nature neuroscience

Mechanisms of stress in the brain

Bruce S McEwen[1], Nicole P Bowles[1], Jason D Gray[1], Matthew N Hill[2], Richard G Hunter[3], Ilia N Karatsoreos[4] & Carla Nasca[1]

The brain is the central organ involved in perceiving and adapting to social and physical stressors via multiple interacting mediators, from the cell surface to the cytoskeleton to epigenetic regulation and nongenomic mechanisms. A key result of stress is structural remodeling of neural architecture, which may be a sign of successful adaptation, whereas persistence of these changes when stress ends indicates failed resilience. Excitatory amino acids and glucocorticoids have key roles in these processes, along with a growing list of extra- and intracellular mediators that includes endocannabinoids and brain-derived neurotrophic factor (BDNF). The result is a continually changing pattern of gene expression mediated by epigenetic mechanisms involving histone modifications and CpG methylation and hydroxymethylation as well as by the activity of retrotransposons that may alter genomic stability. Elucidation of the underlying mechanisms of plasticity and vulnerability of the brain provides a basis for understanding the efficacy of interventions for anxiety and depressive disorders as well as age-related cognitive decline.

The brain is the central organ of stress and adaptation to social and physical stressors because it determines what is threatening, stores memories, and regulates the physiological as well as behavioral responses to stressors that may be damaging or protective[1]. The physiological responses that produce adaptation through 'allostasis' include not only the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system, but also their nonlinear interactions with the metabolic system and the pro- and anti-inflammatory components of the immune defense system[1,2]. Exposure to multiple stressors and the dysregulation of the nonlinear interactions (such as failure to turning on or off responses efficiently) lead to wear and tear on the body and brain that is termed allostatic load and overload[1,3].

Allostasis is the active process of adapting to stressors via mediators such as cortisol and the autonomic, metabolic and immune system that act together in a nonlinear fashion to maintain homeostasis[2]. Allostatic load refers to the cumulative effect of multiple stressors as well as the dysregulation of the nonlinear network of allostasis (for example, production of cortisol, adrenalin or inflammation in response to a challenge). Allostatic overload refers to the cumulative pathophysiology that can result from this dysregulation and excess stress. Allostasis, and allostatic load and overload, are more precise biological concepts than 'stress' to describe adaptation and maladaptation to 'stressors', and they include the physiological effects of health-promoting and health-damaging behaviors as well as stressful experiences[1,2]. Health behaviors (such as smoking, alcohol, poor diet, or lack of sleep), resulting from the experience of stress, also have a role and contribute to allostatic load and overload[1,3].

[1]Laboratory of Neuroendocrinology, The Rockefeller University, New York, New York, USA. [2]Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada. [3]Department of Psychology, University of Massachusetts Boston, Boston, Massachusetts, USA. [4]Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, Washington, USA. Correspondence should be addressed to B.S.M. (mcewen@rockefeller.edu).

Received 19 April; accepted 8 July; published online 25 September 2015; doi:10.1038/nn.4086

'Stress' can be divided into 'good stress', 'tolerable stress' and 'toxic stress'[4]. Early life stress can alter neural architecture to increase adverse reactions to stressors, leading to toxic stress[4]. 'Biological embedding'[4,5] of these effects during critical or sensitive periods of early development has lasting effects through the life course[6,7]. Among the most important early life experiences are those that involve abuse and neglect, on the one hand, versus the establishment of strong, positive attachment of child to caregiver; these alter the ability of the individual to engage in cooperative social experiences or to feel excluded and hostile to the social environment later in life[8].

The brain is a target of stressful experiences, and glucocorticoids, along with excitatory amino acid neurotransmitters, alter neuronal architecture by causing dendritic retraction or expansion and decreased or increased synapse density, depending on the brain region, along with inhibition of dentate gyrus neurogenesis[9–11]. Many intra- and intercellular mediators and processes are involved in changing the brain during stress and recovery from stressful experiences[12,13] (**Box 1**).

This Review provides an overview of the mechanisms and mediators through which stressors alter brain structure and function. It does so by focusing primarily on three brain regions, the hippocampus, amygdala and prefrontal cortex (PFC), although in full recognition of the fact that stress has widespread effects throughout the brain. This Review also emphasizes the complex nonlinear interactions between different stress mediators that are central to the concept of allostasis and allostatic load and overload[3], in which nonlinearity applies not only to systemic hormones but also to intra- and extracellular mediators in the brain. Because of this, the many changes caused by stress often result in an inverted-U-shaped dose-response relationship, as represented in **Figure 1**.

Mechanisms underlying stress effects on the brain

Stressors alter gene expression through multiple mechanisms, including direct effects of glucocorticoids on gene transcription as well as the activation of epigenetic mechanisms in which histone modifications and methylation and hydroxymethylation of CpG residues in

Box 1 Examples of molecules that are necessary or permissive for remodeling

Brain-derived neurotrophic factor (BDNF)[93,94]
- Facilitator of plasticity or growth
- Overexpression occludes effects of chronic stress
- Haploinsufficiency prevents stress-induced plasticity

Tissue plasminogen activator (tPA)[73,74]
- Secreted signaling molecule and protease
- Required for stress-induced spine loss in hippocampus and medial amygdala
- Required for acute stress-induced increase in anxiety; secretion activated by CRF
- In amygdala, regulates tPA release

Corticotropin-releasing factor (CRF)[74,135]
- Secreted in hippocampus by interneurons
- Downregulates thin spines via RhoA signaling

Lipocalin-2
- Secreted protein of previously unknown function[77,78]
- Induced by acute stress
- Downregulates mushroom spines
- Knockout increases neuronal excitability and anxiety

Endocannabinoids[136–138]
- Induced via glucocorticoids
- Regulate emotionality and HPA habituation and shutoff
- CB_1 receptor knockout increases anxiety and basolateral amygdala dendrite length and causes stress-like retraction of prefrontal cortical dendrites, likely through the regulation of glutamatergic transmission
- Fatty acid aminohydrolase (FAAH) is a key regulator of endocannabinoid action

DNA have roles leading to repression and activation of genetic factors, including retrotransposons[14,15]. Glucocorticoids are not the sole mediators of these effects, in which excitatory amino acids and many other cellular mediators also play important parts (**Box 1**). These mediators span influences from extracellular adhesion molecules to cytoskeletal elements and at least one nuclear pore complex protein.

In addition to their critical role in complex behavior and cognition, the hippocampus, amygdala and PFC are important in regulating the autonomic and HPA stress response, and they are the main focus of this review (**Box 2**).

Stress effects on gene expression in an ever-changing brain. As the first extra-hypothalamic brain structure recognized to have receptors for adrenal steroids[16], the hippocampus has been an important gateway to understanding the effects of glucocorticoids and stress on gene expression in the brain. Recent technological advances have allowed high-throughput analysis of gene expression changes in response to stress[17]. For example, microarray analysis of whole hippocampus after acute stress, chronic stress and stress recovery in mice revealed that acute and chronic stress modulate a core set of genes, but that numerous changes are exclusive to each condition, highlighting how duration and intensity of stress alters reactivity[18]. Furthermore, corticosterone injections do not produce the same expression profile as acute stress, suggesting that *in vivo* stressors activate a diverse set of pathways independent of glucocorticoid receptor (GR) activation[18] (**Fig. 2**). Finally, characterization of expression profiles after extended recovery from 21 d of chronic stress showed that, despite a normalization of anxiety-related behaviors, recovery does not represent a return to the stress-naive baseline but rather a new state in which reactivity to a novel stressor produces a unique expression profile[18]. Studies in rats confirm that gene expression profiles can change significantly from the immediate end of stress to 24 h later[19] and that chronic stress can alter the transcriptional response to an acute corticosterone injection in dentate gyrus[20] (**Fig. 2**). Together, these studies demonstrate that a history of stress exposure can have a lasting impact on future stress reactivity and hippocampal function.

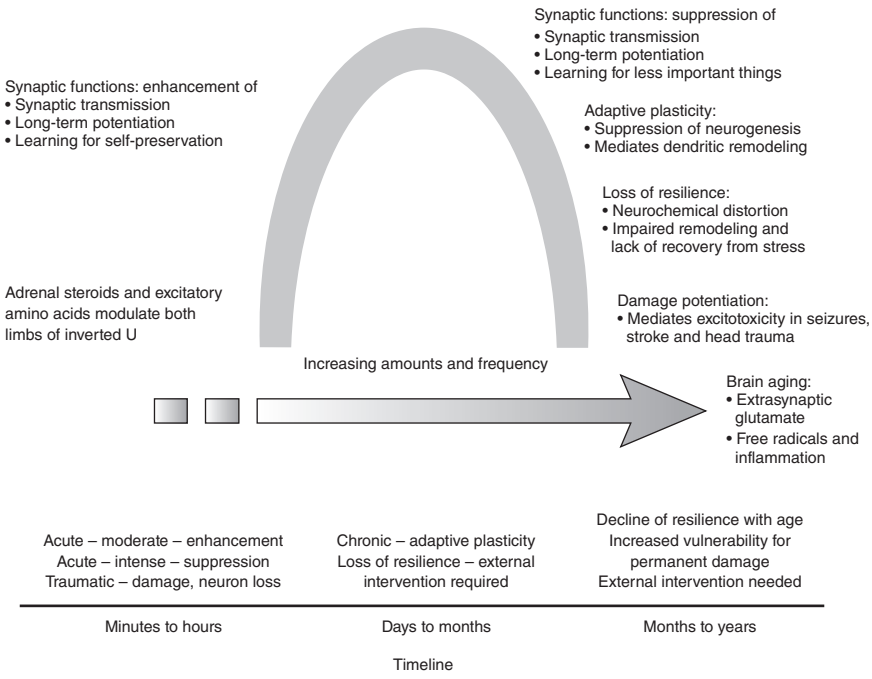
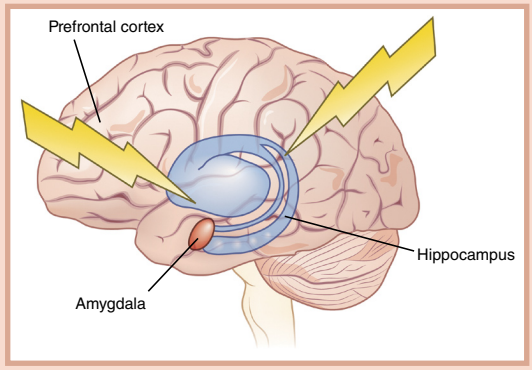


Figure 1 Effects of acute and chronic stress, mediated in part by glutamate and glucocorticoids as well as other molecules described in the text and in **Box 1**. These effects follow an inverted U-shaped curve in dose and time. The timeline shows how acute and chronic stress and aging interact with the intensity and duration of stressor.

