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ICV-CRH Alters Stress-Induced Freezing Behavior Without Affecting Pain Sensitivity[1]

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SHERMAN, J. E. AND N. H. KALIN. *ICV-CRH alters stress-induced freezing behavior without affecting pain sensitivity.* PHARMACOL BIOCHEM BEHAV **30**(4) 801–807, 1988.—Freezing is an adaptive response often induced by stressful, fear-eliciting stimuli. Three experiments with rats investigated the effects of intracerebroventricular (ICV) administration of corticotropin-releasing hormone (CRH) on freezing behavior and pain sensitivity. Experiments 1 and 3 demonstrated that ICV-CRH (300 ng) enhanced shock-elicited freezing. In Experiment 1, ICV-CRH also enhanced recovery from shock-elicited freezing, suggesting that the peptide has a biphasic effect. Experiments 2 and 3 established that CRH-induced freezing was not caused by heightened pain sensitivity. Interestingly, in Experiment 2, hot-plate exposure produced increased freezing that was attenuated by ICV-CRH. Thus, the direction of the ICV-CRH effect on freezing was found to depend on the nature of the stressor. These results suggest that endogenous CRH systems modulate stress-induced freezing.

Freezing behavior Intracerebroventricular (ICV) Corticotropin-releasing hormone (CRH) Pain sensitivity Stress

HYPOTHALAMIC corticotropin-releasing hormone (CRH) potently stimulates the release of ACTH, initiating the hormonal response to stress [32]. Evidence suggests that CRH also plays a role in organizing behavioral systems that complement its endocrine role in the stress response. CRH and its receptors are found in brainstem regions associated with behavioral arousal and anxiety [7, 23, 28], and CRH administered intracerebroventricularly (ICV) increases neuronal activity in these brain regions [33] and produces electroencephalographic indices of arousal [8]. In rats, ICV-CRH produces stress-related behavioral changes—namely, increased grooming and decreased eating [4, 5, 20, 22, 24–27]. In partially restrained rhesus monkeys it evokes vocalization, head-shaking, and struggling [18]. Administration of a CRH antagonist partially reverses stress-induced reduction in food intake [20] and the anxiolytic chlordiazepoxide attenuates anxiety-like effects of ICV-CRH in an operant conflict test [6]. There is also evidence that ICV-CRH potentiates the rat's acoustic startle response, a reflexive behavior sensitive to stress [29].

In a recent study [26], we observed the effects of ICV-CRH on behavior in novel and familiar test environments. CRH and the environmental manipulation independently influenced behavior; there was no evidence that CRH selectively enhanced novelty-induced behavioral changes. This finding suggests that if CRH systems modulate environmentally induced, stress-related behavior, the involvement of CRH systems may be limited by the nature of the stressor and/or class of behavior(s) elicited by the stressor.

The present study further examined the role of CRH in the rats behavioral response to a different environmental stress. The first experiment explored the effects of ICV-CRH on shock-elicited freezing. In the rat, footshock reliably elicits freezing, a response characterized by a period of crouching and immobility [1–3]. Evidence suggests that freezing is mediated by the expectation of danger [3,9], and when elicited by predators and aggressive conspecifics it presumably reduces the probability of attack. ICV-CRH has already been shown to potentiate the rat's acoustic startle response [29], a reflex potentiated by stress, which is highly correlated with freezing [21]. Consequently, we anticipated that ICV-CRH would enhance shock-elicited freezing. Because changes in pain sensitivity may alter responsiveness to footshock and subsequent freezing [12], we also examined the effects of ICV-CRH on both baseline and shock-elicited changes in pain sensitivity.

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METHOD

Experimental Subjects

Subjects for Experiments 1, 2, and 3 were 66, 28 and 36 Sprague-Dawley albino rats, respectively, weighing 180–200 g at the time of delivery from Sasco-King Laboratories (Oregon, WI). Rats were housed individually in standard stainless steel cages with unrestricted access to food and water. All procedures were conducted one to two weeks after rats arrived at our animal care facility, between 0900 and 1600 hr during the light component (0600–1800 hr) of the 24-hr light-dark cycle.

