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Gerard Sanacora, Zhen Yan & Maurizio Popoli

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Gerard Sanacora[1], *Zhen Yan*[2] *and Maurizio Popoli*[3]✉

Abstract | Stress is a primary risk factor for several neuropsychiatric disorders. Evidence from preclinical models and clinical studies of depression have revealed an array of structural and functional maladaptive changes, whereby adverse environmental factors shape the brain. These changes, observed from the molecular and transcriptional levels through to large-scale brain networks, to the behaviours reveal a complex matrix of interrelated pathophysiological processes that differ between sexes, providing insight into the potential underpinnings of the sex bias of neuropsychiatric disorders. Although many preclinical studies use chronic stress protocols, long-term changes are also induced by acute exposure to traumatic stress, opening a path to identify determinants of resilient versus susceptible responses to both acute and chronic stress. Epigenetic regulation of gene expression has emerged as a key player underlying the persistent impact of stress on the brain. Indeed, histone modification, DNA methylation and microRNAs are closely involved in many aspects of the stress response and reveal the glutamate system as a key player. The success of ketamine has stimulated a whole line of research and development on drugs directly or indirectly targeting glutamate function. However, the challenge of translating the emerging understanding of stress pathophysiology into effective clinical treatments remains a major challenge.

Adverse life events, often termed 'stress' in common parlance, are generally accepted as the primary risk factor for numerous neuropsychiatric disorders. Although this gives the word 'stress' a negative connotation, it is important to note that the stress response is a physiological reaction subserving the appropriate adaptation to changes in the environment (including those that are, or may seem, menacing). In this respect, it is not only the nature of the stressor (the event underlying the stress) itself but also the physiological response that may generate deleterious consequences for health[1–6]. Our understanding of how events occurring in the environment (or possibly internally through self-generated mental functions) interact with an organism's physiology and behaviour has markedly evolved since Walter Cannon first introduced the term 'homeostasis' to describe the adaptive mechanisms that preserve functional stability in the face of environmental change nearly a century ago[7]. In 1946, Hans Selye expanded on this theme, introducing the concept of the general adaptation syndrome describing a triphasic process consisting of an initial alarm phase followed by a resistance phase characterized by change and adaptation, which eventually gives way to a maladaptive exhaustion phase[8]. More recently, the concepts of allostasis (the pro-adaptive process of maintaining homeostasis through periods of change) and allostatic load (the price the body pays for maintaining allostasis) have been developed to describe the fundamental processes through which organisms actively adapt to environmental events. An appropriate stress response facilitates the achievement of a new level of adaptation (allostatic change), whereas a maladaptive response sets the system off balance, favouring the onset of psychopathology (allostatic overload)[9]. Led by the work of Bruce McEwen and others, we have started to gain significant insights into the various forms of stress and the factors moderating allostasis and allostatic load (FIG. 1). A more complete understanding of the mechanisms transducing the conversion of stress to psychopathology could greatly improve our ability to develop prophylactic and therapeutic treatment strategies, helping to alleviate the suffering and burden associated with

[1]*Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA.*

[2]*Department of Physiology and Biophysics, State University of New York at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, NY, USA.*

[3]*Laboratory of Neuropsychopharmacology and Functional Neurogenomics, Department of Pharmaceutical Sciences, University of Milano, Milan, Italy.*

✉*e-mail: maurizio.popoli@unimi.it*

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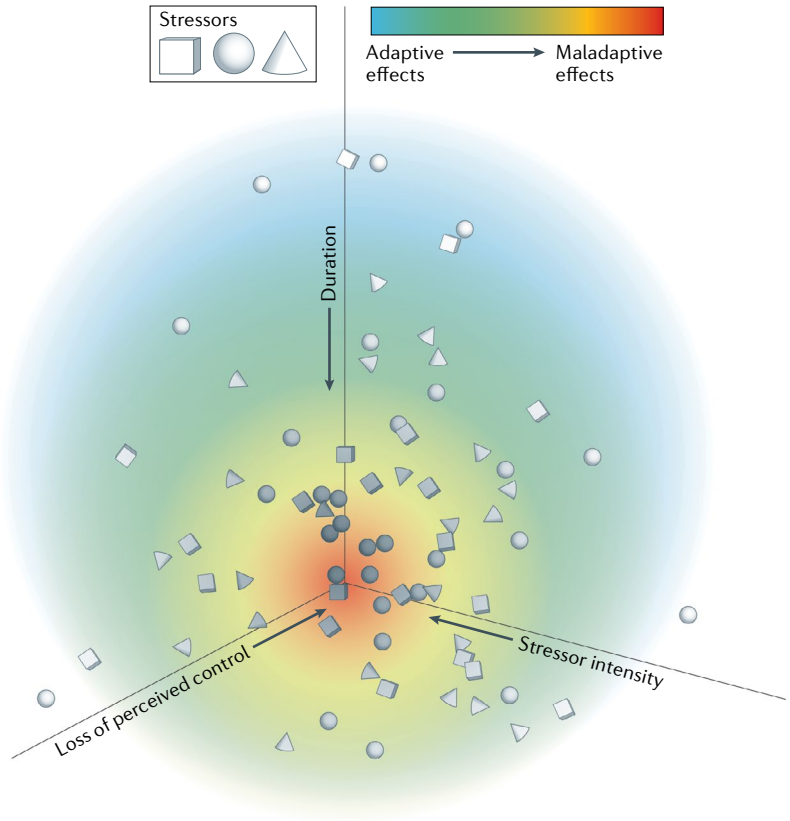

Fig. 1 | **Dimensions of stress impact on brain adaptation.** 'Stress' can be generated through many different events or even internal perceptions and, depending on several factors, can have various effects on brain function. In general, allostatic changes induced by stress typically follow a hormetic-type pattern. Most exposures to stressors result in pro-adaptive processes aiding in survival. However, when pushed to the extremes of intensity or duration, or under specific contexts such as being perceived as uncontrollable or unpredictable[221], they are more likely to cause maladaptive effects[222] (illustrated by changing background colour heat). Degree of resilience or sensitivity to the stress is moderated by developmental stage[223,224], sex[225], genetics[226] and previous experiences[227], and can vary by class of underlying stressor (illustrated by intensity of grey for the individual stressors).

these stress-related disorders. This Review is a 10-year follow-up on an original review highlighting the central role played by the glutamatergic system in mediating these effects[3]. A central, recurrent theme throughout this article will be a thorough analysis of the effects of different forms of stress at the synaptic level, particularly on glutamate synapses[1–6], and of how this is related to the different outcomes of stress exposure.

Stress shapes the brain and its function

Stress may shape the brain for better or worse, depending on whether the stress response brings about pro-adaptive or maladaptive consequences (see previous section). For obvious reasons, maladaptive consequences of stress have been investigated more than pro-adaptive consequences, although understanding resilience signatures is important and may provide valuable insights into which mechanisms could be targeted by treatments[10,11]. Over the past several years, a great deal of work focused on identifying the pathophysiological mechanisms of the stress response that contribute to the development of neuropsychiatric disorders. Although a comprehensive understanding of the underlying pathogenic processes is lacking, mounting evidence demonstrates broad-ranging chronic stress-induced effects on brain structure and function, affording us the opportunity to parse different components in a scalar approach, from the molecular level up to large-scale brain networks. For the sake of simplicity, factors related to synaptic function and plasticity, especially those related to glutamatergic function[1–6], are most prominently addressed here; other major factors in pathophysiology (for example, genetic background, hypothalamic–pituitary–adrenal (HPA) axis imbalance, inflammation and so on), although quite important, are only cursorily addressed here.

As illustrated in FIG. 2, the structural and functional changes associated with chronic stress and major depressive disorder (MDD), used here as a representative stress-related disorder, are evident at several levels of anatomical scale: the molecular and cellular levels, particularly at glutamatergic synapses (rodents); activation or inhibition of relevant circuitry (rodents); neuroarchitecture of relevant brain areas (rodents and/or humans) where glutamatergic neurons/synapses are predominant; and unique large-scale brain networks (humans). The evidence arises from both rodent stress models and from clinical studies of MDD. Using a translational approach, it is possible to integrate the different stress-related pathophysiological processes to suggest a sequence of events evolving through the different levels. However, the present state of knowledge limits our ability to clearly define the chain of events moving from stress exposure to psychopathology.

Chronic stress: molecular changes. Chronic stress induces changes in pathways active at synapses, particularly at glutamate synapses. An array of changes have been implicated, but one pathway, centred on mechanistic target of rapamycin complex 1 (mTORC1), has been shown to play a key role in the process (FIG. 2a). The mTORC1 pathway is a master regulator of protein synthesis-dependent synaptic plasticity and formation of new synapses. Early studies showed that mTOR is localized in the postsynaptic compartment of glutamate synapses and that rapamycin can block long-term potentiation elicited by either high-frequency stimulation or brain-derived neurotrophic factor (BDNF) treatment[12], highlighting its critical role in the process. Chronic mild stress (CMS) protocols, in particular the chronic unpredictable stress (CUS) version (BOX 1), induce anhedonic behaviour (a core symptom of depression), reduce spine density in the prefrontal cortex (PFC) and increase expression of regulated in development and DNA damage responses 1 (REDD1), a glucocorticoid-inducible factor that inhibits mTORC1 and mTORC1-dependent protein synthesis and cell growth. In line with preclinical evidence, post-mortem studies showed that REDD1 was increased whereas mTOR and downstream effectors were reduced in the PFC of people with MDD[13,14]. Further validation of mTORC1's role in mediating structural changes came from studies demonstrating that mTORC1 activation was a necessary step in ketamine's

