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Naloxone Reduces Social Locomotor Activity in Rats

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DOKLA, C. P. J. *Naloxone reduces social locomotor activity in rats.* PHARMACOL BIOCHEM BEHAV **43**(4) 1183–1193, 1992.—Naloxone, a nonspecific opioid antagonist, has been found to decrease the activity and social behavior of rats tested in pairs but the effects on individual locomotor activity have been equivocal. In the present study, groups of male Long-Evans hooded rats received naloxone (1 or 4 mg/kg, IP) or vehicle alone (isotonic saline) 30 min prior to testing sessions. Individual locomotor activity was measured in two activity boxes (41-cm^3) equipped with two infrared photobeams using daily 30-min testing sessions for 5 consecutive days. Following a 1-week washout period (no testing), activity and social attraction (paired distance and contact) were examined in pairs of rats from each group using daily 15-min testing sessions for 4 consecutive days. Locomotor activity and its habituation were not significantly affected by naloxone in rats tested individually. However, both doses of naloxone significantly reduced paired locomotor activity compared to the control group. Measures of social attraction were not significantly affected by naloxone. The present findings suggest that naloxone does not produce nonspecific depressant effects on activity but rather may antagonize opioid release in situational contexts of high arousal (e.g., social activity) with consequent reduction of activity.

Naloxone Opiate antagonist Opioids Locomotor activity Paired activity Spontaneous activity
Exploration Social behavior Habituation Novelty Rat

PIONEERING studies of Hughes and associates (38,39) demonstrated the existence of two endogenous opioid pentapeptides in the brain, [Met]-enkephalin and [Leu]-enkephalin, with potent opiate agonist activities similar to morphine, an opiate alkaloid derived from the poppy, *Papaver somniferum*. Ensuing research isolated a host of additional endogenous opioid peptides sharing some common structural characteristics and belonging to one of three families of opioid precursors, pro-enkephalin, pro-dynorphin, and pro-opiomelanocortin, each of which is coded by a separate mRNA and gene [for reviews, see (2,4,5)]. Radioimmunoassay and immunohistochemical studies have described the widespread distribution and regional anatomic characteristics of opioid peptides in the brain and peripheral nervous system, and in vitro and in vivo pharmacological studies have revealed multiple forms of opioid receptors: μ, κ, and δ (5).

The opioids play diverse and active roles in a number of behavioral phenomena and have critical actions in mediating pain (analgesia) and global responses to stressors [for reviews, see (5,6,54,62)]. As putative neurotransmitters, the enkephalins have been implicated in both learning and memory processes [for reviews, see (51,52,62)]. However, especially pertinent to the present study, opioid peptides have been found to be important in mediating locomotor activity, exploratory behavior, and aspects of social behavior in infrahumans [for reviews, see (13,46)].

Naloxone, a prototype opiate antagonist has strong antagonist properties not only at μ-receptor sites but also antagonizes κ- and δ-receptors as well, and at high doses has been reported to manifest agonist-like properties resembling morphine [for review, see (68)]. Nonspecific opioid antagonism using naloxone has been a standard research protocol for determining the effects of opioid peptides on locomotor activity, exploration, and social behavior in rodents.

The effects of naloxone on individual locomotor activity in rats have yielded mixed results. However, a number of variables can critically affect locomotor activity levels, including the time of testing (light–dark phase); task novelty or habituation; apparatus type and lighting; testing parameters (trial frequency, session length, etc.), and sundry subject variables (79); these variables may interact with the pharmacokinetics of drugs in simple or complex ways. Decreased individual locomotor activity has been suggested to be dependent upon conditions of task novelty during testing in rats pretreated with naloxone (46) and this contention is supported by several studies (1,9,10,20,42,48,65). However, decreased activity in rats habituated to testing conditions has been found following naloxone pretreatment (3,7,27,32,35,57,64,70,72, 78,81). Further, many studies have found no significant effects of naloxone on individual locomotor activity under either conditions of novelty (8,23–28,30,37,53,55) or habituation (16–19,31,36,44,56,73). Few studies have attempted to manipulate task conditions of novelty and habituation within the same study using rats. File (25) found that naloxone (2 mg/kg) reduced paired activity under both novel and habituated testing conditions, while Galina and Amit (27) found that

naloxone (2 mg/kg) decreased individual activity in habituated rats but not under novel testing conditions.

Conflicting findings have been reported for other variables in regard to individual locomotor activity in rats, as well. For example, behavioral activation/arousal occurs during the dark phase of the light–dark photoperiod and most likely when testing is conducted under so-called "dark conditions" (no light or "red bulb" illumination). Several studies have reported decreased activity levels in rats pretreated with naloxone under these testing conditions (3,48,64,65) but others have not (17,56). Naloxone dose and injection parameters are relevant for the expression of behavioral effects. High doses of naloxone have been reported to decrease activity in rats—16 mg/kg (72), 30 mg/kg (35), and 100 mg/kg (42)—but not in all cases (36). A further complication is the lack of dose-dependent effects of naloxone on individual locomotor activity reported in many studies (65).

Comparatively fewer studies have examined individual locomotor activity in rats using naltrexone, a structural analog of naloxone, with potent and long-lasting effects in brain, but contradictory results typify the reported studies. Naltrexone has been found to either decrease activity (15,45,47,48,69) or have nonsignificant effects (11,40,58,74). The importance of multiple relevant variables interacting is indicated in a study by Roth et al. (67) in which naltrexone significantly decreased activity under conditions of novelty after 60 min of stress induction but not zero or 5 min.

Exploratory activity has been often distinguished from simple locomotor activity (61) and in particular instances may represent behavior that is engaged in for purposes of reduction of discomfort due to information inadequacy, that is, "curiosity," stimulus seeking for purposes of novelty, complexity, or environmental change, etc. (14). Exploratory behaviors have typically been quantified in rats by nose-poke, head-dip (66), and similar behaviors in apparatus characterized by stimulus variety or complexity. Using a complex, nine-compartment apparatus, Arnsten and associates reported in a series of studies (8–10) that naloxone (0.25–0.50 mg/kg) increased exploratory activity (e.g., head-dip responses) in rats, and such exploratory increases may account for locomotor activity decreases in comparable, complex testing situations (9,57). However, other studies, typically employing much higher doses of naloxone, have found either decreased exploratory responses in somewhat simpler task environments (25,26,48) or nonsignificant effects (30).

