Exploring effects of stress from a cellular and molecular perspective
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Summary and Conclusions

“Imagination is more important than knowledge”

Albert Einstein
Current treatments for depression are inadequate for many individuals and progress in understanding the neurobiology of this or other affective disorders is slow. Nevertheless, several promising hypotheses concerning the mechanisms underlying stress-induced pathology and antidepressant response have recently been formulated. These have been largely based on the dysregulation of the HPA axis and implicate abnormal glucocorticoids, neurotrophic factors and CREB regulation. The data presented in this thesis seem to corroborate many of these aspects, although our findings also suggest some surprising effects of stress with regard to gender discrepancies. The fact that stress, particularly when prolonged and severe, affects both functional and structural neuronal integrity has been well established and is illustrated in chapter 1. However, the molecular mechanisms involved in these stress-mediated adaptations appear to differ between male and female rats as presented in chapter 2. Nevertheless, following long-term antidepressant treatments, females seem to express, similar to males, attenuation of some of the deleterious consequences associated with sustained stress exposure (chapter 3).

![Figure 1](image)

**Figure 1**

With respect to the HPA axis, CREB and neurotrophin aspects of stress-induced pathology, the data illustrated in chapter 1 seems in line with reports in support of the neurotrophin hypothesis of depression (figure 1). Repeated stress, in fact, selectively targeted the regulation of (BDNF)-ERK1/2-CREB cascade in male rats. In general chronic stress has been proposed to cause neuronal abnormalities by impairing coordinated HPA axis regulation 1-3. Accordingly, neuroendocrine changes observed in chronically stressed males were in line with persistent HPA axis hyperactivity. One might speculate that prolonged elevation of glucocorticoid concentrations could represent a crucial predisposing factor in the development of multiple abnormalities in the regulation of
BDNF-ERK1/2-CREB pathway, such as persistent, and possibly uncontrolled, ERK1/2 activation and reduced CREB phosphorylation.

ERK1/2 and CREB play fundamental roles in the transduction of neurotrophin-related signals ⁵⁻⁹. Recent studies however, have also implicated these proteins in the modulation of cognitive processes and the induction of long-lasting neuronal plasticity¹⁰⁻¹³. A crucial aspect of these complex processes involves activity-driven induction of new gene expression. This in turn is required for long-lasting potentiation of synaptic transmission associated with learning and memory ⁹,¹⁴⁻¹⁶. Transcription of genes such as BDNF is regulated by patterns of CREB phosphorylation ⁹.

Due to the dependency between CREB phosphorylation and BDNF expression, we propose that the observed reduction of phosphorylated CREB levels might result in downregulated BDNF transcription, thereby depriving neurons of this fundamental neurotrophic factor (figure 2). CREB phosphorylation depends by the activation of selective protein kinases such as ERK-1 and -2 ¹⁷,¹⁸ and an abnormal interaction between these kinases and their effector may also result in a reduction of BDNF expression. In accordance, prolonged ERK phosphorylation has been reported to negatively regulate CREB expression and phosphorylation ¹⁹. Furthermore, due to the role of ERK1/2 in the regulation of cytoskeletal integrity, persistent and uncontrolled activation of these kinases may lead to hyperphosphorylation of various cytoskeletal proteins that could ultimately weaken dendritic structure, especially in synaptic terminals where cytoskeletal proteins are particularly abundant ²⁰⁻²². In conclusion, chronic stress-induced phospho-ERK1/2 and phospho-CREB abnormalities may limit BDNF availability, disrupting neuronal plasticity and facilitating the development of neuronal defects.

![Figure 2](image_url)
To date, the exact mechanisms involved in the development of stress-induced ERK1/2 and CREB abnormalities remain largely unknown. As previously stated however, an important factor in this process includes the persistent elevation of circulating glucocorticoids. Adrenal steroids have been reported to inhibit BDNF expression 23 and although short-term reductions of BDNF availability may be compensated for by alternative adaptations, we believe that prolonged downregulation of this neurotrophin results in cellular dysfunctions. When such disturbances continuously occur in the BDNF pathway, this could in turn lead to persistently decreased BDNF synthesis and release and, ultimately, reduced neuronal plasticity.

In human studies, prolonged stress exposure has been associated with impaired neuronal plasticity and psychopathology. The link between these two phenomena has always been fragmentary, although it has been well established that stress represents an important predisposing factor for the development of a wide variety of psychiatric illnesses, including anxiety and depression. These disorders are characterized by multiple serotonergic/noradrenergic defects and marked gender-related prevalence. It is of interest to note that CREB and BDNF play a central role in the expression of two enzymes involved in serotonin and norepinephrine synthesis, such as tryptophan and tyrosine hydroxylase 24-26 (figure 3). In addition to direct negative effects on neuronal plasticity (reduced CREB and BDNF expression), chronic stress may also affect neurotransmitter synthesis, which could indirectly lead to impaired serotonergic and noradrenergic function. Recent reports of significantly reduced BDNF concentrations in the CSF of depressed subjects support the link between decreased availability of this neurotrophin and associated psychopathology.