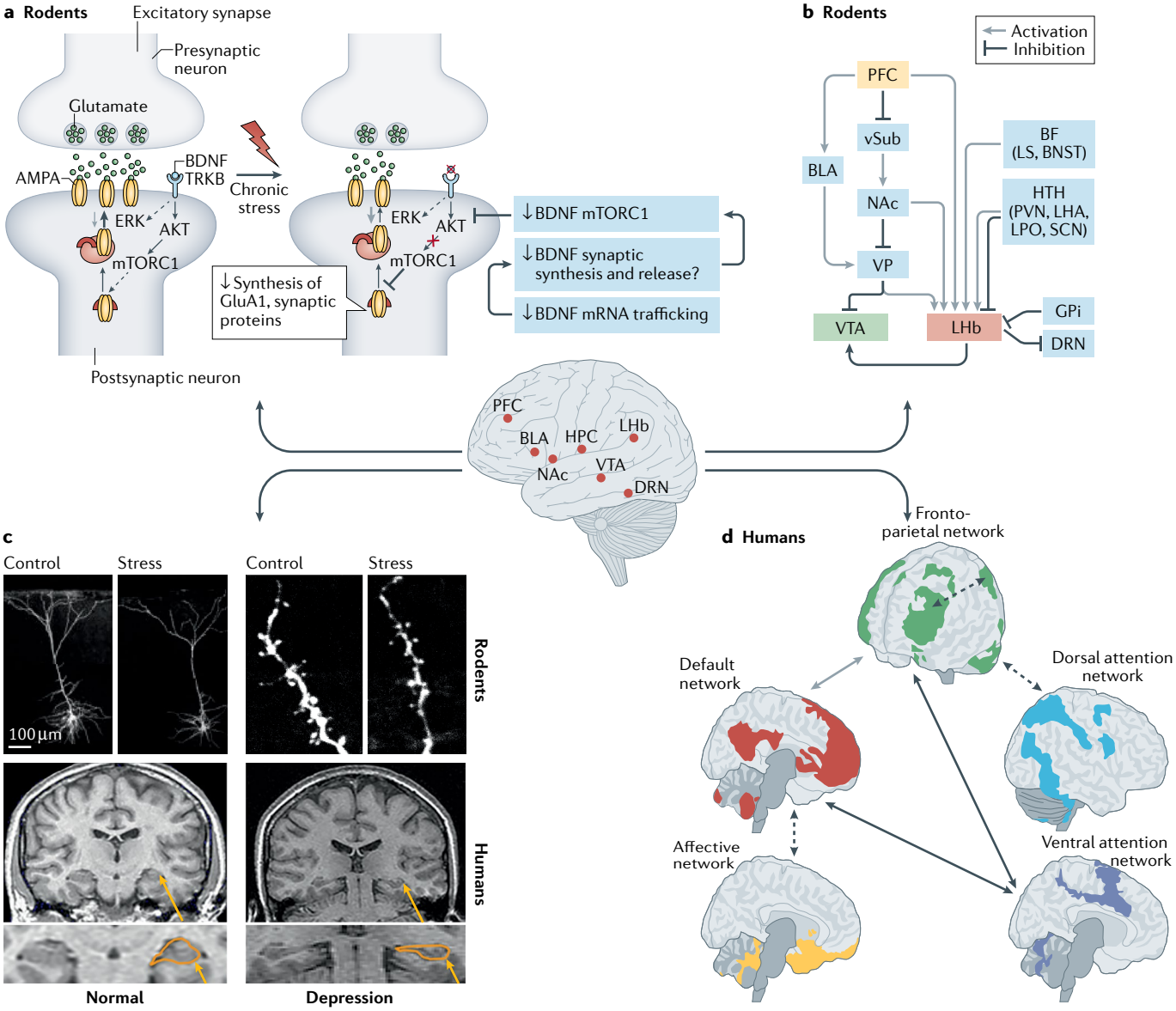

Fig. 2 | **Major putative maladaptive changes associated with chronic stress and depression: a scalar approach.** Chronic stress induces maladaptive changes in the brain at different levels, which, interacting with genetic background and previous adverse life events, may favour development of psychiatric disorders. Major brain areas involved in stress-related disorders by preclinical/clinical research are indicated in the centre. Four different levels of changes are depicted, observed in rodent stress models and human subjects with major depression (a representative stress-related disorder): data from chronic stress rodent models (parts **a**,**b**); data from rodent models and neuroimaging studies in people with major depressive disorder (MDD) (part **c**); and data from resting-state functional connectivity studies in people with MDD (part **d**). **a** | Molecular/cellular changes at synapses, mainly from studies analysing prefrontal cortex (PFC) and hippocampus (HPC). Normally, synaptic transmission keeps excitatory synapses in homeostatic conditions, with presynaptic release of glutamate, activation of postsynaptic glutamate AMPA receptors and depolarization of postsynaptic neuron. Intracellular pathways, essential for homeostatic regulation of synapses, are activated, including mechanistic target of rapamycin complex 1 (mTORC1) and brain-derived neurotrophic factor (BDNF)–TRKB signalling pathways (with related kinases AKT and ERK). Chronic stress decreases BDNF and mTORC1 signalling, and reduces presynaptic release of glutamate and synthesis/insertion of AMPA receptors onto postsynaptic membrane, thereby reducing excitatory synaptic transmission. mTORC1 pathway is also regulated by glucocorticoids, via glucocorticoid receptor (GR), oestrogens, mGlu receptors and insulin, via PI3K and AKT (not shown). **b** | Synaptic and circuitry changes. Neural circuitries regulating the ventral tegmental area (VTA) and lateral habenula (LHb), which undergo changes with chronic stress in rodents. **c** | Neuroarchitecture changes. Upper panel: retraction of apical dendrites and loss of synaptic spines in pyramidal neurons (layer V) of medial PFC of representative rat subjected to 7 days of restraint stress. Lower panel: magnetic resonance imaging (MRI) scan of brain from representative healthy control and depressed subject, with reduced volume of HPC in the depressed subject. **d** | Large-scale brain network dysfunction in depression: fronto-parietal network, default network, dorsal attention network, affective network and ventral attention network. Dotted arrows indicate hypoconnectivity; grey arrows, hyperconnectivity; and black arrows, generally abnormal (both hypoconnectivity and hyperconnectivity). BF, basal forebrain; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; DRN, dorsal raphe nucleus; GPi, globus pallidus; HTH, hypothalamus; LHA, lateral hypothalamic area; LPO, lateral preoptic area; LS, lateral septum; NAc, nucleus accumbens; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; VP, ventral pallidum; vSub, ventral subiculum. Part **c** is reprinted from REF.[5], Springer Nature Limited, and reprinted with permission from REF.[228], Cambridge University Press. Part **d** is adapted with permission from REF.[55], American Medical Association.

Box 1 | **Animal models of chronic stress and brain pathology**

Rodent models of chronic stress are widely used to study the effects of different stressors on the brain. No particular model can be individually related to a single pathology, for the obvious reason that no animal model can entirely reproduce human psychopathology[59,229,230]. Rather, they help us understand mechanisms of pathology, as for neuroarchitecture changes and synaptic disconnection, where they complement clinical neuroimaging studies. One of the most popular chronic stress protocols is the chronic mild stress (CMS), also referred to as chronic unpredictable stress (CUS), model[231,232]. These models have been used by several groups to reproduce features of human depression and to study antidepressant mechanisms. Basically, the protocol consists of numerous mild stressors, randomly applied to rats or mice for weeks (between 3 and 5 weeks). CMS/CUS shows various neurobiological effects, most reversed by chronic antidepressants, including anhedonia, a core symptom of depression. Noteworthy, only a subpopulation shows behavioural/neurobiological changes, which allows them to be deemed susceptible or resilient to stress[21,231,232].

Chronic social defeat stress (CSDS) is a widely used protocol of psychosocial stress, based on the subordination of a C57Bl/6J mouse by a larger, aggressive CD-1 mouse. Each physical bout (up to 10 min) is followed by sensory contact with the aggressor through a plastic partition for 24 h, repeated for 10 days. Following CSDS, a proportion of mice (susceptible) develop social avoidance, anhedonia and dysregulated feeding, features resembling depression/anxiety, reversed by chronic antidepressants[82].

Restraint stress is the most commonly used immobilization protocol and consists of keeping rodents in a semi-cylindrical acrylic tube with proper holes for ventilation. Due to its simplicity, it is one of the most widely used protocols, often with daily sessions for several days. Early evidence for stress-induced dendritic atrophy was obtained with this protocol[233,234].

The learned helplessness protocol is also a well-validated model in depression studies, due to its good construct validity. It is not a chronic protocol proper but is included here because helpless animals show features of depression, including anhedonia, cognitive dysfunction and hypothalamic–pituitary–adrenal (HPA) axis imbalance[235]. The protocol consists of a first session of inescapable footshock and a second session 24 h later in which the animals are tested for avoidance of the shock. Although learned helplessness behaviour only lasts 7–10 days, it has been used to investigate the mechanism of antidepressants.

mechanism of reversing stress-induced synaptic spine loss[15] (see the section Pharmacologically targeting stress).