Paired locomotor activity testing and measures of social behavior (play fighting, active social interaction, and positional distance, etc.) have typically been assessed by testing two rats in the same apparatus. Although the number of studies using this paradigm have been far fewer than those using individual activity testing, naloxone has been found to significantly decrease paired locomotor activity in rats (20,25) and attenuate certain social behaviors, in particular, play fighting (12,25,70,71). Only a single study has reported nonsignificant effects on paired activity and low doses (0.03, 0.125, and 0.5 mg/kg) of naloxone were used (60), while another study (59) found no significant effects on social interaction using two doses of naloxone (1 and 10 mg/kg) and a rather long drug injection to testing interval (60 min).

The present study further examined the effects of naloxone on individual locomotor activity, paired activity, and social behavior in rats. Drug dose and injection-to-testing parameters (71) were selected to be consistent with several previous studies that reported either significant individual activity decreases (1) or significant paired activity decreases and nonsignificant effects on individual activity (20). The effects of naloxone on habituation of activity have not been thoroughly examined because the large majority of studies of individual locomotor activity have used only a single testing session and only one study on paired activity (20) used multiple testing days. In addition, a single naloxone injection has been reported to have delayed behavioral effects on a retest (24 h postinjection) of individual locomotor activity (64). Therefore, the present investigation examined the effects of naloxone on individual and paired activity over repeated days of testing and assessed both activity and social behavior using both intersession and intrasession analyses (79). It was hypothesized that naloxone would significantly decrease paired activity and social attraction but not individual locomotor activity in rats over repeated days of testing.

METHOD

Subjects

Eighteen male Long-Evans hooded rats obtained from Blue Spruce Farms, Inc. (Altamont, NY) were used. The experimentally naive rats were approximately 56 days of age and weighed between 228–265 g (mean = 247 g, SD = 12.82) at the beginning of testing. Rats were housed individually in stainless steel cages under constant fluorescent illumination in a climate-controlled room maintained at 24°C and were allowed food (Rodent Laboratory Chow, No. 5001, Ralston Purina Co., Inc.) and water ad lib throughout the study.

Apparatus

Two identical 41 × 41 × 41-cm activity boxes constructed of three 3/16-in. clear Plexiglas, with exteriors covered by gray matte cardboard (21) were used. Two sets of parallel infrared light sources and photocells (Quad Photo-Relay, Model 1535, Hunter Mfg. Co., Inc., Iowa City, IA) were situated 2.54 cm above the 1/2-in. wire mesh floor of each box. The activity boxes were housed in separate, adjacent cubicles supplied with 2 ft-c of fluorescent illumination and 65-dB white-noise masking and monitored through an overhead videocamera.

Procedure

Experiment 1. All rats were allowed to adapt to individual home cages for 12 days following delivery from the supplier. Daily during this period, rats were weighed and then handled individually for at least another 2 min. The day after the conclusion of adaptation, individual activity testing began. Rats were randomly assigned to one of three groups and (-)naloxone HCl (E. I. Du Pont Pharmaceuticals, Glenolden, PA) was administered 30 min prior to testing to two naloxone-treated groups—1 mg/kg IP (NAL-1, $n = 6$) and 4 mg/kg IP (NAL-4, $n = 6$)—while a control group (CONT, $n = 6$) received 0.9% saline (vehicle) only. Naloxone dose is expressed in terms of the weight of its salt.

Rats were carried, two at a time, to the testing room from an adjacent colony room in separate stainless steel transport cages. The two rats were placed simultaneously in separate activity boxes; placement interrupted the rear photobeam of each box and activated electromechanical programming and recording equipment. Photobeam interruptions were recorded in 60-s bins from each box. Individual activity was measured daily for 30 min for 5 consecutive days of testing.

Experiment 2. Following a 7-day washout period (no drugs or testing), paired locomotor activity testing was conducted.

Rats were tested in pairs, that is, two rats per activity box, according to the following procedure: a) Pairs were randomly constituted from within the same groups used in Experiment 1, that is, three pairs per group; b) pairs were maintained intact throughout Experiment 2; c) each pair was tested in the same activity box over days; d) drug dose and injection procedure were identical to those in the previous experiment, and both members of each pair received the same drug dose (or control injection). Paired locomotor activity was measured daily using 15-min testing sessions for 4 consecutive days.

In both experiments, testing was conducted between 1100 and 1800 h, and each rat was tested at approximately the same time each day to reduce the risk of diurnal variation of endogenous opioid levels (80). Paired activity trials were videotaped for subsequent behavioral analyses.

Statistical Analyses

Individual locomotor activity scores and paired activity scores (mean photobeam interruptions per 60-s intervals) were arranged in three (treatment groups) × five (days) and three (treatment groups) × four (days) factorial designs, respectively, and analyzed using two-way mixed-design analysis of variance (ANOVA) with repeated measures on days. Intrasession (within days) analyses for individual activity and paired activity scores were performed using two-way mixed-design ANOVA (repeated measures on trial blocks). A measure of individual locomotor activity habituation recovery (retention) was calculated using the Leibrecht-Askew index (22) and analyzed using a two-way mixed-design ANOVA (repeated measures on days). Defecation (fecal bolus) frequency per testing session for individual locomotor activity and paired activity was analyzed using two-way mixed-design ANOVA (repeated measures on days). The distance between pairs of rats, a measure of social attraction (49,50), was determined from the videotaped recordings using 10-s sampling intervals (43). In addition, the presence or absence of physical contact (43) between pairs of rats was recorded at each of the sampling intervals using a blind procedure. Intersession and intrasession analyses of distance scores (cm) and contacts were conducted using two-way mixed-design ANOVA (repeated measures on days and trial blocks). Significant main or interaction effects were further analyzed using Newman-Keuls posthoc analyses. Analyses for all the dependent variables were performed using both the raw and transformed ($\log_{10}$ and square root) scores.

RESULTS

Preliminary analyses revealed no significant differences between analyses performed using the raw scores and those using the transformed data; therefore, all analyses are presented for the raw score data only.

Experiment 1

Figure 1 depicts the mean individual locomotor activity/min for each of the three groups over the 5 days of testing.

