

Chapter 3

Synaptic Stress, Changes in Glutamate Transmission and Circuitry, and Psychopathology

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Abstract Dysfunction of the glutamate system is increasingly considered a core feature of stress-dependent neuropsychiatric disorders. Clinical neuroimaging studies have shown consistent volumetric changes in limbic and cortical areas, while preclinical studies with stress protocols in rodents found dendritic remodeling and reduction of synapses in the same areas, suggesting that destabilization of glutamate release/transmission, in turn induced by stress and glucocorticoids, is crucial for cognitive function and neural architecture. We found that acute stress rapidly enhances depolarization-evoked glutamate release/transmission in prefrontal and frontal cortex (PFC/FC), an effect mediated by stimulation of synaptic corticosterone receptors. Corticosterone rapidly increases the readily releasable pool of glutamate vesicles, through activation of synaptic receptor-mediated nongenomic mechanisms in PFC/FC. Moreover, we have shown that chronic antidepressants are able to prevent the enhancement of glutamate release induced by acute stressors in these areas.

While the predominant effect of acute stress is an enhancement of synaptic transmission, repeated exposure to stress brings about atrophy and remodeling of dendrites, loss of synapses, and reduction of synaptic transmission (except perhaps in amygdala). Understanding the mechanisms and effectors involved in this biphasic action of stress is essential to the development of new diagnostic and therapeutic means for stress-related disorders. Select brain-derived neurotrophic factor (BDNF) transcripts and their translation at synapses could be among these key effectors.

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Abbreviations

AMPA	α -Amino-3-hydroxy-methyl-4-isoxazole propionic acid
BDNF	Brain-derived neurotrophic factor
EM	Electron microscopy
FS	Footshock
GR	Glucocorticoid receptors
LTP	Long-term potentiation
MR	Mineralocorticoid receptors
MRI	Magnetic resonance imaging
mPFC	Medial prefrontal cortex
PFC/FC	Prefrontal and frontal cortex
PPF	Paired-pulse facilitation
RRP	Readily releasable pool
SNARE	Soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor
TIRF	Total internal reflection fluorescence
NMDA	N-Methyl-D-aspartate

3.1 Introduction: The Role of Synaptic Stress in Pathophysiology of Stress-Related Neuropsychiatric Disorders

A stressor is an event or experience that threatens the ability of an individual to adapt and cope. The impact of different behavioral stressors on cognitive/affective functions may vary depending on type, intensity, and duration of stress, and is influenced by genetic background. The outcome of stress may range from plasticity enhancing effects and improved cognition, when stress response is efficiently turned on and shut off, to noxious effects, when the response is dysregulated. A maladaptive stress response can be associated with impaired function and triggering of brain, systemic, and metabolic disorders. Recent lines of evidence have shown how tracing the effects of stress at synaptic level and on neurochemical mechanisms may supply essential information as to how different stressors affect the brain, induce adaptive or maladaptive changes, and trigger brain and metabolic disorders (Fig. 3.1; McEwen 2005; Gorman and Docherty 2010; Sanacora et al. 2012; Popoli et al. 2012; Sousa and Almeida 2012; Tokita et al. 2012; Gray et al. 2013).

3.1.1 *Changes in Volume of Brain Areas and Neuronal Architecture in Stress-Related Neuropsychiatric Disorders*

Stressful life events impact on memory and cognition and are known to precipitate neuropsychiatric diseases, in particular mood and anxiety disorders. Half a century after the monoamine hypothesis, which assigned a key role in pathophysiology

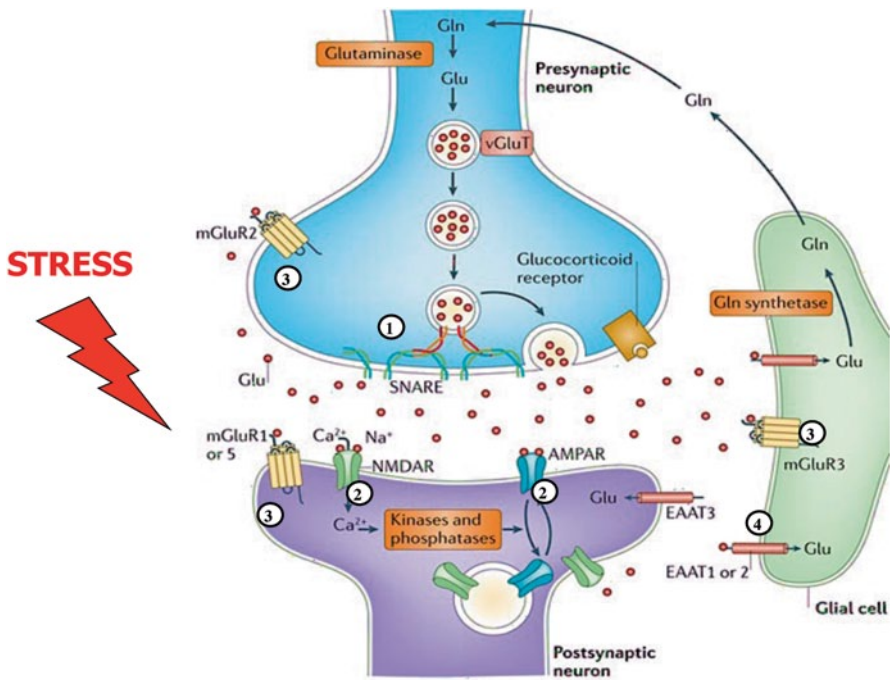


Fig. 3.1 Sites of action (targets) of stress in the glutamate synapse. Several sites/mechanisms of regulation of the glutamate synapse have been shown to be targets of stress: 1 presynaptic release of glutamate; 2 postsynaptic ionotropic receptors for glutamate (N-methyl-D-aspartate receptors (NMDARs) and α -amino-3-hydroxy-methyl-4-isoxazole propionic acid receptors (AMPA)s); 3 metabotropic glutamate receptors (*mGluR*); and 4 glial-specific glutamate transporters. See text for details. Ca^{2+} calcium ions, EAAT excitatory amino-acid transporter, Gln glutamine, Na^+ sodium ions, SNARE soluble N-ethylmaleimide-sensitive factor attachment protein receptor, vGluT vesicular glutamate transporter, Glu glutamate. (Adapted from Popoli et al. 2012)

to reduced availability of monoamine transmitters (Heninger et al. 1996), it has become increasingly acknowledged that maladaptive changes in the structure and function of excitatory/inhibitory circuitry (representing the vast majority of neurons and synapses in the brain) have a primary role in the pathophysiology of mood and anxiety disorders, particularly major depression (McEwen 2005; Gorman and Docherty 2010; Sousa and Almedia 2012; Tokita et al. 2012; Musazzi et al. 2013).