Another activity-dependent pathway that is convergent on the mTORC1 pathway is that of BDNF. BDNF is released at glutamate synapses upon neuronal activation, largely via Ca^{2+} signalling and activation of Ca^{2+}–cAMP response elements, and is essential for synaptic plasticity and formation of new synapses. A common outcome of different chronic stress protocols is a reduction of BDNF expression in the PFC and hippocampus (HPC)[16,17]; conversely, in other areas (for example, amygdala and nucleus accumbens (NAc)) chronic stress increases BDNF expression in a manner believed to be related to maladaptive consequences[18,19]. BDNF, acting through its receptor TRKB, activates downstream protein kinases, including AKT and ERK, which in turn phosphorylate and activate mTOR. Therefore, reduction of BDNF binding to TRKB receptors (as a consequence of chronic stress) would, in turn, reduce activation of mTORC1 (FIG. 2a). A corollary of BDNF and mTORC1 pathway regional deactivation by chronic stress is a reduction of glutamate release and/or transmission. Indeed, a reduction of basal and depolarization-evoked glutamate release after CMS (BOX 1) has been found in the HPC, and there is independent evidence for reduced synaptic activity in the HPC and PFC with chronic stress[1,20,21]. However, although total BDNF expression is reduced by chronic stress (and increased by antidepressants), the connection with maladaptive plasticity at synapses could be more complex. Indeed, converging evidence has shown that local synaptic translation and release of BDNF from dendrites is crucial for synaptic plasticity[22]. Select splice variants of BDNF mRNA are transported along dendrites to support putative synaptic BDNF translation required for synaptic plasticity. It has been shown that CMS (BOX 1) impairs anterograde trafficking of BDNF mRNA in dendrites of the CA3 HPC region, along with induction of anhedonia and impairment of glutamate release, all rescued by ketamine in just 24 h[21]. Interestingly, both chronic antidepressants and prolonged physical exercise increase dendritic trafficking of *BDNF* mRNA, whereas the trafficking is impaired in homozygous mice carrying the human $BDNF^{V66M}$ polymorphism (a vulnerability factor for brain disorders)[23,24]. Therefore, as shown in FIG. 2a, the impairment of BDNF dendritic trafficking by chronic stress could reduce synaptic translation and release of BDNF, in turn reducing stimulation of mTORC1 signalling and downstream synthesis of synaptic proteins, as well as trafficking and insertion of AMPA receptors onto postsynaptic membrane. What is still missing in this framework is a clear-cut demonstration of dendritic release of BDNF[22].

Chronic stress: circuit-level changes. The circuit-level changes induced by chronic stress consist of increased or decreased activation of select neuronal projections within and among different areas of rodent brain. In many cases, the stress-induced changes in these circuits are associated with the development of depressed-like or anxious-like behaviours in the animals (FIG. 2b). In this particular field, the use of chronic stress protocols (BOX 1), coupled with the advent of optogenetic techniques allowing selective stimulation or inhibition of neuronal circuits, has made possible the functional dissection of the circuitry in relation to the behavioural changes induced by stress. This has a high translational value, affording the possibility of linking specific circuit dysfunctions to select symptoms of psychiatric pathology and aiding in the identification of new putative targets for treatment. Two examples briefly discussed here are the dopamine system and reward pathways, and the lateral habenula (LHb) and related pathways.

Anhedonia, a core symptom of depression, is defined as disruption of the anticipation, motivation and decision-making processes involved in obtaining a reward. Converging preclinical and clinical evidence has linked anhedonia to dysfunctions in the reward pathways, in particular the dopamine system[25]. Reduced dopamine release in the NAc and reduced expression of dopamine receptors has been observed in the CMS and learned helplessness protocols (BOX 1). Both protocols resulted in reduced activation of the mesolimbic dopamine pathway, with significant reduction in the number of spontaneously active dopamine neurons. It has been found that stress-induced deactivation of the dopamine system is due to hyperactivation of the infralimbic PFC, which in turn inhibits dopamine neurons of the ventral tegmental area (VTA) via hyperactivation of the infralimbic PFC–basolateral amygdala (BLA)–ventral pallidum (VP) pathway and disruption of

synaptic plasticity in the ventral subiculum (vSub)–NAc pathway[26,27] (FIG. 2b). In line with these findings, independent works have shown that optogenetic inhibition of dopamine neurons in the VTA induced specific depression-like behaviours and that depression-like behaviours induced by CMS were reversed by selective activation of the same dopamine neurons in the VTA, linking these dopamine neurons to neural encoding and expression of depression-related behaviour[28]. However, a different stress protocol (chronic social defeat stress (CSDS)) (BOX 1) was shown to increase phasic bursting and excitability of dopamine neurons in VTA in susceptible mice. Optogenetic phasic stimulation of VTA dopamine neurons also quickly induced a susceptible phenotype in mice previously resilient to CSDS, establishing a link between VTA dopamine neuronal firing patterns and susceptibility to a depression-related phenotype[29,30]. The different effects of stress on VTA dopamine neurons may be explained by various factors, including the different stress protocols, different populations of dopamine neurons and different modes of stimulation (for example, phasic versus tonic). Indeed, these studies have shown that optogenetic manipulation of one brain region may produce different behavioural responses. This is consistent with the notion that distinct depression-related behaviours may be mediated by different circuits and exemplifies the complexity of human mental illness[31–33].

The LHb, part of the habenula nucleus, located at the posterior end of the thalamus, is the only brain region that showed consistently heightened activity in animal models of depression. Habenula output is tightly regulated by a balance of excitatory and inhibitory drive mediated by the glutamatergic and GABAergic inputs that appear to be altered in animal models of depression and attenuated by antidepressant medications[34]. Further suggesting the region plays a central role in the depression-like behaviours, optogenetic manipulations enhancing or suppressing LHb activity in rodents resulted in depressive-like or antidepressant-like effects, respectively[35–37]. However, a study employing CMS found that the LHb played a smaller role in the CMS-induced attenuation of dopamine neuron activity[38]. In humans, functional imaging studies of this structure revealed increased volume and hyperactivity in depression[39]. It was shown that habenula activity is elevated in MDD and exhibits the most significant covariation with depression rating scales across the whole brain[40]. The LHb is a critical node that interconnects forebrain and midbrain monoaminergic nuclei, exerting control on both the dopaminergic and serotonergic systems. Therefore, the LHb is strategically positioned to integrate the brain's motivational, memory and motor systems. The rapid antidepressant action of ketamine is also mediated by suppression of the bursting activity of the LHb neurons associated with depressive-like behaviour[37].

Chronic stress: neuroarchitecture changes. Neuroarchitecture modifications have been observed in various brain areas after stress exposure. Indeed, a large number of rodent studies over the years have consistently shown that chronic stress reduces the apical dendrite length and branching of medial PFC (layers II/III and V) and HPC CA3 pyramidal neurons, while increasing dendritic density in the basolateral amygdala (FIG. 2c). In many cases, dendritic atrophy in the PFC and HPC is accompanied by reduction of synaptic spine density, suggesting that chronic stress induces a 'synaptic disconnection' syndrome within and between these areas[1,4,6,21,41–44]. Most of these changes in the PFC and HPC involve glutamatergic neurons and synapses. These results were obtained using different protocols of psychosocial and environmental stress, including CMS, CUS, CSDS and chronic restraint stress (BOX 1). A primary event responsible for the dendritic shrinkage and spine loss is suggested to be the abnormal enhancement of glutamate release induced by stress/corticosterone (see the section Chronic versus acute stress). This enhancement of excitatory transmission, especially if repeated or sustained over time and reinforced by impairment of glial glutamate transport and recycling[44,45], alters mTORC1 and BDNF pathways (FIG. 2a), resulting over time in a reduction of local synthesis of proteins essential for consolidation of synaptic spines and plasticity.

The studies on chronic stress-induced structural changes in rodents nicely complemented the clinical neuroimaging studies showing structural changes in brains of psychiatric patients (particularly those with depression or post-traumatic stress disorder (PTSD)). Early magnetic resonance imaging (MRI) studies showed a reduced volume of HPC and PFC in people with MDD (FIG. 2c). Numerous meta-analyses have summarized and integrated the results of nearly three decades of neuroimaging studies on depression[46–48]. Taken together, preclinical and clinical data suggested that, in both rodents and humans, the neuroarchitecture changes (for example, reduced dendrite and spine densities) are the primary reason for the volumetric changes described by neuroimaging[1,5,6,17,41,42]. Indeed, a few clinical post-mortem studies observed reduced density of dendrites and synaptic spines in the HPC and PFC, allowing merging of preclinical and clinical evidence[49,50]. A recent large-scale meta-analysis, while confirming significantly lower hippocampal volumes in people with MDD, did not detect significant differences for other subcortical volumes. Instead, they found subtle cortical thickness alterations in 13 of 68 cortical regions in MDD, with the largest effect size observed in the orbitofrontal cortex[51]. Overall, preclinical and clinical results suggest that neuroarchitecture changes in discrete brain areas are a biological correlate of stress-related neuropsychiatric disorders.

Chronic stress: brain network changes. The next level of change above circuits, and a further level of complexity, is observed in large-scale brain networks. Over the years, it has become increasingly apparent that mapping dysfunctional cognitive and emotional processes associated with neuropsychiatric disorders onto individual brain areas or circuits is overly simplistic. Several complementary lines of evidence support the notion that dysfunction of different circuits of the brain is involved in psychopathology, and that distinct depressive symptoms may be encoded by differential changes in brain

Box 2 | **Acute stress is not acute: study of trajectories of resilience or vulnerability**

Pilot work has dissected the short-term and long-term response to acute stressors. Early studies found that acute stress/corticosterone administration rapidly increase extracellular glutamate in the prefrontal cortex (PFC) and hippocampus (HPC)[236,237], confirmed by studies using purified synaptic terminals (synaptosomes) in superfusion[3]. Acute footshock stress rapidly enhances depolarization-evoked release of glutamate in the PFC, confirmed by patch clamp recordings and prevented by chronic antidepressants[238]. Corticosterone binds synaptic receptors and induces rapid, non-genomic, enhancement of the trafficking of synaptic vesicles into the readily releasable pool, dependent on phosphorylation of Ser[9] in synapsin I[239], with a surge of glutamate probably amplified by malfunction of glutamate clearance[240,241]. A popular hypothesis suggests that excitatory/inhibitory imbalance induced by stress/corticosterone results in dendritic atrophy, reduced spine density and synaptic disconnection, when stress is repeated or sustained over time[1,4,6,41,42,44]. In line with the hypothesis, blockade of NMDA receptors during restraint stress prevented apical dendritic atrophy[242].

