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Behavior of Monkeys During Opiate Withdrawal and Locus Coeruleus Stimulation

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GRANT, S. J., Y. H. HUANG AND D. E. REDMOND, JR. *Behavior of monkeys during opiate withdrawal and locus coeruleus stimulation.* PHARMACOL BIOCHEM BEHAV **30**(1) 13–19, 1988.—The noradrenergic nucleus locus coeruleus (LC) has been implicated in morphine withdrawal. The behavioral effects of opiate antagonist-precipitated morphine withdrawal in chair-restrained *Macaca arctoides* were therefore compared with LC electrical field stimulation. Both continuous LC stimulation and administration of low doses of naloxone to morphine pellet implanted monkeys produced a significant increase in the same group of behaviors reported previously to follow activation of the LC, without significant increases in general activity or distress behaviors. Signs of autonomic hyperactivity and distress were observed at high doses of naloxone, but not during LC stimulation. Monkeys which had not received morphine treatment did not exhibit significant changes in any of the behaviors following naloxone administration. Thus, the same group of behaviors specifically increased during low intensity LC stimulation is also selectively increased during naloxone-precipitated morphine withdrawal. These data are consistent with and suggest a behavioral consequence of the interactions of opioids with the LC reported at the molecular, intracellular, and cellular level.

Addiction Locus coeruleus Morphine withdrawal Naloxone Norepinephrine

INCREASED central noradrenergic function has been hypothesized to contribute to the development of many of the physiological and behavioral manifestations of morphine withdrawal [2, 18, 34, 38]. Several lines of convergent evidence support this hypothesis. Naloxone-precipitated morphine withdrawal increases biochemical and electrophysiological indices of central noradrenergic function in both rodent and nonhuman primate species [2, 13, 39]. In human opiate-dependent subjects, MHPG, the principal metabolite of brain noradrenaline (NA), is increased during precipitated morphine withdrawal [9]. In addition, clonidine, an alpha$_2$ adrenergic agonist which attenuates noradrenergic function by suppressing both release of transmitter from noradrenergic terminals and noradrenergic neuron impulse activity, is efficacious in suppressing morphine withdrawal symptoms in humans [10, 11, 18] and morphine withdrawal behaviors in animals [6, 15, 42]. Clonidine's antiwithdrawal activity is thought to be due, at least in part, to the suppression of noradrenergic function of the nucleus locus coeruleus (LC), the largest of the central noradrenergic nuclei [2,33].

The clinical effects of clonidine along with experimental physiological and biochemical data have suggested the hypothesis that opiate withdrawal symptoms are a function of hyperactivity of noradrenergic systems such as the LC. Although similarities have been noted between behavioral effects of electrophysiological activation of the LC and precipitated morphine withdrawal, they have not been directly compared experimentally [33]. If the LC hyperactivity seen during morphine withdrawal is responsible for all or part of the morphine withdrawal syndrome, then the behavioral pattern associated with low intensity activation of the LC should also occur during precipitated morphine withdrawal.

In order to have a common basis to assess the behavioral consequences of both continuous low intensity electrical field stimulation of the LC and naloxone-precipitated morphine withdrawal, an ethogram previously developed specifically for chair-restrained stumptailed monkeys was employed [36,37]. This ethogram was derived from a list of every behavior in chair-restrained monkeys which could be quantitatively rated with acceptable interrater reliability.

The ethogram consists of several subsets of behaviors. One subset of behaviors was reliably associated with activation of the central noradrenergic system either pharmacologically or via electrical field stimulation of the nucleus locus coeruleus [32, 36, 37]. This set consisted of oral activity (e.g., chew, tongue movements, yawn), manipulation of the skin and hair (scratch, hairpull, perineal inspection), and several other behaviors (clutch body, grasp chair, jumpy). These behaviors were given the neutral designation of GROUP I. A second groups of behaviors was found to be reliably associated with the administration of sedative drugs (SEDATION). Other behavioral groups consisted of nonspecific

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motor activity (MOVEMENT: body turn, head turn, hand move) and behaviors commonly associated with pain or distress (DISTRESS: vocalize, struggle, grimace). Some behaviors (eat, groom, freeze) were considered individually.

