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Stress Produces Opioid-Like Effects on Investigatory Behavior[1]

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ARNSTEN, A. F. T., C. BERRIDGE AND D. S. SEGAL. *Stress produces opioid-like effects on investigatory behavior.* PHARMACOL BIOCHEM BEHAV 22(5) 803–809, 1985.—Stimulation of opioid systems with opiate agonists produce characteristic alterations in the investigatory behavior exhibited by rats in a novel environment. As numerous reports now indicate that opioid systems can be activated by exposure to stress, the following study examined whether exposure to stressors could produce opiate-like alterations of investigatory behavior. Naive rats were exposed to one of three stressors (restraint, tailpinch pressure, high intensity white noise) or to control procedures, and were observed in a novel environment. The frequency and duration of a wide range of behavioral activities were recorded. All three stressors were found to produce morphine-like alterations of investigatory behavior. The average time an animal spent per contact with stimuli in the environment was decreased significantly by stress, with greater reductions being associated with locomotor hypoactivity. The stress-induced reductions of investigatory behavior were blocked by very low doses of the opiate antagonist naloxone (0.1–0.25 mg/kg). These results are consistent with an activation of opioid systems underlying some of the changes in investigatory behavior produced by exposure to stress.

Stress Investigatory behavior Opioid systems

ACCUMULATING evidence indicates that exposure to many stressors can activate endogenous opioid systems. Stressors such as limb fracture, footshock, heat stress, analgesia testing, etherization, and restraint have been reported to decrease pituitary beta-endorphin-like immunoreactivity and to produce a parallel increase in plasma beta-endorphin and adrenocorticotropin hormone (ACTH) levels [6, 7, 14, 22, 23, 26]. One report related the degree of stress to the amount of beta-endorphin-like immunoreactivity released [22]. In a study of human stress responding, students were found to have increased plasma beta-endorphin-like immunoreactivity immediately before taking an exam compared to basal levels determined one week earlier [27]. Opioid peptides have also been shown to be released concurrently with epinephrine and norepinephrine from the adrenal medulla [29,32]. In brain, stressors have also been found to produce alterations in enkephalin levels [1,24] and in number of opiate binding sites [8,28].

The involvement of endogenous opioid systems in stress effects is further indicated by observations that exposure to stressors can produce opiate-like alterations in behavior which are reversed by the opiate antagonist naloxone (NAL). For example, inescapable shock was found to produce a NAL-reversible reduction of responsivity to noxious stimuli (analgesia) in both rats [19] and humans [31]. In accord with these observations, human subjects who were rated as "stressed" exhibited hyperalgesic response after administration of NAL [12]. In rats, exposure to a mild stressor, repeated tail pinch pressure has been reported to produce a NAL-reversible analgesia [13,21]. This stressor also induced eating which was blocked by NAL [21]. Prior exposure to high intensity white noise (95 dB) produced opiate-like locomotor activation and grooming which were reduced by opiate antagonists [25].

We have observed previously that opiates produce characteristic, dose-related alterations of investigatory behavior in a novel environment [5] which are not found with other classes of drug [30]. Low doses of morphine (0.5–1.0 mg/kg) significantly reduced the average time a rat spent per contact with stimuli in the environment, and tended to make the animals hyperactive. Higher doses of morphine (2.0–5.0 mg/kg) produced still greater reductions in the time spent per contact with stimuli, as well as decreasing the total duration of contact, and the number of contacts with stimuli, and reducing locomotor activity. These morphine effects were blocked completely by concomitant treatment with NAL. Naloxone by itself markedly prolonged the average time a rat spent per contact with stimuli; these effects on stimulus interaction were stereospecific and were accompanied by hypoactivity at higher doses (0.5–5.0 mg/kg). The findings that morphine decreases, while NAL prolongs, contact with stimuli, strongly suggest that endogenous opioid activation can result in reduced interaction with stimuli in the environment.