Clinical neuroimaging studies have shown consistent volumetric changes in brain areas where glutamate neurons and synapses predominate. Significant volumetric reduction of hippocampus and prefrontal cortex has been shown in MRI studies of patients with mood and anxiety disorders (Campbell and MacQueen 2006; Konarski et al. 2008; Koolschijn et al. 2009; Lorenzetti et al. 2009; Woon et al. 2010). For hippocampus, correlation of volume reduction with length of depressive episodes was found (Frodl et al. 2006; MacQueen et al. 2003; Sheline et al. 2003). Conversely

volumetric enlargement was found in amygdala, at least in the early course of illness (Lorenzetti et al. 2009).

In parallel, rodent studies have shown that chronic stress protocols induce dendritic atrophy, reduction of synapses number, and volumetric reductions resembling those observed in patients with mood and anxiety disorders (McEwen 2005, 2010; Gorman and Docherty 2010; Sanacora et al. 2012; Popoli et al. 2012; Sousa and Almeida 2012; Musazzi et al. 2013). Although dendritic atrophy and remodeling is considered a main mechanism for brain volumetric reduction, there are other factors that have been involved, including glial cell loss, particularly in PFC of depressed patients (Rajkowska et al. 1999), and reduction of neurogenesis in hippocampus (Duman 2004). A current hypothesis states that neuronal dendritic remodeling is mainly responsible for volumetric reduction; this is inferential evidence, because it explains the volumetric reduction seen in humans (and partly in rodents) with the dendritic remodeling induced by repeated stress in rodents (for a discussion see Sanacora et al. 2012). However, recent work has brought new evidence in favor of this hypothesis. Kang et al. (2012) found a reduced synapse number in the postmortem dorsolateral PFC of patients with major depression. Ansell et al. (2012) found that cumulative adverse life events (mostly stressful episodes) were associated in humans (with no psychiatric diagnosis) with smaller gray matter volume of medial PFC and other cortical and limbic areas, as measured with MRI. This last finding established a clear correlation between repeated stress and volumetric reduction. Finally, Kassem et al. (2013) reported that mice subjected to chronic (21 days) restraint stress showed significant gray matter loss in anterior cingulate cortex and hippocampus, measured with MRI. All these lines of evidence clearly support a correlation between stress, dendritic remodeling, and volumetric reduction.

A major role in this process is attributed to elevation of glucocorticoid hormones by stressors, which enhance glutamate release/transmission, in turn purported to induce retraction of dendrites. Converging evidence from various groups has shown that enhancement of glutamate release/transmission in cortical/limbic areas, in turn induced by stress and glucocorticoids, is crucial for these structural/functional changes (Fig. 3.2; Musazzi et al. 2013).

3.2 Acute Stress Enhances Glutamate Release and Transmission in Cortical and Limbic Brain Areas

Several studies have shown in the past that exposure of rodents to acute stressors or administration of glucocorticoids rapidly and transiently increase the level of extracellular glutamate, measured with microdialysis *in vivo*, in cortical and limbic brain areas (Bagley and Moghaddam 1997; Lowy et al. 1993; Moghaddam 1993; Venero and Borrell 1999). However, the nature and origin of extracellular glutamate measured by microdialysis has been questioned, because the pool of glutamate released at presynaptic terminals is only a small part of the total glutamate pool, which is largely made up of metabolic glutamate (for a discussion see: van der

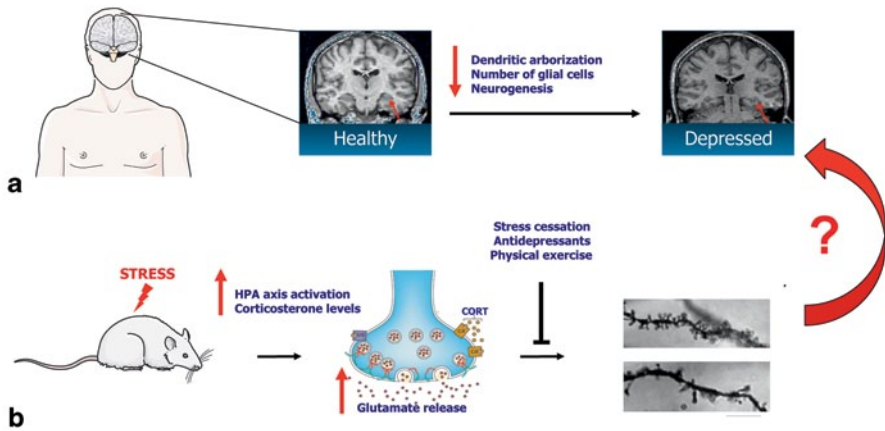


Fig. 3.2 Relationship between stress, glutamate system dysfunction and structural brain changes in stress-related neuropsychiatric disorders: a theoretical model. **a** Neuroimaging studies have consistently shown volumetric reduction of cortical and limbic areas in the brain of patients with mood and anxiety disorders. This has been attributed to several factors, including dendritic atrophy/remodeling, loss of glial cells and reduction of neurogenesis (in hippocampus). **b** Preclinical studies in rodents have shown that stress, through the action of glucocorticoids and other neurochemical/neuroendocrine mediators, may induce abnormal enhancement of glutamate release and excitatory transmission in select brain areas, including amygdala, hippocampus, and prefrontal cortex. If repeated or protracted, stress induces atrophy and remodeling of dendritic arbor at various locations in these areas (except for the amygdala), with loss of dendritic spines and synapses. In turn, dendritic/circuitry remodeling is envisaged as a major causal factor for volumetric changes, observed with magnetic resonance imaging in patients. The evidence that stress and consequent changes in excitatory transmission cause dendritic remodeling comes mostly from rodent studies, while volumetric changes have been observed in humans. The structural/functional changes induced by stress protocols in rodents are reversible with cessation of stress, and are prevented or reversed by pro-adaptive treatments, such as chronic antidepressant treatments and voluntary physical exercise. *HPA* hypothalamic-pituitary-adrenal, *CORT* corticosterone. (Adapted from Musazzi et al. 2013)