The present experiments tested the hypothesis that the behavioral pattern associated with low intensity activation of the LC, specifically selective increases in GROUP I behaviors, should also occur during precipitated morphine withdrawal.

METHOD

Subjects

Ten female stumptailed macaque (*Macaca arctoides*) were studied altogether. Seven of the monkeys were used as subjects in the precipitated withdrawal experiment (4 morphine-treated, 3 controls). Three other monkeys were used in the LC stimulation experiments. All of the monkeys were individually caged between sessions, and all had free access to food and water in their cages.

Chair Adaptation

All of the subjects were well adapted to sitting in a primate restraining chair in a sound-dampened chamber for 2–3 hours a day. "Pink" noise (64 dB) was used to provide further sound masking in the chambers. Chair adaptation sessions began at least 60 days prior to the start of the experimental sessions. Daily chair sessions continued throughout the entire experiment except for days in which morphine pellets were implanted.

Electrode Implantation and Stimulation

Three monkeys were implanted bilaterally under barbiturate anesthesia with bipolar stimulating electrodes in the LC using a previously developed procedure. This procedure uses the anatomical relationship between the LC and the motor nucleus of the trigeminal nerve (Motor V) to guide the placement of the electrodes [23,24]. Electrodes, cable connectors, and a protective nylon cap were fixed to the skull with dental acrylic.

LC stimulation was delivered under computer control via a cable attached to a connector on the monkey's skull. In previous studies of chair-restrained monkeys, short, intermittent periods of LC stimulation were used [36,37]. In contrast, administration of naloxone to rodents given chronic morphine treatment produces a sustained activation of LC neurons [2]. Therefore, in order to provide a more direct approximation of the pattern of LC hyperactivity during morphine withdrawal, continuous, low intensity electrical field stimulation of the LC (50 Hz biphasic pulses 0.8–1.2 mA peak to peak and 0.5 msec in duration) was delivered continuously over a 30-minute period. Only a single, low intensity stimulus was used so as to limit current spread and produce the most specific behavioral effects. The intensity was adjusted for each individual monkey based on previous experiments in these subjects using short, intermittent stimulus trains [36,37].

Following the final stimulation session, the monkeys were sacrificed by an overdose of pentobarbital, and then perfused via the heart with saline and 10% formalin. Electrode placement was confirmed histologically through location of marker lesions made immediately prior to sacrifice. Lesions were localized in 50 μm frozen sections stained with Cresyl Violet.

TABLE 1

BEHAVIORAL CATEGORIES FOR CHAIR RESTRAINED *MACACA ARCTOIDES*

Group I	Group II	Group III
Scratch*	MOVEMENT	SEDATION
Hand wring*	Head turn†	Drowsy‡
Hair pull*	Body turn†	Eyes closed‡
Self mouth*	Hand move†	
Tongue move*		
Chew*	DISTRESS	Freeze‡
Yawn*	Vocalize*	
Jumpy†	Struggle*	
Clutch†	Grimace†	
Grasp chair†		
Perineal inspection*	Eat*	
	Groom*	

Note. If none of the behaviors are scored, the monkey has its eyes open except for normal eye blinks and small movements may be occurring.

*Scored per 1 sec duration. †Scored once per episode. ‡Must last at least 5 sec and then is scored per second duration.

Morphine Treatment and Withdrawal Schedule

Slow release morphine pellets (75 mg/pellet; NIDA) were implanted subcutaneously under ketamine anesthesia (10 mg/kg) along the backs and flanks of four subjects. Sham implantation was performed in three subjects using the same procedure but without the placement of any pellets. For the first 30 days pellets were implanted at 4–5 day intervals, and in increasing doses from 2 pellets (150 mg) to 16 pellets (1200 mg) per implantation. Immediately prior to the first experimental session the implantation schedule was changed to every 2–4 days. By the final experimental session, 25 pellets (1875 mg) were used per implantation. This progression was equivalent to a dosage range of 11.5 to 144.5 mg/kg. Following the last experimental session the implantation sites were irrigated with saline to eliminate residual pellets.

Naloxone-precipitated withdrawal began after 30 days of pellet implantation. A single dose (IM) of naloxone or saline was administered during a given experimental session. Naloxone was given in an ascending dosage schedule of 2 μg/kg, 4 μg/kg, 8 μg/kg, and 16 μg/kg during the second to the fifth experimental sessions. To control for effects of injection, equal volumes of saline were given during the first (0_1) and last (0_2) experimental sessions.

