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Cellular adaptations of dorsal raphe serotonin neurons associated with the development of active coping in response to social stress

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Abstract

Background—Social stress is a risk factor for affective disorders for certain vulnerable individuals. Stress and depression are linked in part through regulation of the dorsal raphe (DR)-serotonin (5-HT) system by the stress-related neuropeptide, corticotropin-releasing factor (CRF). Here we used a rat social stress model that shows individual differences in coping strategies to determine whether differences in CRF-5-HT interactions underlie individual differences in the vulnerability to social stress.

Methods—Rats were exposed to the resident-intruder model of social stress for 5 days. In vivo single unit recordings assessed DR-5-HT neuronal responses to CRF and immunoelectron microscopy assessed CRF₁ and CRF₂ cellular localization 24 h after the last stress.

Results—Rats responded to social stress passively, assuming defeat with short latencies (SL, 48%) or actively with proactive behaviors and longer defeat latencies (LL, 52%). Whereas CRF (30 ng, intra-DR) inhibited 5-HT neuronal activity of control and SL rats, it activated 5-HT neurons of LL rats, an effect that was CRF₂ mediated. Consistent with this, social stress promoted CRF₁ internalization together with CRF₂ recruitment to the plasma membrane of DR neurons selectively in LL rats.

Conclusions—These data suggest that a proactive coping strategy towards social stress is associated with a redistribution of CRF₁ and CRF₂ in DR-5-HT neurons that primes the system to be activated by subsequent stress. The lack of this adaptation in passive coping rats may contribute to their depressive-like phenotype. These studies provide a cellular mechanism for individual differences in stress responses and consequences.

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Keywords

resident-intruder; corticotropin-releasing hormone; receptor internalization; antisauvagine-30; CRF; social defeat

INTRODUCTION

Repeated stress is associated with the development of psychiatric disorders such as depression anxiety, and drug abuse (1–6). One neuropeptide known to link stress and psychiatric disorders is corticotropin-releasing factor (CRF). In addition to initiating the hypothalamic-pituitary-adrenal axis response to stress, CRF actions in extrahypothalamic regions mediate behavioral, cognitive and autonomic responses to stress (7–11). Overproduction of CRF as evidenced by increased CRF levels in cerebrospinal fluid, increased CRF expression in paraventricular hypothalamic neurons and increased CRF-immunoreactivity in the noradrenergic nucleus locus coeruleus (LC), the serotonin (5-HT)-containing dorsal raphe nucleus (DR), and prefrontal cortex have been associated with depressive disorders (12–15). CRF exerts its effects through CRF₁ and CRF₂ receptor subtypes (16, 17). Generally, CRF₁ has been associated with anxiogenic and depressive-like responses in animal models (18–21) whereas the consequences of CRF₂ activation are equivocal (22–28). CRF targets monoaminergic systems implicated in affective disorders including the LC (29), and the DR (30). Within the DR CRF has opposing effects on 5-HT neuronal activity through actions at CRF₁ and CRF₂. At low doses, CRF activates CRF₁ which enhances GABAergic inhibition of DR-5-HT neurons and decreases 5-HT extracellular levels in forebrain and limbic targets (31–34). Higher doses of CRF excite 5-HT neurons through CRF₂ activation and increase extracellular 5-HT in forebrain and limbic targets (31–33, 35–39).

Importantly, the dual actions of CRF on the DR-5-HT system are influenced by prior stress exposure. For example, a single exposure to swim stress qualitatively changed the DR-5-HT neuronal responses to CRF from inhibition to excitation (39). This shift was associated with a cellular redistribution of CRF receptors such that CRF₁ became internalized and CRF₂ was recruited to the plasma membrane. This study revealed mechanisms by which prior stress can recruit different receptor subtypes, leading to qualitatively different cellular responses to CRF and ultimately different consequences of subsequent stress.