Zeyden et al. 2008; Musazzi et al. 2011). The effects of stress and glucocorticoids on glutamate release have been substantially confirmed and shown in details more recently by works employing different methodologies, including: (1) electrophysiological recordings; (2) measurement of endogenous glutamate release from synaptic terminals (synaptosomes) in superfusion; (3) measurement from synaptosomes in bulk by enzyme-linked fluorometric assay; and (4) measurement of resting glutamate levels in vivo by enzyme-based microelectrode arrays (Czakoff and Howland 2010; Hascup et al. 2010; Karst et al. 2005; Musazzi et al. 2010; Reznikov et al. 2007; Satoh and Shimeki 2010; Wang and Wang 2009).

In vitro application of 100 nM corticosterone (the major stress hormone in rodents) to hippocampal slices was shown to enhance rapidly the frequency of miniature excitatory postsynaptic potentials in CA1 pyramidal neurons and reduce paired-pulse facilitation, a measure of synaptic facilitation induced by pairs of stimuli applied at increasing interpulse intervals, suggesting that the stress hormone

increases glutamate release probability (Karst et al. 2005). The rapid onset of this effect and its maintenance in the presence of a protein synthesis inhibitor indicated that a nongenomic pathway underlies this action of corticosterone. In a different study, in rats subjected to elevated platform stress for 30 min, PPF was significantly reduced in CA1 hippocampal area, implying increased glutamate release (Czakoff and Howland 2010). These studies showed that both glucocorticoid application in vitro and acute stress increase glutamate release in hippocampus.

Recently, levels of glutamate in PFC have been measured after tail pinch, a mild stressor used previously in several microdialysis studies, by using enzyme-based microelectrode arrays, which allow better temporal resolution compared to microdialysis in vivo (Hascup et al. 2010). The acute stress induced significant transient increase of extracellular glutamate that was completely blocked by local application of tetrodotoxin, suggesting this was due to exocytotic release of glutamate. Finally, different forms of acute stress were shown to enhance NMDA- and AMPA-receptor mediated synaptic currents in PFC of juvenile rats > 1 h after stress, that were sustained for 24 h after cessation of stress. The enhancement was mimicked by short-term treatment of slices with corticosterone and mediated by increased membrane expression of ionotropic glutamate receptors (Yuen et al. 2009, 2011).

3.2.1 Acute Stress Enhances Depolarization-Evoked Glutamate Release in Prefrontal and Frontal Cortex

In rat prefrontal and frontal cortex (PFC/FC), we have shown that acute stress rapidly enhances glutamate release and transmission, an effect mediated by glucocorticoid/mineralocorticoid receptors (GR/MR). We applied inescapable random foot-shock (FS)-stress to rats for 40 min, and then quickly purified synaptic terminals (synaptosomes) from PFC/FC with Percoll gradients (Musazzi et al. 2010). Basal and depolarization-evoked glutamate release was measured by using the technique of isolated synaptosomes in superfusion. This method is performed by applying a thin layer of purified synaptosomes on a microporous filter and a constant up-down superfusion to the samples (Popoli et al. 2012; Bonanno et al. 2005). By this way, endogenous transmitters/modulators released are immediately removed by the superfusion medium before they can be taken up by transporters, or activate autoreceptors/heteroreceptors present on synaptic terminals. Therefore, any indirect effects are minimized or prevented, and the release of a single amino acid transmitter can be measured precisely. We found that acute FS-stress markedly and significantly enhanced depolarization-evoked release of endogenous glutamate, with no changes in stimulated release of gamma-aminobutyric acid (GABA) or in the basal release of the two amino acids (Fig. 3.3). A selective antagonist of GR, injected systemically prior to stress application completely blocked the stress-induced enhancement of glutamate release (not shown).

Looking at the presynaptic machinery of PFC/FC in stressed rats we found a significant increase in the presynaptic membranes of the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) protein complexes

