7 PTSD: From Neurons to Networks

Rajnish P. Rao, Aparna Suvrathan, Melinda M. Miller, Bruce S. McEwen, and Sumantra Chattarji

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Abstract

Animal models of post-traumatic stress disorder (PTSD) must not only capture salient features of the disorder at the behavioral level but also provide insights into the underlying neuronal, physiological, and endocrine mechanisms. The fact that exposure to severe stress leads to the development of PTSD in humans provides the basic rationale for all rodent models of the disorder. The early focus of many of these models was on studying the debilitating effects of chronic or repeated stress on the hippocampus, a key component of the stress-inhibitory circuit that is reduced in volume in PTSD. Neuroimaging and clinical studies, however, also implicate two other brain areas, the amygdala and the prefrontal cortex, in PTSD. Moreover, structural and functional changes in all three brain structures appear to differ from each other in PTSD. These findings

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from human studies pose a challenge for animal models of PTSD: can the same stressful experience elicit contrasting cellular effects in the hippocampus, amygdala, and prefrontal cortex? Another striking characteristic of PTSD, which is not fully reflected in commonly used animal models of chronic stress, lies in the temporal domain. While PTSD is triggered by a single intensely traumatic event, some symptoms persist well beyond the original event. Can animal studies on the delayed, long-term impact of brief, but severe, stressors shed any light on these temporal features of human PTSD? Finally, while the hippocampus, amygdala, and prefrontal cortex are distinct in their associations with the severity of PTSD symptoms, there are significant neuroanatomical interconnections between the three areas. Can stress-induced modulation of structure and function in one of these brain areas affect changes in another? If so, can we construct new animal models that expand the scope of their analyses by studying stress-induced changes distributed across a wider network encompassing all three brain areas implicated in PTSD? In this review, we address these key questions by summarizing findings from various rodent models of stress. We focus on the morphological, electrophysiological, endocrine and molecular effects of stress in the hippocampus, amygdala, and prefrontal cortex. We end by discussing some of the gaps in our current understanding and explore experimental strategies that may lead to more powerful animal models in the future.

Key Words: Amygdala, anxiety, dendritic remodeling, glucocorticoids, hippocampus, post-traumatic stress disorder, prefrontal cortex, stress, synaptic plasticity.

INTRODUCTION

Study of the neurobiological mechanisms of post-traumatic stress disorder (PTSD) has been driven by two complementary lines of investigation. On the one hand, the advent of functional and structural imaging of the human brain, along with careful and detailed clinical assessments of PTSD etiology and psychopathology, have provided valuable information on candidate brain regions and behavioral dysfunction in PTSD. Top-down information gathered from the human clinical realm in turn has aided a second line of inquiry that relies on animal models to study basic mechanisms underlying the development of the disorder. This bottom-up strategy combines a range of neurobiological tools and models to analyze the effects of stress at multiple levels of neural organization—from molecular and cellular correlates at one end to network and behavioral-level analysis at the other. Increasingly, these two approaches are converging to give rise to a comprehensive intellectual framework that bridges the gap between clinical and basic research. This review focuses on the interplay between top-down information gleaned from studies of PTSD subjects and mechanistic insights gained from bottom-up studies using animal models. To this end, we first summarize clinical findings that have helped identify key brain structures implicated in PTSD. This is followed by a detailed description of a range of observations, made from animal models, on the effects of chronic and acute stress on these brain areas. Other sections attempt to

synthesize these findings into cellular models of stress-induced modulation of synaptic structure and function that may mediate the short- and long-terms effects of stress in brain structures affected by PTSD. We conclude by discussing areas of neuroscience research that are likely to play an influential role in shaping the next generation of more powerful and sophisticated animal models of PTSD.

BRAIN REGIONS IMPLICATED IN PTSD

Clinical studies based on structural and functional neuroimaging studies implicate three brain areas in the pathophysiology of PTSD: the hippocampus, amygdala, and medial prefrontal cortex (mPFC). Reduced hippocampal volumes, compared to either trauma-exposed control subjects or trauma-unexposed healthy subjects, have been reported in PTSD patients *(1)*. Importantly, hippocampal volumes have been inversely associated with combat exposure *(2)* and PTSD symptom severity (Fig. 1) *(3,4)*. Taken together, these findings support the view that not only is decreased hippocampal volume linked to cognitive deficits associated with PTSD but also it may act as a risk factor for the development of the disorder *(5)*. These findings on abnormalities in hippocampal structure and

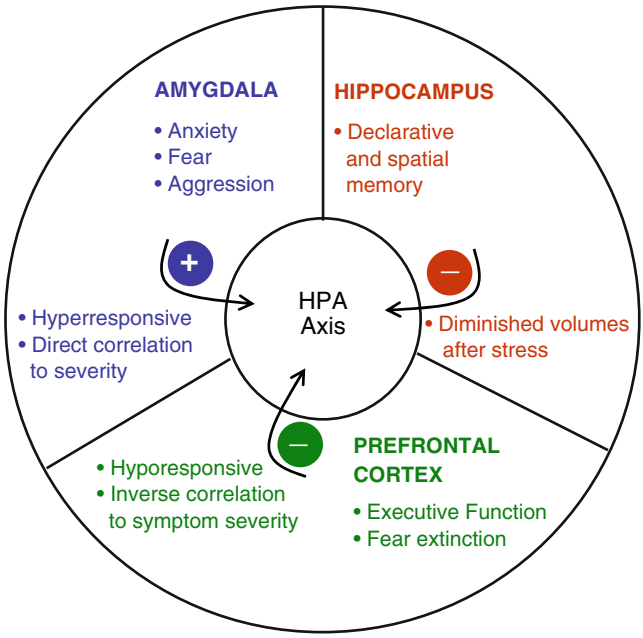

Fig. 1. Brain areas implicated in post-traumatic stress disorder (PTSD). The amygdala, prefrontal cortex, and hippocampus, which are altered structurally and functionally in PTSD, respond differentially to stress-induced adrenal steroids and in turn differentially regulate hypothalamic-pituitary-adrenal (HPA) activity (both positively and negatively). The schematic shows the role of each area in cognitive/emotional function and why it is implicated in PTSD (*See Color Plates*)

function are particularly relevant in light of the central role played by this structure in both the neuroendocrine stress response (Fig. 1) and memory deficits, similar to what has been seen in individuals suffering from PTSD *(6,7)*.

Although neuroimaging studies have not yielded conclusive evidence for structural or volume changes in the amygdala, amygdalar hyperresponsivity has been reported in PTSD patients during the presentation of not only trauma-related visual and auditory cues *(8–15)* but also trauma-unrelated affective cues such as fearful facial expressions *(16–18)*. Strikingly, unlike the hippocampus, amygdala activation is reported to be positively correlated with the severity of PTSD symptoms (Fig. 1) *(8,9,15,19)*. Reports that in PTSD patients the amygdala is more responsive to both traumatic reminders and more general affective stimuli are noteworthy in view of the large body of evidence from animal research demonstrating a pivotal role for the amygdala in Pavlovian fear conditioning *(20)*.

Fear conditioning is a robust learning paradigm in which subjects rapidly learn to associate a previously neutral tone (the conditioned stimulus, CS) with a coincident aversive stimulus (unconditioned stimulus, US), such as electric shock, which invariably evokes an unconditioned response (UCR). Reexposure to the CS alone elicits a conditioned response (CR) that provides a measure for the learned association between CS and US. It is now well established that the amygdala plays an essential role in the acquisition of the tone-shock association, the initial stages of which involve strengthening of thalamic afferent synapses to the lateral amygdala (LA) *(21,22)*.

This form of associative learning paradigm is attractive because it provides a convenient framework for viewing PTSD as fear conditioning gone wrong. Individuals naturally exhibit an initial reaction to a traumatic or severely stressful event (which can be viewed as an US) with arousal and fear (UCR). However, those who eventually develop PTSD continue to show heightened vigilance and avoidance (CR) long after the trauma when they are exposed to cues related to the triggering event (CS). A compelling link between this animal model of fear learning and the affective symptoms of PTSD comes from reports that patients exhibit an increased acquisition of aversive conditioning in the laboratory *(23,24)*. Further, functional imaging studies on abuse survivors demonstrated enhanced responses in the amygdala during the acquisition of fear conditioning *(25)*.

