Biological studies of post-traumatic stress disorder

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Abstract | Post-traumatic stress disorder (PTSD) is the only major mental disorder for which a cause is considered to be known: that is, an event that involves threat to the physical integrity of oneself or others and induces a response of intense fear, helplessness or horror. Although PTSD is still largely regarded as a psychological phenomenon, over the past three decades the growth of the biological PTSD literature has been explosive, and thousands of references now exist. Ultimately, the impact of an environmental event, such as a psychological trauma, must be understood at organic, cellular and molecular levels. This Review attempts to present the current state of this understanding on the basis of psychophysiological, structural and functional neuroimaging, and endocrinological, genetic and molecular biological studies in humans and in animal models.

Skin conductance
A measure of sweat activity recorded from two adjacent fingers and/or the thenar and hypothenar eminences of the palm of the hand. It is thought to be primarily under sympathetic nervous system influence.

Event-related potentials
(ERPs). Electrical potentials that are generated in the brain as a consequence of the synchronized activation of neuronal networks by external stimuli. These evoked potentials are recorded at the scalp and consist of precisely timed sequences of waves or 'components'.

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A day scarcely passes that one does not see a mention of post-traumatic stress disorder (PTSD) in the media. However, this has not always been the case. In American history, post-traumatic psychopathology has been recognized under various names following wars: soldier's heart from the American Civil War, shell shock from the First World War, combat fatigue from the Second World War and delayed stress from the Vietnam War. However, between these wars, the condition was all but forgotten. Spearheaded by Vietnam veterans and their advocates, PTSD finally made its way into the American psychiatric nomenclature as a formal diagnostic entity in 1980 (REF. 1). Because of the political impetus behind its introduction and the fact that PTSD is largely diagnosed on the basis of patients' reports (which may be influenced by secondary motives), the new disorder was initially met with suspicion. Discoveries of biological markers for PTSD, however, have gone a long way to counteracting this scepticism and bolstering the now widespread acceptance of the disorder.

As currently understood, PTSD is a blend of intrusive memories of the traumatic event, avoidance of reminders of it, emotional numbing and hyperarousal[2]. Initially, PTSD was conceptualized nearly entirely in psychological terms, and the PTSD biological literature consisted only of sparse psychophysiological observations[3]. Although purely psychological research into PTSD is important, it is enhanced by an understanding of the neurobiological mechanisms underlying the disorder. The purpose of this article is to review the current state of biological research into PTSD. Such a review is timely and important if the popular understanding of PTSD is not to outstrip its scientific basis and if PTSD is to be grounded in the field of medicine. This article also reviews animal models, which have stimulated hypotheses about PTSD in humans and which point the way to future developments in the field. The ultimate goals of biological research are to identify risk factors, elucidate the mechanisms involved in the development of PTSD, establish biomarkers and generate novel preventive and therapeutic interventions that are aimed at alleviating the substantial suffering and dysfunction this disorder imposes.

It is tempting to assume that because PTSD by definition is caused by a psychologically traumatic environmental event, any biological abnormality found to accompany PTSD must also have been traumatically induced. However, it is also possible that an abnormality pre-dated the traumatic event and came to be associated with PTSD because it increased the risk of development of the disorder upon traumatic exposure — a psychiatric epitome of gene by environment interaction. Readers should keep this in mind as they digest the rich material that follows.

Psychophysiological studies

Measures of heart rate, skin conductance, facial electromyogram (EMG) and cortical electroencephalographic event-related potentials (ERPs) have been extensively applied to the study of PTSD for more than 25 years[4,5]. A robust literature addresses the heightened emotional reactivity to trauma-related cues, exaggerated startle,

Extinction
A procedure by which a conditioned stimulus is repeatedly presented in the absence of the unconditioned stimulus, resulting in diminution of the conditioned response.

P3b
An electroencephalographic event-related potential response that is positive and reaches its maximum deflection approximately 300 ms after a stimulus is presented. It is thought to reflect the amount of attentional resources allocated to the stimulus.

Fear conditioning
An experimental paradigm that teaches an animal or human to associate a previously neutral conditioned stimulus (CS; for example, a light or a tone) with an aversive unconditioned stimulus (for example, an electric shock), the latter of which produces an aversive unconditioned response. Eventually, because of the association, the CS alone comes to elicit a fear response.

Extinction retention
Memory that a conditioned stimulus has been previously extinguished, expressed as a continuing reduction of the conditioned response. It is also called extinction recall.

Fear-potentiated startle
An increased electromyographic responsiveness to a startling stimulus that occurs when an animal or human is afraid.

impaired extinction and increased sensitivity to stimulation observed in people with PTSD, as well as their use in predicting PTSD risk and assessing treatment outcome. A meta-analysis of studies comparing resting psychophysiological levels, trauma-related cue reactivity and exaggerated startle responses between groups of individuals diagnosed with PTSD versus individuals without PTSD attests to the maturation of this field[6].

Psychophysiological markers for post-traumatic stress disorder. Most of the psychophysiological research on PTSD has been performed in cross-sectional studies that compared individuals with PTSD to trauma-exposed or trauma-unexposed individuals without the disorder. One of the earliest and most replicated PTSD findings is that of heightened autonomic reactivity (such as heart rate and skin conductance) and facial EMG reactivity to external, trauma-related stimuli, such as combat sounds and film clips[7], as well as to internal, mental imagery of the traumatic event[8]. Reactivity to trauma-related cues correlates with the severity of the disorder[9,10]. In addition to research focused on reactions to trauma reminders, a substantial body of work has examined exaggerated startle, as measured by eye-blink EMG, to sudden loud sounds[4,6]. Although there is compelling evidence for increased startle in PTSD, it is unclear whether this represents a pre-existing trait, increased sensitivity to contextual threat or sensitization of the nervous system. Patients with PTSD also show heightened heart rate responses to startling stimuli, which appear to be acquired[11,12]. Reduced P3b ERPs to infrequent target stimuli and larger skin conductance responses to novel stimuli[4] could reflect the PTSD symptoms of difficulty concentrating and hypervigilance, respectively.

Psychophysiological assessments of treatment outcome provide more objective information than a patient's report and may be a more sensitive measure of progress. For example, heart rate, skin conductance and facial EMG reactivity during personal traumatic imagery was lower in a group that had received post-trauma propranolol (a β-adrenergic antagonist that has been found to attenuate the consolidation of stressful memories in animal and human studies) compared to placebo, even though groups did not differ in subjective PTSD symptom severity[13]. A recent study that examined psychophysiological responses before and after cognitive behavioural therapy for PTSD found that treatment responders showed a significant reduction in eye-blink EMG, heart rate and skin conductance responses to loud tones, whereas treatment non-responders did not[14]. Understanding discordances between psychophysiological and subjective measures of treatment outcome will require further research. Pretreatment psychophysiological assessments might be examined for their usefulness in guiding treatment selection. For example, individuals who show heightened psychophysiological reactivity to trauma-related cues might benefit from exposure-based therapies that aim to desensitize the emotional arousal associated with traumatic memories.

Conditioning and sensitization. Symptoms related to re-experiencing the traumatic event are a defining feature of PTSD and may be conceptualized within a fear conditioning framework. By contrast, anxiety and hyperarousal in the absence of trauma-related cues may reflect a general sensitization of the nervous system. Alterations in fear conditioning, extinction learning, extinction retention and sensitization are likely to be involved in the development and/or maintenance of PTSD[15]. Recent findings suggest that PTSD is associated with deficits in the ability to extinguish or maintain extinction learning of an acquired fear response, as measured by skin conductance[16] and/or fear-potentiated startle[17]. In addition, PTSD has been found to be associated with extinction failure of a second-order conditioned skin conductance response, after this response was established by pairing a neutral stimulus with a trauma-specific stimulus[18]. A recent study in identical twins discordant for combat exposure (that is, one member of each pair was a Vietnam combat veteran and the other had no combat exposure) found that twins with PTSD showed poorer extinction retention of a conditioned skin conductance response; this appeared to be an acquired rather than a pre-existing PTSD feature because it was not shared by the twins of veterans with PTSD[19]. Impaired extinction or extinction retention would probably interfere with recovery from PTSD symptoms that are based on conditioned fear.

Evidence for increased nervous system sensitization comes from findings of larger heart rate responses to loud-tone stimuli and larger skin conductance orienting responses[4], as well as increased intensity dependence of the P2 ERP response[20]. In a study that involved both startle and conditioned fear assessments, individuals with PTSD were found to show increased eye-blink EMG startle responses during testing that followed a previous session involving aversive fear conditioning[21]. A possible explanation for this finding is that administration of the unconditioned stimulus (a shock) several days earlier sensitized individuals with PTSD, such that they showed a subsequent increase in baseline startle reactivity. Findings from identical twin[11,20] and longitudinal[12,22] studies strongly suggest that the heightened heart rate reactivity to loud tones observed in individuals with PTSD is an acquired feature of the disorder; however, it may not be specific to PTSD[23].

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P2
An electroencephalographic event-related potential response that is positive and reaches its maximum deflection approximately 200 ms after a stimulus is presented. It is thought to reflect 'tuning' of a mechanism that regulates the amount of sensory input to the cortex.

Conditioned fear
Fear that is elicited by a conditioned stimulus (or cue) following fear conditioning. Typical measures include freezing in rodents, skin conductance in humans and potentiated startle in both.

Corrugator EMG
A measure of electromyographic activity associated with contraction of the corrugator supercilii muscle, which draws the inner brow inward and downward during negatively valenced emotion.

Structural MRI
(sMRI). A non-invasive diagnostic and research procedure that uses a magnetic field and radio waves to create detailed sectional images of the internal structure of the body, including the brain.

Ventromedial prefrontal cortex
(vmPFC). A region within the medial wall of prefrontal cortex that roughly corresponds to Brodmann area 10. Some studies treat portions of adjacent Brodmann areas as part of the vmPFC.

CA3
A sector of the cornu ammonis subfield of the hippocampus and a major target of glucocorticoids.

Dentate gyrus
A subfield of the hippocampus that contains adult neural stem cells and is an important site of neurogenesis.

