

Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex

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The hippocampus provided the gateway into much of what we have learned about stress and brain structural and functional plasticity, and this initial focus has expanded to other interconnected brain regions, such as the amygdala and prefrontal cortex. Starting with the discovery of adrenal steroid, and later, estrogen receptors in the hippocampal formation, and subsequent discovery of dendritic and spine synapse remodeling and neurogenesis in the dentate gyrus, mechanistic studies have revealed both genomic and rapid non-genomic actions of circulating steroid hormones in the brain. Many of these actions occur epigenetically and result in ever-changing patterns of gene expression, in which there are important sex differences that need further exploration. Moreover, glucocorticoid and estrogen actions occur synergistically with an increasing number of cellular mediators that help determine the qualitative nature of the response. The hippocampus has also been a gateway to understanding lasting epigenetic effects of early-life experiences. These findings in animal models have resulted in translation to the human brain and have helped change thinking about the nature of brain malfunction in psychiatric disorders and during aging, as well as the mechanisms of the effects of early-life adversity on the brain and the body.

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INTRODUCTION

The field of neuroendocrinology began with the fundamental discovery of the communication between hypothalamus and pituitary by Harris (1970) and this established the basis for understanding brain–body communication via the neuroendocrine system. The hypothalamus and pituitary gland became the focus and led to the discovery of releasing factors in the hypothalamus for pituitary hormones (eg, see Guillemin, 1978; Schally *et al*, 1973; Vale *et al*, 1981) as well as the feedback of hormones on the hypothalamus and pituitary for the purpose of regulating hormone secretion (Meites, 1992). During the same time period, steroid hormones were shown to bind to intracellular receptors that regulate gene expression in tissues such as liver, and the prostate and uterus in the case of sex hormones (Jensen and Jacobson, 1962) and tritium-labeled steroid hormones were used as probes to detect these receptors using cell fractionation (Toft and Gorski, 1966) as well as steroid autoradiography (Pfaff and Keiner, 1973; Stumpf, 1971).

The McEwen laboratory entered this field using tritium-labeled steroids by serendipitously discovering adrenal steroid, and, later, estrogen receptors, in the hippocampal formation of the rat using both steroid autoradiography, as well as cell fractionation methods, and immunocytochemical methods at the electron microscopic and light microscopic levels (Gerlach and McEwen, 1972; Loy *et al*, 1988; McEwen and Plapinger, 1970; McEwen *et al*, 1968; Milner *et al*, 2001; Zigmond and McEwen, 1970). We and others extended these findings to the infrahuman primate brain, as well as to other regions of the brain involved in cognitive and emotional regulation (Gerlach *et al*, 1976). These findings have catalyzed studies that look at actions of hormonal feedback on the brain, not only to regulate hypothalamic functions, but also to influence neurological, cognitive, and emotional functions throughout the entire brain, with translation to the human brain in relation to aging, mood disorders, and the impact of the social environment.

The hippocampus provided the gateway into much of what we have learned about stress and brain plasticity and the initial focus on hippocampus has expanded to other interconnected brain regions, such as the amygdala and prefrontal cortex (PFC). This article describes research in our and other laboratories on these three brain structures that have led to the discovery of structural remodeling of neurons in response to acute and chronic stressors and then led us and others to uncover both epigenetic and non-genomic

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mechanisms by which glucocorticoids and estrogens produce their effects, as well as sex differences in these outcomes.

HIPPOCAMPUS AS THE GATEWAY

Glucocorticoid (GR) and Mineralocorticoid (MR) Receptors and Functions

The discovery of stress hormone receptors acting in the hippocampus has been the gateway to the investigation of other brain regions as well as mechanisms of stress and adrenal steroid action. Work by Reul and DeKloet (1985) demonstrated that there are two types of adrenal steroid receptors, MR (Type 1) and GR (Type 2), in the hippocampus and other brain regions. This was further elaborated by immunocytochemical mapping of the receptors (Ahima *et al*, 1991; Ahima and Harlan, 1990).

Studies in our laboratory and by Diamond *et al* (1992) and Joels (2006) have shown biphasic effects mediated by MR and GR on long-term potentiation and long-term depression (Pavlidis *et al*, 1995). The biphasic effects on excitability are reflected in memory, such that a biphasic corticosterone dose response is seen on object recognition memory (Okuda *et al*, 2004). These effects depend on the state of behavioral arousal in a novel environment, which also implies a synergistic role of adrenaline and the fact that the hippocampus and amygdala work together (Roosendaal *et al*, 1996). The GR is involved in hippocampal-dependent spatial memory via a genomic mechanism as shown in the dimerization-deficient GR mouse, where GR cannot dimerize to bind to glucocorticoid response elements (Oitzl *et al*, 1997). GRs are also involved in contextual fear memory, mediated by hippocampus and amygdala, based upon the finding that Ru486 is able to block it (Pugh *et al*, 1997).

Ultradian fluctuations of glucocorticoids drive GR activation and reactivation, whereas MR occupancy for nuclear activation is more constant and promotes excitability (Stavreva *et al*, 2009) and this has implications for genomic and non-genomic activity of adrenal steroids, as will be discussed below.

Neural Structural Remodeling

Our finding in hippocampus of chronic stress-induced shrinkage of dendrites of hippocampal CA3 and dentate gyrus neurons as well as loss of spines in CA1 neurons (see (McEwen, 1999) revealed aspects of brain structural plasticity (Figure 1). The rediscovery of neurogenesis in the adult as well as developing dentate gyrus of the hippocampal formation (Cameron and Gould, 1994; Gould *et al*, 1992), which had been strongly suggested (Altman and Das, 1965), but largely ignored (Kaplan, 2001) for a number of decades, helped to establish the concept that the adult brain could show remodeling of neuronal architecture not only after stress but also in other conditions.

Moreover, for both chronic stress effects and the ability of estrogens to induce spine synapse formation, it became

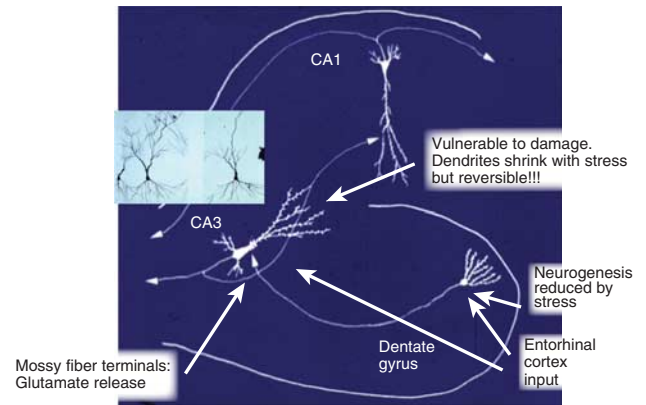


Figure 1. The trisynaptic organization of the hippocampus showing input from the entorhinal cortex to both CA3 and dentate gyrus (DG), with feed forward and feedback connections between these two regions that promotes memory formation in space and time, but at the same time, makes the CA3 vulnerable to seizure-induced excitation (McEwen, 1999). Chronic stress causes apical dendrites of CA3 neurons to debranch and shorten in a reversible manner, and glutamate release by giant mossy fiber terminals is a driving force. Chronic stress also inhibits neurogenesis in DG and can eventually reduce DG neuron number and DG volume (see text).

apparent that hormones did not work alone but involve other mediators, particularly excitatory amino acids (EAAs) and their receptors (Cameron *et al*, 1998; Daniel and Dohanich, 2001; Gazzaley *et al*, 1996; Magarinos and McEwen, 1995; Woolley and McEwen, 1994). For example, for the chronic stress-induced shrinkage of apical dendrites of hippocampal CA3 neurons, the giant mossy fiber terminals (MFTs) have a key role and are a bellwether of the effects of repeated stress (Magarinos *et al*, 1997; Figure 2). Specifically, MFTs from control rats are fully packed with vesicles containing glutamate, whereas after chronic restraint stress, they become depleted of vesicles, but the remaining vesicles are found at the multiple active synaptic zones in these giant presynaptic terminals, along with increased mitochondria in the stressed MFTs. This suggests not an exhausted but a new steady state of increased activity (Magarinos *et al*, 1997).

The CA3 region is not the only region of the hippocampus to show dendritic reorganization with chronic stress. Apical dendrites of rat CA1 neurons were reported to be shorter in adulthood after chronic neonatal bedding stress (Brunson *et al*, 2005), and a comparison of stressors revealed differences within the hippocampus and its functional connectivity to other brain regions (Maras *et al*, 2014). Comparison of a multimodal stress paradigm (concurrent, hours-long light, loud noise, jostling, and restraint) with restraint or loud noise alone revealed severe deficits in hippocampal-dependent object recognition memory after multimodal stress, but fewer deficits after restraint or loud noise alone. These differences in memory were not explained by differences in plasma corticosterone levels or numbers of Fos-labeled neurons in stress-sensitive hypothalamic neurons. Measures of spine density under these different

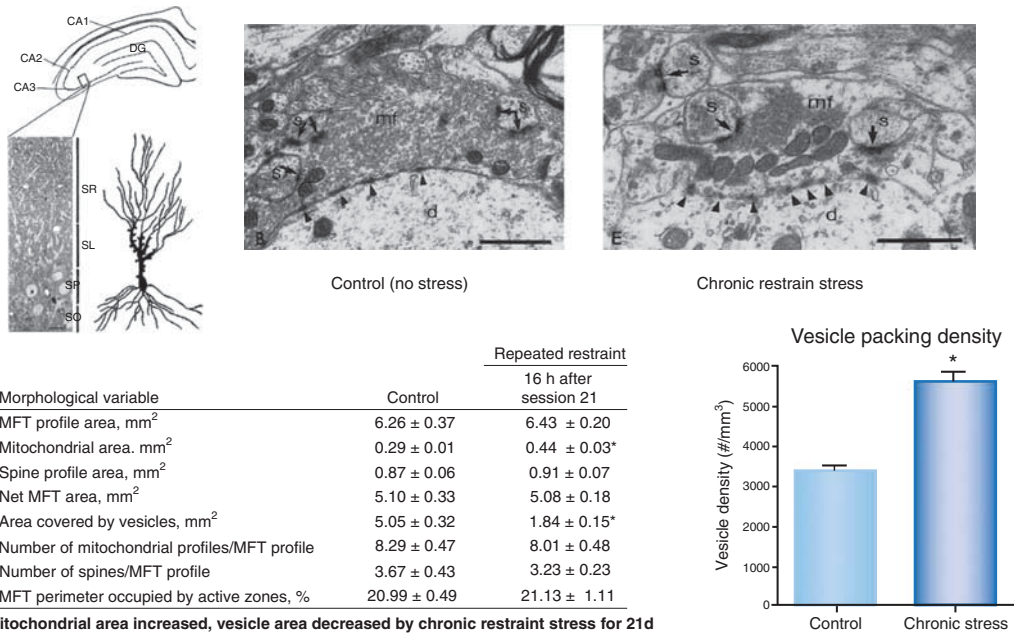


Figure 2. Giant mossy fiber terminals (MFTs) of DG neurons that synapse in stratum lucidum of CA3 have multiple active sites of glutamate release on the thorny excrescences that penetrate the MFTs. Normally fully packed with synaptic vesicles, the MFTs show depletion of vesicles after 3 weeks of chronic restraint stress (CRS), with the remaining vesicles being near active synaptic zones. As shown at the right, vesicle area is reduced by CRS but packing density of the remaining vesicles is increased and the area occupied by mitochondria is also increased. This suggests that the chronically stressed MFT are not exhausted but rather very active after chronic stress (Magarinos *et al*, 1997). Interestingly, by microdialysis, glutamate release in hippocampus caused by restraint stress is abolished by adrenalectomy, indicating involvement of adrenal secretions (Lowy *et al*, 1993). * $P < 0.001$, two-tailed unpaired Student's *t*-test.

conditions revealed that synapses in hippocampal CA3 were reduced by both restraint and multimodal stress, whereas multimodal stress alone reduced synapse numbers severely in dorsal CA1. Ventral CA1 synapses were not significantly affected by any of these stressors. Using c-Fos as a marker of neuronal activity, multimodal stress reduced hippocampal connectivity with septum and thalamus compared with restraint and increased connectivity with amygdala and BST more so than restraint stress (Maras *et al*, 2014).