The third brain region implicated in PTSD is the mPFC. In contrast to amygdalar hyperresponsivity, structural neuroimaging studies have reported decreased volumes of frontal cortex in PTSD. Studies that have specifically focused on the mPFC have shown anterior cingulate (ACg) volumes to be smaller in PTSD compared to trauma-exposed control groups *(26,27)*. Similar to findings on hippocampal volumes, the severity of PTSD symptoms is inversely correlated with ACg volume (Fig. 1) *(26,27)*. Several functional neuroimaging studies have also demonstrated an inverse correlation between PTSD symptom severity and mPFC activation (Fig. 1) *(9,17,18,28)*.

These imaging studies involving the mPFC are particularly relevant in light of another form of learning and memory called *extinction*. Extinction is an inhibitory learning process by which the conditioned fear response (CR) can be attenuated

or extinguished when the CS no longer predicts the US. Importantly, while the fear response (CR) subsides after extinction, the fear memory itself is not erased; it is merely suppressed by the inhibitory memory that is formed during extinction *(22)*. Pharmacological, lesion, and electrophysiological studies in rodents, as well as imaging studies in humans, have established a role for both the amygdala and mPFC in fear extinction *(29–32)*. These studies, taken together with reports that PTSD patients have a diminished extinction of conditioned fear responses in laboratory experiments *(23,33)*, have led to the suggestion that malfunction of the mPFC also contributes to the pathophysiology of PTSD.

NEURAL SUBSTRATES OF PTSD: INSIGHTS GAINED FROM ANIMAL MODELS OF STRESS

Investigations into the neural substrates underlying the cognitive and affective symptoms of PTSD have been driven by the fact that exposure to severe stress triggers the development of PTSD *(34)*. To this end, a variety of animal models of stress have been used to analyze specific cellular and molecular changes in brain areas that are known, especially from imaging studies, to be affected in PTSD. First, as mentioned, reduction in hippocampal and PFC volumes have been reported by many neuroimaging studies *(35,36)*, and these are believed to underlie functional deficits associated with PTSD (Fig. 1). Second, both structures play a pivotal role in negative-feedback regulation of the stress response via the hypothalamic-pituitary-adrenal (HPA) axis (Fig. 1) *(37–40)*. Third, a dominant hypothesis, which looks beyond the genesis of the disorder, suggests that PTSD represents an eventual failure in reinstating physiological homeostasis that was disrupted by traumatic stress *(41,42)*. Thus, as key components of the stress-inhibitory circuit, the hippocampus and PFC have attracted considerable attention in studies using rodent models of stress. Therefore, we first summarize some of the key findings from animal models of chronic stress and their effects on cells and synapses of the hippocampus and PFC and follow with a discussion of stress effects on another brain area that has an opposing influence on the HPA axis: the amygdala.

Effects of Repeated Stress on the Hippocampus and PFC

Earlier investigations into cellular mechanisms underlying stress-induced impairment of hippocampal function have focused on two common metrics of hippocampal plasticity—one structural and the other electrophysiological (reviewed in subsequent sections). Morphological analyses of how stress and stress hormones affect the rat hippocampus revealed that 21 days (6 h per day) of repeated restraint stress produces significant dendritic remodeling in CA3 pyramidal neurons (Fig. 2) *(43–47)*. This dendritic remodeling is characterized by a shortening and debranching of apical dendrites *(48)* and is mediated by mechanisms involving high levels of glucocorticoid secretion, glutamate, and serotonin *(44)*. More recently, it has been shown that even shorter durations of immobilization stress (2 h per day for 10 days) are capable of causing significant atrophy of both apical and basal dendrites

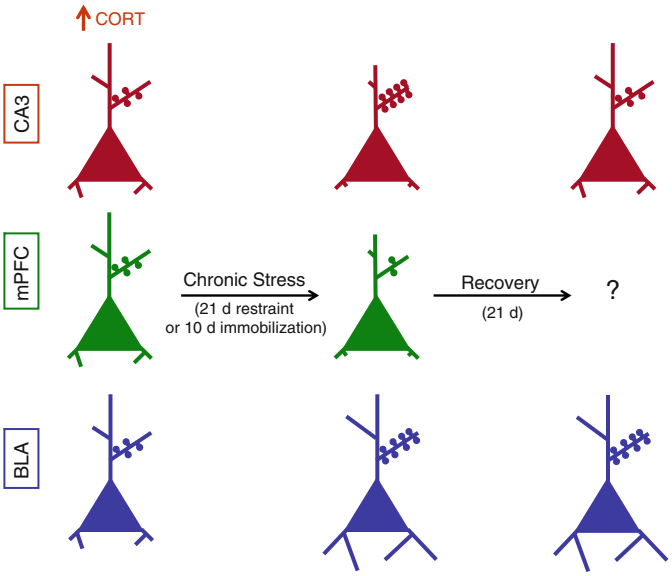

Fig. 2. Chronic stress leads to contrasting patterns of structural plasticity in excitatory pyramidal neurons in the basolateral amygdala (BLA), hippocampal area CA3, and layer II/III of medial prefrontal cortex (mPFC). Repeated exposure to restraint (6 h/day, 21 days) or immobilization (2 h/day, 10 days) stress triggers dendritic atrophy in pyramidal cells in hippocampal area CA3 (*top*) and layer II/III of mPFC (*middle*), which is opposite to the dendritic hypertrophy seen in BLA projection neurons (*bottom*). Chronic stress-induced morphological changes also differ in terms of their temporal longevity—BLA hypertrophy, unlike CA3 atrophy, persists even after 21 days of stress-free recovery. A third point of difference lies at the level of dendritic spine density, which is enhanced in BLA and CA3 neurons but is reduced in mPFC cells (*See Color Plates*)

on CA3 pyramidal cells (Fig. 2) *(49)*. These findings have contributed to rodent models of stress-induced neuronal atrophy that may provide one potential explanation for the hippocampal volume loss associated with PTSD *(2,3)*.

The PFC, which (like the hippocampus) exerts negative-feedback regulation of the stress response *(50,51)*, appears to be affected by chronic stress in a similar fashion. The mPFC and prelimbic cortex exhibit a shortening of dendritic length and a simplification of dendritic branching in layer II/III neurons in response to repeated stress and repeated corticosterone treatment (Fig. 2) *(52–55)*. It is not yet known if the infralimbic cortex (IL) of the mPFC also shows remodeling after 21 days of chronic restraint stress (Fig. 2). This would be important because of the involvement of this region in extinction of fear conditioning *(56)*. These structural changes predict that prefrontal cortical functions in working memory, executive function, and fear memory extinction would be impaired by chronic stress (Fig. 1). Indeed, this has been demonstrated for a form of

cognitive flexibility *(55,57)*. Glucocorticoid treatment and adrenalectomy (ADX) have both been reported to alter the morphology of PFC neurons as well as the cognitive flexibility that is associated with PFC function. Dexamethasone treatment resulted in a pronounced impairment in working memory and behavioral flexibility, effects that correlated with atrophy of layer II of the infralimbic, prelimbic, and cingulate cortices. Exposure to corticosterone produced milder impairments in behavioral flexibility, but not in working memory, and reduced the volume of layer II of all prefrontal areas. These volume reductions were accompanied by atrophy of distal apical dendrites, but also by increased branching of middle dendrites. Interestingly, ADX-induced effects were apparent on reversal of learning and were associated with reduction of length of middle dendrites. None of the experimental procedures influenced the morphology of retrosplenial or motor cortices *(57–59)*.

Dendritic remodeling, through its modulation of postsynaptic dendritic surface, will have a profound impact on the availability of synaptic inputs and thereby synaptic plasticity. Indeed, it has long been hypothesized that morphological and numerical alterations in dendritic spines, the site of excitatory synaptic transmission in the brain, underlie long-term structural encoding of behavioral experiences. In this context, plasticity at the level of dendritic spines can be viewed from two different perspectives. First, spine synapses may act as the primary site of plasticity elicited by stressful experiences. This possibility, a very likely one, is discussed in detail in sections subsequent.