Box 2 Overview of stress effects on the hippocampus, amygdala and prefrontal cortex



Marina Corral Spence/Nature Publishing Group

Three regions of the brain shown in the illustration have important roles in behavior and cognitive function as well as in regulating the autonomic and HPA stress response and are the main focus of this review.

Glucocorticoid and mineralocorticoid receptors were first recognized in the hippocampal formation[16], showing that adrenal steroids affect the brain in more ways than through the hypothalamus, which is now known to include effects on spatial and episodic memory and mood regulation. In the hippocampus, stress and glucocorticoids were first shown to cause dendritic shrinkage and loss of spines. The rediscovery of neurogenesis in the dentate gyrus[11] galvanized widespread interest in the functional role of neuronal replacement in the adult brain. It was in the hippocampus that the role of excitatory amino acids in stress effects was first recognized[9].

Effects of acute and chronic stress on the amygdala differ from those in the hippocampus. Acute traumatic stressors cause increased spine density on basolateral amygdala neurons, and chronic stress leads to the expansion of basolateral amygdala dendrites[139]. Yet the medial amygdala shows a chronic stress–induced loss of spines[75]. These alterations are implicated in increased anxiety and in PTSD-like behaviors[67,139].

Within the prefrontal cortex, chronic stress causes medial PFC neurons to develop debranching and shrinkage of dendrites that is associated with cognitive rigidity, while orbitofrontal cortical neurons expand dendrites that may be related to increased vigilance[122,140]. The PFC under stress has provided important clues to age-related loss of resilience and impaired memory as well as to the effects of circadian disruption and extinction of fear memory[141].

These three brain regions have contributed to our knowledge of cellular and molecular mechanisms and cellular processes that are described in this Review, revealing brain regional specializations as well as common mediators and mechanisms and the complex interactions among the mediators.

Many of the genes altered after glucocorticoid and chronic stress exposure in the hippocampus are known epigenetic regulators[21], providing one possible mechanism underlying the persistent alterations in the expression response beyond the end of stress exposure. The continually changing pattern of gene expression is consistent with the finding that, although stress-induced dendritic retraction in PFC neurons appears to be reversible in terms of dendritic length and branching, the recovered neurons are different, in that dendrites that regrow after recovery from stress are more proximal to the cell body than those that retracted[22].

Epigenetic mediation via post-translational histone modifications. Stress has a clear impact on many types of molecular epigenetic mechanisms, from histone modifications to DNA methylation and hydroxymethylation and expression of noncoding RNA[14,23–25] (**Fig. 3**). For instance, social defeat stress in rodents causes changes in both histone methylation and acetylation[26]. Acute and chronic stress promote histone modifications leading to repression or activation of genes related to memory and other processes. Studies of memory acquired in the forced swim test and Morris water maze uncovered a novel, rapid mechanism: glucocorticoids, via GRs, facilitate signaling of the ERK-MAPK pathway to the downstream nuclear kinases MSK1 and Elk-1 in dentate gyrus granule neurons; and activation of this pathway results in phosphorylation of serine 10 (S10) acetylation of lysine 14 (K14) of histone H3 (H3S10p-K14ac), leading to the induction of the immediate-early gene products c-Fos and Egr-1 (ref. 27).

Unlike that of other immediate-early gene products, FosB and its splice variant ΔFosB increases and remain elevated in the nucleus accumbens (NAc) after social defeat stress and is deficient in those animals that show depressive-like behavior, as well as in human patients with depression postmortem. Moreover, increased FosB/ΔFosB expression in NAc protects animals from the deleterious effects of chronic stress[28]. Epigenetically, the FosB promoter is enriched for dimethylation of H3 lysine 9 (H3K9me2) in the NAc of humans with depression relative to that of controls without depression, implicating this repressive epigenetic modification in the repression of FosB. Moreover, in mice zinc finger protein (ZFP)-induced enrichment of H3K9me2 at FosB in NAc not only was sufficient to reduce FosB/ΔFosB expression, but also induced depression- and anxiety-like behaviors after social stress[28].

A current practical application of this approach is the investigation of rapidly acting antidepressants[28,29] that act, at least in part, via epigenetic mechanisms, as does electroconvulsive therapy[26,30]. An epigenetic mechanism connects excitatory amino acid function with neural remodeling and stress-related behavior in one genetic and one stress-induced rodent model of anxiety- and depressive-like behavior in which downregulation of the presynaptic inhibitor of neuronal glutamate release, the mGlu2 receptors, in hippocampus is a key biomarker[29]. In that connection, drugs that modify glutamate overflow, such as ketamine, acetyl-L-carnitine and riluzole, have been show to exert rapid antidepressant-like effects in animal models[29,31] and in humans[32].

The novel antidepressant candidate acetyl-L-carnitine (LAC) appears to act inside and outside the nucleus to exert fast antidepressant responses: LAC corrects mGlu2 deficits by increasing acetylation of histone H3 lysine 27 (H3K27) bound to Grm2 promoter gene as well as acetylation of the NF-κB p65 subunit[29]. Using the same animal models, 14 d of treatment with the tricyclic antidepressant clomipramine were needed to promote antidepressant responses, which disappeared when the treatment was stopped, whereas antidepressant effects of LAC were still evident after 2 weeks of drug withdrawal. The persistent effects of LAC highlight the involvement of stable molecular adaptations that are reflected at the level of histone modifications in controlling mGlu2 transcription in hippocampus.

Transposons and retrotransposons. Acute restraint stress also has repressive epigenetic effects in the hippocampus and most prominently in the dentate gyrus via trimethylation of H3K27 and H3K9. The latter is associated with repression of a number of retrotransposable elements (RTE) and reduction of the coding and noncoding RNA normally produced by the repressed DNA, so far only in hippocampus[30,33]. This repression is lost with repeated stress, suggesting that those RTEs may impair genomic stability under conditions of chronic stress[15].

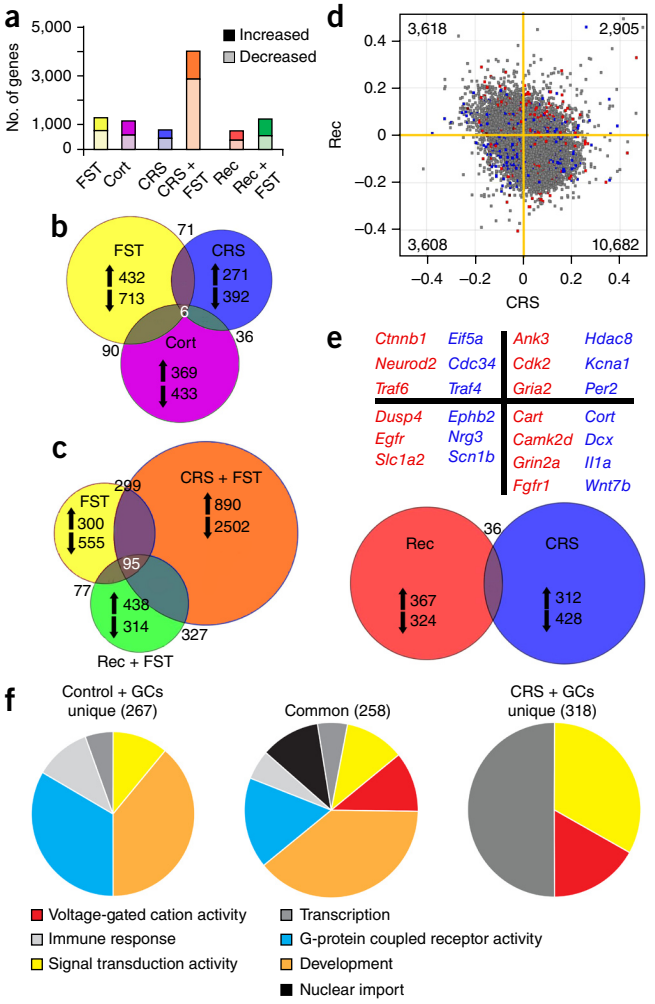


Figure 2 Gene expression changes in hippocampus in response to stress and glucocorticoid challenge depend on the prior stress history of the subject. Hippocampal microarray data reveals stress-induced changes in gene expression. (**a**) Dark colors represent the number of genes with significantly increased expression and pastels represent those with significantly decreased expression as identified by pairwise comparisons of each stress group with age-matched controls. FST, forced swim test; Cort, glucocorticoid injection; CRS, chronic restraint stress; Rec, recovery from CRS. (**b**) Proportional Venn diagram illustrating the genes with expression significantly altered by the acute stress, chronic stress and glucocorticoid (Cort) injection conditions and their overlap. The numbers of genes unique to each comparison whose expression was increased or decreased are listed next to arrows indicating the direction of change. (**c**) Venn diagram of genes whose expression was altered by each FST condition reveals a core of 95 genes that were always changed by this stressor. The large number of unique gene expression changes in each condition shows that the response to FST is altered by the stress history of the group, with the vast majority of changes occurring when an animal is exposed to a novel stressor immediately after a chronic stress exposure, as also shown in **a**. (**d**) Scatter plot of normalized expression values for each microarray probe comparing CRS (*x* axis) with recovery from CRS (*y* axis). For the majority of genes, expression is increased by CRS, but decreased after recovery; however, some probes show expression that is increased by CRS and remains elevated after recovery or that is suppressed by CRS and remains low in recovery. Highlighted probes are those that reached significance when compared with age-matched controls (blue, CRS; red, recovery from CRS; gray, not significant). Several examples of the highlighted genes are listed below the scatter plot by color designation and quadrant. For example, blue points in the lower left quadrant represent genes, such as *Nrg3* and *Scn1b*, whose expression is significantly changed by CRS as compared with that in unstressed controls and is also decreased after recovery from CRS. By contrast, red points in the upper right quadrant represent genes, such as *Cdk2* and *Gria2*, whose expression remains significantly different from that of controls after recovery from CRS and is also increased immediately following CRS. (**e**) Venn diagram illustrating that the genes whose expression was significantly different from that of controls after recovery from CRS are mostly distinct from those whose expression was significantly altered by CRS. Reprinted from ref. 18 with permission from Macmillan Publishers Ltd. (**f**) Pie charts of Gene Ontology (GO) terms that are over-represented among the 576 genes that were differentially expressed upon GC challenge in naive as compared to chronically stressed rats. The differentially expressed genes were divided into groups that responded to GCs in both controls and CRS animals (center) or only in controls (left) or in CRS animals (right). The pie charts represent the GO terms that were overrepresented in the three groups of GC-responsive genes and show that after CRS, GC challenge gives rise to a different gene signature than is seen in control animals. Reprinted from ref. 20 with permission.

