

## Review article

## Role of brain norepinephrine in the behavioral response to stress

David A. Morilak<sup>\*</sup>, Gabe Barrera, David J. Echevarria, April S. Garcia, Angelica Hernandez,  
Shuaike Ma, Corina O. Petre*Department of Pharmacology and Center for Biomedical Neuroscience, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA*

Accepted 26 August 2005

Available online 13 October 2005

---

**Abstract**

The brain noradrenergic system is activated by acute stress. The post-synaptic effects of norepinephrine (NE), exerted at a cellular or neural circuit level, have been described as modulatory in nature, as NE facilitates responses evoked in target cells by both excitatory and inhibitory afferent input. Over the past few years, we have undertaken a series of studies to understand how these cellular modulatory effects of NE, elicited by acute stress, might translate into modulation of the behavioral–affective components of the whole-animal response to stress. Using microdialysis, we have demonstrated that acute immobilization stress activates NE release in a number of stress-related limbic forebrain target regions, such as the central and medial amygdala, lateral bed nucleus of the stria terminalis, medial prefrontal cortex, and lateral septum. Using microinjections of adrenergic antagonist drugs directly into these regions, we have shown that this stress-induced release of NE facilitates a number of anxiety-like behavioral responses that are mediated in these regions, including stress-induced reduction of open-arm exploration on the elevated plus-maze, stress-induced reduction of social interaction behavior, and activation of defensive burying behavior by contact with an electrified probe. Dysregulation of the brain noradrenergic system may be a factor in determining vulnerability to stress-related pathology, or in the interaction of genetic vulnerability and environmental sensitization. Compared to outbred Sprague–Dawley rats, we have shown that the modulatory effect of NE is deficient in Wistar–Kyoto rats, which also exhibit attenuated behavioral reactivity to acute stress, as well as increased vulnerability to stress-induced gastric ulcers and exaggerated activation of the hypothalamic–pituitary–adrenal (HPA) stress axis. Further, repeated exposure to mild intermittent cold stress resulted in a much greater sensitization of both the brain noradrenergic system and the HPA axis in Wistar–Kyoto rats compared to Sprague–Dawley rats. The recruitment of a robust noradrenergic facilitatory influence following repeated cold exposure in this previously deficient strain resulted in an aberrant HPA response, which may be illustrative of the kinds of neurobiological changes that may contribute to the development of stress-related neuropsychiatric disorders such as depression, post-traumatic stress disorder, or other anxiety disorders in predisposed or susceptible individuals. On the other side of the same issue, regulatory alterations in noradrenergic neurotransmission, or in the stress-modulatory functions of NE, may be important in the behavioral effects of chronic antidepressant drug treatment. We present recent preliminary results addressing the effects of chronic treatment with the selective NE reuptake inhibitor, desipramine, on acute behavioral reactivity to stress. A better understanding of the role of NE in adaptive responses to acute stress, the pathological consequences of prolonged, repeated or severe stress, and the mechanisms of action of drugs used to treat stress-related diseases, may contribute to the future development of more effective strategies for the treatment or even prevention of such disorders.

© 2005 Elsevier Inc. All rights reserved.

**Keywords:** Antidepressant; Anxiety; Behavior; Microdialysis; Norepinephrine; Stress

---

**Abbreviations:** ACTH, adrenocorticotrophic hormone; ADs, antidepressant drugs; BSTL, lateral bed nucleus of the stria terminalis; CeA, central amygdala, DMI, desipramine, HPA, hypothalamic–pituitary–adrenal; LC, locus coeruleus; MeA, medial amygdala; mPFC, medial prefrontal cortex; NE, norepinephrine; PTSD, post-traumatic stress disorder; WKY, Wistar–Kyoto.

<sup>\*</sup> Corresponding author. Department of Pharmacology, MC 7764, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA. Tel.: +1 210 567 4174; fax: +1 210 567 4303.

E-mail address: [Morilak@uthscsa.edu](mailto:Morilak@uthscsa.edu) (D.A. Morilak).

## Contents

1. Introduction . . . . .	1215
2. Activation of noradrenergic neurotransmission in response to acute stress . . . . .	1215
3. Modulatory effects of stress-induced noradrenergic neurotransmission on anxiety-like behavioral responses to acute stress . . . . .	1217
4. The noradrenergic system as a potential substrate for stress vulnerability? . . . . .	1219
5. Norepinephrine and the interaction of stress vulnerability and environmental sensitization . . . . .	1220
6. Pharmacological regulation of noradrenergic modulatory effect as a mechanism of therapeutic drug action . . . . .	1221
7. Summary and conclusions . . . . .	1222
Acknowledgements . . . . .	1222
References . . . . .	1222

## 1. Introduction

A diverse range of adaptive behavioral responses are evoked by acute exposure to a variety of stressful stimuli. In coordination with similarly induced neuroendocrine and autonomic responses, such behavioral adaptations, the exact nature of which are presumed to be both specific and appropriate to the provoking stimulus, serve to maintain homeostasis, enhance optimal functioning, and ensure survival in the face of a challenge or threat, either real or perceived. In addition to the primary neural circuits mediating contextually specific responses, acute stress also activates other brain systems that play a more widespread, modulatory role, serving to coordinate the complex, integrated organismic response to any stress. Among the most prominent of these stress-modulatory systems is the brain noradrenergic system.

The noradrenergic system originates in a relatively small number of cells located in the locus coeruleus (LC) and in other cell groups in the medulla and pons that utilize norepinephrine (NE) as a neurotransmitter. Nonetheless, an extensive network of noradrenergic terminals projecting from these few cells innervate essentially the entire neuraxis, from olfactory bulb to spinal cord. This widespread and divergent anatomical organization positions this system to potentially influence the operating characteristics of the entire nervous system under conditions of elevated noradrenergic activity. A seminal series of electrophysiological studies by Woodward and colleagues in the 1980's defined the prototypic neuromodulatory effects exerted by NE on the cellular activity of post-synaptic target neurons. Rather than inducing simple inhibition or excitation, NE altered the "signal to noise ratio" of responses evoked by other afferents, both excitatory and inhibitory, enhancing synaptic transmission in target circuits (Woodward et al., 1991b). Such modulatory effects have since been described for NE in many brain circuits, and have been shown to be mediated, via different transduction mechanisms, by both  $\beta$ - and  $\alpha_1$ -adrenergic receptors (Aghajanian and Rogawski, 1983; Jiang et al., 1996; Waterhouse et al., 1990; Woodward et al., 1991a).

Given the anatomical organization of the central noradrenergic system, it is likely, then, that stimuli that activate the limited population of hindbrain noradrenergic neurons will result in the release of NE in many target regions throughout

the brain, potentially altering the operating characteristics and reactivity of many neural circuits mediating a variety of behavioral and physiological responses. Further, the anatomical organization and modulatory cellular effects of NE suggest that it is unlikely to be either necessary or sufficient for the mediation of any specific responses, but rather may facilitate a number of responses evoked by other afferents. Thus, the observable effect of any widespread elevation in noradrenergic neurotransmission will depend on the set of specific neural circuits recruited, and the set of specific behavioral responses elicited by the stimulus or context which provoked the increase in NE release. Thus, if we are to translate the modulatory influence of NE at a cellular level to a modulatory influence at the circuit and ultimately the behavioral level, we must understand the contexts in which noradrenergic transmission is elevated.

