

Differential Impact of Audiogenic Stressors on Lewis and Fischer Rats: Behavioral, Neurochemical, and Endocrine Variations

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Exposure to intense noise can trigger a cascade of neuroendocrine events reminiscent of a stress response, including activation of the hypothalamic–pituitary–adrenocortical (HPA) axis. Using male Fischer and Lewis rats, which exhibit differences in their corticosterone response to stressors, this investigation assessed effects of acute noise exposure on neurochemical and neuroendocrine responses. In response to the noise exposure, Fischer rats displayed greater plasma adrenocorticotropin-releasing hormone (ACTH) and corticosterone responses than their Lewis counterparts. However, both strains responded with similar increases of plasma prolactin, suggesting that strain differences in the HPA response were not likely because of differences in noise perception. Post-mortem analyses revealed that noise exposure induced strain-dependent variations of corticotropin-releasing hormone (CRH) across several brain regions. These effects were evident irrespective of whether the rats were noise exposed in a familiar (home cage) or unfamiliar environment. *In vivo*, dynamic assessment of immunoreactive (ir)-CRH at the pituitary gland revealed that noise exposure elicited an immediate rise in ir-CRH among Fischer rats, relative to the delayed response in Lewis rats. Similarly, the rise in local interstitial corticosterone was more rapid and pronounced in Fischer rats. In contrast to these differences, ir-CRH released at the central nucleus of the amygdala (CeA) was gradual and protracted following noise exposure in both strains. Behaviorally, the Fischer rats displayed an active stress response, whereas the Lewis strain adopted freezing as a defensive style. The role of CRH in the genesis of the overall strain-dependent response to stressors is discussed.

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INTRODUCTION

In spite of its ubiquitous and intrusive nature, community noise has yet to be firmly established as a significant risk factor for the development of stress-related disease (WHO, 1999). Nevertheless, intense audio signals provoke neuroendocrine variations in humans and experimental animals reminiscent of those associated with stressor exposure, including increased circulating levels of adrenocorticotropin-releasing hormone (ACTH), glucocorticoids, and enhanced secretion of catecholamines, reflecting autonomic activation (Van Raaij *et al*, 1997; Windle *et al*, 1997). As hypothalamic corticotropin-releasing hormone (CRH) is the

primary secretagogue for pituitary ACTH, which in turn evokes the release of adrenal glucocorticoids, it seems likely that CRH contributes to the neuroendocrine effects of noise.

Several studies have demonstrated marked strain differences in response to physical and/or psychological stressors (Anisman *et al*, 1998; Dhabhar *et al*, 1997; Sternberg *et al*, 1992). In this respect, the Lewis rat has been extensively studied as a unique strain demonstrating susceptibility to various inflammatory conditions, including streptococcal cell wall-induced arthritis (Dhabhar *et al*, 1995; Sternberg *et al*, 1989a) and encephalomyelitis (Mason *et al*, 1990). In contrast, the histocompatible Fischer strain is relatively resistant to the deleterious effects of stressor exposure (Dhabhar *et al*, 1993; Moncek *et al*, 2001; Sternberg *et al*, 1989b). In the current study we assessed the neuroendocrine, neurochemical, and behavioral responses to an acute audiogenic stressor in these two rat strains.

In addition to hypothalamic control over the activation of the hypothalamic–pituitary–adrenocortical (HPA) axis, considerable evidence sustains the notion that extrahypothalamic structures, such as the amygdala, may also

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regulate the activation of the HPA axis. Indeed, lesions to the CeA block the HPA responses to acoustic stimuli (Feldman *et al*, 1994) and restraint stressors (Van de Kar *et al*, 1991). Furthermore, the stressor-induced increase of CRH mRNA and release at the CeA (Hsu *et al*, 1998; Merali *et al*, 1998; Pich *et al*, 1995; Richter *et al*, 2000) suggests that previously observed HPA, autonomic, and behavioral responses to stressors in the Fischer and Lewis rats (Glowa *et al*, 1991; Sternberg *et al*, 1989a, 1992) might be associated with differences in the release dynamics of CRH at the CeA. In the present investigation, we determined whether their differential sensitivity would be restricted to HPA factors and/or include extrahypothalamic sites. In addition to immediate effects, we also examined whether noise exposure would induce relatively protracted changes of neuroendocrine and neuropeptide activity in these strains, and whether such effects would be influenced by the environmental context in which the stressor was applied. Although stressor effects are usually transient (Anisman *et al*, 1991), the duration of these effects can be influenced by contextual cues, in this instance administering the stressor in an animal's home cage, as opposed to a similar but novel cage.

In the present investigation, we assessed immunoreactive (ir)-CRH levels in post-mortem brain micropunched tissue of Lewis and Fischer rats that had been exposed to noise. Although micropunch results provide useful information regarding which brain regions may be involved in the stress response, they preclude conclusive interpretations regarding the temporal dynamics associated with the challenge. Therefore, we complemented the post-mortem studies with experiments that assessed interstitial levels using *in vivo* microdialysis and push-pull perfusion, hence providing a more dynamic and clearer mechanistic perspective.

MATERIALS AND METHODS

The Effect of Noise Exposure on Brain ir-CRH and Plasma Hormone Levels

Animals. Male Lewis and Fischer rats (Charles River, Canada), weighing 300–350 g were used. The animals were housed individually in standard rodent cages ($36 \times 31 \times 17 \text{ cm}^3$) with a 12:12 h reversed light-dark cycle (lights off 07:00 h) and temperature maintained at 23–24°C with 60% relative humidity. Animals had *ad libitum* access to Purina™ rat chow and tap water. All experimental procedures met the guidelines on the ethical treatment of animal subjects in experimental research set forth by the University of Ottawa and Health Canada.

Noise exposure and blood collection. To minimize the effects of transporting animals from the housing room to the test area, rats were acclimated to the regimen of daily transportation for 3 weeks prior to the experiment being initiated. On test days (between 08:00 and 12:00 h), animals were transported (in their home cages), from the housing area to a separate room where they were immediately exposed to a 15-min burst of 90 dB continuous white noise, band-limited from 80 Hz to 20 kHz, and equalized in 1/3-octave bands within the cage. Noise was delivered through two professional loud speakers (JBL4892) suspended ~40 cm above the testing cages. Sound pressure level

varied slightly, depending on the position in the cage and the arrangement of bedding chips. Based on measurements from 10 positions within the cage and variations in bedding distribution, overall the standard deviation for the unweighted average sound level was ± 2 dB, and in individual 1/3-octave bands it was ± 4 dB.

Animals were killed (by decapitation) either immediately (~30 s) after the stressor session (ie 15 min after noise/noise onset; $n=9$ /strain/condition) or were returned to their home cages, and then killed 1 h later ($n=9$ /strain-noise; $n=7$ /strain-no noise). Control rats were treated identically, except that the noise exposure was not administered. Brains and trunk blood were collected for radioimmunoassay (RIA) of peptide and hormone content (Palkovits and Brownstein, 1988).

The Impact of Environmental Cues on Protracted Changes of Brain ir-CRH and Plasma Hormone Levels 24 h after Noise Exposure

Animals and procedure. Rats of each strain were brought to the test room, where half of the rats remained in their home cages ($n=10$ /strain), while the remaining rats were placed in a similar cage that contained fresh bedding (novel cage, $n=10$ /strain). The rats were then exposed to the noise stressor as described earlier. Animals exposed to noise in a novel cage were returned to their home cages immediately after noise exposure and all rats were returned to the housing room. Thus, some rats were maintained in the same environment as that in which they were ordinarily housed, while the remaining rats were exposed to noise in a novel environment and then returned to their home cages before returning to the housing room. The following day (24 h after noise termination) rats were transported to the necropsy area where they were killed immediately and brains and trunk blood taken for later neurochemical and neuroendocrine determinations.

Tissue collection. Following decapitation, brains were rapidly removed and placed into a brain matrix (McIntyre, Carleton University, ON) with coronal planes corresponding to the rat brain atlas of Paxinos and Watson (1986). Serial coronal sections (1.5–2.0 mm) were obtained and placed in ice-cold 0.1 M phosphate-buffered saline and a total of 11 discrete brain nuclei known to be sensitive to the effects of stressors were identified and micropunched/dissected. The selected hypothalamic areas included the paraventricular nucleus of the hypothalamus (PVN), and the median eminence-arcuate complex (Me/Arc). In addition, the following extrahypothalamic regions were taken: paraventricular thalamic nucleus (PV), prefrontal cortex (PFC), nucleus accumbens (nAcb), caudate (Cau), dorsal hippocampus (dHipp), central nucleus of the amygdala (CeA), basal lateral nucleus of the amygdala (BLA), medial nucleus of the amygdala (MeA), and locus coeruleus (LC). All nuclei were bilaterally micropunched using a customized 1 mm punch, except for the Me/Arc and PV, which were removed with a single punch, while the dHipp and PFC were microdissected free-hand (see Figure 1). Brain tissue was placed in clean 1.5 ml centrifuge tubes on dry ice and stored at -80°C until processing (within 4 weeks). All samples were collected between 07:00 and 13:00 h.