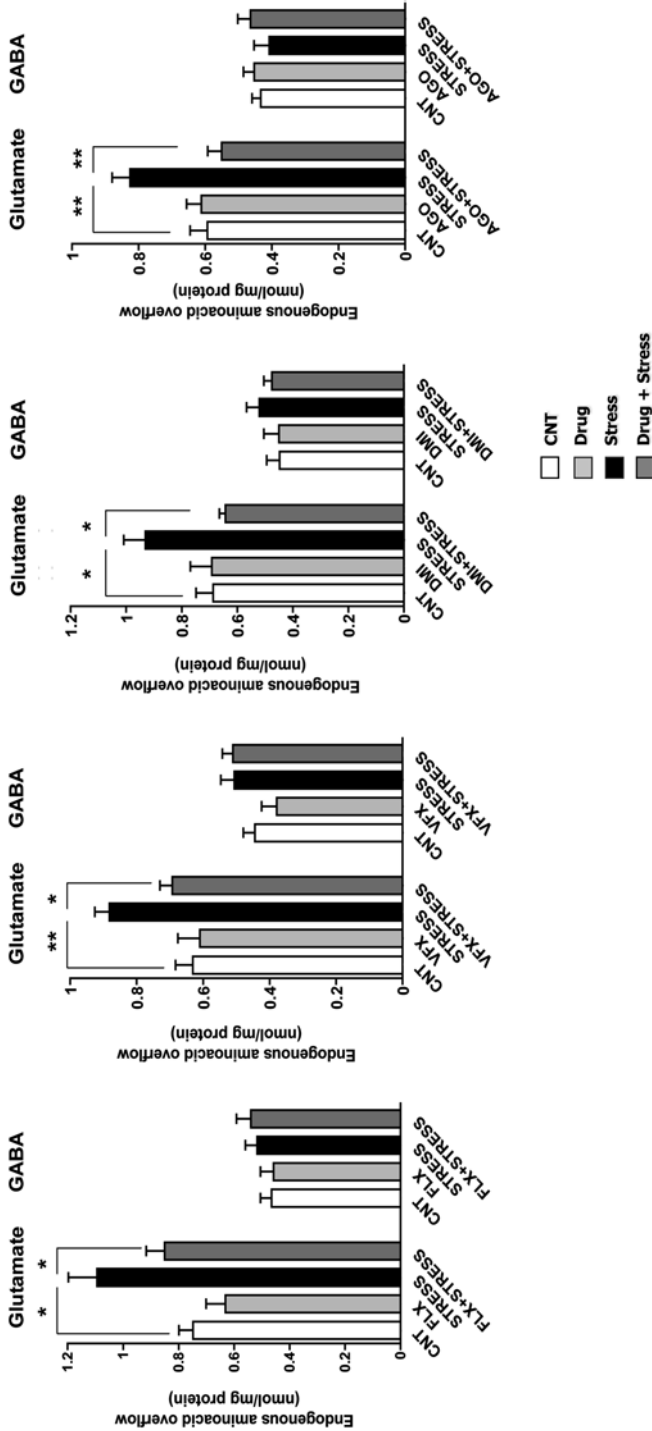


Fig. 3.3 Acute footshock stress enhances depolarization-evoked glutamate release in prefrontal and frontal cortex. Chronic antidepressants prevent the enhancement of release. The bar graphs show glutamate and gamma-aminobutyric acid (GABA) release from prefrontal and frontal cortex of superfused synaptosomes, evoked by 15 mM KCl. The rats were treated chronically (2 weeks) with vehicle (CNT), fluoxetine (FLX), venlafaxine (VF-X), desipramine (DMI), agomelatine (AGO), or subjected to acute footshock stress, or treated chronically with either of the four drugs and then subjected to acute footshock stress. Data are expressed as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$; Newman-Keuls post hoc tests following one-way ANOVA ($n = 6-9$ rats/group). (From Musazzi et al. 2010)

that mediate fusion of synaptic vesicles (Musazzi et al. 2010). Patch-clamp recordings in slices of PFC from stressed rats showed significant increase in the amplitude of spontaneous excitatory postsynaptic potentials, and significant decrease of PPF, which confirmed an enhancement of glutamate release induced by acute stress (not shown). Interestingly, the stress-induced enhancement of glutamate release was abolished if the rats were treated with antidepressant drugs for 2 weeks before the acute stress (Fig. 3.3).

3.2.2 Acute Stress Increases the Number of Vesicles Docked to the Presynaptic Membrane in Perforated Synapses of Medial Prefrontal Cortex

The finding that the number of SNARE protein complexes (which is thought to be a constant number X vesicle in the same terminal) is increased by acute stress in pre-synaptic membranes of PFC/FC suggested that stress may acutely increase the size of the readily releasable pool (RRP) of synaptic vesicles. The RRP is constituted by the vesicles docked onto the presynaptic membrane and ready for fusion when the terminal is stimulated (Rosenmund and Stevens 1996; Lonart and Sudhof 2000; Milanese et al. 2011). First, we sought to assess whether acute stress was able to change the distribution of vesicles in excitatory synaptic terminals and the number of vesicles docked onto the membrane. Therefore, we used a stereological approach for synaptic vesicles quantification, which takes advantage of serial section electron microscopy (Nava et al. 2014; Treccani et al. 2014). We counted the number of total vesicles and the number of vesicles with their membrane overlapping with the presynaptic membrane (membrane-docked vesicles), in asymmetric (excitatory), perforated, and nonperforated medial PFC (mPFC) synapses from control and FS-stressed rats (Fig. 3.4a). Acute stress induced a significant increase in the number of docked vesicles, selectively in perforated but not in nonperforated synapses. The total number of vesicles in both perforated and nonperforated synapses was not changed by stress (Fig. 3.4b). These results were in line with an increase of RRP induced by stress, and suggested that these changes in the distribution of vesicle pools are localized to perforated synapses, which are the synapses undergoing rapid morphological changes, likely under the effect of stress (Treccani et al. 2014).

3.2.3 Corticosterone In Vitro Enhances the Trafficking of Glutamate Vesicles Towards the Presynaptic Membrane: Visualization by TIRF Microscopy

Next, we sought to visualize the trafficking of synaptic vesicles into the RRP, by using an in vitro approach. To this purpose, synaptic vesicles in purified PFC/FC synaptosomes from control rats were labeled with the lipophilic dye FM1-43,

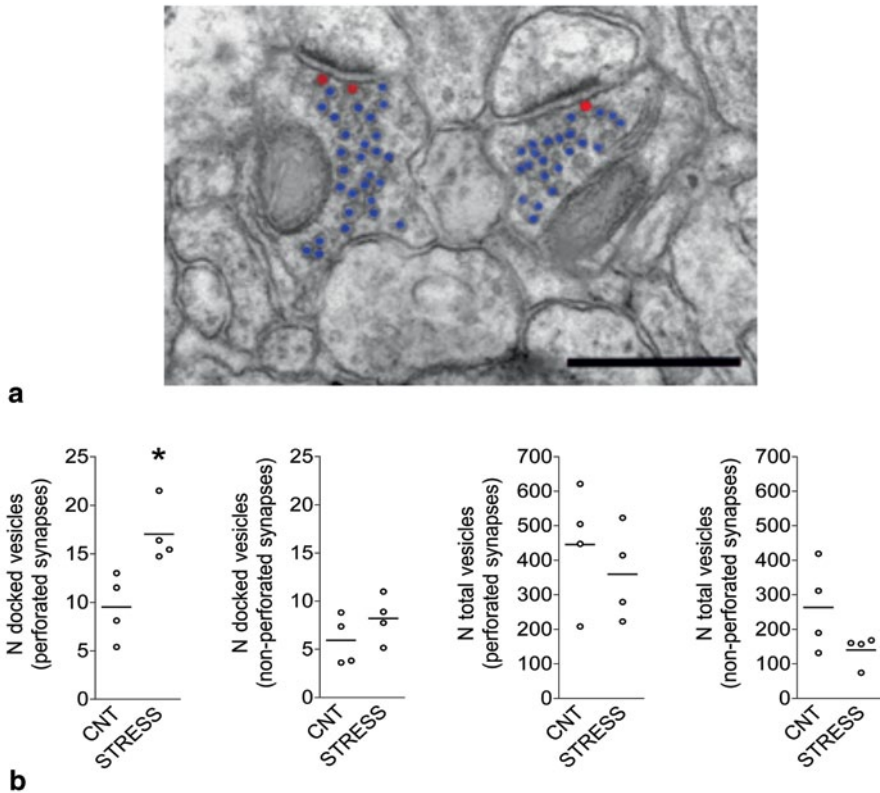


Fig. 3.4 Electron microscopy stereology: Acute stress increases the number of docked vesicles in perforated synapses of medial prefrontal cortex. **a** Representative transmission electron micrograph (EM) of medial prefrontal cortex non-perforated asymmetric synapse, showing docked (*red*) and total (*blue*) vesicles, used for serial reconstruction (28,000X, scale bar 500 nm). **b** Number of docked and total vesicles in perforated and non-perforated synapses of control (*CNT*) and acutely stressed (*STRESS*) rats. Data and statistics as in Fig. 3.3; * $p < 0.05$, $n = 4$ rats/group