In C57Bl6 mice, 10 days of chronic immobilization stress (CIS) induced dendritic retraction of CA3 short-shaft pyramidal neurons, but not CA3 long-shaft pyramidal neurons, along with a robust retraction of dendrites in dorsal CA1 pyramidal neurons (Christian *et al*, 2011). In mice specifically lacking NMDA receptors in CA3 neurons, chronic stress-induced dendritic retraction was not evident in any of the neurons in either CA3 or CA1 and this prevention of dendritic retraction in the mutant mice had a minimal effect on HPA axis activation and behavioral alterations that were induced by chronic stress (Christian *et al*, 2011). In the hippocampus of hibernating animals, rapid shrinkage of CA3 apical dendrites is seen with onset of hibernation, whereas regrowth of those dendrites occurs within hours of termination of hibernation, suggesting that the cytoskeleton can rapidly depolymerize and repolymerize when needed via a mechanism in which phosphorylation of a soluble form of tau is involved as a factor involved in cytoskeletal dissociation (Arendt *et al*, 2003; Magarinos *et al*, 2006; Figure 3).

Besides glucocorticoids and EAA receptors, there is a growing list of mediators implicated in the stress-induced

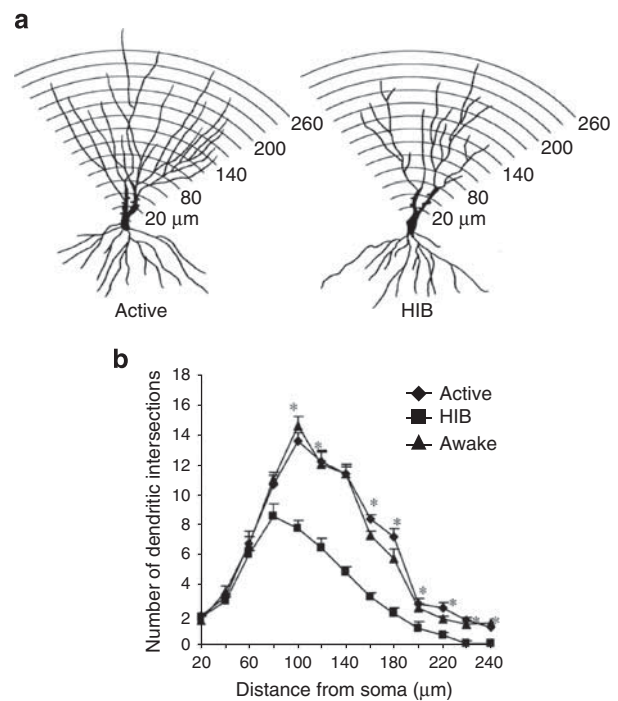


Figure 3. Effect of deep hibernation and induced awakening on the apical dendritic branching density of CA3 pyramidal neurons. (a) Camera lucida drawings of representative CA3 pyramidal neurons from active and hibernating European hamsters. An overlay of concentric rings centered at the cell body was used for Sholl analysis. (b) One-way ANOVA followed by Tukey *post hoc* test revealed statistical differences among experimental groups between 100 and 180 μm from the soma (hibernation (HIB) vs active and awoken groups, $P < 0.001$) and also between 200 and 260 μm from the soma (HIB vs active and awoken groups ($P < 0.005$)). Taken from reference Magarinos *et al* (2006).

TABLE 1 Molecules that are Necessary/Permissive for Remodeling

BDNF: brain-derived neurotrophic factor
Facilitator of plasticity or growth
BDNF overexpression—occludes effects of chronic stress
BDNF haploinsufficiency prevents stress-induced plasticity
tPA: tissue plasminogen activator
Secreted signaling molecule and protease
Required for stress-induced spine loss in hippocampus and medial amygdala
Required for acute stress-induced increase in anxiety; CRF activates tPA secretion
CRF in amygdala regulates tPA release
CRF: corticotrophin-releasing factor
Secreted in hippocampus by interneurons
Downregulates thin spines via RhoA signaling
Lipocalin-2: secreted protein; previously unknown function
Acute stress induces Lipocalin-2
Lipocalin-2 downregulates mushroom spines
Lipocalin-2 KO increases neuronal excitability and anxiety
Endocannabinoids
Induced via glucocorticoids
Regulate emotionality and HPA habituation and shut off
CBI receptor KO increases anxiety and basolateral amygdala dendrite length and causes stress-like retraction of prefrontal cortical dendrites

dendritic remodeling (Table 1). These include trophic factors such as brain-derived neurotrophic factor (BDNF) and the secreted signaling molecule and protease tissue plasminogen activator (tPA), another signaling molecule, lipocalin 2, corticotrophin-releasing factor (CRF), and endocannabinoids. Besides these signaling mediators, cell surface molecules have an important role. Expression of the polysialylated form of neural cell adhesion molecule in the hippocampal formation is increased by stress, whereas PSA removal by Endo-neuraminidase-N (Endo-N) is known to cause the mossy fibers to defasciculate and synapse ectopically in their CA3 target area. Enzymatic removal of PSA by Endo-N produced a remarkable expansion of dendritic arbors of CA3 pyramidal neurons, with a lesser effect in CA1. This expansion eclipsed the CIS-induced shortening of CA3 dendrites, with the expanded dendrites of both no-stress-Endo-N and CIS-Endo-N rats being longer than those in no-stress-control rats and much longer than those in CIS-control rats. As predicted by the hypothesis that Endo-N-induced dendritic expansion might increase vulnerability to excitotoxic challenge, systemic injection with kainic acid, showed markedly increased neuronal degeneration, as assessed by fluorojade B histochemistry, in rats that had been treated with Endo-N compared with vehicle-treated rats throughout the entire hippocampal formation. PSA removal also exacerbated the CIS-induced reduction in body weight and abolished effects of CIS on neuropeptide Y and NR2B mRNA levels (McCall *et al*, 2013; Figure 4).

Much of the structural remodeling described above is mediated by gene expression changes that results from changes in DNA transcription (genomic effects), principally

through binding of activated GRs to glucocorticoid response elements in DNA. However, stress-induced plasticity can also be mediated through non-genomic mechanisms, as described below.

Non-Genomic Effects

Immunocytochemistry at the electron microscopic level revealed specific immunolabeling for epitopes of glucocorticoid and estrogen receptors near the cell surface, in the mitochondria, dendrites, and presynaptic terminals and post-synaptic densities (Johnson *et al*, 2005; Liposits and Bohn, 1993; McEwen and Milner, 2007; Milner *et al*, 2001). Application of steroid autoradiography at the electron microscopic level revealed the putative estrogen receptor sites as having the ability to bind radioactive iodinated estradiol (Milner *et al*, 2008). Along with evidence that steroid receptors worked via non-genomic, rapid signaling mechanisms along with their epigenetic actions to regulate gene expression (Kelly and Levin, 2001). This led to a new view of estrogen and glucocorticoid action via both direct and indirect genomic stimulation as well as a variety of other rapid signaling mechanisms (Popoli *et al*, 2012; see Figure 5).

A powerful example of non-genomic functions of steroid receptors was the finding that the knockout of the MR abolished the ability of corticosterone to rapidly stimulate EAA release, implying a dual role for MR in both genomic and rapid non-genomic signaling (Karst *et al*, 2005). Again, this finding began with studies on hippocampus and it helped explain the adrenal dependency of acute restraint stress (ARS)-induced increases in extracellular glutamate in