Apparatus and Drugs

All experimental manipulations were conducted in a separate room in which white noise (62 dB) was continuously present. Rats were transported to the test room in individual opaque plastic cages. The test chambers consisted of two identical chambers, 29.2 cm long by 25.4 cm wide by 27.3 cm high. The front and back walls and lids were made of clear Plexiglas, the side walls of stainless steel. A speaker 6.24 cm in diameter, attached behind the right side wall, provided white noise. Illumination was provided by a 28 V lamp mounted at the top and center of the Plexiglas rear wall and by two 2.5 V lamps mounted on the Plexiglas front wall spaced at equal intervals from the sides.

The chamber's floor was constructed of 16 stainless steel rods 6.35 mm in diameter, spaced 1.72 cm apart, center to center. These rods were wired to a Davis shock generator/scrambler that provided a one-second constant-voltage shock. The output of the shocker was in series with a 0.47-Mohm resistor to reduce variations in shock amplitude. The prescrambled output, measured with a digital meter, was 0.79 mA. Each shock chamber was housed in a Colbourn sound-attenuating chamber with the front door removed for observation. A fan mounted in the sound-attenuating chamber enhanced ventilation and provided additional background noise. Before each session, the drop pan was cleaned and supplied with fresh wood chips, and the shock chamber was wiped with a 1% solution of acetic acid. For Experiment 3, shock was generated by a constant current shock generator (Colbourn Instruments Model E1 3-08) set at either 0.375 or 0.50 mA; a test chamber was used during testing. In this experiment, a 1.5% solution of acetic acid was used for wiping the chamber.

Assessment of pain sensitivity was conducted with the hot plate apparatus (Colbourn Instruments) previously described [24]. This apparatus heated and circulated 51.4°C water under the surface of an aluminum plate. During preexposure sessions, the temperature of the water was 22–24°C.

CRH solutions were prepared as previously described [18], using synthetic rat CRH (Bachem Co., Torrance, CA). Vehicle was 0.9% sterile saline.

Procedures and Experimental Design

ICV cannula placement, drug administration, and verification of cannula placement followed procedures previously described [25]. The number of rats in each group represent those with correct cannula placements. To reduce potential novelty stress, each rat was placed in the shock chamber for 25 min the day before testing (the sixth postsurgery day). Rats in Experiment 2 were also given a 5-min preexposure to the cool hot-plate surface on this day. On the day of the study, rats were infused with vehicle or CRH and returned to their transport cages. Between 22 and 25 min after infusion, each rat was placed in the test chamber for 4 min of baseline behavioral rating (Table 1).

TABLE 1

BEHAVIORAL CATEGORIES RATED DURING BLOCKS OF BEHAVIORAL TESTING

Behavior Category	Definition
Freezing	Absence of all skeletal and vibrissae movement except that necessary for respiration
Grooming	Licking of body or fur, wiping of body or fur with paws, or scratching with paws
Walking	Locomotion in which movement of hind paws is a component
Self-gnawing*	Stereotyped mouthing of paws or tail
Rearing	Any raising of both forepaws off the grid floor that does not involve grooming or gnawing

*This behavior is typically directed at the paws. Inspection of paws after the test sessions occasionally revealed redness but no break in the skin. The rat characteristically crouches on its haunches while gnawing its paws, and this behavior is almost invariably followed by grooming.

In Experiment 1, three 1.0-sec footshocks (constant-voltage, 0.79 mA) were administered at 20-sec intervals, beginning 20 sec after the 4-min observation period. Control rats received vehicle or CRH treatments, but did not receive shocks. Ten sec after the third shock, or after an equivalent time had elapsed for the nonshocked controls, a 20-min behavioral observation period was begun. The number of rats receiving 0 (vehicle), 100, or 300 ng ICV-CRH in the shock-treated group was 9, 11 and 10, respectively; similarly, in the nonshock-treated group, the number of rats was 10, 13 and 13, respectively.

In Experiment 2, the effects of ICV-CRH on pain sensitivity were assessed before and after footshock. Rats received either 0 or 300 ng ICV-CRH (n=14 in each group), and immediately after the baseline period, all rats were removed from the shock chamber and placed on the hot-plate apparatus for 60 sec. Latency to first paw-lick or jump was recorded. Rats were then returned to the shock chamber for 4 min of behavioral ratings and were then given three footshocks as described in Experiment 1. A second hot-plate test was performed after the last shock was administered.