Psychophysiological risk factors for post-traumatic stress disorder. The possibility that some measures of psychophysiological reactivity represent pre-existing vulnerability traits has stimulated research examining potential predictors of the risk of developing PTSD. A prospective study of firefighter trainees found that increased skin conductance and eye-blink EMG responses to a series of loud tones[24] and slower extinction of fear-conditioned corrugator EMG responses[25] predicted the severity of post-traumatic stress symptoms following subsequent exposure to a traumatic event. Another prospective study also implicated slower extinction of fear-conditioned corrugator EMG responses in predicting post-traumatic stress symptoms[26]. Increased physiological reactivity to trauma-related cues soon after exposure to a traumatic event is predictive of subsequent severity and/or persistence of PTSD symptoms[9,27,28]. For example, women who showed an increased heart rate response while engaging in personal traumatic imagery 2 weeks after trauma exposure had higher symptom severity and were more likely than female non-responders and men to develop PTSD 6 months later[10]. Whether resting heart rate obtained immediately post-trauma predicts the subsequent development of PTSD is currently unclear[27,29].

Sleep. Although sleep disturbance is a PTSD diagnostic criterion, support for it in the polysomnography laboratory has not been as straightforward as might have been expected. However, more stage 1 sleep and less slow-wave sleep — which is indicative of shallower sleeping — as well as greater rapid-eye movement density have been demonstrated in individuals with PTSD[30].

Structural neuroimaging studies

As findings in experimental animals suggested that chronic stress damages the hippocampus[31], this brain structure was a logical starting place for structural MRI (sMRI) studies in patients with PTSD. In fact, the most replicated structural abnormality found in PTSD is a lower volume of the hippocampus. A lower volume of the ventromedial prefrontal cortex (vmPFC), which is another structure that is damaged by chronic stress in animals, has also been reported. However, just because these structures can be damaged by chronic stress in animals does not necessarily mean that this is the origin of their diminutions in PTSD; they may also represent premorbid risk factors. To the extent that diminished volume may underlie diminished function, these findings — whatever their origin — are consistent with a model of PTSD that posits a reduced cortical capacity to inhibit fear and other negative emotional responses. In this view, the hippocampus may fail to use contextual cues in the environment to signal safety, and the vmPFC may fail to adaptively maintain extinction of conditioned emotional responses once traumatic learning is no longer relevant.

Hippocampus. Pioneering sMRI studies found significantly smaller hippocampi in subjects with PTSD compared to trauma-exposed and non-trauma-exposed subjects without PTSD[32–34]. Since then, a large literature has emerged, most of which, along with meta-analyses[35], has provided empirical support for a lower hippocampal volume in PTSD. A recent study that used high-resolution sMRI found the most substantial hippocampal volumetric diminution in the CA3 and dentate gyrus subfields[36]. Recent meta-analyses have concluded that smaller hippocampal volume in PTSD is bilateral[37,38], and they have not provided strong evidence for a gender effect[39]. The severity of PTSD symptoms may be an important factor in determining effect sizes of PTSD-related hippocampal differences[38,40]; studies of adults with PTSD that failed to replicate the finding of smaller hippocampal volumes generally included subjects with less severe or less chronic illness[41,42]. Studies conducted in children with PTSD have often also failed to reveal smaller hippocampi, suggesting a role for neuromaturational factors in the development of hippocampal diminution[43]. Numerous investigations have attempted to control for the impact of confounding comorbid conditions; most have concluded that hippocampal volume differences in PTSD are not accounted for by histories of alcohol abuse or depression[40,44,45]. Moreover, studies using magnetic resonance spectroscopic imaging (MRSI), which quantifies *N*-acetylaspartate as a marker of neuronal density, have also consistently found neuronal reduction in the hippocampus of patients with PTSD[46,47].

Controversy exists as to whether smaller hippocampal size in PTSD is a result of trauma exposure or rather represents a risk factor for PTSD that is of genetic and/or environmental origin. A study of identical twins discordant for combat exposure in Vietnam (FIG. 1) found that the (high-risk) combat-unexposed, non-PTSD co-twins of combat veterans with PTSD had hippocampal volumes that were comparable to that of their twins with PTSD and lower than the hippocampal volume of combat veterans without PTSD and their (low-risk) combat-unexposed co-twins[40]. This finding suggests that hippocampal volume serves as a pre-trauma risk factor for PTSD. Cavum septum pellucidum (the so-called 'fifth ventricle'), which is a neurodevelopmental abnormality in part related to hippocampal maldevelopment, is found more frequently in patients with PTSD[48]. Not all neuroimaging evidence is consistent with a risk factor interpretation of hippocampal diminution[49]. Pharmacological treatment of PTSD with the selective 5-hydroxytryptamine (5-HT; also known as serotonin) reuptake inhibitor (SSRI) paroxetine has been reported to increase hippocampal volume[50], suggesting that reduced hippocampal volume could be an acquired, reversible abnormality; however, even pre-existing risk factors may be malleable. In addition, although subjects with PTSD were found to show diminished hippocampal volume relative to trauma-exposed subjects without PTSD, this difference was smaller than that observed in the comparison between subjects with PTSD and non-trauma-exposed control subjects[37,38]. Similarly, a recent meta-analysis revealed that non-PTSD, trauma-exposed subjects have smaller hippocampi than non-PTSD, non-trauma-exposed subjects[51]. These findings suggest that

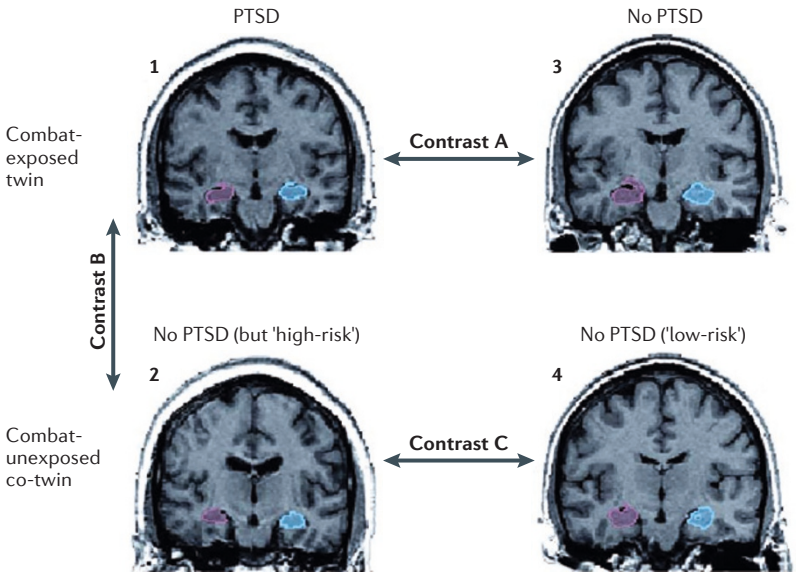

Figure 1 | **Assessing structural abnormalities in post-traumatic stress disorder using a combat-discordant identical-twin design.** Sample coronal structural magnetic resonance images of right (blue) and left (purple) hippocampi in a twin pair consisting of a combat veteran with post-traumatic stress disorder (PTSD) (1) and his combat-unexposed co-twin (2), who has no PTSD but is considered 'high-risk' because his identical twin developed PTSD when exposed to trauma, as well as a control twin pair consisting of a combat-exposed veteran without PTSD (3) and his 'low-risk' combat-unexposed co-twin (4), who also has no PTSD. Contrast A provides a replication test of studies demonstrating smaller hippocampal volume in combat veterans with versus without PTSD. Two additional contrasts can shed light on whether this abnormality is a result of combat exposure leading to PTSD, or whether it represents a pre-existing vulnerability factor. Contrast B compares hippocampal volumes in combat-exposed PTSD veterans with their own high-risk co-twins. If the twin with PTSD (1) has smaller hippocampal volume than his co-twin (2), the trait has probably been acquired. Contrast C compares hippocampal volumes in high versus low-risk co-twins. If the trauma-unexposed, non-PTSD twin of the veteran with PTSD (2) has smaller hippocampal volume than the unexposed, non-PTSD twin of the veteran without PTSD (4), it is likely that the trait represents a pre-existing vulnerability factor. This type of design can also be used to assess the origin of other abnormalities observed in patients with PTSD.

trauma may reduce hippocampal volume regardless of the development of subsequent PTSD or that reduced hippocampal volume may represent a risk factor for trauma exposure. However, trauma-exposed subjects who are not categorically classified as having PTSD may still have some PTSD symptoms, and this could also account for their lower hippocampal volume. In short, the debate between risk factor versus acquired origin of hippocampal diminution in PTSD has not been resolved; it is quite possible that both have a role.

Prefrontal cortex. The PTSD neuroimaging literature also documents volume reductions in prefrontal brain regions. Studies using sMRI have found reduced volume in individuals with PTSD in both the rostral (or pregenual) vmPFC[52] and in the dorsal anterior cingulate cortex (dACC)[53]. Youths with PTSD symptoms (but not with full-blown PTSD) were found to have less total brain tissue and lower total cerebral grey matter volumes compared to healthy controls, with specific diminution in left ventral inferior prefrontal regions[54]. In contrast to the findings in hippocampus, an sMRI study of combat-discordant twins that used voxel-based morphometry suggested that the reduction in ACC volume represents an acquired feature of PTSD rather than a pre-existing vulnerability[52]. MRSI studies have also reported diminished neuronal density in the ACC of patients with PTSD[46,47]. Furthermore, studies using diffusion tensor imaging, which provides a measure of the integrity of white matter tracts, have shown aberrant white matter integrity in the cingulum bundle, a major neuronal tract that connects the ACC with the amygdala[55]. This may be a basis for an impaired inhibitory interaction between these two regions, as further discussed below.

A recent study capitalized on a sample of subjects who had undergone sMRI before an earthquake; 42 subjects returned for re-scanning afterwards. Although none of these subjects developed full-blown PTSD, higher grey matter volume in the right ventral ACC before the earthquake was negatively associated with PTSD symptoms: that is, it conferred resilience. Subjects with more PTSD symptoms showed a greater decrease in grey matter volume in the left orbitofrontal cortex from before to after the earthquake, which was suggestive of an acquired diminution in this brain region[56].

Functional neuroimaging studies

Functional neuroimaging studies using positron emission tomography (PET) or functional MRI (fMRI) have shown altered activity in the amygdala, vmPFC and dACC, as well as in the hippocampus and insular cortex in individuals with PTSD (FIG. 2). During the past two decades, rodent studies have outlined the neural networks involved in fear learning and its extinction. These have shown that the amygdala is a key structure for both the recognition of dangerous stimuli and the coordination of the fear response. Its activity is modulated by higher cortical influences — in rodents, mainly by the infralimbic and prelimbic cortices. Evidence from pharmacological, electrophysiological, stimulation and molecular approaches in animals points to the prelimbic cortex as a brain region that facilitates the expression of conditioned fear, whereas the infralimbic cortex is crucial for the consolidation and expression of fear extinction memory (that is, extinction retention), resulting in lowered fear[57–59]. Fear conditioning and extinction paradigms in humans have identified functionally homologous brain regions in the human brain, with the dACC and vmPFC being putative human homologues of the prelimbic and infralimbic cortices, respectively[59].