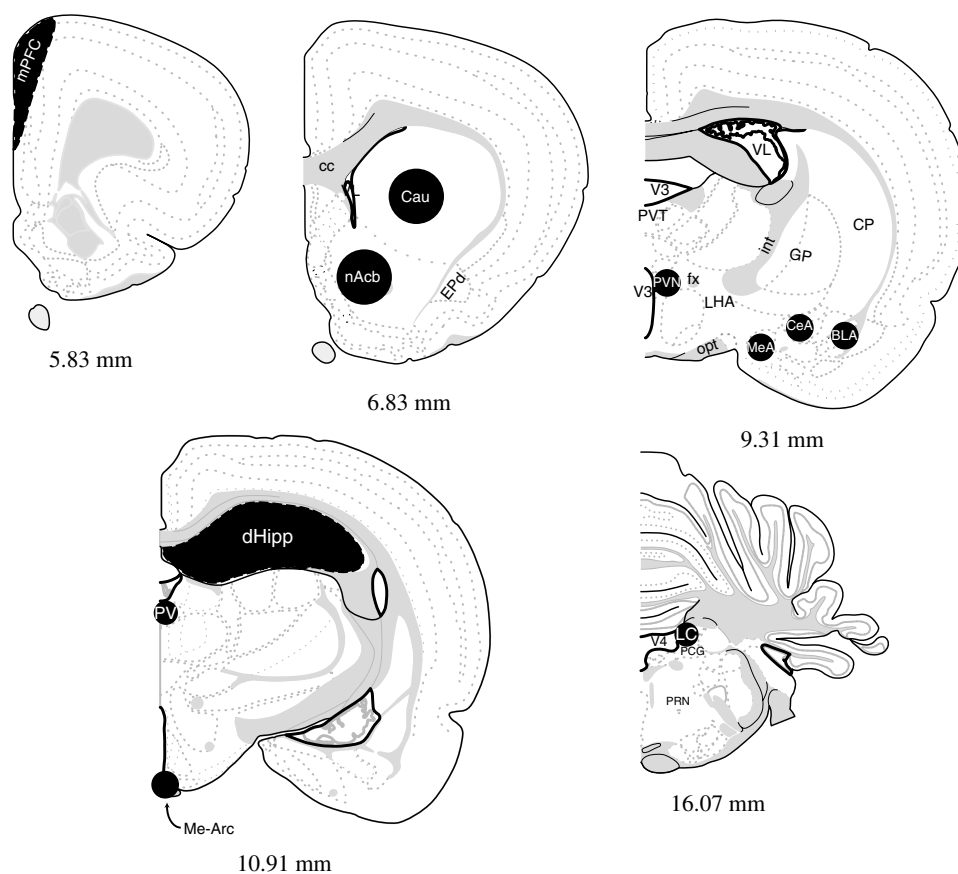


Figure 1 Fresh brains were blocked in an acrylic rat brain matrix and 1.5–2.0 mm coronal sections taken. A series of discrete brain regions were bilaterally punched or free-hand dissected according to the atlas of Paxinos and Watson (1986). Selected brain regions are demarcated with solid shading at the anterior pole of each site. Distances from bregma are indicated below each section. Abbreviations: BLA, basal lateral amygdala; CeA, central amygdala; Cau, caudate; dHipp, dorsal hippocampus; MeA, medial amygdala; Me-Arc, median eminence arcuate complex; nAcb, nucleus accumbens; PFC, prefrontal cortex; PV, paraventricular thalamic nucleus; PVN, paraventricular nucleus of the hypothalamus; LC, locus coeruleus.

Tissue processing. Tissue punches were sonicated (VirSonic 60, Virtus, USA) in 0.5–1.0 ml of Iscove's modified Dulbecco's buffer (GIBCO) containing 0.5% fetal calf serum and a cocktail enzyme inhibitors (in mM: 100 amino-*n*-caproic acid, 10 EDTA, 5 benzamidine-HCl, and 0.2 phenylmethylsulfonyl fluoride) for 10 s. A portion of the homogenate (25 μ l) was then withdrawn for protein determination, and the remainder of the homogenate was centrifuged at 13 000 rpm at room temperature for 9 min. Supernatant was aliquoted into 0.6 ml vials and stored at -80°C for subsequent peptide level determination. Protein assays were performed using bicinchoninic acid with a protein analysis kit (Pierce Scientific, Brockville, ON) using the microtiter plate method.

Plasma ACTH, corticosterone, and prolactin detection. Trunk blood was collected into 5.0 ml glass tubes containing EDTA (Fischer Scientific Ltd, ON), centrifuged at 1500 rpm, and the plasma aliquoted into 1.5 ml vials and stored at -80°C . Plasma ACTH, corticosterone, and prolactin levels were determined, in duplicate, using commercial RIA kits (ICN Pharmaceuticals Inc., Costa Mesa, CA). These assays yielded intra- and interassay variations of less than 10%. The quantification of corticosterone in the perfusates was accomplished using a modification of the commercially available corticosterone RIA protocol

designed to enhance the sensitivity of the assay (Bonsall *et al*, 1996).

The Effect of Noise Exposure on the Release of ir-CRH at the CeA

Animals and surgical procedure. Male Lewis ($n=8$) and Fischer ($n=9$) rats were maintained as described above. Rats were anesthetized (60 mg/kg i.p. pentobarbital) and stereotactically implanted with a 20-gauge stainless-steel guide cannula containing a removable 24-gauge stainless-steel obturator aimed at the dorsal aspect of the CeA. The placement coordinates (Paxinos and Watson, 1986) with skull level were anteroposterior, -2.3 mm; dorsoventral, -7.0 mm; and lateral, ± 4.2 mm. The guide cannula protruding from a custom-manufactured Delrin[®] pedestal was secured to the skull with four stainless-steel screws and dental cement. After surgical recovery (at least 7 days), animals were transferred to individual testing chambers and allowed to acclimate for 48 h before testing. The testing chambers comprised Plexiglas cages ($25 \times 35 \times 34$ cm³) with a stainless-steel grid floor.

In vivo microdialysis. Approximately 24 h prior to testing, rats were lightly restrained manually (~ 20 s), and the obturator within the guide cannula was replaced with a

concentric microdialysis probe, consisting of a 2.0 mm regenerated cellulose active membrane (250 μm outer diameter; 6000 molecular weight cutoff; Spectrum Laboratories, CA). The probe was partially inserted so that the active membrane remained within the guide cannula shaft. On the following day, the anchor screw securing the probe was loosened and the probe was gently inserted into the CeA of the freely behaving animals. Each probe was resecured with the anchor screw and connected via polyethylene tubing (Intermedic, Clay Adams, NJ) to a liquid swivel (Instech Laboratories, PA) and a 2.5 ml gastight infusion syringe attached to a pump (model 22, Harvard Apparatus, MA). Microdialysis probes were perfused at 3 $\mu\text{l}/\text{min}$ with filtered Krebs-Ringer phosphate (KRB) solution consisting of (in mM): 2.7 K^+ , 145 Na^+ , 1.35 Ca^{2+} , 1.0 Mg^{2+} , 150 Cl^- , 0.05 ascorbate, pH 7.4 (Moghaddam and Bunney, 1989), and BSA (0.1%). After the probe insertion, a 60-min stabilization period was allowed, which represented a time interval previously demonstrated to permit stabilization of peptide release (Merali *et al*, 2001). Subsequently, dialysate samples were collected every 20 min ($\sim 60 \mu\text{l}$) and immediately frozen on dry ice and stored at -80°C for 2 weeks until RIA analyses. The efficiency of *in vitro* peptide recovery by the microdialysis probes averaged $3.3 \pm 0.6\%$ (details previously described by Merali *et al*, 1998).