Retrotransposons constitute a tenfold larger fraction of mammalian genomes than protein coding genes and appear to be unusually active in brain and steroidogenic tissues[15]. Consequently, they have recently attracted increasing attention from neuroscientists, who have shown that they contribute to neural diversity, cell fate and development as well as brain disease[15,34–36]. In addition to transposons' mobility, they also seem to contribute the largest fraction of functional elements to what might be referred to as 'the RNA genome'[37–39]. This other genome is composed of genes for noncoding RNAs that are being found to govern a growing number of cellular processes, including development, cell differentiation, chromosome imprinting and the regulation of the epigenetic machinery[40,41]; thus RTE-derived RNAs represent a substantial store of both genetic and epigenetic information.

Barbara McClintock, who discovered transposons over 60 years ago, noted that they were important contributors to an organism's ability to deal with environmental stress[42,43], and this insight appears to hold true with regard to the neurobiology of stress, though as with many other aspects of stress, transposons are likely to have both adaptive[34] and possibly deleterious effects given that their dysregulation has been observed in both humans with post-traumatic stress disorder (PTSD) and animal models of stress disorders[44–46]. Brain transposons therefore appear to represent a significant new frontier for stress research.

Role of excitatory amino acids. Excitatory amino acids, particularly glutamate, play key roles in structural as well as functional changes in the brain (**Fig. 4**). Initial studies of restraint stress, in which chronic stress causes shrinkage of apical dendrites of hippocampal CA3 neurons, showed that acute restraint stress elevates extracellular glutamate levels through a process that is absent in adrenalectomized animals, suggesting a role for the adrenal cortex[47]. Indeed, corticosterone acts directly via membrane-associated mineralocorticoid receptors (MRs) and GRs to cause glutamate release[29,48,49]. Importantly, blocking NMDA receptors and interfering with excitatory stimulation of ion channels blocks stress-induced dendritic remodeling within the hippocampus, an effect similar to that of blockade of adrenal steroid synthesis[50,51]. Similarly, stress-induced NMDA-dependent dendritic remodeling has been reported in medial PFC neurons[52]. Excess glutamatergic activity, without adequate reuptake in the aftermath of trauma from seizures, ischemia and head trauma, leads to permanent neuronal loss by a process that is exacerbated by glucocorticoids[53]. These relationships can be summarized in an inverted-U-shaped dose- and time-response curve (**Fig. 1**).

In that connection, the shrinkage of apical dendrites as a result of stress in CA3 pyramidal neurons can be thought of as a protective mechanism against permanent damage and neuron loss that is caused by the

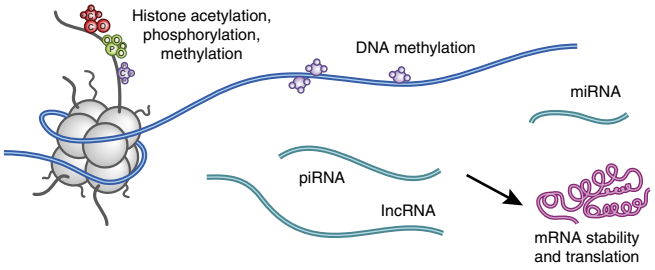


Figure 3 Molecular epigenetic modifications. Among the molecular mechanisms that fall under the epigenetic rubric are covalent modifications of the histone proteins that package and control access to the DNA, which include acetylation, methylation and phosphorylation as well as a growing number of more exotic modifications. The DNA itself may be methylated or hydroxymethylated at cytosine residues. A suite of noncoding RNA species such as microRNA (miRNA), piwi-interacting RNA (piRNA) and long noncoding RNA (lncRNA) also act to convey epigenetic information and to coordinate interactions between DNA and the transcriptional and chromatin modification machinery. Many of these mechanisms seem to have evolved in part from, or as a consequence of the presence of, transposable elements in eukaryotic genomes. Adapted from ref. 24 with permission of Elsevier.

metastable dentate gyrus–CA3 feedforward and feedback circuitry that is the basis of its function[54] and yet makes it vulnerable to seizure-induced damage[55]. This is well illustrated in hibernation, a state of low energy supply to the brain that is accompanied by rapidly reversible (within hours) shrinkage of CA3 apical dendrites in the hippocampus[56,57]. This hypothesis is further substantiated by studies in which removal of polysialic acid residues from neural cell adhesion molecule (NCAM) leads to marked increases in dendritic length of CA3 neurons and increased vulnerability to excitotoxic damage, supporting the notion that shorter dendrites reduce the vulnerability of CA3 neurons to overstimulation[58].

Role of glucocorticoids via multiple intracellular sites and mechanisms. Glucocorticoids produce both genomic and nongenomic effects in the brain through multiple sites and pathways. In addition, glucocorticoids have biphasic effects in which the timing and the level of GR expression are critical[29,59]. Glucocorticoid actions via genomic mechanisms involve both direct interactions with glucocorticoid response elements (GRE) and indirect actions via tethering to other transcription factors[60]. Glucocorticoids can directly stimulate release of excitatory amino acids via membrane-associated receptors, and they can indirectly regulate both glutamate and GABA release through induction of local synthesis of endocannabinoids[61] (see below).

In addition, glucocorticoids can also translocate GRs, along with the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), to mitochondria where they together promote Ca^{2+} sequestration and regulate mitochondrial oxidation, free radical formation and membrane potential, three independent measures of mitochondrial function. Bcl-2 is able to inhibit the formation of Bax-containing pores on the mitochondrial outer membrane and reduce the release of calcium and cytochrome *c* from the mitochondria. Importantly, these three glucocorticoid effects show an inverted U–shaped dose-response curve and are biphasic, and high glucocorticoid levels cause a failure of this mechanism over 72 h, leading to increased free-radical formation[62] (**Fig. 5**).

Just as location of GR action is an essential consideration, the level of expression of GRs is also very important. Genetically induced overexpression of GR in forebrain leads to increased lability of mood-related behaviors, yet also confers greater responsiveness to antidepressant drugs[63], whereas genetic knockdown of GR has the opposite effect[64]. Epigenetic regulation of GR activity also has significant functional implications, as increased CpG methylation within the GR promoter is associated with a suboptimal HPA stress response and with poor maternal care in rodents and early life abuse in human suicide victims[65,66].

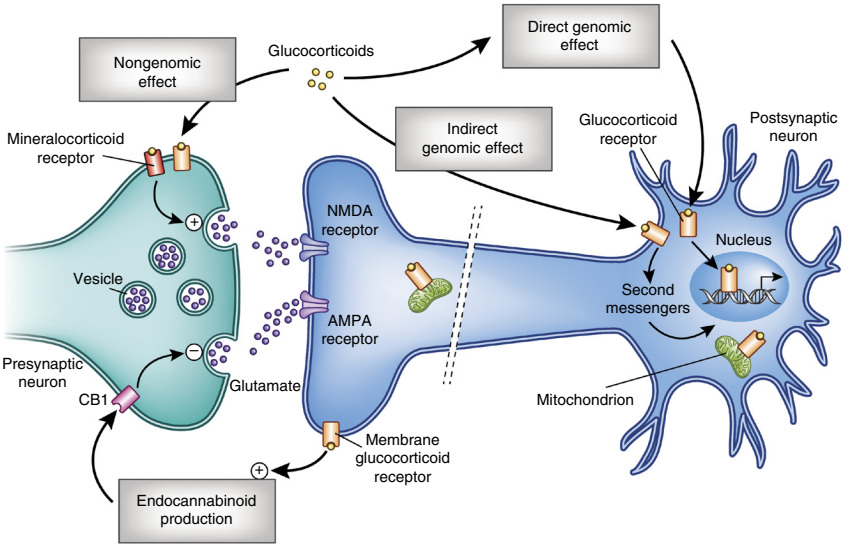


Figure 4 Glucocorticoids are released from the adrenal glands. Basal release varies in a diurnal pattern, and release increases severalfold after exposure to a stressor. Glucocorticoids can bind, with different affinities, to glucocorticoid and mineralocorticoid receptors, which are expressed throughout 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 nongenomic mechanisms (mediated by membrane receptors, see the figure), indirect genomic mechanisms (mediated by membrane receptors and second messengers, see the figure) and genomic mechanisms (mediated by cytoplasmic receptors that move to the nucleus and act as transcription factors; see figure), as seems now to be the case for all steroid hormones. Although 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. In addition, activated glucocorticoid receptors can translocate to mitochondria and enhance their calcium buffering capacity. 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. An indirect way by which glucocorticoids can influence neurotransmission (glutamatergic, as well as GABAergic, cholinergic, noradrenergic and serotonergic) is through cross-talk with the endocannabinoid system. 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 (see the figure). Although a G protein–coupled receptor is implicated in endocannabinoid production, 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. Modified from ref. 32 with permission from Macmillan Publishers Ltd.