If exposure to stressors can activate endogenous opioid systems, stress could have morphine-like effects on inves-

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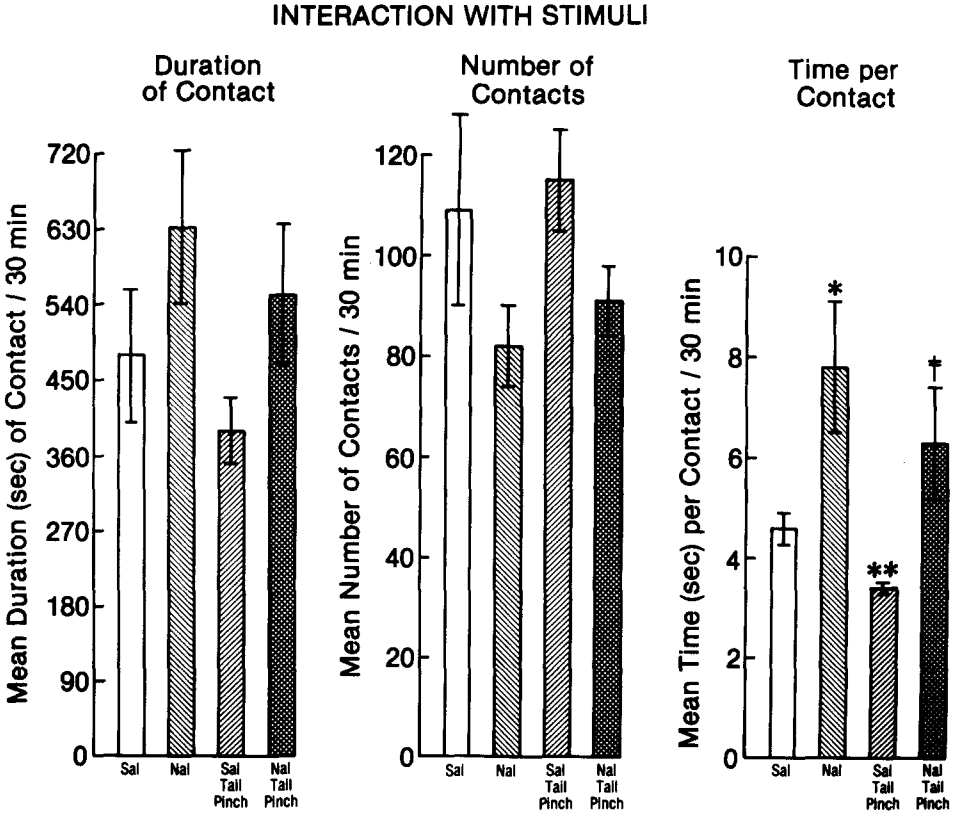

FIG. 1. The effects of repeated exposure to tailpinch on interaction with stimuli in the multicompartment chamber. Values represent duration of contact, mean number of contacts, and mean time per contact ±S.E.M. for the 30-min session; N=8. Significant differences from saline control values: *$p<0.05$; **$p<0.01$; from saline tailpinch values: †$p<0.05$; 2-ANOVA, simple main effects.

tigatory behavior which would be blocked by NAL. This hypothesis was tested in the following experiment in which separate groups of naive rats were exposed to restraint stress, tail-pinch pressure or control handling procedure prior to observation of their investigatory behavior. To address the possibility of stress pretreatment producing transient effects which would not sustain into the test session, an additional group of naive rats was exposed to high intensity white noise (100 dB) during behavioral observation. The three stressors all had been previously shown to either induce beta-endorphin release (restraint stress) or produce NAL-reversible changes in behavior (tailpinch pressure and loud noise). The effects of the three stressors on investigatory behavior were examined for comparison to morphine and for determination of NAL reversibility.

METHOD

Animals

Male Wistar rats (300–325 g) obtained from Charles River Breeding Laboratories (Boston, MA) were group housed under standard conditions for at least one week prior to the start of experimentation. Food and water were available ad lib and lighting was maintained on a 12-hr light-dark cycle (light 600–1800 hr). All rats were used only once in the experiment.