## 2. Activation of noradrenergic neurotransmission in response to acute stress

Before considering any potential role for NE in the acute response to stress, it may be useful first to define, as objectively as possible, what is meant by the term "stress" (please also see related reviews by Herman and Cullinan, 1997; Sawchenko et al., 2000; Day, 2005—this issue). At a conceptual level, stress can be defined as any threat, either real or perceived, to the homeostasis and well-being of an organism. Within this definition, two broad and qualitatively differentiated categories of stressors emerge. The first, termed "systemic" or "physiologic" stress, is a real and imminent physical threat to health and well-being, in which the physical characteristics of the stimulus itself place immediate restorative response demands on the organism (e.g., injury with loss of blood). Conscious awareness or perception of the stimulus by the organism is not required for "stress" to be present, nor for a "stress response" to be initiated. The second category, called processive or psychogenic stress, depends upon perception, cognitive processing and interpretation of the stimulus to confer upon it a stressful quality (e.g., the threat posed by potential loss of a job). In this case, the relative severity of the stressor and its physiologic impact, indeed whether it is even considered to be stressful at all, can vary between individuals. Thus, for purposes of quantification, normalization or experimental

comparison, the severity of stress presented by a stimulus, whether physiologic or psychogenic, has typically been defined in terms of the magnitude of the physiological response it elicits, for instance by measuring activation of the hormonal hypothalamic–pituitary–adrenal (HPA) stress axis, or of the peripheral sympathoadrenal autonomic response system.

Noradrenergic neurons are excited briefly by punctate sensory stimuli of several modalities (Aston-Jones et al., 1991; Rasmussen et al., 1986), suggesting that information from the external as well as internal environment, transduced by a variety of sensory systems, gains access to the noradrenergic system. However, as the behavioral response to such innocuous stimuli habituates, so does the response of noradrenergic neurons (Aston-Jones et al., 1999, 2000; Rasmussen et al., 1986), suggesting also that information regarding the “salience” of the stimulus is an important factor in determining a noradrenergic response. Given this, both electrophysiological and neurochemical studies (i.e., in vivo microdialysis) have

shown that the brain noradrenergic system is phasically and robustly activated by a diverse array of acutely stressful stimuli, including immobilization, loud noise, immune challenge, electric shock, hypoglycemia, hypotension, cold exposure, bladder distension forced activity, and others (Abercrombie and Jacobs, 1987; Cecchi et al., 2002a; Morilak et al., 1987a,b; Pacak et al., 1995; Page et al., 1992; Svensson, 1987; Valentino et al., 1993). Whereas the brief bursts of electrical activity elicited by punctate, innocuous stimuli occur over a range of 100's of milliseconds, phasic activation of noradrenergic neurotransmission by acutely stressful stimuli is much longer lasting, on the order of seconds to minutes or hours, depending on the stimulus, often outlasting the duration of the stimulus itself, and correlated temporally with peripheral physiological indicators of the stress response (Jacobs et al., 1991).

For example, in recent studies we have used in vivo microdialysis to determine the phasic release of NE in limbic brain regions induced by acute immobilization stress, a

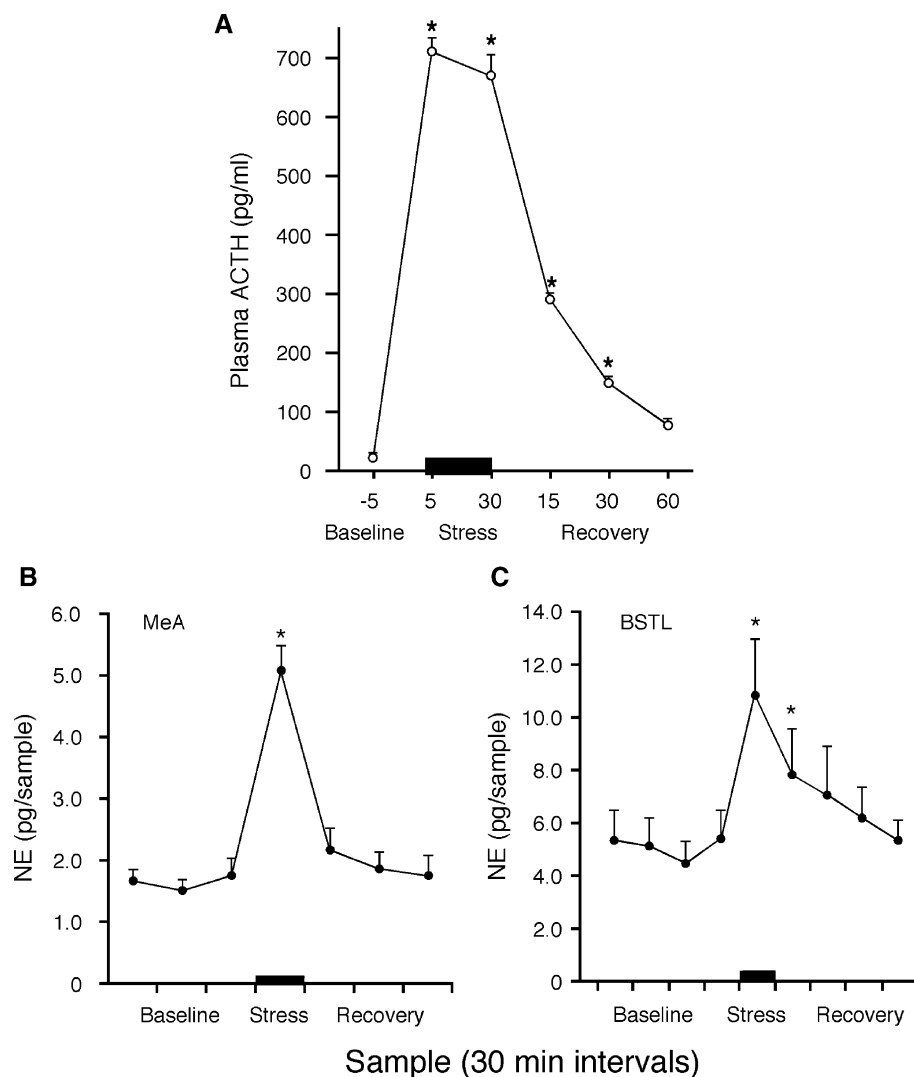


Fig. 1. Activation of NE release in limbic forebrain concurrent with activation of the HPA axis in response to acute immobilization stress. (A) Activation of the HPA axis induced by acute immobilization stress (bar), indicated by a phasic increase in plasma ACTH levels measured by RIA ( $n=8$ ). NE levels in microdialysate samples collected in MeA (panel B,  $n=7$ ) and BSTL (panel C,  $n=6$ ) were significantly increased during acute immobilization stress (Bar). Sample collection time was 30 min. All values expressed as mean  $\pm$  SE. \* $p<0.05$  compared to baseline. Panels A and B reproduced and adapted from (Ma and Morilak, 2005), with permission from Blackwell Publishing.