A second scenario is based on the consideration that repeated application of the same stressor can lead to habituation in the stress response *(60)*. This raises the possibility that although chronic stress triggers dendritic remodeling, it may eventually set in motion adaptive changes that counter the initial effects of stress on dendritic morphology. Such homeostatic mechanisms, triggered by prolonged stress, could be mediated by changing the number of spines, thereby regulating the overall synaptic connectivity in the affected area. This possibility finds support in the observation that dendritic atrophy in hippocampal CA3 pyramidal neurons, caused by repeated restrained stress, is accompanied by a numerical increase in spines (Fig. 2) *(61)*. This is indicative of an adaptive mechanism that may compensate for the loss of dendritic area for synaptic inputs to terminate. If similar adaptive plasticity mechanisms are activated in the mPFC by repeated application of stress, then one would also predict an increase in the number of spines along atrophied dendrites in the mPFC. However, contrary to this scenario, following 21 days of chronic restraint stress, there is a *reduction* in spine density on the apical dendrites of layer II/III neurons (Fig. 2) *(54)*. The net result of these changes is estimated to be a 40% reduction in synaptic inputs to the mPFC (Fig. 2). Thus, while chronic stress elicits similar forms of structural plasticity at the level of dendrites, its impact at the levels of spines may differ between the hippocampus and PFC. These results also raise interesting questions about the relationship between dendritic remodeling and modulation of spine density: for example, is one always accompanied by the other? Does dendritic remodeling follow or precede spine plasticity? Do the rules governing these forms of structural plasticity vary in different brain regions?

Impact of Repeated Stress on the Amygdala

The impetus for the search for cellular correlates of stress in the amygdala came from findings that highlight the contrasting manner in which the hippocampus and amygdala affect the stress response and how their behavioral outputs in turn are modulated by stress. First, there are anatomical data showing that limbic inputs impinging on the paraventricular nucleus (PVN) of the hypothalamus and hypothalamic γ-aminobutyric acid-ergic (GABA-ergic) neurons can be either excitatory from the hippocampus and thereby enhance GABA-ergic tone or inhibitory from the amygdala and thereby reduce GABA-ergic tone *(37–40,62)*. This implies that whereas enhanced hippocampal input would suppress the HPA axis, enhanced amygdalar input could have the opposite effect on HPA activity (Fig. 1). Second, stress facilitates aversive learning but impairs spatial learning in rodents *(63,64)*. Although repeated stress that produces dendritic atrophy in the CA3 region impairs hippocampal-dependent learning *(65)*, the basolateral amygdala (BLA) has been shown to be essential for stress-induced facilitation of aversive learning *(66,67)*. Taken together, these observations highlighted the need to examine the cellular effects of stress in the amygdala.

Applying the same tools of morphometric analysis of Golgi-stained neurons that were earlier used in the hippocampus, the first cellular evidence for contrasting patterns of stress-induced structural plasticity was found in the BLA *(49)*. In this study, chronic immobilization stress (2 h per day for 10 days) induced dendritic atrophy and debranching in CA3 pyramidal neurons of the hippocampus, which is consistent with earlier reports using other forms of repeated stress (Fig. 2). By contrast, principal neurons in the BLA exhibited *enhanced* dendritic arborization in response to the same chronic stress (Fig. 2). This stress-induced enhancement in dendritic arborization was restricted only to pyramidal and stellate neurons of the BLA, which are presumably excitatory projection neurons *(68,69)*. The efficacy of chronic immobilization stress in eliciting dendritic hypertrophy in BLA was also shown to be relevant in terms of its anxiogenic properties.

Chronic immobilization stress also caused a significant increase in anxiety-like behavior *(70)*. On the other hand, chronic unpredictable stress, which failed to enhance anxiety, did not cause any dendritic remodeling of BLA principal neurons *(49)*. Importantly, the positive correlation between stress-induced BLA dendritic hypertrophy and greater anxiety was further strengthened in a subsequent study that also brought into focus the temporal dimension, a key feature of PTSD. Even after 21 days of stress-free recovery following exposure to chronic immobilization stress, animals continued to exhibit enhanced anxiety *(70,71)*. At the cellular level, stress-induced dendritic growth of spiny BLA pyramidal neurons was also as persistent as enhanced anxiety after 21 days of recovery (Fig. 2). Interestingly, BLA hypertrophy is distinct from hippocampal CA3 atrophy, which is reversible within the same period of stress-free recovery (Fig. 2) *(71)*. Further, following 10-day exposure to immobilization stress, dendritic hypertrophy is accompanied by an equally robust increase in spine density that spreads across both primary and secondary dendrites of BLA pyramidal

neurons *(73)*. Thus, chronic stress leads to a significant increase in the structural basis of synaptic connectivity in the BLA, which is opposite to the significant loss of synaptic connectivity in the mPFC (Fig. 2) *(54)*.

In summary, findings on structural plasticity induced by chronic stress highlight important differences between the hippocampus, amygdala, and PFC. Principal neurons in specific subregions of the hippocampus and PFC, both part of the stress-inhibitory circuitry, undergo dendritic atrophy (Fig. 2). But, spine density increases in the hippocampus while it reduces in the mPFC (Fig. 2). In contrast, dendrites grow bigger in the BLA following chronic stress, and these elongated dendrites also possess higher spine densities (Fig. 2). These results from animal models of chronic stress are quite striking because they are consistent with neuroimaging findings from humans with PTSD that also point to a negative correlation between the amygdala on the one hand and the mPFC and hippocampus on the other (Fig. 1). Taken together, these changes in the structural basis of synaptic connectivity will affect information processing by these three brain areas and might contribute significantly to the varied cognitive and affective symptoms in PTSD.

BEYOND CHRONIC STRESS MODELS: BRIEF STRESSORS AND THEIR DELAYED IMPACT

Despite the convergence of findings from animal models and human neuroimaging in identifying key brain areas implicated in PTSD, animal models of PTSD based on chronic stress paradigms have two specific limitations. Two of the defining features of PTSD are not fully reflected in these animal models. First, the most commonly held view of PTSD is that it is triggered by a *single* overwhelmingly traumatic, often life-threatening, event. Second, this disorder is defined as one in which some components of the fear response persist well beyond the original traumatic event. Indeed, symptoms must persist more than 1 month after the trauma for acute PTSD and 3 months after the trauma for chronic PTSD *(34)*. However, the animal models discussed so far have not used brief stressors that better reflect features of traumatic stress, and almost all measurements were carried out soon after the termination of the chronic stress protocol. An acute, but severe, stressor would replicate more accurately the initial triggering event and enable studies on the further cascade of endocrine, cellular, and behavioral changes, especially those manifested well after the initial triggering event. This section focuses on some of these issues in greater detail.

Adding a Temporal Axis

Allostatic overload in an animal or person is normally caused by chronic elevation and dysregulation of stress responses *(74,75)*. Although milder forms of acute stress result in increase in levels of cortisol and other neurochemicals such as epinephrine, norepinephrine, and serotonin, these often serve only to increase available energy and help the animal to escape or respond suitably to the stres-

sor *(76–78)*. Acute stress can also increase memory and attention, which is of adaptive benefit. However, when the stressor is of extreme severity, a cascade of events is triggered that can lead to PTSD in humans. Such an acute stressor would therefore need to be severe enough to result in delayed ill effects.

Several animal models have been developed that allow investigations into the impact of an acute, but severe, stressor over time—especially at time points that are removed from the period of stress itself *(72,79)*. One study, using a single 2-h episode of immobilization stress, has reported a delayed increase in anxiety-like behavior that is paralleled by an increase in spine density in principal neurons of the BLA. These findings are particularly striking because the increase in anxiety and BLA spine density is evident only 10 days after the acute stress and not the day after *(72)*. Further, the newly formed spines are localized proximal to the cell soma, also in the absence of any dendritic remodeling. This study also suggests that BLA spinogenesis in itself may be adequate to increase behavioral anxiety, a correlation that has also emerged from another study in which BLA spinogenesis, caused by transgenic overexpression of brain-derived neurotrophic factor (BDNF), leads to enhanced anxiety *(80)*.

Taken together, these findings raise the possibility that an acute episode of severe stress initiates plasticity mechanisms culminating in delayed and restricted spinogenesis, and this in itself may be sufficient to modulate anxiety-like behavior. But, repeated exposure to the same stressor pushes the same cellular machinery to scale up to a greater magnitude of spinogenesis along with enlargement of the dendritic tree *(49)*. Although hippocampal volume loss has been the traditional focus of human studies on PTSD *(1)*, these findings on the delayed manifestation of enhanced anxiety and BLA spinogenesis, triggered by a single temporally restricted episode of stress, may provide a new framework for studying cellular mechanisms of PTSD in the amygdala. The delayed buildup of spines and anxiety after exposure to acute stress also highlights the unique temporal characteristics of stress-induced structural plasticity in the amygdala. An earlier report using 10-day chronic immobilization stress showed that BLA hypertrophy and anxiety endure for a number of weeks after termination of the stressor (Fig. 2) *(71)*. Thus, the amygdala appears to have special features, especially with respect to its temporal manifestation and persistence, which fit well with the delayed and prolonged enhancing effects on fear and anxiety observed in PTSD *(81)*.