which intercalates with plasma membranes and allows monitoring vesicle trafficking (Cochilla et al. 1999). After labeling with FM1-43, live synaptosomes were incubated for 10 min with different concentrations of corticosterone (100 nM, 10 μ M) to assess whether the hormone was able, by local action, to change vesicle trafficking. We used total internal reflection fluorescence (TIRF) microscopy, an imaging technique that allows the study of events occurring in or immediately beneath the plasma membrane (about 100 nm; Groves et al. 2008; Perego et al. 2012). Corticosterone application caused a time-dependent increase in the number of fluorescent spots in the TIRF field, which indicates a time-dependent accumulation of fluorescent vesicles in close proximity to the membrane (Fig. 3.5a). This increase was significant for both corticosterone concentrations, and started immediately after its

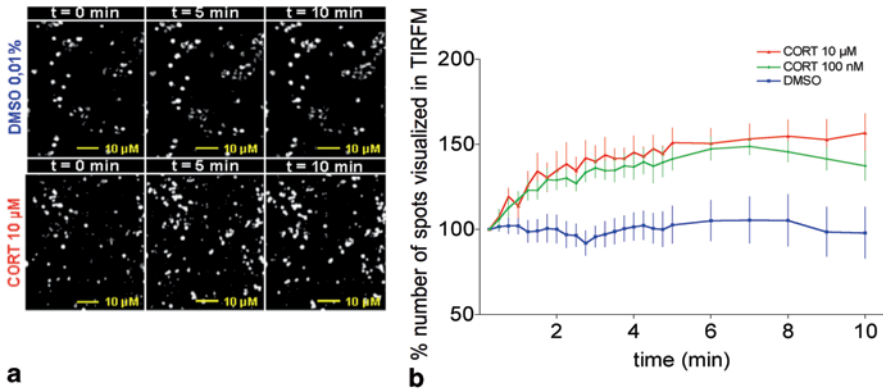


Fig. 3.5 Total internal reflection fluorescence (*TIRF*) microscopy of prefrontal and frontal cortex synaptosomes. Corticosterone (*CORT*) application *in vitro* increases the trafficking of FM1–43 fluorescent vesicles near the presynaptic membrane. **a** Representative *TIRF* images (magnification 100X) at $t=0$, $t=5$, $t=10$ min of live synaptosomes stained with FM1–43 and incubated with 0.01% dimethyl sulfoxide (*DMSO*, top) or 10 μM *CORT* (bottom). The synaptosomes incubated with *CORT* show an increase in the fluorescent spots appearing in *TIRF* field after 5 and 10 min, compared with the number of spots at $t=0$. Scale bar: 2 μm . **b** Graph showing the number of fluorescent spots visualized in the *TIRF* field (expressed as percentage vs the number of spots at $t=0$) during 10 min of *in vitro* incubation with *DMSO*, 100 nM or 10 μM *CORT*. $n=6$ –11 recordings, four independent experiments. The area under the curve of the recording curves for 100 nM and 10 μM *CORT* were significantly different from *DMSO* (control), for time ($p<0.0001$), treatment ($p<0.01$) and interaction ($p<0.0001$). One-way ANOVA followed by Newman–Keuls post hoc test

application; the fluorescent spots were maximal after 3–5 min incubation and remained constant for up to 10 min of recording (Fig. 3.5b). These results showed that *in vitro* incubation of PFC/FC synaptosomes with corticosterone induces rapid mobilization of vesicles towards the presynaptic membrane, consistent with an increase in the RRP size.

In separate experiments with purified synaptosomes in superfusion we found that both acute stress (*ex vivo*) and corticosterone application *in vitro* increased the RRP of glutamate (Treccani et al. 2014), measured after pulse stimulation with hypertonic sucrose, which is the standard method to measure RRP (Rosenmund and Stevens 1996; Lonart and Sudhof 2000; Milanese et al. 2011). We also observed that the rapid action of corticosterone on the trafficking of synaptic vesicles was mediated by local MR/GR, present on isolated synaptosomes, because the concomitant application with corticosterone of either MR/GR selective antagonist (spironolactone or RU486) blocked the translocation of FM1–43 fluorescent vesicles near the presynaptic membrane, observed with *TIRF* microscopy (not shown).

However, corticosterone application together with (15 mM KCl)-containing depolarizing buffer did not enhance glutamate release (not shown), as observed *ex vivo* with synaptosomes freshly isolated from acutely stressed rats (see Fig. 3.3). This finding clearly showed that, although corticosterone mediates an early effect of

stress (e.g., the translocation of vesicles into the RRP), it is not able to fully replicate the effect of acute stress inducing the release of glutamate. This was confirmed by electrophysiological recordings in mPFC, which showed that application of 1 or 10 μM corticosterone to brain slices did not change synaptic transmission for up to 20 min (Treccani et al. 2014).

