone doses up to 50 mg/kg on stimulation "on" or "off" times at lateral hypothalamic and periaquaductal gray sites. These results appear at odds with the present report showing naloxone blockade of brain stimulation duration at the central gray site. However, total duration of brain stimulation in the present study depends both on the number of responses and the duration of each response. The former data is reported as mean seconds per crossing to the "on" or "off" side and does not reflect the number of responses. Since the present study finds no significant effect of naloxone on number of responses we conclude that the duration must have been reduced.

Only one previous investigation [20] reports a dose-related decrease in rate of brain stimulation responding on an FR 15 schedule at moderate naloxone doses. This result contradicts the present lack of effect of moderate doses on self-stimulation rate. However, the present study employed a reinforcement schedule where rate was deliberately confounded with the self-selected current durations. Nevertheless, increasing current monotonically increased both rate of responding and duration at both sites. Naloxone only produced significant dose related effects on duration of responses. Our finding that the effect on duration was most

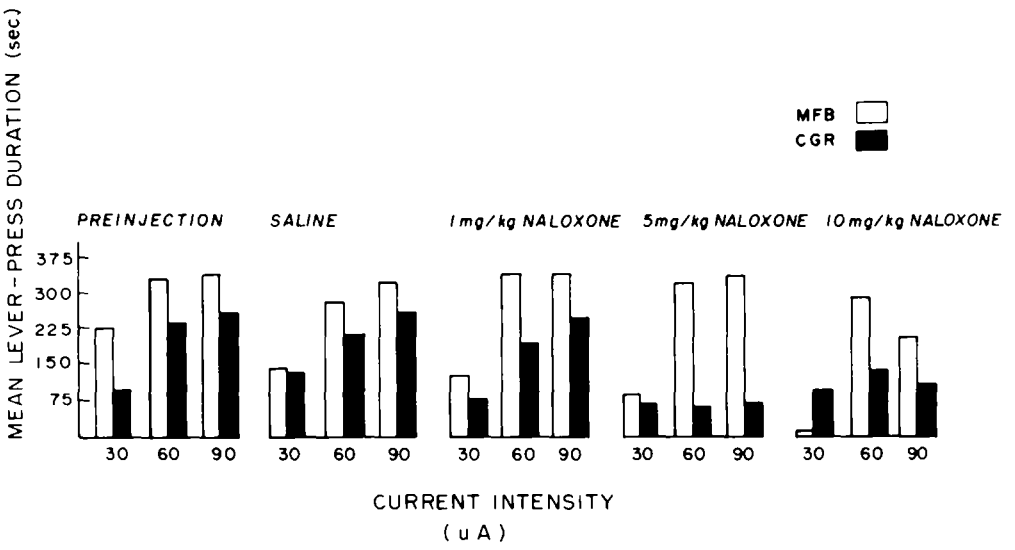

FIG. 3. Total lever press duration per 10 min in MFB and CGR implanted rats as a function of current intensity during various non-drug and drug treatments.

pronounced at the CG site is consistent with the previous finding [20] that CG is more sensitive than MFB to the rate reducing effects of naloxone.

In agreement with most previous studies, the present study shows no effect of moderate naloxone doses on response rate [4, 10, 11, 14]. However, the same moderate doses in the present study attenuated response durations in a dose-related fashion for both sites. Response durations generally increased as a direct function of current intensity across drug treatment conditions for MFB. In CGR, however, injections of 5 or 10 mg/kg naloxone eliminated the effect of current on duration, producing short duration responses at all levels of current. Considering the high concentration of opiate-mu receptors in the periaqueductal gray, it seems reasonable to conclude that the reported naloxone effects are mediated via a particular subset of opiate receptors. Moreover, the normal role of these receptors in BSR is to inhibit the aversive consequences of stimulation at these sites rather than elicit or mediate rewarding components.

When stimulus durations are subject-controlled, rats produce stable lever-press durations for a given set of stimulation parameters. Systematic changes in stimulation parameters produce systematic changes in lever press duration and are sensitive to drug administration [24,32]. In terms of reinforcement processes these stable "preferred durations" support the hypothesis that both the onset and termination of brain stimulation may be rewarding, as suggested by the fact that rats will terminate, but not avoid, long durations of stimulation [3,19]. Others have suggested that two conflicting processes are activated by the stimulation [12,29]: a reward process activated by current onset and a time-dependent aversive process when current durations are forced on the animal.

The onset of stimulation, or the initial lever-press, is an approach (positive BSR) behavior reflected in rate measures, i.e., the more rewarding the onset of stimulation, the higher the rate of responding. The offset of stimulation, or release of the lever, is a termination (negative BSR) behavior reflected in duration data, i.e., the greater the aversive buildup, the shorter the duration of lever presses.

Several general processes have been hypothesized to underly the termination of stimulation. That is, rats may terminate stimulation due to decreasing reward or adaptation, due to increasing valency of aversive elements temporally summated (i.e., punishment) over the course of long durations of stimulation [5,9], or interference of competing behavior induced by the stimulation. The termination of stimulation could therefore be viewed as a reduction in reward value of the present stimulus, increase in aversive or interfering components, or due to a contrast between the relatively greater reward value of stimulation onset compared to offset. The present experiment does not easily discriminate these alternatives. However, the dose and site dependent effects of naloxone on duration rather than response rate indicates that enkephalins released by the stimulation may act to prevent termination due to aversive buildup rather than enhance pleasurable consequences of stimulus onset.

The site stimulated is an important consideration as to whether adaptive or punishing influences are the substrate for termination of stimulus trains [24]. In the first place, there is a large disparity between MFB and CGR opiate receptor [21] and enkephalin [22,27] concentrations. Moreover, stimulation of MFB yields relatively "pure" positive BSR [16] while stimulation of CGR yields mixed positive/aversive BSR components [11,31]. It follows that current should more likely be terminated at CGR than MFB due to the gradual incorporation of these aversive components. However, the pre-injection data suggest that MFB rats made a greater number of short duration responses than the CGR rats. This observation, is not easily explainable within a reward punishment paradigm and suggests that both the onset and termination of current is rewarding.

The present study indicates that positive BSR, behav-

iorally evidenced by the onset of stimulation, is mediated by a non-enkephalin, possibly catecholamine (CA), system. First, MFB (high CA, low enkephalin) subjects responded at much higher rates than CGR (high enkephalin, low CA) subjects; and, while both groups displayed enhanced response rates as current intensity increased, this effect was greater at MFB sites. Second, naloxone failed to affect response rates in a dose-related manner, from either electrode site, but did block long durations of stimulation at the CGR site suggesting that the rewarding qualities of stimulus onset are not subserved by enkephalins, but that the aversive consequences of prolonged stimulation of enkephalin rich sites are enhanced by naloxone.

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