A single prolonged stress, consisting of 2-h restraint stress followed by 20-min forced swimming and ether anesthesia, is another animal model that has been proposed for PTSD *(82)*. In this model, rats showed enhanced inhibition of the HPA axis and potentiated acoustic startle response 1 week, but not 1 day, after single prolonged stress *(79)*. This form of stress also impaired spatial memory, which paralleled the deficits in hippocampal long-term potentiation (LTP), a candidate synaptic plasticity mechanism for learning and memory. This study also reported enhanced contextual fear memory and impaired LTP in the amygdala in the rats 1 week after stress exposure. Consistent with the acute stress findings mentioned, many of the behavioral and synaptic changes were

not evident a day after single prolonged stress, but a week later when plasma corticosterone had recovered from an initial increase caused by the stress *(79)*.

In contrast to the acute stress models discussed, an alternative approach has employed more ethologically relevant paradigms in which rodents are subjected to their natural predators to examine long-term behavioral and neurochemical changes. Adamec et al. *(83)* reported a long-lasting increase in anxiety-like behavior and a decrease in risk assessment in the elevated plus maze following a single 5-min direct exposure of a rat to a cat. These effects could be seen 30 min to 1 h after predator exposure and persisted for at least 3 weeks. Likewise, acute predator stress increased acoustic startle response and decreased entries into the light box of the light-dark box paradigm *(84)* and potentiated neural transmission both to and from the amygdala 10 days poststress *(83)*. Acute predator exposure has also been shown to impair long-term memory in rats exposed to a cat immediately prior to water maze training when tested 24 h later. Further, the learning-induced increase in basal dendritic spines on CA1 pyramidal neurons, typically seen 24 h after water maze training, was inhibited in the stressed animals *(85)*. Rodents, even those bred and raised in a laboratory, show an innate and immediate stress response to odors produced in the urine, hair, and scent glands of their predators. Cat odors alone, using odorants coming from a used collar worn by a cat *(86)*, a ball of cat fur *(87)*, or used cat litter *(88)*, have also been shown to cause both immediate and long-term changes to behavior commensurate with those seen after actual predator exposure.

Finally, there is an important methodological issue that is often overlooked in the process of designing animal models of PTSD; this has to do with the animals themselves. While some studies have chosen to focus on the entire group of stressed animals, others have used behavioral measures to differentiate animals displaying the most extreme behavioral changes. Some rat strains are known to have higher baseline anxiety than others. For example, the Lewis rat strain, an inbred strain developed from the Sprague-Dawley (SD) rats, has been proposed as a model for PTSD because a higher percentage of these develop severe stress-induced anxiety-like behaviors *(88)*. Furthermore, Lewis rats have been characterized by their abnormal HPA stress response. Lewis rats have normal basal blood corticosterone levels, as compared to their SD cousins, but have a hypoactive response to stress. This is relevant in light of clinical evidence that individuals who produce lower-than-average levels of cortisol after a traumatic event may have a higher probability of later developing PTSD *(42,89)*. In comparison with other strains of rats, Lewis rats exhibited greater baseline anxiety behaviors and greater stress-induced increases in anxiety. According to studies by Cohen and colleagues *(88,90)*, 1 week after a cat odor exposure paradigm, the majority of animals demonstrated "extreme behavioral responses," such as never entering the open arms of the plus maze, and heightened acoustic startle that did not habituate. An increase in acoustic startle response has also been seen in patients with PTSD *(91)*.

In summary, animal studies using chronic or repeated stress have led to models of acute stress that can capture salient features of PTSD, especially symptoms

that become evident with a time delay. Importantly, these models enable more detailed examination of the cellular and molecular changes that gradually develop after the initial endocrine and physiological response triggered during, and immediately after, the severe stress. The study of such cellular changes, however, also poses a challenge in that it becomes hard to distinguish changes underlying the development of a disease state from those that are adaptive (i.e., help the organism reestablish homeostasis). This fundamental challenge in turn highlights the need for careful sequential analysis of how key markers of neural change—molecular, cellular, or behavioral—evolve over time. Moreover, analysis of this progression of the initial stress response into a psychiatric condition would also provide valuable insights into possible time points for therapeutic interventions after the initial traumatic event.

SYNAPTIC AND MOLECULAR CORRELATES OF STRESS EFFECTS ON KEY BRAIN REGIONS

Animal models of PTSD of the kind discussed so far are aimed at uncovering important cellular and molecular mechanisms underlying the disorder that can also be targeted for pharmacological interventions. Indeed, these models have given rise to a wide range of findings that not only implicate endocrinological and physiological factors directly linked to the immediate response to stress, but also changes in excitatory and inhibitory synaptic transmission that eventually give rise to a variety of plasticity mechanisms that may underlie a range of PTSD symptoms at the behavioral level. Since a comprehensive review of all these changes is beyond the scope of the present discussion, we focus on the actions of stress hormones and how these influence synaptic transmission and plasticity in brain circuits that play a major role in the stress models discussed.

Mechanisms of Adrenal Steroid Actions

Exposure to stressful events leads to glucocorticoid release by the activation of the HPA axis. The action of these stress hormones in conjunction with other stress mediators (epinephrine/norepinephrine, parasympathetic nervous system, cytokines) is key in facilitating an adaptation to the stressor, thereby restoring homeostasis *(92)*. However, prolonged exposure to these hormones is damaging to several brain areas, especially the hippocampus under conditions of ischemia and seizures, and this is thought to underlie some of the adverse behavioral and physiological effects of chronic stress *(92)*.

A shared feature of the mediators of stress and adaptation is that they operate as a nonlinear network, with each mediator having regulatory actions on the production and activity of the other *(92)*. Some mediators, such as adrenal steroids, act in a dose-dependent manner along an inverted U-shaped curve *(85,93)* involving the mineralocorticoid receptors (MRs) *(94)* and the glucocorticoid receptors (GRs) *(95)*. Using overexpression *(96–98)* or knockout mouse models *(99–100)*, MRs have been shown to be actively involved in the stability

of neuronal networks and hippocampal cell survival *(6,101,102)*. As a consequence of stress, when glucocorticoid levels rise, the MRs begin to saturate, and the GRs take over as the chief mediators of glucocorticoid action and thus are key to the feedback inhibition of the HPA axis *(93)*.

There are other factors that add to the subtlety of adrenal steroid actions. The first is the diversity of transcriptional regulation. On binding of the hormone, the corticosteroid receptors are translocated to the nucleus, where they bind as homodimers to the DNA *(103)* or interact with a whole host of transcription factors (AP-1, nuclear factor kappa B [NFκB], cyclic adenosine monophosphate response element-binding [CREB], STATs) *(104)* as monomers. Different brain regions, which not only have different MR/GR ratios and expression of splice variants of the receptors but also express various combinations of these transcriptional coactivators and corepressors, can thus contribute to differential gene expression. This in turn may produce a diverse array of receptor-mediated actions. Second, local intracellular concentrations of glucocorticoids can be regulated by the expression of modifying enzymes such as 11-β-steroid dehydrogenase 1 and 2 *(105–107)*, 3-α- and 5-α reductases *(105)*. Also, by the action of the multidrug resistance (MDR) pump, cortisol, but not corticosterone, has been shown to be extruded from cells *(108)*. Finally, MRs have an unusual role: as determined by genetic deletion of the MR, they are essential for a rapid nongenomic effect of corticosterone in the mouse hippocampus that increases excitatory postsynaptic potentials (EPSPs) *(109)*. A similar rapid corticosterone effect on glutamate levels has been reported for the rat hippocampus, and this action is not blocked by RU486 and hence does not appear to be mediated by the glucocorticoid (GR or type II) receptors *(110)*.