However, this did not explain how acute stress may induce similar neuroarchitecture changes to chronic stress. An explanation came from the finding that after acute footshock stress glutamate release in the PFC is strongly enhanced for 24 h whereas, at the same time, atrophy of apical dendrites starts and lasts for at least 2 weeks[63,243]. These results confirmed that acute stress may induce long-term neuroarchitecture changes, linked to sustained glutamatergic activation, consistent with the notion that psychopathology may be triggered by acute traumatic stress (for example, post-traumatic stress disorder (PTSD)). As several studies found reduction of glutamate release/transmission after chronic stress[1,20,21,73,244], the changes might follow a biphasic process, during which increased excitatory activation turns into its opposite, producing progressive exhaustion of the system, in turn resulting in impairment of excitatory function and neuroarchitecture[245,246].

The footshock stress protocol has been recently implemented to allow distinguishing trajectories of resilience versus susceptibility. Rats are deemed resilient or susceptible 24 h after footshock stress with sucrose intake test (SIT), as for chronic mild stress (CMS)[21]. By measuring various read-outs in the two groups, this approach is used to identify biomarkers of resilience versus vulnerability[59] (see the figure). This protocol represents a simpler alternative to classical chronic stress protocols to study pathophysiology and test novel pharmacological approaches.

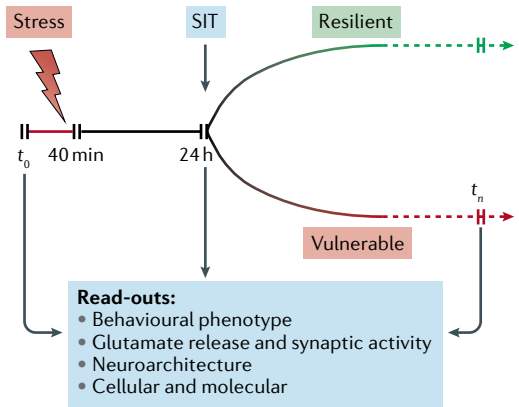

Figure is adapted from REF.[59], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

circuits. This is in line with the observation that psychiatric conditions encompass multiple and heterogeneous behavioural phenotypic features[52]. The study of brain networks is mainly carried out using functional MRI studies, which calculate temporal correlations between spontaneous activity of blood oxygen level-dependent signals coming from different brain regions. Data from rodent models using MRI to examine circuit and network changes with stress are beginning to appear but still remain relatively limited[53]. However, a recent surge of studies on functional connectivity among brain networks has demonstrated that the human brain is intrinsically organized into coherent large-scale functional networks. Different groups have consistently found that abnormal activation/deactivation within single networks and among different networks plays a role in several neuropsychiatric disorders[54,55]. FIGURE 2d depicts dysfunctional networks implicated in MDD: the fronto-parietal network (FN; also called the central executive network), involved in top-down regulation of attention and emotion; the default network (DN) and the dorsal attention network, involved in internally or externally oriented attention, respectively; and the affective network and the ventral attention network (often called together the salience network), involved in processing emotion or monitoring for salient events. As illustrated in FIG. 2d, reduced connectivity within FN control systems and imbalanced connectivity between FN and networks involved in internal (DN) or external (dorsal attention network) attention may reflect depressive biases towards internal thoughts (rumination). Moreover, reduced connectivity between the affective network and medial PFC regions of the DN may reflect impaired ability to upregulate or downregulate emotions or arousal, whereas abnormal connectivity between the ventral attention network and posterior regions of the FN may reflect altered or biased salience monitoring. Dysfunction of the same brain networks have also been investigated under stress — limited to acute stress thus far — and show increased activity and/or connectivity in the salience network and in the DN; most studies show no changes in the central executive network[56]. Overall, these studies support the notion that MDD is associated with hypoconnectivity within and between some networks and hyperconnectivity in others, which contributes to overall dysregulation of cognitive and/or emotional information processing and symptomatology such as rumination.

Chronic versus acute stress

Most rodent models employed in preclinical studies are chronic stress protocols, but what about acute stress? Acute stress usually consists of a single, time-limited exposure to stressors, with the severity of stressors often similar to that of chronic protocols. Moreover, it is well known that acute traumatic stress may have long-term consequences, such as depression and PTSD[6,57,58]. Furthermore, a limitation of chronic stress models is that, as a rule, they assess the different changes at one end point, after days or weeks of repeated stress. Using this approach we may obtain a clear picture of the end point changes but just miss what happened during the long-term stress response, making it difficult to understand how the changes develop over time. Longitudinal studies would be required to overcome this limitation, but this can be complex, costly and require a large number of animals. However, a valid alternative to chronic models is represented by longitudinal studies of stress response after acute protocols, which are proving themselves quite useful for dissection of pathophysiological changes and identification of novel targets for treatment[6,59] (BOX 2).

Indeed, a few studies assessing the effects of acute or subacute stress on neuroarchitecture showed that brief stress exposure may reproduce the sustained structural changes typical of chronic stress. Different protocols of acute or subacute stress (FSWS, footshock stress, short

multimodal stress) (BOX 3) have been found to induce loss of synaptic spines or atrophy of dendrites as early as 24 h after stress[60–63]. With acute footshock stress, the rapid change in neuroarchitecture was also sustained for 2 weeks[63]. Taken together, these studies demonstrated that acute stress may induce rapid and also sustained neuroarchitecture changes in the brain. How this may be possible is addressed in BOX 2.

Sex differences of stress

Significant sex differences exist in stress-associated diseases in humans. For example, the prevalence of aggression/violence is significantly higher in men, whereas women have a higher vulnerability to develop depression or PTSD[64,65]. Stress exposure during the prenatal period and early postnatal life leaves males with higher risks of developing diseases involving socialization (for example, autism spectrum disorder) and attention (for example, attention deficit hyperactivity disorder), whereas stress exposure during puberty puts females at an increased risk of developing emotion-related illnesses (for example, depression and PTSD)[66,67]. Revealing sex differences in stress responses may hold the key to understanding the neurobiological underpinnings of the sex bias of different psychopathology.

Sex-specific behaviour changes to stress. Although most animal studies on stress have focused on males, some reports have shown sex-specific effects of various stressors, suggesting that stress exerts diverse behavioural and emotional impact in males and females[66,68,69]. A review of behavioural findings found that robust sex differences exist in models of all psychiatric disorders, including depression, anxiety, PTSD, addiction, obsessive-compulsive disorder, schizophrenia, bipolar disorder and autism[70].

Box 3 | **Animal models of acute stress**

Protocols of acute stress are used to investigate short-term and long-term effects of different stressors.

Acute forced swim stress has been widely used either as a stress protocol or as a test for behavioural despair (interpreted as equivalent to depressive-like behaviour)[247]. The latter is a test for antidepressant action, although it provides little construct or face validity[248]. In a typical protocol of forced swim stress, mice are forced to swim in a cylinder 20 cm in diameter filled halfway with water for 10 min. This protocol may be used once or repeated on subsequent days, as in REF.[61].

Short multimodal stress lasts 5 h, in which mice are exposed to different stressors simulating intense real-life stress, and results in defective learning/memory functions. Within hours after stress, impairment of long-term potentiation and reduction of synaptic spine density in the hippocampus (HPC) are observed. Spine loss was positively correlated with memory impairment[60].

Acute restraint stress is carried out as in the chronic protocol (BOX 1), with a single session of variable length.

Acute inescapable footshock stress is another widely used protocol, both in a single session or in the context of the learned helplessness protocol (BOX 1). In a typical setting, a single session lasts 40 min, with 20 min of actual random shocks (0.8 mA, 2–8 s). Footshock stress has been used in connection with depression and post-traumatic stress disorder (PTSD), because it induces long-term behavioural changes, including social avoidance, defensive behaviour, hypervigilance, sleep disturbances and generalization of fear[249,250]. Acute footshock stress induces, among other changes, impairment of cognitive function at 24 h, rapid and sustained (24 h) enhancement of glutamate release, and apical dendrite atrophy in the prelimbic prefrontal cortex (PFC) from 24 h up to 2 weeks[59,63,239,243].

Early life stress (ELS), such as maternal separation (that is, between postnatal days 2 and 14), renders male mice to have significantly disrupted locomotor and exploratory activity, and female mice to have increased anxiety and social behaviour[71]. Prepubertal psychogenic stress (for example, exposure to predator odour) induces sex-specific effects on auditory and contextual fear conditioning: males have increased conditioned fear and impaired extinction to the tone, whereas females have reduced freezing responses to the context[72]. Prepubertal male rats exposed to 1-week repeated restraint stress exhibit the impairment of PFC-mediated cognitive function[73], whereas stressed females show no negative effects on cognition[74]. Prolonged stress exposure during adolescence and early adulthood usually induces heightened and sustained aggression in male rodents[75–77], but triggers depression-like behaviours in females[78–81].

Since the introduction of CSDS (BOX 1) for studying emotional homeostasis[82], most studies have used male mice repeatedly subjected to bouts of social defeat by larger and aggressive male mice, which results in the development of a depressive-like syndrome characterized by the enduring deficits in social interactions[83]. Recently, it was found that female mice exposed to vicarious social defeat stress also display a behavioural profile that mimics symptoms of depression, including the decreased social behaviour and sucrose preference, along with increased immobility in the tail suspension test[84].

Sexual dimorphic effects of stress on behavioural phenotypes have been linked to sex-specific changes in neuronal structure. Within 24 h after one acute stressful event of intermittent tailshocks, spine density is enhanced in the male HPC but reduced in the female HPC[85]. ELS decreases juvenile social behaviour and impairs reversal learning in females, but not males, which is found to be associated with the sex-specific alteration of dendritic arborization and the decreased expression and density of parvalbumin-positive interneurons in the frontal cortex[86,87]. Moreover, the antidepressant ketamine exerts sex-specific effects: reversing the decline of synaptic proteins and spine density in male rats exposed to chronic isolation stress, but failing to do so in stressed females[88], suggesting that distinct mechanisms underlie the efficacy of ketamine in the two sexes. However, the existence of sex differences in ketamine's (or esketamine's) effectiveness in treating MDD has not been observed in the larger-scale clinical trials completed to date.