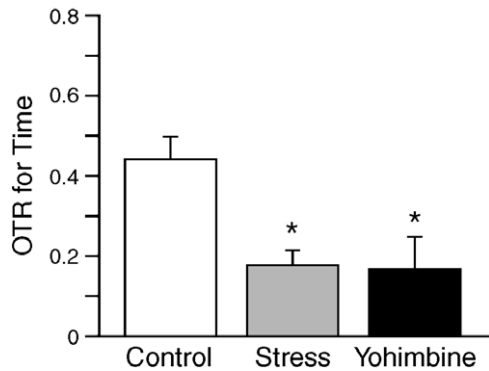


Fig. 2. Effects of acute immobilization stress (5 min), or administration of the  $\alpha_2$ -adrenergic autoreceptor antagonist yohimbine (2.5 mg/kg, i.p.) on anxiety-like behavioral reactivity on the elevated plus-maze. Exploratory activity on the open arms was measured as OTR for time (ratio of time spent in open arms relative to total time spent in all four arms). Values represent the mean  $\pm$  SE ( $n=6-18$  per group). \* $p<0.05$  compared to controls. Adapted from (Khoshbouei et al., 2002), with permission from Elsevier.

prototypic psychogenic stressor. For the stress, rats were placed prone on a flat, plastic rack large enough to support their entire body, with their limbs taped gently but securely to the rack with medical adhesive tape, and strips of tape placed across the body and the back of their head to prevent excessive head movements. In these neurochemical experiments, the period of immobilization was 30 min, after which the stress was terminated by gently removing the animal, and measurement of NE release continued for several hours thereafter. Plasma ACTH secretion, taken as an indicator of HPA activation in response to this stimulus, showed robust elevation within 5 min of stress onset, and was maintained at an elevated level for the duration of the stimulus, returning to baseline levels over 15–30 min following termination of the stress period (see Fig. 1A). Using microdialysis, we have measured the immobilization stress-induced activation of NE release in a number of limbic

forebrain regions thought to be involved in mediating a variety of behavioral, cognitive, affective, autonomic and neuroendocrine responses to stress, including regions such as the central amygdala (CeA), medial amygdala (MeA), lateral bed nucleus of the stria terminalis (BSTL), lateral septal nucleus (LS) and medial prefrontal cortex (mPFC) (Cecchi et al., 2002a,b; Garcia et al., 2003; Ma and Morilak, 2005; Pardon et al., 2002; Petre et al., 2004). The noradrenergic innervation of these regions range in density from quite high (BSTL) to sparse (CeA), and the origin of their noradrenergic innervation varies from those arising primarily from neurons in the A1 and A2 medullary cell groups (BSTL), to those receiving input from both medullary cells and the pontine LC (MeA), to those arising exclusively from the LC (mPFC). In all regions, acute immobilization stress elevated the release of NE, reflected in an increase in NE levels measured in the dialysis sample collected during exposure to stress (Fig. 1B and C). Extracellular NE levels returned to baseline levels over the 30 min following stress termination.

### 3. Modulatory effects of stress-induced noradrenergic neurotransmission on anxiety-like behavioral responses to acute stress

The facilitating effects that have been demonstrated for NE on evoked synaptic responses at the cellular and circuit level suggest that NE may facilitate specific behavioral responses mediated by those circuits. For example, it has been shown previously, that increasing noradrenergic transmission in the 5th cranial nerve motor nucleus, either pharmacologically or by acute stress exposure, facilitated the masseteric jaw closure reflex, a simple sensorimotor reflex mediated in that nucleus (Morilak and Jacobs, 1985; Stafford and Jacobs, 1990).

The integrated response to acute stress includes a prominent behavioral–affective component resembling anxiety, which has

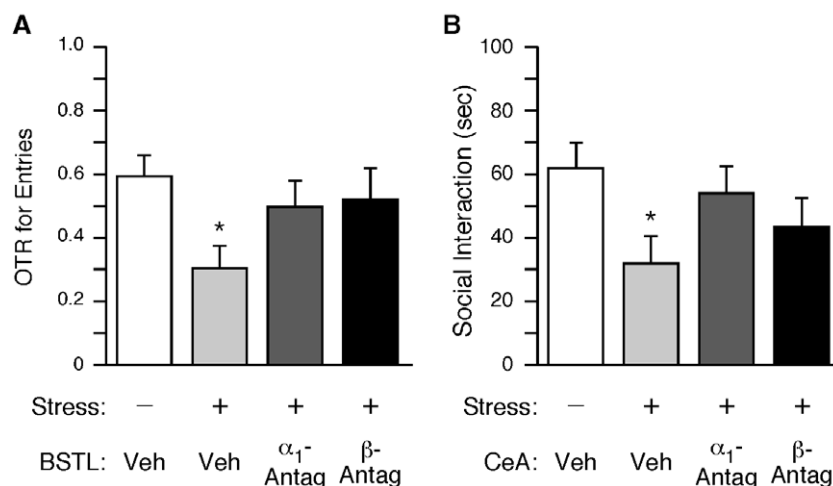


Fig. 3. Modulatory effects of NE on acute anxiety-like behavioral reactivity. A) Bilateral microinjection of either the  $\alpha_1$ -noradrenergic receptor antagonist benoxathian (2.0 nmoles) or a cocktail of  $\beta_1/\beta_2$ -antagonists betaxolol+ICI 118,551 (1 nmole each) into BSTL attenuated acute anxiety-like behavioral reactivity on the elevated plus-maze in response to 5 min immobilization stress ( $n=6-8$  per group). Exploratory behavior on the open arms was expressed as OTR for Entries. Adapted from (Cecchi et al., 2002a), with permission from Elsevier. B) Similarly, bilateral microinjection of benoxathian (2.0 nmole) into CeA blocked the reduction in social interaction time induced by 5 min acute immobilization stress ( $n=6-8$  per group). Adapted from (Cecchi et al., 2002b), with permission from Elsevier. All values expressed as mean  $\pm$  SE; \* $p<0.05$  compared to vehicle-microinjected controls.

been modeled using a number of well-validated behavioral tests in rats, such as the social interaction test, elevated plus maze, and shock-probe defensive burying test (File, 1995, 1988; Pellow et al., 1985; Treit et al., 1981). Acute pharmacological elevation of noradrenergic neurotransmission, for instance by systemic administration of the  $\alpha_2$ -adrenergic autoreceptor antagonist yohimbine, a known anxiogenic agent (Charney et al., 1987), can promote anxiety-like behavioral responding on such tests, similarly to the effects of acute stress (Fig. 2). This suggests that activation of NE release in the limbic forebrain by acute stressors may similarly facilitate anxiety-like behavioral responsivity in such anxiety-provoking situations.

We have tested this hypothesis in a recent series of experiments. Behavioral stress responsivity was defined

operationally as a change in behavior induced after a brief (5 min) exposure to immobilization stress as compared to the behavior of unstressed control rats. Selective adrenergic receptor antagonist drugs were microinjected into CeA or BSTL immediately prior to stress, followed by behavioral testing in the social interaction (SI) and elevated plus maze tests (Cecchi et al., 2002a,b). In these experiments, the behavioral response to acute immobilization stress was seen as a reduction in social behavior on the SI test, and a specific reduction in open-arm exploration on the elevated plus-maze (Fig. 3A, 3B). Blockade of  $\alpha_1$ -adrenergic receptors in the CeA attenuated the stress-induced reduction in social behavior, but  $\beta$ -antagonists had no effect, suggesting that stress-induced NE release acted specifically on  $\alpha_1$  receptors in CeA to facilitate the behavioral response seen on the SI test (Fig. 3B). By