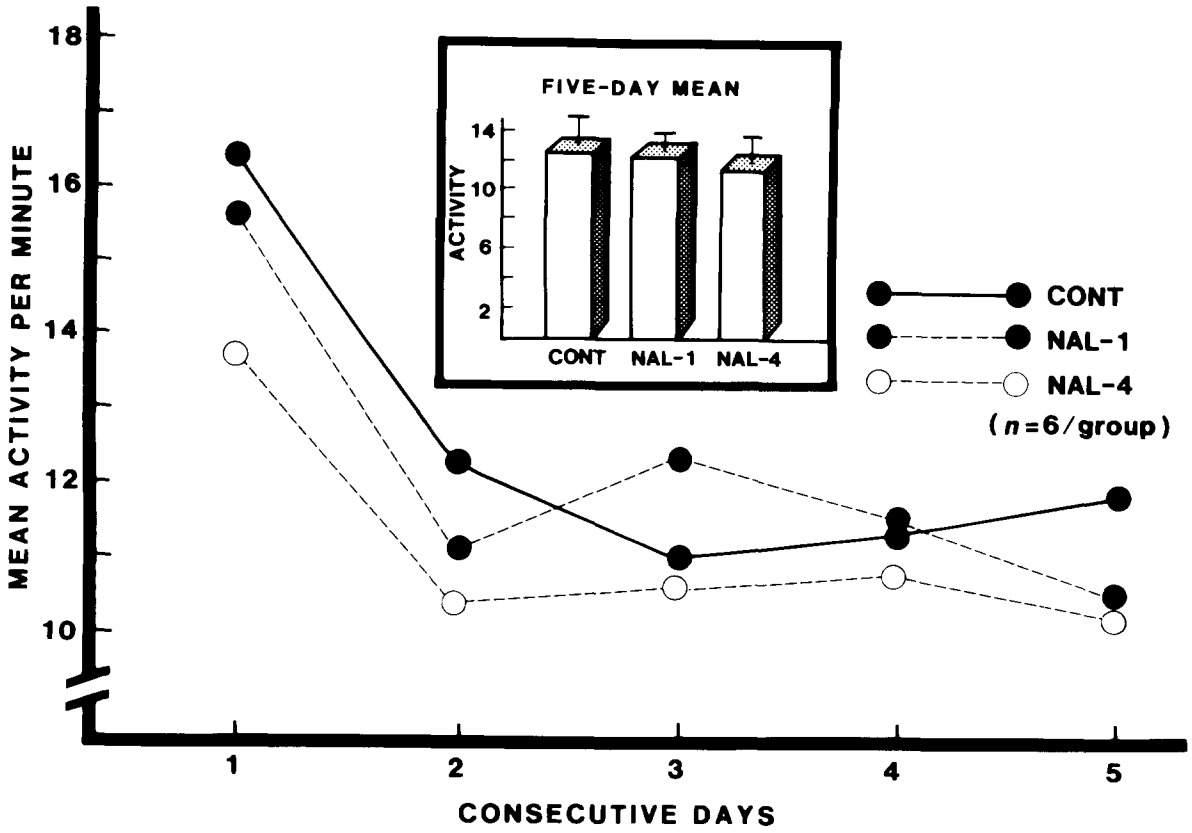

FIG. 1. Mean individual locomotor activity per minute (photobeam counts) for each of the three groups on each of the 5 days of testing; for purposes of graphic clarity, the SEM bars (± 1.04 to ± 2.51 activity scores) have been omitted. The inset depicts the 5-day mean activity per min (± SEM) for each of the groups. CONT, control; NAL, naloxone.

The nonsignificant drug × days interaction, $F(8, 60) = 0.59$, $p > 0.05$, indicated comparable rates of activity habituation between the groups over days. Similarly, the main effect for drug, $F(2, 15) = 0.21$, $p > 0.05$, also proved nonsignificant. Only the main effect for days, which indicated decreasing activity over days (especially the first two) across groups, was significant, $F(4, 60) = 14.33$, $p < 0.001$.

Figure 2 presents the mean individual locomotor activity/min for each of the three groups over the five blocks of 6-min intervals on day 1 of testing. Intrasession habituation of activity was not significantly affected by naloxone, as indicated by the drug × session blocks interaction, $F(8, 60) = 1.48$, $p > 0.05$. Although the NAL-4 group had somewhat lower activity overall compared to the other groups (see Fig. 2 inset), the main effect for drug, $F(2, 15) = 0.61$, $p > 0.05$, was nonsignificant. Only the main effect for session blocks, $F(4, 60) = 30.0$, $p < 0.001$, proved significant. Intrasession analyses for the other days of testing yielded the same pattern of results as for day 1.

Figure 3 presents the mean percent recovery of activity habituation for individual locomotor activity. The drug × days interaction, $F(6, 45) = 0.89$, $p > 0.05$, indicated that naloxone did not significantly affect habituation recovery over testing. Although the NAL-1 group had somewhat lower 5-day habituation retention (see Fig. 3 inset) than the other groups, the main effect for drug, $F(2, 15) = 3.66$, $p = 0.051$, just missed significance. Only the main effect for days, $F(3, 45) = 4.70$, $p < 0.01$, proved significant.

Figure 4 depicts the mean defecation (fecal bolus) frequency of the three groups on each of the 5 days of individual locomotor activity testing. The drug × days interaction, $F(8, 60) = 0.82$, $p > 0.05$, and the main effect for drug, $F(2, 15) = 0.69$, $p > 0.05$, indicated that naloxone had no significant effect on defecation frequency. Only the main effect for days was significant, $F(4, 60) = 3.43$, $p < 0.025$.

Experiment 2

Figure 5 presents the mean paired locomotor activity/min for each of the three groups over the 5 days of testing. Both the NAL-1 and NAL-4 groups displayed lower activity across days than the CONT group. Although the main effect for drug, $F(2, 6) = 18.50$, $p < 0.005$, proved significant, the nonsignificant drug × days interaction, $F(6, 18) = 0.61$, $p > 0.05$, and main effect for days, $F(3, 18) = 1.11$, $p > 0.05$, indicated a stable reduction of activity over days. A Newman-Keuls posthoc analysis on the significant drug main effect indicated that the NAL-1 and NAL-4 groups both had