In Experiment 3, the effects of ICV-CRH were assessed at different intensities of footshock. Rats received either 0 or 300 ng ICV-CRH following the same procedure as described for Experiments 1 and 2. However, following the baseline period, rats were given either a 0.375 or 0.50 mA shock (constant current) as described above and were behaviorally rated for 12 minutes while they were in the shock chambers. Immediately thereafter, rats were placed on the hot-plate and latency to paw-lick or jump was recorded. The number of rats treated with vehicle that received the 0.375 or 0.50 mA shock was 11 and 8, respectively; similarly, the number of rats treated with 300 ng ICV-CRH that received the 0.375 or 0.50 mA shock was 10 and 7, respectively.

Behavioral rating following time-sampling protocols described previously [24]. At the end of each 10-sec interval of testing, one of the behaviors defined in Table 1 was scored.

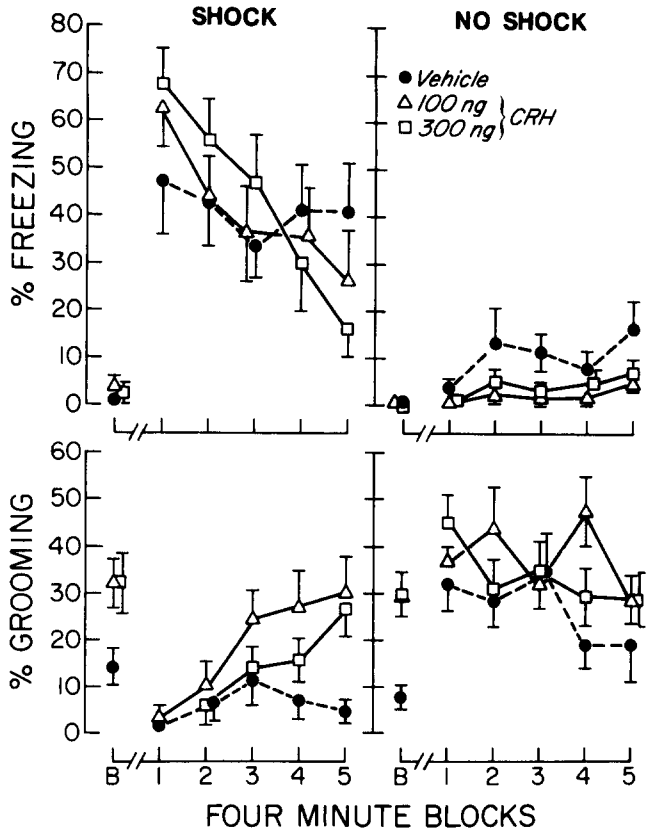

FIG. 1. Mean percent ±SEM of freezing and grooming scored for each 4-min block of testing at baseline (B) and postbaseline. Behavior was sampled at 10-sec intervals. In footshocked rats, CRH initially enhanced freezing and later facilitated recovery from freezing; it did not influence this behavior in nonshocked rats. CRH enhanced grooming at baseline. Grooming was suppressed by shock, but greater recovery of this behavior was observed in CRH-treated rats.