Experimental Sessions

During each experimental session the behavior of the subject was videotaped with superimposed coded identification and timing signals. Videotaping began 60 minutes after the subject was placed in the experimental chamber. Videotaping lasted for at least one hour, which included a 15-minute baseline period and a 30–45-minute posttreatment period.

Data Collection

Each videotaped session was rated on an ethogram of 40 behaviors developed in previous studies out of a list of every recognizable behavior of chair-restrained stumptailed monkeys that can be reliably defined and scored from videotape. These behaviors and their groupings are listed in Table 1.

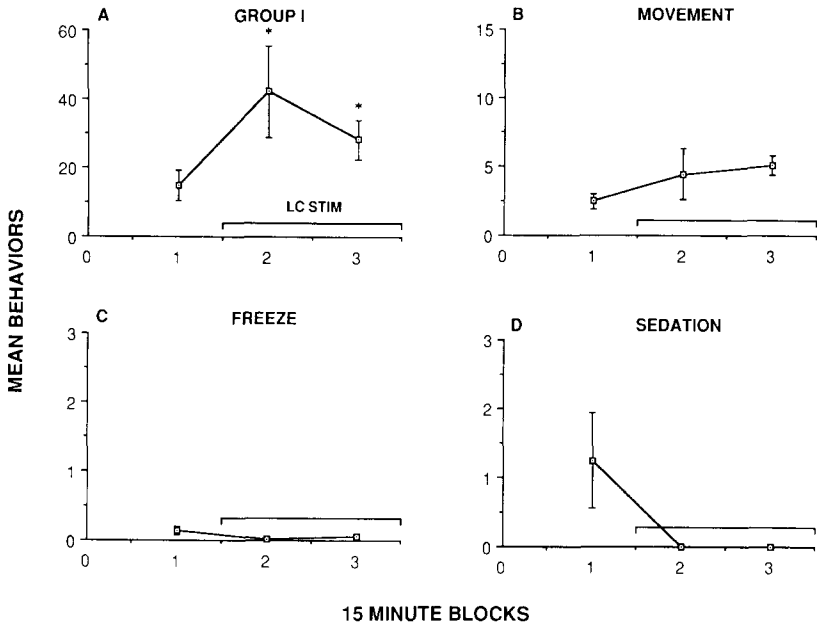

FIG. 1. Behavioral effects of continuous, low intensity electrical field stimulation of the nucleus locus coeruleus (LC) (50 Hz, 0.4–1.2 mA, 0.5 msec pulse width, in three chair-restrained *M. arctoides*. Data points represent the mean number of behaviors ±standard error of the mean (s.e.m.) for the three monkeys during consecutive 15 minute blocks. Duration of the stimulation is indicated by the brackets (BLOCKS 2–3). *$p<0.05$, Dunnett's test.

In addition to the items on the ethogram, the occurrence of autonomic withdrawal signs commonly associated with morphine withdrawal (hyperventilation, lacrimation, salivation, piloerection, and rhinorrhea) were also noted if present during the session [1, 5, 41, 43, 47].

Ratings of the videotaped records were made by experienced personnel, who were blind to the dose of naloxone administered in that session or to the presence or absence of LC stimulation.

Data Analysis

Raw counts of individual behaviors were summed to produce the groups outlined above, although some behaviors (eat, groom, freeze) were considered individually. Group totals were collapsed into 15-minute blocks since initial inspection of the data indicated that peak effects were generally seen within the first 15 minutes posttreatment. Thus, each session consisted of a pretreatment baseline block (BLOCK 1) and 2 or 3 posttreatment blocks (BLOCKS 2–4).

Data were analyzed using repeated measures ANOVA or ANCOVA designs with the SAS statistical package. The response to LC stimulation or naloxone administration over time was examined by plotting the mean behaviors for behavioral groups over the course of each session. Effects seen within a single session were analyzed using repeated measures ANOVA. Direct comparisons across naloxone doses were made with respect to changes from the pretreatment baseline (BLOCK 1) for each session. This allowed evaluation of dose-related effects of naloxone independent of changes in the preinjection baseline (BLOCK 1) behavioral levels due to prior naloxone administration. The data were analyzed by an analysis of covariance (ANCOVAR) using BLOCK 1 as the prescore or covariate since simple change scores or percentages can introduce statistical errors [12,27]. The results from the ANCOVAR were used to construct a dose response curve using the least-squares adjusted mean for each group.