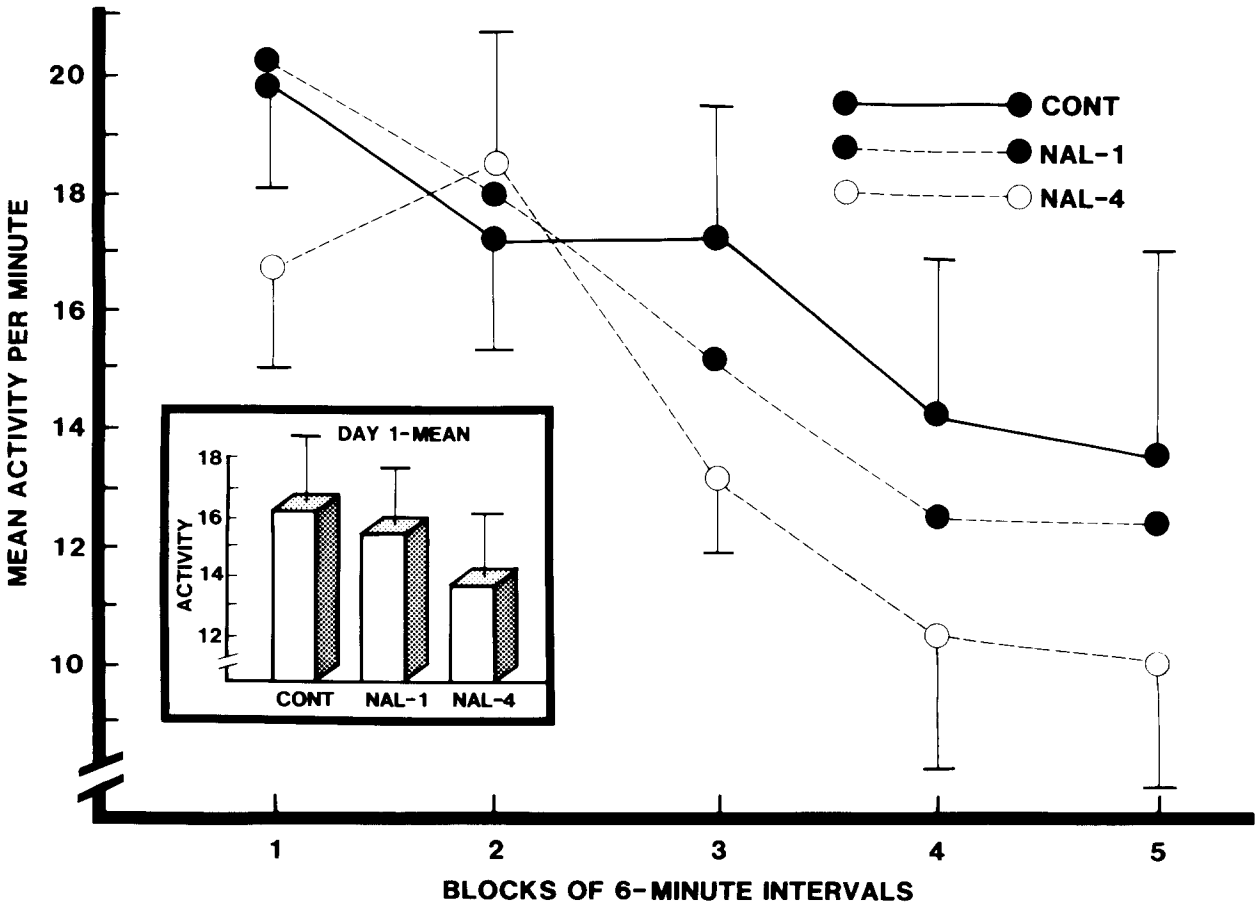

FIG. 2. Mean individual locomotor activity per minute (±SEM) for each of the three groups on each of the five blocks of 6-min intervals on day 1 of testing. The inset depicts the day 1 mean activity per min (±SEM) for each of the groups. CONT, control; NAL, naloxone.

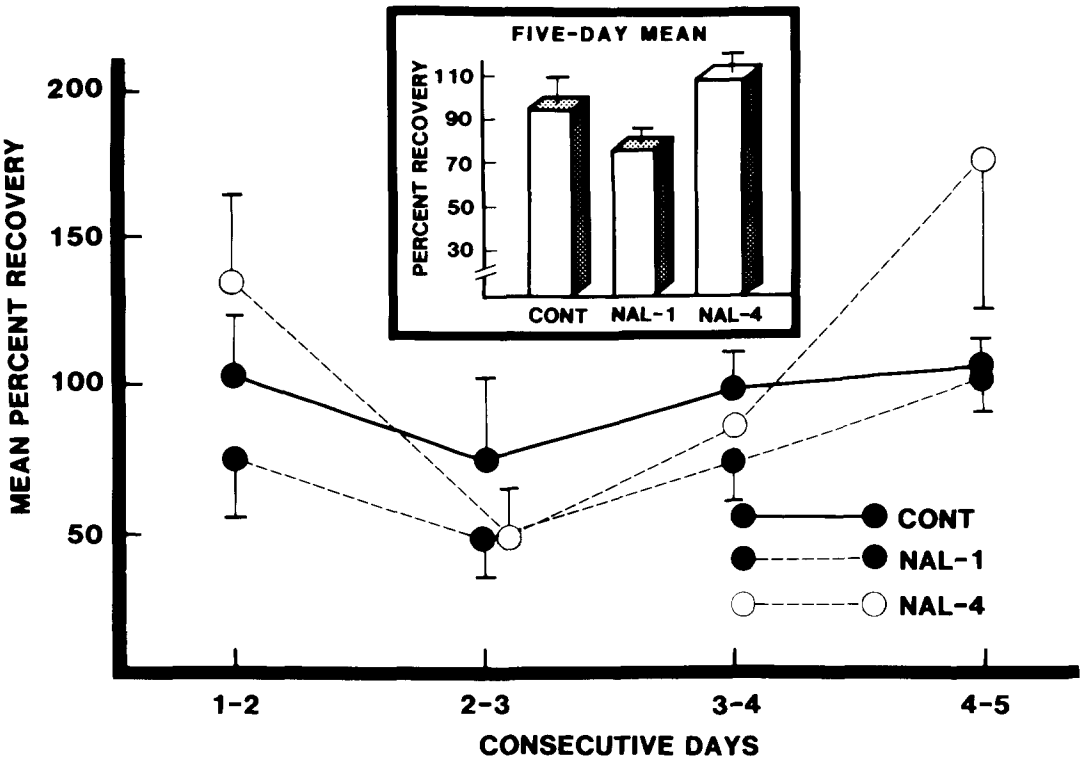

FIG. 3. Mean percent recovery (±SEM) of individual activity habituation (Leibrecht & Askew index) for each of the three groups for consecutive 2-day blocks of testing. The inset depicts the 5-day mean percent recovery of activity habituation (±SEM) for each of the groups. CONT, control; NAL, naloxone.

lower activity than the CONT group ($p < 0.01$) but were not significantly different from one another ($p > 0.05$).