Amygdala. The amygdala has a crucial role in the detection of threat, fear learning, fear expression and heightening memory for emotional events. Functional neuroimaging studies have reported exaggerated amygdala activation in response to trauma-related stimuli (for example, narratives, photographs, odours and sounds)[60] as well as generic stimuli (for example, fearful facial expressions, emotional photographs and tones) in patients with PTSD compared to control subjects[61]. Patients with PTSD also show greater amygdala activation during the acquisition of conditioned fear[62].

Magnetic resonance spectroscopic imaging
(MRSI). A non-invasive research and diagnostic technique that is similar to MRI but uses a stronger field to detect regional body chemistry at the cellular level. It is also called ^{1}H-nuclear magnetic resonance spectroscopic imaging and proton magnetic resonance spectroscopic imaging.

N-acetylaspartate
A putative marker of neuronal integrity thought to be present predominantly in neuronal cell bodies. It emits the largest signal in magnetic resonance spectroscopic imaging of the human brain.

Dorsal anterior cingulate cortex
(dACC). A cortical area that roughly corresponds to Brodmann area 24. It may also be called anterior the mid-cingulate cortex.

Voxel-based morphometry
An automated neuroimaging analytic technique that allows the investigation of focal differences in brain anatomy using the statistical approaches of statistical parametric mapping and smoothing applied to structural images.

Diffusion tensor imaging
A structural MRI-based technique that tracks the diffusion of water molecules within a closed space, usually a tube such as a neural axon. It is useful in revealing white matter fibre structure and providing information regarding regional brain connectivity.

Positron emission tomography
(PET). A functional neuroimaging technique that uses radioactive isotopes to quantify regional cerebral blood flow, glucose metabolism or receptor occupancy.

Functional MRI
(fMRI). A functional neuroimaging technique that uses a magnetic field and radio waves to measure the blood-oxygenation-level-dependent signal, which serves as an index of regional brain activation.

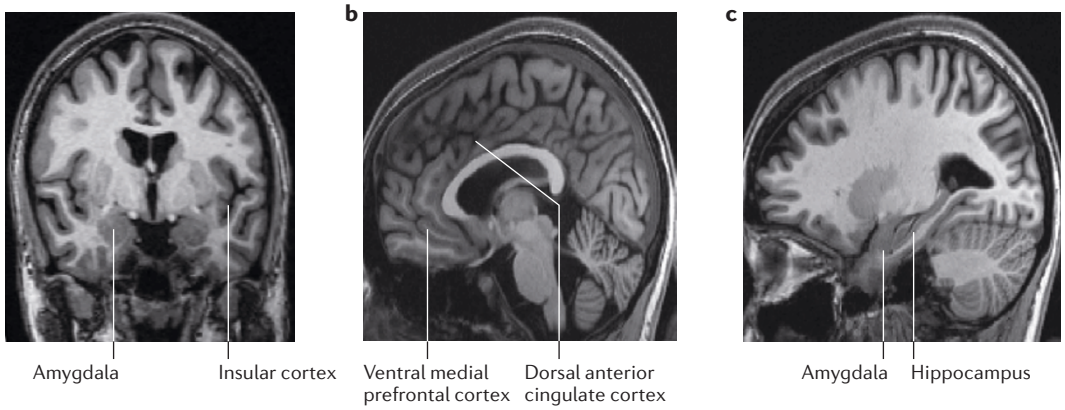

Figure 2 | **Brain regions implicated in post-traumatic stress disorder functional neuroimaging studies.** The amygdala (shown in panels **a** and **c**) is involved in recognizing both conditioned and unconditioned stimuli signalling danger, as well as in expressing the fear response. Amygdala reactivity is exaggerated in individuals with post-traumatic stress disorder (PTSD) and is positively correlated with symptom severity. The insular cortex (**a**) and dorsal anterior cingulate cortex (**b**) are also hyper-reactive in PTSD; these structures may modulate (in these cases enhance) the amygdala's expression of fear. By contrast, activation in the ventromedial prefrontal cortex (**b**), which also modulates (in this case reduces) the amygdala's expression of the fear response, is diminished in PTSD; ventromedial prefrontal cortex activity is also negatively correlated with symptom severity. Functional neuroimaging findings in the hippocampus (**c**), which is involved in recognizing both safe and dangerous contexts, have been mixed in PTSD, with both hypo- and hyper-reactivity observed.

Ventral medial prefrontal cortex. Areas in the vmPFC (including the rostral ACC), subcallosal cortex and medial frontal gyri show decreased activation in subjects with PTSD during tasks that use either trauma-related[63] or trauma-unrelated[64,65] stimuli. Activation of the vmPFC during the recollection of personal traumatic events negatively correlates with PTSD symptom severity[66]. Furthermore, the degree of symptomatic improvement after cognitive behavioural therapy has been positively correlated with increases in rostral ACC activation[67]. In subjects with PTSD, failure to recall extinction learning is accompanied by lower vmPFC activation[68] (FIG. 3). Subjects with PTSD also show deficient vmPFC activation during emotional cognitive interference tasks[69].

Dorsal anterior cingulate cortex. The dACC subserves response selection, error detection, pain perception and fear learning and expression. Activation in the dACC is increased in PTSD versus control groups during fear conditioning[70], recall of extinction learning[68] (FIG. 3) and auditory oddball tasks[71,72] (which measure responses to infrequent or novel stimuli). Such functional abnormalities are positively associated with PTSD symptom severity[73]. Individuals with PTSD as well as their identical (high-risk) co-twins showed greater resting dACC glucose metabolism in a PET study[74] and greater dACC activation during a non-emotional cognitive interference task in an fMRI study[75] compared to trauma-exposed individuals without PTSD and their identical (low-risk) co-twins; these latter findings suggest that increased dACC activity is a biomarker reflecting familial risk of developing PTSD after trauma.

Hippocampus. The hippocampus is involved in the encoding and recognition of episodic memories and environmental cues (for example, contexts), including those that are present during fear learning and extinction. Findings from functional neuroimaging studies in the hippocampus in patients with PTSD have been mixed, with some studies reporting less activation[76] and others reporting more activation[77] than in comparison groups. These different findings could be due to differences in the types of paradigms and/or analyses conducted across studies. In subjects with PTSD, failure to recall extinction learning is associated with lower hippocampal activation[68]. Whether potential functional abnormalities in the hippocampus in PTSD are linked to smaller hippocampal volume is yet to be determined.

Insular cortex. The insular cortex is involved in monitoring internal bodily states. Individuals with PTSD exhibit greater insular cortex activation — possibly reflecting heightened detection of bodily arousal — during the anticipation of aversive images and in response to fearful facial expressions, painful stimuli and memories[78–80]. Insular cortex activation appears to be positively correlated with PTSD symptom severity[78]. Increased insular cortex activation has also been observed in other anxiety disorders[61] and therefore is not specific to PTSD.

Neurocircuitry of post-traumatic stress disorder. A recent meta-analysis of 79 functional PTSD neuroimaging studies found that the mid-ACC, dACC and bilateral amygdala were the most hyperactivated regions, whereas the vmPFC and inferior frontal gyrus were the most hypoactivated regions. Decreased vmPFC activity was associated with increased amygdala activity[81]. Neurocircuitry models of PTSD[82,83] posit that the vmPFC fails to inhibit the amygdala, leading to attentional bias towards threat, increased fear responses, impaired extinction of traumatic memories and its retention, and deficits in emotion regulation. The protracted neurodevelopment of the PFC relative to the amygdala may

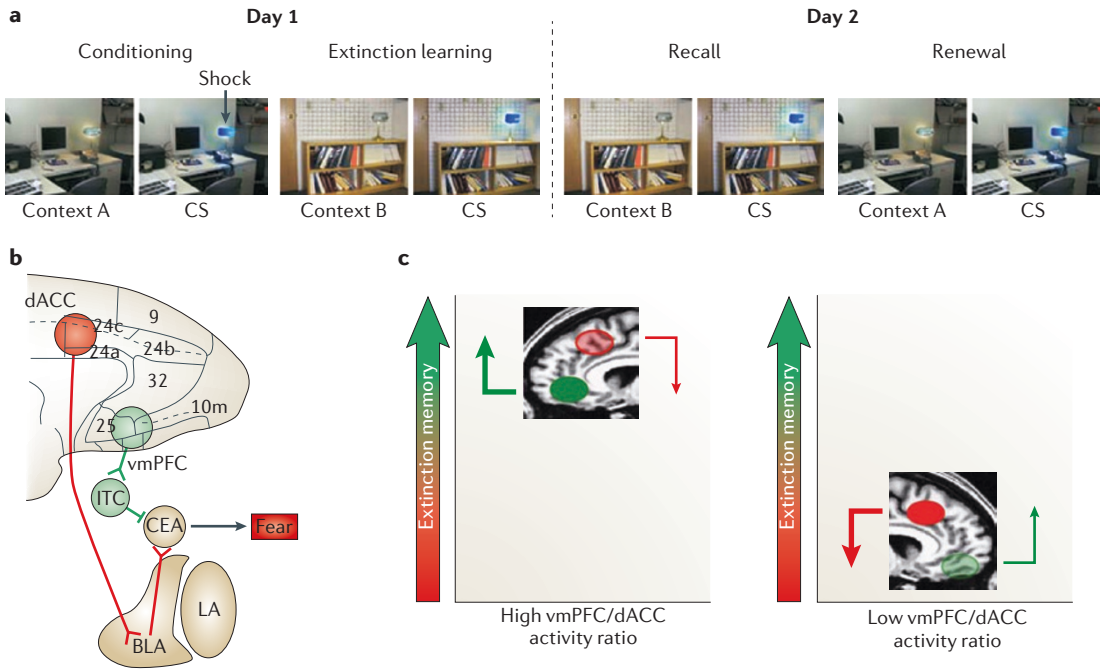

Figure 3 | **Contributions of prefrontal regions to fear regulation and expression.** **a** | Activation in the human brain during fear conditioning and extinction can be investigated in a Pavlovian fear conditioning and extinction paradigm. During conditioning, a conditioned stimulus (CS; a coloured light) is paired with a mild electric shock to the fingers in a particular context (context A). The acquisition of conditioned fear in this paradigm can be measured by the skin conductance response to the light. During extinction learning, the light is subsequently repeatedly presented without the shock in a different context (context B). This leads to extinction of the conditioned response. The next day, the light is again presented in the absence of the shock in context B (extinction recall), and then again in context A (fear renewal). Greater retention of extinction learning is associated with a lower fear response during extinction recall. Extinction retention is context-dependent: the hippocampus is thought to recognize whether a context is safe (B) or dangerous (A) and to communicate this information to other structures in the fear network. **b** | Functional MRI studies have shown that activation of the ventromedial prefrontal cortex (vmPFC; shown in green) during extinction recall is positively correlated with extinction retention[213], whereas activation of dorsal anterior cingulate cortex (dACC; shown in red) is negatively correlated with extinction retention[214]. The vmPFC sends excitatory glutamatergic projections to GABAergic intercalated cells (ITC) in the amygdala, which in turn inhibit the expression of the fear response by the central nucleus of the amygdala (CEA). By contrast, the dACC sends excitatory glutamatergic projections to the amygdala's basolateral nucleus (BLA), which in turns activates the expression of the fear response by the CEA. **c** | Non-PTSD subjects (left) have a relatively high vmPFC/dACC activation ratio during extinction recall, which tips the balance towards better extinction retention and less fear expression. By contrast, PTSD subjects (right) have a lower vmPFC/dACC activation ratio, which tips the balance towards less extinction retention and more fear expression[68]. LA, lateral amygdala nucleus. Part **a** is modified, with permission, from REF. 213 © (2007) Elsevier.