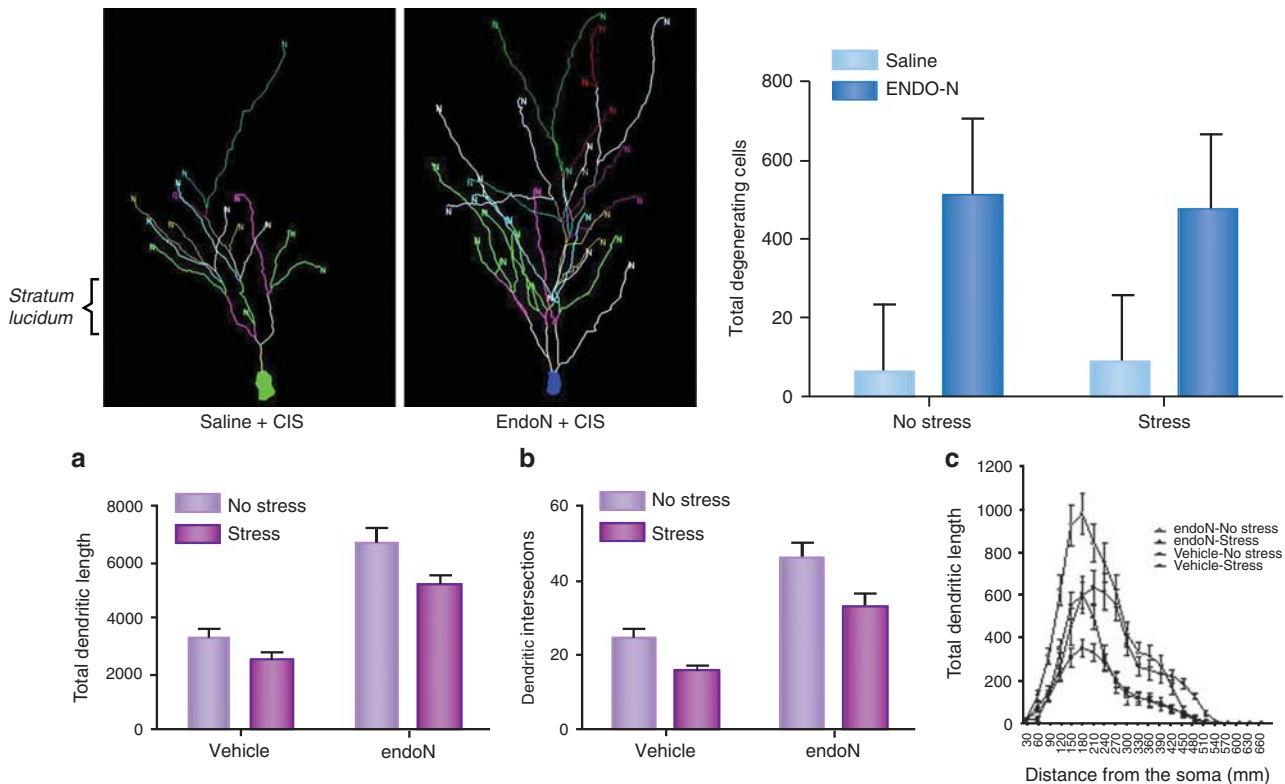


Figure 4. Polysialylate removal from NCAM neural cell adhesion molecule antagonized the effect of chronic immobilization stress (CIS) to shorten dendritic branches of CA3 neurons. Top: (left) Representative camera lucida traces from vehicle and endo-N-treated rats that were exposed to 10 days of CIS. The dendritic arbors were larger in endo-N-treated animals. (right) Excitotoxic challenge causes greater neuron loss in endo-N treated hippocampus compared to saline control. Bottom: (a) CIS shortened the total length of CA3 dendritic arbor, whereas PSA removal produced an increase in these measures. With combination of CIS and endo-N, the total arbor length was shorter than with endo-N alone but still larger than control values. (b) Interestingly, the effects on numbers of branching points followed a similar pattern. (c) Sholl analysis revealed that PSA removal alone produced an elongation of branches located at all distances from the soma (from 0 to 550 μm approximately), whereas CIS alone specifically shortened branches located near the soma (approximately between 100 and 250 μm). Interestingly, PSA removal antagonized this CIS-induced dendrite atrophy. Data are presented as means (\pm SEM) $N=9-10/\text{group}$. Taken from reference McCall *et al* (2013).

hippocampus (Lowy *et al*, 1993), which are likely to underlie the ability of chronic restraint stress to cause dendritic remodeling in hippocampus (McEwen, 1999).

Hippocampal involvement in mood disorders and age-related memory loss

The hippocampus has long been implicated in learning and memory; however, it also has an important role in the regulation of mood. In addition to the structural remodeling described earlier, the dentate gyrus is a region that exhibits adult neurogenesis, which is regulated by adrenal steroid levels (Cameron and Gould, 1994; Gould *et al*, 1992). Subsequent findings have shown that antidepressants increase neurogenesis, and this, in turn, provided a novel mechanism for their action that brought in the hippocampus as a brain structure involved in mood disorders (Duman *et al*, 2001). Indeed, the dentate gyrus undergoes reduced cell number under chronic stress (Pham *et al*, 2003) and in response to corticosterone levels (Sousa *et al*, 1999), whereas physical activity and an enriched environment increase dentate gyrus volume and neuron number (Kempermann *et al*, 1997; van Praag *et al*, 1999). Hippocampal neurogenesis

has also been linked to BDNF levels, which are highly dynamic in response to chronic stress, where initial decreases have been observed (Smith *et al*, 1995), but with recovery after stress can return to baseline (Lakshminarasimhan and Chattarji, 2012). And direct infusion of BDNF has been shown to increase hippocampal neurogenesis (Scharfman *et al*, 2005).

Subsequent work has identified the ventral hippocampus as the major target of these effects (Jayatissa *et al*, 2006; Sahay and Hen, 2007). Yet, the dentate gyrus and neurogenesis are not the sole explanation for depressive behavior and its treatment by antidepressants because there are behavioral endpoints of antidepressant action that occur when the possibility of neurogenesis is suppressed, eg, by X-irradiation (David *et al*, 2009); rather, antidepressant actions upon only certain aspects of depressive-like behavior require neurogenesis, whereas other actions involve dendrite remodeling and synapse turnover (Bessa *et al*, 2008). This fits with human autopsy data on brains from depressed individuals that show no neuronal loss in hippocampus but reduced cell nuclear size of hippocampal pyramidal neurons and reduced glial cell number; glial cell reduction fits with loss of dendritic length and branching (Stockmeier *et al*, 2004).

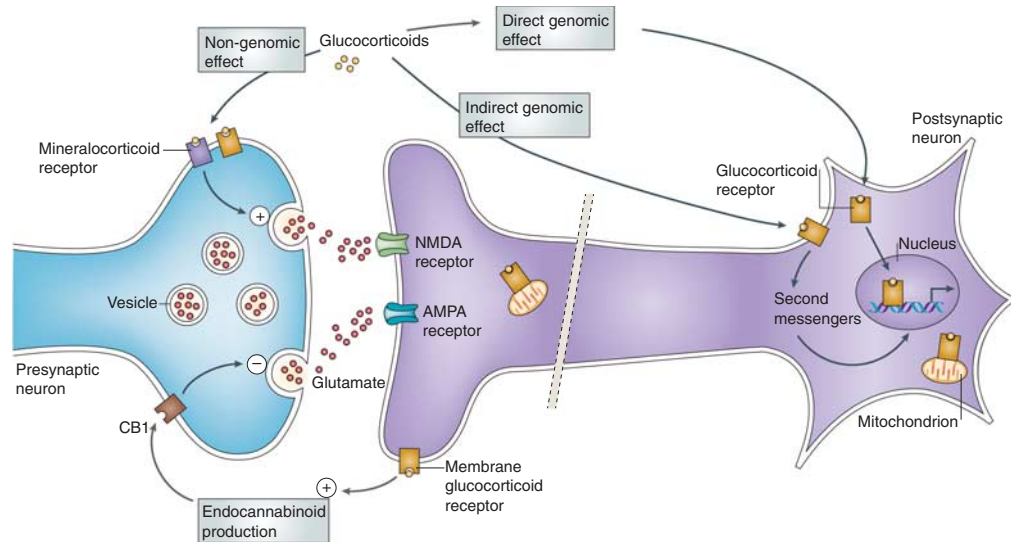


Figure 5. Basal release of glucocorticoids varies in a diurnal pattern, and release increases several fold after exposure to a stressor. Glucocorticoids can bind with different affinities to glucocorticoid and mineralocorticoid receptors, which are expressed throughout the brain and seem to exist in both membrane-bound form and nuclear form. Adrenal steroids can have both rapid and delayed effects. The effects can result from non-genomic mechanisms (mediated by membrane-associated receptors, see the figure; Karst *et al*, 2005; Kelly and Levin, 2001), indirect genomic mechanisms (mediated by membrane receptors and second messengers) and genomic mechanisms (mediated by cytoplasmic receptors that move to the nucleus and act as transcription factors; Yamamoto, 1985). Although classical mineralocorticoid and glucocorticoid receptors seem to mediate many of these effects, other membrane-associated receptors, including G-protein-coupled receptors, may also be involved in some of these actions (Orchinik *et al*, 1992; Tasker *et al*, 2006). In addition, activated glucocorticoid receptors can translocate to mitochondria and enhance their calcium buffering capacity (Du *et al*, 2009). Glucocorticoids rapidly induce glutamate release in the hippocampus through a mechanism that is absent when the mineralocorticoid receptor is deleted and that may involve a membrane-associated form of the mineralocorticoid receptor (Karst *et al*, 2005; Lowy *et al*, 1993). An indirect way by which glucocorticoids can influence neurotransmission (glutamatergic, as well as GABAergic, cholinergic, noradrenergic, and serotonergic) is through crosstalk with the endocannabinoid system (Katona and Freund, 2008). They rapidly stimulate endocannabinoid production in the brain, whereupon endocannabinoids bind to cannabinoid receptor 1 (CB1) and transient receptor potential cation channel subfamily V member 1 (TRPV1), and inhibit neurotransmitter release (Chavez *et al*, 2010; Hill and McEwen, 2010). Although a G-protein-coupled receptor is implicated in endocannabinoid production (Di *et al*, 2009), there is also evidence for a mechanism blocked by Ru486—a selective antagonist of the classical cytoplasmic glucocorticoid receptor—in the rapid actions of glucocorticoids in prefrontal cortex (Hill and McEwen, 2010). Reprinted from reference Popoli *et al*, 2012 with permission.

The hippocampus is affected by aging. Memory impairment is seen in rats as they age and there is a link between rising glucocorticoid levels, EAAs, and memory impairment (Landfield *et al*, 1978; Sapolsky *et al*, 1986). Pharmacological treatment with drugs that affects EAA actions has been shown in several cases to retard the memory loss, using hippocampal-dependent spatial memory as a measure. One study found beneficial effects of a positive allosteric modulator of AMPA receptors (Ampakine) that selectively enhance fast excitatory neurotransmission in the brain and increase overall neuronal excitability and exert some neuroprotective activity (Bloss *et al*, 2008). Treatment of aging rats by oral administration of the Ampakine S18986 (Servier, France) from 14 to 18 months of age increased locomotor activity and improved performance in a spatial memory task involving the hippocampus. In addition, chronic S18986 treatment retarded the decline of forebrain cholinergic neurons and midbrain dopaminergic neurons by ~40% and attenuated the age-related increase in the expression of a microglial marker indicative of neural inflammation in the hippocampus (Bloss *et al*, 2008).

In another study involving modulation of excitatory amino-acid actions (Pereira *et al*, 2014), riluzole was used

because it increases glutamate uptake through glial transporters and is thought to decrease glutamate spillover to extrasynaptic NMDA receptors while increasing synaptic glutamatergic activity. Aging rats treated between 10 and 14 months of age were protected against the age-related cognitive decline displayed in non-treated aged animals. Memory performance on tasks involving hippocampal spatial and episodic memory correlated with density of thin spines on apical dendrites in the CA1 region, although not with mushroom spines. Furthermore, riluzole-treated rats had an increase in clustering of thin spines that correlated with memory performance and was specific to the apical, but not the basilar, dendrites of CA1. Clustering of synaptic inputs is thought to allow nonlinear summation of synaptic strength (Pereira *et al*, 2014).

Hippocampal GRs and Effects of Early-Life Experiences

Another 'gateway' function of research on the hippocampus was to focus attention on the neural effects of early-life experiences. Meaney and colleagues showed that the effects of 'neonatal handling' on emotionality (Levine *et al*, 1967)

were due to maternal care after the pups were returned to the nest after a brief separation from the dam (Francis, 1999; Meaney *et al*, 1988). Rats that received poor maternal care showed deficits in GRs in the hippocampus (Liu *et al*, 1997) and impaired shut off of the HPA stress response that may result, at least in part, from those deficits (Caldji *et al*, 2000). Thyroid hormone and serotonin were shown to be involved in maintaining adequate hippocampal GR levels (Meaney *et al*, 2000), but the methylation of CpG DNA elements in the promotor region of the GR was found to decrease GR expression and, indeed, it was found to be increased in pups receiving poor maternal care (Meaney and Szyf, 2005). Poor HPA shut off in rats that received poor maternal care could be reinstated in adult rats that had received good maternal care as infants by inducing the methylation of adult GR CpG elements in adult animals (Weaver *et al*, 2005). These seminal findings have catalyzed a huge field of research in the growing field called 'epigenetics' that now includes translational studies such as the one showing increased GR promotor CpG methylation in individuals who were abused as children (McGowan *et al*, 2009).