Figure 6 presents the mean paired locomotor activity/min for each of the three groups over the five session blocks of 3-min intervals on day 1 of testing. Both the NAL-1 and NAL-4 groups displayed lower activity across the session compared to the CONT group, as indicated by the significant main effect for drug, $F(2, 6) = 13.25$, $p < 0.01$. However, the nonsignificant drug × session blocks interaction, $F(8, 24) = 0.50$, $p > 0.05$, indicated comparable rates of habituation of paired activity among the groups, while the session blocks main effect, $F(4, 24) = 7.70$, $p < 0.0005$, indicated that paired activity decreased over the session across groups. A Newman-Keuls posthoc analysis on the significant main effect for drug indicated that the NAL-1 and NAL-4 groups both had lower paired activity than the CONT group ($p < 0.01$ and $p < 0.02$, respectively) but were not significantly different from each other ($p > 0.05$). Intrasession analyses for other days of testing revealed nonsignificant effects for drug (all $p > 0.05$).

Figure 7 presents the mean distance (cm) per 10-s sampling interval between pairs of rats during paired locomotor activity testing for each of the 4 days of testing. The drug × days interaction, $F(6, 18) = 1.24$, $p > 0.05$, and the main effects for drug, $F(2, 6) = 2.78$, $p > 0.05$, and days, $F(3, 18) = 1.35$, $p > 0.05$, all proved nonsignificant. Intrasession analyses for each of the days of testing revealed nonsignificant main and interaction effects. Intersession and intrasession analyses for contacts revealed a similar pattern to distance score analyses; nonsignificant effects for drug were found in all analyses. Also, defecation frequency during paired testing was not significantly affected by naloxone.

DISCUSSION

The main findings of the present investigation may be summarized as follows: a) Naloxone did not significantly affect individual locomotor activity or its habituation; b) naloxone significantly decreased paired activity—the effect was nondose dependent and was stable over repeated days of testing; c) naloxone did not significantly affect habituation of paired activity; d) social attraction (distance and contact) during paired activity was not significantly affected by naloxone.

The present results serve to corroborate those of File (25) and DeRossett and Holtzman (20). File (25) found that Long-Evans hooded rats that received 2 mg/kg naloxone 30 min pretesting showed significantly decreased paired (but not individual) locomotor activity and reduced social interaction during a single 10-min trial under either conditions of task novelty or habituation in a photocell-type activity apparatus. DeRossett and Holtzman (20) reported significantly decreased paired locomotor activity in Sprague-Dawley rats that received 0.1, 1, and 10 mg/kg naloxone 30 min pretesting to three 30-min sessions (alternate days) under conditions of task novelty in a jiggle-type (electromagnetic) activity apparatus. However, Oka and Hosoya (60) found no significant effects on paired locomotor activity in Wistar rats that received 0.03125, 0.125, or 0.5 mg/kg naloxone immediately before a single 60-min trial under conditions of task novelty in a photocell-type activity apparatus. These results are puzzling especially because

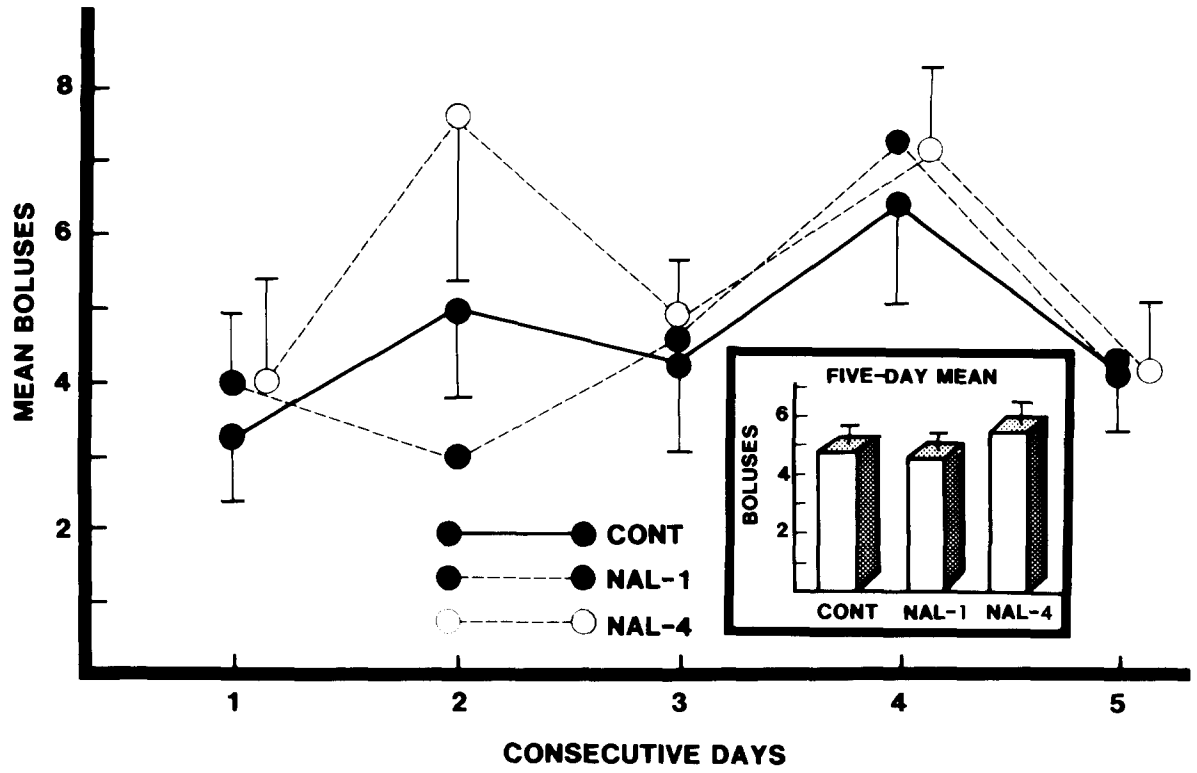

FIG. 4. Mean defecation (fecal bolus) frequency (±SEM) for each of the three groups on each of the 5 days of individual activity testing. Inset depicts the 5-day mean defecation frequency per day (±SEM) for each of the groups. CONT, control; NAL, naloxone.