Noise exposure. Following the collection of six baseline dialysate samples, a single 15-min burst of 90 dB continuous white noise (as described previously) was delivered via speakers (Voxtek 85 S, 8 Ω 0.5 W) affixed to the top of the test cage. Following noise exposure, 12 postnoise dialysate samples were collected.

The Effect of Noise Exposure on ir-CRH and Corticosterone Availability at the Anterior Pituitary

Animals and surgical procedure. Male Lewis ($n=9$) and Fischer ($n=12$) rats, maintained as previously described, were anesthetized with pentobarbital (60 mg/kg i.p.) and stereotaxically implanted with a 20-gauge custom-made stainless-steel guide cannula containing a removable 24-gauge stainless-steel obturator aimed at the anterior lobe of the pituitary gland. The placement coordinates (Paxinos and Watson, 1986) with skull level were anteroposterior, -5.3 mm; dorsoventral, -11.0 mm; and lateral, ± 0.9 mm. The guide cannula was secured to the skull with four stainless-steel screws and dental cement. After surgical recovery (at least 7 days), animals were transferred to individual testing chambers and allowed to acclimate for 24 h before being placed individually in the noise room, where they remained for another 24 h prior to testing. Rats were tested individually in test chambers identical to those used in the microdialysis experiments.

In vivo push-pull perfusion. Push-pull probes were custom manufactured using a 24-gauge stainless-steel outer cannula as the 'pull' aspect of the probe, while a concentric silica tubing (75 μm ID \times 150 μm OD, Polymicro Technologies, AZ) protruding 0.4 mm beyond the outer cannula constituted the 'push' element of the probe. On the morning of testing the probes were calibrated for 20 min *ex vivo* to

ensure the stability of the push-pull dynamics. The obturator was then carefully removed from the guide cannula of freely moving rats and replaced with the probe so that the silica tubing entered into the anterior lobe of the pituitary gland (permitting a free exchange between the KRB and the interstitial fluid). Push-pull probes were secured with polyethylene tubing to a dual channel liquid swivel (Instech Laboratories, PA) and then connected to separate peristaltic pumps (Minipulse 3, Gilson, WI) that were used to perfuse the probes with KRB (as described above). Typically, the 5-min sampling interval yielded 110 μl of perfusate, which was aliquoted into two separate sample vials. These were immediately placed on dry ice and later stored at -80°C until CRH and corticosterone assays were conducted, 2–3 weeks later.

Noise exposure. Following the insertion of the probe 12 baseline samples were collected (over a 1 h period), followed by a 15-min burst of 90 dB continuous white noise identical to that described in the previous experiments. In all, three 5 min samples were collected during noise exposure followed by an additional 12 postnoise samples.

Strain-Dependent Behavioral Response to Noise Exposure

Behavioral monitoring. During the push-pull experiment, the occurrence of various behaviors was assessed using a time-sampling procedure (Merali and Banks, 1994). Specifically, during the 15-min period prior to noise onset, during the 15-min noise exposure period, and for an equivalent time following noise termination, the occurrence of a predefined behavior(s) was recorded during each 5-s observation interval. The cumulative frequency for each of the following behaviors is reported for each 15-min observation interval. *Sleeping/resting*: lying still with eyes completely or partially closed; *exploration*: actively moving about the cage; *sniffing*: vibrissal movements accompanied by head movements directed toward the floor or walls of the chamber; *rearing*: forelimbs off the cage floor in the absence of grooming; *grooming*: forelimbs are wiped over the face or ventral regions of abdomen and thorax; *freezing*: rigid posture, complete absence of visible movement, including vibrissae, with rapid respiration (Goldstein *et al*, 1996; Horvitz *et al*, 2001). In our laboratory, this procedure had previously been found to yield better than 90% agreement between raters (Merali and Banks, 1994).

Histology. At the end of the microdialysis and push-pull studies, animals were briefly anesthetized with halothane and then killed so that their brains could be extracted, sectioned, and stained for histological verification of probe placement. The correct placement of the push-pull probe into the anterior lobe of the pituitary gland was readily verified in the freshly extracted whole pituitary gland using low magnification without staining. Only data from correctly placed probes were used for statistical analyses.

RIA. The detection and quantification of ir-CRH was achieved using a solid-phase high-sensitivity adaptation or modification (Maidment and Evans, 1991) of the double-antibody liquid phase RIA originally described by Vale *et al*

(1983). Briefly, protein A/G- (Calbiochem Corp., CA) coated immulon-4 wells (Dynatec Laboratories Inc., VA) were incubated with anti-CRH serum (rC70, kindly provided by W Vale, The Salk Institute, La Jolla, CA) for 2 h at 20°C. Samples, standards (diluted in tissue-processing medium, ranging from 0.05 to 250 fmol/well), and blanks were incubated for 24 h at 4°C. Next, 25 µl of assay buffer containing 5000–6000 cpm of ^{125}I -[Tyr⁰]-rCRF (Amersham, ON) was added to each well and incubated for an additional 18 h at 4°C. Finally, the wells were rinsed and separated, and their residual radioactivity was counted in a gamma counter (Cobra II Auto gamma, Model D5002, Packard Instrument Company, CT). A four-parameter logistic curve fit model was used for interpolation of the standard curve. Sensitivity of the assay was typically ~0.1 fmol/well.

Statistical analyses. All results are expressed as means ± SEM. The plasma ACTH, corticosterone and prolactin levels, and the brain tissue levels of CRH were analyzed by a series of 2 (Strain) × 2 (Noise condition) × 2 (Time: immediate vs 1 h) analyses of variance (ANOVA). Similarly, the data from rats killed 24-h postnoise exposure in their home vs novel cage were analyzed by a 2 (Strain) × 2 (Stressor condition: home cage vs novel environment) ANOVA. Subsequent comparisons of means of significant main effects or simple effects of significant interactions were analyzed using Bonferroni corrected *t*-tests. At times, the degrees of freedom varied because of missing samples.

For the analysis of the *in vivo* microdialysis and push-pull results, ir-CRH and corticosterone release, the 3 baseline values preceding noise exposure from each animal were averaged and defined as 100%. All values were then expressed as a percentage of that baseline value. A significant main effect of Sample was followed up with Bonferroni corrected *t*-tests at relevant sample points. For the behavioral data obtained during the push-pull study, separate mixed measures ANOVAs with Sample blocks (baseline, noise, and postnoise) as the within-group variable were performed for each behavior, with Strain (Fischer vs Lewis) serving as the between-group factor.

RESULTS

Strain-Dependent Behavioral Response to Noise Exposure

Figure 2 depicts the behavior of rats at baseline, during noise exposure, and postnoise. These behavioral data were collected in the push-pull study but are presented first to illustrate the robust strain-dependent behavioral effects of the stressor. Individual ANOVAs of the frequency of sniffing, grooming, and exploration revealed significant Strain × Sample Blocks interactions, $F_{(2,38)} = 11.41$, 8.95, and 9.91, p 's < 0.001, respectively. *Post hoc* analyses revealed that sniffing, grooming and exploration were significantly elevated from baseline during noise exposure in the Fischer, but not in the Lewis rats (see Figure 2a–c). In each instance, these behaviors in the Fischer rats were significantly more frequent than in the Lewis rats during the noise period.

Freezing varied as a function of the interaction between Strain and Sample Blocks, $F_{(2,38)} = 14.15$, $p < 0.001$, that was because of a marked increase of immobility during noise

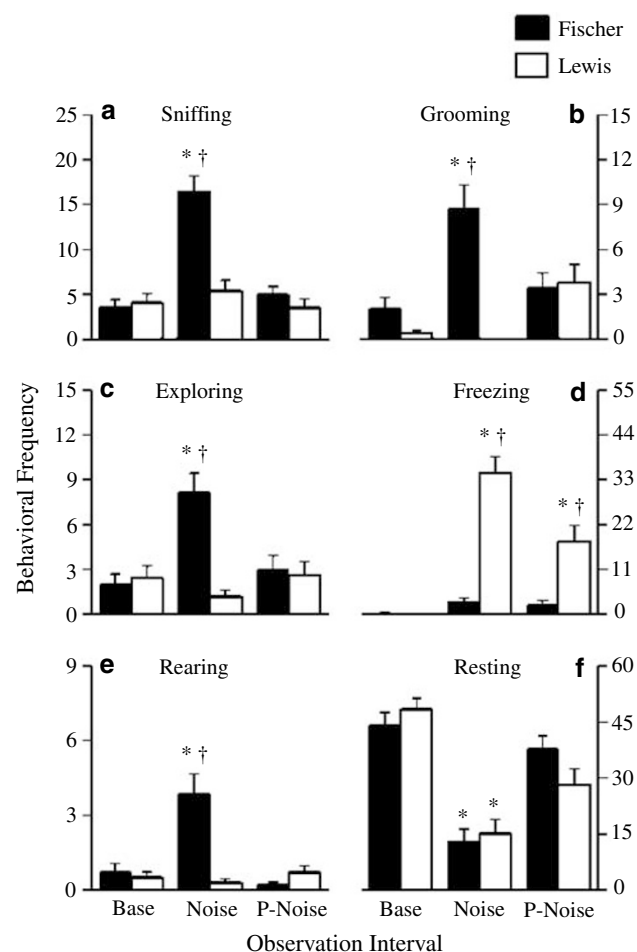


Figure 2 The frequency of sniffing (a), grooming (b), exploring (c), freezing (d), rearing (e), and resting (f) was observed before noise exposure (base) during noise exposure (noise) and following 15 min of noise exposure (P-noise) in Fischer and Lewis rats. *Significant within-strain difference from baseline condition, $p < 0.05$. †Significant between strain difference from matched time observation period, $p < 0.05$.