Given this array of factors, it may be more understandable that adrenal steroids appear to play contradictory roles in the actions of stress on hippocampus, PFC, and amygdala. In the hippocampus, low levels of adrenal steroids have trophic effects in maintaining dendritic branch patterns in the dentate gyrus and may do so via MRs *(111)*. Similar effects have been reported in the PFC *(57,58)*. Yet, in both hippocampus and PFC, repeated stress and high-dose corticosteroid treatment promotes dendritic remodeling *(46,52,54,112)*, and we know for the hippocampus that these actions require excitatory amino acids and N-methyl D-aspartate (NMDA) receptors (*44,123–125*). In the amygdala, adrenal steroids are implicated in dendritic lengthening in neurons of the basolateral nucleus as well as increased anxiety *(113)*. Yet, at the same time adrenal steroids are also implicated in the ability of an acute stress prior to acute immobilization to reduce the immobilization-induced anxiety and induction of excitatory spine synapses *(114)*. Thus, it appears that the actions of adrenal steroids are very much dependent on their concentration, as well as on time and the activities of other mediators such as excitatory amino acids and serotonin. For example, adrenal steroids are required for serotonin to exert gating effects on synaptic transmission in the BLA *(115)*, while, as described in another section, acute corticosteroid treatment enhances excitability of BLA neurons and reduces inhibitory tone *(116)*.

Effects on Excitatory Synaptic Transmission

Stress has a profound impact on transmission and plasticity at excitatory glutamatergic synapses in two ways. First, stress leads to significant increase in the extracellular levels of glutamate in the hippocampus, amygdala, and PFC (Fig. 3) *(117,118)*. This suggests that at least one of the immediate consequences of stress is similar in all three brain areas that subsequently exhibit contrasting patterns of structural plasticity of dendrites and spines. In other words, two key factors—glucocorticoids and glutamate—are both elevated following exposure to stress (Fig. 3). This implies that cellular mechanisms more downstream of these short-term changes may hold the key to the differential response of the

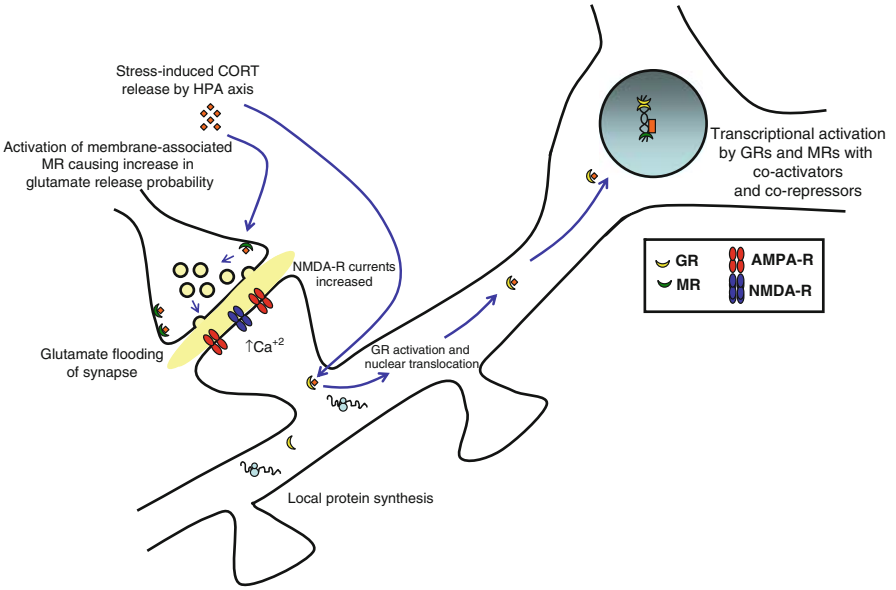

Fig. 3. The effect of adrenal steroids (via glucocorticoid receptors [GRs] and mineralocorticoid receptors [MRs]) on excitatory amino acid neurotransmission through *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (NMDA-R and AMPA-R, respectively). Stress-induced elevation of corticosterone *(44)* leads to the rapid activation of the presynaptic membrane-associated MR, which results in an increase in the glutamate release probability *(109)* and subsequent abundance of glutamate in and around synapses *(117,118)*. This surge of glutamate in turn leads to activation of postsynaptic AMPA and NMDA receptors. Calcium influx through NMDA receptors *(120,121)*, in addition to triggering intracellular signaling cascades related to synaptic plasticity, causes protein synthesis at the synaptic level that is essential for dendritic remodeling *(193)*. Corticosterone (CORT) also binds to the GRs, which then translocate to the nucleus and trigger gene expression *(95)* in coordination with a host of cell-specific transcriptional coactivators and corepressors *(93)* (*See Color Plates*)

three brain areas to stress. This in turn points to the second major effect of glucocorticoids: their impact on glutamate receptor subtypes and synaptic plasticity mechanisms mediated by them.

Both stress and glucocorticoid treatment cause enhanced expression of NMDA receptors in the hippocampus (Fig. 3) *(119,120)*, and this is another potential mechanism by which adrenal steroids are involved in the dendritic remodeling described. Chronic stress also increases the deactivation time constant and the amplitude of NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) in the CA3, further supporting increased NMDA receptor signaling in response to stress *(121)*. It is puzzling, however, that NMDA receptors are not expressed in the stratum lucidum, where mossy fibers terminate on hippocampal CA3 pyramidal cells *(122)*, given the evidence cited for the importance of this innervation for dendritic atrophy. The presence of NMDA receptors on the more distal aspects of CA3 dendrites suggests that the mossy fiber activation of glutamate release triggers a much more widespread activity of excitatory amino acids affecting the entire dendritic tree of the CA3 pyramidal neurons. Following up on the widespread activation of NMDA receptors, the increased levels of intracellular calcium (Fig. 3) may make the dendritic cytoskeleton become depolymerized or undergo proteolysis *(46)*.

The effects of stress on the hippocampus are dependent on the NMDA subtype of glutamate receptors as NMDA antagonism blocks the stress-induced hippocampal plasticity *(44,123–125)*, including impairment of neurogenesis in the dentate gyrus. Conversely, direct in vitro application of corticosterone enhances NMDA currents (Fig. 3) *(126)*, and glucocorticoids increase NR2A and NR2B subunit expression in the hippocampus after chronic administration *(120)*. The enhancement of amygdala-dependent aversive learning is also blocked if the NMDA channel is blocked transiently during the stress episode *(67)*. Therefore, NMDA conductance serves as a gating mechanism for the cascade of stress responses that eventually result in both impaired hippocampal and amygdalar plasticity and memory tasks.

Besides regulating NMDA receptors, stress and glucocorticoids also regulate excitatory amino acid transporters in the hippocampus *(127,128)*. Whereas chronic stress that causes dendritic remodeling in the CA3 region increases glutamate transporter Glt1a and Glt1b expression, adrenal steroids appear to suppress the expression of Glt1a, as evidenced by studies with ADX and adrenal steroid replacement *(128)*. This counterregulation by adrenal steroids is similar to what happens with inflammatory responses, which are suppressed by adrenal steroids *(92)*.

Effects on Plasticity at Excitatory Synapses

Adrenal steroids acutely modulate synaptic plasticity in hippocampal neurons, commonly assayed by the measurement of LTP *(129)*. In the hippocampal CA1 field and the dentate gyrus, acute stress and acute glucocorticoid elevation impairs LTP or its close relative, primed-burst potentiation (PBP). Further, there

is a U-shaped dose-response curve, with low levels of corticosterone facilitating PBP and high levels inhibiting PBP in the CA1 region (for review, see Refs. *92* and *130)*.Glucocorticoids also lower the NMDA-dependent long-term depression (LTD) of the synapses *(125)* and increases voltage-gated calcium currents *(131)* in the hippocampus. Glucocorticoids act on neurons in both the hippocampus and the BLA to increase the amplitude of high-voltage activated calcium currents without changing any of their passive properties *(132)*. However, glucocorticoids have also been shown to cause an increase in the excitability of LA neurons *(116)*. Unlike the hippocampus, the amplitude of the low afterhyperpolarization potentials are not changed in the BLA, possibly due to differences in subunit expression of calcium channels *(133)*.