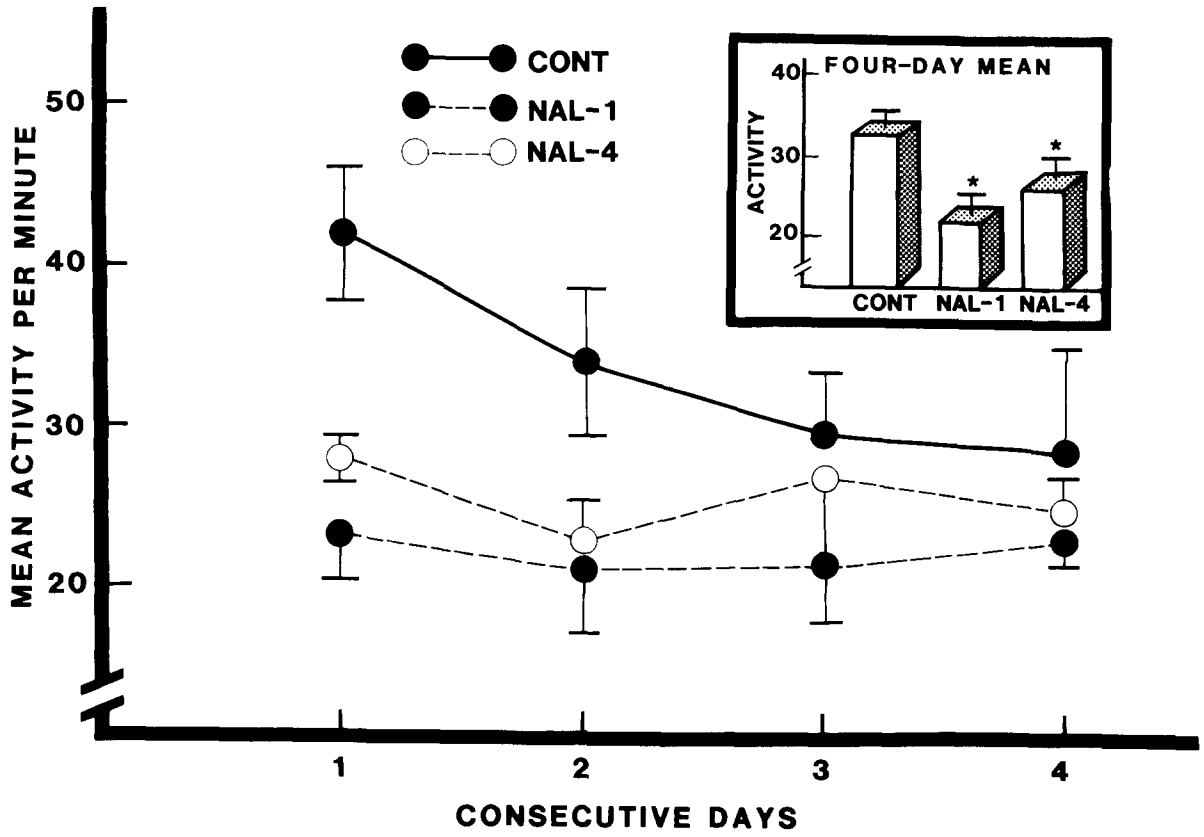

FIG. 5. Mean paired locomotor activity per minute (±SEM) for each of the three groups on each of the 4 days of testing. The inset depicits the 4-day mean activity per min (±SEM) for each of the groups; $*p < 0.01$, compared to control (CONT) group. NAL, naloxone.