3.2.4 Both Acute Stress and Corticosterone In Vitro Rapidly Increase the Readily Releasable Pool of Vesicles but only Stress Rapidly Increases Glutamate Release. Implications for the Mechanism of Acute Stress in Prefrontal and Frontal Cortex

Overall, our results showed that both acute stress and application of corticosterone in vitro to synaptosomes rapidly increase the RRP, but only stress rapidly increases glutamate release in PFC/FC. This effect of stress is seemingly mediated by a non-genomic action of the hormone, through the activation of synaptic MR/GR (Karst et al. 2005; Musazzi et al. 2010; Joëls et al. 2012). The presence of membrane-associated receptors for corticosterone has been shown in amygdala and PFC/FC (Treccani et al. 2014; Prager et al. 2010). However, the rapid synaptic action of corticosterone is necessary, but not sufficient, to increase glutamate release/transmission in PFC/FC, which likely requires activation of delayed, genomic, mechanisms. This is consistent with a previous work, which found that brief application of corticosterone to rat brain slices enhanced synaptic transmission only after 1 h (Yuen et al. 2011). The mechanism of stress seems to be different in PFC/FC compared to hippocampus, where local application of corticosterone is sufficient to induce rapid enhancement of glutamate release and synaptic transmission (Karst et al. 2005; Joëls et al. 2012).

Therefore, although the enhancement of glutamate release induced by acute stress in PFC/FC appears to be mediated by corticosterone, the hormone seems necessary but not sufficient for this effect (Fig. 3.6). Corticosterone binds local MR/GR located at or near presynaptic terminals, and rapidly, by nongenomic action, increases the trafficking of synaptic vesicles into the RRP. The RRP increase is localized mostly to perforated synapses, which are the synapses undergoing rapid plastic changes under the effect of stress. This buildup of RRP primes the terminals for the enhancement of glutamate release, which may be delayed by about 1 h, for the subsequent involvement of unknown genomic effects of corticosterone and possibly additional effectors, including retrograde messenger(s) (Popoli et al. 2012; Treccani et al. 2014). Chronic antidepressant treatments mostly or completely prevent the stress-induced enhancement of glutamate release (see Fig. 3.3), suggesting that stabilization of glutamate release/transmission is a relevant part of their therapeutic action (Musazzi et al. 2013). This drug action may protect from buildup of dangerous concentrations of synaptic (or extrasynaptic) glutamate and contribute to preventing or reversing the dendritic remodeling and synaptic disconnection

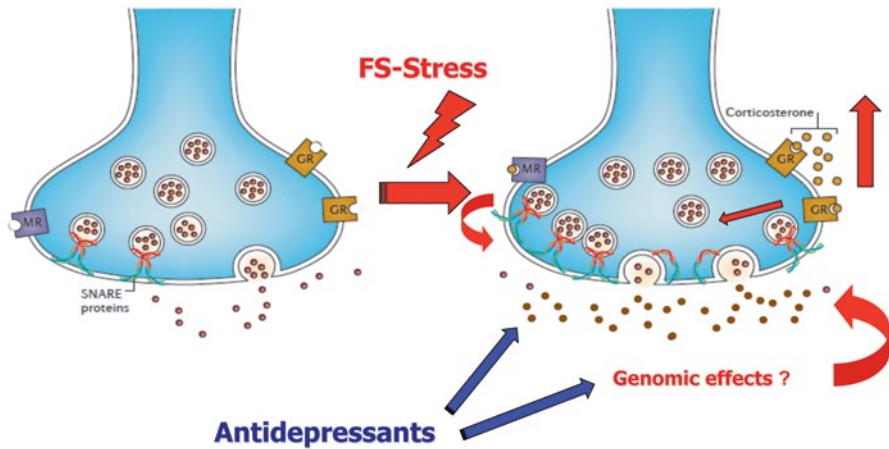


Fig. 3.6 Acute stress enhances glutamate release in prefrontal and frontal cortex. Corticosterone increases the readily releasable pool of glutamate vesicles by local synaptic action, and is necessary but not sufficient for stress-induced enhancement of glutamate release in cortical areas. Acute footshock stress enhances depolarization-evoked release of glutamate from presynaptic terminals of prefrontal and frontal cortex. The acute stress response involves a rapid increase of circulating levels of corticosterone, which binds to putative presynaptic membrane-associated receptor (*GR* and *MR*), in turn inducing increased trafficking of vesicles into the readily releasable pool (RRP). Corticosterone *in vitro* increases the RRP in purified synaptosomes, but does not enhance glutamate release for up to 20 min; evidence obtained by: purified synaptosomes in superfusion, EM-stereology of asymmetric synapses, TIRF microscopy of purified synaptosomes (see Musazzi et al. 2010; Treccani et al. 2014). In prefrontal and frontal cortex, different from hippocampus, corticosterone seems necessary but not sufficient to induce enhancement of glutamate release (at least in the first 20 min). The effect of corticosterone on RRP is likely a rapid non-genomic effect. Delayed, perhaps genomic, effects of corticosterone are necessary for completion of corticosterone action and enhancement of glutamate release and transmission (see Yuen et al. 2011). Previous chronic antidepressant treatments block the stress-induced enhancement of glutamate release. The mechanism of this drug action is not clear yet, but could be related to the delayed genomic effects of corticosterone on glutamate synapses. *SNARE*: N-ethylmaleimide-sensitive fusion protein attachment protein receptor.

which is thought to be a major factor in stress-related neuropsychiatric disorders (Sanacora et al. 2012; Musazzi et al. 2013). The mechanism of this anti-stress action of antidepressants is not clearly understood at present. The drugs prevent the enhancement of glutamate release in PFC/FC, but not the rise of corticosterone levels and the increase of number of SNARE complexes in presynaptic membranes in the same areas (Musazzi et al. 2010). Therefore, the action of these drugs seems to be downstream of the early action of corticosterone and could be related to the delayed, genomically mediated, effects that bring about the enhancement of glutamate release (Fig. 3.6). Further research is under way to dissect this mechanism, which may serve for the identification of new drug targets.

3.3 Structural/Functional Changes Induced by Chronic Stress

Acute stress protocols, as shown above (Sect. 3.2), may allow a careful dissection of the mechanisms whereby stress triggers the modifications in the glutamate synapses. However, experimental protocols employing repeated stress are more often used for animal models of neuropsychiatric pathology, mainly because stress is considered a major predisposing factor in psychopathology, such as for mood and anxiety disorders. There is a considerable literature which analyzed in rodents the effects of stress on: (1) structural features of synapses and circuitry and (2) synaptic transmission and plasticity. We have addressed the first issue in Sect. 3.1.1. For a detailed discussion see: McEwen 2005, 2010; Sanacora et al. 2012; Sousa and Almeida 2012.