Sex-specific genetic changes by stress. Emerging evidence suggests that stress exerts diverse transcriptional impact in males and females[79,89,90]. Transcriptomic analysis of humans with MDD finds limited overlap between males and females in MDD-induced rearrangement of transcriptional patterns[89]. Manipulation of an identified key regulator of female MDD gene networks, Dusp6, validates its sex-specific impact on stress susceptibility and transcriptional remodelling[89]. Large-scale gene expression meta-analysis across three cortico-limbic regions confirms the great sex differences in the transcriptional profile of MDD, with multiple transcriptional changes

in opposite directions between men and women with MDD, for example, synapse-related genes are decreased in MDD men but increased in MDD women[91].

Using a 'two-hit' stress model, genome-wide analysis across three brain reward regions (VTA, NAc and PFC) has found that, depending on an ELS history, experience of the second stress in adulthood induces distinct transcriptional patterns in male and female mice[90]. Common pathways related to neuronal outgrowth and synapse signalling are enriched in stress-induced transcriptomic changes across sexes and regions; however, some of these pathways show opposite associations with stress resilience or susceptibility in males and females, raising the possibility of prominent sex differences in molecular alterations associated with stress[90].

Epigenetic enzymes and transcription factors have been implicated in sex-specific effects of stress on gene expression and behavioural manifestation. Females are less vulnerable to prenatal insults than males, and the high level of histone H3 trimethylated at lysine 27 (H3K27me3), a repressive histone modification, in the female placenta is found to render female resilient to the alteration of neurodevelopmental programming associated with prenatal stress[92]. Females are more vulnerable to subchronic variable stress than males, and RNA sequencing analysis has revealed a sex-specific transcriptional profile in the NAc, with a greater induction of DNA methyltransferase 3a (*Dnmt3a*) mRNA in the NAc of stressed females, and *Dnmt3a* knockout in the female NAc partially shifts the transcriptome towards the pattern of stressed male NAc and induces behavioural resilience to stress in females[79]. By contrast, in male mice exposed to CSDS, a reduction of *Dnmt3a* in the medial PFC is found to accompany anxiety-like behaviours, and overexpression of *Dnmt3a* in the medial PFC attenuates stress-induced anxiety in males[93]. More findings on epigenetic changes by stress are detailed in the next section.

In male mice, the developmental transcription factor Otx2, which regulates transcriptional programming in the brain reward region VTA, is identified as a key mediator of the enduring effect of ELS on lifelong stress susceptibility[94]. However, Otx2 does not serve as an important upstream regulator of ELS-induced transcription in females[90]. Sexually dimorphic expression of the immediate-early gene early growth response 1 (EGR1), a key player in transcriptomic regulation of the PFC in both males and females[95], is identified as a crucial factor controlling sex-specific social anxiety-like behaviours[96].

Several key molecules regulating sex differences in stress responses have been identified, including the gonadal hormones testosterone and oestradiol, as well as genes on the sex chromosomes[97]. Oestrogen is found to protect against the detrimental effects of repeated restraint stress on glutamatergic transmission in PFC pyramidal neurons and PFC-dependent cognition in young female rats[74]. In search of upstream regulators of genes differentially expressed after CSDS, ERα is identified as the top regulator of pro-resilient transcriptional changes in the NAc[98]. Dysregulation of the corticotropin-releasing factor (CRF)-mediated stress neurocircuitry has been implicated in psychiatric disorders that are more prevalent in women, such as depression and PTSD[99]. The reason why females are more sensitive to CRF and less adaptable to excess CRF is found to be related to sex differences in CRF receptor signalling and trafficking[100]. The neuropeptide Orexin, which plays a key role in regulating arousal, vigilance, endocrine homeostasis, fear and anxiety[101–103], is found to be critical for protecting against depressive reactions to stressful events[104]. The level of orexin is significantly decreased in suicidal patients with MDD[105] and combat veterans with chronic PTSD[106], but is elevated in individuals with panic anxiety[107], implicating its complex role in psychiatric disorders. After repeated restraint stress, only female rats show the increased orexin expression and activation, as well as deficits in cognitive flexibility[108]. In male rats exposed to predator-scent stress, only resilient animals display significantly activated orexin system, and increasing brain orexin levels in vulnerable animals reduces the prevalence of the PTSD phenotype[109]. These data suggest that orexin is important for promoting adaptive responses to stress in both males and females.

Epigenetics of stress

As noted earlier, the mechanisms mediating the long-lasting effects of stress on brain structure and function, which ultimately lead to the development of stress-related disorders, remain an intriguing question. Epigenetic regulation of gene expression has emerged as a key player underlying the persistent impact of stress[110]. Stress directly influences epigenetic marks for fine-tuning of genes involved in brain plasticity across the lifespan[4]. The stress-induced epigenetic modification of HPA axis-associated genes that govern homeostatic levels of glucocorticoids, as well as many other genes important for neuronal functions, can determine stress adaptation or maladaptation that shapes the trajectories to health or disease[73,111–118] (FIG. 3).

Histone modification. Increasing evidence suggests that aberrations in chromatin remodelling and subsequent effects on gene expression within limbic regions contribute to the pathogenesis of stress-related disorders[118]. In male mice subjected to CSDS (BOX 1) and human subjects with depression, the reduced expression of RAC1, a key regulator of actin cytoskeleton and synaptic structure, is identified in the NAc, which is associated with the repressive chromatin state at *RAC1* promoter[119]. Inhibition of class I histone deacetylases (HDACs) rescues the CSDS-induced decrease of *RAC1* transcription and depression-related behaviours, highlighting the epigenetic regulation of *RAC1* as a disease mechanism in depression[119]. In blood samples from patients with schizophrenia who have encountered ELS, the level of HDAC1 is increased, and the ELS-induced schizophrenia-like phenotype in mice is also correlated with the increased expression of HDAC1 (REF.[120]). Moreover, the detrimental effects of ELS are mimicked by HDAC1 overexpression in medial PFC neurons and rescued by an HDAC inhibitor, pointing to HDAC1 as a key player linking ELS to schizophrenia[120].

The increased level of SIRT1, a class III HDAC, is also found in the NAc of male mice after CSDS, and

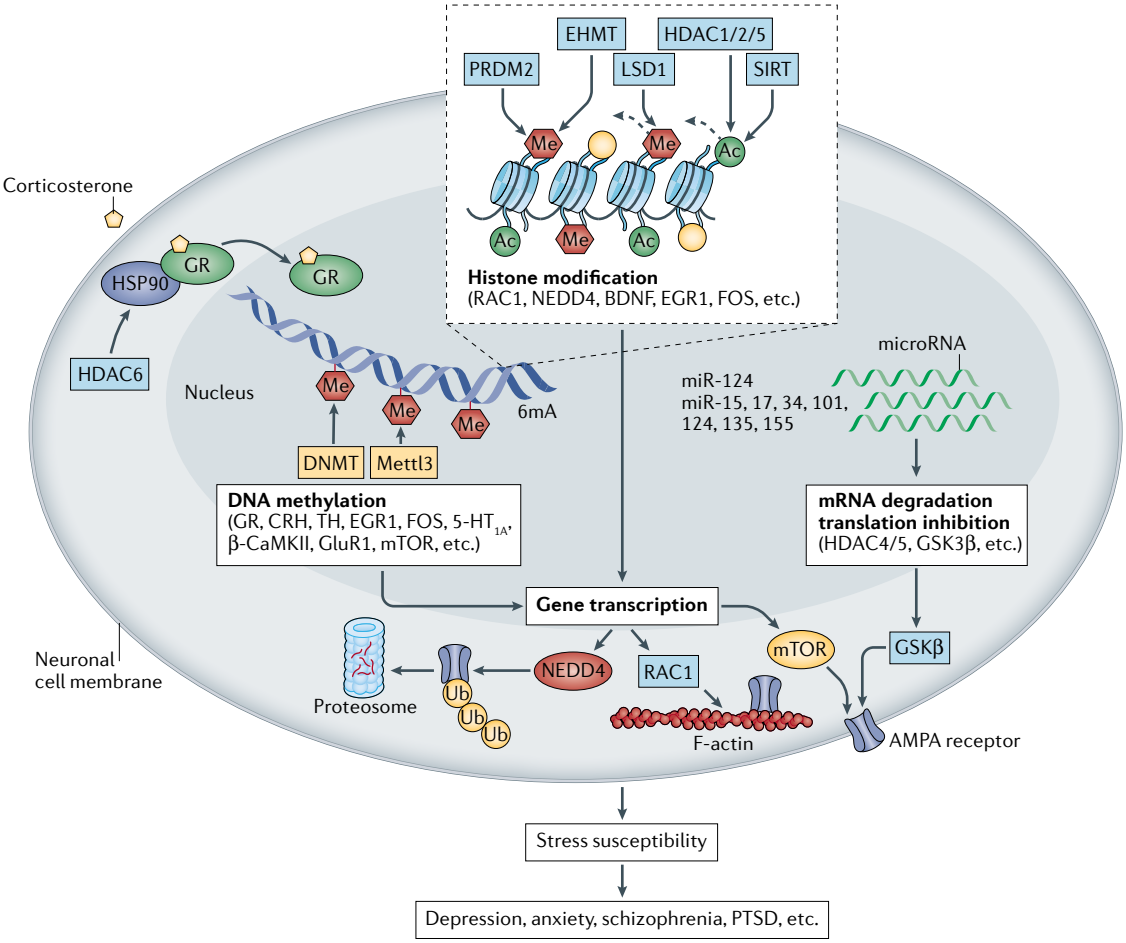

Fig. 3 | **Major epigenetic changes associated with chronic stress.** Stress exposure leads to changes in epigenetic enzymes, causing aberrant DNA methylation and histone modification at specific genes and microRNA alterations in target regions including prefrontal cortex (PFC), nucleus accumbens (NAc), ventral tegmental area (VTA), hippocampus (HPC) and other brain areas. Consequently, transcription and translation of molecules involved in stress response, synaptic plasticity and neuromodulator signalling are disrupted, resulting in maladaptive changes and stress-related mental disorders. Ac, histone acetylation; BDNF, brain-derived neurotrophic factor; CRH, corticotropin-releasing hormone; DNMT, DNA methyltransferase; EGR1, early growth response 1; GR, glucocorticoid receptor; HDAC, histone deacetylase; LSD1, lysine-specific demethylase 1; 6mA, N^6-methyladenine; Me, histone methylation; PTSD, post-traumatic stress disorder; TH, tyrosine hydroxylase; Ub, ubiquitin.

intra-NAc infusion of a SIRT1 antagonist reduces depression-like and anxiety-like behaviours[121], consistent with the association of the SIRT1 locus with major depression in humans[122]. Earlier studies have found a decreased level of HDAC2 and HDAC5 in the NAc in stressed animals or depressed humans[123,124], which seems to represent positive neuronal adaptations, as HDAC inhibitors exert robust antidepressant effects and reverse the effects of CSDS on global patterns of gene expression[124,125].