In a manner similar to chronic stress, a single stress episode affects synaptic plasticity in the hippocampus: LTP is impaired, and LTD is enhanced *(134–137)*. This defect in synaptic plasticity correlates with the stress-induced impairment of hippocampus-dependent memory *(124)*. It is well documented that stress interferes with performance of hippocampus-dependent tasks but facilitates tasks such as eyeblink conditioning in both rats *(63)* and humans *(138)*. Hippocampus-dependent spatial learning tasks are impaired by chronic stress, and these changes endure well beyond the duration of the stressor *(139)*. Unlike chronic stress, however, the changes in plasticity caused by at least certain types of acute stress do not last long *(136)*.

If the effects of acute stress in animal models show any parallel with the human disorder of PTSD, behavioral impairments should be expressed long after an acute stress episode. Although there is no evidence of a delayed onset of aberrant hippocampus-dependent memory tasks, there are experiments showing that anxiety-like behavior can have a delayed onset after a single stressful experience *(72,79)*. In this context, the interaction between the amygdala, where the effects of chronic stress can be persistent *(71)*, and the hippocampus may explain the temporal evolution of the behavioral changes *(140)*.

Mechanistically, the effects on the hippocampus are known to be dependent, at least in part, on glucocorticoids *(141,142)*. Although both the hippocampus and the amygdala have high levels of MRs and GRs, agonists of the GRs are known to enhance retention memory for learning tasks by acting on the BLA *(103,143–145)*. While the stress effects are dependent on glucocorticoids, there is evidence that this is not sufficient to explain the behavioral enhancement of memory seen after stress, and that this enhancement may be selective for certain forms of memory *(146,147)*.

In the amygdala, as in the hippocampus, mechanisms of plasticity are impaired by stress in a glucocorticoid-dependent manner *(148)*, even though amygdala-dependent fear conditioning learning is enhanced *(63)*. Yet, the populations of cells recruited by acute versus chronic stress may be different *(149)*. Acute stress causes a suppression of unit activity in the BLA, and reexposure to the stressful context also causes a decrease in activity in an NMDA-dependent manner. This is similar to the recall and reactivation hypothesis in PTSD *(150)*.

Effects on Inhibitory Synaptic Transmission

As for inhibitory neurotransmission, the link between stress-induced anxiety disorders and the GABA-ergic system in the amygdala is a well-established one *(151,152)*. Stress and glucocorticoids reduce inhibitory currents in the BLA (Fig. 4) *(116,153)* and in the PVN of the hypothalamus *(154)* and reduce extracellular GABA in the hippocampus *(155)*. A study by Duvarci and Pare provided valuable insights into the direct synaptic impact of glucocorticoid action on intrinsic excitability and inhibitory synaptic transmission in the LA (Fig. 4). Direct wash in of glucocorticoids onto a brain slice resulted in an increase in excitability in amygdalar neurons and a reduction in evoked inhibitory postsynaptic potentials (IPSPs) *(116)*.

Acute stress results in an immediate, short-lived decrease in spontaneous miniature inhibitory postsynaptic currents (mIPSCs) (Fig. 4) *(156)*. It has also

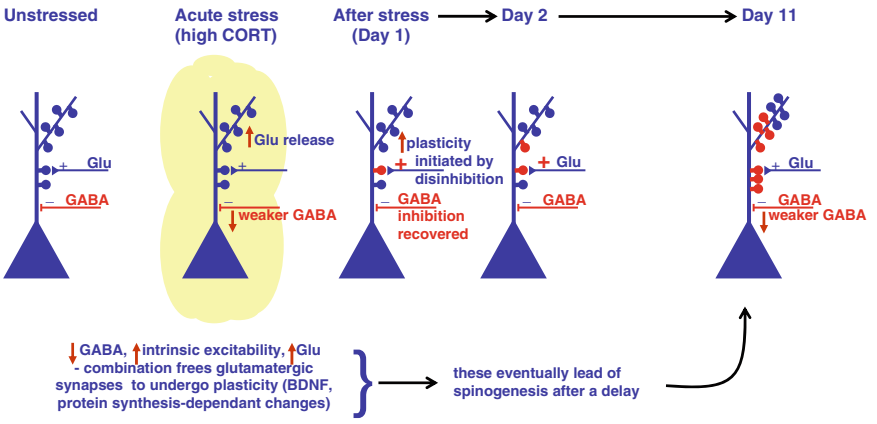

Fig. 4. Effects of acute stress on basolateral amygdala (BLA) principal neurons. Brief but severe stress, such as 2 h of immobilization, triggers a surge of high corticosterone (CORT) and glutamate release (*yellow cloud*) *(117,118)*. High CORT in turn causes a reduction in γ-aminobutyric acid-ergic (GABA-ergic) inhibitory synaptic inputs to BLA cells, which also exhibit enhanced intrinsic excitability by way of enhanced action potential firing *(116)*. Stress-induced disinhibition frees up the excitatory glutamatergic synapses to undergo plasticity, which eventually leads to a delayed strengthening of these inputs through biochemical signaling mechanisms. These plasticity mechanisms, once triggered, continue despite restoration of normal levels of inhibition, glutamate, and CORT after the termination of acute stress. Eventually, this leads to strengthening of the structural basis of synaptic connectivity that is manifested as newly formed spines in the BLA *(72)*, which are restricted to dendritic segments that are closer to the soma. However, as noted in the text, CORT elevation prior to the time of the severe stress has the ability to prevent the increased anxiety and increased synaptogenesis *(114)* via mechanisms still to be elucidated (*See Color Plates*)

been found that acute stress results in a delayed structural change in the form of increase in spine density in the LA 10 days later *(72)*. This change in excitatory synaptic connections is similar to that caused by 10 days of chronic stress and is similarly accompanied by a decrease in mIPSCs (Fig. 5). Although the delayed structural effects of acute stress are less widespread than those of chronic stress *(49)*, they are similar in terms of modified amygdalar output. The original period

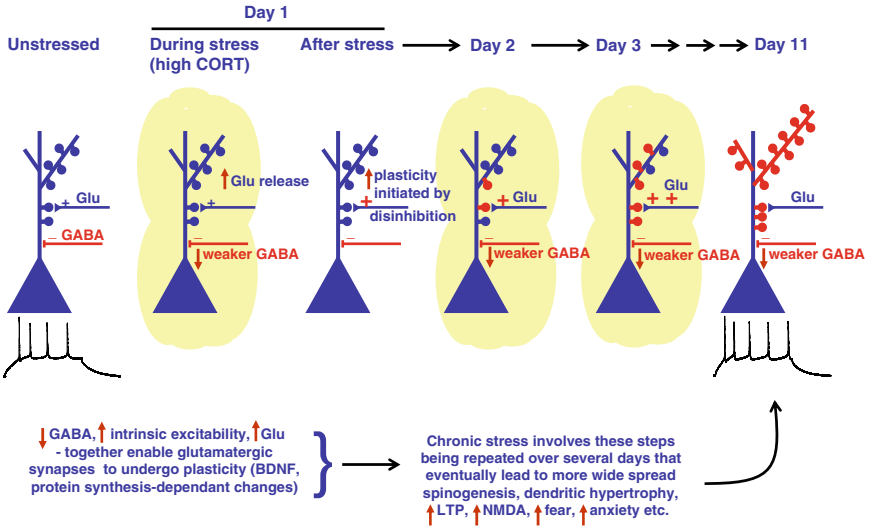

Fig. 5. Effects of chronic stress on basolateral amygdala (BLA) principal neurons. The first episode of stress (on day 1) elicits a reduction in γ-aminobutyric acid-ergic (GABA-ergic) inhibition as well as an increase in excitability *(116)*. Although this inhibition is reduced *(153,156)* only during and short periods after stress-induced increases in corticosterone (CORT) and glutamate release (*yellow cloud*), this brief window of disinhibition sets in motion plasticity mechanisms in the excitatory synapses that persist beyond the duration of the first stress episode (see Fig. 4). Exposure to repeated stress on subsequent days has a cumulative effect *(157)* in that the same single stress episode-induced changes in synaptic inhibition and excitation now act on a cellular substrate that is already undergoing plasticity as a result of earlier exposure to stress. Thus, chronic exposure to stress acts on a sliding, and continuously strengthening, baseline of plastic inputs that quickly add up to give rise to more robust and widespread structural changes (*new spines in red*). These plastic changes are eventually manifested as extensive spinogenesis across both primary and secondary dendrites (as opposed to more localized spinogenesis that is triggered by a single episode, as depicted in Fig. 4). This enhanced structural connectivity also supports enhanced long-term potentiation (LTP) *(157)*, possibly mediated by larger *N*-methyl-D-aspartate (NMDA) receptor-mediated currents *(157)*. Finally, chronic stress-induced strengthening of the physiological and structural basis of synaptic connectivity may also lead to dendritic elongation. As noted in the text, CORT has the ability to mimic, and therefore possibly to mediate, the dendritic elongation *(113)* (*See Color Plates*)

of low inhibition may therefore set off a cascade of synaptic and molecular events that result, 10 days later, in the changes in both excitatory and inhibitory synapses in the LA (Fig. 4).