For humans, a prevalent stressor is social stress and this has been modeled in rodents using the resident-intruder stress (40, 41). We previously identified two phenotypes in the same strain of rats exposed to this stressor (42). One phenotype exhibits a passive coping strategy in response to the stress that is characterized by a short latency to be defeated (SL rats) and develops stress-induced behavioral, neuroendocrine, and cardiovascular endpoints similar to those associated with depression (18, 42). The other phenotype adopts a proactive coping strategy with increased defeat latencies (LL rats) and does not develop the same pathological consequences as the SL rats (18, 42). To determine the role of CRF-5-HT interactions in these phenotypes 5-HT neuronal sensitivity to CRF was assessed in rats exposed to repeated social stress using electrophysiological approaches. Additionally, the cellular localizations of CRF₁ and CRF₂ were visualized using immunogold labeling and electron microscopy. The results identified a cellular adaptation that occurs selectively in active coping rats that qualitatively changes the response of DR-5-HT neurons to CRF and may underlie the resilience of this phenotype.

METHODS AND MATERIALS

Subjects

Male Sprague Dawley rats (275–300g) were used as control or social stress “intruder” rats, and male Long-Evans retired breeders (550–850g) served as residents (Charles River, Wilmington, MA). Rats were singly housed in a climate-controlled room with a 12 hour light-dark cycle (lights on at 0700 hours). Food and water were freely available. A total of 92 rats were used in these studies. Care and use of animals was approved by the Children’s Hospital of Philadelphia Institutional Animal Care and Use Committee and was in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Social Stress

The social stressor utilized in these studies was modified from the resident-intruder model originally developed by Miczek et al. (40). Rats were randomly assigned to either a control or social stress exposure for 30 minutes on five consecutive days identical to Wood et al. (18, 42). Social stress exposure elicited intruder subordination, termed defeat, and was operationally defined by the intruder exhibiting a supine posture for approximately 3 seconds. Once this occurred or 15 min elapsed, the intruder was placed behind a wire mesh partition in the same cage as the resident that prevented physical contact between the resident and intruder. The latency to exhibit a supine posture in the presence of the resident aggressor was recorded and averaged over the 5 exposures. Controls were placed behind the partition in a novel cage for 30 minutes daily. Rats were returned to their home cage after each session. Twenty-four hours after the 5th exposure to social stress or control manipulation, rats were either subjected to electrophysiological recording or tissue was harvested for electron microscopic or western blotting studies.

Electrophysiological recordings in DR

Extracellular single unit activity from DR-5-HT neurons was recorded in the isoflurane-anesthetized state as previously described (31, 39). Double barrel glass micropipettes were used to record DR neuronal activity and simultaneously microinfuse CRF (30 ng in 30 nl artificial cerebrospinal fluid) adjacent to the recording electrode as previously described (31). Spontaneous discharge rate was recorded for 3–5 minutes immediately preceding the microinfusion and for 5–15 minutes after CRF infusion. In a separate cohort of rats, the CRF₂ antagonist, antisauvagine-30 (3 µg in 3 µL) was administered through an intracerebroventricular (i.c.v.) cannula 10 minutes before CRF. Only one microinjection of CRF was administered to an individual rat. For detailed methods see Supplement 1.

Histology

At the end of the electrophysiological recording the recorded neurons were juxtacellularly labeled with neurobiotin (1.5%, Vector Laboratories-SP-1120) as previously described (43, 44). Neutral red dye (3 µl, i.c.v.) was injected for later verification of the i.c.v. cannula placement and the rats were transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Rats remained anesthetized from the beginning of the experiment to termination.

Immunohistochemistry

Following perfusion, brains were removed and post-fixed for 1 hour at 4°C, and stored in 20% sucrose solution containing 0.1% sodium azide at 4°C. Coronal sections (30 µm) through the DR were cut on a cryostat. Sections were processed to visualize neurobiotin and 5-HT (see methods in Supplement 1). Only data from juxtacellularly labeled neurons that were 5-HT-immunoreactive were used in the analysis.

Immunoelectron microscopy

For immunoelectron microscopy, rats were transcardially perfused with 3.8% acrolein and 2% paraformaldehyde in 0.1M PB 24 h after the last manipulation. Coronal sections (40 μ m) through the DR were cut on the Vibratome and processed for CRF₁ and CRF₂ using immunogold detection identical to methods previously described (39, 45, 46). See details in supplement 1.