help to explain the increased risk of PTSD associated with younger age in combat veterans[84]. In contrast to the vmPFC, the dACC appears to promote fear expression. Hippocampal dysfunction may be associated with memory impairments for neutral material and deficits in recognizing safe contexts. A hyperactive insular cortex, which may reflect heightened interoceptive awareness, may confer proneness to anxiety.

Receptor imaging. A functional imaging technique that has been less often applied in PTSD studies uses selective, exogenously administered radioligands to assess the binding and distribution of various neurotransmitter receptors in the brain via PET. One such study found a reduction in GABA type A ($GABA_A$) receptor binding throughout the cortex, hippocampus and thalamus in veterans with PTSD compared to veterans without PTSD[85], which is suggestive of globally diminished inhibitory brain function. The fact that the changes were so widespread prohibits relating this finding to any specific PTSD neurocircuitry. Another study of non-combat-related PTSD found decreased 5-HT transporter binding in the amygdala[86], which the authors suggested is consistent with findings of amygdala hyperactivity upon exposure to trauma- or fear-related stimuli in PTSD (reviewed above). However, the absence of a trauma-exposed, non-PTSD control group limited inferences regarding the specificity of this finding to PTSD. Indeed, in two other studies using an exogenous ligand — one that found reductions in μ-opioid receptor binding potential in various limbic and paralimbic brain regions[87], and another that found reduced $5\text{-}HT_{1B}$ receptor expression in the caudate, amygdala and ACC[88] — abnormalities occurred both in subjects with PTSD and in trauma-exposed subjects without PTSD, raising the possibility that these abnormalities reflect the effect of trauma exposure on the brain rather than specific aspects of PTSD pathophysiology.

The interpretation of results of exogenous ligand studies is often obscured by incomplete knowledge of the underlying microanatomy. (For example, is the neuron an excitatory projection neuron or an inhibitory interneuron? Is the receptor a postsynaptic receptor, an autoreceptor or a heteroceptor?) It is also obscured by the underlying physiology. (For example, is the binding lower because receptor number and/or affinity is reduced or because the receptor is already occupied with endogenous ligand? Is the downregulation (or upregulation) primary or compensatory?) Variations in these details can have different, or even opposite, implications.

Neuroendocrinological studies

Abnormalities in catecholamine and cortisol levels were among the first findings in individuals with PTSD. Subsequent studies have revealed changes in many other hormonal and neuroregulatory factors among populations of patients with PTSD as well.

Catecholamines. Numerous studies have provided compelling evidence for the presence of sympathetic nervous system hyper-reactivity in PTSD[89]. It has been suggested that an excessively strong adrenergic response to the traumatic event may mediate the formation of the durable traumatic memories that in part characterize the disorder[90]. Factors that may contribute to the increased release of noradrenaline in response to sympathetic nervous system activation in PTSD include genetic or stress-induced decrements in neuropeptide Y (NPY)[91], which inhibits noradrenaline release, as well as a lower number[92] or affinity[93] of α2-adrenergic autoreceptors. Noradrenergic hyper-reactivity in patients with PTSD is associated with hyperarousal and re-experiencing symptoms, including trauma-related nightmares, flashbacks, intrusive memories, and emotional and physiological reactions to traumatic cues[94–97]. In addition, sympathetic nervous system activation induced by administration of yohimbine (an α2-adrenergic autoreceptor antagonist) decreased orbitofrontal and prefrontal cortical metabolic activity in subjects with PTSD, whereas it increased metabolic activity in these regions in control subjects[98]. Studies demonstrating the efficacy of prazosin (a postsynaptic α1-noradrenergic receptor inhibitor) for the treatment of nightmares or daytime hyperarousal and re-experiencing symptoms of PTSD[99,100] are consistent with these findings. Results of early studies suggested that the β-adrenergic antagonist propranolol had value in the acute prevention of PTSD[13,101], but these results have not been consistently replicated[102,103].

Indoleamines. The 5-HT system also appears to be implicated in both the acute mediation of PTSD symptoms and the modulation of PTSD risk, as neuropharmacological, treatment and genetic epidemiological studies have indicated. Administration of meta-chlorophenylpiperazine (mCPP), which acts as a 5-HT receptor agonist, resulted in acute anxiety, panic attacks and PTSD symptoms (including flashbacks) in a subgroup of male combat veterans with PTSD[104]. Interaction of mCPP with the 5-HT transporter[105] and multiple 5-HT receptor subtypes results in increased extracellular 5-HT levels, as well as behavioural, psychological and cognitive effects reminiscent of PTSD that are reversible by administration of mixed 5-HT_{1C}–5-HT_2 receptor antagonists[106]. This suggests that a phasic increase in 5-HT acting at postsynaptic 5-HT_{1C}–5-HT_2 receptors may induce PTSD symptom expression, which is supported by findings in the rodent single prolonged stress (SPS) model of PTSD (see below). It also accords with the potential clinical benefits of chronic use of SSRIs, which appear to result in 5-HT_{2C} receptor desensitization[107]. Further evidence for a role of the 5-HT system in PTSD is provided by studies of the 5-HT transporter gene (see below).

Neuropeptide Y. Research has demonstrated that NPY has protective effects during stress, which are likely to be mediated by the modulation of sympathetic responses[91] and antagonism of the anxiogenic effects of corticotropin-releasing hormone (CRH; also known as CRF)[108]. Humans possessing *NPY* gene variants associated with increased gene expression showed lower levels of trait anxiety and less amygdala reactivity to emotionally provocative stimuli[109]. Similarly, male military personnel who exhibited higher plasma NPY levels during intense training stress showed less distress and dissociation, and superior performance[110]. By contrast, lower cerebrospinal fluid (CSF) and resting plasma NPY levels and/or a blunted NPY response to sympathetic activation have been associated with more severe PTSD symptoms[91,111]. In addition, a retrospective study in male veterans showed that lower plasma NPY levels were associated with less improvement in PTSD symptoms over time[112]. Efforts to develop pharmacological agents with clinically relevant, circumscribed NPY receptor-mediated effects have so far been unsuccessful. Manipulating the NPY system epigenetically may have greater promise as a strategy for the development of NPY-based therapeutics.

Corticotropin-releasing hormone. CRH has anxiogenic physiological and behavioural effects[113]. Increased CRH levels have been found in the CSF and/or blood of several different cohorts of patients with PTSD[114,115]. However, a recent study found that CRH levels in the CSF decreased among patients with PTSD watching a trauma-related video[116], indicating that much is yet to be learned about the dynamics of CRH in the CNS during stress. Although recent clinical treatment trials of newly developed CRH antagonists have had to be aborted owing to hepatic toxicity, interest in pharmacological treatments for PTSD directed at the CRH system continues.

Cortisol. Early research produced the paradoxical finding of abnormally low cortisol levels in PTSD[117], although this finding has not been consistently replicated in large studies. Nevertheless, sufficient evidence has accumulated to conclude that PTSD is not characterized by increased tonic cortisol levels[118], as might be expected in a state of chronic stress. By contrast, greater suppression of plasma cortisol after a low dose

Phasic
Designating intermittent signalling, usually in response to a stimulus.

Dissociation
The splitting off of a mental process or group of mental processes from the main body of consciousness.

Tonic
Designating continuous, steady or baseline signalling.

of dexamethasone, which has been reported in some studies of PTSD[119], is consistent with excessive shutting down of the hypothalamus–pituitary–adrenal cortical (HPA) axis owing to enhanced sensitivity to negative feedback. Research has supported a higher number of glucocorticoid receptors in lymphocytes of such subjects with PTSD[120]. These findings are thought to represent, at least in part, a pre-trauma risk factor for the disorder[121]. A gene of current high interest related to PTSD risk, *FKBP5* (FK506 binding protein 5), is a co-chaperone of the glucocorticoid receptor, and polymorphisms in this gene have been related to glucocorticoid receptor supersensitivity and risk of PTSD[122]. An exception to the absence of increased cortisol in patients with PTSD may be presented by people, mainly women, with comorbid major depressive disorder[106,123,124]. Premenopausal women with PTSD also exhibited increased phasic cortisol responses to CRH and adrenocorticotropin[125].

The proposition that a cortisol deficit may have a role in the pathogenesis of PTSD has been supported by findings that administration of supplemental doses of cortisol to acutely ill medical patients reduces the PTSD outcome[126]. A recent preliminary study found that high-dose cortisol administration within hours of a traumatic event reduced the subsequent development of PTSD[127]; these encouraging therapeutic results call for testing in larger samples. Another study found beneficial effects of cortisol administered to patients who already had PTSD, which was attributed to the well-recognized inhibitory effect of cortisol on memory retrieval (in this case, traumatic memory retrieval)[128]. However, the possibility that cortisol could worsen PTSD in some cases, for example, in patients without a cortisol deficit, should not be dismissed.