The link between enhanced excitability and decreased inhibition in the amygdala after stress is of relevance in the action of anxiolytics such as benzodiazepines, which act by enhancing inhibition *(151,152,157)*. Stress-induced anxiety may therefore be tightly coupled to levels of inhibition in the amygdala and possibly in other brain areas. In parallel with the increase in NMDA currents *(120,121)*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) binding *(158)*, and extracellular glutamate *(117,118)* caused by stress or glucocorticoid application, this reduction in inhibition may be shifting the balance away from homeostasis into a disease state (Figs. 4 and 5). Both from the point of view of therapeutic drugs and from a possible role in gating the cascade of stress-induced changes seen in PTSD, the inhibitory GABA-ergic system is very likely to be a key player.

Besides excitation, inhibitory mechanisms are also modulated by adrenal steroids. For example, low levels of corticosterone alter messenger ribonucleic acid (mRNA) levels for specific subunits of $GABA_A$ receptors in hippocampal area CA3 and the dentate gyrus of ADX rats *(159)*, whereas stress levels of corticosterone have produced different effects on $GABA_A$ receptor subunit mRNA levels and receptor binding in hippocampal subregions, including CA3 *(160)*. Therefore, it appears that corticosterone may alter the excitability of hippocampal neurons through regulation of $GABA_A$ receptor expression as well as excitatory amino acid expression, but it remains to be seen if the corticosteroid effects on neuronal morphology involve changes in the number or pharmacological properties of $GABA_A$ receptors.

SYNTHESIS AND FUTURE DIRECTIONS

Animal models of stress, aimed at explaining many of the symptoms of PTSD, have relied on a wide range of analyses spanning multiple levels of neural organization—from behavior, through cells and synapses, down to molecules. These studies have yielded a rich collection of findings in three brain structures that have also been implicated in clinical and neuroimaging analyses of PTSD in humans. As discussed, two broad themes have emerged from these findings in the hippocampus, amygdala, and PFC. First, although many of the mechanistic details are yet to be fully elucidated, there is growing evidence pointing to a common set of endocrinological and physiological changes that are triggered in all three brain areas during and immediately after stress (Fig. 3). Second, despite sharing common features in their genesis, the plasticity mechanisms that eventually take shape in cells and synapses across these three areas exhibit strikingly different patterns (Fig. 2), which in turn may explain the diversity of symptoms of PTSD. A major challenge for future research will be to unravel the precise mechanisms and spatiotemporal features of how these diverse patterns emerge over time. Three issues, discussed next, are particularly important in this regard.

Differential Modulation of Synaptic Signaling During and Immediately After Stress

While elevation in the levels of glucocorticoids and glutamate appears to be one of the earliest changes induced by stress, there may be subtle differences in how such an increase affects excitatory and inhibitory synaptic transmission in the hippocampus, amygdala, and PFC. For example, evidence from the hippocampus suggests that there is an inverted U-shaped curve (for an example of "hormesis," see Ref. *161)*, in which low physiological levels of adrenal steroids enhance excitability and memory processes and exert trophic and protective effects on hippocampal neurons. It is not clear if a similar profile of glucocorticoid dependence is in play in the amygdala as well. However, clinical studies on PTSD treatment are strongly suggestive of a protective role for cortisol in alleviating symptoms of PTSD. Adrenal glucocorticoids have a powerful effect on the human brain. In Cushing's disease, there is hippocampal atrophy, depressed mood, and impaired cognitive function that can be at least partially reversed by reducing the hypercortisolemia *(162,163)*.

In contrast, there are reports that individuals having lower levels of salivary or urinary cortisol are more susceptible to developing PTSD *(89)*. This has been studied therapeutically in a clinical research setting so that patients who receive stress levels of cortisol as part of their treatment in medical intensive care units (ICUs) have a lower probability of developing ICU-related PTSD symptoms *(164–168)*. These data suggest that glucocorticoids may protect against the development of stress-related disorders. Work with abuse victims has shown that there is an exaggerated adrenocorticotropic hormone (ACTH) response to stress or corticotropin-releasing hormone (CRH), suggesting that hypocortisolism in these people is an adaptation of the adrenal gland to the exaggerated response to stress at the level of the hypothalamus/pituitary. In response to this decreased production of cortisol, there is an increased responsiveness of GRs in PTSD patients *(169)*. An interesting theory put forward to give an evolutionary basis for this phenomenon posits that glucocorticoid insufficiency might be adaptive in that it allows inflammatory healing to occur by favoring innate immunity mechanisms. Also, reduced glucocorticoid signaling would favor an enhancement of noradrenergic function, which is key to the consolidation of emotionally laden experiences *(170)*, and might increase arousal and facilitate emotional memory formation.

Preliminary studies using animal models of acute stress suggest a similar protective role for corticosterone in the amygdala. As described, rats exposed to acute immobilization stress exhibit a delayed increase in anxiety-like behavior *(72)*. Using this same acute stress model, it has been reported that oral administration of corticosterone prior to acute stress prevents the delayed increase in anxiety *(114)*. Furthermore, whereas either corticosterone administration or repeated restraint stress will cause dendrites to be shortened in the CA3 region, the combination of the two treatments nullifies the dendritic remodeling in CA3 *(171)*. And, as noted, adrenal steroids are implicated in

the ability of chronic stress to cause dendritic lengthening in BLA neurons as well as increased anxiety *(113)*. Yet, at the same time, adrenal steroids are also implicated in the ability of an acute stress prior to acute immobilization to reduce the immobilization-induced anxiety and spinogenesis in the BLA *(114)*.There are also paradoxes in the role of adrenal steroids in inhibition in amygdala. On the one hand, an electrophysiological study has demonstrated that in vitro application of stress levels of corticosterone leads to a reduction in GABA-ergic inhibitory synaptic transmission, along with an increase in intrinsic excitability of excitatory principal neurons, in brain slices of the LA (Fig. 4) *(116)*. There is also evidence for an inverse relationship between GABA-ergic inhibitory tone in the BLA and behavioral anxiety *(151,152)*. On the other hand, in vivo recordings and immunocytochemical labeling have also shown that the ability of serotonin to inhibit glutamatergic activity in the LA is dependent on the presence of corticosterone and possibly glucocorticoid activation. While serotonin inhibited both synaptically and glutamate-evoked action potentials in LA neurons, it failed to do so in ADX rats. Strikingly, high, but not low, corticosterone doses given to ADX animals reinstated the inhibition of excitatory transmission of serotonin *(115)*. These findings raise the intriguing possibility that varying levels of corticosterone may have different effects on amygdalar function and its behavioral consequences, possibly following a U-shaped dose-response curve. In other words, very high levels of corticosterone (e.g., triggered by severe and chronic stress in rats), as well as very low levels (e.g., insufficient cortisol in ICU patients), may cause an imbalance between excitation and inhibition in the amygdala, thereby eliciting affective symptoms (Figs. 4 and 5). Taken together, these findings highlight the need for future studies to examine the precise dose dependence of how glucocorticoids modulate excitatory and inhibitory synaptic transmission in the amygdala and PFC and how these compare with the hippocampus.

Similarly, the availability of large amounts of extracellular glutamate after stress could have a profound impact on glutamate receptor activation and consequent plasticity (Fig. 3). For example, the dominant form of Hebbian LTP that is impaired by stress depends on the activation of postsynaptic NMDA receptors in the hippocampus *(172)*. Strikingly, the LA also supports a form of associative Hebbian LTP that requires the activation of presynaptic, and not postsynaptic, NMDA receptors. Would the surge in extracellular glutamate triggered by stress affect these two forms of Hebbian NMDA receptor-dependent LTP differently? Indeed, LTP itself appears to be quite different in terms of its ease of induction in the hippocampus versus the amygdala. In the amygdala, GABA-ergic inhibition exerts a potent regulatory influence on LTP induction, which is not the case in the hippocampus *(173)*. In other words, the differential modulation of GABA-ergic modulation by stress and stress hormones could serve as an early point of divergence between the hippocampus and amygdala. These early differences in turn could trigger biochemical signaling cascades that eventually set the plasticity mechanisms in excitatory glutamatergic synapses on a different course in the different brain areas.