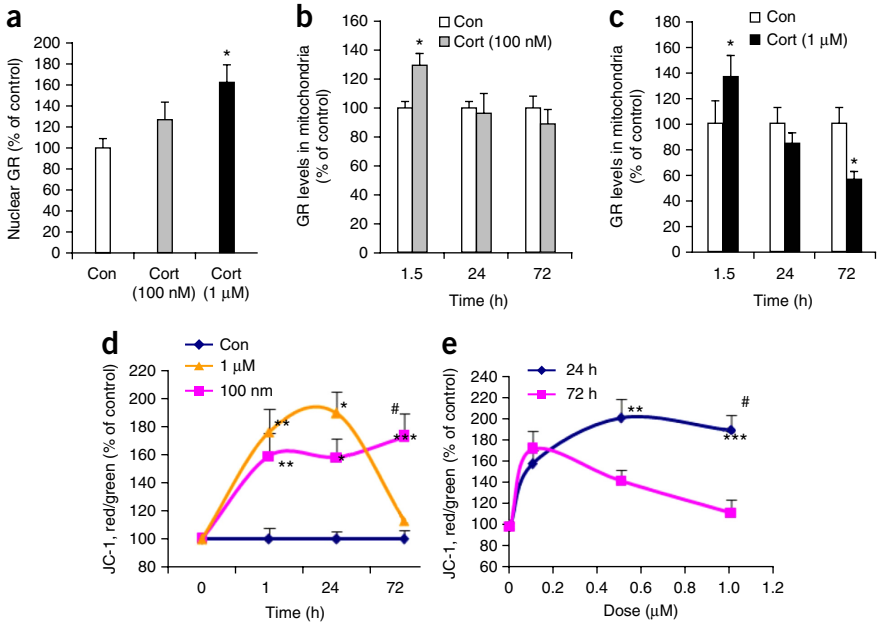


Figure 5 Biphasic effect of glucocorticoids (Cort) in regulating mitochondrial function. (**a**) Cort readily penetrate the cell membrane and interact with cytoplasmic glucocorticoid receptors (GRs), causing a dose-dependent increase in GR translocation into cell nuclei. Con, vehicle control. (**b**) Translocation of GRs into mitochondria as a complex with the anti-apoptotic protein Bcl-2, where they upregulate mitochondrial calcium levels, membrane potential and oxidation; this is stabilized at a 100 nM dose of Cort (**b**) and decreases with time at the high, 1 μM Cort dose (**c**), where, after a 3-d treatment, high Cort leads to decreased abundance of GR and Bcl-2 in mitochondria. (**d**,**e**) Cort modulates membrane potential, measured by Janus-1 (JC-1) staining, in a dose- and time-dependent manner. Time course of JC-1 staining after Cort treatment shows sustained potential at 100 nM dose and loss of potential at 1 μM dose (**d**); dose-dependent curve for JC-1 staining after Cort treatment shows that both low and high Cort maintain potential at 24 h, but high Cort causes failure of membrane potential at 72 h (**e**). This regulation of mitochondrial function by Cort parallels neuroprotection: that is, treatment with low doses of Cort has a neuroprotective effect, whereas high Cort enhances kainic acid–induced toxicity of cortical neurons[62,133], consistent with the "glucocorticoid endangerment" hypothesis[134]. Error bars, s.e.m.; $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. Modified from ref. 62 with permission from Macmillan Publishers Ltd.

The actions of glucocorticoids are biphasic, as illustrated above for mitochondria[62], and their timing is important. For example, in several animal models of traumatic stress–induced PTSD-like delayed anxiety and traumatic stress–induced spine synapse formation in basolateral amygdala (BLA), a timed elevation of glucocorticoids, before the induction of stress, prevents development of the anxiety and synapse formation[67]. Data on human PTSD supports a protective role for adequate glucocorticoid levels at time of traumatic stress[68,69]. Yet repeated high-dose glucocorticoid treatment mimics chronic stress and induces dendritic lengthening in BLA[70], a result that emphasizes the differences between acute and chronic elevations of glucocorticoids.

Regarding MRs, which exert both genomic actions and nongenomic actions to stimulate glutamate release[48], mice that spontaneously show increased anxiety have elevated expression of hippocampal MR, which mediate a stress-induced suppression of mGlu2 expression and increased levels of anxiety- and depression-like behavior[71]. Importantly, blocking MR receptors and interfering with glucocorticoid stimulation of glutamate activity blocks stress-induced mood abnormalities. The nature of the experiences of the animals that develop higher MR is not known, but it may involve epigenetic influences early in life, such as maternal care and stressors in the neonatal nesting environment[72]. The epigenetic allostasis model points to developmental origins 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 (**Fig. 6**).

Involvement of secreted signaling molecules. In addition to glucocorticoids, secreted signaling molecules have important roles in the remodeling of neural tissue during stress (**Box 1**). Corticotropin-releasing factor (CRF), which is better known for its role in governing the secretion of adrenocorticotropic hormone (ACTH) and glucocorticoids, plays a key part in stress-induced dendritic remodeling in the CA1 region of the hippocampus[73,74]. Findings over the past decade have also implicated new players in the regulation of dendritic remodeling. For instance, tissue plasminogen activator (tPA), a secreted signaling molecule as well as protease, is implicated in stress-induced dendritic remodeling and spine loss in medial amygdala as well as in the CA1 hippocampus. Specifically, tPA-knockout mice fail to show chronic stress impairment of memory and spine reduction in CA1 (refs. 73,75). Linking these two factors together, there is evidence that in the amygdala tPA release is stimulated by CRF[76]. Similarly, lipocalin-2 is a novel modulator of spine plasticity with different effects in amygdala and hippocampus[77,78]. Acute stress increases lipocalin-2 levels, and lipocalin-2 downregulates mushroom spines and generally inhibits actin motility in hippocampus. Remarkably, deletion of lipocalin-2 increases neuronal excitability and anxiety, and, in amygdala, the absence of lipocalin-2 increases the basal number of spines and prevents a stress-induced increase in spine density.

Endocannabinoids are another class of signaling molecules that importantly regulate multiple aspects of the stress response. In addition to contributing to the termination[79] of the acute response to stress, as well as habituation to repeated stress[80], endocannabinoids also seem to be important for the regulation of structural plasticity under conditions of repeated stress (**Box 1**). For example, cannabinoid 1 (CB_1) receptor–deficient mice exhibit reductions in prefrontal cortical dendritic length and complexity, while having enhanced and more complex dendritic arbors within the BLA, both of which effects parallel the effects of chronic stress[81,82]. More importantly, chronic stress and corticosterone treatment are both known to impair endocannabinoid signaling at multiple levels, through both a down-regulation of the CB_1 receptor[83] and a reduction in the levels of the endocannabinoid anandamide that is mediated by an increase in its hydrolysis by the enzyme fatty acid amide hydrolase (FAAH)[84,85].

Given the parallels between genetic deletion of the CB_1 receptor and the ability of chronic stress to impair endocannabinoid signaling, it is interesting to note that elevation of anandamide via CB_1 receptor signaling, through genetic or pharmacological impairment of FAAH, retards the ability of chronic stress to produce dendritic hypertrophy in the BLA as well as concomitant changes in emotional behavior[85–88,89]. Collectively, these data indicate that endocannabinoid signaling buffers against many of the effects of stress and seems to be important for

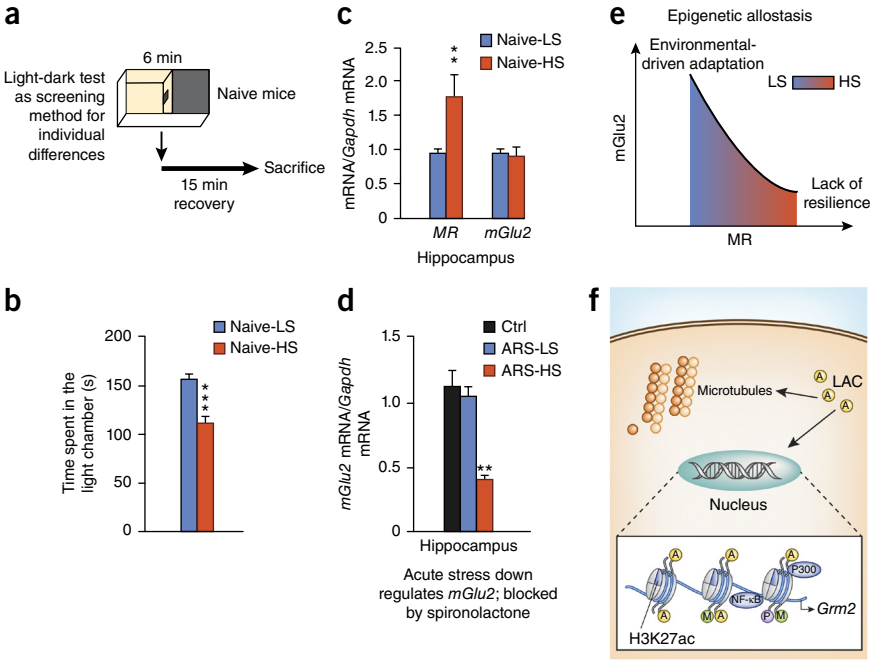


Figure 6 Individual differences in naive C57Bl6 mice in anxiety-related behavior reveal animals more sensitive to stress-induced downregulation of hippocampal mGlu2 expression, a biomarker of depressive-like behavior and antidepressant response. (**a**–**c**) The use of the light-dark test as a screening method (**a**) allows identification of clusters of animals with a different baseline anxiety profile (**b**) along with differences in mineralocorticoid receptor gene (*MR; Nr3c2*) transcript levels in hippocampus (**c**). Error bars, s.e.m.; $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. (**d**) The susceptible (HS) mice, which are characterized by higher baseline *MR* transcript levels, show reduced hippocampal *mGlu2* (*Grm2*) transcript expression associated with exacerbation of anxious and of depressive-like behaviors after acute and chronic stress, respectively. Conversely, individuals with lower baseline *MR* transcript levels (low susceptible, LS) cope better with stress and show adaptation in mGlu2 receptor expression in hippocampus. Ctrl, unstressed sex and age-matched control animals; ARS, acute restraint stress. (**e**) The epigenetic allostasis model points to the developmental origins of these individual differences, suggesting that as-yet-unknown epigenetic influences early in life may lead to alterations in hippocampal *MR* transcript levels[71]. (**f**) Representative mechanisms of action of acetyl-L-carnitine (LAC): work in our and other laboratories has shown that a decrease in mGlu2 receptors either following stress exposure or occurring in a genetic animal model of depression is rapidly corrected by 3 d of intraperitoneal administration of the novel antidepressant candidate LAC via acetylation of either the H3K27 bound to the *Grm2* promoter, which encodes mGlu2 receptors, or the NF-κB p65 subunit[30]. A, acetyl; M, methyl; P, phosphate; MR (Nr3c2), MGI 99459; mGlu2 (Grm2), MGI 1351339.