Behavioral Testing

The experimental chamber (79×79×84 cm) consisted of nine interconnecting compartments. Each compartment (25×25 cm) contained a wire mesh stimulus recessed in a 3-cm hole below the floor. Rats were monitored individually for a 30-min period between 1100 and 1700 hr by trained observers who were unaware of the treatment conditions. The frequency and duration of a wide spectrum of behavioral activities were indicated on a keyboard connected to a Sierra 3000 computer for storage and further processing. The following behavioral activities were recorded:

Measures of Physical Contact with Stimulus: (a) Duration of Contact; (b) Number of Contacts; (c) Time per Contact (duration/number of contacts).

Measures of Locomotor Activity: (a) Compartment Entries—Entry of head and both forelimbs into a compartment; (b) Rearings—Lifting of both forepaws from the floor; (c) Inactivity—Absence of bodily movement and sniffing.

Treatments

Tailpinch pressure. Mild tailpinch pressure was applied 1″ from the tip of the tail by means of a clamp according to the procedure described by Antelman and Caggiula [2]. Each of the 10 daily tailpinch sessions consisted of four 1-min appli-

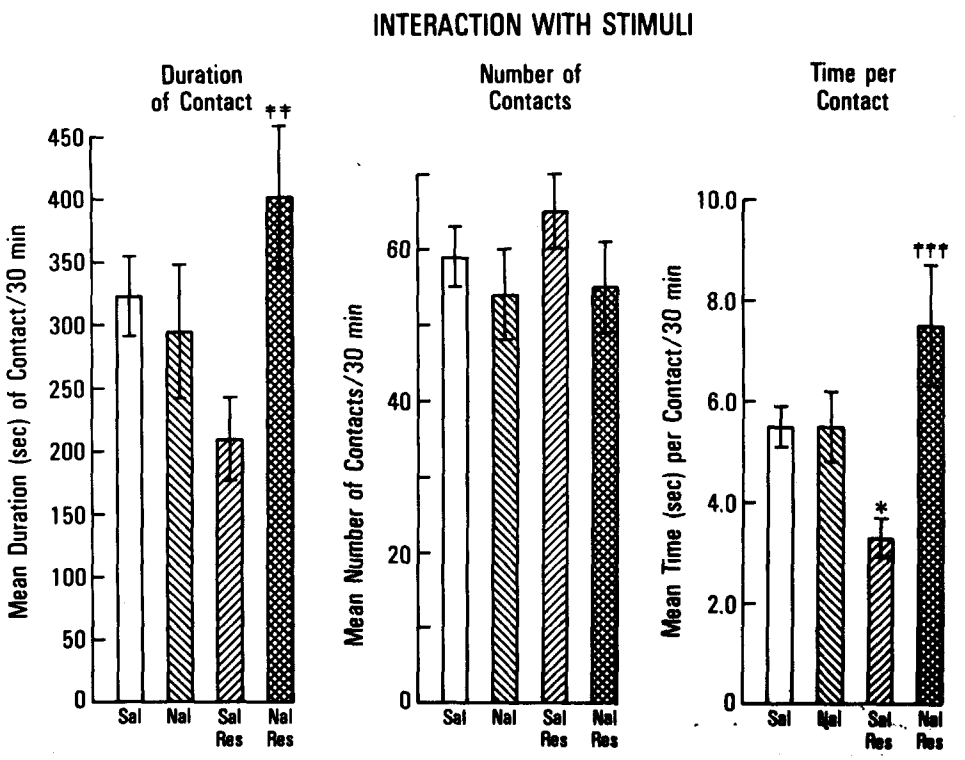

FIG. 2. The effects of repeated exposure to restraint stress on interaction with stimuli in the multicompartment chamber. Values represent duration of contact, mean number of contacts, and mean time per contact ±S.E.M. for the 30-min session; N=8. Significant differences from saline control values: *$p<0.05$; from saline restraint values: ††$p<0.02$; †††$p<0.01$; 2-ANOVA, simple main effects.

cations of the clamp separated by 15 min. As previously observed [2], tailpinch produced gnawing of food pellets. This response was pronounced in all animals by the fourth day. Control rats were treated similarly except that tailpinch pressure was not applied. Immediately after the tenth tailpinch session animals were injected with saline or NAL (0.25 mg/kg, subcutaneous, SC) and then placed directly into the multicompartment chamber for observation. As the 0.25 mg/kg dose was found to have effects on control behavior, the NAL dose was lowered in subsequent experiments.