Differences in Network Architectures

It is quite possible that the contrasting patterns of plasticity in the three brain areas (Fig. 2) are not all caused by intrinsic differences in the early steps of synaptic signaling triggered by stress. It is also possible that the common set of early changes triggered by stress elicits plasticity mechanisms that do not differ between the three areas—and that the difference lies in their drastically different neuronal circuitry. Indeed, there is evidence for very different forms of stress-induced structural plasticity even within the amygdala. For example, while repeated restraint stress for 21 days leads to the formation of spine synapses in the BLA, it causes the opposite effect—a loss of spines—in the medial amygdala (MeA). Furthermore, stress-induced spine loss in the MeA depends on the extracellular matrix protease tPA, which plays no role in spine formation in the BLA *(174,175)*. Further, stress-induced upregulation of tPA is restricted to the MeA and central nucleus of the amygdala (CeA), the two major noncortical output nuclei of the amygdala. While these observations highlight differences between input and output nuclei of the amygdala, there may also be important differences between the output nuclei themselves. For example, studies using the startle reflex indicated that the bed nucleus of stria terminalis (BNST) in the so-called extended amygdala may be involved in processing signals more akin to cue-nonspecific fear or anxiety, whereas the CeA is more involved in cue-specific fear *(176,177)*. Therefore, rules governing neuronal plasticity that vary even between microcircuits located in the same brain structure could also contribute to the divergent effects of stress.

Interconnectivity and Interdependence

In earlier reports of the specific findings described, stress-induced plasticity in different brain regions was treated as stand-alone effects manifested as properties intrinsic to individual structures. However, a large body of neuroanatomical data also points to extensive interconnections among the hippocampus, amygdala, and PFC *(130,178,179)*. This raises the intriguing possibility that some of the structural and physiological changes triggered by stress in one brain area may, at least in part, influence changes in other areas.

In a series of influential studies, McGaugh and colleagues reported that pharmacological perturbations in the amygdala, affecting synaptic transmission mediated by GABA, opioid, norepinephrine, and acetylcholine, can facilitate or impair the formation of hippocampal memory *(180–182)*. Lesions and drug infusions targeting the amygdala, as well as stimulation of the amygdala, have also been shown to modulate the magnitude of LTP in the dentate gyrus *(183)*. These findings raise the possibility that the amygdala is in a position to play a significant role in mediating the effects of stress on hippocampal function.

Consistent with this view, Kim and colleagues reported that electrolytic lesions of the amygdala before exposure to uncontrollable restraint-tail shock stress prevent impairment of hippocampal LTP and spatial memory in rats *(184)*. More recent experiments by the same group, using microinfusions of the $GABA_A$ receptor agonist muscimol into the amygdala before stress, prevented stress-induced

impairment of LTP in hippocampal slices *(185)*. Consistent with these physiological effects, at the behavioral level, stress failed to impair spatial memory in the Morris water maze task in animals receiving muscimol infusions in the amygdala. Importantly, muscimol infusions into the amygdala immediately after stress did not rescue stress-induced deficits in LTP and spatial memory *(184)*.

These studies, which provide compelling evidence for a role of the amygdala in mediating the effects of stress on hippocampal LTP and memory, have also been extended to one of the earliest reported cellular correlates of stress-induced plasticity in the hippocampus—dendritic atrophy of CA3 pyramidal neurons. A report suggested that 21-day restraint stress failed to elicit dendritic atrophy in hippocampal CA3 cells in rats with lesions of the BLA *(186)*. Taken together, these physiological and behavioral findings highlight the role of amygdalar neuronal activity in the impairment of hippocampal synaptic plasticity and memory caused by stress. Similar studies will be needed to examine if stress-induced changes in the PFC contribute to, or are influenced by, changes in the amygdala and hippocampus.

Future Directions: Investigating the Spatiotemporal Dynamics of Stress Effects Across Distributed Networks

As informative as the studies reviewed here have been in constructing a powerful framework for animal models of PTSD, our current understanding is limited by the fact that function is inferred from analysis at the cellular and behavioral levels without any online readout of dynamic changes in neuronal activity in the intact animal. In other words, although a large body of evidence has been gleaned from snapshots at fixed time points in various brain regions, much less is known about stress effects on neuronal activity in the hippocampus, amygdala, or PFC while animals perform behavioral tasks that are known to be affected by stress. The next generation of animal studies aimed at investigating neural mechanisms of PTSD will have to bridge this crucial gap in knowledge. Rapid advances in powerful multichannel single-unit recording tools for behaving animals, first in the hippocampus *(187)* and more recently in the amygdala and PFC *(188,189)*, provide an ideal foundation to extend the scope of current animal models of PTSD in this direction. Indeed, one recent study *(190)* did this by analyzing the effects of audiogenic stress on hippocampal "place cells," the firing of which indicates a specific location in the environment of the rat and has been shown to encode memories of familiar spatial locations *(187,191,192)*. This study reported for the first time that, in addition to impairing the consolidation of spatial memory and hippocampal LTP in vitro, audiogenic stress impairs the stability of firing rates of place cells recorded from area CA1 in rats foraging freely on a novel open-field platform. These observations raise the possibility that stress-induced impairment in synaptic plasticity may block the storage of stable "rate maps" by hippocampal place cells, and this may underlie spatial memory deficits triggered by stress *(190)*.

In vivo electrophysiological recording techniques in awake, behaving rodents that enabled detailed studies linking spatial memory and place cells in the hippocampus

have also been very valuable in establishing that neurons in the LA encode aversive memories during the acquisition and extinction of Pavlovian fear conditioning (reviewed by Maren and Quirk in Ref. *22)*. Further, in vivo unit recordings in behaving animals, combined with lesions and pharmacological inactivation, have shown that extinction of fear memory reduces associative plasticity in the LA and involves the hippocampus and PFC. In addition to the inherent power of these in vivo multielectrode recording studies in providing valuable insights into the encoding of information in neuronal networks implicated in PTSD, they also bring us back to the dominant theme emerging from human neuroimaging studies: interactions between activity in the hippocampus, amygdala, and PFC.

We began this chapter by summarizing results from neuroimaging research that revealed enhanced responsivity of the amygdala and diminished responsivity of the mPFC. Further, while amygdala responsivity is positively associated with symptom severity in PTSD, mPFC responsivity has the opposite association *(35,36)*, which points to a functional relationship between these two regions. There is also evidence for reduced hippocampal volumes and function in PTSD. In this context, several recent in vivo recording studies are particularly relevant. For example, simultaneous recordings of electrical activity have provided evidence for an increase in rhythmically synchronized activity at theta frequencies between the LA and hippocampal area CA1 in freely behaving mice during fear memory retrieval *(188)*. Further evidence highlighting the importance of synchronization of neuronal activities between networks comes from another study that used simultaneous tetrode recordings from the CA1 area and mPFC in rats *(189)*. This study showed that correlated firing in the two brain areas is selectively enhanced during behavior that recruits spatial working memory, allowing the integration of hippocampal spatial information into a broader, decision-making network. Importantly, the increased correlations are paralleled by enhanced coupling of the mPFC and CA1 area in the theta frequency range, the same range earlier shown to play a role in synchronization of activities in the amygdalo-hippocampal network in fear-conditioned mice *(188)*. These studies suggest that neural synchrony may represent a more general mechanism through which brain structures that are differentially affected in PTSD encode information independently and interact selectively according to specific needs imposed by any given behavioral task (e.g., decision making based on efficient recall of a particular cue or context related to a fearful experience).

The power of these in vivo electrophysiological recording methods opens up new avenues to investigate how the hippocampus, PFC, and amygdala interact dynamically during and after stress. Such studies will also help us understand the precise role for the amygdala in plasticity mechanisms triggered in other brain regions, possibly revealing a common target for clinical interventions for PTSD. Furthermore, future studies will have to analyze how stress-induced modulation of cellular and synaptic mechanisms in these three brain areas affects neural synchrony both within and across these structures. Finally, it will be particularly interesting to examine if and how such network effects manifest themselves as disruption of cognitive and emotional function at the behavioral level.

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