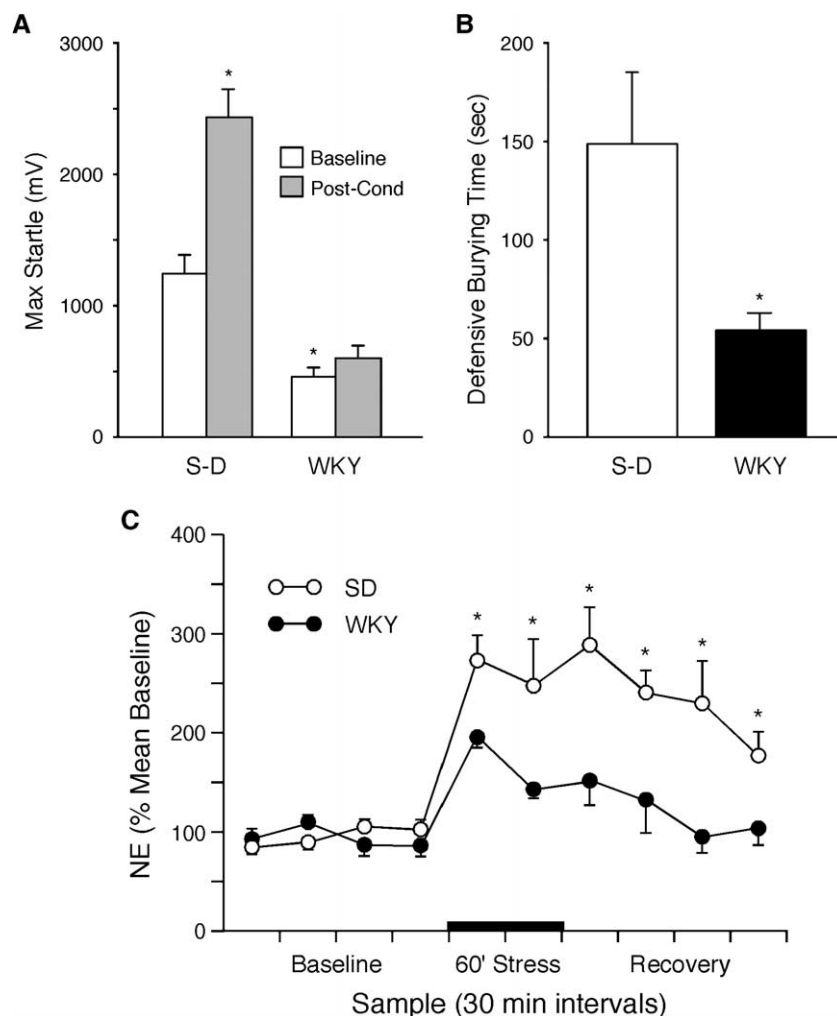


Fig. 4. WKY rats exhibit lower behavioral reactivity and reduced noradrenergic reactivity to acute stress compared to Sprague–Dawley (SD) rats. A) WKY rats showed lower baseline acoustic startle responding (white bars) and a lack of fear-potentiation of startle responding in trials preceded by presentation of a light that had been previously paired to a shock (gray bars). Startle amplitude (mV) is expressed as mean  $\pm$  SE ( $n=6-9$  per group). \* $p<0.05$  compared to unconditioned SD baseline. Adapted from (Pardon et al., 2002), with permission from Elsevier. B) WKY rats exhibited less shock-probe defensive burying behavior than SD rats. Burying time (measured during a 900 s test) is expressed as mean  $\pm$  SE ( $n=15-16$  per group); \* $p<0.05$ , (Ma and Morilak, unpublished data). C) Differences between WKY and SD rats in the acute increase in NE release induced in BSTL by 60 min immobilization stress (bar), measured in microdialysis samples collected every 30 min. To compare the relative magnitude of the stress-induced increase in NE levels, all values were converted to a percent of the mean pre-stress baseline level in each rat, expressed as mean  $\pm$  SE for the group ( $n=7$  per group). Both strains showed a significant increase in NE levels relative to their baseline in response to acute immobilization stress ( $p<0.05$ , not indicated for the sake of clarity). The response in WKY rats was, however, of lesser magnitude and shorter duration compared to SD rats. \* $p<0.05$  comparing the two strains at the same time point. Adapted from (Pardon et al., 2002), with permission from Elsevier.



contrast, adrenergic receptor blockade in the CeA had no effect on the stress-induced reduction in open-arm exploration on the elevated plus-maze. The opposite was true, however, when adrenergic antagonists were administered into BSTL, where blockade of either  $\alpha_1$  or  $\beta$  receptors attenuated the reduction in open-arm exploration on the plus-maze, but had no effect on the stress-induced reduction in social behavior (Fig. 3A).

These results might be interpreted to suggest that stress-induced NE release in the CeA and BSTL facilitated specific behavioral responses mediated by circuitry involving these regions. However, the response to stress in both of these tests represents an inhibition of ongoing behavior — open arm exploration in the elevated plus maze, and social behavior in the social interaction test. It is therefore possible that NE preferentially facilitated or biased its target response circuits toward inhibitory responses, rather than facilitating evoked responses in general. To address this possibility, it was important to use the same strategy to test the effects of NE on a response that represented an elicitation of active behavioral responding that would not otherwise occur in the absence of stress. Thus, in a recent preliminary study, we have also demonstrated release of NE in the lateral septum (LS) upon exposure to an electrified shock-probe, and showed that microinjection of both  $\alpha_1$ - and  $\beta$ -receptor antagonists into LS attenuated the active defensive burying behavioral response elicited in this test as well (Petre et al., 2004).

Thus, stress-induced activation of NE neurotransmission in CeA, BSTL and LS facilitated behavioral responses evoked by acute stress, whether these responses represented an inhibition of ongoing behavior, or a de novo activation of behavior that would not otherwise occur in the absence of acute stress. Moreover, it did so in a region-specific, receptor-specific and response-specific fashion. Importantly, none of the antagonists in any of these regions had any effect on baseline unstressed behavioral activity, suggesting that it was specifically the phasic, stress-activated noradrenergic neurotransmission that facilitated these acute stress-evoked responses, but that tonic, basal noradrenergic neurotransmission had little effect on baseline, “unstimulated” activity that may be mediated by these behavioral response circuits.

#### **4. The noradrenergic system as a potential substrate for stress vulnerability?**

An inability to appropriately initiate or regulate aspects of the stress response has been proposed as a potentially critical factor in the pathophysiology of various stress-related disorders (Gold and Chrousos, 1999; Johnson et al., 1992). Dysregulation of noradrenergic neurotransmission has been implicated in stress-related psychiatric diseases such as depression, post-traumatic stress disorder (PTSD) and other anxiety disorders (Schatzberg and Schildkraut, 1995; Southwick et al., 1993; Sullivan et al., 1999). However, although stress is a precipitating factor in a number of such psychiatric disorders, not all individuals exposed to an equivalent level of stress develop pathological consequences. Even for PTSD, in which a clear connection exists between an identifiable

trauma and the subsequent development of inappropriate responses to neutral or mild stimuli, not all survivors of the same trauma exhibit the disorder. Thus, individual vulnerability factors, including, for example, genetic predisposition, must also be involved. The degree and nature of any stress-related psychopathology must then result from an interaction of this predisposition with exposure to a sufficiently sensitizing environmental stimulus. We have recently examined this interaction, and the possibility that endogenous differences in the reactivity or regulation of the brain noradrenergic system might contribute to stress vulnerability, using rat strains defined by differences in their stress response and their vulnerability to certain stress-related illness. The inbred Wistar–Kyoto (WKY) rat strain in particular has been proposed as a model of stress susceptibility, as they develop stress-induced gastric ulcers more readily than their parent Wistar strain (Redei et al., 1994), and show exaggerated HPA responses to acute stress (Pare and Redei, 1993; Redei et al., 1994).