Glucocorticoids and HPA reactivity are also indicators of the quality of maternal care and the response of the offspring to that care. Building upon the importance of maternal care, Tang *et al* (2014) have developed the 'maternal modulation' concept in which consistency of maternal care and not absolute quantity are important factors, along with exposure to novelty, leading to better social and cognitive development; one measure of the efficacy of good maternal care is maternal stress self-regulation, referring to low basal CORT and a robust increased CORT secretion in response to a stressor.

Epigenetics, Stress, and Mood-Related Behaviors: Search for Rapidly Acting Treatments

After its original definition, as the emergence of characteristics of an organism during development that were not evident at earlier stages (Waddington, 1942), 'epigenetics' now refers to events 'above the genome' that regulate expression of genetic information without altering the DNA sequence. Besides the CpG methylation described above, other mechanisms include histone modifications that repress or activate chromatin unfolding (Allfrey, 1970) and the actions of non-coding RNAs (Mehler, 2008). Again the hippocampus is providing important information. For example, Reul and colleagues have shown that the forced swimming-induced behavioral immobility response requires histone H3 phospho-acetylation and c-Fos induction in distinct dentate granule neurons through recruitment of the NMDA/ERK/MSK 1/2 pathway (Chandramohan *et al*, 2008).

Another histone mark changed in hippocampus, most prominently in the dentate gyrus, is the dramatic induction by an ARS of trimethylation of lysine 9 on histone H3, which is associated with repression of a number of retrotransposon elements and reduction of the coding and non-coding RNA normally produced by the repressed DNA

(Hunter *et al*, 2009). This repression is lost with repeated stress, suggesting the possibility that those retrotransposon elements may impair genomic stability under conditions of chronic stress (Hunter *et al*, 2015).

A current practical application is the search for rapidly acting antidepressants, because classical antidepressants work very slowly and are not effective on every depressed individual. Epigenetic processes are likely involved in the chronic relapsing nature of major depression, the strikingly higher incidence of depression in women after puberty, the high discordance rates between monozygotic twins, and the individual responsivity to stress that precipitates mood-related behaviors in susceptible individuals. In the course of these studies, we are learning more about epigenetic mechanisms that connect EAA function with neural remodeling and stress-related behavior. The identification of the fast antidepressant effects of ketamine, an NMDA receptor blocker, has resulted in a paradigm shift toward the discovery of a new generation of rapidly acting antidepressants (Li *et al*, 2010).

Recently, our laboratory and other groups have found that the naturally occurring compound acetyl-L-carnitine (LAC) shows fast antidepressant efficacy in genetic and environmentally induced animal models of depression through the epigenetic modulation of the metabotropic glutamate receptor, mGlu2, in hippocampus (Cuccurazzu *et al*, 2013; Nasca *et al*, 2013). mGlu2 is known to exert an inhibitory tone on glutamate release from synapses and pharmacological modulators for this receptor are under clinical development to treat stress-related mood disorders, such as anxiety and depression (Nicoletti *et al*, 2015). Using the same animal models, 14 days of treatment with the tricyclic antidepressant clomipramine were needed to promote antidepressant responses, which disappeared when the treatment was stopped. In contrast, LAC antidepressant effects were still evident after 2 weeks of drug withdrawal (Nasca *et al*, 2013). The persistent effects of LAC suggested the involvement of stable molecular adaptations that may be reflected at the level of histone modifications in controlling mGlu2 transcription in the hippocampus. Indeed, LAC increases levels of mGlu2 receptors by acetylation of the histone H3K27 among other mechanisms (discussed below).

These findings support previous studies that have shown that histone deacetylase inhibitors, given intraperitoneally, normalize gene expression profiles in vulnerable brain regions, such as hippocampus, amygdala, and nucleus accumbens, to promote fast antidepressant responses following stress (Covington *et al*, 2011; Tsankova *et al*, 2006). The use of agents like LAC that act on histone remodeling to regulate transcription of the mGlu2 gene offers alternative and complementary strategies to ketamine and histone deacetylase inhibitors with safer profiles and lower potential for drug dependence (Nicoletti *et al*, 2015).

In the course of this work, we have become aware of individual differences among inbred mice and rats (Cavigelli and McClintock, 2003; Freund *et al*, 2013; Miller *et al*, 2012). Using a simple light-dark test to rapidly screen naïve mice

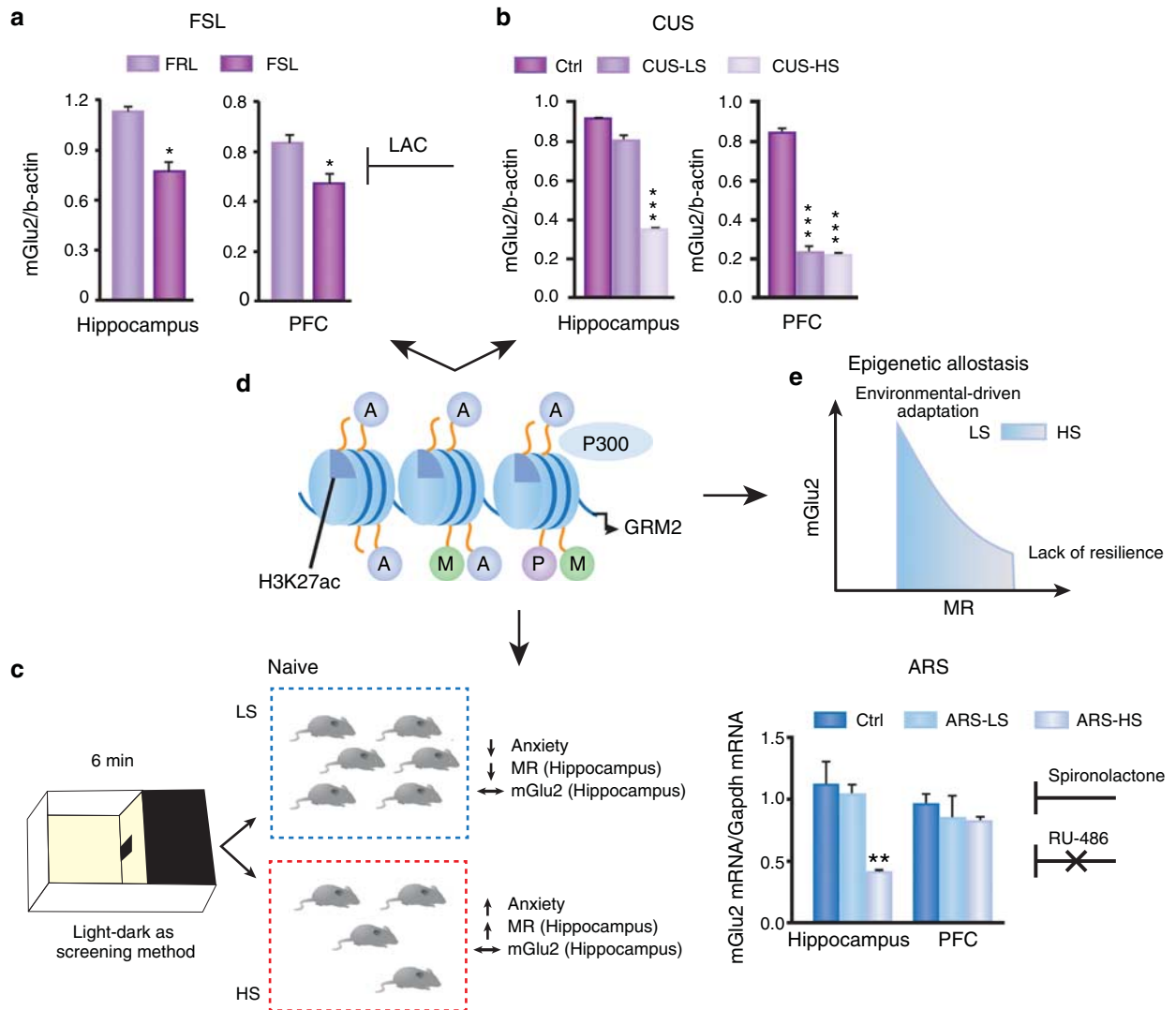


Figure 6. Lower mGlu2 in hippocampus is a biomarker of anxious and depressive-like behaviors and response to rapidly acting antidepressants. (a) mGlu2 receptor expression is reduced in the hippocampus and prefrontal cortex of depressed Flinders Sensitive Line (FSL) rats compared with their controls (Flinders Resistant Line, FRL). These changes are rapidly corrected by acetyl-L-carnitine (LAC), whose effects endure for 2 weeks after drug withdrawal. $*P < 0.05$, two-tailed unpaired Student's *t*-test. (b) Chronic unpredictable stress (CUS) in susceptible individuals results in depressive-like behaviors that are rapidly corrected by LAC (Nasca *et al*, 2013). Interestingly, only susceptible individuals show reduced mGlu2 protein levels within the hippocampus. $***P < 0.001$, one-way analysis of variances followed by Tukey's test for the *post hoc* analysis. (c) The recently introduced screening method using a light–dark chamber allows identification of high (HS) and low (LS) susceptible individuals, which are characterized by baseline differences in anxiety and in the levels of mineralocorticoid receptors (MRs), but not mGlu2 in the hippocampus. When stressed, HS mice show decreased mGlu2 levels in hippocampus and exacerbation of the baseline anxiety-like behavior compared with LS mice, which cope better with stress. These changes are prevented by a single injection of spironolactone (a MR antagonist), but not RU486 (a GR antagonist). $**P < 0.01$, one-way analysis of variances followed by Tukey's test for the *post hoc* analysis. (d) Acetylation of histone H3K27, which is regulated by P300, is a key mediator of mGlu2 regulation in response to stress and antidepressant treatment. (e) The MR-driven downregulation of mGlu2 expression is summarized in the epigenetic allostasis model, which suggests that individual differences in stress responsivity may originate from unknown epigenetic influences early in life (Nasca *et al*, 2014).

(Nasca *et al*, 2014), we found that a subset of mice shows elevated hippocampal MR levels and that this baseline difference makes those mice with higher MR show greater stress-induced reduction in mGlu2 accompanied by more anxiety and depressive-like behaviors. How MR activation does this is not yet clear, but it activates a mechanism that is opposite to that of LAC, which has been shown to use the acetyltransferase P300 to acetylate lysine 27 on histone H3

(Nasca *et al*, 2013). Likewise, the nature of the experiences of the animals that develop higher MR is also not yet known but may involve maternal care and stressors in the neonatal nesting environment (Francis *et al*, 1999). The epigenetic allostasis model points to a developmental origin of individual differences in the responses to stress and implies that unknown early-life epigenetic influences program each individual to different trajectories of behavioral and

physiological responses to later stressful life events. In line with this model, previous studies have also associated increased hippocampal MR levels in juvenile animals with anxiety-like behavior in adulthood (Brydges *et al*, 2014; Korte *et al*, 1995; Figure 6).