Despite the findings mentioned above, there still exists preclinical evidence suggesting the possibility that increased responses of this stress hormone could have a role in PTSD pathogenesis. Cortisol, like adrenaline, potentiates memory consolidation[129]; an acute cortisol response to the traumatic event could contribute to the formation of a durable traumatic memory. Whether this possibility is relevant to PTSD remains to be directly tested. Baseline levels, phasic responses to stress, dosage (physiological versus pharmacological), target of action and the possibility of an inverted U-shaped dose–response curve may all be important parameters to address in resolving the mixed cortisol findings in PTSD. Indeed, both too high and too low a level of glucocorticoids can interfere with frontal lobe-mediated working memory and long-term potentiation (LTP) in the hippocampus, in part through dose- and time-dependent effects on glutamatergic neurotransmission during learning[130]. However, glucocorticoids are crucial for stress adaptation, and many genes with relevance to resilience, including those encoding enzymes involved in the synthesis of allopregnanolone (see below) and NPY, contain glucocorticoid response elements[131].

In future clinical research, it will be important to evaluate cortisol (and CRH) levels in relation to other neuroendocrinological factors that it regulates (for example, allopregnanolone and NPY) or that confer protection from its potentially deleterious effects (for example, dehydroepiandrosterone (DHEA)). It will also be important to take into account specific psychiatric comorbidities (such as depression and nicotine[132] and other substance dependence), gender, reproductive and menstrual phase, and genetic polymorphisms known to affect HPA axis regulation. (These considerations may apply to other endocrinological variables as well.) It also will be important to study central as well as peripheral cortisol regulation in relation to PTSD symptoms. Results of one study suggest that cortisol regulation in the CNS may differ from that in the periphery. Plasma cortisol levels were comparable between male veterans with PTSD and healthy comparison subjects, whereas CSF cortisol levels (which are arguably a better reflection of the level of brain exposure to cortisol) were higher in the veterans with PTSD and were correlated with CRH levels in the CSF[114]. Recent work has also highlighted the possible importance of performing cortisol measurements by gas chromatography with mass spectrometry because standard cortisol measurement by radioimmunoassay (RIA) does not discriminate between cortisol and its inactive metabolites. For example, a relationship between low total glucocorticoid species (measured simply as 'cortisol' by RIA) and resistance to prolonged exposure therapy for PTSD was attributable to low levels of an inactive cortisol metabolite synthesized by 5-α reductase rather than to low levels of cortisol per se[133].

Dehydroepiandrosterone. Adrenally derived DHEA, which is the immediate precursor of the androgens, is secreted synchronously with cortisol and is also thought to be the source of its active sulphated metabolite, DHEAS[134]. In the brain, DHEA and DHEAS both antagonize $GABA_A$ receptors and facilitate NMDA receptor function, which in the amygdala is essential to both fear conditioning and fear extinction. In the hippocampus, DHEA reverses cortisol-induced impairments in LTP, protects against excitatory amino acid-induced and oxidative stress-induced neuronal damage, regulates programmed cell death and promotes neurogenesis[134]. Such regional neuroprotective effects may be conferred by the anti-glucocorticoid properties of DHEA[135,136]. Although clinical studies have demonstrated increases in adrenal DHEA release and increased plasma DHEA and DHEAS levels in individuals with PTSD, they paradoxically have also shown negative relationships between these indices and general severity of PTSD and comorbid negative mood symptoms[137–140]. In addition, there are studies showing a positive relationship between DHEA or DHEAS levels or the ratio of DHEA to cortisol and stress resilience in active duty military personnel[141,142] and in long-term recovery from PTSD in male veterans[143]. These findings could suggest that stress-associated increases in DHEA confer resilience, but such an interpretation may need qualification. For example, sleep disturbance in the context of PTSD has been associated with high DHEA responses to adrenal activation[139] and with high baseline blood DHEAS levels[138]. It may be that the balance between the levels

Extrasynaptic
Located outside the synapse. Extrasynaptic receptors can be accessed by neuromodulatory factors derived from the periphery and circulating in the cerebrospinal fluid.

Heritable
Capable of being passed from one generation to the next via DNA.

Single-nucleotide polymorphism
(SNP). A variation in a DNA sequence in which a single nucleotide (A, C, G or T) at a specific locus differs between members of the same biological species or between paired chromosomes of an individual.

of this neuroprotective, excitatory neuroactive steroid and other neuroendocrine factors, such as cortisol, or the inhibitory GABAergic neurosteroids, such as allopregnanolone[144] (see below), is relevant. In humans, administration of DHEA reduces blood levels of cortisol and increases levels of allopregnanolone, testosterone and oestrogen. These effects may be variously beneficial in PTSD[145]. Chronic DHEA administration has also been found to have antidepressant effects in multiple clinical trials[146] but, to date, no trials have assessed its therapeutic effects in PTSD.

Allopregnanolone and pregnanolone. CSF levels of the adrenal- and brain-derived neuroactive steroids allopregnanolone and its equipotent enantiomer pregnanolone (together termed ALLO) relate strongly and negatively to re-experiencing and depressive symptoms in PTSD[144]. The ratio of the level of allopregnanolone to the level of its progesterone precursors is also low in the CSF and serum of patients with PTSD, suggesting that ALLO synthesis is deficient. ALLO is the most potent and selective positive endogenous modulator of GABA action at brain $GABA_A$ receptors. At extrasynaptic $GABA_A$ receptors, ALLO maintains a tonic inhibitory conductance that moderates gain in neuronal output during periods of increased excitation, such as that which occurs during stress[147]. Functionally, ALLO provides long-loop negative feedback at the HPA axis and confers anxiolytic, sedative, anaesthetic, neuroprotective and regenerative effects. Results of an animal study suggest that the effects of SSRIs (the current drug treatment of choice for PTSD) may be due to ALLO increases rather than to 5-HT reuptake blockade[148]. It is possible that a deficiency in ALLO synthesis accounts for the substantial rate of SSRI treatment resistance in PTSD, but this remains to be determined.

Putative brain-state shift in post-traumatic stress disorder. The development of PTSD may involve a shift in brain state from high-level processing of multimodal contextual and mnemonic stimuli (dependent on hippocampus and PFC-mediated working memory) (FIG. 4a) to more primitive amygdala-mediated formation of time-locked sensory associations and expression of the species-specific defence response (FIG. 4b). Individual constitutional and personality (for example, intelligence and neuroticism) differences may influence the stimulus threshold at which this shift occurs and thereby affect vulnerability. A number of neuroregulatory and neuroendocrinological factors discussed above may influence this 'brain-state shift'; these factors vary across individuals due to genetic and epigenetic influences, as well as within individuals over time due to environmental influences, such as intervening stressful events. These neuroendocrine factors probably interact in both counter-regulatory and synergistic manners that are likely to influence PTSD risk, symptom profiles and severity, as well as capacity for recovery, thus providing potentially exploitable pharmacological and epigenetic targets for the development of new PTSD treatments.

Genetic studies

Genetic influences account for 30%[149,150] to 72%[151] of vulnerability to PTSD. These estimates take into account genetic factors that may contribute to exposure to traumatic events, such as combat or interpersonal violence[150,152,153]. Genetic influences on exposure to trauma are thought to largely function through heritable personality traits. Genetic risk factors that are common to major depression, generalized anxiety disorder and panic disorder also account for most of the genetic variation in PTSD identified to date. Thus, genes that affect the risk of developing PTSD also influence the risk of developing other psychiatric disorders, and vice versa. As with other mental disorders[153–156], influences on PTSD are probably polygenic; at least 17 gene variants have been associated with PTSD in at least one published study (TABLE 1; Supplementary information S1 (table)). These include genes involved in dopaminergic and 5-HT systems, HPA axis regulation, the locus coeruleus–noradrenergic system and those encoding neurotrophins.

Recent studies have attempted to identify the mechanisms by which gene variants influence PTSD risk. For example, a single-nucleotide polymorphism (SNP) in a putative oestrogen response element within the gene encoding adenylyl cyclase-activating polypeptide 1 (pituitary) receptor type I (*ADCYAP1R1*; also known as *PACAPR1*) predicted PTSD diagnosis and symptoms in females only[157]. This SNP was associated with brain *ADCYAP1R1* mRNA expression and fear discrimination. However, the genetic association reported in this paper was not replicated in two large independent samples[158].

As in other psychiatric disorders, the candidate gene approach has not yet been successful in leading to the identification of robust genetic variants that confer vulnerability to (or resilience against) PTSD.

Gene expression. A growing literature has provided evidence for gene expression patterns that distinguish between individuals with and without PTSD. An early microarray study of RNA derived from peripheral blood cells identified 656 transcripts involving immune and hormonal systems that were differentially expressed in acutely traumatized people who went on to develop PTSD[159]. Another study found 19 differentially expressed transcripts involving immune functions or reactive oxygen species, five of which were upregulated and 14 downregulated in people with PTSD compared to individuals without PTSD who had been exposed to the same traumatic event almost 20 years earlier[160]. More recently, 16 distinct genes involved in signal transduction, neuronal signalling and survival, immune cell function and HPA axis activity were found to be differentially expressed in individuals with PTSD versus individuals without PTSD who had been exposed to the terrorist attacks of 11 September 2001 (REF. 161). The gene encoding mannosidase, alpha, class 2C, member 1 (*MAN2C1*) showed the largest expression difference and had not previously been linked to PTSD. Another recent study found methylation differences in this gene in blood cells of patients with PTSD[162].