In behavioral tests, when compared to the outbred Sprague–Dawley strain, WKY rats exhibited a high degree of passivity, immobility, and an overall deficit of baseline behavioral activity, perhaps indicative of a lower level of baseline behavioral arousal. Evidence of this tendency for behavioral passivity was seen in their reduced baseline exploratory activity in both the elevated plus maze and the open field, depressed baseline startle reactivity, and reduced baseline social behavior (Pardon et al., 2002). Similarly, when acute behavioral reactivity was tested, WKY rats exhibited a lack of fear-potentiation of the acoustic startle response, a lack of acute stress-induced reductions in both social behavior and open-arm exploratory behavior on the plus-maze (Pardon et al., 2002), and reduced defensive burying activity (Pare, 1994; Ma and Morilak, unpublished data), all indicating an attenuation of acute behavioral stress responsivity (Fig. 4A, 4B). Consistent with these behavioral observations, microdialysis experiments revealed a corresponding attenuation of phasic stress-induced NE release in the BSTL of WKY rats. Thus, the reduced basal activity and blunted acute behavioral stress responsivity seen in WKY rats were associated with deficient brain noradrenergic reactivity (Fig. 4C).

Such a deficit in noradrenergic modulatory function might hinder the ability of WKY rats to cope with stress, which may explain not only their increased susceptibility to stress-related pathology, but also their exaggerated HPA responses. Thus, despite the facilitation of HPA activity shown previously for NE in BSTL (Cecchi et al., 2002a), these data would suggest that the exaggerated HPA responses to acute stress seen in WKY rats are not attributable to elevated noradrenergic facilitation, but rather may be secondary to a deficit of adequate coping capability, resulting in an increase in the relative “impact” of stress. Consistent with this interpretation, adrenergic antagonist administration in BSTL failed to reduce stress-induced ACTH secretion in WKY rats as it did in Sprague–Dawley rats (Pardon et al., 2003).

In a subsequent study to begin to address the possible neurobiological mechanisms underlying dysregulation of the

stress response in WKY rats, we investigated potential strain differences in stress-induced c-Fos expression in brain regions that are both involved in regulating behavioral and neuroendocrine stress responses, and are also related to the noradrenergic system, either as targets of noradrenergic modulation or as sources of afferent innervation of noradrenergic neurons. In this study, we found that stress-induced c-Fos expression was lower in WKY rats compared to Sprague–Dawley rats in the LC and also in the MeA, whereas there were no strain differences in c-Fos induction in other regions examined, including the BST, CeA and PVN (Fig. 5) (Ma and Morilak, 2004). The MeA has been shown to be involved in the activation of brainstem noradrenergic neurons in response to psychogenic stress (Dayas and Day, 2002). Thus, a reduction in stress-induced activation of the MeA could be a potential mechanism to account for the reduction in stress-induced activation of the noradrenergic system in WKY rats. However, in addition to providing a descending excitatory input to the brainstem noradrenergic system, the MeA is also a target of ascending noradrenergic innervation. Thus, from these data alone, it is not possible to determine whether the attenuated activation of MeA in WKY rats may represent an upstream mechanism contributing to the reduced noradrenergic activation seen in this strain, or if it may instead represent a potential downstream consequence of that reduced noradrenergic activation.

### 5. Norepinephrine and the interaction of stress vulnerability and environmental sensitization

Having demonstrated a strain difference in both noradrenergic and behavioral stress reactivity, we have recently begun to investigate how such genetically determined differences might interact with sensitizing environmental stimuli to produce aberrant changes in the stress response that could potentially contribute to an increased vulnerability to stress-related pathology. Chronic intermittent exposure to cold has

been shown to sensitize subsequent activation of the HPA axis by a novel acute stressor (Bhatnagar and Dallman, 1998). More severe and prolonged cold exposure has also been shown to enhance excitation of the electrophysiological activity of noradrenergic neurons in LC (Mana and Grace, 1997), and acute stress-induced activation of NE release in the forebrain (Jedema et al., 1999; Nisenbaum et al., 1991). Using a mild cold stress protocol (7 days, 4 h/day, 4 °C), we compared the sensitivity of WKY and Sprague–Dawley rats to the sensitizing influence of chronic intermittent cold exposure on HPA and noradrenergic stress reactivity, and also on noradrenergic facilitation of the HPA response. We found that chronic cold exposure enhanced acute immobilization stress-induced release of NE in the BSTL of WKY rats but not Sprague–Dawley rats (Fig. 6A). Chronic cold also potentiated activation of the HPA axis by acute immobilization stress in both strains, although to a greater extent in WKY rats (Fig. 6B). Moreover, the sensitized noradrenergic response in WKY rats had a significant impact on their neuroendocrine response to stress. As we had shown previously,  $\alpha_1$  receptor blockade in BSTL of non-cold-exposed WKY rats was ineffective in altering the HPA response, indicating a lack of noradrenergic facilitation in this strain. However, the enhanced ACTH response to acute stress in WKY rats after cold-sensitization was attenuated by microinjection of an  $\alpha_1$  antagonist into BSTL (Fig. 6B). By contrast,  $\alpha_1$  adrenergic receptor blockade in BSTL produced a similar degree of attenuation of the HPA response of both cold-exposed and control Sprague–Dawley rats. Thus, chronic cold exposure not only sensitized the stress-induced release of NE in WKY rats, it also induced noradrenergic facilitation of their HPA response. Such functional sensitization of a previously deficient facilitatory system may thus be one mechanism whereby exposure to repeated or severe stress may induce pathologic dysregulation of the stress response in susceptible individuals. To date, because of the profound inhibition of baseline behavioral activity exhibited by unstressed WKY rats relative to the comparator Sprague–Dawley strain, we have not

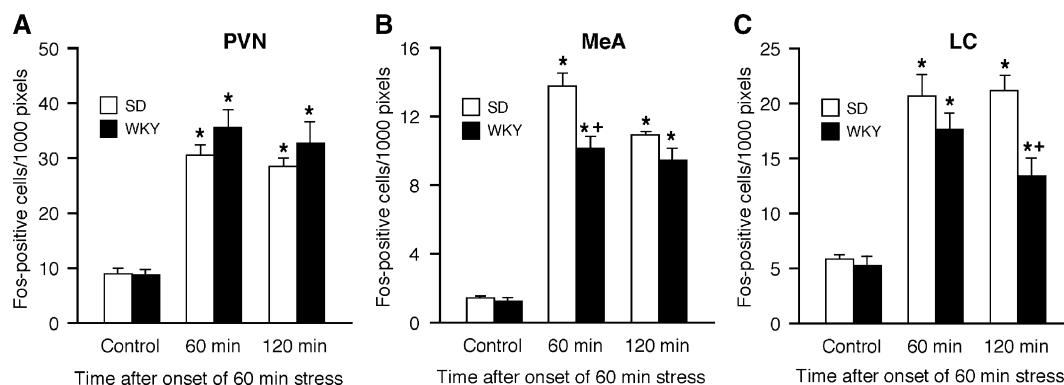


Fig. 5. Differential Fos induction in select brain regions of WKY and SD rats by acute stress. A) A similar degree of Fos induction was seen in PVN of SD and WKY rats after 60 min immobilization stress. Data expressed as number of Fos-immunopositive cells/1000 pixels<sup>2</sup> (mean ± SE,  $n=4-5$ /group). B) Fos expression was induced in MeA of both strains by acute immobilization stress, with significantly more Fos induction in MeA of SD rats compared to WKY rats at the end of 60 min stress. No strain difference was seen after 60 min post-stress recovery (ie, 120 min after stress onset). C) Immobilization stress also induced a significant increase in Fos expression in the LC of both SD and WKY rats, although Fos induction was greater in the LC of SD rats, the difference being significant at 120 min ( $n=4-5$ /group). In all panels: \* $p<0.05$  compared to same-strain baseline; + $p<0.05$  between strains at the same time point. Figures reproduced from (Ma and Morilak, 2004), with permission from Elsevier.