Restraint stress. Animals were firmly wrapped in cloth towels for 60 min. After removal from restraint, rats were injected immediately with saline or NAL (0.1 mg/kg, SC) and placed in the multicompartment chamber for testing.

White noise. Rats were injected with saline or NAL (0.1 mg/kg, SC) 10 min prior to placement in the multicompartment chamber. The test session was conducted either under standard conditions or in the presence of white (wide band) noise maintained at 100 + 5 dB throughout the multicompartment chamber. This intensity was selected on the basis of results from stress experiments in humans in which exposure to white noise at less than 95 dB did not consistently alter behavior.

RESULTS

All three stressors produced morphine-like effects on stimulus interaction in the multicompartment chamber. Repeated tailpinch pressure and restraint stress significantly reduced the average time an animal spent per contact with the stimuli (Figs. 1 and 2). These reductions were associated with a non-significant decrease in the duration and an increase in the number of contacts made with the stimuli. Tailpinch and restraint stressed animals tended to be hyperactive (Figs. 3 and 4). This response profile resembled that produced by low doses of morphine (0.5–1.0 mg/kg) [5].

Like the other stressors, exposure to 100 dB white noise during the test session markedly reduced the average time an animal spent per contact with stimuli (Fig. 5). The duration and number of contacts with stimuli were also significantly reduced by noise. With regard to locomotor measures, noise-exposed animals made fewer compartment entries and spent more time inactive (Fig. 6). This response profile, i.e., a depression of both stimulus interaction and locomotor activity, is similar to that produced by intermediate doses of morphine (2.0–5.0 mg/kg) [5].

Naloxone completely reversed the reduction in stimulus interaction produced by exposure to these three stressors. In tailpinched animals, 0.25 mg/kg NAL significantly increased the time spent per contact with stimuli to control levels of responding (Fig. 1). In addition, the tailpinch-induced increase in compartment entries was significantly reduced by NAL (Fig. 3). Thus, NAL reversed both the decreased stimulus interaction and the tendency towards hyperactivity in tailpinch stressed rats. However, this dose of NAL produced a similar pattern of behavior in nonstressed controls (Figs. 1 and 3), thus complicating the interpretation of this interaction. A lower dose of NAL (0.1 mg/kg), which by itself had no significant effects on behavior in the multicompartment chamber, reversed the restraint stress response with respect to stimulus interaction (Fig. 2) and locomotor activity (Fig. 4).