exposure in the Lewis rats (see Figure 2d). The elevated levels of freezing were still evident during the postnoise period. As a result, during the noise and the postnoise period, freezing in the Lewis rats significantly exceeded that in the Fischer rats. Strain × Sample Blocks interactions were also evident for rearing, $F_{(2,38)} = 14.28$, $p < 0.000$, and resting, $F_{(2,38)} = 3.33$, $p < 0.05$. *Post hoc* analyses indicated that while both strains showed almost no rearing during baseline, this behavior increased significantly with noise exposure only in the Fischer rats (see Figure 2e.), resulting in a more frequent rearing than in the Lewis strain. The Strain × Sample Blocks interaction for resting derived from a slightly higher amount of resting following noise exposure among the Fischer rats (see Figure 2f).

The Effect of Noise Exposure on Brain ir-CRH and Plasma Hormone Levels

Figure 3a depicts the changes of plasma ACTH associated with noise exposure. The ANOVA revealed that ACTH levels varied as a function of the Noise × Time and the

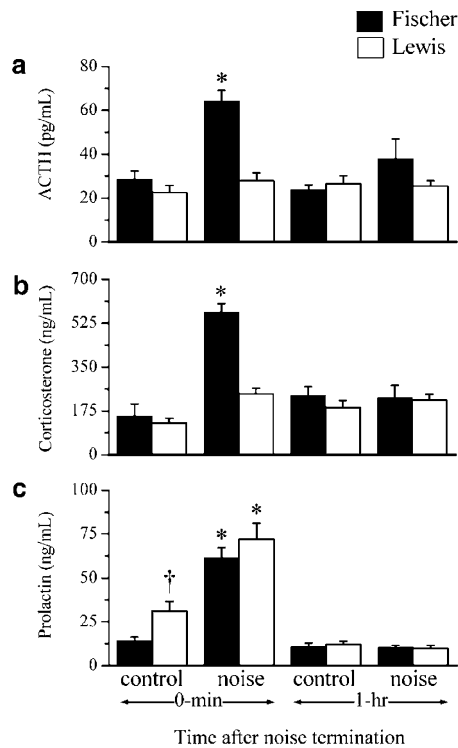


Figure 3 Variations of plasma hormone levels at 0 min and 1 h after 15 min of noise exposure are depicted as means (\pm SEM) for ACTH (a), corticosterone (b), and prolactin (c). *Significant within-strain difference from matched time control group, $p < 0.0001$. †Significant between strain difference at matched time, $p < 0.05$.

Noise \times Strain interactions, $F_{(1,60)} = 4.37$ and 5.96 , p 's < 0.05 , respectively. The comparisons of the means comprising the simple effects of these interactions confirmed that immediately after noise exposure the ACTH concentrations were elevated in the Fischer rats but not in the Lewis strain. Within 1 h of stressor termination ACTH levels in the Fischer rats had returned to control levels. The effects of the noise exposure on plasma corticosterone levels essentially paralleled the ACTH changes (see Figure 3b). The ANOVA revealed a significant Strain \times Noise \times Time interaction, $F_{(1,60)} = 11.36$, $p < 0.01$. The multiple t -tests indicated that corticosterone levels were elevated in Fischer rats immediately after noise exposure, but not at the 1 h interval. In the Lewis rats, in contrast, the noise stressor failed to alter significantly corticosterone levels.

Figure 3c shows that basal plasma prolactin in Lewis rats was slightly, but significantly, higher than in the Fischer rats, $F_{(1,60)} = 4.57$, $p < 0.05$. As well, prolactin levels varied as a function of the Noise \times Time interaction, $F_{(1,60)} = 45.89$, $p < 0.01$. Unlike ACTH and corticosterone, the level of prolactin was comparably elevated in both strains immediately after the noise exposure. In plasma taken 1 h later, prolactin had returned to basal levels.

Hypothalamic-pituitary ir-CRH variations following noise exposure. Figure 4 shows the changes of ir-CRH within the PVN (upper panel) and Me-Arc (lower panel). At the PVN the levels of ir-CRH varied as a function of the Noise \times Time interaction, $F_{(1,50)} = 16.14$, $p < 0.001$. Immediately after the noise exposure, ir-CRH was slightly

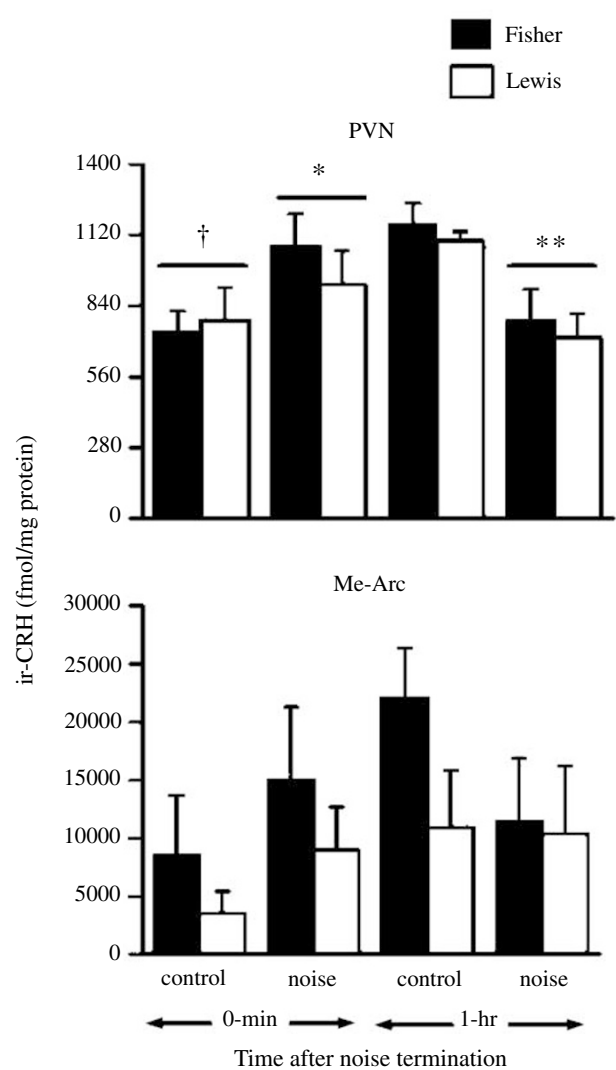


Figure 4 Variations of ir-CRH tissue levels (mean \pm SEM) at 0 min and 1 h after 15 min of noise exposure at the PVN (upper panel), and Me-Arc (lower panel). PVN, paraventricular nucleus of the hypothalamus; Me-Arc, median eminence-arcuate complex. *, **Significant difference from matched time control group, $p < 0.05$ and 0.01 , respectively. †Significant difference from 1-h time point, $p < 0.05$.

elevated in both strains, although this effect was marginal in the Lewis strain. In noise-exposed rats killed 1 h later, ir-CRH levels were lower than among nonstressed controls. The latter effect was because of a significant increase of ir-CRH in control animals killed at the 1 h interval, relative to control groups killed at the earlier time point (see Figure 4, upper panel). There was a tendency for the ir-CRH levels at the Me-Arc to be higher in the Fischer rats; however, because of the high variability, these effects failed to reach statistical significance (see Figure 4, lower panel).