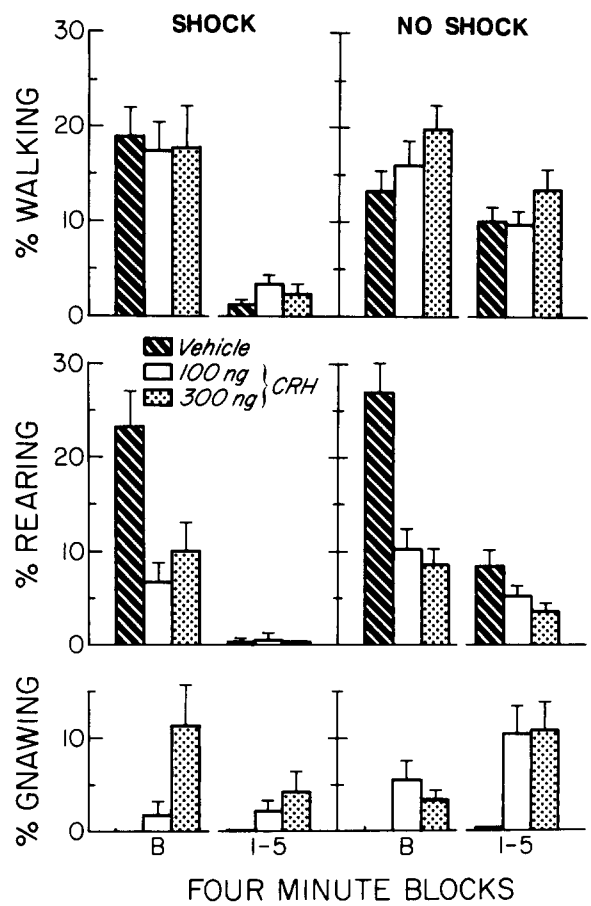

FIG. 2. Mean percent ±SEM of walking, rearing, and gnawing scored for the 4-min baseline (B) and postbaseline (averaged across the five 4-min blocks). Behavior was sampled at 10-sec intervals. Postbaseline data were averaged across blocks because there were no significant interactions of dose or shock condition with blocks. CRH attenuated rearing, enhanced gnawing, and was without effect on walking. Shock reduced all of these behaviors.

Behavior was scored by trained observers unaware of the drug treatment.

Statistical Analysis

Baseline data analysis was based on the percentage of total possible counts (24) scored during the 4-min test period for each dependent measure. In Experiment 1, percentage scores also were calculated for successive 4-min blocks during the 20-min postshock or nonshock observation period.

Analyses of treatment effects (CRH dose and shock) were conducted for each dependent behavior by analysis of variance (ANOVA). For Experiment 1 baseline, scores were submitted to a two-way ANOVA (0, 100, and 300 ng CRH) × shock (shock vs. no shock). Postshock scores were submitted to the same analysis including a repeated measures factor (five blocks of 4-min scores). For Experiment 2, effect of dose on each of the rated behaviors was compared at baseline (pre hot-plate) and after the first hot-plate test with a mixed design ANOVA; the between-S factor was dose (0 vs. 300 ng) and the within-S factor was time of testing (pre and post initial hot-plate test). The same design was employed for hot-plate latency, except that the within-S factor referred to pre- and postshock administration.

Analyses for Experiment 3 were conducted comparable to those for Experiment 1. For baseline, a two-way ANOVA (dose × shock intensity) was conducted for the behavioral ratings. After shock, a repeated measures factor was added (4-min blocks). The hot-plate test was analyzed via a two-way ANOVA. In all experiments, post-hoc statistical comparisons were conducted with the protected least significant difference test ($p<0.05$) [19].

RESULTS

Experiment 1

The effects of test block significantly interacted with dose and/or shock treatment for the freezing and grooming measures, but not for the other measures. Therefore data are presented for each block of testing for freezing and grooming (Fig. 1). For the remaining dependent measures, the effects of shock and dose are averaged across the five 4-min blocks of testing (Fig. 2).

Freezing. There was little evidence of spontaneous freezing at baseline (B) (Fig. 1). Shock dramatically increased freezing, and CRH produced a biphasic change in shock-

elicited freezing. Immediately after shock (Block 1), CRH enhanced freezing; but later (Block 5) it facilitated recovery from freezing. Nonshocked rats showed no evidence of CRH-enhanced freezing. In fact; there was a tendency for CRH to suppress spontaneous freezing.

At baseline, statistical analyses revealed no significant effects of dose, assignment to shock or nonshock condition, or interaction of these factors. However, for freezing behavior after shock, there was a significant main effect of shock, $F(1,60)=67.7$, $p<0.0001$ and an interaction of shock condition $\times$ dose $\times$ test block, $F(8,240)=2.66$, $p<0.01$. To characterize this three-way interaction, separate analyses of dose and block effects were conducted for the shock and no-shock conditions. A significant dose $\times$ block interaction was obtained for the shock condition, $F(8,240)=5.28$, $p<0.0001$, but no effect of dose or dose $\times$ block was obtained for the no-shock condition.

Further analysis of the dose $\times$ block interaction of the shock condition showed that rats receiving 300 ng of CRH displayed more freezing in Block 1 than rats receiving vehicle ($p<0.03$) but not more than rats receiving 100 ng of CRH. Rats treated with 100 ng of CRH displayed more freezing than vehicle-treated controls, but this difference was not statistically significant ($p=0.075$). At no other block did the 300-ng dose produce significantly more freezing than did vehicle. At block 5, shocked rats given 300 ng of CRH froze significantly less than vehicle-treated rats ($p<0.02$).