As illustrated above, chronic stress reduces excitatory transmission in select brain areas, including the PFC. A prominent loss of AMPA receptor signalling and GluR1 expression is observed in PFC pyramidal neurons of repeatedly stressed male rats, which is attributable to the increased ubiquitin–proteasome-mediated degradation of GluR1 that is controlled by the E3 ubiquitin ligase NEDD4 (REF.[73]). The transcription of *Nedd4* is found to be upregulated by repeated stress via an epigenetic mechanism involving the elevated HDAC2 (REF.[116]). HDAC2 inhibitors block the impairment of glutamatergic transmission and recognition memory in the PFC of chronically stressed animals[116]. These results suggest that targeting HDAC offers a potential therapeutic strategy for stress-induced cognitive and emotional impairment.

Other than histone acetylation, histone methylation also plays a key role in sculpting the response to stressful stimuli. G9a (also known as EHMT2), the histone methyltransferase that catalyses H3K9me2 (a repressive histone mark), has been found to mediate cocaine-induced vulnerability to stress[126]. CSDS (BOX 1) induces lasting downregulation of Bdnf III and IV transcripts in the HPC, which is accompanied by the increased repressive histone methylation at their corresponding promoters[127]. Moreover, CSDS-resilient versus susceptible male mice have distinct regulation of BDNF transcripts and epigenetic enzymes in the PFC and HPC[128]. The stress-induced epigenetic regulation

of *BDNF* transcription provides a critical mechanism underlying the vital role of BDNF in stress responses (see the section Chronic stress: molecular changes). In addition, a history of alcohol dependence persistently decreases the PFC expression of PRDM2, a histone methyltransferase that catalyses H3K9me1, which leads to the alteration of genes involved in synaptic communication and the enhancement of stress-induced relapse of alcohol seeking[129]. The transcriptional co-repressor lysine-specific demethylase 1 (LSD1) that removes the permissive histone mark H3K4me2 is identified as a molecular transducer of stressful stimuli and a stress-response modifier: psychosocial stress transcriptionally upregulates and changes LSD1 mRNA splicing, resulting in the alteration of target genes EGR1 and FOS, which govern anxiety-like behavioural phenotypes[130].

These studies have indicated that a diversity of histone-modifying enzymes work collectively to maintain the proper balance of histone acetylation and methylation at genetic loci. Stress often disrupts this balance at various sensitive neural circuits, leading to aberrant upregulation or downregulation of key genes involved in synaptic plasticity and intracellular signalling, facilitating the transition from an acute adaptive response to a chronic psychiatric illness (FIG. 3).

DNA methylation. DNA methylation, the chemical modifications on DNA molecules, such as 5-methylcytosine and 5-hydroxymethylcytosine, at key stress-response genes influences susceptibility or resilience to environmental stressors[131]. Early life adversity has been found to increase the DNA methylation of a neuron-specific exon 17 promoter of the glucocorticoid receptor (GR; also known as NR3C1), which leads to persistent HPA axis sensitivity and stress maladaptation[132]. A human study also reveals the hypermethylation of GR in depressed and bullied adolescents[133]. On the other hand, augmented maternal care induces persistent alterations of DNA methylation and associated changes in histone acetylation of GR promoter, as well as better HPA responses to stress, in the offspring, all of which are blocked by central infusion of a HDAC inhibitor[134] or methionine that serves as the donor of methyl groups for DNA methylation[135]. Enriched neonatal experience also recruits the transcriptional repressor NRSF (neuron-restrictive silencing factor) to chromatin, which leads to the alteration of DNA methylation and expression of target genes highly relevant to stress resilience in hypothalamic neurons[136,137]. In animals exposed to CSDS (BOX 1), the reduced DNA methylation of the corticotropin-releasing hormone (CRH) gene and the elevated expression of CRH are found to be linked to stress vulnerability and stress-induced social avoidance[138].

In addition to stress-response genes, many other genes involved in synaptic plasticity, neuronal activity and receptor signalling are also subject to DNA methylation changes, which are associated with stress responsivity[117]. For example, in a mouse model carrying a genetic risk factor for neuropsychiatric disorders, adolescent isolation stress (3 weeks) induces DNA hypermethylation of the tyrosine hydroxylase (TH) gene in mesocortical dopaminergic neurons, resulting in reduced TH expression and dopamine release, as well as behavioural abnormalities, all of which are blocked by a GR antagonist[139]. In an animal model of PTSD, a psychosocial stress regimen significantly increases *Bdnf* DNA methylation and decreases *Bdnf* mRNA in the dorsal HPC[140]. Similarly, the decreased *Bdnf* expression in the HPC and amygdala of prenatally stressed rats at weaning and in adulthood is accompanied by the increased DNA methylation in *Bdnf* exon IV[141]. In animals exposed to an acute stressor, DNA demethylation at FOS and EGR1 promoters and the induction of these immediate early genes are observed in the dentate gyrus, which is associated with the impaired behavioural immobility response[142]. In animals exposed to CUS (BOX 1), the expression of 5-HT_{1A}, a negative regulator of serotonergic activity, is increased in the PFC, which is paralleled by a specific increase in DNA methylation of the 5-HT_{1A} promoter site that is repressed by SP4 transcription factor[110]. By contrast, a significant decrease of DNA methyltransferase 1 (Dnmt1) and DNA hypomethylation is observed in the LHb of chronically stressed animals, which results in the increased transcription of β-CaMKII and GluR1 in the LHb and the attenuation of 5-HT release in the dorsal raphe nucleus[143]. These results have highlighted the role of cell type-specific DNA methylation of specific genes in controlling stress responses and emotional state.

A novel DNA adenine modification, N^6-methyladenine (6mA), is found to be significantly elevated in the mouse brain upon stress, which is associated with the transcriptional alteration of neuronal genes, and genes bearing stress-induced 6mA changes significantly overlap with loci associated with neuropsychiatric disorders[144]. Consistently, regulation of 6mA is impaired in patients with MDD following glucocorticoid stimulation[145]. The methyltransferase METTL3 and the demethylase FTO control the 6mA epitranscriptome, which regulates transcriptome response to fear and fear memory[145].

Genome-wide profiling of the DNA methylome and transcriptome in peripheral blood cells of humans with MDD has revealed 39 differentially methylated regions and 30 differentially expressed genes, which are enriched in signalling pathways related to stress responses, mTOR signalling and neuron apoptosis[146], suggesting that altered DNA methylation could serve as an epigenetic marker for MDD and related disorders (FIG. 3).

microRNA. The small, regulatory, non-coding microRNAs (miRNAs), which act as a molecular switch of gene expression, have been found to have a pivotal role in stress-associated neuronal plasticity. The brain-enriched miR-124 is reduced in the HPC of mice exposed to chronic stress, and miR-124 overexpression in hippocampal neurons confers behavioural resilience to stress[147]. Moreover, HDAC4, HDAC5 and GSK3β are identified as targets of miR-124, which mediates the effect of miR-124 on stress sensitivity and depression-like behaviours[147]. The expression of miR-124-3p is highly dysregulated in stressed rodents and antidepressant-free subjects with MDD, and the interaction of miR-124-3p with the RNA-induced silencing complex (RISC) is

compromised in people with MDD[148]. Recent small-RNA sequencing has revealed the persistently changed miRNAs, including miR-135b, in the basolateral amygdala complex of a PTSD mouse model and in serum from military veterans with PTSD[149]. Overexpression of basolateral amygdala complex miR-135b in stress-resilient animals enhances remote fear memory expression, whereas inhibition of basolateral amygdala complex miR-135b in stress-susceptible animals promotes the resilient-like phenotype[149].

More miRNAs have been found to play a causal role in regulating anxiety-like behaviours, such as miR-15a, miR-17-92, miR-34, miR-101, miR-124, miR-135 and miR-155, and their targets include those associated with the HPA axis, synaptic plasticity and neuromodulator signalling[150]. Moreover, miRNAs are regulated in distinct brain regions by various anxiolytic strategies, including antidepressant treatments, as well as non-pharmacological interventions, such as fear extinction therapy or exposure to environmental enrichment[150]. These studies have opened up possibilities for miRNAs to be used as biomarkers and drug targets for depression, PTSD and anxiety-related disorders (FIG. 3).

Pharmacologically targeting stress

The growing understanding of mechanisms linking stress and psychopathology has stimulated attempts to target components of the stress response in developing treatments for stress-related disorders such as MDD and anxiety. Early attempts to target the HPA axis have included pharmacological blockade of cortisol synthesis and antagonists of the CRF1 receptor and GR. Despite promising preclinical and early-phase clinical studies, larger late-phase clinical trials have consistently yielded disappointing results[151]. The inability to demonstrate clinical effectiveness with this broad class of drugs has caused consternation, leading some to question the general approach of targeting stress-response mechanisms for the development of therapeutics. Others believe unresolved issues related to the heterogeneity and complex developmental staging of the disorders, in addition to the practical challenges of achieving adequate drug levels in the brain, are possible explanations for the findings in the larger trials.