Regarding the second issue, the outcome of acute stress episodes on the plasticity of glutamate synapses has been thoroughly analyzed, particularly in hippocampus, by measuring Long-Term Potentiation (LTP), an activity-dependent enhancement of synaptic strength that represents the most studied cellular process linked to memory and learning (Citri and Malenka 2008). Briefly, current hypotheses suggest that stress initially induces activation of synaptic transmission in the forebrain and facilitation of LTP, a phase that is partly coincident with rapid nongenomic action of corticosteroid hormones. The early enhancement of synaptic plasticity in hippocampus may have a role in the formation of traumatic memories that are saved in an individual's experience. The early enhancement is followed by a phase of inhibition of synaptic plasticity, in which the threshold for induction of LTP is raised, corresponding to delayed genomic-mediated action of corticosteroids, probably to consolidate the memory related to stressful events and avoid interference of subsequently formed memories (Kim and Diamond 2002; Diamond et al. 2007; Joëls 2008; Krugers et al. 2010). However, a high level of emotional arousal may impair proper evaluation and processing of experience by interfering with hippocampal function. Detailed information on the effects of stress on synaptic plasticity can be found in Sect. 2 of this chapter.

Regarding the effects of chronic stress on synaptic function, i.e., presynaptic glutamate release and function or membrane insertion of postsynaptic glutamate receptors, less information is available. Early evidence was provided by microdialysis studies, which found selective changes in the adaptation of glutamate release in hippocampus and PFC after application of a few consecutive stressors (Moghadam 2002). Little or no evidence, obtained with later technologies (see above, Sect. 3.2), is available for glutamate release in rodents subjected to chronic stress protocols. However, recently it was found that depolarization-evoked glutamate release, measured in superfused synaptosomes from ventral hippocampus, was reduced in rats subjected to prenatal stress, which showed an anxious behavioral phenotype (Marrocco et al. 2012). In a different study, it was found that glutamate release induced by BDNF in slices of the PFC was attenuated in rats subjected to chronic restraint stress, coupled with anxious/depressive phenotype and downregulation of

GR (Chiba et al. 2012). These works suggest that the consequences of chronic stress protocols on glutamate release may be different from acute stress.

Moreover, recent work has shown that the outcome of chronic stress on the function and membrane expression of ionotropic glutamate receptors may be the opposite of the action of acute stress (Yuen et al. 2009, 2011, 2012). While acute stress was shown to enhance NMDA- and AMPA-receptor mediated synaptic currents in PFC of juvenile rats, repeated restraint or unpredictable stress caused marked reduction of ionotropic glutamate receptors mediated currents, due to ubiquitin/proteasome-mediated degradation of GluR1 and NR1 subunits. This effect of repeated stress was linked to GR activation and concomitant to significant impairment of temporal order recognition memory, a cognitive process controlled by the PFC. For more details and discussion see Chap. 4, this volume.

3.3.1 Structural/Functional Changes Induced by Stress in Glutamate Synapses and Circuitry: A Biphasic Process?

A remarkable feature of excitatory and inhibitory synapses is their continuous reorganization, with changes in morphology and stability, as well as the birth of new synapses and elimination of old ones (Holtmaat and Svoboda 2009; Yoshihara et al. 2009; Caroni et al. 2012). This phenomenon is regulated by synaptic activity, and the size of spine heads has been shown to be correlated with synaptic strength, such as in LTP and learning. Some studies have tried to correlate the time-dependent enhancement of transmission with spine growth and synaptogenesis. Thus, it has been suggested that synapses involving new spines are assembled within 12–18 h. However, recent evidence suggest that activity-dependent formation of new synaptic spines could be much faster. It has been shown in hippocampal slice cultures that new spines stimulated by glutamate uncaging may become functional within 10 min and show features of morphologically mature synapses already after 1.5 h (Nägerl et al. 2007; Zito et al. 2009).

As shown above, although the evidence is far from conclusive, the outcome of acute and repeated (chronic) stress on structural and functional features of the glutamate system could be different and often opposite. While acute stressors enhance glutamate release and excitatory transmission in select areas of the forebrain, chronic stress has been shown to reduce excitatory transmission and to induce consistently atrophy/remodeling of dendrites and loss of synapses, in line with reduction of excitatory transmission in the same areas. It is not known whether acute stress induces rapid morphological changes in synapses and circuitry, although hippocampal spinogenesis induced by stress has been reported (Shors et al. 2001; Diamond et al. 2006). Recently, it was shown that infradian corticosterone peaks promote learning-dependent formation of new spines in motor cortex, and application of corticosterone in vitro to hippocampal slices increased the density of spines in CA1 area after 1 h (Komatsuzaki et al. 2012; Liston et al. 2013). On the other hand, even single stress episodes, if measurements are performed at least 24 h later, induce loss

of spines or dendritic retraction (Izquierdo et al. 2006; Hajszan et al. 2009). Currently, more work is under way to understand whether and how acute stress induces rapid changes in synaptic morphology.

Considering the different effects of acute and chronic stress in hippocampus and PFC on synaptic function and plasticity, and on synaptic spines reorganization, it may be conceived that, during the stress response, the early enhancement of glutamate transmission (perhaps coupled with an early increase of spines and synapses number) can be turned with time into its opposite. Repeated stressors, or the delayed consequences of acute stressors, seem to bring about destabilization of neuronal architecture and loss of synaptic connections in some pathways, with diffuse alterations in areas and circuits mediating cognitive and emotional behaviors (e.g., hippocampus, PFC). Therefore, the structural and functional changes in excitatory circuitry may follow a biphasic process (Fig. 3.7; see Popoli et al. 2012), with the exception of basolateral amygdala, where enhancement of excitatory transmission seems to prevail for a longer time. This is also mirrored by the effects of stress and physiological levels of glucocorticoids on related cognitive functions, which may be enhanced by acute stress and impaired by repeated stress (see Chap. 4, this volume). The stress response is a complex physiological process involving hormonal, neurochemical, and metabolic mechanisms. As it is often observed in pathophysiology, it can be envisaged that a continuum exists between physiological mechanisms of plasticity, allowing adaptation to the environmental stimuli, and pathological mechanisms, turning a normal response into maladaptive structural/functional changes. In this framework, the main target for research should be the identification of cellular effectors mediating the crucial passage from the early effects into later effects of stress (also linked with the action of repeated stress) on glutamate synapses and circuitry. If early enhancement of excitatory transmission (perhaps coupled with early increase of spines and synapses number) is recorded in the first few minutes and hours from the beginning of stress episodes, and delayed inhibitory effects (with atrophy and remodelling of dendrites) can be detected as early as 24 h after the stress episode (Izquierdo et al. 2006; Hajszan et al. 2009), the turning point in plasticity must be somewhere along this time frame. A better knowledge of the cellular effectors involved in this biphasic effect would be quite useful for a better understanding of stress-related pathophysiology. If early activity-dependent morphological changes at synapses can be observed in a matter of minutes (see above), it is possible that they are carried out by changes in local protein translation at dendrites, which is also consistent with early effects being linked to nongenomic effects of glucocorticoids (Joels et al. 2012, see also above). Later changes, particularly atrophy and remodelling of dendrites, must instead be linked to more robust changes in gene expression, involving both transcription and translation, as well as trafficking of signalling molecules.