Western Blotting

Brains were flash frozen in isopentane and stored at -80°C . Coronal sections were cut on the cryostat to the approximate rostral-caudal level of -8.00 mm from Bregma according to Paxinos and Watson Rat Brain Atlas. A 2 mm deep section of tissue containing the DR was microdissected using a trephine. Samples were homogenized and centrifuged. Protein content was quantified using the BCA method for each sample. Protein extracts (10 μ g) were subjected to SDS-PAGE gel electrophoresis and proteins were transferred to polyvinylidene fluoride membranes (Immobilon-FL) as previously described (47). CRF₂ has two functional splice variants, α and β (48, 49). CRF_{2 α} is the major CRF₂ splice variant expressed in rodent brain (50). Therefore, membranes were probed for the full length CRF₂ receptor (CRF_{2 α}) using an antibody directed against the transmembrane domain of CRF_{2 α} (CRF_{2 α} (TMD), Santa Cruz (W-17)). To examine the possible contribution of a CRF_{2 α} splice variant that is not inserted into the plasma membrane (sCRF_{2 α}) blots were probed with an antibody directed against the unique C-terminus (49) of sCRF_{2 α} (Wylie Vale, The Salk Institute, La Jolla, CA). Membranes were also probed with an antibody detecting β -arrestin2 (sc-13140). All bands were expressed as a ratio of the corresponding loading controls COX IV (ab14744) or GAPDH (Sigma, G9545). Odyssey Infrared Imaging system and software were used to determine the molecular weight and integrated intensity of each band as previously described (42). Detailed methods are provided in Supplement 1.

Data analysis

A cluster analysis (JMP 9.0) was applied to defeat latencies of all rats exposed to social stress that were used in electrophysiological and protein studies to define phenotypes by defeat latency. Because brains for electron microscopy studies were collected at different times separate cluster analyses were applied to these. Neuronal discharge rates were determined over 120 seconds before the CRF infusion as the mean basal discharge rate. Discharge rate was determined in 30 second intervals after CRF infusion and expressed as a percentage of the mean basal discharge rate. The mean basal rates were compared between groups using a One-way ANOVA and the effect of CRF on DR activity over time in different experimental groups were compared using a two-way repeated measures ANOVA with Bonferroni's post-hoc test.

Quantification of CRF₁ and CRF₂ receptor distribution for the immunoelectron microscopy study was completed in the midcaudal dorsomedial and ventromedial subregions of the DR extending from approximately -7.80 to -8.3 mm from Bregma as previously detailed (39). Differences in CRF₁ and CRF₂ receptor trafficking between control, SL and LL rats were quantified in immunolabeled dendritic processes by calculating the percentage of cytoplasmic to total gold particles (percent cytoplasmic) for all sampled dendrites in each rat. The mean percentage was determined for each rat and this was averaged for all rats in a group for a single group mean. Group means were compared by a one-way ANOVA followed by Tukey's multiple comparison post hoc test.

The Odyssey Infrared Imaging software was used to quantify CRF_{2 α} , sCRF_{2 α} and β -arrestin2 protein levels as the integrated intensity of each protein band of interest as a ratio

to the corresponding loading control (COX IV or GAPDH). Mean ratios were compared between groups using One-way ANOVA's.

Drugs

Ovine CRF (American Peptide Co., Sunnyvale, CA) and antisauvagine-30 (Sigma Chemical, St. Louis, Missouri) were diluted in dH₂O (1mg/ml), and 10 μ L aliquots were concentrated, stored at -80°C and reconstituted in artificial cerebrospinal fluid (aCSF) on the day of the experiment.

RESULTS

Individual differences in the behavioral response to social stress

Consistent with our previous findings (42), the out-bred population of Sprague Dawley rats was bimodally distributed with respect to average defeat latency (Fig. 1). Two clusters could be identified with a cutoff of 416 s to classify behavioral reactivity to social defeat. A subpopulation of 22 rats (48%) assumed a submissive posture in <416 s and were classified as SL rats. The other subpopulation resisted defeat resulting in an increased latency to exhibit the submissive posture (>416 s) and were classified as LL rats ($n=24$, 52%). Within all rats exposed to social defeat, the SL and LL populations had significantly different mean $\pm$ SE defeat latencies of 271 ± 20 s and 627 ± 23 s, respectively ($t_{(44)}=11.7$; $p<0.0001$).