Lessons of an Ever-Changing Brain from Gene Expression

The hippocampus has been an important gateway to understanding the effects of glucocorticoids and stress on gene expression. Recent advances in technology have allowed for high-throughput analysis of gene expression changes in response to stress (Rubin *et al*, 2014). For example, microarray analysis of whole hippocampus after acute and chronic stress, as well as recovery from stress in mice, has revealed a number of insights surrounding stress-induced neuroplasticity (Gray *et al*, 2014). Although acute and chronic stress modulate a core set of genes, there are numerous expression changes that are exclusive to each condition, highlighting how the duration and intensity of stress alters reactivity. Further, corticosterone injections did not yield the same expression profile as acute stress, suggesting that *in vivo* stressors are able to activate a diverse set of pathways independent of GR activation. Finally, characterization of expression profiles after an extended recovery from chronic stress (21 days) revealed that, despite a normalization of anxiety-related behaviors, recovery did *not* represent a return to the stress-naïve baseline, but rather represented a new state in which reactivity to a novel stressor produced a unique expression profile (Gray *et al*, 2014). Studies in rats have also confirmed that gene expression profiles can vary significantly from the immediate end of stress (1 h) to 24 h after the end of stress (Wang *et al*, 2010), and that chronic stress can alter the transcriptional response to an acute corticosterone injection in dentate gyrus (Datson *et al*, 2013). These studies demonstrate that a history of stress exposure can have a lasting impact on future stress reactivity and hippocampal function. Many of the genes identified as changed after chronic stress by Datson and DeKloet are known epigenetic regulators, providing one possible mechanism underlying the persistent alterations in the expression response beyond the end of stress exposure.

Identification of New Gene Candidates

One of the benefits of gene expression studies is the discovery of new gene markers and pathways that contribute to important functions. A recent example is the unexpected role of a cell nuclear pore complex protein, NUP-62, in the stress-induced dendritic remodeling in the CA3 region of hippocampus (Kinoshita *et al*, 2014). First identified as a gene that was downregulated in the PFC of depressed patients (Tochigi *et al*, 2008), it was also found to be reduced in response to chronic stress in CA3 neurons of rodents (Kinoshita *et al*, 2014). Importantly, the levels of other

nuclear pore complex genes were unchanged with chronic stress, supporting the specificity of its role in stress remodeling. Subsequent *in vitro* studies confirmed that the downregulation of NUP-62 is associated with dendritic retraction and this effect is regulated at the molecular level by NUP-62 phosphorylation at a PYK2 site, which results in its retention in the cytoplasm (Kinoshita *et al*, 2014).

Pathway analysis of high-throughput gene expression data sets allows for a fresh look at known pathways implicated in the stress response. For example, inflammation has been associated with chronic stress and recent microarray data from whole mouse hippocampus have helped to confirm that NF- κ B/TNF- α and IL-6 signaling are in fact some of the most affected pathways (Gray *et al*, 2014). Acetylation of p65, a transcription factor in NF- κ B signaling, has also been associated with the rapid antidepressant effects of LAC (Nasca *et al*, 2013; Wang *et al*, 2014), which supports a growing literature that underscores an important role for the inflammatory response in the pathophysiology and treatment of depression (Dantzer *et al*, 2008). Similarly, LAC is a nonspecific acetylating agent and its rapid antidepressant effects may be mediated by acetylation of other proteins outside the nucleus (Russo and Charney, 2013). This raises the possibility that regulation of cytoskeleton genes is presumably necessary for promoting rapid antidepressant effects and for the dendritic remodeling of neurons to occur (Figure 7). High-throughput data sets that can measure the expression levels of the entire transcriptome now allow for the visualization of genes that are changed from the cell surface receptors to the cytoskeleton itself (Figure 7). The data reveal changes in both predictable regulators, such as ROCK1/2, and other signaling intermediates that might not have been anticipated.

Translation to the Human Hippocampus

The hippocampus has also been a gateway to translating animal model findings in order to begin to elucidate the effects of stress and stress-related disorders on the human brain, starting with the hippocampus (McEwen, 2007; McEwen and Gianaros, 2011; McEwen and Morrison, 2013). These include changes in brain structure and functional activity in depression, PTSD, Cushing's disease, and Type 2 diabetes, as well as effects of jet lag and shift work, chronic life stress, perceived stress, and the beneficial effects of physical activity (McEwen and Gianaros, 2011; Sheline, 2003). Circadian disruption, as in shift work, causes dendritic atrophy and impairs cognitive flexibility while also promoting obesity and insulin and leptin resistance (Karatsoreos *et al*, 2011). Regarding physical activity, previously sedentary older adults who walk 1 h a day for 6 months to 1 year show enlargement of the hippocampal formation (Erickson *et al*, 2011) and this is likely due, at least in part, to the increased dentate gyrus neurogenesis that is stimulated by exercise and by an enriched environment (Kempermann *et al*, 1997; van Praag *et al*, 1999). It is also noteworthy that hippocampal volume increases

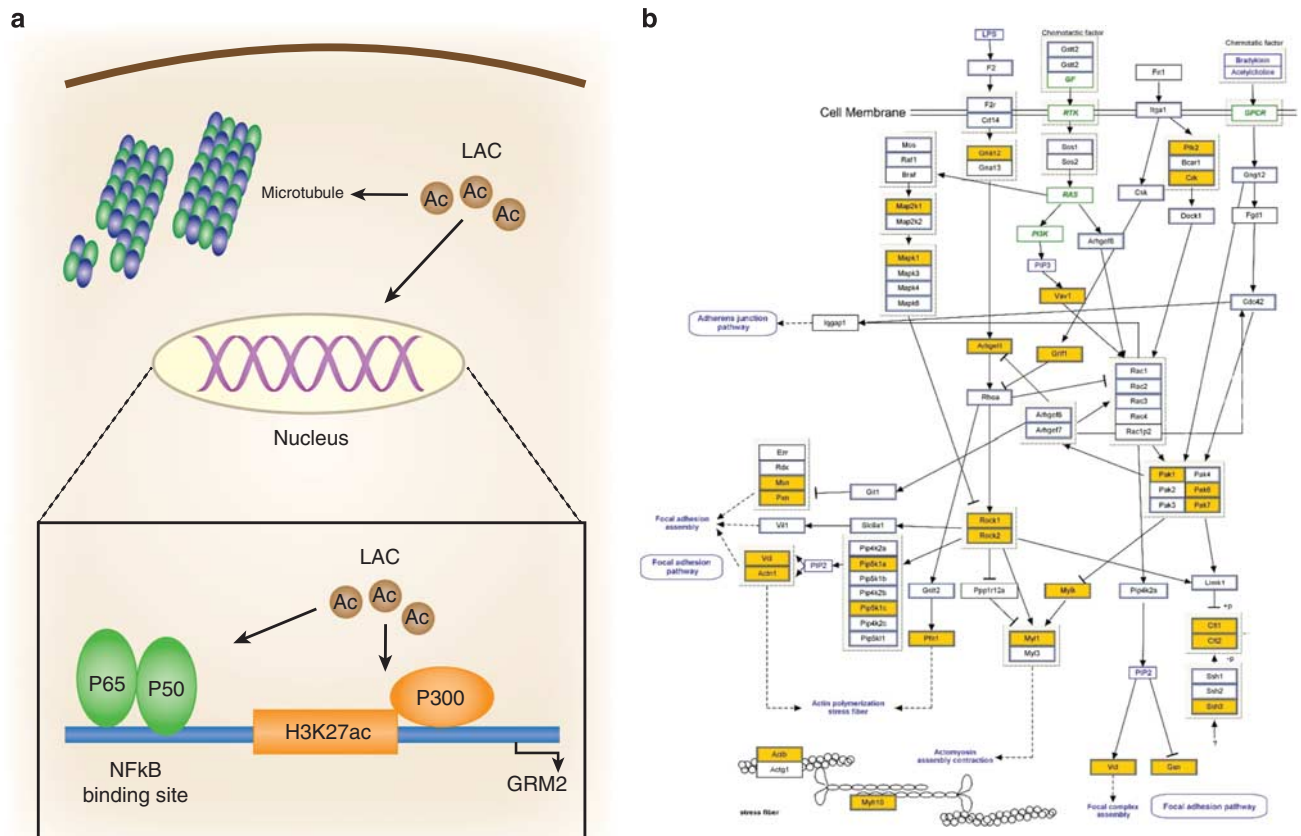


Figure 7. Potential cellular mechanisms for rapid antidepressant action. (a) Acetyl-L-carnitine (LAC) may act inside and outside the nucleus to promote rapid antidepressant responses. Inside the nucleus, LAC increases mGlu2 transcription by enhancing acetylation of the NF- κ B and histone H3K27 bound to mGlu2 promoter gene. Outside the nucleus, LAC may control stability of the neuronal cytoskeleton to regulate dendritic remodeling (Nasca *et al*, 2013). (b) Graphical representation of known intracellular signaling pathways involved in the regulation of the actin cytoskeleton (adapted from WikiPathways) in which genes altered by acute stress, chronic stress, or after recovery from stress are highlighted (yellow) based on microarray data derived from Gray *et al*. (2014).

with intense learning (Draganski *et al*, 2006) but is also decreased in Cushing's disease (Starkman *et al*, 1992), and chronic jet lag without adequate time for recovery is associated with cognitive impairment, dysregulated cortisol secretion, and a smaller temporal lobe (Cho, 2001). In relation to aging, cortisol levels in aging humans predict memory impairment over 5 years and aged humans with significantly prolonged cortisol elevations showed reduced hippocampal volume and deficits in hippocampus-dependent memory tasks compared with normal cortisol controls. Moreover, the degree of hippocampal atrophy correlated strongly with both the degree of cortisol elevation over time and current basal cortisol levels (Lupien *et al*, 1998).

Taken together, information gained from across all subregions of the hippocampus has led to the inverted U shape dose-time response concept summarized in Figure 8. Acute stress, mediated by glucocorticoids and EAAs along with other mediators, increases excitability at moderate physiological levels but can have the opposite effect at higher levels of activity. Chronic stress produces the largely reversible, adaptive plasticity described above in which the

retraction of dendrites and reduced synapse density may subserve a protective function against permanent damage, whereas sudden traumatic events such as head trauma, seizures, and ischemia do not give the hippocampus an opportunity to adapt and lead to permanent damage and neuron loss. However, when resilience is lacking after the stressor is over, cognitive impairment and anxiety or depression may persist and require external interventions. In all of this, it should be kept in mind that at each stage of experience, the brain is changing even if there is apparent recovery of morphological and neurochemical changes produced by stressors.