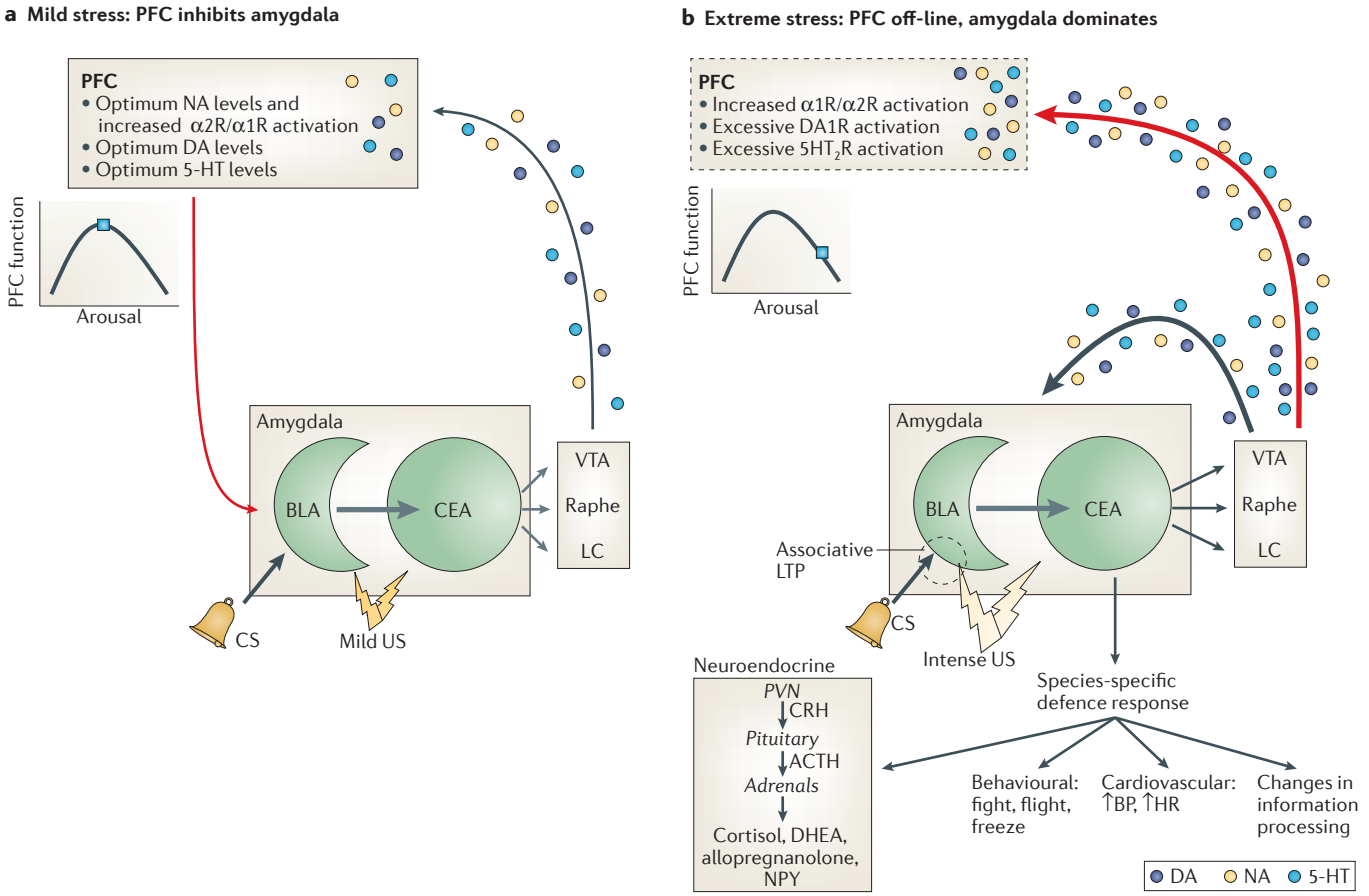

Figure 4 | **Putative brain-state shift relevant to post-traumatic stress disorder.** The figure shows two different brain states. The first (**a**) shows a resilience state. This reflects the response of previously non-traumatized individuals who have been exposed to mild or moderately arousing unconditioned threat stimuli, of resilient individuals who are resistant to the arousing effects of more extreme unconditioned threat and of individuals with post-traumatic stress disorder (PTSD) after undergoing extinction and recovery so that conditioned threat stimuli are no longer highly arousing. The second (**b**) shows a risk state. This reflects the response of previously non-traumatized individuals exposed to unconditioned threat that is highly arousing, of individuals with PTSD who are re-exposed to trauma-related cues (conditioned threat) before extinction and recovery, and of individuals with PTSD who are resistant to recovery. **a** | Neuromodulation contributing to relative prefrontal cortical dominance (resilience). Mild to moderately arousing conditioned stimuli (CS) or unconditioned stimuli (US) activate the central nucleus of the amygdala (CEA), which projects both directly and indirectly to brainstem monoaminergic cell body regions to activate mesocorticolimbic dopamine (DA) pathways emanating from the ventral tegmental area (VTA), as well as more widely disseminating noradrenaline (NA) and 5-hydroxytryptamine (5-HT) pathways emanating from the locus coeruleus (LC) and medial and dorsal raphe nuclei, respectively (pathways shown together in black)[215]. In the prefrontal cortex (PFC), the resulting mild to moderate increases in synaptic levels of these monoamines engage high-affinity receptors (for example, α2-noradrenergic receptors (α2Rs)) to enhance working memory[216] and to activate glutamatergic pyramidal output neurons that project output (shown in red) back to the amygdala. There, glutamatergic activation of GABAergic interneurons in the basolateral amygdala (BLA) and/or intercalated nuclei (not shown) suppresses associative learning and inhibits excitatory BLA pyramidal cell projections to the CEA and the expression of the species-specific defence response (see below)[217]. **b** | Neuromodulation contributing to relative amygdala dominance (risk). Strongly arousing unconditioned threat stimuli (due to objective threat characteristics or to an individual's increased sensitivity to objective threat) or conditioned threat stimuli in people with PTSD activate the amygdala to a greater degree, which in turn excites brainstem mesocorticolimbic monoaminergic neurons more vigorously[57,215] — a process that is likely to be facilitated by reductions in GABAergic neurotransmission within the amygdala[218,219]. This results in strong DA, 5-HT and NA output to the PFC. In the PFC, higher synaptic levels of these monoamines engage low-affinity α1Rs, as well as DA1 and 5-HT_2 receptors (DA1Rs and 5-HT_2Rs, respectively), resulting in working memory impairment and a reduction in PFC-mediated inhibition of the amygdala[216]. The consequent lifting of the PFC 'brake' reduces GABAergic tone within the BLA and intercalated nuclei of the amygdala to enable associative pairing of US and convergent contextual CS or later reconsolidation of CS, as well as activation of the species-specific defence response, which includes increases in blood pressure and heart rate, hypothalamus–pituitary–adrenal (HPA) axis activation, engagement in reflexive defensive behaviours (such as fight, flight and freezing) and restriction of high-level information processing to enable efficient focus on survival-relevant phenomena. Direct catecholamine effects in the amygdala also facilitate defensive responding: activation of D1 receptors on the terminals of PFC projection neurons inhibits glutamate activation of GABAergic interneurons, whereas activation of postsynaptic D2Rs on BLA-to-CEA pyramidal projection neurons increases their excitation by convergent US–CS inputs, as well as adventitious sensory stimuli. This probably facilitates or maintains associative fear conditioning and may contribute to a generalization of fear[220]. By contrast, neuronal activity in the BLA is generally suppressed by α2R and α1R stimulation. The α1R effect, which is mediated by enhanced terminal release of GABA, is reduced by extreme or chronic stress[221], whereas β-noradrenergic receptor activation is excitatory and enhances US–CS pairing[222]. Black arrows indicate an activating effect and red arrows indicate an inhibitory effect. ACTH, adrenocorticotropic hormone; BP, blood pressure; CRH, corticotropin-releasing hormone; DHEA, dehydroepiandrosterone; HR, heart rate; LTP, long-term potentiation; NPY, neuropeptide Y; PVN, paraventricular nucleus of the hypothalamus.

Neuromodulation
An alteration in the response of a neuron induced by a substance that would not, by itself, affect neuronal firing rate.

DNA methylation
The modification of a strand of DNA in which a methyl group is added to a cytosine molecule that stands directly before a guanine molecule in the same chain. It has the effect of reducing gene expression.

Epigenetic mechanisms. The lack of consistency of associations between specific genetic variants and PTSD may be explained by epistatic effects (modification of genetic effects by other genes) and/or by environmental factors not accounted for across studies. For example, some studies have found significant effects for specific genetic variants only under conditions of extreme traumatic stress[163] or a history thereof[164]. The environment modifies genetic effects through epigenetic mechanisms such as DNA methylation. PTSD has been distinguished by methylation profiles suggesting upregulation in immune system-related genes and downregulation in genes involved in neurogenesis and the startle response[165,166]. Methylation of *ADCYAP1R1*, which appears to be an important gene in fear learning, in peripheral blood was associated with PTSD[157].

A joint consideration of genotype and methylation patterns may clarify inconsistencies in the PTSD genetics literature. *SLC6A4* (solute carrier family 6 (neurotransmitter transporter, serotonin, member 4); also known as *SERT*, *HTT, 5-HTT* and *5-HTTLPR*), which regulates synaptic 5-HT reuptake and thereby influences emotional behaviour, has been the most studied gene in relation to PTSD. Published findings for this locus

Table 1 | **Summary of candidate genes studied in relation to post-traumatic stress disorder***

Gene	Common name	Location	Total number of published reports	Significant findings	Null findings
DRD2 (also known as *D2R* and *D2DR*)	Dopamine receptor D2	11q23	6	4	2
DRD4 (also known as *D4DR*)	Dopamine receptor D4	11p15.5	1	1	0
SLC6A3 (also known as *DAT1*)	Dopamine transporter	5p15.3	4	3	1
DBH	Dopamine β-hydroxylase	9q34	1	0	1
SLC6A4 (also known as *HTT*, *5-HTT*, *SERT* and *5-HTTLPR*)	5-hydroxytryptamine (serotonin) transporter	17q11	16	12	4
HTR2A (also known as *5-HT2A*)	5-hydroxytryptamine receptor 2A	13q14–q21	1	1	0
FKBP5	FK506 binding protein 5	6p21	4	4	0
GCCR (also known as *NR3C1*)	Glucocorticoid receptor	5q31.3	1	0	1
CRHR1	Corticotropin-releasing hormone receptor 1	17q12–22	1	1	0
RGS2	Regulator of G protein signalling 2	1q31	1	1	0
CNR1 (also known as *CB1* and *CNR*)	Cannabinoid receptor 1 (brain)	6q14–q15	1	0	1
APOE	Apolipoprotein E	19q13	1	1	0
BDNF	Brain-derived neurotrophic factor	11p13	3	0	3
NPY	Neuropeptide Y	7p15.1	1	0	1
GABRA2	GABA type A receptor α2	4p12	1	1	0
COMT	Catechol-*O*-methyltransferase	22q11	2	2	0
ADCYAP1R1	Receptor for adenylate cyclase-activating polypeptide 1	7p14	2	1	1
DTNBP1	Dystrobrevin-binding protein 1	6p22	1	1	0
CHRNA2	Cholinergic receptor, neuronal nicotinic, α-polypeptide 5	15q25.1	2	1	1
PRKCA	Protein kinase Cα	17q22–q23.2	1	1	0
TPH1	Tryptophan hydroxylase 1	11p15.3–p14	1	1	0
TPH2	Tryptophan hydroxylase 2	12q21.1	1	1	0

SLC6A3, solute carrier family 6 (neurotransmitter transporter, dopamine), member 3; *SLC6A4*, solute carrier family 6 (neurotransmitter transporter, serotonin), member 4. *A referenced version of this table appears in Supplementary information S1 (table).