Variations of ir-CRH levels at amygdaloid nuclei following noise exposure. Figure 5 represents changes in the content of ir-CRH at the amygdala. The ir-CRH changes at the CeA varied as a function of a significant Noise \times Time interaction, $F_{(1,48)} = 21.18$, $p < 0.01$. In both strains, the level of ir-CRH immediately after stressor exposure did not differ from that of controls. However, 1 h after noise exposure the

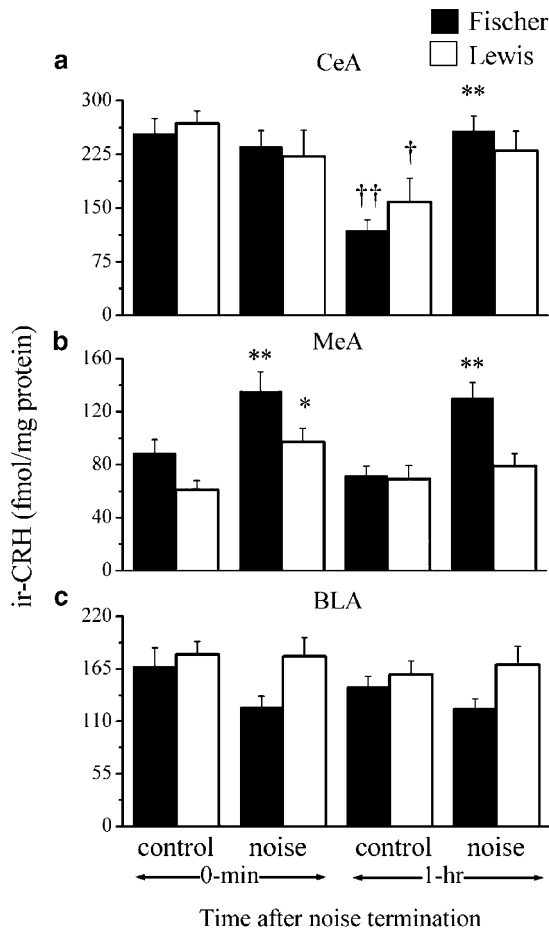


Figure 5 Variations of ir-CRH tissue levels (mean \pm SEM) at 0 min and 1 h after 15 min of noise exposure at the CeA (a), MeA (b), and BLA (c). CeA, central nucleus of the amygdala; MeA, medial nucleus of the amygdala; BLA, basal lateral nucleus of the amygdala. *, **Significant within-strain difference from matched time control group, $p < 0.02$ and $p < 0.01$, respectively. †, ††Significant within-strain difference from 0-min time point, $p < 0.02$ and $p < 0.001$, respectively.

levels of ir-CRH in nonstressed animals were reduced relative to that of rats that had been exposed to noise or to control conditions 1 h earlier (see Figure 5a).

At the MeA, ir-CRH varied as a function of the Strain \times Noise interaction, $F_{(1,58)} = 3.93$, $p < 0.05$. As seen in Figure 5b, in the absence of noise the levels of ir-CRH were comparable in the two strains. The level of ir-CRH in Fischer rats was elevated both immediately and 1 h following exposure to noise. Immediately after noise exposure ir-CRH was increased in the MeA of Lewis rats, but this elevation was not evident 1 h after noise exposure. Finally, at the BLA the level of ir-CRH was greater in Lewis than in Fischer rats, $F_{(1,59)} = 8.06$, $p < 0.01$, although the magnitude of this effect was relatively small and unrelated to the stressor condition (see Figure 5c).

Variations of ir-CRH levels at other stressor-sensitive brain regions following noise exposure. The levels of ir-CRH within the LC varied as a function of the Strain \times Noise interaction, $F_{(1,39)} = 8.54$, $p < 0.01$. The t -tests confirmed that there was no change of ir-CRH in the Lewis rats. However, while the measured levels of ir-CRH

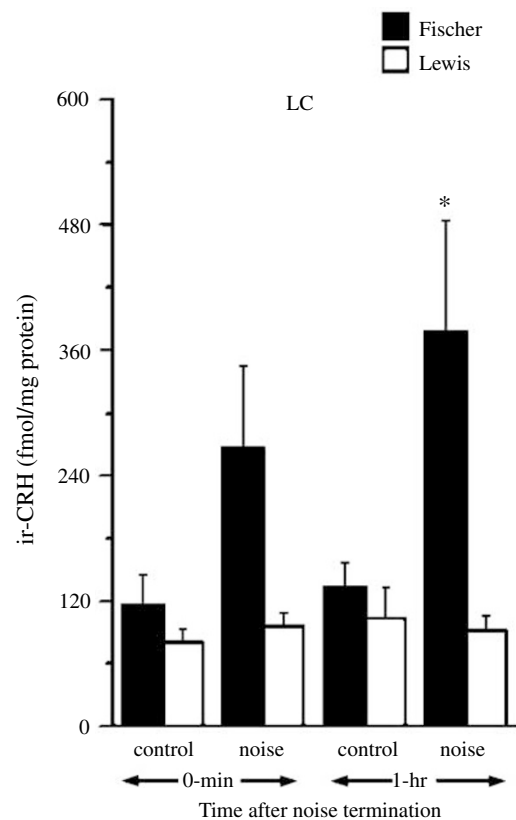


Figure 6 Variations of ir-CRH tissue levels (mean \pm SEM) at 0 min and 1 h after 15 min of noise exposure at the LC. LC, locus coeruleus. *Significant within-strain difference from matched time control group, $p < 0.05$.

increased appreciably at both time points in the Fischer rats, they only reached statistical significance 1 h after noise exposure (see Figure 6).

Noise exposure failed to alter the levels of ir-CRH at the PV and dHipp in either strain (data not shown); however, under control conditions Lewis rats had significantly elevated ir-CRH content at the nAcb, but reduced levels at the Cau at the 0-min time interval. Finally, at 1 h following noise exposure Fischer rats exhibited significant increases of ir-CRH tissue levels at the nAcb, whereas no change was observed in the Lewis rats (see Table 1).

The Impact of Environmental Cues on Protracted Changes in Brain ir-CRH and Plasma Hormone Levels 24 h Following Noise Exposure

Plasma ACTH, corticosterone, and prolactin levels were unaffected by noise exposure administered 24 h earlier, irrespective of whether the stressor was administered to rats in their home cages or in novel (distinct) environments (data not shown). Similarly, ir-CRH levels within the PVN and Me-Arc at 24 h were unaffected by the environment in which the noise had been applied (data not shown). Within the CeA the level of ir-CRH 24 h after noise exposure varied as a function of the interaction between the Strain and the environment in which the noise was administered, $F_{(1,29)} = 22.51$, $p < 0.01$. Noise applied to Lewis rats while in the home cage resulted in ir-CRH levels at the CeA

Table 1 Variations of ir-CRH Levels at Stressor-Sensitive Brain Regions 0 min and 1 h after 15 min of Noise Exposure

| Strain | Region | Time after noise termination | | | |
|---------|--------|------------------------------|--------------|----------------------------|----------------------------|
| | | 0 min | | 1 h | |
| | | Control | Noise | Control | Noise |
| Fischer | nAcb | 51.4 ± 9.1 | 94.8 ± 19.2 | 62.9 ± 18.9 | 130.8 ± 19.4* |
| | Cau | 57.8 ± 4.5 | 45.9 ± 3.5 | 46.2 ± 2.7 [†] | 52.3 ± 4.9 |
| | PFC | 668.3 ± 31.9 | 717.2 ± 84.4 | 420.6 ± 67.6 ^{††} | 414.4 ± 55.9 ^{††} |
| Lewis | nAcb | 134.1 ± 28.1 ^{§§} | 85.6 ± 18.3 | 70.9 ± 11.7 | 72.2 ± 10.5 [§] |
| | Cau | 39.4 ± 2.9 ^{§§} | 41.0 ± 5.7 | 52.5 ± 5.2 [†] | 41.9 ± 4.9 |
| | PFC | 528.8 ± 62.9 | 521.5 ± 49.2 | 400.5 ± 25.1 | 515.8 ± 43.3* |

*, **Significant within-strain difference from same time control condition. [†], ^{††}Significant within-strain difference from 0 min after noise termination. [§], ^{§§}Significant between-strain difference. All effects are $p < 0.05$ and $p < 0.02$, respectively. Data are presented as fmol/mg protein (Mean ± SEM).

Caudate (Cau), paraventricular thalamic nucleus (PV), nucleus accumbens (nAcb), prefrontal cortex (PFC), and dorsal hippocampus (dHipp).