Ketamine-induced optimism. The greatest success to arise, at least in part from attempts at targeting stress-related physiological mechanisms, has been the discovery of ketamine's antidepressant effects[152]. Early studies showing abnormalities in glutamatergic neurotransmission associated with rodent stress models contributed to the hypothesis that NMDA receptor antagonism could produce antidepressant actions[153,154]. The rapid onset of antidepressant effects following sub-anaesthetic ketamine dosing is now well established[155], and (*S*)-ketamine (the enantiomer with greatest affinity for the NMDA receptor) has received US Food and Drug Administration (FDA) indications for treatment-resistant MDD and MDD associated with suicidal ideation and behaviour[156]. The discovery of ketamine's unique antidepressant properties has inspired a wave of back-translational studies that have dramatically expanded our understanding of the mechanisms mediating the antidepressant response and the relationship of these antidepressant mechanisms to stress-related pathophysiology.

Interestingly, many of the structural and/or functional changes observed following ketamine treatment appear closely related, and often in opposition to the changes induced by chronic stress. As noted above and highlighted in FIG. 4, ketamine has several effects on the glutamate neurotransmission that result in mTORC1 activation, enhancement of local protein synthesis and regional changes in dendritic spine density[15,157]. Ketamine's effects on mTORC1 function and, ultimately, neuroarchitecture appear to be mediated through a transient surge in glutamate release from pyramidal cells within the PFC, and potentially other brain regions, and stimulation of AMPA receptors[158–161]. Whereas the precise mechanisms driving the enhanced release of glutamate following ketamine administration are not yet fully understood, evidence suggests that a preferential blockade of NMDA receptors present on inhibitory GABA interneurons contributes to the surge in glutamate efflux[162,163]. This effect may be related to ketamine's affinity for the GluN2D subunit of the NMDA receptor, which is highly expressed on the GABAergic interneurons[164]. The transient increase in glutamate release is postulated to result in activation of AMPA receptors and increase in BDNF release that, ultimately, provides positive feedback on the system giving rise to increased synaptic connectivity. Alternatively, it has also been proposed that ketamine's antidepressant effect can be mediated by selective blockade of activity-independent NMDA receptors involved in spontaneous synaptic transmission. These receptors function to deactivate calcium/calmodulin-dependent kinase eukaryotic elongation factor 2 kinase (eEF2K), releasing eukaryotic elongation factor 2 (eEF2) to facilitate translation elongation at the ribosome[165]. Thus, ketamine's antagonism of the activity-independent NMDA receptor results in increased local protein synthesis and the subsequent release of a suppressed BDNF protein synthesis in brain regions critical to the pathology of depression. Similarly, it is possible that ketamine's blockade of extrasynaptic NMDA receptors that typically reduce local protein synthesis through mTOR-dependent mechanisms may also contribute to the drug mechanism of action[166]. Based on rodent studies suggesting metabolites of ketamine with very low affinity for the NMDA receptor retain antidepressant-like effects, others suggest ketamine's antidepressant-like effects in rodent models may, in fact, be independent of NMDA receptor antagonism[167,168].

Mechanistic role of BDNF and mTORC1. In contrast to the disagreement surrounding the proximal events initiating ketamine's antidepressant-like action, there is converging evidence demonstrating the critical roles of BDNF expression, regionally enhanced local protein synthesis and increased synaptic plasticity in mediating the antidepressant-like effects in these models (see above). Another recent report suggests that ketamine, as well as other antidepressant drugs, may allosterically

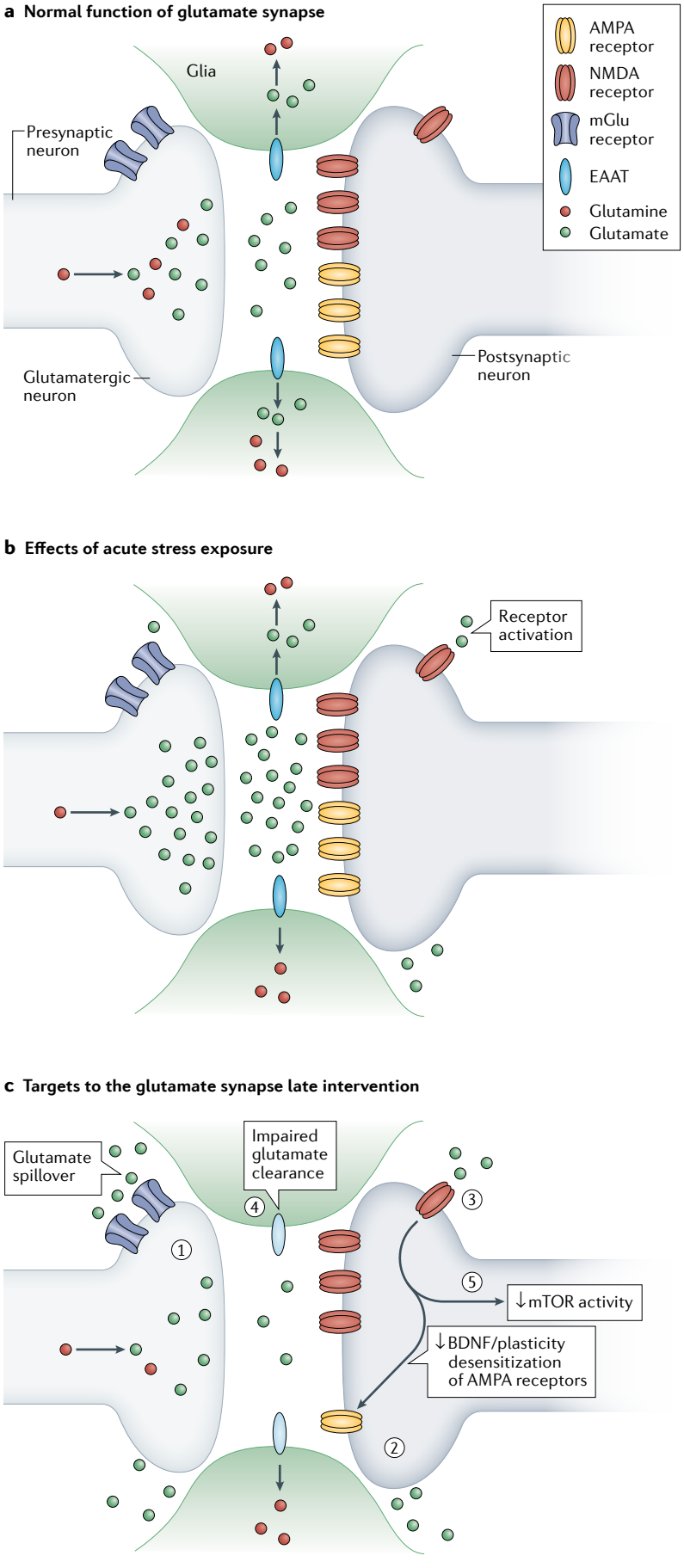

Fig. 4 | **Putative maladaptive stress-induced changes at the tripartite synapse and identified drug targets.** **a** | Normal functioning of tripartite glutamatergic synapse, with clearance tightly matching release, thus limiting synaptic spillover. **b** | Transitions observed in synapse following acute stress exposure in rodent models. Increased release of glutamate and increased signalling through extrasynaptic receptors found following acute stress exposure. **c** | More persistent effects of chronic stress, or possibly even sustained impact of certain classes of single stress exposure, including impaired glutamate clearance through excitatory amino acid transporters (EAATs), increased activation of extrasynaptic NMDA receptors and downstream effects resulting in changes in neurotrophic and metaplastic potential, and effects on group II metabotropic receptors limiting phasic glutamate release and altering glutamatergic system's effective signal-to-noise ratio. Also highlighting how these sites provide potential targets for pharmacological intervention. Drugs capable of modulating glutamate release either by acting on ion channels, vesicular proteins and group II metabotropic receptors or, indirectly, by modulating inhibitory tone on the presynaptic neuron (target 1). Drugs potentiating activation of synaptic ion channels such as AMPA receptors offer a means of enhancing the signal-to-noise ratio disturbed by the relatively increased levels of extrasynaptic glutamate (target 2). Drugs modulating sensors of extrasynaptic spillover signalling by targeting group I and group II metabotropic glutamate receptors and extrasynaptic NMDA receptors may protect against excitotoxic effects (target 3). Various treatments facilitating extracellular glutamate clearance by enhancing expression and function of EAATs could offer protection and help normalize the signal-to-noise aspects of glutamate signalling that are disrupted following stress exposure (target 4). Treatments more directly targeting intracellular pathways such as mechanistic target of rapamycin complex 1 (mTORC1) that lie downstream of the more proximal sensors of stress signalling may allow for alternative approaches to drug development (target 5). BDNF, brain-derived neurotrophic factor.

facilitate BDNF signalling by directly binding to the TRKB receptor, and thereby facilitating synaptic localization enhancing BDNF activation[169]. However, despite the emerging consensus arising from the rodent models, inconsistencies can be found in the translational human studies exploring the role of BDNF expression and mTORC1 activation in ketamine's antidepressant action. Although an initial study showed that, similar to the rodent studies, people with MDD carrying the lower-expressing V66M SNP of BDNF were less responsive to ketamine treatment[170], later studies failed to replicate this finding[171,172]. Nevertheless, the SNP may have effects on the dose–response relationship and some specific symptoms[173]. Another recent study exploring the role of mTORC1 in mediating ketamine antidepressant response by pretreating patients with rapamycin prior to ketamine administration did not show any diminution in the antidepressant effect[174]. In fact, the post hoc secondary analysis found unexpected evidence of an extended response when rapamycin was administered prior to ketamine. Although potential confounds, such as poor rapamycin brain penetration and rapamycin-related effects on immune function that potentially promote antidepressant actions, have limited

the interpretation of the study, the difficulty in clearly identifying critical factors mediating the antidepressant effect in humans has frustrated attempts to ascertain a more complete understanding of ketamine's mechanism of antidepressant action at the cellular/molecular levels.