Allele
One or more alternative forms of a genetic locus or a gene.

Epigenesis
One or more mechanisms that regulate gene function without altering the underlying DNA sequence.

Face validity
The degree to which a model or a term appears to measure what it is supposed to measure.

Construct validity
The degree to which a model or a term corresponds to or reflects an underlying theory.

Glucocorticoid negative feedback
A negative-feedback phenomenon by which cortisol reduces its own release through inhibition of the hypothalamus–pituitary–adrenal cortical axis.

have been contradictory, with most studies implicating the short (S) allele, which is associated with decreased gene expression, but others implicating the long (L) allele, which is associated with increased expression. Several studies have found significant genotype by environment interactions[154]. Childhood adversity appears to be a particularly potent modifier of genetic risk for PTSD[164,167,168]. Social context also appears to modify PTSD risk; for example, the S allele was associated with decreased PTSD risk in environments with low crime and unemployment rates but with increased PTSD risk in opposite environments[169]. Emerging evidence suggests that methylation at downstream CpG sites modifies *SLC6A4* expression[170]. A recent study showed that *SLC6A4* methylation modified the effect of the number of traumatic events on PTSD after controlling for *SLC6A4* genotype[171]. Specifically, people who had a greater number of experienced traumatic events were at greater risk for PTSD only if *SLC6A4* methylation levels were low. By contrast, high *SLC6A4* methylation levels seemed to protect people who had experienced a greater number of traumatic events from developing PTSD. These findings suggest that both genotype and gene-specific methylation patterns contribute to either PTSD vulnerability or resilience. Histone deacetylation is another molecular epigenetic mechanism that may be involved in PTSD pathogenesis[172].

Collectively, the above studies suggest that genotype, DNA methylation and histone deacetylation and gene expression differences influence or accompany the development of PTSD. However, we are unaware of any study that has incorporated all three forms of genetic information into one study. Nor are there definitive findings for any one gene or gene system in the aetiology of PTSD. Importantly, epigenetic changes and gene expression in living humans currently can only be assessed in peripheral blood cells, which severely limits the interpretation of the findings. For example, the frequent finding of genetic immune system alterations in PTSD could be an artefact of the tissue used in these analyses, *viz.*, white blood cells, a chief function of which is to regulate the immune response. The most important tissue, *viz.*, that from brain regions reviewed in this article, in which variations in molecular epigenesis and gene transcription are most likely to be relevant to PTSD, is currently off limits (except in post-mortem tissue, which is not without problems[173]). Technological developments that could make live human brain tissue accessible for DNA methylation, histone deacetylation and gene transcript research could lead to breakthroughs, but such developments may be a long way off. Meanwhile, this points to the need for animal models.

Animal models of post-traumatic stress disorder

A full explanation of the mechanisms involved in the development of PTSD requires prospective studies. Experimentally inducing stressors of the magnitude capable of causing PTSD is ethically impermissible in humans, so researchers have relied on animal models. Such studies have identified candidate brain circuits and cellular and molecular processes involved in PTSD pathophysiology and tested molecular therapeutic targets and specific interventions.

Early animal models of PTSD were largely based on face validity. Some used experimental manipulations that seemed as if they would be 'traumatic' to the animals, and some were nonspecific stress models, such as inescapable shock. Although these approaches were useful, they provided limited PTSD-specific information. Similarly, exposure to fear conditioning alone, being a normative animal and human phenomenon, is insufficient to produce the PTSD phenotype[174]. Newer models have incorporated construct validity by capitalizing on the increasing understanding of the pathophysiology of PTSD. These models have used one or more 'PTSD-specific' end points, such as abnormal fear learning, exaggerated acoustic startle response, enhanced glucocorticoid negative feedback and exaggerated catecholamine release. Here, we review only studies that have used models with both satisfactory face and construct validity. These models include predator exposure (PredEx), exposure to SPS and exposure to footshock with additional stressors.

PredEx models expose animals (for example, rats) to a natural predator (for example, cats or ferrets) or to predator scents (for example, cat litter or fox urine) in an environment from which the animal cannot escape. Exposed animals develop enhanced acoustic startle and show increased behavioural manifestations of anxiety[175,176]. Some studies have added behavioural cut-off criteria that capitalize on individual variability in stress reactivity in order to identify animals that exhibit extreme responses thought to be analogous to PTSD[177]. In some instances, a subsequent psychosocial stress is added to the predator exposure[178].

SPS paradigms involve serial exposure to multiple stressors (for example, restraint, a cold swim and ether anaesthesia) that independently activate the HPA axis, followed by a 1-week 'no-touch' sensitization period. The sensitization period is necessary for the development of the enhanced glucocorticoid negative feedback[179] and increased acoustic startle response[180] reported in PTSD (FIG. 5). Time-dependent sensitization and stress–restress can be seen as variants of the SPS model, with stress–restress adding another stressful exposure (or electric shock) at the end of the sensitization period.

Footshock with additional stress (here called footshock-plus (FS+)) models use a single session of exposure to an electric shock (sometimes paired with a conditioned stimulus, in which case it is a fear conditioning paradigm) followed by additional shocks[181] or contextual reminders[182]. A variant of this model pre-exposes animals to repeated stress before fear conditioning. Using post-conditioning reminders leads to enhancement of acoustic startle[181,182], whereas using pre-conditioning stress leads to enhanced fear conditioning and sensitization[183].

These animal models have been important in identifying the PTSD-specific neurocircuitry described in the sections above. Animal learning studies lead to hypotheses regarding fear conditioning and/or extinction abnormalities in PTSD, and sensitization studies

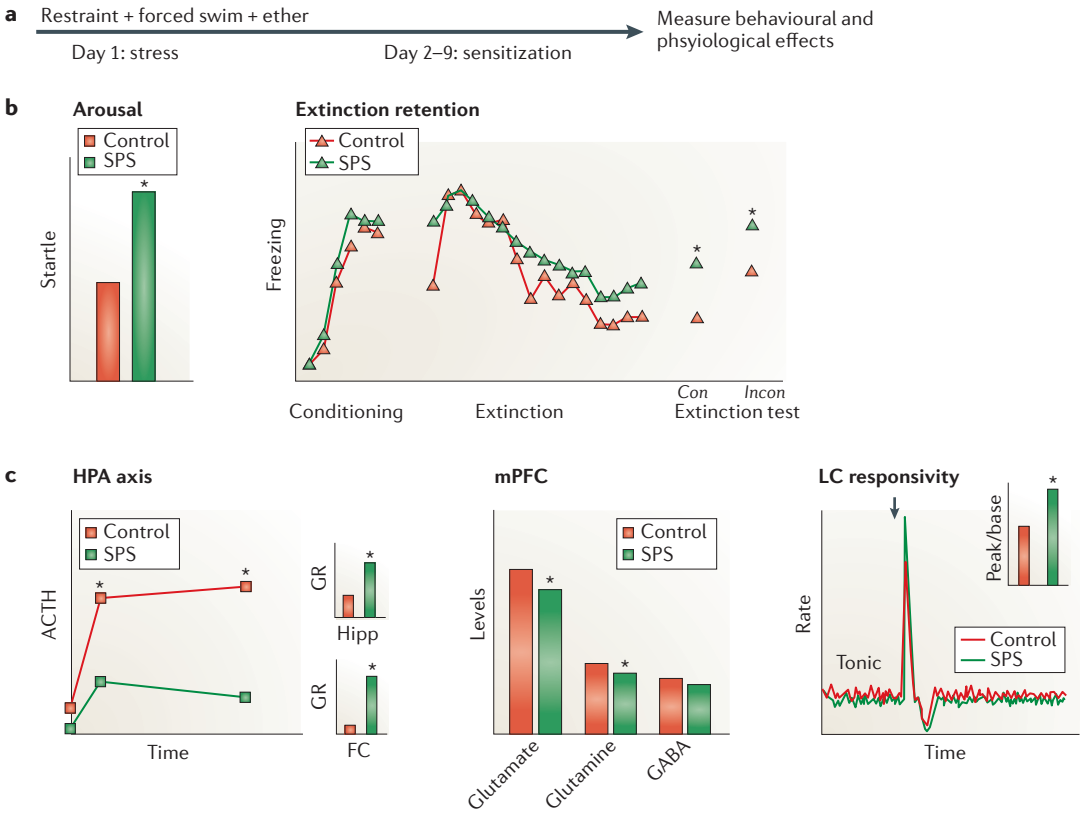

Figure 5 | **Behavioural and physiological changes in a post-traumatic stress disorder animal model.** **a** | A prototypic rodent model of post-traumatic stress disorder (PTSD): single prolonged stress (SPS). This model involves serial exposure to multiple stressors (for example, restraint, a cold swim and ether anaesthesia), followed by a 1-week 'no-touch' sensitization period, after which behavioural and physiological effects of the stress exposure are measured. **b** | Behavioural effects of exposure to SPS include increased arousal as reflected in the startle response (left) and decreased extinction retention in contexts that are either consistent (Con) or inconsistent (Incon) with the extinction context (right). **c** | Physiological effects of exposure to SPS include enhanced glucocorticoid receptor (GR) expression in the hippocampus (Hipp) and frontal cortex (FC) and negative feedback in the hypothalamus–pituitary–adrenal (HPA) axis (left), decreased excitatory tone in the medial prefrontal cortex (mPFC) (middle), and decreases in tonic as well as increases in phasic responses to stimulation (arrow) of locus coeruleus (LC) neurons (right). Note: data are illustrative. * indicates statistical significance. ACTH, adrenocorticotropic hormone.

lead to hypotheses regarding abnormal unconditioned response in PTSD, such as startle. Results of studies using these animal models have also implicated cellular processes such as protein synthesis and apoptosis[184] in brain regions within this circuitry, suggesting novel interventions targeting these processes. However, the major contribution of animal models to date has been the identification of molecular biological processes that may be involved in PTSD pathophysiology, which may be targets for interventions too. These include alterations in neuroendocrine receptor systems (HPA), neurotransmitter receptor systems (glutamatergic, serotonergic and catecholaminergic) and cellular brain-derived neurotrophic factor (BDNF)–TrkB tyrosine kinase (also known as NTRK2) receptor systems.