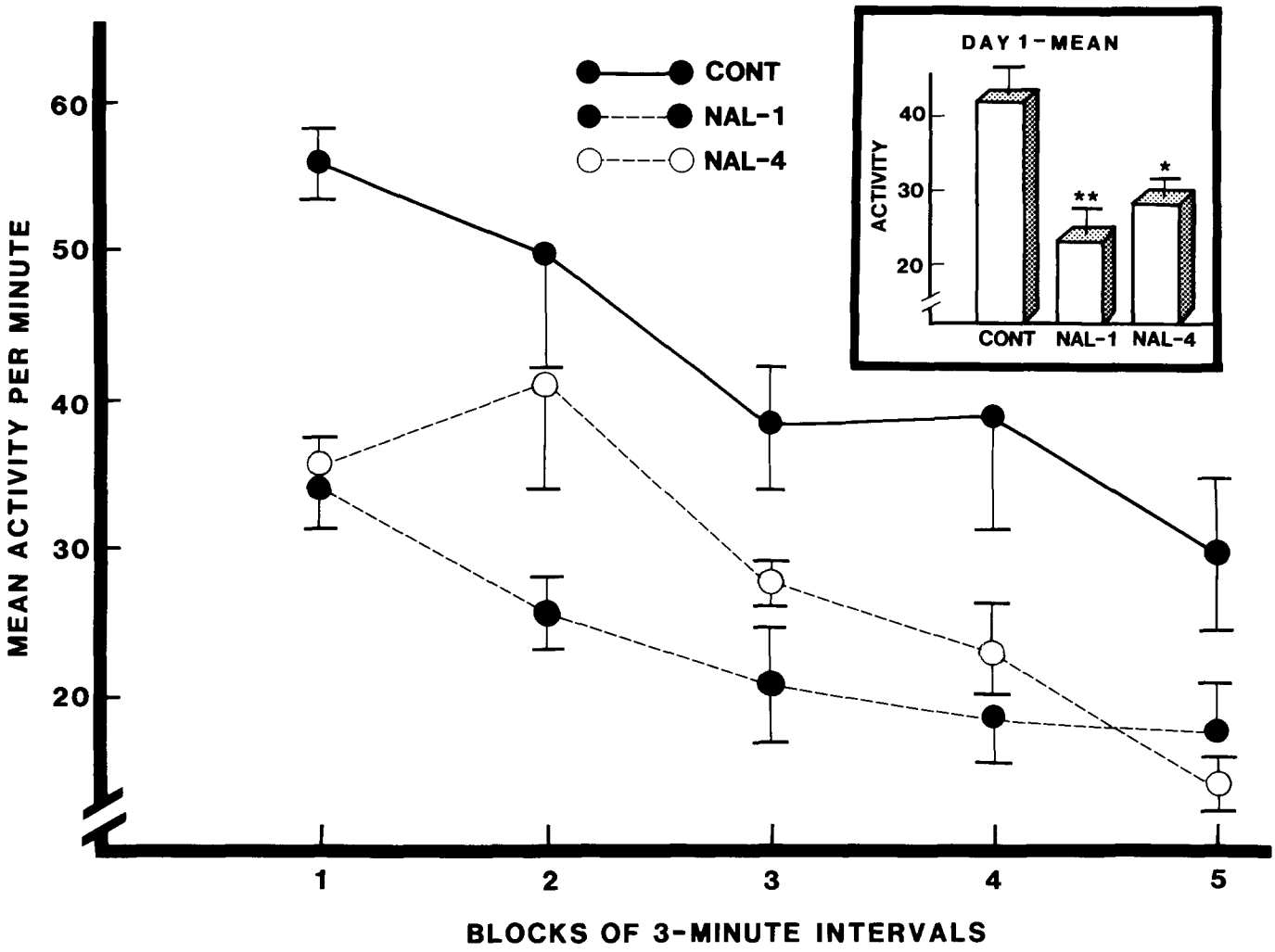

FIG. 6. Mean paired locomotor activity per minute (±SEM) for each of the three groups on each of the five blocks of 3-min intervals on day 1 of testing. The inset depicts the day 1 mean activity per min (±SEM) for each of the groups; $*p < 0.02$; $**p < 0.01$, compared to control (CONT) group. NAL, naloxone.

DeRossett and Holtzman (20) found effects at the 0.1-mg/kg dose; however, strain differences at this dose level may be a significant factor. Nonetheless, it is clear that naloxone at doses between 1 and 10 mg/kg can decrease paired locomotor activity under conditions of task novelty and also habituation.

The present results provide further insights into the nature of the paired activity deficit due to the repeated-measures design and analysis of intrasession results. Although DeRossett and Holtzman (20) used three testing sessions, data were reported collapsed across sessions, thereby prohibiting interpretation of possible habituatory effects of naloxone on activity. In the present study, naloxone produced a significant and rather stable, non-dose-dependent reduction of activity over the testing sessions, whereas the control group exhibited significantly higher activity than the two drug groups on the first day of testing (as shown by intrasession analysis) and then progressively decreased activity over days. Further, intrasession habituation does not appear to have been affected by naloxone, as demonstrated by the absence of significant interaction effects in these analyses. These findings suggest that opioid peptides probably do not play a critical role in habituation of locomotor activity. Further, the absence of significant behavioral change over the testing days would seem to limit the possibility of naloxone-induced receptor changes, that is, upregulatory supersensitivity, at these dosing parameters. Upregulatory changes following chronic but not acute infusion of naloxone have been observed previously in rats (82).

The present study also examined social interactions using procedures similar to those of Latane and Glass (49,50). Rats maintain closer than chance distance when tested in pairs and also make significantly more contacts with another rat than with an inanimate object or an anesthetized rat (50). Distance and contact ratings have proved useful in distinguishing the social behavior of septal-lesioned and amygdalectomized rats from sham-operated normals (43). The intersession and intrasession analyses of the present study provided no significant evidence that distance and contact frequency were altered by naloxone in paired rats. The sensitivity of the distance and contact measures may be dependent upon both the degree of social isolation prior to testing (70) and possibly apparatus

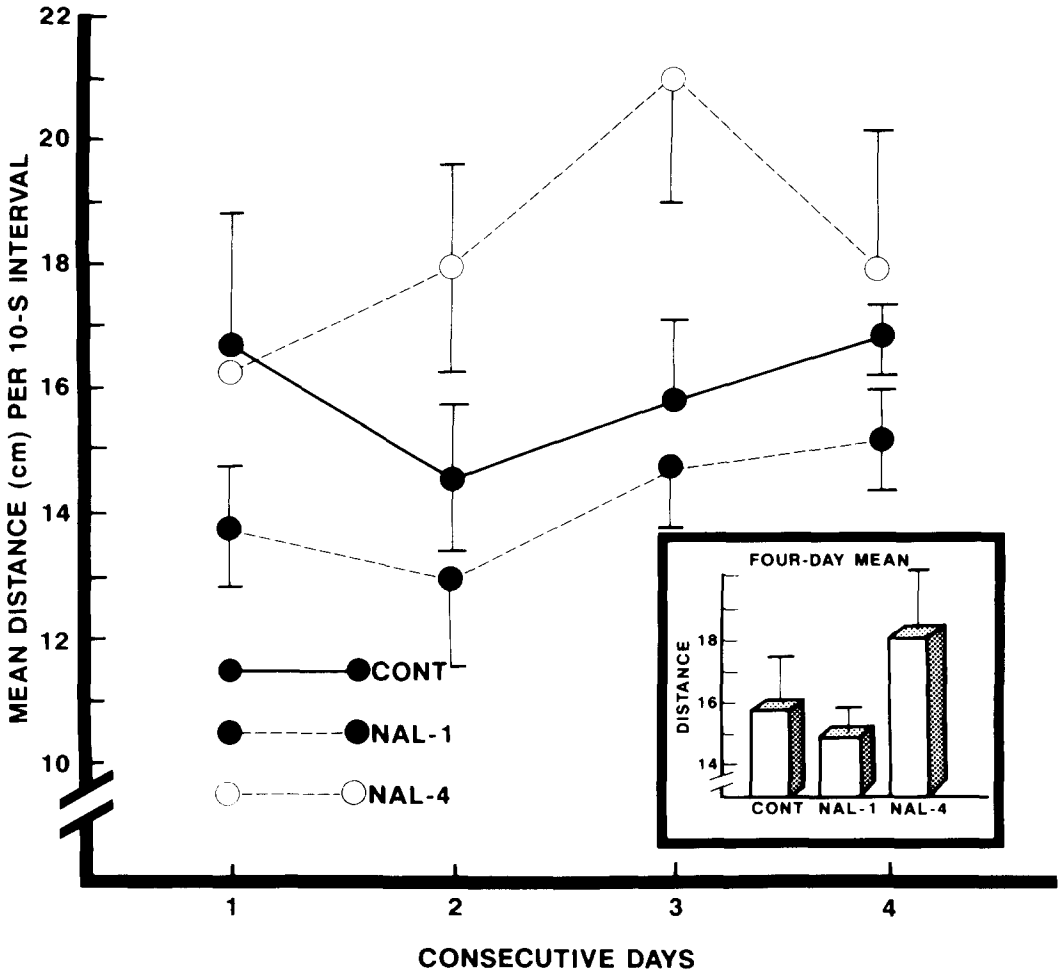

FIG. 7. Mean distance (cm) per 10-s sampling interval (±SEM) between pairs of rats during activity testing for each of the 4 days of testing. The inset depicts the 4-day mean distance per 10-s sampling interval (±SEM) for each of the groups. CONT, control; NAL, naloxone.

size. The previously cited studies have used large circular open fields with floor areas of 11,675 cm^2 (49,50), whereas the floor area of each of the apparatuses used in the present study was only 1,681 cm^2, a sevenfold difference. A smaller surface area may have contributed to reduced opportunity for pairs of rats to remain apart during testing.