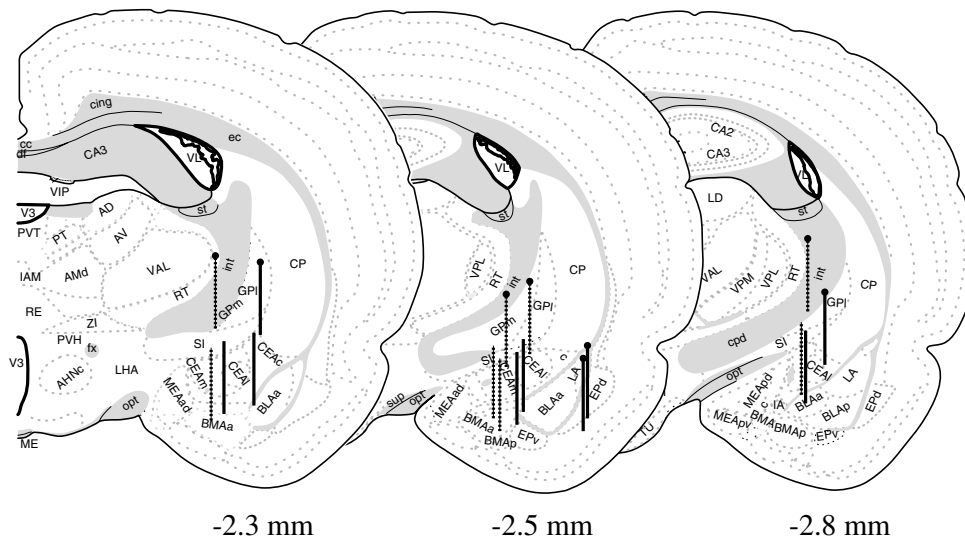


Figure 7 Anatomical localization of the microdialysis probe membranes aimed at the central nucleus of the amygdala in animals exposed to noise. A series of consecutive brain sections bearing the trace of microdialysis probes were stained, the most ventral location of the probe tip was determined, and a 2.0 mm line was drawn vertically, tracing the estimated location of the active region of the probe. Solid and dashed vertical lines represent Fischer and Lewis rats, respectively, included in the analyses. Pin-head traces represent their corresponding 'off-site' placements omitted from the overall analyses.

exceeding that of similarly treated Fischer rats and Lewis rats exposed to noise in the novel cage (respective means: 325 ± 33 vs 199 ± 12 vs 192 ± 12). Finally, there was a tendency towards higher ir-CRH levels in Fischer rats exposed to noise in the novel cage compared to their home-cage counterparts; however, this difference was not statistically significant (respective means, 247 ± 13 vs 199 ± 12).

In the remaining brain regions (MeA, BLA, dHipp, PFC, nAcb, Cau, and PV), the exposure environment had no effect on the levels of ir-CRH 24 h after the noise exposure (data not shown).

The Effect of Noise Exposure on the Release of ir-CRH from the CeA

Figure 7 depicts the histological verification of the microdialysis probe placements. An initial comparison of

the raw ir-CRH values from the dialysates constituting the baselines from animals with correctly positioned probes ($n = 5$ /Fischer; $n = 4$ /Lewis) revealed a significant difference between the two strains (respective means; 19.2 ± 5.2 and 217.3 ± 17.5 pg/45 μ l, $p < 0.0001$). An ANOVA of the percent change from baseline revealed that interstitial ir-CRH varied as a function of Sample, $F_{(12, 84)} = 14.95$, $p < 0.001$. *Post hoc* analyses revealed that noise exposure was associated with an immediate (during noise exposure) rise of ir-CRH concentration in the dialysates collected from the Fischer rats, which persisted until the end of the collection period (see Figure 8). In the Lewis rats ir-CRH levels increased during noise exposure but did not reach statistical significance ($p = 0.06$) until the third postnoise sample. Thereafter, ir-CRH concentrations were significantly higher than baseline values until the end of the experiment (see Figure 8). Further, between-group comparisons at each sample time confirmed that although ir-CRH in the Lewis

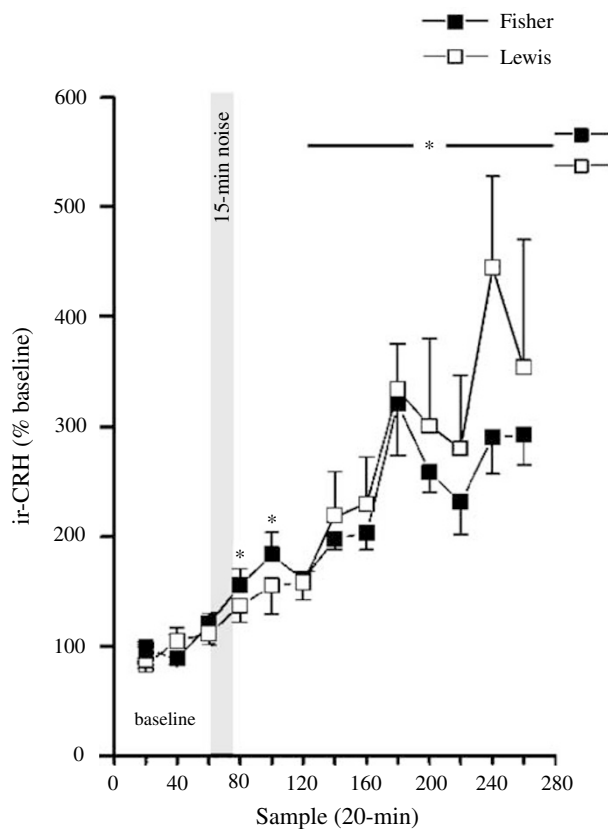


Figure 8 Levels of ir-CRH at the CeA as measured by *in vivo* microdialysis under baseline condition and following 15 min of noise exposure in Fischer and Lewis rats. The baseline samples from each subject were averaged and defined as 100%. All values were then expressed as a percent of that baseline (actual values were 4.04 ± 1.1 and 45.7 ± 3.7 fmol/sample, for Fischer and Lewis strains, respectively). *Significant difference from baseline condition at $p < 0.05$.

strain was elevated relative to that seen in Fischer rats, this difference was not statistically significant.

The Effect of Noise Exposure on ir-CRH and Corticosterone Availability at the Anterior Pituitary

Figure 9 (top panel insert) depicts the typical probe placement within the anterior pituitary. The variability in push-pull probe placement within the anterior pituitary was visually assessed in freshly extracted whole pituitary glands under low-level magnification. The assessment of probe placement was readily apparent in this manner. With the exception of a single Fischer rat, all probes were correctly positioned at the anterior pituitary gland. An initial comparison of the raw ir-CRH values from the perfusates constituting the baselines from animals with correctly positioned probes ($n = 11$ /Fischer; $n = 9$ /Lewis) revealed no strain differences (respective means; 29.9 ± 4.3 and 21.5 ± 2.5 pg/45 μ l, $p = 0.4$). An ANOVA of the percent change from baseline revealed a significant Sample \times Strain interaction, $F_{(17,323)} = 2.92$, $p < 0.001$. *Post hoc* analyses indicated that during the first 10 min of the 15-min noise exposure, ir-CRH concentrations were significantly elevated above baseline in the Fischer rats (p 's < 0.05) and decreased