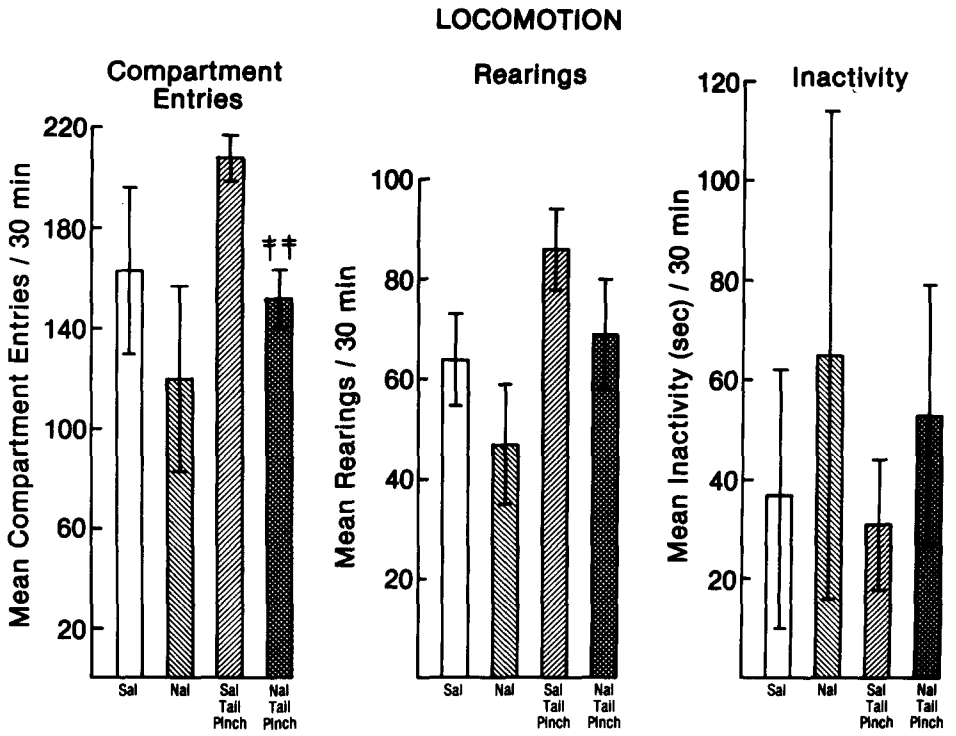

FIG. 3. The effects of repeated exposure to tailpinch on locomotion in the multicompartment chamber. Values represent mean compartment entries, mean rearings, and mean duration of inactivity ±S.E.M. for the 30-min session; N=8. Significant differences from saline tailpinch values: ††$p<0.02$; 2-ANOVA, simple main effects.

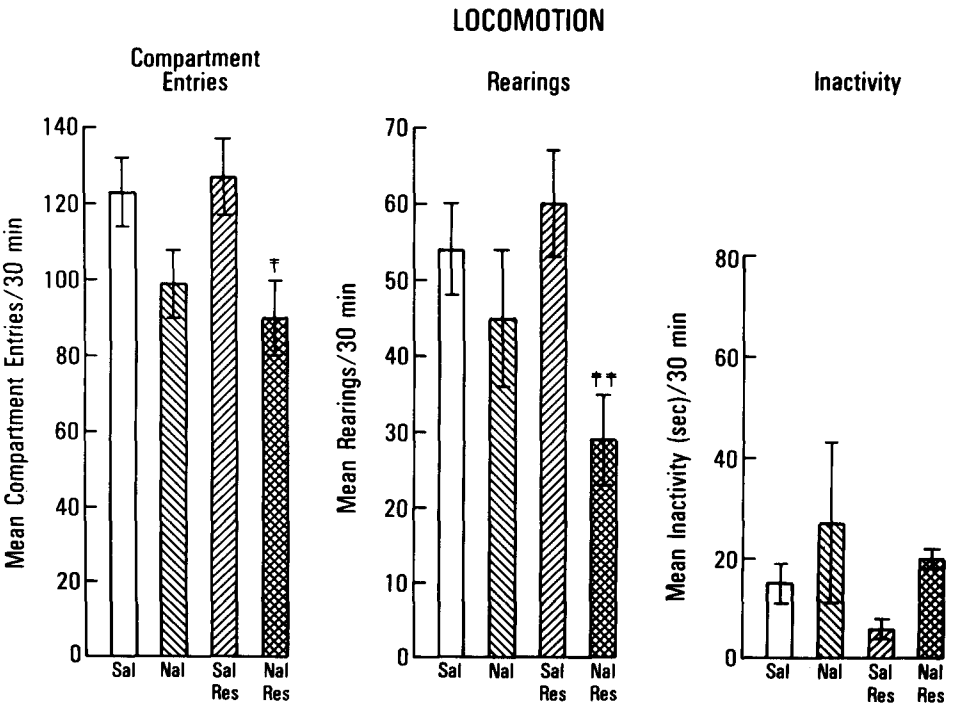

FIG. 4. The effects of repeated exposure to restraint stress on locomotion in the multicompartment chamber. Values represent mean compartment entries, mean rearings, and mean duration of inactivity ±S.E.M. for the 30-min session; N=8. Significant differences from saline restraint values: †$p<0.05$; ††$p<0.02$; 2-ANOVA, simple main effects.