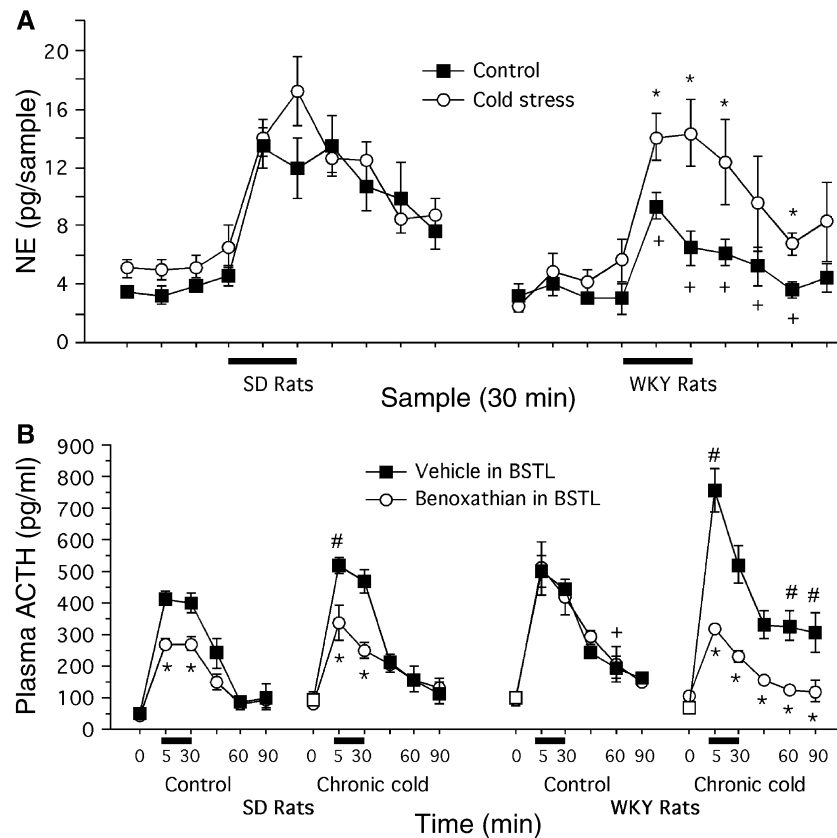


Fig. 6. Strain differences in sensitization of the response of brain NE and the HPA axis to acute stress following chronic intermittent cold exposure. A) In all groups, 60 min acute immobilization stress (bars) induced a significant elevation in NE release measured in BSTL by microdialysis relative to their respective baselines ( $p < 0.05$ , not indicated for the sake of clarity). Chronic cold exposure produced no change in the acute stress-induced increase in NE release in BSTL of SD rats, but significantly sensitized the response in WKY rats compared to their non-cold-exposed controls. Samples collected every 30 min; NE levels expressed as pg/sample (mean  $\pm$  SE,  $n = 5-6$  per group). \* $p < 0.05$  compared to control rats of the same strain at the same time point; + $p < 0.05$  control WKY compared to control SD rats at the same time point. B) Pharmacological results revealing the differential sensitization of noradrenergic modulation of the HPA stress response in the BSTL of SD and WKY rats by chronic intermittent cold exposure. As shown previously, the baseline HPA response to acute immobilization stress (bars), measured by plasma ACTH levels, was slightly greater and longer lasting in WKY rats than in SD rats (+ $p < 0.05$ ). Further, although chronic cold exposure enhanced the acute stress-induced ACTH response in both strains, the sensitizing effect of chronic cold was greater in WKY rats, both in magnitude and duration (# $p < 0.05$  comparing cold-exposed rats to control rats of the same strain at the same time points). To reveal the degree of noradrenergic facilitation of the HPA response, bilateral microinjections of vehicle or the  $\alpha_1$ -adrenergic receptor antagonist benoxathian (2 nmol) were made into BSTL prior to acute immobilization stress. As also shown previously, blockade of  $\alpha_1$ -receptors in BSTL of control SD rats attenuated stress-induced ACTH secretion by approximately 40%, but had no effect in control WKY rats, indicating a lack of noradrenergic modulatory influence in this strain. However, after cold sensitization, benoxathian significantly attenuated the HPA response in WKY rats as well (\* $p < 0.05$  comparing benoxathian-treated rats to vehicle-treated rats of the same strain and cold condition at the same time point), suggesting that some of the cold-induced sensitization may be attributable to a recruitment of noradrenergic facilitatory influence in this previously deficient strain. Plasma ACTH is expressed as mean  $\pm$  SE pg/ml ( $n = 6-7$  per group). Reproduced from (Pardon et al., 2003), with permission from Elsevier.

yet examined the effects of chronic cold sensitization on specific stress-induced behavioral responses in WKY rats, although we are actively pursuing methods to address this issue in ongoing experiments.

## 6. Pharmacological regulation of noradrenergic modulatory effect as a mechanism of therapeutic drug action

Whether primary dysregulation of noradrenergic function is a factor in the etiology or manifestation of stress-related psychiatric disorders or not, noradrenergic neurotransmission is a regulatory target of many of the drugs used to treat such disorders. Alterations in noradrenergic neurotransmission are important in the actions of many classes of antidepressant drugs (ADs), including tricyclic ADs, many of which block the reuptake of both NE and serotonin, and a newer generation of

dual uptake inhibitors as well as selective NE reuptake inhibitors. These drugs are effective in alleviating depressed mood, social withdrawal and other “inhibitory” symptoms of depression. In addition, anxiety is a prominent component of depression, and these drugs are also effective at alleviating anxiety and other symptoms of depression that may be indicative of behavioral or emotional “activation”, including aggression, agitation, psychomotor tension, distress, etc. There is even some limited evidence that they may be effective in treating certain anxiety disorders as well (Ferguson et al., 2002; Nelson, 1999; Versiani et al., 2002).

Chronic treatment with drugs that block NE reuptake produce a tonic elevation in the extracellular levels of NE in target brain regions, and it seems likely that this elevated level of NE might contribute in some way to many of the antidepressant behavioral effects of such drugs, particularly



those involving an enhancement of affective or cognitive functioning, e.g., increases in arousal, attention, psychomotor activity, etc. However, as was shown in the experiments described above, the dynamically activated increases in noradrenergic transmission that are induced by acute stress exposure facilitate anxiety-like behavioral responding to such stimuli, and such acute behavioral responses were apparently not influenced by baseline levels of noradrenergic transmission in the absence of acute stress. Thus, if changes in noradrenergic neurotransmission also play a role in the anxiolytic efficacy of antidepressant drugs in the context of treating depression, then chronic treatment with such drugs must not only produce an increase in tonic extracellular levels of NE, but they must also somehow attenuate the acute stress-induced activation of noradrenergic transmission that contributes to anxiety-like behavioral responding. In a recent series of pilot studies, we have obtained evidence that such regulatory alterations may indeed occur following chronic treatment with the selective NE reuptake blocker desipramine (DMI). After rats were treated for 3 weeks with DMI (15 mg/kg/day, delivered continuously by osmotic minipump), we observed a reduction in the defensive burying response, and also an attenuation of the acute stress-induced reduction in open arm exploration on the elevated plus maze (Garcia et al., 2004), both of which had been shown previously to be facilitated by stress-induced NE release (see above). Understanding the mechanisms by which chronic NE reuptake blockade can differentially regulate tonic steady-state NE levels and acute stress-activated noradrenergic function, reducing acute facilitatory activity in the face of tonically elevated basal activity, may provide important insights into the mechanisms by which antidepressant drugs more generally can produce their clinically relevant behavioral effects. Indeed, the mechanisms by which chronic treatment with antidepressants of many drug classes can regulate monoaminergic neurotransmission, and how such alterations in neurotransmitter function can produce the behavioral alterations that contribute to the clinical efficacy of these drugs have generated a tremendous amount of intense research effort. Understanding how these drugs exert their behavioral effects is likely to lead not only to the development of more effective, rapid or specific treatment strategies, but also to a better understanding of the etiology of stress-related psychiatric disorders themselves, which have thus far remained elusive at best.