Distinct electrophysiological responses of 5-HT neurons to CRF in SL and LL rats

A total of 79 cells were labeled with neurobiotin and 56 cells were 5-HT immunoreactive. Figure 2 shows examples of neurobiotin labeling of a 5-HT cell, as indicated by double labeling (top panel), and a non-5-HT cell indicated by the absence of double labeling (bottom panel). The mean basal discharge rate of 5-HT neurons in untreated control ($n=23$), SL ($n=10$) and LL ($n=23$) rats were 2.3 ± 0.2 , 1.6 ± 0.2 and 1.7 ± 0.2 respectively. Although the basal discharge rates tended to be lower in stressed compared to control rats, these did not quite reach statistical significance ($F_{(2,45)}=3.1$; $p=0.056$).

Figure 3 shows representative records of the effect of CRF (30 ng) on DR-5-HT neuronal activity in rats of the three experimental groups. As previously reported in unstressed rats (31, 39) CRF inhibited DR-5-HT neuronal activity (Fig. 3A1) and had mixed effects, including activation of 2 of 5 non-5-HT cells (Fig. 3A2). CRF produced a similar inhibition of 5-HT cells of SL rats (Fig. 3A3, 3B1). In contrast, CRF produced a robust excitation of 5-HT neurons of LL rats (Fig., 3A4, 3B1). A repeated measures ANOVA revealed a significant group $\times$ time interaction ($F_{(14,151)}=2.8$; $p=0.001$) and Bonferroni posthoc tests determined that 5-HT neuronal activity was significantly increased by CRF in LL rats compared with controls ($p<0.001$) and SL rats ($p<0.001$). There was no difference between SL rats and controls.

Previous studies indicated that the inhibitory effect of CRF on 5-HT neurons is mediated by CRF₁ and excitatory responses are consistent with CRF₂ receptor activation (31, 39). Administration of the CRF₂ antagonist, antisauvagine-30 (ASV; 3 μ g in 3 μ L) prevented the CRF-induced excitation of 5-HT cells of LL rats, consistent with a CRF₂-mediated effect (Fig. 3B2). In all ASV-treated rats, regardless of group, CRF inhibited 5-HT neurons indicated by a significant effect over time ($F_{(8,239)}=2.3$; $p=0.025$). However, Bonferroni post-hoc tests revealed that LL rats exhibited significantly less inhibition compared with control rats at 90 ($p=0.008$) seconds after CRF infusion. A two-way repeated measures ANOVA comparing only LL rats left untreated with those receiving ASV treatment revealed a significant treatment $\times$ time interaction ($F_{(7,119)}=4.11$; $p<0.001$) Bonferroni post-hoc

analysis confirmed these differences between LL rats that did or did not receive ASV at the time points of 30–210 seconds ($p < 0.001$).

Distinct cellular localization of CRF receptors in DR neurons in SL and LL rats

Immunogold-silver labeled CRF₁ was relatively equally distributed between the plasma membrane and cytoplasmic compartments of dendrites in control rats consistent with previous reports in unstressed rats (39) (Figure 4A1 and A2). Exposure to social stress did not alter CRF₁ distribution in SL rats. However, repeated social stress exposure resulted in greater CRF₁ localization in the cytoplasm in LL rats, consistent with internalization (Figure 4A1 and A2). Quantification of the percentage of internalized CRF₁ (percentage of cytoplasmic to total CRF₁) confirmed a stress-induced increase in internalization in LL rats ($n=3$; $F_{(2,8)}=12.1$; $p=0.0078$) compared with control ($n=3$; $p<0.05$) and SL rats ($n=3$; $p<0.01$). In contrast to CRF₁, CRF₂ was predominantly localized within the cytoplasm of DR neurons of control rats (Figure 4B1 and B2), as previously reported in unstressed rats (39). Social stress did not affect CRF₂ distribution in SL rats, indicated by a comparable percentage of cytoplasmic CRF₂ compared to controls. Notably, the percentage of cytoplasmic CRF₂ was less in LL rats ($n=5$) compared to controls or SL rats, suggesting a recruitment of CRF₂ to the plasma membrane ($F_{(2,11)}=23.8$; $p=0.0003$) compared with controls ($p<0.001$; $n=4$) and SL rats ($p<0.01$; $n=3$; Figure 4B2).