RESULTS

LC Stimulation

The monkeys with implanted LC electrodes had no complications following implantation surgery, and remained in good health until sacrifice. All of the electrodes were localized within the confines of the compact cell body portion of the LC, which in the monkey is composed almost entirely of noradrenaline containing neurons [3, 14, 19].

During electrical field stimulation of the LC, significant increases were seen only in GROUP I, $F(2,4)=7.41$, $p<0.05$, as shown in Fig. 1. Peak behavioral effects were seen during the first 15 minutes of stimulation (BLOCK 2) and declined slightly over the remainder of the 30-minute stimulation period. This temporal pattern may simply reflect a decline in the efficacy of noradrenergic transmission during sustained stimulation of LC neurons. Noradrenergic neurons possess a number of intrinsic mechanisms which would tend to counteract the effects of prolonged activation including depletion of noradrenaline from the nerve terminals, decreased axonal velocity mechanisms, autoreceptor-mediated inhibition of impulse flow and transmitter release as well as postsynaptic adaptive processes such as receptor desensitization [3, 19, 40].

Other behaviors did not change significantly during electrical field stimulation of the LC. Although there was an increase in MOVEMENT over the entire session, this trend was not significant at the single intensity employed,

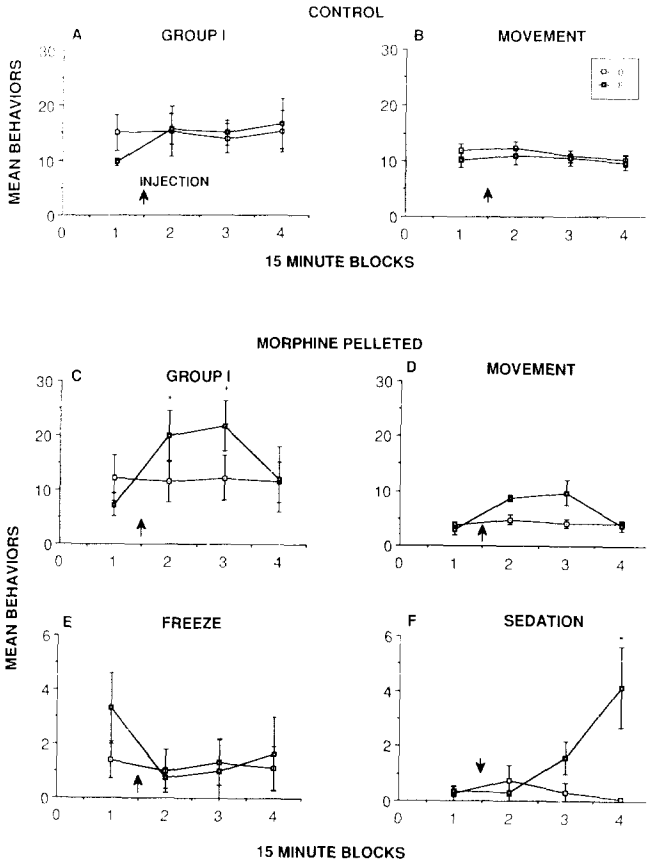

FIG. 2. Behavioral effects over time of saline and 8 μg/kg of naloxone in three CONTROL (sham pelleted) (A–B) and four MORPHINE PELLETED (C–F) chair-restrained *M. arctoides*. Data points represent the mean number of behaviors ±standard error of the mean (s.e.m.) for each group of monkeys during consecutive 15 minute blocks. Drug injection was made at the beginning of BLOCK 2 as indicated by the arrowhead. Dose 0 represents the first saline (sham) session. GROUP I: $F(3,9)=5.13$, $p<0.05$. MOVE: $F(3,9)=2.18$, p=n.s. FREEZE: $F(3,9)=3.3$, $p<0.07$. SEDATION: $F(3,9)=4.8$, $p<0.05$. *$p<0.05$, Dunnett's test.