## 7. Summary and conclusions

We are just beginning to understand how the widespread and divergent anatomical organization of the brain noradrenergic system, together with the cellular modulatory influence exerted by NE on its post-synaptic targets, might serve to enhance a number of behavioral responses elicited specifically by stress, and mediated by a variety of neural circuits throughout the brain. More generally, a growing body of research suggests that the activation of brain noradrenergic neurotransmission by acute stress acts to facilitate an array of neuroendocrine, autonomic, behavioral and cognitive components of the integrated, organismic response to stress. Given

such a widespread modulatory effect influencing a number of systems comprising the adaptive response to stress, it is likely that dysregulation of the brain noradrenergic system may also represent a potential substrate for the interaction between environmental sensitization and individual vulnerability leading to stress-related psychopathology. Moreover, whether or not a primary dysfunction of noradrenergic modulatory activity is a factor in the development of specific psychiatric disorders, the pharmacological regulation of noradrenergic modulatory function represents a potentially powerful and important mechanism whereby psychotherapeutic drug intervention can alleviate many of the symptoms of stress-related psychiatric illnesses such as depression, PTSD or other anxiety disorders. It is therefore important to continue to clarify the exact role played by this system in behavioral adaptation to stress, as well as in the development of maladaptive consequences of chronic, repeated or severe stress, and also to better understand how the regulation of this system by pharmacological or other therapeutic interventions may contribute to the successful alleviation of such disorders.

## Acknowledgements

Original research conducted in the authors laboratory was supported by research grants from the National Institute of Mental Health (MH60118 and MH53851), and from the Veterans Administration and Department of Defense. All experiments and procedures described were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and were consistent with NIH guidelines for the care and use of laboratory animals. The authors would like to acknowledge the contributions made to original research described in this paper by Dr. Marco Cecchi, Dr. Habibeh Khoshbouei, Dr. Marie-Christine Pardon, and Ms. Lauren Phillips. Some of the material in this review has been modified from material presented in a previous review chapter by Morilak, D.A., "Norepinephrine and Stress", in *Norepinephrine: Neurobiology and Therapeutics*, (G. Ordway, M. Schwartz and A. Frazer, eds.), Cambridge Press, *in press*.

## References

- Abercrombie, E.D., Jacobs, B.L., 1987. Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats: I. Acutely presented stressful and nonstressful stimuli. *J. Neurosci.* 7, 2837–2843.
- Aghajanian, G.K., Rogawski, M.A., 1983. The physiological role of  $\alpha$ -adrenoceptors in the CNS: new concepts from single-cell studies. *Trends Pharmacol. Sci.* 4, 315–317.
- Aston-Jones, G., Chiang, C., Alexinsky, T., 1991. Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. *Prog. Brain Res.* 88, 501–520.
- Aston-Jones, G., Rajkowski, J., Cohen, J., 1999. Role of locus coeruleus in attention and behavioral flexibility. *Biol. Psychiatry* 46, 1309–1320.
- Aston-Jones, G., Rajkowski, J., Cohen, J., 2000. Locus coeruleus and regulation of behavioral flexibility and attention. *Prog. Brain Res.* 126, 165–182.
- Bhatnagar, S., Dallman, M., 1998. Neuroanatomical basis for facilitation of hypothalamic–pituitary–adrenal responses to a novel stressor after chronic stress. *Neurosci.* 84, 1025–1039.