Grooming. CRH enhanced the frequency of preshock baseline grooming compared with vehicle (Fig. 1, bottom half). Shock suppressed both spontaneous and CRH-induced grooming. After shock, CRH-treated rats showed an enhanced recovery of grooming, especially at the 100-ng dosage. CRH increased grooming in nonshocked rats as well. As in shocked rats, the 100-ng dose produced the clearest effect.

Analysis of baseline data revealed significant effects of dose, $F(2,60)=11.41$, $p<0.0002$, but not assignment to shock condition or interaction of these two factors. Both CRH-treated groups displayed significantly more grooming than vehicle controls, $Fs(1,60)\geq 17.26$, $ps<0.002$, but they did not differ from one another.

Analysis of postbaseline grooming indicated a significant effect of shock, $F(1,60)=40.59$, $p<0.0001$, shock $\times$ block interaction, $F(4,240)=8.92$, $p<0.0001$, dose, $F(2,60)=4.77$, $p<0.02$, and dose $\times$ block interaction, $F(8,240)=2.22$, $p=0.03$. Because dose did not significantly interact with shock condition, dose effects were analyzed for each block of testing averaged over the shock and no-shock conditions. On blocks 1 through 3 there were no reliable differences between groups. On block 4, only rats receiving 100 ng of CRH groomed significantly more than vehicle-treated controls ($p<0.02$). On block 5, both groups of CRH-treated rats displayed more grooming than vehicle-treated controls ($p<0.02$) but did not differ from each other. These results show that both CRH and shock exerted effects on grooming although in opposite directions and not in interactive fashion.

Walking, rearing and gnawing. Results for walking, rearing and gnawing (Fig. 2) are averaged across the postshock test period because the effects of shock and CRH dose did not interact with block of testing. Baseline levels of walking were not influenced by dose, shock assignment, or interaction of these two factors. Shock suppressed walking, $F(1,60)=52.61$, $p<0.0001$, but there was no effect of CRH dose.

Baseline levels of rearing were not affected by shock group assignment or interaction of shock group with dose, although a main effect of dose was obtained, $F(2,60)=25.48$, $p<0.0001$. Both CRH-treated groups reared less then untreated controls, $Fs(1,60)\leq 37.03$, $ps<0.0001$, but there was no difference between the two dosage groups. Analysis of postshock data revealed a significant shock condition $\times$ dose interaction, $F(2,60)=3.62$, $p<0.04$. CRH depressed rearing in nonshocked rats, $F(2,60)=7.22$, $p<0.002$, but, because of the nearly complete suppression of rearing by shock, had no effect in shocked rats.

Vehicle-treated rats rarely engaged in self-gnawing, whereas CRH-treated rats displayed this behavior. At baseline, an overall effect of dose was obtained, $F(2,60)=6.20$, $p<0.004$. This effect was clearest for rats assigned to the shock treatment, $F(2,60)=4.86$, $p<0.02$ (Fig. 2). After baseline, there was less self-gnawing in shocked rats than in nonshocked rats, $F(1,60)=8.05$, $p<0.006$. There was an overall effect of CRH dose, $F(2,60)=6.42$, $p=0.003$; both doses of CRH produced more self-gnawing than vehicle, $Fs(1,60)\geq 8.12$, $ps<0.006$, but did not differ from each other.

TABLE 2

LATENCY RESPONSE TO HOT-PLATE TESTING (EXPERIMENT 2)

Drug Administered	Response Time, Seconds (Mean ± SEM) Before Shock	After Shock
CRH	13.14 ± 2.1	31.4 ± 3.5
Vehicle	16.00 ± 2.0	35.6 ± 3.3

Experiment 2

The most striking result of Experiment 1 was that ICV-CRH enhanced freezing elicited by footshock without influencing freezing in the absence of shock. Because freezing is directly related to shock intensity [10], heightened sensitivity to pain might account for the increased freezing observed in Experiment 1. Experiment 2 was designed to test whether the effects of CRH observed in Experiment 1 were mediated by changes in the rats' pain sensitivity. If ICV-CRH heightened responsiveness to pain by modulating the processing of nociceptive information, it may have enhanced freezing by increasing the perceived intensity of shock rather than by acting directly on the freezing response. Alternatively, while not influencing baseline sensitivity to pain per se, it is possible that CRH combined with shock resulted in enhanced sensitivity to pain. That is, CRH might have sensitized, or primed, processing of nociceptive information only after the first painful footshock was received, resulting in enhanced reactivity to subsequent shocks.