STRUCTURAL REMODELING IN THE AMYGDALA

The hippocampus provided a gateway into the study of another important brain region involved in stress and stress-related disorders. In 2002, a landmark study showed that while CIS (more severe than restraint and hence effective in 10 vs 21 days) caused shortening of dendrites in the CA3 region of rats, the

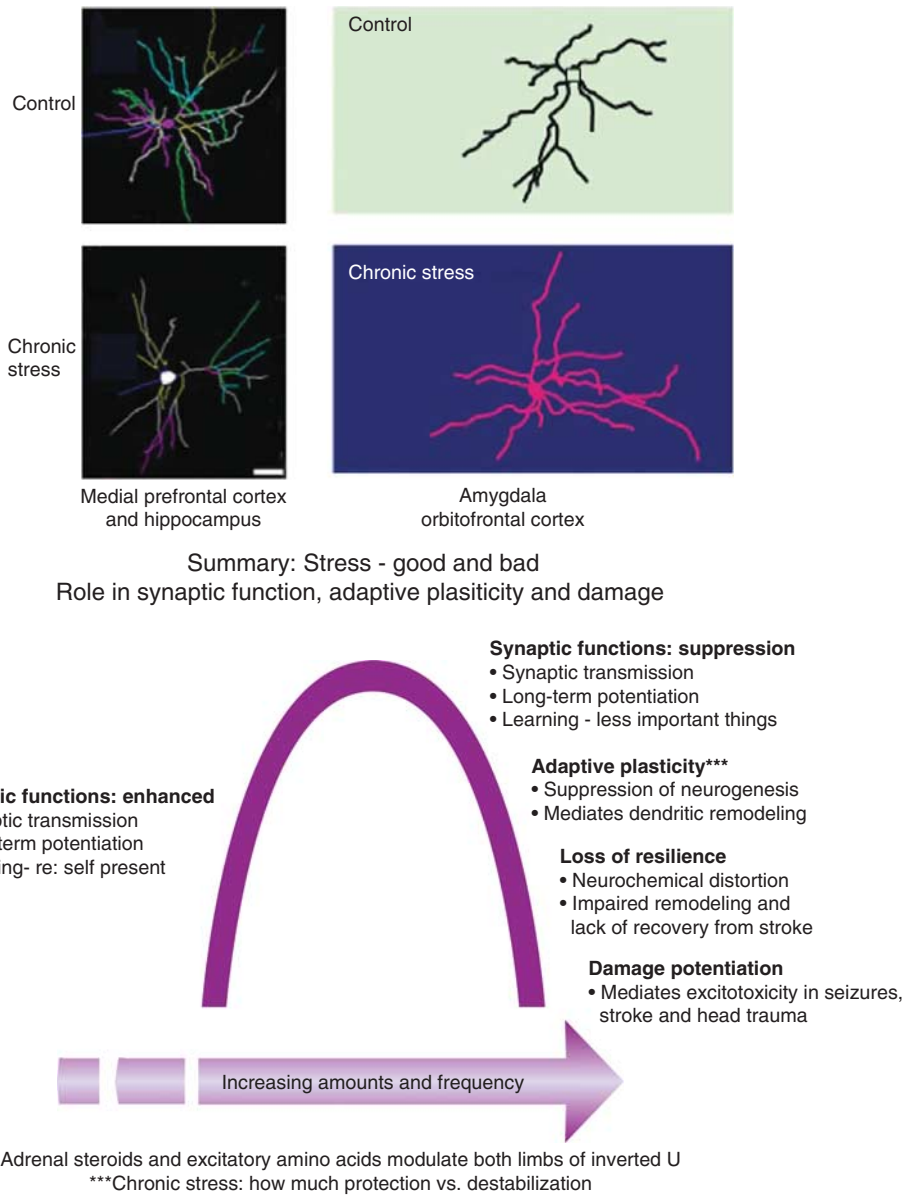


Figure 8. Chronic stress causes remodeling of dendrites and synaptic connections in many brain regions, including not only hippocampus but also amygdala and medial prefrontal and orbitofrontal cortex (top panel). Effects of acute and chronic stress operate in space and time in an inverted U-shaped manner (bottom panel). Acute stress, mediated by glucocorticoids, and excitatory amino acids and other mediators (see Table 1), can enhance excitability and promote memory over minutes to hours as long as the stressor is not overly intense; intense stress can have the opposite effect. Chronic stress causes neuronal remodeling as depicted in top panel in a largely reversible manner, promoting adaptation (eg, increase vigilance and anxiety in a dangerous environment). Yet, if there is no reversal of the stress-induced changes in neuronal architecture, an outside intervention with pharmaceutical agents and behavioral therapies may be needed to correct the imbalance. Finally, seizures, ischemia, and head trauma can trigger uncontrolled activation of excitatory amino acids leading to free radicals and inflammatory tone potentiated by glucocorticoids.

basolateral amygdala (BLA) responded by an expansion of dendrites (Vyas *et al*, 2002). At the same time, spine density was downregulated by CIS in medial amygdala, where it was dependent on one of the modulators in Table 1, namely, tPA, as shown in tPA knockout mice; dendrite expansion in the BLA was independent of tPA mediation (Bennur *et al*, 2007). Increased anxiety after acute stress was also shown to be dependent on tPA (Pawlak *et al*, 2003) where tPA release was found to be stimulated by CRF via CRF1 receptors (Matys *et al*,

2004). Interestingly, the ability of chronic stress to reduce spine density on hippocampal CA1 neurons was also dependent on tPA and tPA-KO mice also were not impaired by chronic stress in a spatial memory task in contrast to wild-type mice (Pawlak *et al*, 2005). Because CRF is also known to regulate spine density in the hippocampus, it is likely that it was activating the tPA release there (Regev and Baram, 2014). A recent study using targeted deletion of GR showed that tPA-BDNF-TrkB signaling altered fear conditioning (Revest *et al*, 2014)

A Paradoxical Role of BDNF in Acute and Chronic Stress

BDNF also has an important role in dendritic remodeling in both hippocampus and BLA. Overexpression of BDNF in mice increases dendritic length in both CA3 and BLA and occludes the effects of chronic stress to decrease dendritic branching in CA3 and increase it in BLA (Govindarajan *et al*, 2006). Without such overexpression, chronic stress causes a downregulation of BDNF in CA3 of hippocampus and an upregulation of BDNF in the BLA, and the effect in BLA persists after 21 days post stress, whereas that in CA3 has normalized (Lakshminarasimhan and Chattarji, 2012). Moreover, acute stress with a 10-day delay, which causes increased density of spines in BLA neurons (Mitra *et al*, 2005), caused BDNF expression to rise and stay elevated for 10 days, whereas levels in CA3 fell after acute stress but did so only transiently (Lakshminarasimhan and Chattarji, 2012). Corticosterone levels increased after both acute and chronic stress and remained elevated after chronic, but not after acute stress. Importantly, Lakshminarasimhan and Chattarji (2012) point out how elevated glucocorticoids and glutamate after stress lead to contrasting patterns of BDNF expression and structural plasticity, suggesting there may be a signaling intermediate that could regulate the differential region-specific response. However, the mechanisms regulating this (possibly epigenetic or post-translational) remain to be identified.

Interestingly, beyond the epigenetic action on the acetylated H3K27 to correct glutamate activity in hippocampus, LAC also increases BDNF brain and serum levels to promote antidepressant responses, supporting the existence of an intermediate key mediator between the glutamate system and BDNF signaling in the etiology and treatment of stress-related mood disorders (Nasca *et al*, 2013).

Traumatic Stress Effects and Paradoxical Actions of Glucocorticoids

Another paradoxical effect of a mediator of stress and adaptation to stressors involves glucocorticoid actions in relation to acute vs chronic stress effects upon the amygdala. Consistent with the elevation of corticosterone after acute and chronic stress accompanying the increase of dendritic length in BLA, as noted above (Lakshminarasimhan and Chattarji, 2012), a single large bolus of corticosterone mimics the ability of 10 consecutive days of CIS to increase anxiety and dendritic length in BLA (Mitra and Sapolsky, 2008). Furthermore, a single traumatic stressor causes a naïve rat to develop anxiety and increased spine density on BLA neurons, but with no increase in BLA dendritic length, with a delay of 10 days as noted above (Mitra *et al*, 2005).

But a timed elevation of a low to moderate dose of corticosterone at the time of the traumatic stressor prevents the increased anxiety and increased BLA spine density 10 days later (Rao *et al*, 2012). A similar protective effect of corticosterone has been reported for a different acute traumatic stress paradigm (Zohar *et al*, 2011). One

possibility currently under investigation is that corticosterone stimulation of endocannabinoid production may be involved (Hill *et al*, 2010a), as endocannabinoids have an important role in the amygdala regulating basal and chronic stress levels of HPA activity (Hill and McEwen, 2009; Hill *et al*, 2010b) and endocannabinoids are known to modulate amygdala dendritic structure (Hill *et al*, 2013).

Translation to Human Amygdala: Mood Disorders and PTSD

The protective effects of a timed elevation of glucocorticoids in several animal models of traumatic stress have a human counterpart, as both epidemiologic studies and clinical research on patients undergoing cardiovascular surgery indicate that low glucocorticoid levels at the time of trauma increase probability of PTSD symptoms (Schelling *et al*, 2004; Yehuda *et al*, 1998). Moreover, administration of a glucocorticoid within an hour after a traffic accident was reported to reduce subsequent PTSD symptoms (Zohar *et al*, 2011).

Amygdala overactivity is also associated with mood disorders (Drevets and Raichle, 1992) and amygdala enlargement is reported in children of chronically depressed mothers (Lupien *et al*, 2011). Therapeutically, individuals with a chronic anxiety disorder have been shown to benefit from mindfulness-based stress reduction and when anxiety is reduced there is a reported decrease in amygdala volume (Holzel *et al*, 2010).

STRESS INDUCES STRUCTURAL REMODELING IN THE PFC

Findings in the hippocampus have also provided a gateway into another important brain region involved in stress and stress-related behaviors, namely, the PFC, which is important for working memory, executive function, and self-regulatory behaviors and shows sex differences in response to stressors (McEwen and Morrison, 2013).

Stress and Glucocorticoids have Biphasic Effects on PFC Functions

As is the case for the hippocampus, glucocorticoids also have biphasic effects on the PFC, namely, in relation to working memory and recognition memory. Acute stress caused a long-lasting potentiation of NMDAR- and AMPAR-mediated synaptic currents via glucocorticoid receptors in PFC pyramidal neurons, accompanied by increased surface expression of NMDAR and AMPAR subunits. Acute stress enhanced working memory via a GR-dependent mechanism (Yuen *et al*, 2009). Moreover, acute stress or short-term corticosterone treatment *in vitro* induced a delayed and sustained potentiation of the synaptic response and surface expression of NMDA and AMPA receptors in PFC pyramidal neurons through a mechanism depending on the induction of serum- and glucocorticoid-inducible kinase

and the activation of Rab4, which mediates receptor recycling between early endosomes and the plasma membrane. *In vivo*, a serum- and glucocorticoid-inducible kinase mechanism is involved in facilitating working memory (Yuen *et al*, 2011a). Yet, chronic stress significantly reduced AMPA and NMDA receptor-dependent synaptic transmission and cell surface expression. This reduction was related to ubiquitin/proteasome-dependent degradation of GluR1 and NR1 subunits controlled by E3 ubiquitin ligase Nedd4-1 and Fbx2 and inhibition of proteasomes or knockdown of Nedd4-1 or Fbx2 prevented the chronic stress effects on recognition memory and glutamatergic function (Yuen *et al*, 2012).