limiting the effects of chronic stress on structural plasticity within these identified limbic circuits. At a mechanistic level, this is likely to be due to the ability of CB_1 receptor signaling to gate glutamatergic release, as it has been demonstrated that CB_1 receptor–deficient mice show greater changes in glutamatergic signaling and excitotoxicity within the PFC following chronic stress[90]. Moreover, in a pattern similar to the protective effects of CB_1 receptor activation identified within the amygdala, administration of a CB_1 receptor agonist during repeated stress can reduce the increase in glutamatergic signaling, the induction of pro-inflammatory cytokines and lipid peroxidation within the PFC[90]. Thus, the release of endocannabinoids during stress may temper changes in structural plasticity by limiting the magnitude of glutamate release in response to stress; and under conditions of chronic stress, when this system becomes compromised, the loss of this endogenous buffer facilitates excess glutamate release and the ensuing changes in dendritic morphology. Linking this model with the factors previously described, it is interesting to note that in addition to promoting tPA release, CRF has also been found to induce anandamide hydrolysis by FAAH[91], suggesting that CRF could act as an orchestrator of multiple signaling molecules, all of which converge in structural changes within the brain following chronic stress.

Recent studies have suggested that blood-based biomarkers may be able to predict aspects of brain signaling associated with trauma-related effects in both males and females, specifically with respect to convergence onto GR signaling pathways. After a predator-scent-stress (PSS) exposure, male and female rats were classified into vulnerable ("PTSD-like") and resilient (minimally affected) phenotypes on the basis of their performance on a variety of behavioral measures[92]. Genome-wide expression profiling in blood, amygdala and hippocampus indicated that glucocorticoid signaling was the only convergent pathway associated with individual differences in susceptibility. Moreover, corticosterone treatment 1 h after PSS exposure prevented anxiety and hyperarousal 7 d later in both sexes, consistent with prior findings in the same as well as in another PTSD animal model[67,68], confirming the involvement of the GR in sequelae of traumatic stress.

Roles of brain-derived neurotrophic factor (BDNF). BDNF plays an important role in dendritic remodeling in both hippocampus and BLA (**Box 1**). BDNF-overexpressing mice show increases dendritic length in both CA3 and BLA, which occludes the effects of chronic stress in decreasing dendritic branching in CA3 and increasing it in BLA[93]. On the other hand, in WT animals, chronic stress causes a downregulation of BDNF in CA3 hippocampus and an upregulation of BDNF in the BLA. Although the increase in BLA persists beyond 21 d after the stress, the effect in CA3 normalizes[94]. These intriguing timing issues become even more interesting when one considers that after a single acute stress, BDNF expression in the BLA rises and stays elevated for 10 d, while that in CA3 shows only a transient increase[94]. This increase in the BLA is associated with both increased anxiety and increased density of spines in BLA neurons[85]. The mechanism of these effects on BDNF remain enigmatic, but they are not entirely mediated by glucocorticoid actions as corticosterone levels increase after both acute and chronic stress and remained elevated after chronic, but not acute, stress. Thus, it is clear that BDNF-mediated signaling is involved in the structural effects of stress, but that the direction and nature of signaling is region specific and stress specific and is influenced by epigenetic modifications[89] along with post-translational modifications[94,95]. The epigenetic mechanisms controlling BDNF expression are influenced by maternal separation early in life, which, in turn, leads to changes in BDNF expression and epigenetic regulation via histone acetylation and methylation over the life course, with consequences for anxiety-like behaviors[96].

Cellular processes in remodeling of neural architecture. The neuronal surface, cytoskeleton and nuclear envelope are each implicated

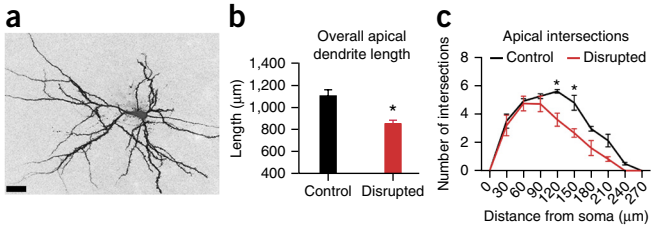


Figure 7 Mice cannot adjust to a 10 h light/10 h dark cycle, as indicated by body temperature and locomotor activity rhythms. This circadian disruption, as in humans performing shift work, leads to increase body fat and leptin and insulin resistance, along with remodeling of apical dendrites of prefrontal cortical neurons and indications of cognitive rigidity. (**a**–**c**) Shown are a representative neuron (**a**), overall dendritic length (**b**) and a Sholl analysis (**c**). Error bars, s.e.m. Data from ref. 123.

in the mechanisms of stress-induced retraction and expansion of dendrites and synapse turnover. The polysialylated form of neural cell adhesion molecule (PSA-NCAM) is expressed in the CA3 and DG regions of the hippocampus and is believed to denote the capacity for adaptive structural plasticity in many parts of the CNS[97–99]. Repeated stress causes retraction of CA3 hippocampal dendrites accompanied by a modest increase in PSA-NCAM expression, possibly as the result of glucocorticoid mediation[100]. Using endoneuraminidase N (EndoN) to remove PSA from NCAM, Sandi reported impairment of consolidation of contextual fear conditioning[101]. Using the same treatment, we observed considerable expansion of the dendritic tree in both CA3 and CA1 and a marked increase in excitotoxicity and damage to CA3 neurons; repeated stress still caused some dendrite retraction after PSA removal[58]. Thus, although PSA-NCAM is a facilitator of plasticity, the PSA moiety appears to also limit the extent of dendritic growth and yet is not necessary for dendritic retraction under stress.

Two other classes of cell adhesion molecules are reported to change with chronic stress, with behavioral consequences. Neuroligins (NLGNs) are important for proper synaptic formation and functioning and are critical regulators of the balance between neural excitation and inhibition (E/I), and chronic restraint stress reduces hippocampal NLGN-2 levels, in association with reduced sociability and increased aggression[102,103]. This occurred along with a reduction of NLGN-2 expression throughout the hippocampus, detectable in different layers of the CA1, CA3 and DG subfields. Intrahippocampal administration of neurolide-2, which interferes with the interaction between NLGN-2 and neurexin, led to reduced sociability and increased aggression, thus mimicking effects of chronic stress[102].

Chronic restraint stress also increases activity of matrix metalloproteinase-9 (MMP-9) in the CA1. MMP-9 carries out proteolytic processing of another cell adhesion molecule, nectin-3. Chronic stress reduced nectin-3 in the perisynaptic CA1, but not in the CA3, with consequences for social exploration and social recognition and for a CA1-dependent cognitive task. Implicated in this is a stress-related increase in extracellular glutamate and NMDA receptor mediation of MMP-9 (ref. 104). These findings are reminiscent of the CA1-specific effects of tPA in mediating stress effects on spine density in CA1 (ref. 73).

Actin polymerization plays a key role in filopodial extension and spine synapse formation as well as in plasticity within the synapse itself[105], and cytoskeletal remodeling is an important factor in the effects of stress and other environmental manipulations. Hibernation in European hamsters and ground squirrels results in rapid retraction of dendrites of CA3 pyramidal neurons, and an equally rapid expansion occurs when hibernation torpor is reversed[56,57]. The retraction of dendrites is accompanied by increases in a soluble phosphorylated form of tau that may indicate disruption of the cytoskeleton, which permits the dendrite shortening and possible protection from excitotoxicity; at the same time, PSA-NCAM expression is lost during hibernation torpor, reducing the capacity for plasticity[106]. This model highlights the important role that tau plays in normal cytoskeletal function, a fact that should be emphasized when attempting to understand its role in pathology[107].

Even though dendrite retraction and regrowth would appear to involve a reversible depolymerization and repolymerization of the cytoskeleton, there are other processes that point to the importance of nuclear factors. A recent example is the unexpected role of a cell nuclear pore complex protein, NUP-62, in stress-induced dendritic remodeling in the CA3 region of the hippocampus[108]. First identified as the product of a gene whose expression was downregulated in the prefrontal cortex of depressed patients[109], NUP-62 was also found to be reduced in response to chronic stress in CA3 neurons of rodents[108]. 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 that this effect is regulated at the molecular level by NUP-62 phosphorylation at a PYK2 site which results in its retention in the cytoplasm[108]. A role of NUP-62 in maintaining chromatin structure for transcription is suggested as well as in nucleocytoplasmic transport[108].

Stress: not always what one thinks it is

Just as stress is not a unitary phenomenon at the cell and circuit level, neither is it one at the level of the whole organism. As noted in the introduction, a key aspect of stress effects on the brain and body is the nonlinear interaction of multiple mediators of stress and adaptation that is part of the concept of allostasis[1], which refers to the active process of maintaining homeostasis through the output of hormones and ANS activity along with immune and metabolic system mediators and the mediators in the brain that are the main focus of this review. When one mediator system changes, the others adjust, and the resulting output can be distorted, as in chronic inflammation or a flat cortisol diurnal rhythm caused by sleep deprivation or depression[2]. Moreover, the actions of any one mediator may depend on the actions of other mediators. For example, glucocorticoids and excitatory amino acids are both involved in stress-induced suppression of neurogenesis, which has been found not only in rodents but also in tree shrews and rhesus monkeys[110–112]. Yet, glucocorticoid levels alone do not predict the direction of neurogenesis, as shown by studies of male sexual behavior, which results in increased neurogenesis but also high glucocorticoid levels; in this scenario oxytocin appears to play an important role, emphasizing the importance of understanding the interaction of these distinct signaling molecules[113,114].