Figure 3

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Despite a growing body of literature that emphasizes the greater female sensitivity to stress \(^{27-29}\) and the higher prevalence of psychiatric disorders in women \(^{30,31}\), our results suggest a more complex situation. Whereas chronically stressed males revealed increased cortical-limbic FOS-ir, abnormal prefrontocortical ERK1/2, and reduced cortical and subcortical phospho-CREB expression, stressed females only showed a decreased cortical-limbic FOS-ir. These findings might thus implicate that repeated footshock exposure represents a more severe stressor for male than for female rats. As it has been previously suggested that responses to stress could be stressor-specific \(^{32,33}\), it cannot be excluded that the differential gender-dependent patterns of protein expression and phosphorylation seen here are specific to this particular paradigm and/or the immunohistochemical markers used in this analysis. Studies reporting higher stress sensitivity in female rats and greater prevalence of stress-related psychiatric disorders in women \(^{30,31}\), are thus in stride with the data presented in this thesis which indicate milder stress-induced abnormalities in cyclic female rats (chapter 2). A putative explanation for these conflicting results is provided by the presence, in females, of ovarian hormones, which may attenuate some of the deleterious effects of stress on neuronal plasticity. Sex steroids have been shown to protect neurons from a wide variety of insults and their intracellular actions appear to be mediated by the same signaling pathways underlying neuronal plasticity such as the MAPK cascade. Accordingly, estrogen has also been reported to stimulate CREB phosphorylation \(^{34-36}\). It is thus possible that the negative influences of repeated footshock exposure on BDNF expression, ERK1/2 activation and CREB phosphorylation might have been attenuated by the presence of ovarian hormones (chapter 2). The protective effects of estrogen upon increased severity of the stressor, due to longer exposure to aversive conditions, may however be insufficient to counteract or override the negative influences under more severe stressful circumstances (chapter 3). Remarkably, the established gender-related prevalence of affective disorders begins with puberty and continues until menopause, a period during which ovarian hormones cyclically fluctuate. Despite their reported neuroprotective effects, sex hormones have also been considered as main candidates for this differential susceptibility to psychiatric illnesses, yielding a paradox.

Despite extensive research during the past two decades, our knowledge of the etiological factors and molecular mechanisms underlying the differential sensitivity to stress between males and females still remains fragmentary as well as the link between ovarian hormones and psychopathology. Although implicated, ovarian hormones do not account for all of the differences between male and female brains. Other factors besides simple hormonal fluctuations may also play a contributing role, such as:
• gender-related structural and functional differences between the male and the female brain;
• gender-related differences in intracellular transduction pathways modulating specific neuronal functions;
• greater HPA axis activity and higher glucocorticoid levels in females.

Antidepressants such as selective monoamine reuptake inhibitors represent the treatment of choice for affective disorders such as anxiety and depression. Selective serotonin and norepinephrine reuptake inhibitors (SSRIs and NARIs) have received particular interest since stimulation of serotonergic and/or noradrenergic transmission has been associated with clinical recovery 37. Recently however, newer medications characterized by atypical pharmacological profiles (i.e. tianeptine) have been added to the already large number of drugs available for the treatment of mood disorders. Altering monoamine availability in the synaptic cleft represents only one of the mechanisms by which these compounds exert their beneficial effects. By modifying monoamine levels, antidepressants also enhance neurogenesis and neuronal plasticity38,39, which may in turn help to correct functional and structural dysfunctions involved in the development and/or maintenance of affective disorders 40-43. It is interesting to note that although characterized by different and, sometime, opposite mechanisms of action, citalopram, tianeptine, and reboxetine share surprisingly similar clinical efficacy. This suggests that different antidepressants may correct complex neuronal dysfunctions by acting at different levels. Tianeptine, for instance, a serotonin reuptake enhancer, appears to selectively target the HPA axis and the hippocampus, limiting stress-induced activation of the former and protecting the latter from the deleterious consequences of elevated glucocorticoid concentrations. Similarly, our findings show that citalopram, a serotonin reuptake inhibitor, was able to attenuate stress-induced abnormal HPA axis regulation. Besides the hippocampus however, citalopram may exert its beneficial effects by correcting malfunctions in the neurocircuitry underlying the modulation of this critical stress response system. Reboxetine, a norepinephrine reuptake inhibitor, appears instead to exert its positive action by counteracting the negative influence of repeated stress on neuronal plasticity, an effect that appears to be mediated by the stimulation of CREB phosphorylation. These findings suggest that antidepressants characterized by different pharmacological profiles may all target those “state-related functional and/or structural abnormalities” involved in the development and/or maintenance of psychopathology.

It is clear from this discussion that although research is progressing, we are still a long way from fully understanding the neurobiological substrates controlling mood and emotions under normal and pathological conditions. Given the pervasive deficits that characterize affective disorders, it is likely that the mechanisms by which currently
available treatments attenuate depressive symptoms involve the modulation of the activity of numerous brain regions, neurotransmitter systems, and various peptides besides the ones discussed here. Progress in functional brain imaging might represent a powerful tool to identify differential activation patterns and gross circuits in the brain that are affected in depressed subjects. In addition to gender-related dimorphisms in the healthy and pathological brain, the ability to image the living human brain might one day even allow us to investigate BDNF, CREB and newly born neurons, thereby helping direct research into the molecular and cellular mechanisms involved. Ultimately however, the key to elucidating the riddle of the specific disorder mysteries lies in genetics and prevention. Due to the heterogeneous nature of such disorders, many patients remain treatment-resistant. Identifying specific genetic variations that confer risk or resistance for depression will likely represent the essential step in categorizing depression based on underlying biology. In turn, these advances will lead to new approaches to stress-pathology research and perhaps development of definite treatments and eventually cures or, even better, preventive measures.
References