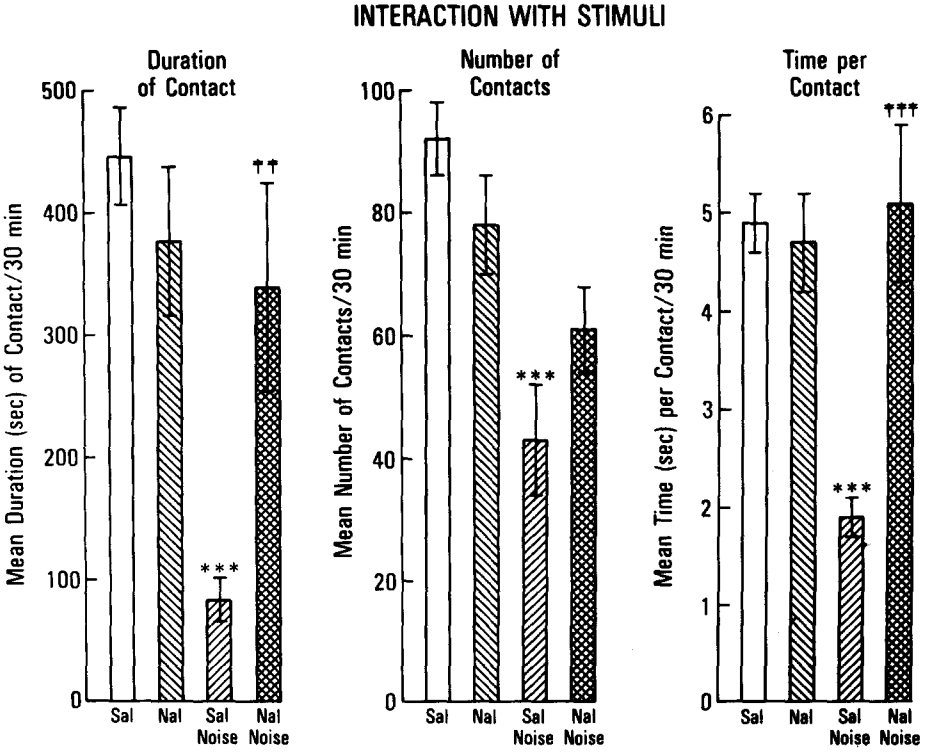

FIG. 5. The effects of repeated exposure to noise stress on interaction with stimuli in the multicompartment chamber. Values represent duration of contact, mean time per contact ±S.E.M. for the 30-min session; N=8. Significant differences from saline control values: ***$p<0.01$; from saline noise values: ††$p<0.02$; †††$p<0.01$; 2-ANOVA, simple main effects.