Social stress does not significantly impact protein expression of CRF_{2α}, alternatively spliced CRF_{2α} (sCRF_{2α}) or β-arrestin2 in the dorsal raphe

To determine whether the distinct CRF₂-mediated excitation of 5-HT neurons observed in LL rats was related to differences in protein expression of CRF_{2α}, a soluble splice variant of the CRF_{2α} receptor (sCRF_{2α}), or β-arrestin2, the protein initiating CRF receptor internalization, these proteins were quantified using western blot (Table 1). Exposure to social stress did not significantly alter CRF_{2α} protein expression detected by CRF_{2α} (TMD) ($p=0.39$). The alternatively spliced sCRF_{2α} that lacks a TMD was also not significantly impacted by stress ($p=0.67$), nor was the ratio of full length CRF_{2α} to sCRF_{2α} ($p=0.42$). Furthermore, β-arrestin2 levels were not significantly altered by stress exposure in SL or LL rats compared with controls ($p=0.92$).

DISCUSSION

As previously reported, two populations of rats emerged following repeated social stress as defined by their active or passive coping strategy (42). Repeated social stress resulted in dynamic changes in the DR-5-HT system only in rats that adopted an active coping strategy. In these rats social stress promoted the cellular redistribution of CRF₁ and CRF₂ such that CRF₂ was more prominent on the plasma membrane and CRF₁ was internalized. This translated to a qualitative shift in the response of 5-HT neurons to CRF from inhibition to a CRF₂ mediated excitation. The lack of adaptation in rats that were readily defeated and that typically have depressive-like characteristics (42) suggests that these cellular adaptations to social stress may promote resilience to the potential pathological consequences of social stress.

CRF interactions with the DR-5-HT system in stress

The DR-5-HT system is targeted by CRF released during stress and CRF actions within the DR are thought to mediate behavioral responses to stress (26, 31, 34, 35, 39, 51, 52). The finding that basal DR-5-HT discharge rate was not altered by a history of social stress is consistent with previous findings in rats with a history of swim stress (39). The results indicate that CRF is not tonically released in the DR in rats with a history of stress, but rather that prior stress alters postsynaptic mechanisms that mediate neuronal responses to

CRF. CRF has complex dose-related effects on the DR-5-HT system because of the neurochemical heterogeneity of the DR and the existence of multiple CRF receptor subtypes that are differentially localized within the DR and that have different affinities for CRF (31, 35, 53). CRF₂ is the prominent receptor subtype and is present on both GABAergic and 5-HT neurons as well as in axon terminals (39, 54). CRF₂ is activated by relatively high levels of CRF and excites DR-5-HT neurons and increases 5-HT extracellular levels in forebrain regions (36, 37, 44). CRF₁ expression in the DR is much lower and evidence supports a localization that is primarily on GABA neurons through which CRF inhibits DR-5-HT neurons and 5-HT release in forebrain regions (34, 54). Excitatory CRF₂-mediated effects have been linked to the development of learned helplessness and increased immobility during swim stress (26, 39, 55). Similar to the results reported here for LL rats, acute swim stress causes a redistribution of CRF₂ to the plasma membrane and a switch to CRF-mediated excitation of DR-5-HT neurons 24 h after swim stress, at a time when a subsequent swim would increase immobility (39). Conversely, CRF₁ mediated inhibition of this system has been suggested to increase active behaviors in response to swim stress, defensive burying and to protect against the development of learned helplessness (55, 56). With regard to social stress, either one or two exposures to social defeat has been reported to increase c-fos expression in rat DR-5-HT neurons indicative of excitation and consistent with the present findings in LL rats (57). However, in contrast to the present findings, this study associated 5-HT excitation with reactive defensive behaviors rather than proactive behaviors. These previous studies would predict that CRF-induced activation of DR-5-HT neurons would occur in the more defensive SL rats rather than the proactive LL rats. However, a major difference between the studies cited above is the more chronic nature of the social stress used in the current study. It is possible that an enhanced CRF₂ influence on the DR-5-HT system in the long term promotes active coping perhaps as a result of other molecular changes that require a longer duration of exposure. CRF₂ excitation of the DR-5-HT system has also been implicated in conditioned fear that develops in hamsters that have been exposed to social defeat whereby their territorial aggression becomes diminished (51). Notably, in these animals CRF₂ mediates an active fleeing response.