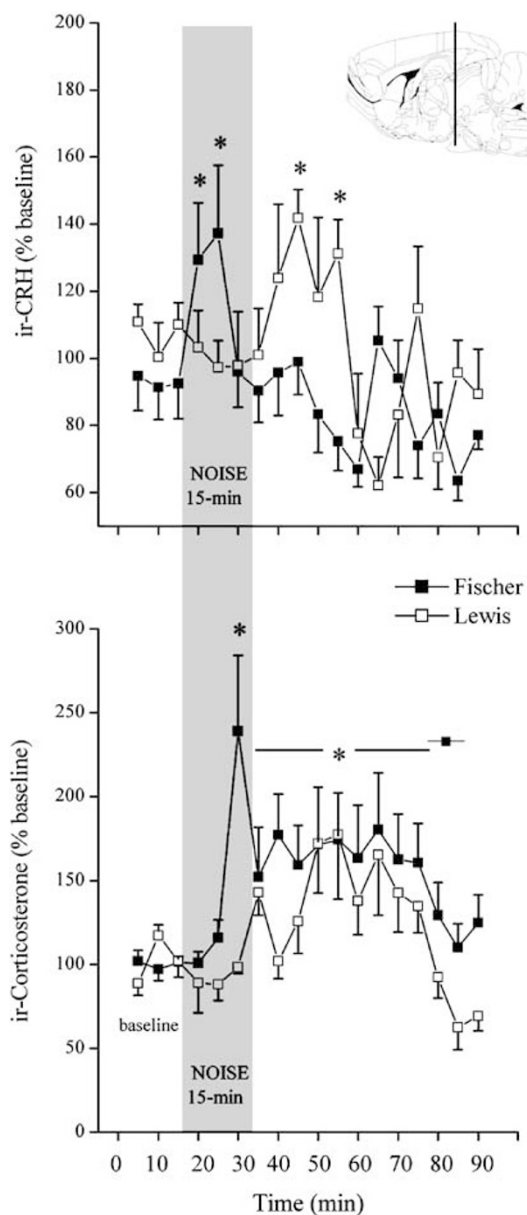


Figure 9 Levels of ir-CRH at the anterior pituitary gland (top panel) and corticosterone (bottom panel) as measured by *in vivo* push-pull perfusion under baseline condition and following 15 min of noise exposure in Fischer and Lewis rats. The baseline samples from each subject were averaged and defined as 100%. All values were then expressed as a percent of that baseline (actual ir-CRH values were 6.3 ± 0.9 and 4.5 ± 0.5 fmol/sample, for Fischer and Lewis strains, respectively and corticosterone values were 8.7 ± 0.9 and 4.0 ± 0.6 fmol/sample, respectively). *Significant difference from baseline condition at $p < 0.05$.

thereafter to basal levels (see Figure 9, top panel). In the Lewis rats, noise was associated with a more delayed rise of ir-CRH concentrations, beginning at 25 min following noise onset, and reaching a statistically significant increase above baseline by 30 min ($p < 0.01$). The ir-CRH remained elevated until about 45 min following stressor onset, and then declined (see Figure 9, top panel).

An initial comparison of the means constituting the baselines for both strains revealed that corticosterone levels in the perfusates collected from the Fischer rats were

significantly higher than those of the Lewis rats (respective means; 3.0 ± 0.3 and 1.4 ± 0.2 pg/45 μ l, $p < 0.05$). The levels of corticosterone varied as a function of the Sample, $F_{(17,272)} = 3.83$, $p < 0.0001$. During the last 5 min of the 15-min noise exposure, corticosterone levels were significantly elevated in the Fischer rats and remained so for an additional nine samples (45 min) (see Figure 9, bottom panel). In the Lewis strain noise exposure failed to increase significantly corticosterone levels in the perfusates. However, by the eighth postnoise sample (40 min postonset), corticosterone levels were elevated and approached statistical significance ($p = 0.07$) (see Figure 9, bottom panel).

DISCUSSION

The present study demonstrated that relative to male Lewis rats, Fischer rats exhibited a more robust corticosterone and ACTH response to noise exposure. This is concordant with earlier reports using female rats (Windle *et al*, 1998) and suggests that enhanced HPA reactivity of the Fischer rat (as compared to Lewis) is gender independent. While noise did not increase plasma corticosterone in Lewis rats in the present investigation, certain other stressors, including saline injection and restraint, have been reported to increase plasma corticosterone in both strains, although this effect was blunted and transient in the Lewis rats (Baumann *et al*, 2000; Stohr *et al*, 2000). It is possible that under our sampling schedule (immediately, 1 and 24 h postexposure) noise-induced changes of ACTH and corticosterone went undetected in the Lewis rats. However, as ACTH peaks earlier than corticosterone following stressor exposure, it seems rather unlikely that we would have failed to detect changes in either of these hormones, particularly 15 min after stressor onset.

Consistent with elevated circulating corticosterone levels following noise exposure, marked strain differences were evident with respect to interstitial levels of corticosterone at the anterior pituitary, with noise exposure markedly elevating corticosterone availability in the Fischer, but not in the Lewis rats. Corticosterone-induced suppression of the HPA axis occurs both at pituitary and suprapituitary levels (Mahmoud *et al*, 1984; Widmaier and Dallman, 1984). The finding that male Fischer and Lewis rats display similar sensitivity to peripherally administered dexamethasone (Gomez *et al*, 1998) suggests similar glucocorticoid receptor function between these strains. Thus, the possibility that the elevated corticosterone binding globulin observed in Fischer relative to Lewis rats (Dhabhar *et al*, 1993) hinders this hormone from accessing the central target(s) involved in the inhibition of the HPA axis should be considered. In contrast to the strain-dependent effects of the stressor on ACTH and corticosterone, noise exposure was associated with a comparable increase of plasma prolactin in both strains. Most stressors inhibit the activity of tuberoinfundibular dopamine neurons in the arcuate nucleus, thus disinhibiting the release of prolactin from the anterior pituitary (Freeman *et al*, 2000). Thus, the blunted ACTH and corticosterone responses in Lewis rats are likely attributable to central mechanisms that specifically trigger the activation of the HPA axis in response to stressful stimuli and not to strain-specific differences of stressor

perception. Indeed, the more pronounced freezing observed in the Lewis strain suggests that their auditory perception was not impaired. However, to our knowledge data are unavailable assessing differences in auditory acuity between these two rat strains.

Noise exposure was associated with site-specific alterations of tissue ir-CRH concentrations in Fischer and Lewis rats at the LC, PVN, and Me-Arc as well as in other related limbic structures, such as the amygdala. The regionally distinct effects may be related to several factors, including (a) whether the region comprises cell bodies (CeA and PVN) or terminal regions (LC and Me-Arc) and (b) region-specific differential turnover rates, that is, changes in the level of the peptide could reflect the combined effects of utilization as well as the rate of replenishment. Accordingly, changes in the peptide levels at these regions ought to be interpreted cautiously and, as indicated earlier, *in vivo* dynamic assessments are fundamental in order to delineate the processes related to CRH variations. In the Fischer rats, and to a lesser extent in the Lewis strain, the content of ir-CRH at the PVN 15 min following noise onset was elevated compared to control conditions. By 1 h following noise exposure, ir-CRH levels were lower in both strains compared to the control levels; however, at this time control levels of ir-CRH were greater than that seen in control animals 1 h earlier. One possible interpretation for this pattern of change might be that both the noise exposure and control conditions (which entailed transportation of rats to the test room) imparted changes of PVN ir-CRH content, but that these changes were more persistent in rats exposed to noise. While efforts were made to minimize the potential effects associated with the transportation of the animals to the test area (ie exposing animals to daily transportation to and from the experimental test room, for over 3 weeks), it seems that CRH variations at the PVN were still evident in response to potential situational variables. Furthermore, one cannot discount the possibility that other factors, such as the potential interaction between time since stressor exposure and the animal's response to handling, may have influenced peptide tissue levels. Similarly, although efforts were made to minimize diurnal fluctuations of CRH by maintaining a narrow temporal testing window, this does not eliminate the potential peptidergic fluctuations that may occur within this time frame (Ixart *et al*, 1993). Likewise, Windle *et al* (1998) have recently demonstrated that the pulsatile release of plasma corticosterone is not only different between female Fischer and Lewis rats, but that noise exposure only evoked an increase in plasma corticosterone in the Lewis rats if the stressor coincided with the increasing phase of endogenous corticosterone release. In contrast, the Fischer rats responded with a surge of corticosterone irrespective of the directional phase of corticosterone at the time of noise exposure. These results suggest that stressor-induced changes could be undermined or exaggerated depending on the specific sampling time. In addition, it ought to be considered that experimental conditions, in general, may have influenced the extent of the strain and stressor effects observed in the present investigation. For instance, stressed and control animals were housed in the same room and the possibility exists that olfactory cues and/or ultrasonic vocalizations of stressed rats could have influenced the control animals.