In seeking to understand where and how stress affects neural circuits, it has become evident that when these mediators act is also an important consideration. In most vertebrate species, plasma glucocorticoids rise just before the active phase. This rhythm is largely driven by changes in the amplitude and frequency of the ultradian secretion of glucocorticoids[115]. Indeed, the natural ultradian fluctuations of glucocorticoids mediate turnover of a subset of synapses in cerebral cortex; and inhibiting the fluctuations with a minimal dose of dexamethasone impairs spine turnover[116]. Moreover, these diurnal changes in spine formation and removal are important for motor learning[117].

In addition to ultradian pulses, circadian (or diurnal) rhythms are a crucial factor that impact the stress response. Rhythmic HPA function seems to be necessary for the normal initiation and

termination of the stress response of ACTH, cortisol and other mediators[118]. Epidemiologically, disrupted sleep and circadian rhythms lead to increased risk for development of psychiatric, cardiovascular or other physiological syndromes in shift workers or populations undergoing chronic circadian disruption[119]. Housing mice in a light-dark cycle of 20 h (10 h light/10 h dark), rather than standard 24-h cycles, to drive circadian disruption results in metabolic signs of allostatic load[120], with increased weight, adiposity and leptin levels, as well as an imbalance between insulin and plasma glucose suggesting a pre-diabetic state[121]. The metabolic changes are accompanied by changes in PFC cellular morphology, mirroring those observed in chronic stress[122], with circadian-disrupted animals having shrunken and less complex apical dendritic trees of cells in layer II/III of the medial PFC[123] (**Fig. 7**). In addition, circadian-disrupted mice show altered responses to endotoxin challenge with lipopolysaccharide[124], highlighting the similarities between chronic circadian disruption and chronic stress. The mechanisms by which these systems interact is not yet fully understood, but they do not appear to be driven simply by elevation of glucocorticoids.

Intriguingly, glucocorticoids are able to regulate the expression of circadian clock genes in several brain regions[125] as well as in liver[126]. As such, disruption of normal oscillatory profiles of glucocorticoids could lead to desynchronized activity between different brain regions as well as peripheral organ systems. This dissonance is thought to contribute to several pathologies that are similar to the effects of chronic stress, including obesity and metabolic syndrome[119]. Thus circadian disruption is both a 'stressor', in that it increases allostatic load or overload, and a risk factor for other stressful experiences, emphasizing the importance of timing in glucocorticoid actions through the brain and body.

Conclusions and future directions

The response of the brain to stressors is a complex process involving multiple interacting mediators that utilizes both genomic and nongenomic mechanisms, from the cell surface to the cytoskeleton to epigenetic regulation via the cell nucleus. Resilience in the face of stress is a key aspect of a healthy brain, even though gene expression shows a brain that continually changes with experience[127]. Therefore, recovery from stress-induced changes in neural architecture after stress is not a 'reversal' but a form of neuroplastic adaptation that also may be impaired in mood disorders and reduced with aging (**Fig. 1**). Resilience may be thought of as an active process that implies ongoing adaptive plasticity without external intervention[128].

On the other hand, resilience is decreased and vulnerability is increased by adverse childhood experiences that lead to 'biological embedding' of trajectories of response to stressful life events[4] throughout the life course[6], which contribute disproportionately to allostatic overload in the form of physical and mental health disorders over the life course[7]. Evidence from CpG methylation of DNA indicates the embedded influence of early adversity[66]. From the original definition of epigenetics[129] as the emergence of characteristics of each individual of each species, not evident from prior stages of development, interventions to counteract adverse childhood experiences cannot 'roll back the clock' but rather may be able change the trajectory of brain and body development in a more positive direction.

Can the effects of stress on the brain be treated even though there are no 'magic bullets' like penicillin for stress-related disorders[6]? For psychiatric illnesses such as depression and anxiety disorders, including PTSD, it is necessary to complement and even replace existing drugs and adopt strategies that center around the use of targeted behavioral therapies along with treatments, including pharmaceutical agents, that open up 'windows of plasticity' in the brain and facilitate the efficacy of the behavioral interventions[5,130,131]. To that extent, meeting the demands imposed by stressful experiences through various coping resources can lead to growth, adaptation and learning to promote resilience and improved mental health[128,132].

ACKNOWLEDGMENTS

The McEwen laboratory acknowledges research support from US National Institutes of Health (R01 MH41256), and the Hope for Depression Research Network and the American Foundation for Suicide Prevention to C.N. and National Research Service Award F32 MH102065 to J.D.G.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. McEwen, B.S. Protective and damaging effects of stress mediators. *N. Engl. J. Med.* **338**, 171–179 (1998).
2. McEwen, B.S. Protective and damaging effects of stress mediators: central role of the brain. *Dialogues Clin. Neurosci.* **8**, 367–381 (2006).
3. McEwen, B.S. & Wingfield, J.C. The concept of allostasis in biology and biomedicine. *Horm. Behav.* **43**, 2–15 (2003).
4. Shonkoff, J.P., Boyce, W.T. & McEwen, B.S. Neuroscience, molecular biology, and the childhood roots of health disparities. *J. Am. Med. Assoc.* **301**, 2252–2259 (2009).
5. McEwen, B.S. Brain on stress: how the social environment gets under the skin. *Proc. Natl. Acad. Sci. USA* **109** (suppl. 2): 17180–17185 (2012).
6. Halfon, N., Larson, K., Lu, M., Tullis, E. & Russ, S. Lifecourse health development: past, present and future. *Matern. Child Health J.* **18**, 344–365 (2014).
7. Felitti, V.J. *et al.* Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am. J. Prev. Med.* **14**, 245–258 (1998).
8. Tost, H., Champagne, F.A & Meyer-Lindenberg, A. Environmental influence in the brain, human welfare and mental health. *Nat. Neurosci.* **18**, 1421–1431 (2015).
9. McEwen, B.S. Stress and hippocampal plasticity. *Annu. Rev. Neurosci.* **22**, 105–122 (1999).
10. McEwen, B.S. & Chattarji, S. in *Handbook of Neurochemistry and Molecular Neurobiology* (A. Lajtha & G. Baker, eds.) 572–593 (Springer-Verlag, 2007).
11. Cameron, H.A. & Gould, E. in *Receptor Dynamics in Neural Development Pharmacology and Toxicology: Basic and Clinical Aspects* (ed. C.A. Shaw) 141–157 (CRC Press, 1996).
12. McEwen, B.S. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* **87**, 873–904 (2007).
13. McEwen, B.S. Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. *Ann. NY Acad. Sci.* **1204** (suppl.): E38–E59 (2010).
14. Meaney, M.J. & Szyf, M. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin. Neurosci.* **7**, 103–123 (2005).
15. Hunter, R.G., Gagnidze, K., McEwen, B.S. & Pfaff, D.W. Stress and the dynamic genome: steroids, epigenetics, and the transposome. *Proc. Natl. Acad. Sci. USA* **112**, 6828–6833 (2015).
16. McEwen, B.S., Weiss, J. & Schwartz, L. Selective retention of corticosterone by limbic structures in rat brain. *Nature* **220**, 911–912 (1968).
17. Rubin, T.G., Gray, J.D. & McEwen, B.S. Experience and the ever-changing brain: what the transcriptome can reveal. *BioEssays* **36**, 1072–1081 (2014).
18. Gray, J.D., Rubin, T.G., Hunter, R.G. & McEwen, B.S. Hippocampal gene expression changes underlying stress sensitization and recovery. *Mol. Psychiatry* **19**, 1171–1178 (2014).
19. Wang, K. *et al.* Transcriptome profiling analysis reveals region-distinctive changes of gene expression in the CNS in response to different moderate restraint stress. *J. Neurochem.* **113**, 1436–1446 (2010).
20. Datson, N.A. *et al.* Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus. *Endocrinology* **154**, 3261–3272 (2013).
21. de Kloet, E.R., Fitzsimons, C.P., Datson, N.A., Meijer, O.C. & Vreugdenhil, E. Glucocorticoid signaling and stress-related limbic susceptibility pathway: about receptors, transcription machinery and microRNA. *Brain Res.* **1293**, 129–141 (2009).
22. Goldwater, D.S. *et al.* Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience* **164**, 798–808 (2009).
23. Hunter, R.G. & McEwen, B.S. Stress and anxiety across the lifespan: structural plasticity and epigenetic regulation. *Epigenomics* **5**, 177–194 (2013).
24. Griffiths, B.B. & Hunter, R.G. Neuroepigenetics of stress. *Neuroscience* **275**, 420–435 (2014).
25. Reul, J.M. Making memories of stressful events: a journey along epigenetic, gene transcription, and signaling pathways. *Frontiers Psychiatry* **5**, 5 (2014).