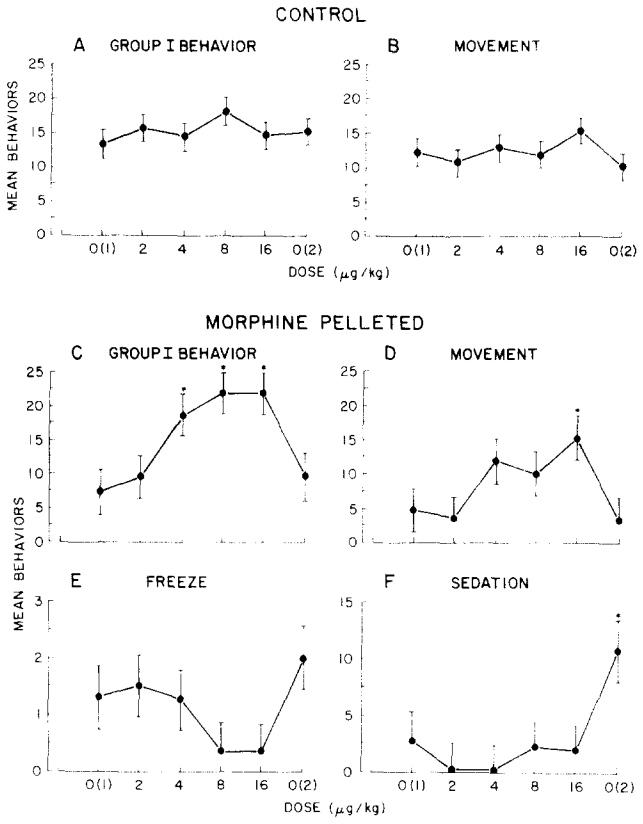

FIG. 3. Behavioral dose response curves for CONTROL (sham pelleted) (A–B) and MORPHINE PELLETED (C–F) *M. arctoides* across four doses of naloxone and 2 sham (saline) injections (0_1 and 0_2). Plotted points represent least squares adjusted (LS) mean values ±s.e.m. of the first 15 minute period (BLOCK 2) following the injection derived from an analysis of covariance, with the preinjection baseline period (BLOCK 1) used as the covariate. *$p<0.05$, Fisher's t-test.

$F(2,4)=1.50$, p=n.s. During the stimulation period both FREEZE and SEDATION were abolished. However, neither change was statistically significant [FREEZE: $F(2,4)=2.26$, p=n.s.; SEDATION: $F(2,4)=1.1$, p=n.s.] probably due to the low prestimulation (BLOCK 1) levels for FREEZE and the absence of SEDATION behaviors in 2 out of the 3 monkeys. There was no occurrence of any of the other rated behaviors either prior to or during stimulation.

Precipitated Morphine Withdrawal

All monkeys survived the entire period of pellet implantation, naloxone-precipitated withdrawal, and final spontaneous withdrawal procedure with no medical complications.

Changes in the rated behaviors over time are illustrated in Fig. 2. Changes in rated behaviors were only observed in the morphine-treated monkeys. The effects of naloxone appeared within the first 15 minutes postinjection (BLOCK 2), lasted an additional 15 minutes, and returned to baseline levels by the end of the session. In general, both GROUP I and MOVEMENT increased following naloxone but not after saline injections (Fig. 2A,B). SEDATION and FREEZE tended to decrease during the first 30 minutes postinjection (BLOCKS 2–3), but only SEDATION increased afterwards (Fig. 2C,D). The recovery of the behaviors to preinjection base line (BLOCK 1) levels by the end of each session is consistent with the relatively short duration of action of naloxone [26].

The control group did not show significant changes in any of the behavioral measures across any of the doses [GROUP I: $F(5,19)=0.63$, p=n.s.; MOVEMENT: $F(5,9)=0.90$, p=n.s.] as indicated by the flat dose-response curves for GROUP I and MOVEMENT in Fig. 3A,B. The control group did not exhibit any appreciable incidence of the other behaviors either during baseline periods or following saline or naloxone injections.