Chronic Stress Induced Remodeling of PFC Neural Architecture

As in hippocampus, chronic stress also causes reversible structural remodeling of medial prefrontal cortical neurons that is reversible after the termination of chronic stress in young animals (Cook and Wellman, 2004; Liston *et al*, 2006; Radley *et al*, 2004, 2005), but not as readily reversible in middle aged and less so in aged animals (Bloss *et al*, 2010). However, one aspect of reversibility is that dendrites that shrink are distal to the cell body, whereas those that grow back after termination of stress are more proximal (Goldwater *et al*, 2009), suggesting that those neurons are different after stress, perhaps in their connectivity and most probably in terms of gene expression as described for hippocampus (Gray *et al*, 2014). EAAs are mediators of the stress-induced remodeling in PFC as they are in hippocampus (Martin and Wellman, 2011). Likewise, corticosterone administration alone also causes dendritic remodeling in medial PFC (mPFC), just as it does in the CA3 region (Cerqueira *et al*, 2005; Watanabe *et al*, 1992; Wellman, 2001). Endocannabinoids can also mediate stress-induced remodeling, as shown by the finding that mice lacking the endocannabinoid CB1 receptor have shorter dendrites in mPFC and respond with more dendritic shrinkage to chronic stress compared with wild-type mice (Hill *et al*, 2011a). Endocannabinoids in the medial PFC also have a role in the termination of the HPA stress response (Hill *et al*, 2011b).

In contrast to stress effects on the mPFC, the orbitofrontal cortex (OFC) shows expansion of dendrites after the same chronic stress that causes dendrite shrinkage in mPFC and hippocampus and dendrite expansion in the BLA (Liston *et al*, 2006). Unlike the mPFC under stress where impaired cognitive flexibility after chronic stress resembles lesions to mPFC, but in a manner that is reversible in young animals after termination of the chronic stress (Birrell and Brown, 2000; Liston *et al*, 2006), the functional significance of neuronal remodeling in OFC is less clear. However, one can speculate that it is a reflection of the role of the OFC in determining the salience of reward or punishment and thus part of an adaptive mechanism to chronic stress to increase vigilance to possible new stressors (McEwen and Morrison, 2013).

Epigenetics for PFC that Complement and Contrast with Hippocampus

In contrast to stress effects on hippocampus in susceptible individuals, in the PFC, ARS fails to decrease mGlu2 receptors, nor are any changes in the histone H3K27ac observed in the PFC as well as no change in the epigenetic regulator, P300, in the layers I–II and III of the orbitomedial PFC and prelimbic cortex, as determined by immunocytochemistry (Figure 9). Nevertheless, in the PFC, ARS results in strong upregulation of the postsynaptic mGlu5 receptors and decrease of the subunit NR1 of NMDA receptors along with no significant changes in the transcription of the metabotropic mGlu3 receptors and glial transporters xCT and Glt-1 (Figure 9). The ARS-induced decrease in expression of the NR1 subunit that would decrease ionotropic actions of glutamate and the concomitant increase in postsynaptic mGlu5 receptor expression, which would potentiate overall glutamate signaling, may represent an adaptive, homeostatic response to alterations in neurotransmitter overflow. In contrast, after ARS, the hippocampus shows evidence of an excessive EAA tone along with a decrease of mGlu2 (Nasca *et al*, 2014). The different responses of the hippocampus and PFC may reflect their respective roles in higher cognitive function, as the hippocampus encodes memories related to spatial orientation and daily events (McEwen, 1999), whereas the PFC has an important role in working memory and executive function as well as in self-regulatory behaviors (McEwen and Morrison, 2013), all of which are affected to some degree by acute and more prolonged stressors.

Translation to the Human PFC

The PFC in humans is affected by stressors and the psychosocial environment. Severe acute stressors impair cognitive function largely via adrenergic mechanisms (Arnsten, 2009). Yet, glucocorticoids facilitate working memory in young people (Lupien *et al*, 2002), but impair it in older subjects where basal cortisol levels and reactivity are higher (Lupien *et al*, 1997), which implies a biphasic dose response as well as a possible aging effect. For perceived stress, medical students who had high scores on the 10-item perceived stress scale of Sheldon Cohen, of Carnegie Mellon University, showed impaired functional connectivity by fMRI in a brain circuit involving the PFC as well as impaired performance on a test of mental flexibility; these effects were reversed by a month vacation (Liston *et al*, 2009). Thus, the young adult human PFC reflects the effects of chronic stress by showing impaired cognitive flexibility and reduced functional connectivity that parallels the effects of stress in the young adult rat brain, including the reversibility after the end of the stressful period as described above.

The long-term psychosocial environment also affects the PFC and its ability to exert control over amygdala activity. Low perceived social standing is associated with reduced PFC gray matter volume as well as with an increased systemic inflammatory tone and altered white matter structure

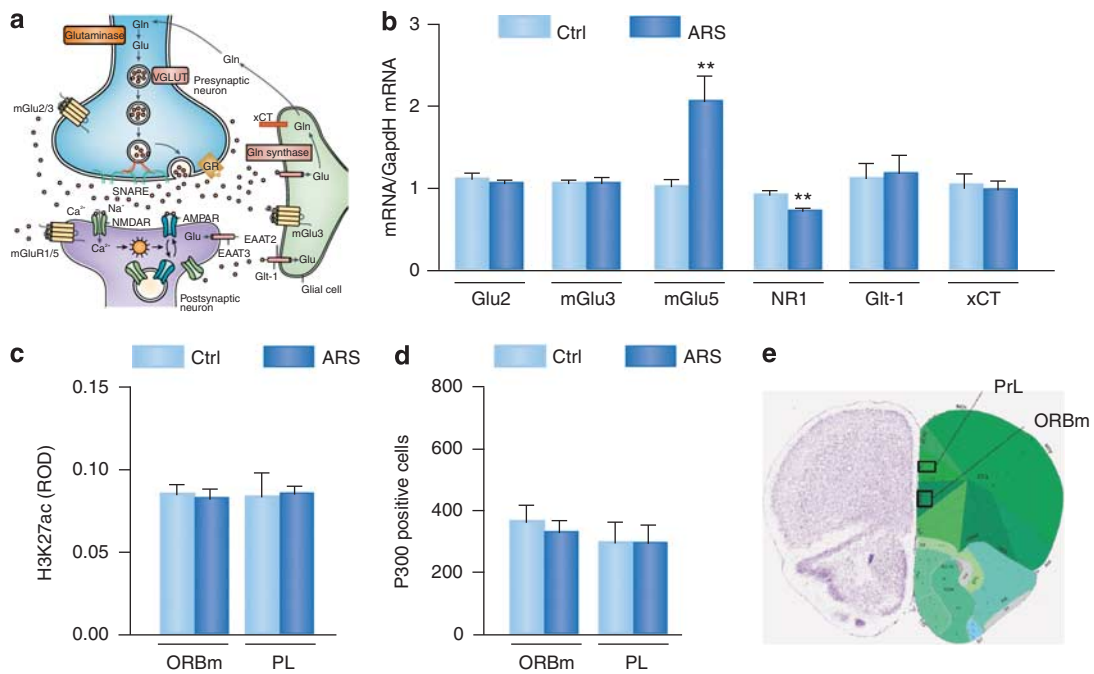


Figure 9. Epigenetics coupled to the glutamatergic gene expression profile in the PFC in response to stress. (a) The tripartite glutamatergic synapse. Adapted and modified by Popoli *et al* (2012) by permission. (b) Glutamatergic gene expression profile in unstressed age-matched mice and in mice subjected to acute restraint stress within the PFC. Glt-1: principal glutamate transporter, which removes glutamate from the neuronal synaptic cleft into neuroglia and neurons; xCT: the cystine–glutamate exchanger that facilitates glutamate exchange from glial cells to the perisynaptic space; mGluRs: metabotropic glutamate receptors, which mainly inhibit the release of glutamate into the synaptic space and generate excitatory responses; NR1, essential subunit of NMDA receptors: ionotropic glutamate receptors, which mainly regulate mechanisms of synaptic plasticity. Bars represent mean±SEM, significant comparisons with corresponding controls, ** $P < 0.01$ (t -test). (c) ROD analysis of H3K27ac immunoreactivity showing the lack of ARS effects in the subregions (ORBm and PrL) of the PFC. (d and e) Cell count of P300-positive cells showing the lack of ARS effects in the layers I–II and III of either the ORBm or PrL subregions of the PFC. Image credit: Allen Institute for Brain Science (e).

throughout the brain (Gianaros *et al*, 2007, 2013) and preclinical atherosclerosis is associated with increased amygdala reactivity to stimuli such as sad and angry faces and reduced inhibitory input from the PFC (Gianaros *et al*, 2009).

The functional connectivity between PFC and amygdala develops first as a positive influence and then as an inhibitory one (Gee *et al*, 2013b). Under typical conditions, mPFC connections with the amygdala are immature during childhood and become adult-like during adolescence, and rodent models have shown that maternal deprivation accelerates this development. Previously institutionalized youths, who experienced early maternal deprivation, exhibited atypical amygdala–mPFC connectivity, in that they failed to show the positive amygdala–mPFC coupling of children with good maternal attachment. Rather, these children with a history of early adversity showed mature, negative amygdala–mPFC coupling and thus, resembled the adolescent phenotype. Cortisol is implicated as a mediator suggesting a developmental role of the deprivation stress. Despite being age-atypical, negative amygdala–mPFC coupling conferred some degree of reduced anxiety, although anxiety was still significantly higher in the previously institutionalized group indicating only partial compensation for lack of strong maternal attachment, and this suggests that

accelerated amygdala–mPFC development is an ontogenetic adaptation in response to early adversity (Gee *et al*, 2013a).

Plasticity and resilience in the PFC are enhanced by regular moderate aerobic exercise, which increases blood flow to this brain region and improves executive function (Colcombe *et al*, 2004; Kramer *et al*, 1999). Cognitive behavioral therapy has been shown to increase gray matter volume in the PFC when it is able to reduce symptoms of chronic fatigue (de Lange *et al*, 2008).

SEX DIFFERENCES

The hippocampus has also been a gateway for the understanding of the actions of ovarian hormones on neural and cognitive functions over and above the actions that subservise reproduction. The discovery of genomic and non-genomic forms of the estrogen receptor in spines, dendrites, mitochondria, presynaptic terminals, and astrocytes paved the way for discovery of such non-nuclear estrogen receptor localization in other brain regions (McEwen and Milner, 2007). This revelation occurred along with the discovery of ovarian hormone turnover of spine synapses in the CA1 region of the female rat hippocampus. The identification of

signaling processes involving estradiol stimulation via PI3 kinase of phosphorylation of LIMK1 and 4E-BP1 leads to actin polymerization and translation of PSD95 mRNA, respectively (Akama and McEwen, 2003; Dumitriu *et al*, 2010; Yuen *et al*, 2011b). This is a sexually differentiated response and male rats do not show synapse induction in response to estradiol, but do so in response to testosterone (Leranth *et al*, 2003).