Previous studies have not used distance and contact measures of social attraction to assess naloxone's effects on social behavior but have relied instead upon measures of observed social behaviors such as following responses; play fighting: kicking, boxing, pinning, etc.; and climbing over or under a partner (25). Both File (25) and Beatty and Costello (12) found that naloxone decreased both composite and individual social behaviors in rats. Niesink and Van Ree (59) found no significant effects of either 1 or 10 mg/kg naloxone on the composite social behavior of rats; however, this study used an unusually long injection to testing interval: 60 min. Although pharmacokinetic data indicate that the half-life of naloxone in blood and the brain is quite short, ca. 20–35 min, behavioral effects of naloxone have nonetheless been demonstrated to occur as long as 3 h postinjection at 5 mg/kg (IP) (71), which encompasses the testing duration used in the majority of studies, including the present one. Whether these long-duration effects are directly opioid mediated or not is still unresolved.

Pinning behavior, a type of play-fighting in which one rat is forced onto its dorsal surface in submission by another rat, has been reported to be reliably decreased by naloxone (70,71). Inspection of the videorecordings of the present study revealed that pinning behavior occurred with too low frequency for reliable statistical analysis. Presumably age differences are critical in meditating the response. Previously reported studies have used juvenile rats (ca. 20–30 days old) in assessing naloxone effects, whereas the present study used rats more than twice as old.

Beatty and Costello (12) proposed two hypotheses in their assessment of naloxone effects on play-fighting, and both of these hypotheses may be extended to the present discussion. First, naloxone may serve to block the reward value of social activity directly, consistent with the findings of Herman and Panksepp (34). In the present study, distance and contact measures of social behavior proved insensitive to the effects of a limited number of doses of naloxone. Second, naloxone may have general depressant effects on activity. This hypothesis is not supported by the present findings because naloxone only

decreased paired activity and not individual activity. In man, naloxone has been reported to produce electroencephalograph (EEG) slowing, that is, decreased α-activity, in a manner similar to low doses of the agonist morphine (77). δ-Slow-wave activity and total spectral power in the rat EEG have been found to be increased by bolus (IV) infusion of naloxone during periods of high arousal but not during periods of low arousal (29). Further, a single naloxone injection (1 mg/kg) produced similar effects when given prior to the onset of the dark phase of the photoperiod (a time of high arousal/activity) but not during light-phase periods of low arousal/activity (29). Thus, the presumably high arousal conditions engendered by paired testing may serve to promote endogenous opioid release; diminished arousal following opioid antagonism may contribute to reduced paired activity. However, as per individual activity testing, defecation frequency was not significantly affected by naloxone during paired activity testing in the present study. Although the frequency of social behaviors was not decreased by naloxone (due to the conditions of testing, e.g.) the intensity or vigor of these behaviors may have been reduced. Decreased intensity of social interactions could be reflected in reduced activity apparatus counts. In well-handled, docile, and exploratory rats, as employed in the present study, the novelty effects of individual activity box testing may have produced only modest increases in arousal or stress; this may have been the case, especially because the apparatus was small and the conditions of illumination low. Under such conditions, it would be predicted that opioid release would be relatively unchanged from basal levels. Thus, the present findings are in general consistent with the results of Grasing and Szeto (29).

An alternative explanation for the present results might be simply that enhanced activity during paired testing provided a more stable baseline for revealing the inhibitory effects of naloxone than individual testing did. However, activity counts (corrected for differential sample size) were quite comparable between paired activity testing and individual testing.

The molecular pharmacological substrates for the observed effects of naloxone on paired activity are still unclear. Naloxone is a nonspecific opioid antagonist; it fails to distinguish between receptor subtypes and specific anatomic loci (4). Selective antagonists could be used to evaluate if the behavioral effects are μ-, δ-, or κ-dependent. For example, the nonopioid δ-receptor antagonist naltrindole has high affinity, high selectivity, and potent central activity following peripheral administration (41,63). Naltrindole might profitably be used in assessing the role of the δ-receptor in mediating the behavioral effects. However, even the use of selective ligands may be complicated if multiple opioids interact at a given receptor site in vivo [for discussion, see (4)].

A number of studies have examined the relationship between opioid receptors, the mesolimbic and nigrostriatal dopamine (DA) pathways, and GABA in mediating the effects on individual locomotor activity. The nigrostriatal DA pathway is important in regulating motor responses, but as Vaccarino and Corrigall (75) have shown opioid antagonism using naltrexone (0.3, 1.0, and 3.0 μg) attenuated heroin-induced hyperactivity only when administered directly into the nucleus accumbens and not into the periaqueductal gray of the midbrain. Havemann and Kuschinsky (33) found that locomotor activity stimulation is dependent upon δ-receptor activation in the nucleus accumbens, whereas depressant effects on activity were related to μ-receptor activation. A participatory role for GABA in these effects is suggested by the research of Agmo and Tarasco (3). Naloxone (0.8 and 3.2 mg/kg) significantly decreased activity in rats tested during the fourth to eighth hour of the dark phase, a period of high activity and presumably high opioid release. The GABA antagonists bicuculline and picrotoxin reversed the locomotor activity deficits. It was suggested that naloxone-induced opioid disinhibition of GABAergic neurons, and not GABA antagonistic effects of naloxone, resulted in decreased DA activity (3). This suggestion is consistent with the finding that GABAergic basket cells in the hippocampus are inhibited by opioid receptors; stimulation of these opioid receptors results in a disinhibition of cholinergic pyramidal cells and excitatory effects (76). These latter effects are also interesting from another standpoint. The hippocampus has been assigned an important role in mediating exploratory responses (61), and opioid receptors may be important in modulating these behaviors.

It is clear that further research is needed and warranted to determine the role of the mesolimbic DA pathway and the nucleus accumbens, in particular, on paired locomotor activity and social behavior following peripheral and intracranial administration of selective opioid agonists and antagonists. In addition, the electrophysiological consequences of these manipulations need evaluation in conjunction with the behavioral responses being recorded in different situational contexts.

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