We are currently investigating molecular effectors, which may be responsible for rapid changes induced in synaptic morphology by acute stress. The main target here may be the neurotrophin BDNF, which is encoded by different splice variant mRNAs, assembling together the mRNA transcribed from the 3' coding exon, with one of the transcripts of at least eight 5' noncoding exons (Aid et al. 2007). The protein product is the same, but it has been shown that different splice variants code for

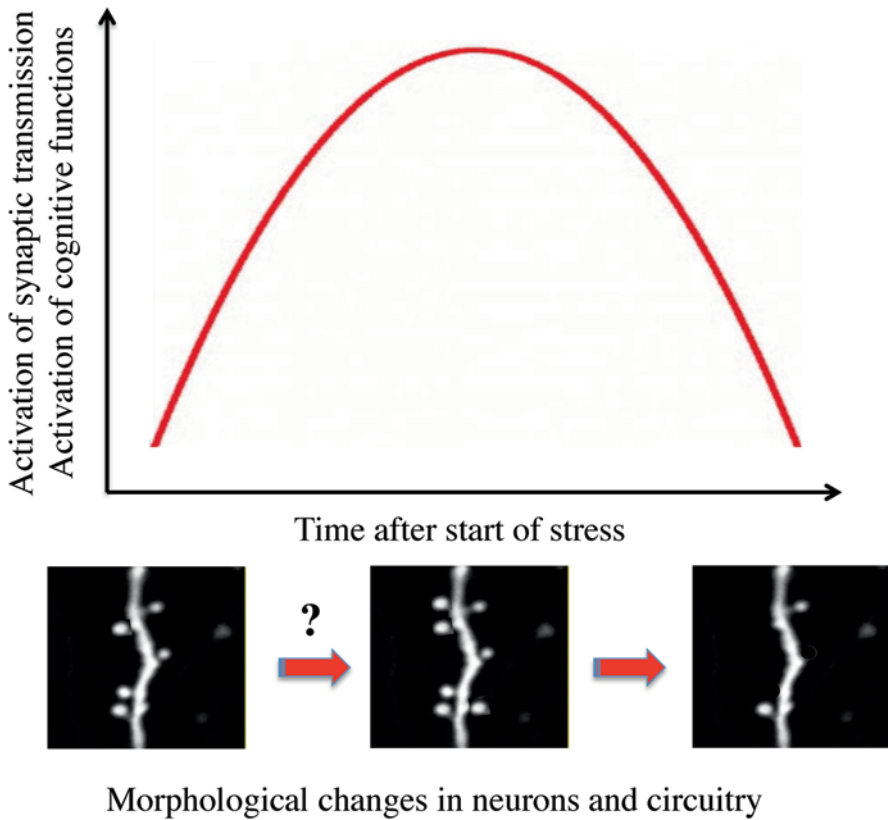


Fig. 3.7 Hypothetical scheme of structural/functional changes induced by stress in the glutamate system: a biphasic process. Acutely, e.g., in the first several minutes and hours, stress induces enhancement of excitatory synaptic transmission (often accompanied by cognitive enhancement). It is not clear yet if this is accompanied by an increase in the number of spines and synapses, although corticosterone was shown to exert rapid effect on spines morphology (Komatsuzaki et al. 2012; Liston et al. 2013). Later on, at least 24 h after application of the stressor (Izquierdo et al. 2006; Hajszan et al. 2009), a phase of inhibition follows, with reduction of synaptic transmission and related structural changes: dendritic atrophy and remodeling, loss of spines and synapses. This phase brings about destabilization of the glutamate system, with negative effects on cognitive functions. It is conceivable that, to a certain point, this is a compensatory physiologically adaptive process during the stress response. However, if the stress response is not correctly turned on and then shut off, because the stressor overcomes the coping capability of the system (stress is uncontrollable, too long, repeated, hits individual vulnerability, etc.) the structural/functional changes may become more stable or permanent, with the possible development of stress-related pathology (see text for explanation; McEwen 2005, 2010; Popoli et al. 2012). The rendering of dendrites at bottom emphasizes the biphasic changes in morphology over time

cellular localization of mRNAs, with a few of them targeted in activity-dependent fashion to dendrites, to subservise local dendritic translation of BDNF, and synaptic plasticity (Chiaruttini et al. 2008; Baj et al. 2011). We have recently shown that both voluntary physical exercise and chronic antidepressant treatments, two types of environmental factors that enhance adaptive neuroplasticity, increase expression and trafficking of select BDNF transcripts to hippocampal dendrites in rodents (Baj et al. 2012). In addition, we found that acute stress blocks the increase of total and dendritic BDNF expression induced by physical exercise, as well as the positive effect of physical exercise on dendritic trafficking of BDNF (not shown). Overall, these results point to BDNF dendritic transcripts as crucial mediators of adaptive/maladaptive changes in activity-dependent synaptic plasticity. In turn, BDNF regulates the trafficking of additional dendritic mRNAs and their translation at synapses, by selectively promoting the translation of a subset of dendritic mRNAs, including cytoskeletal proteins involved in synaptic rearrangement (Gray et al. 2013; Leal et al. 2013; Ruiz et al. 2013). Therefore, the investigation of BDNF and related pathways will supply essential information as to the nature of rapid versus delayed changes induced by stress in excitatory synapses and circuitry.

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