- Cecchi, M., Khoshbouei, H., Javors, M., Morilak, D.A., 2002a. Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. *Neurosci.* 112, 13–21.
- Cecchi, M., Khoshbouei, H., Morilak, D.A., 2002b. Modulatory effects of norepinephrine, acting on  $\alpha_1$ -receptors in the central nucleus of the amygdala, on behavioral and neuroendocrine responses to acute immobilization stress. *Neuropharmacology* 43, 1139–1147.
- Charney, D.S., Woods, S.W., Goodman, W.K., Heninger, G.R., 1987. Neurobiological mechanisms of panic anxiety: biochemical and behavioral correlates of yohimbine-induced panic attacks. *Am. J. Psychiatry* 144, 1030–1036.
- Day, T.A., 2005—this issue. Defining stress as a prelude to mapping its circuitry: No help from allostasis. *Prog. Neuropsychopharmacol. Biol. Psychiatr.* 29, 1195–1200.
- Dayas, C.V., Day, T.A., 2002. Opposing roles for medial and central amygdala in the initiation of noradrenergic cell responses to a psychological stressor. *Eur. J. Neurosci.* 15, 1712–1718.
- Ferguson, J.M., Mendels, J., Schwart, G.E., 2002. Effects of reboxetine on Hamilton Depression Rating Scale factors from randomized, placebo-controlled trials in major depression. *Int. Clin. Psychopharmacol.* 17, 45–51.
- File, S., 1995. Animal models of different anxiety states. *Adv. Biochem. Psychopharmacol.* 48, 93–113.
- File, S.E., 1988. How good is social interaction as a test of anxiety? In: Simon, P., Soubrie, P., Widlocher, D. (Eds.), *Selected Models of Anxiety, Depression and Psychosis*. Karger, Basel, pp. 151–166.
- Garcia, A., Barrera, G., Burke, T., Hensler, J., Morilak, D.A., 2003. Autoreceptor-mediated inhibition of norepinephrine release in rat medial prefrontal cortex is maintained after chronic desipramine treatment. *Abstr. - Soc. Neurosci.* 29 (Online: Program no. 793.2.)
- Garcia, A.S., Barrera, G., Morilak, D.A., 2004. Chronic treatment with desipramine attenuates acute anxiety-like behavioral reactivity on the elevated plus-maze and shock-probe defensive burying test. *Abstr. - Soc. Neurosci.* 30 (Online: Program no. 466.16).
- Gold, P.W., Chrousos, G.P., 1999. The endocrinology of melancholic and atypical depression: relation to neurocircuitry and somatic consequences. *Proc. Assoc. Am. Physicians* 111, 22–34.
- Herman, J.P., Cullinan, W.E., 1997. Neurocircuitry of stress: central control of the hypothalamo–pituitary–adrenocortical axis. *Trends Neurosci.* 20, 78–84.
- Jacobs, B.L., Abercrombie, E.D., Fornal, C.A., Levine, E.S., Morilak, D.A., Stafford, I.L., 1991. Single-unit and physiological analyses of brain norepinephrine function in behaving animals. *Prog. Brain Res.* 88, 159–165.
- Jedema, H.P., Sved, A.F., Zigmond, M.J., Finlay, J.M., 1999. Sensitization of norepinephrine release in medial prefrontal cortex: effect of different chronic stress protocols. *Brain Res.* 830, 211–217.
- Jiang, M., Griff, E.R., Ennis, M., Zimmer, L.A., Shipley, M.T., 1996. Activation of locus coeruleus enhances the responses of olfactory bulb mitral cells to weak olfactory nerve input. *J. Neurosci.* 16, 6319–6329.
- Johnson, E.O., Kamilaris, T.C., Chrousos, G.P., Gold, P.W., 1992. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. *Neurosci. Biobehav. Rev.* 16, 115–130.
- Khoshbouei, H., Cecchi, M., Dove, S., Javors, M., Morilak, D.A., 2002. Behavioral reactivity to stress: amplification of stress-induced noradrenergic activation elicits a galanin-mediated anxiolytic effect in central amygdala. *Pharmacol. Biochem. Behav.* 71, 407–417.
- Ma, S., Morilak, D.A., 2004. Induction of Fos expression by acute immobilization stress is reduced in locus coeruleus and medial amygdala of Wistar–Kyoto rats compared to Sprague–Dawley rats. *Neurosci.* 124, 963–972.
- Ma, S., Morilak, D.A., 2005. Norepinephrine release in medial amygdala facilitates activation of the HPA axis in response to acute immobilization stress. *J. Neuroendocrinol.* 17, 22–28.
- Mana, M.J., Grace, A.A., 1997. Chronic cold stress alters the basal and evoked electrophysiological activity of rat locus coeruleus neurons. *Neurosci.* 81, 1055–1064.
- Morilak, D.A., Jacobs, B.L., 1985. Noradrenergic modulation of sensorimotor processes in intact rats: the masseteric reflex as a model system. *J. Neurosci.* 5, 1300–1306.
- Morilak, D.A., Fornal, C.A., Jacobs, B.L., 1987a. Effects of physiological manipulations on locus coeruleus neuronal activity in freely moving cats: II. Cardiovascular challenge. *Brain Res.* 422, 24–31.
- Morilak, D.A., Fornal, C.A., Jacobs, B.L., 1987b. Effects of physiological manipulations on locus coeruleus neuronal activity in freely moving cats: III. Glucoregulatory challenge. *Brain Res.* 422, 32–39.
- Nelson, J.C., 1999. A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. *Biol. Psychiatry* 46, 1301–1308.
- Nisenbaum, L.K., Zigmond, M.J., Sved, A.J., Abercrombie, E.D., 1991. Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. *J. Neurosci.* 11, 1478–1484.
- Pacak, K., Palkovits, M., Kopin, I.J., Goldstein, D.S., 1995. Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary–adrenocortical and sympathoadrenal activity: in vivo microdialysis studies. *Front. Neuroendocrinol.* 16, 89–150.
- Page, M.E., Akaoka, H., Aston-Jones, G., Valentino, R.J., 1992. Bladder distension activates noradrenergic locus coeruleus neurons by an excitatory amino acid mechanism. *Neurosci.* 51, 555–563.
- Pardon, M.-C., Gould, G.G., Garcia, A., Phillips, L., Cook, M.C., Miller, S.A., Mason, P.A., Morilak, D.A., 2002. Stress reactivity of the brain noradrenergic system in three rat strains differing in their neuroendocrine and behavioral responses to stress: implications for susceptibility to stress-related neuropsychiatric disorders. *Neurosci.* 115, 229–242.
- Pardon, M.-C., Ma, S., Morilak, D.A., 2003. Chronic cold stress sensitizes brain noradrenergic reactivity and noradrenergic facilitation of the HPA stress response in Wistar Kyoto rats. *Brain Res.* 971, 55–65.
- Pare, W.P., 1994. Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats. *Physiol. Behav.* 55, 433–439.
- Pare, W.P., Redei, E., 1993. Depressive behavior and stress ulcer in Wistar Kyoto rats. *J. Physiol.* 87, 229–238.
- Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* 14, 149–167.
- Petre, C.O., Barrera, G., Morilak, D.A., 2004. Noradrenergic facilitation of shock-probe defensive burying in lateral septum. *Abstr. - Soc. Neurosci.* 30 (Online Program no. 466.18).
- Rasmussen, K., Morilak, D.A., Jacobs, B.L., 1986. Single unit activity of locus coeruleus neurons in the freely moving cat: I. During naturalistic behaviors and in response to simple and complex stimuli. *Brain Res.* 371, 324–334.
- Redei, E., Pare, W.P., Aird, F., Kluczynski, J., 1994. Strain differences in hypothalamic–pituitary–adrenal activity and stress ulcer. *Am. J. Physiol.* 266, R353–R360.
- Sawchenko, P.E., Li, H.-Y., Ericsson, A., 2000. Circuits and mechanisms governing hypothalamic responses to stress: a tale of two paradigms. *Prog. Brain Res.* 122, 61–78.
- Schatzberg, A.F., Schildkraut, J.J., 1995. Recent studies on norepinephrine systems in mood disorders. In: Bloom, F.E., Kupfer, D.J. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press Ltd., New York, pp. 911–920.
- Southwick, S.M., Krystal, J.H., Morgan, C.A., Johnson, D., Nagy, L.M., Nicolaou, A., Heninger, G.R., Charney, D.S., 1993. Abnormal noradrenergic function in posttraumatic stress disorder. *Arch. Gen. Psychiatry* 50, 266–274.
- Stafford, I.L., Jacobs, B.L., 1990. Noradrenergic modulation of the masseteric reflex in behaving cats: II. Physiological studies. *J. Neurosci.* 10, 99–107.
- Sullivan, G.M., Coplan, J.D., Kent, J.M., Gorman, J.M., 1999. The noradrenergic system in pathological anxiety: a focus on panic with relevance to generalized anxiety and phobias. *Biol. Psychiatry* 46, 1205–1218.
- Svensson, T.H., 1987. Peripheral, autonomic regulation of locus coeruleus noradrenergic neurons in brain: putative implications for psychiatry and psychopharmacology. *Psychopharmacology* 92, 1–7.
- Treit, D., Pinel, J.P.J., Fibiger, H.C., 1981. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. *Pharm. Biochem. Behav.* 15, 619–626.

- Valentino, R.J., Foote, S.L., Page, M.E., 1993. The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. *Ann. N. Y. Acad. Sci.* 697, 173–188.
- Versiani, M., Cassano, G., Perugi, G., Benedetti, A., Mastalli, L., Nardi, A., Savino, M., 2002. Reboxetine, a selective norepinephrine reuptake inhibitor, is an effective and well-tolerated treatment for panic disorder. *J. Clin. Psychiatry* 63, 31–37.
- Waterhouse, B.D., Azizi, S.A., Burne, R.A., Woodward, D.J., 1990. Modulation of rat cortical area 17 neuronal responses to moving visual stimuli during norepinephrine and serotonin microiontophoresis. *Brain Res.* 514, 276–292.
- Woodward, D.J., Moises, H.C., Waterhouse, B.D., Yeh, H.H., Cheun, J.E., 1991a. The cerebellar norepinephrine system: inhibition, modulation, and gating. *Prog. Brain Res.* 88, 331–341.
- Woodward, D.J., Moises, H.C., Waterhouse, B.D., Yeh, H.H., Cheun, J.E., 1991b. Modulatory actions of norepinephrine on neural circuits. *Adv. Exp. Med. Biol.* 287, 193–208.