Females Respond Differently to Chronic Stress

All of the animal model studies of stress effects summarized above were carried out on male rodents, and female rodents do not show the same pattern of neural remodeling after chronic stress as do males. The first recognition of this sex difference was in the hippocampus, in which the remodeling of CA3 dendrites did not occur in females after CRS, even though all the measures of stress hormones indicated that the females were experiencing the stress as much as males (Galea *et al*, 1997). Females and males also differ in the cognitive consequences of repeated stress, with males showing impairment of hippocampal-dependent memory, whereas females do not (Bowman *et al*, 2001; Luine *et al*, 1994, 2007).

In contrast, acute tail shock stress during classical eyeblink conditioning improves performance in males, but suppresses it in females (Wood and Shors, 1998) by mechanisms influenced by gonadal hormones in development and in adult life (Shors and Miesegaes, 2002; Wood *et al*, 2001). However, giving male and female rats control over the shock abolishes both the stress effects and the sex differences (Leuner *et al*, 2004). These findings suggest that sex differences involve brain systems that mediate how males and females interpret stressful stimuli and that a sense of control is paramount to coping with those stimuli.

There are sex differences in response to chronic stress in mPFC neurons, in which female rats with intact ovaries or estrogen treatment after ovariectomy showed an expansion of dendrites, whereas males show a chronic stress-induced retraction (Garrett and Wellman, 2009). Refining this in terms of where mPFC neurons project, Shansky showed that female rats fail to show the mPFC dendritic remodeling seen in males after CRS in those neurons that do not project to amygdala. Those neurons that project to amygdala do not change in males with chronic stress but, in females with estrogens, there is a chronic stress-induced expansion of the dendritic tree in the subset of neurons that project to the BLA (Shansky *et al*, 2010). Moreover, ovariectomy prevented these chronic stress effects on dendritic length and branching. Furthermore, estradiol treatment of ovariectomized females increased spine density in mPFC neurons, irrespective of where they were projecting (Shansky *et al*, 2010).

Recent behavioral work on male and female rats undergoing fear conditioning and extinction involving, respectively, the prelimbic and infralimbic PFC (Santini *et al*, 2008) has revealed sex differences based upon individual differences in efficacy of extinction (poor extinction, HF; good

extinction, LF) that were evident in both males and females (Gruene *et al*, 2014). Despite no overall sex differences in freezing behavior, the HF/LF phenotypes emerged in males during extinction, but in females it emerged during fear conditioning, which does not involve infralimbic-BLA neurons. HF vs LF males exhibited neuroanatomical distinctions in dendrites that were not observed in HF vs LF females, namely differences in prefrontal cortical dendritic length that may have either preceded or resulted from the extinction. The authors speculate that, in females, the sex differences in PFC to amygdala circuitry (Shansky *et al*, 2010) underlie the female HF vs LF difference at the time of conditioning. The fact that estrogen's and androgen's effects are widespread in the central nervous system via both genomic and non-genomic receptors (McEwen and Milner, 2007), there are likely to be many more examples of sex \times stress interactions related to many brain regions and multiple functions, as well as developmentally programmed sex differences that affect how the brain responds to stress, eg, in the locus coeruleus (Bangasser *et al*, 2010, 2011).

Emergence of Sex Differences in Response to Stressors After Puberty

Although the sensitive period for testosterone effects on sexual differentiation is perinatal in rodents, sex differences in response to chronic stressors manifest themselves gradually over the pubertal transition. Demonstration of this was accomplished using a rodent model of chronic restraint stress in juvenile rats daily from postnatal days 20 to 41 (Eiland *et al*, 2012). Chronic stress produced depressive-like behavior and significant neuronal remodeling of brain regions likely involved in these behavioral alterations: the hippocampus, PFC, and amygdala. Both chronically stressed males and females exhibited anhedonia, increased locomotion when exposed to novelty, and altered coping strategies when exposed to acute stress. Chronic stress produced shrinkage of dendrites in the hippocampus and PFC and concurrent hypertrophy of dendrites in the amygdala, with a trend for males to show more robust responses than females (Eiland *et al*, 2012).

Even then, in prepubertal female rats there are already sex differences in that young female rats exposed to 1 week of repeated restraint stress show no negative effects on temporal order recognition memory, a cognitive process controlled by the PFC, in contrast to impairment in temporal order recognition memory observed in stressed males (Wei *et al*, 2014). Chronically stressed females also showed normal glutamatergic transmission and glutamate receptor surface expression in PFC pyramidal neurons, in contrast to impairment seen in stressed males. However, inhibition or knockdown of PFC estrogen receptors revealed chronic stress-induced impairment in females, and estradiol administration to stressed males prevented the inhibitory effects of estradiol. Furthermore, blocking aromatase in females also causes chronic stress to have deleterious effects in these young females. Thus, already at 4–5 weeks of age, before the

onset of puberty, estrogens already have protective effects in both chronically stressed females and males (Wei *et al*, 2014).

Translation to the Human Brain

The impact of sex and sex differences has undergone a revolution and much more is to come (Cahill 2006; McCarthy *et al*, 2012; McCullough *et al*, 2014; McEwen and Lasley, 2005), including insights into X and Y chromosome contributions to brain sex differences (Carruth *et al*, 2002). In men and women, neural activation patterns to the same tasks are quite different between the sexes even when performance is similar (Derntl *et al*, 2010). This leads to the concept that men and women often use different strategies to approach and deal with issues in their daily lives, in part because of the subtle differences in brain architecture. Nevertheless, from the standpoint of gene expression and epigenetic effects, the principles of what we have learned in animal models regarding plasticity, damage, and resilience are likely to apply to both males and females.

SUMMARY AND CONCLUSIONS

The hippocampus has provided the gateway into much of what we have learned about stress and brain structural and functional plasticity not only in adult life, but also as a result of stressors early in life. The initial focus on hippocampus has expanded to interconnected brain regions such as the amygdala, PFC, and nucleus accumbens. Moreover, the initial focus on gene regulatory effects has broadened with the recognition that steroid hormone receptors also mediate rapid signaling pathways and that both direct and indirect genomic and non-genomic actions of steroids involve non-linear interactions with other intra- and extracellular mediators (Table 1). The resulting epigenetic effects result in ever-changing patterns of gene expression, in which there are important sex differences that need further exploration. Translation of these findings from animal models to human brain function is changing thinking about the nature of brain malfunction in psychiatric disorders and during aging, as well as the mechanisms of the effects of early-life adversity on the brain and the body.

One outcome of the epigenetic perspective is the new possibility for interventions that help the brain, as the master organ of stress and adaptation to stressors, to change itself.

As suggested in the life course developmental perspective (Halfon *et al*, 2014), the brain changes that occur as a result of adverse experiences may be amenable to intervention that epigenetically changes brain structure and function to remediate those early events, even though true ‘reversal’ is not possible. Some of these interventions that have been shown to change brain structure and function are summarized in Table 2. The key may be agents—pharmaceutical or behavioral like physical activity—that facilitate plasticity so that behavioral intervention may be effective, such as the recently reported ability of fluoxetine to enhance recovery from stroke (Chollet *et al*, 2011). The main goal of this new approach (Castren and Rantamaki, 2010) is to open a ‘window of increased plasticity’ that may be capitalized by a positive behavioral intervention, eg, behavioral therapy in the case of depression or the intensive physiotherapy to promote neuroplasticity to counteract the effects of a stroke. This is consistent with animal model work that shows that ocular dominance imbalance from early monocular deprivation can be reversed by patterned light exposure in adulthood that can be facilitated by fluoxetine (Vetencourt *et al*, 2008), but also by caloric restriction as well as by every-other-day glucocorticoids in the drinking water (Spolidoro *et al*, 2011). These glucocorticoid actions bring into focus work showing that ultradian pulsatility of glucocorticoids promotes turnover of a subset of synapses throughout the cortex that is involved in motor learning, among other functions (Liston *et al*, 2013; Liston and Gan, 2011). Investigations of underlying mechanisms for the re-establishment of a new window of plasticity are focusing on the balance between excitatory and inhibitory transmission and removing molecules that put the ‘brakes’ on such plasticity (Bavelier *et al*, 2010).

It is important to reiterate that successful behavioral therapy, which is tailored to individual needs, can produce volumetric changes in both PFC in the case of chronic fatigue (de Lange *et al*, 2008), and in amygdala, in the case of chronic anxiety (Table 2; Holzel *et al*, 2010). This reinforces two important messages: (i) that plasticity-facilitating treatments should be given within the framework of a positive behavioral or physical therapy intervention; and (ii) that negative experiences during the window may even make matters worse (Castren and Rantamaki, 2010). In that vein, it should be noted that excess BDNF also has the ability to promote pathophysiology, such as seizures in some instances (Heinrich *et al*, 2011; Kokaia *et al*, 1995; Scharfman, 1997).

TABLE 2 Interventions that help the brain change itself

Regular physical activity

Increased hippocampal volume and PFC blood flow and improved executive function and memory (Erickson *et al*, 2011).

Mindfulness-based stress reduction

Reducing anxiety decreases amygdala volume in those individuals who responded to a mindfulness-based stress reduction therapy (Holzel *et al*, 2010).

Social support and integration

Experience Corps for elderly volunteers, who showed improved executive function, increased blood flow in PFC, and better overall health with slower decline.

Meaning and purpose (eudaimonia) are a likely component along with social support and increased physical activity (Carlson *et al*, 2009).

FUTURE DIRECTIONS

For real progress in diagnosing and treating mental and physical health disorders, it is essential that the life-course developmental perspective on the epigenetic embedding of early-life experience (Halfon *et al*, 2014) help redefine thinking about the origins of health and disease throughout the life course. The discovery of antibiotics for infectious disease promoted the notion that a pill could cure a disease ('magic bullets'). Although this may be true for infectious diseases, the recognition of the non-communicable diseases such as diabetes and heart disease has introduced preventative health behaviors such as diet and exercise (Engel 1977) where drugs are not 'magic bullets' but rather can help correct some physiological imbalances such as high cholesterol and low HDL. The life-course developmental perspective adds the important notion of the biological embedding of early-life experiences that carry over throughout the life course as well as the concept that via epigenetics the physical and social environment is continually changing the brain and the body. This dynamic interaction opens the possibility of redirecting toward a more positive trajectory via behavioral interventions like physical activity and certain pharmaceutical agents that open windows of plasticity for a behavioral intervention to be effective. Nowhere is this more important than for anxiety and depressive disorders as the brain is the central organ of adapting to stressors and is a vulnerable organ that is, however, subject to changes from external behavioral intervention, aided in some cases by pharmaceutical agents that open windows of plasticity.

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