As alluded to earlier, tissue peptide release or utilization can change without peptide levels being altered, as increased release may be accompanied by an equal rate of synthesis, which would not be detected in post-mortem analyses of tissue peptide levels. Thus, the use of *in vivo* sampling techniques has distinct advantages in detecting potential strain differences with respect to the impact of various challenges. The current study represents the only direct assessment of noise-associated alterations in the availability of ir-CRH at the anterior pituitary in the Fischer and Lewis rats. Push-pull perfusion at the anterior pituitary revealed that while noise exposure provoked a rapid rise of interstitial ir-CRH levels in Fischer rats, this change was delayed in Lewis rats. It might have been expected that since ir-CRH availability increased at all in Lewis rats, plasma corticosterone should likewise have increased. In fact, the rise of corticosterone availability in the Lewis rats appeared to follow the delayed rise of ir-CRH; nevertheless, this blunted increase failed to reach statistical significance. Accordingly, other factors that might influence the stimulatory effect of CRH on ACTH release should be considered. It is of interest that cultured pituitary cells taken from female Lewis rats are reportedly more sensitive to the suppression of CRH-induced secretion of ACTH by dexamethasone and corticosterone (Zelazowski *et al*, 1992). It might thus be argued that corticosterone availability at the anterior pituitary in Lewis rats does not need to reach the same concentrations as it does in the Fischer rats in order to inhibit further activation of the HPA axis. This contention is consistent with the finding that, in males, central administration of dexamethasone blunts cocaine-induced corticosterone secretion to a greater extent in the Lewis than in the Fischer rat (Simar *et al*, 1997).

Several stressors, including noise, increase the neuronal firing rate and release of various neurotransmitters at the LC (Singewald *et al*, 1999; Singewald and Philippu, 1998). To date, the release of CRH at the LC has been alluded to, but never directly measured. Nevertheless, several indirect approaches support the hypothesis that CRH may serve as an excitatory neurotransmitter at this site (Valentino *et al*, 1998; Valentino and Wehby, 1988; Van Bockstaele *et al*, 1996, 1998). In the current study, we report that noise exposure was associated with elevated ir-CRH tissue content at the LC among Fischer rats, but not in the Lewis strain. If lower tissue peptide levels in the Lewis rats reflect enhanced release, then it would be consistent with the finding that *in vitro* firing rate of LC neurons is greater in the Lewis as compared to the Fischer rats (Guitart *et al*, 1993). Ultimately, it will be necessary to assess stressor provoked *in vivo* CRH release at the LC in the two rat strains.

Considerable efforts have been made towards characterizing the role of the amygdala in the cognitive, behavioral, and physiological responses to emotionally salient stimuli (Feldman *et al*, 1994; Kalin *et al*, 2001; Swanson and Petrovich, 1998), including noise (Galeno *et al*, 1984). The current micropunch data suggest nucleus- and peptide-specific alterations at the amygdaloid complex. Immediately after noise exposure a marked elevation of ir-CRH was evident at the MeA in both strains, while no differences were noted between the control and noise-exposed groups at either the CeA or the BLA. These data appear to support

the proposition that the MeA is more closely aligned with the generation of the neuroendocrine response to emotional stressors, relative to either the CeA or the BLA (Dayas *et al*, 1999). However, ir-CRH tissue levels at the CeA 1 h following control conditions (which included transport to and from the test room) was markedly reduced in comparison with the control group killed at the earlier time interval. Furthermore, ir-CRH levels at the CeA taken from animals killed at 1-h poststressor exposure were similar to both control and noise-exposed groups killed at the 15-min sampling time. At this juncture, it is difficult to discern why higher CRH levels at the PVN are evident in control rats 1 h after exposure to control conditions, while in the CeA the CRH levels were reduced in the same group of animals. One possibility is that the transportation of the rats to the test area acted as a mild stressor, but still sufficient to influence amygdaloid ir-CRH at the earlier sampling time, after which the peptide reverted to prestress levels. In contrast, with the more severe noise stressor the elevated peptide levels were more persistent. The finding that the profiles in the PVN and CeA were opposite of one another may be related to the fact that PVN CRH turnover is particularly rapid and consequently replenishment of the peptide levels is not evident at short intervals following a stressor experience. Indeed, it is not unusual for stressors not to alter PVN CRH levels despite the fact that these treatments are associated with elevated pituitary ACTH and adrenomedullary glucocorticoid release (Makino *et al*, 1999, 1994).

The results from the microdialysis study support the notion that the CeA is involved in noise stressor reactivity (Galeno *et al*, 1984), as the concentration of ir-CRH in the dialysates collected from the CeA increased following noise exposure in both strains. This is consistent with reports that microinjections of CRH into the CeA decreased immobility, but increased exploration and rearing (Wiersma *et al*, 1997, 1995), whereas CRH receptor antagonists attenuated behaviors associated with stressors, including freezing (Swiergiel *et al*, 1993).

The observed behavioral response to noise exposure in the present study was very similar to that reported by Windle *et al* (1998) where female Fischer and Lewis rats displayed striking differences in their behavioral response to loud noise. We noted that, in general, the Fischer rats displayed a more active coping style (increased exploration and grooming), whereas the Lewis strain adopted a more defensive coping strategy (freezing). It was likewise reported that in a forced swim test, the Fischer rats struggled more and showed less immobility than their Lewis counterparts (Lahmame *et al*, 1997). Interestingly, in the current study extracellular levels of CRH at the CeA in the Lewis rats were approximately 10-fold greater than in the Fischer strain. Although this finding is based on a small number of animals, and hence should be regarded as provisional, it is possible that these basal differences of CRH might be related to the observed strain differences in the behavioral response to noise. In spite of the basal differences, upon noise exposure both strains showed a similar CRH release profile at the CeA suggesting that it is not the relative release of CRH at the CeA that subserves the behavioral response to noise. Lahmame *et al* (1997) likewise concluded that there was a dissociation between CRH

activity (based on tissue content) and the expression of depression-like behavior in the forced swim test.

The apparent disparity between CRH alterations and behavioral responses suggests that either the diverse behavioral profiles may reflect (1) equivalent anxiety, but expressed in a strain-dependent fashion, (2) that CRH at the CeA is not a contributing factor in the behavioral response to the noise stressor, or (3) that the sensitivity to CRH varies between the two strains. The latter possibility is supported by the finding that the Fischer and Lewis rats show behavioral differences in response to centrally applied CRH (Glowa *et al*, 1991), such that exogenously administered CRH makes the Fischer rats behaviorally similar to non-CRH-exposed Lewis rats. Indeed, female Fischer rats show an immediate rise of plasma corticosterone following centrally applied CRH, compared to the relatively delayed increase observed in female Lewis rats (Windle *et al*, 1998). Likewise, it has been demonstrated that female Fischer rats are more sensitive to CRH-induced ACTH augmentation than Lewis rats (Spinedi *et al*, 1994). These findings, taken together with the strain differences we observed in both CRH and corticosterone availability at the anterior pituitary gland, suggest that at least one of the underlying mechanisms mediating the strain differences may be related to the apparent strain-dependent sensitivity to the effects of CRH, which would then impact stressor-associated HPA activation and behavioral change (Berridge and Dunn, 1989).

In summary, the current neurochemical, neuroendocrine, and behavioral data provide evidence that the apparent hypoadrenal response in the Lewis rats exposed to stressors (noise in this instance) is not simply a function of altered CRH release, but may have more to do with altered sensitivity at the receptor and postreceptor level. In this regard, the strain differences reported here, as well as by others (Glowa *et al*, 1991; Gomez *et al*, 1998; Windle *et al*, 1998), may be reflective of strain differences in the number and/or sensitivity of pituitary corticotrophs to endogenous CRH and/or glucocorticoids, as suggested by *in vitro* findings in females of these strains (Zelazowski *et al*, 1992). Our results also make it tempting to suggest that noise exposure might have protracted effects on CRH changes related to the exposure environment and that Lewis rats might be more sensitive to these cues compared to Fischer rats. Such findings, along with those that suggest that stressors might sensitize processes associated with neurotransmitter release (Berridge and Dunn, 1989; Zhang *et al*, 1995) or alter co-expression of neuropeptides within hypothalamic neurons (Bartanusz *et al*, 1993; Schmidt *et al*, 1996), indicate that aversive events, such as environmental noise exposure, may have long-term repercussions that could impact on behavioral outputs. However, it is likely that the impact of such events may be dependent on individual characteristics, including genetic disposition.

The present investigation makes it clear that a noise stressor profoundly influences corticosterone and ACTH release and may affect neuropeptide activity at hypothalamic and extrahypothalamic sites. Moreover, among the Lewis rats, ir-CRH changes at the CeA were evident as long as 24 h after the stressor experience, provided that the animals were housed in the environment where the stressor had been applied. The data further indicate that the well-characterized corticosterone and ACTH responses to

stressors in Fischer and Lewis rats were also evident with respect to ir-CRH availability at the anterior pituitary and, to a lesser extent, release at the CeA. Finally, it appeared that a mismatch existed between behavioral coping responses and the neuroendocrine alterations in these strains; while the Fischer rat reacts to stressors with active responses, the Lewis rats assumed a passive defensive strategy in response to noise.

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