CRF receptors in the DR: active vs. passive coping, vulnerability vs. resilience

The role of CRF receptors in the DR in mediating active vs. passive coping behaviors is relevant because the emergence of a passive strategy to cope with stress is associated with vulnerability to affective disorders in humans (58, 59). In our rodent model, SL rats that are readily submissive develop an endocrine and behavioral depressive-like profile (42). In contrast, a proactive strategy is associated with resilience in both clinical and preclinical studies (18, 42, 58, 59). Therefore, cellular adaptations associated with the development of active coping responses to stress may provide critical insight into mechanisms mediating resilience. In our model, during the first few exposures to social stress, the different phenotypes are not apparent and latencies to defeat are similar for all rats. It is only after 4 or more exposures that phenotypic distinctions are clear. Daily administration of a CRF₁ antagonist shifts the behavioral response to social stress from passive to active coping strategies, resulting in greater time spent in a defensive upright posture and increased defeat latencies (18), consistent with the present finding that CRF₁ internalization is associated with the proactive phenotype. CRF₁ antagonism also prevents the depression-like consequences of repeated social stress including immobility in response to swim stress, adrenal hypertrophy and decreases in heart rate variability (18). Thus CRF₁ internalization selectively seen in LL rats can account for the development of proactive behaviors and a more resilient phenotype with regard to certain endpoints. However, the more prominent effect produced by repeated social stress in LL rats was the shift from CRF-induced inhibition of DR-5-HT neurons to a robust activation as a result of CRF₂ recruitment to the plasma membrane. This would predict that LL rats respond to stressors with an increase

rather than the decreased 5-HT release that would be seen in SL rats. Although increased 5-HT in response to stress has been implicated in passive behavior in certain models such as learned helplessness, other studies support the facilitation of active coping by increased 5-HT extracellular levels in some brain regions (60, 61). Analogous to the present study, in a psychosocial stress model in nonhuman primates that results in clusters of stress sensitive and stress resilient animals, stress resilient animals were characterized by increased CRF₂ expression in the DR and markers of 5-HT function (62). Moreover, treatment of the stress sensitive population with antidepressants increased CRF₂ expression in DR and reversed the stress sensitive phenotype. Taken with these findings in the nonhuman primate social stress model, our current results in rats underscore a role for increased CRF₂ function in DR-5-HT neurons with stress resilience.

Mechanisms of underlying CRF receptor redistribution

The qualitative change in DR-5-HT responses to CRF in LL could be attributed to the cellular redistribution of CRF₁ and CRF₂ in DR neurons. However, the molecular mechanisms underlying this redistribution are currently unknown. Differences in β -arrestin2, which is responsible for internalization of G protein coupled receptors, could account for differential receptor trafficking of the CRF receptor in the SL and LL phenotypes (63–65). In the present study, however, differences in β -arrestin2 expression levels were not observed, nor were there significant differences in protein expression of the full length CRF_{2a} receptor. An alternative explanation could be the emergence or suppression of an alternatively spliced variant of the CRF_{2a} receptor (sCRF_{2a}) in which exon 6 is deleted from the gene encoding for CRF_{2a} resulting in the absence of a transmembrane spanning domain (49). Consequently, sCRF_{2a} does not traffic to the plasma membrane (66). In the present studies, the SL and LL phenotypes did not differ significantly in their sCRF_{2a} protein expression. Recently it was reported that while sCRF_{2a} did not serve as a decoy receptor, potentially sequestering free CRF, the unproductive splicing of the CRF_{2a} pre-mRNA to sCRF_{2a} may serve to alter levels of full length CRF_{2a} and thus functional protein levels (66). In the present studies, significant differences in the ratio of the full length CRF_{2a} (as measured by an antibody generated near the internal transmembrane domain) to the expression of the s CRF_{2a} were also not evident.

CRF₂ is unique in possessing an N-terminal pseudo signal peptide that remains uncleaved and is maintained as part of the mature protein (67, 68). This results in low cell surface expression as was apparent in the electron photomicrographs from control and SL rats. We speculate that repeated social stress alters the function of this peptide in certain individuals such that the mature protein can be inserted into the plasma membrane as a functional receptor. Studies exploring the molecular switch promoting the internalization of CRF₁ and recruitment of CRF₂ to the plasma membrane in select individuals will shed light on critical mechanisms of stress vulnerability and resiliency.