The morphine-treated subjects did have significant changes in many of the behaviors (Fig. 3C–F). GROUP I exhibited a clear dose-response relationship, $F(5,14)=4.90$, $p<0.01$. Significant increases (Fisher's t-test) relative to the first saline session (0_1) were found at 4 μg/kg ($p=0.02$), 8 μg/kg ($p<0.01$), and 16 μg/kg ($p=0.005$) of naloxone. In contrast, the increase in MOVEMENT was not quite significant, $F(5,14)=2.52$, $p=0.075$. Only the 16 μg/kg dose was significantly different from saline administration (0_1) ($p<0.05$),

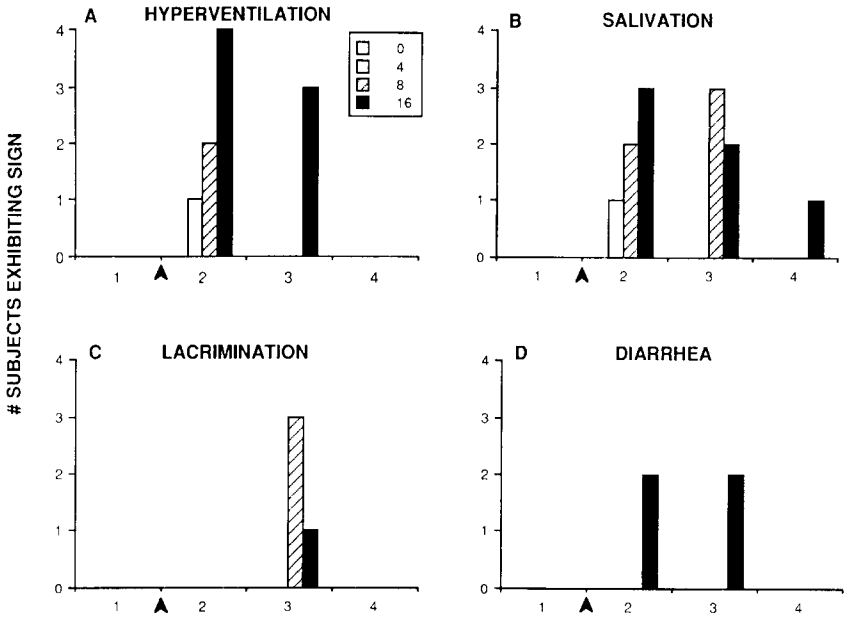

FIG. 4. Autonomic morphine withdrawal signs observed in morphine-treated *M. arctoides* (N=4) following administration of naloxone. Signs were noted as either present or absent during each 15 min block. The number of subjects exhibiting a given sign is plotted for three doses of naloxone (4, 8, 16 μg/g) and saline (0). Injections were given after a 15 min baseline period (BLOCK 1), indicated by arrows. Control subjects not treated with morphine did not exhibit any autonomic withdrawal signs.

while the 4 μg/kg dose was marginal ($p=0.13$). Overt DISTRESS behaviors (vocalization, grimace and struggling) were only observed in one monkey and only at the highest naloxone dose (16 μg/kg).

There were no significant changes in SEDATION, $F(5,14)=4.67$, $p=0.10$, or FREEZE overall, $F(5,14)=1.60$, $p=$n.s. However, SEDATION did show a marginal increase during the final saline session (0_2) relative to the initial saline session (0_1) ($p=0.06$).

Increases in the autonomic signs (hyperventilation, lacrimation, salivation, and diarrhea) were only observed in the morphine-treated animals and only following naloxone administration. The occurrence of these signs increased as a function of the dose of naloxone, both in terms of the number of monkeys exhibiting a given sign and their duration during a session (Fig. 4).

DISCUSSION

Since LC stimulation produces specific behavioral effects and morphine withdrawal activates noradrenergic impulse flow, it was postulated that morphine withdrawal and LC stimulation would have similar behavioral profiles. The results of this study demonstrate that both naloxone-precipitated morphine withdrawal and continuous electrical field stimulation of the LC produce selective increases in the same group of behaviors (GROUP I) associated in previous studies with either short, intermittent periods of LC stimulation or pharmacological activation of central noradrenergic neurons [36]. Although GROUP I behaviors reached higher absolute levels during electrical field stimulation, the increases in BLOCK 2 relative to the prestimulation period (BLOCK 1) in both treatments were similar (186% for LC stimulation vs. 176% at 8 μg/kg naloxone).