26. Tsankova, N.M. *et al.* Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat. Neurosci.* **9**, 519–525 (2006).
27. Reul, J.M.H.M. & Chandramohan, Y. Epigenetic mechanisms in stress-related memory formation. *Psychoneuroendocrinology* **32**, S21–S25 (2007).
28. Heller, E.A. *et al.* Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. *Nat. Neurosci.* **17**, 1720–1727 (2014).
29. Nasca, C. *et al.* L-Acetylcarnitine causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors. *Proc. Natl. Acad. Sci. USA* **110**, 4804–4809 (2013).
30. Dyrvig, M., Gotzsche, C.R., Woldbye, D.P. & Lichota, J. Epigenetic regulation of Dnmt3a and Arc gene expression after electroconvulsive stimulation in the rat. *Mol. Cell Neurosci.* **67**, 137–143 (2015).
31. Hunter, R.G., McCarthy, K.J., Milne, T.A., Pfaff, D.W. & McEwen, B.S. Regulation of hippocampal H3 histone methylation by acute and chronic stress. *Proc. Natl. Acad. Sci. USA* **106**, 20912–20917 (2009).
32. Popoli, M., Yan, Z., McEwen, B.S. & Sanacora, G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat. Rev. Neurosci.* **13**, 22–37 (2012).
33. Monteggia, L.M. & Zarate, C. Jr. Antidepressant actions of ketamine: from molecular mechanisms to clinical practice. *Curr. Opin. Neurobiol.* **30**, 139–143 (2015).
34. Erwin, J.A., Marchetto, M.C. & Gage, F.H. Mobile DNA elements in the generation of diversity and complexity in the brain. *Nat. Rev. Neurosci.* **15**, 497–506 (2014).
35. Reilly, M.T., Faulkner, G.J., Dubnau, J., Ponomarev, I. & Gage, F.H. The role of transposable elements in health and diseases of the central nervous system. *J. Neurosci.* **33**, 17577–17586 (2013).
36. Hunter, R.G., McEwen, B.S. & Pfaff, D.W. Environmental stress and transposon transcription in the mammalian brain. *Mob. Genet. Elements* **3**, e24555 (2013).
37. Khalil, A.M. *et al.* Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc. Natl. Acad. Sci. USA* **106**, 11667–11672 (2009).
38. Johnson, R. & Guigo, R. The RIDL hypothesis: transposable elements as functional domains of long noncoding RNAs. *RNA* **20**, 959–976 (2014).
39. Kelley, D. & Rinn, J. Transposable elements reveal a stem cell-specific class of long noncoding RNAs. *Genome Biol.* **13**, R107 (2012).
40. Khalil, A.M. & Rinn, J.L. RNA-protein interactions in human health and disease. *Semin. Cell Dev. Biol.* **22**, 359–365 (2011).
41. Pauli, A., Rinn, J.L. & Schier, A.F. Non-coding RNAs as regulators of embryogenesis. *Nat. Rev. Genet.* **12**, 136–149 (2011).
42. McClintock, B. Chromosome organization and genic expression. *Cold Spring Harb. Symp. Quant. Biol.* **16**, 13–47 (1951).
43. McClintock, B. The significance of responses of the genome to challenge. *Science* **226**, 792–801 (1984).
44. Rusiecki, J.A. *et al.* DNA methylation in repetitive elements and post-traumatic stress disorder: a case-control study of US military service members. *Epigenomics* **4**, 29–40 (2012).
45. Ponomarev, I., Rau, V., Eger, E.I., Harris, R.A. & Fanselow, M.S. Amygdala transcriptome and cellular mechanisms underlying stress-enhanced fear learning in a rat model of posttraumatic stress disorder. *Neuropsychopharmacology* **35**, 1402–1411 (2010).
46. Ponomarev, I., Wang, S., Zhang, L., Harris, R.A. & Mayfield, R.D. Gene coexpression networks in human brain identify epigenetic modifications in alcohol dependence. *J. Neurosci.* **32**, 1884–1897 (2012).
47. Lowy, M.T., Gault, L. & Yamamoto, B.K. Adrenalectomy attenuates stress-induced elevations in extracellular glutamate concentrations in the hippocampus. *J. Neurochem.* **61**, 1957–1960 (1993).
48. Karst, H. *et al.* Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proc. Natl. Acad. Sci. USA* **102**, 19204–19207 (2005).
49. Treccani, G. *et al.* Stress and corticosterone increase the readily releasable pool of glutamate vesicles in synaptic terminals of prefrontal and frontal cortex. *Mol. Psychiatry* **19**, 433–443 (2014).
50. Magariños, A.M. & McEwen, B.S. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience* **69**, 89–98 (1995).
51. Watanabe, Y., Gould, E. & McEwen, B.S. Stress induces atrophy of apical dendrites of hippocampus CA3 pyramidal neurons. *Brain Res.* **588**, 341–345 (1992).
52. Martin, K.P. & Wellman, C.L. NMDA receptor blockade alters stress-induced dendritic remodeling in medial prefrontal cortex. *Cereb. Cortex* **21**, 2366–2373 (2011).
53. Sapolsky, R.M., Krey, L.C. & McEwen, B.S. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr. Rev.* **7**, 284–301 (1986).
54. Lisman, J.E. Relating hippocampal circuitry to function: recall of memory sequences by reciprocal dentate-CA3 interactions. *Neuron* **22**, 233–242 (1999).
55. McEwen, B.S. Stress and hippocampal plasticity. *Annu. Rev. Neurosci.* **22**, 105–122 (1999).
56. Magariños, A.M., McEwen, B.S., Saboureau, M. & Pevet, P. Rapid and reversible changes in intrahippocampal connectivity during the course of hibernation in European hamsters. *Proc. Natl. Acad. Sci. USA* **103**, 18775–18780 (2006).
57. Popov, V.I., Bocharova, L.S. & Bragin, A.G. Repeated changes of dendritic morphology in the hippocampus of ground squirrels in the course of hibernation. *Neuroscience* **48**, 45–51 (1992).
58. McCall, T. *et al.* Depletion of polysialic acid from neural cell adhesion molecule (PSA-NCAM) increases CA3 dendritic arborization and increases vulnerability to excitotoxicity. *Exp. Neurol.* **241**, 5–12 (2013).
59. Joëls, M. Corticosteroid effects in the brain: U-shape it. *Trends Pharmacol. Sci.* **27**, 244–250 (2006).
60. Revollo, J.R. & Cidlowski, J.A. Mechanisms generating diversity in glucocorticoid receptor signaling. *Ann. NY Acad. Sci.* **1179**, 167–178 (2009).
61. Hill, M.N. & McEwen, B.S. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **34**, 791–797 (2010).
62. Du, J. *et al.* Dynamic regulation of mitochondrial function by glucocorticoids. *Proc. Natl. Acad. Sci. USA* **106**, 3543–3548 (2009).
63. Wei, Q. *et al.* Glucocorticoid receptor overexpression in forebrain: a mouse model of increased emotional lability. *Proc. Natl. Acad. Sci. USA* **101**, 11851–11856 (2004).
64. Jacobson, L. Forebrain glucocorticoid receptor gene deletion attenuates behavioral changes and antidepressant responsiveness during chronic stress. *Brain Res.* **1583**, 109–121 (2014).
65. Szyf, M., Weaver, I.C.G., Champagne, F.A., Diorio, J. & Meaney, M.J. Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front. Neuroendocrinol.* **26**, 139–162 (2005).
66. McGowan, P.O. *et al.* Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat. Neurosci.* **12**, 342–348 (2009).
67. Rao, R.P., Anilkumar, S., McEwen, B.S. & Chattarji, S. Glucocorticoids protect against the delayed behavioral and cellular effects of acute stress on the amygdala. *Biol. Psychiatry* **72**, 466–475 (2012).
68. Zohar, J. *et al.* High dose hydrocortisone immediately after trauma may alter the trajectory of PTSD: interplay between clinical and animal studies. *Eur. Neuropsychopharmacol.* **21**, 796–809 (2011).
69. Schelling, G., Roozendaal, B. & De Quervain, D.J.-F. Can posttraumatic stress disorder be prevented with glucocorticoids? *Ann. NY Acad. Sci.* **1032**, 158–166 (2004).
70. Mitra, R. & Sapolsky, R.M. Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. *Proc. Natl. Acad. Sci. USA* **105**, 5573–5578 (2008).
71. Nasca, C., Bigio, B., Zelli, D., Nicoletti, F. & McEwen, B.S. Mind the gap: glucocorticoids modulate hippocampal glutamate tone underlying individual differences in stress susceptibility. *Mol. Psychiatry* **20**, 755–763 (2015).
72. Francis, D., Diorio, J., Liu, D. & Meaney, M.J. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* **286**, 1155–1158 (1999).
73. Pawlak, R. *et al.* Tissue plasminogen activator and plasminogen mediate stress-induced decline of neuronal and cognitive functions in the mouse hippocampus. *Proc. Natl. Acad. Sci. USA* **102**, 18201–18206 (2005).
74. Chen, Y., Fenoglio, K.A., Dube, C.M., Grigoriadis, D.E. & Baram, T.Z. Cellular and molecular mechanisms of hippocampal activation by acute stress are age-dependent. *Mol. Psychiatry* **11**, 992–1002 (2006).
75. Bennur, S. *et al.* Stress-induced spine loss in the medial amygdala is mediated by tissue-plasminogen activator. *Neuroscience* **144**, 8–16 (2007).
76. Matys, T. *et al.* Tissue plasminogen activator promotes the effects of corticotropin releasing factor on the amygdala and anxiety-like behavior. *Proc. Natl. Acad. Sci. USA* **101**, 16345–16350 (2004).
77. Mucha, M. *et al.* Lipocalin-2 controls neuronal excitability and anxiety by regulating dendritic spine formation and maturation. *Proc. Natl. Acad. Sci. USA* **108**, 18436–18441 (2011).
78. Skrzypiec, A.E. *et al.* Stress-induced lipocalin-2 controls dendritic spine formation and neuronal activity in the amygdala. *PLoS ONE* **8**, e61046 (2013).
79. Hill, M.N. *et al.* Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. *J. Neurosci.* **31**, 10506–10515 (2011).
80. Hill, M.N. *et al.* Endogenous cannabinoid signaling is essential for stress adaptation. *Proc. Natl. Acad. Sci. USA* **107**, 9406–9411 (2010).
81. Hill, M.N., Hillard, C.J. & McEwen, B.S. Alterations in corticolimbic dendritic morphology and emotional behavior in cannabinoid CB1 receptor-deficient mice parallel the effects of chronic stress. *Cereb. Cortex* **21**, 2056–2064 (2011).
82. Lee, T.T., Filipski, S.B., Hill, M.N. & McEwen, B.S. Morphological and behavioral evidence for impaired prefrontal cortical function in female CB1 receptor deficient mice. *Behav. Brain Res.* **271**, 106–110 (2014).
83. Hill, M.N. *et al.* Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. *Neuropsychopharmacology* **30**, 508–515 (2005).
84. Bowles, N.P. *et al.* Chronic, noninvasive glucocorticoid administration suppresses limbic endocannabinoid signaling in mice. *Neuroscience* **204**, 83–89 (2012).
85. Mitra, R., Jadhav, S., McEwen, B.S., Vyas, A. & Chattarji, S. Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proc. Natl. Acad. Sci. USA* **102**, 9371–9376 (2005).
86. Lomazzo, E. *et al.* Therapeutic potential of inhibitors of endocannabinoid degradation for the treatment of stress-related hyperalgesia in an animal model of chronic pain. *Neuropsychopharmacology* **40**, 488–501 (2015).