In summary, the present study suggests a cellular mechanism whereby exposure to the same stressor can lead to divergent adaptations promoting resiliency in one individual while rendering another susceptible. Elucidating the basis for individual differences in the expression of this cellular mechanism will illuminate mechanisms by which stress leads to disease in vulnerable individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Hierarchical cluster analysis of defeat latencies. The defeat latencies of all rats used for electrophysiological and Western blot studies were subject to cluster analysis using JMP software. The red and green depict the distinct clusters. Lines are ordered going from the shortest to longest latency.

Figure 2.

Neurochemical identification of recorded neurons in the dorsal raphe nucleus. Fluorescent photomicrographs of sections through the DR showing of neurobiotin labeled neurons (red) and 5-HT immunohistochemically labeled neurons (green). The top panel displays a neuron that is double labeled for neurobiotin and 5-HT-immunoreactivity seen in the merged image as yellow. The bottom panel displays a non-5-HT recorded cell, as indicated by the absence of double labeling in the merged image. The arrows in the top 5-HT panel point to the 5-HT immunoreactive neuron that also is neurobiotin labeled. The arrowheads in the bottom 5-HT panel point to the 5-HT neurons that are adjacent to the neurobiotin labeled neuron in the merged panel.

Figure 3.

Social stress results in a qualitative change in corticotropin-releasing factor (CRF) effects on dorsal raphe (DR)-5-HT neuronal activity. (A1–A4) Traces indicate the mean frequency of an individual DR-5-HT unit of a control rat (A1), a non-5-HT neuron in a control rat (A2), and 5-HT neurons in an SL (A3) and an LL (A4) rat. Arrowheads indicate the administration of 30 ng CRF. Notably, CRF administration had divergent effects in 5-HT neurons of LL social stress rats versus control and SL rats. B) Time course of the effect of CRF on DR-5-HT neuronal activity in control (n=7), SL (n=6), and LL rats (n=6) in the absence (B1) and presence of antisauvagine-30 administered 10 minutes prior to CRF (B2, control: n=11, SL: n=7, LL: n=9). The abscissa indicates the time after CRF injection and the ordinate indicates the mean discharge rate expressed as a percentage of baseline (the average rate of 120 seconds before the injection). Bonferroni posthoc: ***p<0.001 vs. control, ###p<0.001 vs. SL.

Figure 4.

Immunoelectron microscopic evidence of a stress-induced shift in CRF₁ and CRF₂ receptor localization in DR dendrites. Representative dendritic profiles showing immunogold-silver labeling for the CRF₁ (A1) and CRF₂ (B1) receptor on the plasma membrane (arrowheads) and in the cytoplasm (arrows) of a control, SL and LL rat. Bar graphs indicate the percentage of cytoplasmic CRF₁ (A2) and CRF₂ (B2) for each condition (each group consists of data from 3–4 rats and mean per rat generated from at least 125 dendritic profiles). LL rats exhibited greater internalization of CRF₁ receptors compared with SL and controls and less internalization (more plasma membrane bound) of CRF₂ receptors compared with SL and controls. Tukey's posthoc: * $p < 0.05$, *** $p < 0.001$ vs. control, ## $p < 0.01$ vs. SL.

Table 1

Mean ratio of the integrated density of each western blot band of CRF_{2α}, sCRF_{2α} or β-arrestin2 in to the corresponding loading control

	CON (n=9)	SL (n=11)	LL (n=5)
CRF _{2α} (TMD): COX IV	0.102 ± 0.01	0.089 ± 0.01	0.099 ± 0.01
sCRF _{2α} : COX IV	0.098 ± 0.01	0.112 ± 0.01	0.102 ± 0.02
sCRF _{2α} : CRF2 (TMD)	1.04 ± 0.16	1.31 ± 0.15	1.05 ± 0.16
β-arrestin2: GAPDH	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.02

CRF_{2α}(TMD) antibody raised against the internal transmembrane domain of CRF_{2α} (amino acid 180–230). sCRF_{2α} generated to the unique sCRF_{2α} C-terminus,