To test these possibilities, rats were given a hot-plate test for pain sensitivity at a time after CRH administration that corresponded to the receipt of shock in Experiment 1. Approximately 5 minutes later, rats were retested after receipt of three shocks. Table 2 presents the results of hot-plate testing. Statistical analyses of the effect of dose on latency to respond failed to reveal a significant main effect of dose or interaction of dose with time of testing (before or after shock). However, consistent with the known literature on stress-induced analgesia [30], there was a significant increase in latency to respond on the hot-plate after exposure to shock, $F(1,26)=78.84$, $p<0.0001$. Thus, the dose of CRH that

TABLE 3
OBSERVED BEHAVIORS BEFORE AND AFTER FIRST HOT-PLATE TEST (EXPERIMENT 2)

Drug Administered	Percent Observed Behavior (Mean ± SEM)				
	Freeze	Groom	Gnaw	Rear	Walk
		Before Hot-Plate Exposure			
CRH	0.0	22.9 ± 3.5	5.1 ± 2.3	11.3 ± 2.4	20.0 ± 2.2
Vehicle	0.0	11.3 ± 3.2	0.0	28.9 ± 3.6	14.8 ± 2.5
		After Hot-Plate Exposure			
CRH	12.0 ± 6.3	21.7 ± 3.4	4.2 ± 2.1	3.6 ± 1.3	14.0 ± 2.3
Vehicle	46.7 ± 10.1	11.9 ± 4.7	0.0	4.5 ± 1.4	9.3 ± 2.5

produced increased freezing in Experiment 1, failed to significantly alter pain sensitivity.

Table 3 presents the results of the behavioral ratings before the first hot plate test (time corresponding to baseline in Experiment 1), and the results averaged over a 4-min period after hot-plate testing but before shock treatment. Analysis of baseline alone revealed the same constellation of behavioral effects produced by 300 ng ICV-CRH at baseline in Experiment 1; i.e., increased grooming, $F(1,26)=5.93; p<0.02$, increased self-gnawing, $F(1,26)=4.94$, $p<0.04$, and decreased rearing, $F(1,26)=16.18$; $p<0.0001$, and no statistically significant effects on walking or freezing. However, analysis of the changes after the first hot-plate test as a function of dose revealed significant dose × hot-plate test interactions for rearing, $F(1,26)=12.58$; $p<0.002$, and freezing, $F(1,26)=8.01; p<0.009$. Subsequent comparisons revealed that after the first hot-plate test, both groups displayed a significant decrease in rearing relative to baseline ($p<0.03$). An unexpected finding was that vehicle-treated rats displayed significantly *more* freezing after the hot-plate test than rats given CRH, $F(1,26)=8.08; p<0.008$.

Experiment 3

Because CRH unexpectedly attenuated hot-plate-induced freezing in Experiment 2, we repeated the experimental design from Experiment 1 in Experiment 3. We assessed the effects of CRH on shock-induced freezing, followed by an assessment of pain sensitivity. This experiment provided the opportunity to replicate the enhancement of shock-elicited freezing by CRH found in Experiment 1 and to further investigate the absence of a CRH effect on pain sensitivity which was noted in Experiment 2. In addition, we used two shock levels to study whether intensity of shock is an important variable.

Figure 3 presents (a) the effects of CRH on freezing before and after footshock and (b) the latency of response to the hot-plate test. At baseline, there were no differences in freezing as a function of dose, assignment to shock conditions, or interaction of these two factors.

Analysis of the postshock freezing results revealed a significant effect of dose (CRH vs. vehicle), $F(1,32)=5.0$; $p<0.03$, and shock intensity (0.375 vs. 0.50 mA), $F(1,32)=7.32; p<0.01$. Both of these factors significantly interacted with block of testing, $F(\leq 2,64)>3.77; p<0.03$. As in Experiment 1, the effect of dose was clearest on the first block, whereas the overall effect of shock intensity was clearest on the third block (Fig. 3a). Although the results suggest a more robust effect of dose at the 0.375 mA shock, the interaction of dose and shock intensity was not statistically significant, $F(1,32)=2.29$; $p<0.14$; neither was the three-way interaction with block of testing. In contrast to Experiment 1, there was no evidence that ICV-CRH exerted a biphasic effect on freezing in Experiment 3. However, examination of the results of Experiment 1 (Fig. 1) show that enhanced recovery from freezing occurred at blocks 4 and 5. In Experiment 3, only 3 blocks of testing were conducted so that pain sensitivity could be assessed under conditions in which an enhancement of freezing were still evidenced. Results of experiments 1 and 3 were highly comparable for the first three blocks of testing.