Ketamine alters connectivity/circuitry. At the circuit level, there is strong evidence demonstrating that sub-anaesthetic treatment with ketamine has region-specific effects on brain function as evidenced by brain metabolism studies in rodent models. Autoradiography and magnetic resonance studies using blood oxygen level-dependent MRI show broad but region-specific effects of ketamine on brain metabolism in rodents shortly after administration[175]. The effects are seen in limbic-related regions of the medial PFC and HPC, and appear to be highly dose dependent, with lower sub-anaesthetic doses increasing and higher doses reducing metabolism globally. More recent studies show ketamine can rapidly alter the metabolism and function of several rodent brain regions commonly associated with cognitive, sensory, subcortical emotion and reward-related circuitry[176]. Other work has shown that ketamine, by acting on the dopamine system, restores synaptic plasticity of the HPC–accumbens pathway disrupted by chronic stress[177]. Reviewing existing human neuroimaging studies, ketamine's effects on metabolism and activations were most notably found in the subgenual anterior cingulate cortex, posterior cingulate cortex, PFC and HPC[178].

Related to the reduced synaptic connectivity associated with chronic stress, studies show consistent effects of ketamine inducing changes in functional connectivity between regions of the PFC and HPC in both rodents and humans that appear related to the sustained antidepressant action[179]. These effects also appear dependent on the dose used, the time of measurement after exposure and, possibly, even the pretreatment status/condition of the subject. Ketamine has repeatedly been shown to reverse prefrontal dysconnectivity for a period of up to 24 h following an infusion[180,181]. Ketamine's effects on functional connectivity suggest a pattern of enhanced top-down control, shifting from internal connectivity towards external connectivity between executive regions and primary cortices[182,183].

Other glutamatergic targets

In addition to targeting the NMDA receptor, there have been several attempts to target other components of the glutamatergic synapse to develop novel therapeutics for stress-related neuropsychiatric illnesses. Several attempts have been made at targeting the group II metabotropic receptors in efforts to modulate glutamate release and improve clinical symptoms. Following from the belief that the rapid surge in glutamate excitation in several critical brain regions could reactivate areas atrophied or damaged by chronic stress, drugs acting to decrease mGlu2/3 receptor activity, and thereby decrease the inhibition of glutamate release, have been studied[184,185]. In general, there is strong preclinical evidence suggesting that this approach could produce beneficial effects, reversing many of the cellular and behavioural effects induced by stress; however, the limited clinical trials to date have failed to provide additional support for their use in treating the disorders[184]. More recent work suggesting unique and region-specific physiological roles for the mGlu2 receptors in modulating glutamate release and mGlu3 receptors in modulating postsynaptic plasticity suggest that more specific drug targeting of the receptors could be employed in different approaches to treat the disorders at various stages of development[186]. Also, indicating varying effects of the targeted mGlu2/3 treatments related to the timing of administration, mice with reduced hippocampal mGlu2 expression were found to be more susceptible to CUS. This suggests that mGlu2 function may play a critical role in modulating stress susceptibility, and that increased activation of the receptor could protect against stress-related pathology by limiting stress-induced glutamate release[187]. However, there are contradictory findings suggesting that blockade of the mGlu2/3 receptors enhance stress resilience[188]. The complex interaction of the mGlu2/3 receptors with stress-response physiology may help explain the seemingly paradoxical findings of antidepressant-like responses associated with both positive and negative allosteric mGlu2 modulators in a rodent model[184].

Similarly, there have been efforts to target the group I mGlu5 receptor in developing novel treatments for various neuropsychiatric disorders. Postsynaptic mGlu5 receptors are functionally linked to both NMDA and AMPA receptors, and have effects on local protein synthesis and synaptic plasticity that could be central to the production of antidepressant and anxiolytic-like behaviours in rodent models[189]. There are numerous studies demonstrating the antidepressant and anxiolytic effects of various mGlu5 receptor antagonist and negative modulating drugs in a range of rodent models[190]. Again, however, the limited clinical trials conducted to date with mGlu5 negative allosteric modulators have failed to provide further evidence to support their clinical utility[184]. As with the group II metabotropic drugs, there is evidence of both negative and positive mGlu5 receptor modulation having antidepressant-like properties in rodent models. Again, this highlights the complexity of the system and the likely impact of region, dose and timing of drug administration on the physiological and behavioural effects.

An alternative mechanism of targeting the stress-induced changes occurring within the glutamatergic system has focused on modifying the efficiency of extracellular glutamate clearance. If, as several models suggest, the damaging effects of stress-related toxicity are related to excessive extrasynaptic glutamatergic activation[191], then limiting the extrasynaptic spread of glutamate could provide a means of reducing, and possibly reversing, the pathological effects of stress exposure. A cadre of excitatory amino acid transporters (EAATs) are responsible for maintaining low levels of glutamate outside the synaptic space. EAAT2, the transporter primarily responsible for clearance of extracellular glutamate in most regions of the brain, is largely located on astroglial cells abutting glutamatergic synapses. Changes in astrocytic structure and function at the synapse have been shown to modulate synaptic function and, possibly,

stabilize several forms of learning[192–195], including threat conditioning[196] that is commonly associated with stress-related neuropsychiatric conditions. Impaired EAAT2 function in the primate subgenual cingulate cortex specifically enhances the cardiovascular, behavioural and neural responses to threat, possibly contributing to the changes in basic physiological processes commonly associated with stress-related disorders[197]. Interesting work now shows that the level of EAAT2 is subject to epigenetic regulation, including histone modification and DNA methylation[198], suggesting that previous life experiences could dramatically impact future stress response and resilience.

Riluzole, a drug originally developed as a treatment for amyotrophic lateral sclerosis, has been shown to enhance the expression and function of EAAT2 and has been demonstrated to have neuroprotective effects in a wide array of stress and injury models[199–203]. Riluzole has specifically been demonstrated to attenuate many of the molecular, cellular and behavioural effects related to CUS (BOX 1) in rodent models[45]. Clinical trials attempting to use riluzole as a treatment for mood and anxiety disorders have yielded mixed results to date. Several early studies suggested potential benefits in depression and obsessive-compulsive disorder, and, possibly, symptom-limited effects in PTSD, but no clear evidence of clinical efficacy has been demonstrated in the larger studies completed to date[204–208]. However, perhaps the most striking example of riluzole's protective effects is found in its ability to shield against the age-related decrements in spine density and cognitive function[209], and attenuation of the cognitive decline in several murine models of dementia and Alzheimer disease[210–214]. Highlighting the potential interaction between stress, glutamate toxicity and age-related cognitive decline, riluzole was recently shown to attenuate the deficits in long-term potentiation related to ELS in an EAAT2-dependent manner within a genetic rodent model of Alzheimer disease[215].

Conclusions and future perspectives

In summary, our understanding of the pathophysiology of stress-related disorders has seen great advancement, with the discovery of several mechanisms directly or indirectly related to changes in the function and plasticity of glutamatergic synapses. These mechanisms have been summarized here in a scalar approach, going from molecular/cellular level to circuitry, neuroarchitecture and large brain networks. The crucial role of epigenetic mechanisms in mediating the effects of stress and the additional role of sex has also been illustrated. The many lines of evidence have stimulated increasing efforts for the development of therapeutics for various neuropsychiatric disorders. Although there is good evidence for effectiveness of several approaches targeting the HPA axis and glutamatergic systems in animal models, there remains limited evidence of efficacy in clinical trials. At present, only esketamine has demonstrated clinical benefit and relative safety sufficient to receive approval for the treatment of depression by the FDA. Several other drugs targeting various aspects of stress responses, including orexins[216], neurosteroids[217] and intracellular modulators of mTORC1 function[218], are currently being tested for a range of stress-related disorders. The increased awareness of the interaction between stress physiology, reward-related circuitry impairments and dopamine neurotransmission has also helped to spur renewed interest in exploring the potential clinical benefits of dopamine targeting drugs such as the D3-preferring dopamine agonist pramipexole[219] or the κ-opioid receptor antagonist JNJ-67953964 (REF.[220]). As we better understand the varied systems contributing to stress physiology, the possible targets will increase. Well-designed clinical trials will be required to tell whether this refocused effort can truly yield clinically meaningful advances in our treatment of neuropsychiatric disorders.

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Author contributions
All authors contributed equally to the preparation of this manuscript.

Competing interests
G.S. has served as a consultant for Allergan, Alkermes, AstraZeneca, Avanier Pharmaceuticals, Axsome Therapeutics Biohaven Pharmaceuticals, Boehringer Ingelheim International GmbH, Bristol-Myers Squibb, Clexio, Denovo Biopharma, Engrail Therapeutics, EMA Wellness, Epiodyne, Intra-Cellular Therapies, Janssen, Lundbeck, Merck & Co., Navitor Pharmaceuticals, Neurocrine, Novartis, Noven Pharmaceuticals, Otsuka, Perception Neuroscience, Praxis Therapeutics, Sage Pharmaceuticals, Taisho Pharmaceuticals, Valeant, Vistagen Therapeutics, and XW Labs over the past 36 months; has received research contracts from AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Johnson & Johnson, Hoffman La-Roche, Merck, Naurex, Servier and Usona over the past 36 months; holds equity in BioHaven Pharmaceuticals; and is a co-inventor on US Patent 8,778,979 held by Yale University and a co-inventor on US Provisional Patent Application No. 047162-7177P1 (00754) filed on 20 August, 2018 by Yale University Office of Cooperative Research. Z.Y. has no competing interests. M.P. has received research contracts from Rodin Therapeutics (now Alkermes) in the last 36 months; and has received research contracts from Merck, GlaxoSmithKline, Servier, Sigma-Tau, Fidia and Abbott.

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