The behavioral effects of naloxone were dose-dependent and were only observed in monkeys given chronic morphine. This indicates that the behavioral effects were due to antagonism of morphine at opiate receptors and not due to nonspecific pharmacological actions of naloxone, the injection procedure, or the morphine pelleting procedure. The threshold dose for producing a significant increase in GROUP I behaviors (4 μg/kg) was approximately equal to the threshold dose of naloxone for producing withdrawal in addicted humans [26]. On the other hand, autonomic signs of withdrawal and overt DISTRESS behaviors only became prominent at higher doses. Thus, GROUP I behaviors appear to be more sensitive in detecting the effects of naloxone-precipitated morphine withdrawal in nonhuman primates than the autonomic signs usually rated alone. This finding is consistent with and provides quantitative assessment of an early observation that the first indications of the onset of morphine withdrawal in primates consist of signs of "apprehension and irritability" ([41], p. 152).

At higher doses of naloxone (>8 μg/kg), however, there were significant increases in MOVEMENT and the emergence of autonomic signs and DISTRESS behaviors. These additional effects seen during severe morphine withdrawal could be the result of two, not necessarily mutually exclusive processes: (1) increasing levels of activation of the same system (LC) or (2) emerging contributions from other NA or nonNA systems [6]. Significant increases in MOVEMENT and DISTRESS can be obtained at higher intensities of LC stimulation than used here [33, 36, 37]. Because the interpretation of high stimulation intensities is confounded by potential current spread into surrounding brainstem structures only a single stimulation intensity, chosen to produce maximal behavioral specificity, was used in this study. This

precluded direct comparison of higher intensity electrical field stimulation with high naloxone doses. On the other hand, even though autonomic withdrawal signs were not observed during low intensity LC stimulation in this study, some of these autonomic signs are known to be reduced by clonidine administration suggesting a significant contribution of NA systems (e.g., bowel motility and diarrhea) [28,33].

The similarities between LC stimulation and naloxone-precipitated morphine withdrawal may therefore be a direct consequence of the LC hyperactivity seen during morphine withdrawal [2, 13, 39] and the quasi-morphine withdrawal syndrome [13, 17, 20, 46] in animals. A related increase in noradrenergic function during morphine withdrawal has been shown in humans based on increased concentrations of MHPG in plasma [9]. Conversely, administration of clonidine, an alpha$_2$ adrenergic agonist which inhibits noradrenergic release and impulse flow, attenuates morphine withdrawal both clinically [11,18] and experimentally in animals [6, 15, 33, 42, 45], as does acute inhibition of norepinephrine synthesis [22]. Clonidine also antagonizes the behavioral effects of electrical stimulation of the LC [33,36], supporting the interpretation that the behavioral effects of stimulation of the LC are under the control of alpha$_2$ adrenergic receptors in the region of the LC or its terminal fields.

On the other hand, the activity of many brain regions other than the LC is certainly altered during morphine withdrawal [49]. However, many of the brain areas which have been suggested to be critical for the expression of morphine withdrawal behaviors receive a prominent noradrenergic input, e.g., amygdala [4, 7, 44, 49], hippocampus [25, 44, 49], periaquaductal grey [48], serotonin neurons [8], and spinal sympathetic neurons [16]. In addition, postsynaptic adrenergic receptors become hypersensitive during chronic morphine administration [21, 29–31] which would further amplify the net effects of the increased noradrenergic impulse flow in these areas during morphine withdrawal relative to electrical stimulation of the LC in nondependent subjects.

In conclusion, the present results provide evidence for a behavioral similarity between naloxone-precipitated morphine withdrawal and low intensity electrical field stimulation of the locus coeruleus. These results are consistent with previous studies demonstrating an activation of noradrenergic neurons during morphine withdrawal and the amelioration of morphine withdrawal behaviors by the alpha$_2$ adrenergic agonist clonidine. Increases in GROUP I behaviors may therefore serve as a sensitive behavioral index of NA contributions to the expression of morphine withdrawal in primates. However, in order to determine the specific contribution of the LC-NA system to morphine withdrawal in primates, further studies using specific, direct reversible inactivation of the locus coeruleus during morphine withdrawal would be necessary.

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