The results of hot-plate testing (Fig. 3b) revealed that the higher shock intensity produced significantly longer latencies on the hot-plate test, $F(1,32)=5.49; p<0.025$. Although there was a tendency for CRH to decrease latency of response, this was not statistically significant, $F(1,32)=2.94$; $p<0.10$. There was no interaction of dose and shock intensity. The effects of CRH on grooming, gnawing, rearing, and walking were comparable to those observed in Experiments 1 and 2. Thus, this experiment confirmed that ICV-CRH enhances shock-elicited freezing without significantly altering sensitivity to pain.

DISCUSSION

Rats administered ICV-CRH (300 ng) displayed more shock-elicited freezing than rats administered vehicle (Experiments 1 and 3). Experiment 3 demonstrated that shock intensity may be an important variable. When shock resulted in too much freezing (as seen with 0.5 mA), we could not detect further enhancement by CRH. Of importance, ICV-CRH enhanced shock-elicited freezing without altering freezing under conditions of no shock (Experiment 1). Because magnitude of freezing is related to shock intensity [10], we tested the possibility that the enhancement of freezing by ICV-CRH was due to increased sensitivity to pain. Relative to controls, hot-plate tests given before shock (Experiment 2) or after shock (Experiment 2 and 3) failed to reveal statistically significant alterations in pain sensitivity. Thus, neither baseline levels of pain sensitivity or changes in pain sensitivity elicited by footshock were influenced by CRH.

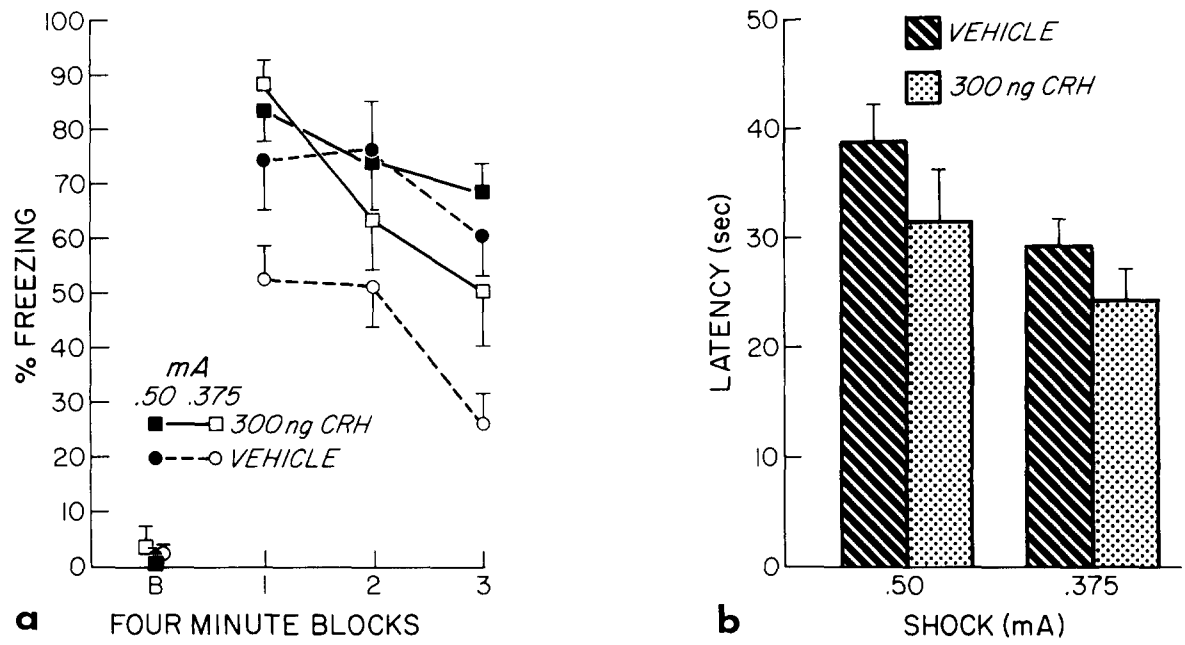

FIG. 3. Mean percent ±SEM of freezing averaged across baseline (4 min) and after shock (12 min). (a) Behavior sampled at 10-sec intervals. ICV-CRH had no effect on baseline freezing. (b) Mean latency of response ± SEM after hot plate exposure. The 0.5 mA shock yielded longer latencies than did 0.375 mA. ICV-CRH did not significantly affect latency to respond.