87. Bortolato, M. *et al.* Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. *Biol. Psychiatry* **62**, 1103–1110 (2007).
88. Rossi, S. *et al.* Preservation of striatal cannabinoid CB1 receptor function correlates with the antianxiety effects of fatty acid amide hydrolase inhibition. *Mol. Pharmacol.* **78**, 260–268 (2010).
89. Tsankova, N., Renthal, W., Kumar, A. & Nestler, E.J. Epigenetic regulation in psychiatric disorders. *Nat. Rev. Neurosci.* **8**, 355–367 (2007).
90. Zoppi, S. *et al.* Regulatory role of cannabinoid receptor 1 in stress-induced excitotoxicity and neuroinflammation. *Neuropsychopharmacology* **36**, 805–818 (2011).
91. Gray, J.M. *et al.* Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety. *J. Neurosci.* **35**, 3879–3892 (2015).
92. Daskalakis, N.P., Cohen, H., Cai, G., Buxbaum, J.D. & Yehuda, R. Expression profiling associates blood and brain glucocorticoid receptor signaling with trauma-related individual differences in both sexes. *Proc. Natl. Acad. Sci. USA* **111**, 13529–13534 (2014).
93. Govindarajan, A. *et al.* Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. *Proc. Natl. Acad. Sci. USA* **103**, 13208–13213 (2006).
94. Lakshminarasimhan, H. & Chattarji, S. Stress leads to contrasting effects on the levels of brain derived neurotrophic factor in the hippocampus and amygdala. *PLoS ONE* **7**, e30481 (2012).
95. Gray, J.D., Milner, T.A. & McEwen, B.S. Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. *Neuroscience* **239**, 214–227 (2013).
96. Suri, D. *et al.* Early stress evokes age-dependent biphasic changes in hippocampal neurogenesis, BDNF expression, and cognition. *Biol. Psychiatry* **73**, 658–666 (2013).
97. Seki, T. & Arai, Y. Different polysialic acid-neural cell adhesion molecule expression patterns in distinct types of mossy fiber boutons in the adult hippocampus. *J. Comp. Neurol.* **410**, 115–125 (1999).
98. Rutishauser, U. Polysialic acid in the plasticity of the developing and adult vertebrate nervous system. *Nat. Rev. Neurosci.* **9**, 26–35 (2008).
99. Theodosis, D.T., Bonhomme, R., Vitiello, S., Rougon, G. & Poulain, D.A. Cell surface expression of polysialic acid on NCAM is a prerequisite for activity-dependent morphological neuronal and glial plasticity. *J. Neurosci.* **19**, 10228–10236 (1999).
100. Pham, K., Nacher, J., Hof, P.R. & McEwen, B.S. Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *Eur. J. Neurosci.* **17**, 879–886 (2003).
101. Sandi, C. Stress, cognitive impairment and cell adhesion molecules. *Nat. Rev. Neurosci.* **5**, 917–930 (2004).
102. van der Kooij, M.A. *et al.* Impaired hippocampal neuroligin-2 function by chronic stress or synthetic peptide treatment is linked to social deficits and increased aggression. *Neuropsychopharmacology* **39**, 1148–1158 (2014).
103. Wood, G.E., Young, L.T., Reagan, L.P. & McEwen, B.S. Acute and chronic restraint stress alter the incidence of social conflict in male rats. *Horm. Behav.* **43**, 205–213 (2003).
104. van der Kooij, M.A. *et al.* Role for MMP-9 in stress-induced downregulation of nectin-3 in hippocampal CA1 and associated behavioural alterations. *Nat. Commun.* **5**, 4995 (2014).
105. Matus, A., Brinkhaus, H. & Wagner, U. Actin dynamics in dendritic spines: a form of regulated plasticity at excitatory synapses. *Hippocampus* **10**, 555–560 (2000).
106. Arendt, T. *et al.* Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *J. Neurosci.* **23**, 6972–6981 (2003).
107. Morris, M., Maeda, S., Vossel, K. & Mucke, L. The many faces of tau. *Neuron* **70**, 410–426 (2011).
108. Kinoshita, Y. *et al.* Role for NUP62 depletion and PYK2 redistribution in dendritic retraction resulting from chronic stress. *Proc. Natl. Acad. Sci. USA* **111**, 16130–16135 (2014).
109. Tochigi, M. *et al.* Gene expression profiling of major depression and suicide in the prefrontal cortex of postmortem brains. *Neurosci. Res.* **60**, 184–191 (2008).
110. Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A.M. & Fuchs, E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J. Neurosci.* **17**, 2492–2498 (1997).
111. Gould, E., Tanapat, P., McEwen, B.S., Flugge, G. & Fuchs, E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc. Natl. Acad. Sci. USA* **95**, 3168–3171 (1998).
112. Cameron, H.A. & Gould, E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* **61**, 203–209 (1994).
113. Leuner, B., Glasper, E.R. & Gould, E. Sexual experience promotes adult neurogenesis in the hippocampus despite an initial elevation in stress hormones. *PLoS ONE* **5**, e11597 (2010).
114. Leuner, B., Caponiti, J.M. & Gould, E. Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. *Hippocampus* **22**, 861–868 (2012).
115. Lightman, S.L. *et al.* The significance of glucocorticoid pulsatility. *Eur. J. Pharmacol.* **583**, 255–262 (2008).
116. Liston, C. & Gan, W.B. Glucocorticoids are critical regulators of dendritic spine development and plasticity *in vivo*. *Proc. Natl. Acad. Sci. USA* **108**, 16074–16079 (2011).
117. Liston, C. *et al.* Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance. *Nat. Neurosci.* **16**, 698–705 (2013).
118. Akana, S.F., Jacobson, L., Cascio, C.S., Shinsako, J. & Dallman, M.F. Constant corticosterone replacement normalizes basal adrenocorticotropin (ACTH) but permits sustained ACTH hypersecretion after stress in adrenalectomized rats. *Endocrinology* **122**, 1337–1342 (1988).
119. Karatsoreos, I.N. & McEwen, B.S. Timing is everything: a collection on how clocks affect resilience in biological systems. *F1000 Res.* **3**, 273 (2014).
120. Seeman, T., Epel, E., Gruenewald, T., Karlamangla, A. & McEwen, B.S. Socio-economic differentials in peripheral biology: cumulative allostatic load. *Ann. NY Acad. Sci.* **1186**, 223–239 (2010).
121. Karatsoreos, I.N. & McEwen, B.S. Psychobiological allostasis: resistance, resilience and vulnerability. *Trends Cogn. Sci.* **15**, 576–584 (2011).
122. Radley, J.J. *et al.* Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience* **125**, 1–6 (2004).
123. Karatsoreos, I.N., Bhagat, S., Bloss, E.B., Morrison, J.H. & McEwen, B.S. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc. Natl. Acad. Sci. USA* **108**, 1657–1662 (2011).
124. Phillips, D.J., Savenkova, M.I. & Karatsoreos, I.N. Environmental disruption of the circadian clock leads to altered sleep and immune responses in mouse. *Brain Behav. Immun.* **47**, 14–23 (2015).
125. Lamont, E.W., Robinson, B., Stewart, J. & Amir, S. The central and basolateral nuclei of the amygdala exhibit opposite diurnal rhythms of expression of the clock protein Period2. *Proc. Natl. Acad. Sci. USA* **102**, 4180–4184 (2005).
126. Reddy, A.B. *et al.* Glucocorticoid signaling synchronizes the liver circadian transcriptome. *Hepatology* **45**, 1478–1488 (2007).
127. McEwen, B.S., Gray, J. & Nasca, C. Recognizing resilience: learning from the effects of stress on the brain. *Neurobiol. Stress* **1**, 1–11 (2015).
128. Russo, S.J., Murrough, J.W., Han, M.H., Charney, D.S. & Nestler, E.J. Neurobiology of resilience. *Nat. Neurosci.* **15**, 1475–1484 (2012).
129. Waddington, C.H. The epigenotype. *Endeavour* **1**, 18–20 (1942).
130. Bavelier, D., Levi, D.M., Li, R.W., Dan, Y. & Hensch, T.K. Removing brakes on adult brain plasticity: from molecular to behavioral interventions. *J. Neurosci.* **30**, 14964–14971 (2010).
131. Castrén, E. & Rantamaki, T. The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Dev. Neurobiol.* **70**, 289–297 (2010).
132. McEwen, B.S. & Gianaros, P.J. Stress- and allostasis-induced brain plasticity. *Annu. Rev. Med.* **62**, 431–445 (2011).
133. Yan, X.-X., Toth, Z., Schultz, L., Ribak, C.E. & Baram, T.Z. Corticotropin-releasing hormone (CRH)-containing neurons in the immature rat hippocampal formation: light and electron microscopic features and colocalization with glutamate decarboxylase and parvalbumin. *Hippocampus* **8**, 231–243 (1998).
134. Hill, M.N. & McEwen, B.S. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **34**, 791–797 (2010).
135. Gunduz-Cinar, O., Hill, M.N., McEwen, B.S. & Holmes, A. Amygdala FAAH and anandamide: mediating protection and recovery from stress. *Trends Pharmacol. Sci.* **34**, 637–644 (2013).
136. Hill, M.N. *et al.* Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. *Mol. Psychiatry* **18**, 1125–1135 (2013).
137. Vyas, A., Mitra, R., Rao, B.S.S. & Chattarji, S. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J. Neurosci.* **22**, 6810–6818 (2002).
138. Liston, C. *et al.* Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J. Neurosci.* **26**, 7870–7874 (2006).
139. McEwen, B.S. & Morrison, J.H. The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course. *Neuron* **79**, 16–29 (2013).
140. Du, J., McEwen, B.S. & Manji, H.K. Glucocorticoid receptors modulate mitochondrial function. *Commun. Integr. Biol.* **2**, 350–352 (2009).
141. Armanini, M.P., Hutchings, C., Stein, B. & Sapolsky, R. Glucocorticoid endangerment of hippocampal neurons is NMDA-receptor dependent. *Brain Res.* **532**, 7–12 (1990).