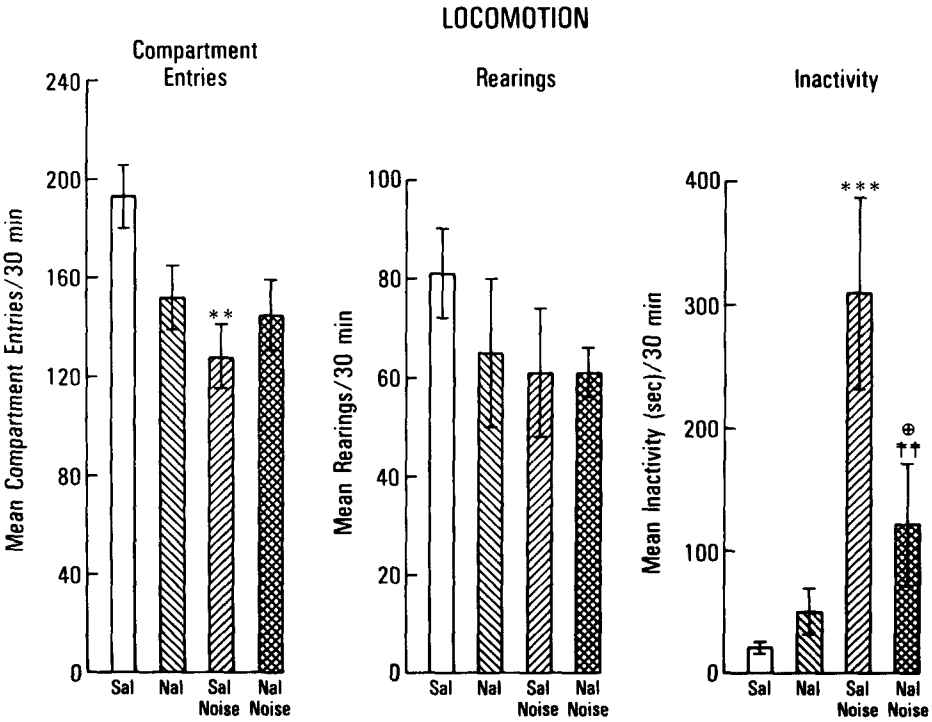

FIG. 6. The effects of repeated exposure to noise stress on locomotion in the multicompartment chamber. Values represent mean compartment entries, mean rearings, and mean duration of inactivity ±S.E.M. for the 30-min session; N=8. Significant differences from saline control values: **$p<0.02$; ***$p<0.01$; from saline noise values: ††0.005; 2-ANOVA, simple main effects. Significant differences from saline control values: †$p<0.01$; two-tailed t-test.

The reduction in stimulus interaction produced by 100 dB white noise also was blocked completely by pretreatment with 0.1 mg/kg NAL (Fig. 5). However, the diminished locomotor activity was not completely reversed by this dose of NAL (Fig. 6). It is possible that a higher NAL dose is necessary to block the locomotor depressant effects of loud noise stress, consistent with our observation that stimulus-directed behavior appears to be more sensitive to opioid alterations than is locomotion [3,4]. Alternatively, it is possible that components of the noise stress-induced locomotor depression involve nonopioid mechanisms which would be unaffected by opiate receptor antagonism.

DISCUSSION

In summary, all three stressors significantly reduced the time an animal spent per contact with stimuli in the multicompartment chamber. The smaller stess-induced decreases in stimulus interactions were associated with a tendency towards hyperactivity (i.e., tailpinch and restraint), while the larger reduction was accompanied by hypoactivity (noise). This behavioral response pattern corresponds to the dose response profile produced by morphine [5]. Low doses of NAL completely reversed the reductions in stimulus interaction induced by either morphine [5] or stress. The 0.1 mg/kg NAL dose had no significant effect on control behavior thus precluding an additive effect between stress and NAL. These data indicate an opioid involvement in stress-induced alterations of investigatory behavior.

Furthermore, the relative effects of the two doses of NAL suggest that different opioid systems may be activated as a function of the intensity or quality of the stressor. That is, the lower NAL dose (0.1 mg/kg) had potent effects on the behavior of stressed animals, but no effect on control animals. In contrast, the higher NAL dose (0.25 mg/kg) altered the behavior of both control and stressed groups. Thus, the lower dose was only effective under conditions where there was presumably more, not less, endogenous opioid activation. These results are not consistent with NAL acting as a competitive antagonist on a single opioid system. Rather, it is conceivable that multiple opioid mechanisms underlie regulation of stress-related stimulus-directed behavior, and that with increasing levels of stress, those opioid systems which are relatively more sensitive to NAL are activated. Since the doses of NAL used in this study have been reported to block the effects of opiate agonists which are relatively selective for the mu opioid system [10, 11, 20], it appears that moderated levels of stress and mu opiate agonists such as morphine may be affecting behavior through a common mechanism.

In humans, stress appears to produce impairment of selective attention as manifested by increased distractibility and the reduced ability to focus on relevant cues [18]. Particularly relevant to our results, exposure to 100 dB white noise has been shown to impair the ability to ignore distracting stimuli [9,15], especially during the performance of complex tasks [16]. Our results demonstrate that in rats this stressor impairs stimulus-directed behavior, an effect which appears to involve opiate receptor activation. It is conceivable, therefore, that a similar mechanism may underlie stress-induced impairment of selective attention in humans. An opioid role in the regulation of attention is consistent with reports that morphine impairs concentration [17], whereas NAL increases measures of selective attention in humans [4]. This evidence suggests that opiate receptor antagonists may be effective in alleviating deficits in selective attention associated with stress.

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