The absence of an effect on the hot-plate test does not seem to reflect the sensitivity of our measurement protocol because prior research using this protocol [24–26] has demonstrated it to be sensitive to experimental manipulations. In fact, the hot-plate test employed in Experiment 3 yielded statistically significant effects induced by small differences in shock intensity (0.375 vs. 0.5 mA).

It should be noted that in this study and previous studies conducted in our laboratory [25,26], ICV/CRH-treated rats consistently, although nonsignificantly, displayed shorter hot-plate and tail-flick test latencies than vehicle controls. The consistency of this finding suggests that nociceptive systems might be altered by ICV-CRH but not of the magnitude to yield significant effects in any single experiment in which conventional numbers of subjects are used. These considerations suggest that the enhancement of shock-elicited freezing demonstrated in Experiments and 1 and 3 are probably not mediated by alterations in nociceptive systems.

An unexpected finding in present study was that ICV-CRH attenuated freezing induced by exposure to the thermal stress of the hot-plate apparatus (Experiment 2). This contrasted with the enhancement of shock-elicited freezing seen in Experiments 1 and 3. This result is important and suggests that ICV-CRH dose not under all circumstances enhance stress-induced freezing. Factors that might account for the differences in the effects of CRH on freezing include (1) the shock was brief and intermittent (three 1-sec burst 20 sec apart), whereas the thermal stimulus was longer (up to 60 sec) and continuous; (2) the shock was not likely to be modified by the rats' behavior, whereas paw licking and jumping could produce immediate, short-lived relief from the thermal stimulus; and (3) shock was administered in the same environment as the behavioral testing, but the thermal stimulus was presented elsewhere, necessitating additional handling of the animal. Because testing was conducted in the same environment where shock was given, the rat may have developed a conditioned fear to the environment, which mediated the effects of shock [9]. It is of interest that Galina *et al.* showed that brief exposure to a hot-plate produces activation of the HPA system, transient analgesia [14,15], and decreased activity [16]. The reduction in activity may be mediated by ACTH, since it was blocked by hypophysectomy but not adrenalectomy, and in the hypophysectomized animals the behavior was reinstated by peripheral administration of ACTH[4–10]. Although ICV-CRH can increase plasma ACTH concentrations [18], the effects of ICV-CRH that we observed were opposite to those reported for ACTH[4–10].

Finally, in Experiment 1 ICV-CRH both enhanced shock-elicited freezing and facilitated recovery from freezing. This recovery may be attributable to the reemergence of CRH-induced grooming. Before shock was administered, CRH increased grooming, which was suppressed by shock and gradually returned to preshock levels by the fifth block of testing (Fig. 1). For CRH-treated rats, return of grooming behavior was associated temporally with recovery from freezing. The effect of ICV-CRH on freezing may be considered selective in that it occurred in the context of a relevant environmental demand (footshock), but not otherwise. In contrast, the facilitation of recovery from freezing probably results from the general tendency of ICV-CRH to increase grooming (a behavior incompatible with freezing), regardless of environmental demands. Consistent with this, we have observed that grooming is enhanced by ICV-CRH under other environmental conditions where grooming is normally suppressed [26].

Although increased grooming seemingly may reflect a maladaptive behavior in contexts where freezing would seem

more appropriate, it is possible that grooming is in fact an adaptive response to the heightened internal arousal induced by CRH. Evidence suggests that under conditions of heightened arousal, certain behaviors of animals and humans occur out of their normal context [17,31]. Such "displaced" behaviors reduce arousal, as indexed by endocrine and autonomic measures [17]. From this perspective, grooming may be a displacement activity that reduces CRH-induced arousal and may be inhibited, at least temporarily, by more immediate motivationally significant events in the environment such as footshock.

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