HPA axis. Increased hippocampal glucocorticoid receptor levels have been found in SPS[179] and PredEx (using behavioural cut-off criteria)[185] models, as has an increased ratio of hippocampal glucocorticoid receptors over mineralocorticoid receptors in SPS[179,186]. Pretreatment with the glucocorticoid receptor antagonist RU40555 prevented SPS-induced changes in fear conditioning and LTP in the hippocampal CA1 region[180]. Similarly, glucocorticoid receptor antagonism (in combination with a β-noradrenergic blocker)[187], CRH1 receptor antagonism[188] and early treatment with high-dose corticosterone[189] prevented the development of a PTSD-like phenotype in a PredEx model. Together, these findings implicate hippocampal glucocorticoid receptors in the development of post-traumatic psychopathology, a role that is supported by recent evidence that high-dose glucocorticoid administration shifts appropriate contextual conditioned responding to generalized, context-inappropriate responding. Moreover, this shift was accompanied by c-FOS expression changes in the dorsal CA1 and ventral dentate gyrus regions of hippocampus[190]. These animal data are consistent with the glucocorticoid receptor hypersensitivity found in patients with PTSD and support further exploration of glucocorticoid receptor-targeted interventions as potential PTSD prevention and/or treatment approaches.

Brain-derived neurotrophic factor
(BDNF). A protein that is often released from a neuron, and that is involved in growth and the differentiation of new neurons and synapses.

TrkB
A membranous tyrosine kinase receptor that binds brain-derived neurotrophic factor and other neurotrophic factors (also known as neurotrophins).

Glutamate and NMDA. Abnormal vmPFC glutamate levels and hippocampal NMDA receptor levels have been found in SPS[191] and stress–restress[192] models, respectively. In addition, SPS models showed alterations in the level of the NMDA receptor modulator glycine and glycine transporter mRNA in the hippocampus[193]. Because glutamatergic projections from the vmPFC and the hippocampus are crucial in the modulation of fear extinction and contextual fear conditioning, decreased glutamatergic signalling could contribute to impaired extinction recall or enhanced contextual fear. Indeed, the NMDA receptor modulator D-cycloserine prevented SPS-induced changes in fear extinction and hippocampal NMDA receptor mRNA expression[194], and the NMDA antagonist CPP blocked the effects of predator stress on phosphorylated cAMP response element-binding protein (pCREB)-like immunoreactivity in brain areas implicated in fear behaviour[195]. These findings offer specific molecular targets for therapeutic strategies aimed at addressing fear memories and intrusive recollection in patients with PTSD, in addition to shedding light on pathophysiology.

5-hydroxytryptamine. The 5-HT system has a key role in regulating mood, anxiety and stress responses. 5-HT transporter knockout mice show an enhanced vulnerability to predator stress[196]. Changes in hippocampal 5-HT levels have been implicated in a stress–restress model[197], and an SSRI reversed behavioural changes in a PredEx model[198]. Increases in 5-HT_{1A} receptor levels in the dorsal raphe nucleus[199] and hippocampus[200] have been reported in SPS and time-dependent sensitization models, respectively, and the 5-HT_{1A} receptor antagonist WAY-100635 inhibited SPS-induced increases in glucocorticoid receptor and CRH expression in the hippocampus and hypothalamus[201]. In an SPS model, amygdala 5-HT_{2C} receptor gene expression was increased, and administration of a $5HT_{2C}$ receptor antagonist decreased SPS-induced contextual fear-related freezing[202]. Although the exact role of 5-HT systems in trauma-related psychopathology awaits further clarification, the animal data suggest that targeting potential upregulation of 5-HT_{1A} and 5-HT_{2C} receptors might prove a useful strategy in expanding the PTSD treatment and/or prevention arsenal.

Catecholamines. Noradrenergic transmission has a key role in arousal regulation and, together with glucocorticoids, in memory processes. Abnormal hippocampal noradrenaline levels have been implicated in late effects of SPS[197].

Animals exposed to PredEx and chronic stress show an abnormal behavioural and endocrine response to the α2-adrenergic autoreceptor antagonist yohimbine[176]. Noradrenergic abnormalities have also been reported in a modified FS+ reminders model[203]. β-adrenergic blockers prevent the anxiogenic effects of PredEx[187], and blockade of postsynaptic α1-adrenoreceptors normalizes the behavioural abnormalities in a modified FS+ reminders model[203]. Together with human data, these findings suggest that noradrenergic signalling might be a useful target for the development of treatment strategies aimed at decreasing arousal and stress reactivity.

Brain-derived neurotrophic factor–TrkB tyrosine kinase. BDNF–TrkB signalling is critical for hippocampal-dependent memory, so associated abnormalities could contribute to PTSD symptomatology. Changes in levels of BDNF mRNA and protein and in levels of the BDNF receptor TrkB have been reported in SPS and PredEx models. Interestingly, hippocampal TrkB levels were increased in both models, whereas BDNF levels were increased in SPS[204] but downregulated in PredEx[205]. *BDNF* DNA methylation may be differentially regulated in hippocampal subfields[206], which might explain this discrepancy. These findings are particularly interesting because there have been no identified effective neurotrophic factor-based treatment strategies to date. As these novel approaches become available, their applicability to PTSD will need to be examined.

Data from the above animal models suggest that multiple molecular pathways may be involved in PTSD pathophysiology. This is hardly surprising considering the fact that, on the one hand, PTSD symptomatology is heterogeneous, and, on the other hand, three of the five systems identified (glucocorticoid, catecholamine and 5-HT systems) interact and often regulate or modulate each other's activity. These models also have identified intriguing changes in glutamatergic and BDNF–TrkB systems that warrant further exploration as potential novel targets for prevention and intervention. Further research is needed to narrow these foci and translate potential targets into effective therapeutic strategies.

General conclusions

The research findings reviewed in this article suggest that PTSD has become one of the better understood psychiatric disorders from a biological standpoint, although much work remains. A substantial achievement has been the identification of core biological abnormalities that cut across a wide variety of traumatic events, ranging from child abuse to military combat. Part of the reason for the impressive progress in biological PTSD research has been the fact that, unlike in many psychiatric conditions, the causal environmental event, and hence the onset of the pathophysiological process, are considered to be known. This has provided a jump-start for investigating in both human patients and animal models the organic, cellular and molecular pathophysiological processes set in motion by the traumatic event.

PTSD biomarkers. As reviewed above, a number of biological abnormalities have been found statistically to discriminate PTSD from non-PTSD control groups in various studies; on this basis, they may loosely be regarded as biomarkers. However, none of them possesses the specificity and sensitivity that is necessary to be used as a stand-alone diagnostic test for PTSD. The current 'gold standard' for a PTSD diagnosis are the diagnostic criteria set forth in the fourth edition

of the *Diagnostic and Statistical Manual of Mental Disorders*[2], which rely heavily on patients' subjective reports. The reliability of subjectively reported symptoms directly affects the sensitivity and specificity of PTSD biomarkers because the latter can be no more reliable or accurate than the diagnostic standard. Someday, a biomarker (or combination of biomarkers) may be the gold standard for PTSD diagnosis against which the accuracy of subjective measures will be judged.

From an applied standpoint, biomarkers that are likely to represent pre-existing risk factors might be used to identify individuals who are at an especially high risk of PTSD for preventive intervention. However, no putative biomarker has yet progressed to the point of practical use. Acquired biomarkers (that is, biomarkers of the disorder itself rather than of vulnerability for the disorder) offer promise as targets of therapeutic intervention. Importantly, however, blood, CSF and/or brain neuroimaging markers that are collected during a resting state may turn out to be inadequate to fully characterize PTSD, given that a central feature of the PTSD diagnosis is an abnormal reactivity to both trauma-related and trauma-unrelated stimuli. Measures acquired under conditions of symptom provocation (for example, responses to traumatic reminders) may offer more promise as PTSD diagnostic biomarkers[207].

Translational post-traumatic stress disorder research. It is scarcely possible to read a grant proposal nowadays without encountering the buzz word 'translational'. If translational is taken to mean the cross-fertilization that can be achieved when results from animal research are used to inform human studies and vice versa, then PTSD research has been highly fruitful in this regard. However, if translational is taken to mean the conversion of research results into novel and effective treatments, the story is different. The currently most effective treatment for PTSD, *viz*., cognitive behavioural therapy, was conceived entirely on psychological grounds. Trials of the most effective drugs for PTSD, *viz*., SSRIs, were based on these drugs' observed antidepressant effect; the recognition that the 5-HT system is involved in the biology of PTSD only came afterwards. Indeed, despite the abundance of biological insights into PTSD that have been achieved, it is difficult to think of examples of any of these having improved treatment.

A recent example of translational research that could potentially lead to a clinical application is the preliminary finding, which has been described above, that a high dose of cortisol given shortly after the traumatic event reduced the probability of developing PTSD[127]. This study was inspired by observations that the natural cortisol response to a traumatic event may be lower in people who go on to develop PTSD[208,209]. If the results of this preliminary study are replicated, high-dose cortisol may become part of psychiatrists' and emergency room physicians' armamentarium for preventing this disorder.

An example of a potential translational treatment for PTSD is blockade of traumatic memory reconsolidation[210,211]. This approach is based on animal findings that a fear memory may not necessarily last forever but may be susceptible to pharmacological intervention after it has been activated. However, insufficient human studies exist to conclude that this intervention is efficacious in PTSD. Non-pharmacological memory updating procedures based on reconsolidation may also offer promise, but these have only been studied preclinically[212]. To date, the development of PTSD treatments has not been different from the history of much of medicine: effective agents are discovered by serendipity, and their biological mechanisms of action clarified later.

Future directions. A conspicuous limitation in PTSD research to date has been reliance on cross-sectional (that is, correlational) designs in the overwhelming majority of studies in patients with PTSD. There is currently a dearth of pre-trauma prospective studies, which are required to establish causation. That such studies are expensive and difficult to perform is the likely reason they are few in number, but they will be required if biological PTSD research is to advance above the plateau posed by cross-sectional designs. Prospective treatment studies that attempt to identify biomarkers that inform prognosis and aid in treatment selection, about which little guidance currently exists, will be especially useful. Novel molecular targets implicated by validated animal models (for example, neurotrophic factors) should be examined as potential biomarkers, as well as used to further dissect the molecular processes involved. It will be necessary to continue to move from serendipitous discovery to testing translational hypotheses derived from preclinical animal and human work, as guided for example by the US National Institute of Mental Health's recent emphasis on 'research domain criteria'. Progress may be also expected from capitalizing on technological advances, for example, use of mass spectography instead of RIA to better characterize critical molecules; the development of radioligands with greater receptor specificity; improved neuroimaging resolution that is capable of measuring, for example, activity in functionally different amygdala substructures; finding a way to perform DNA analyses on live brain tissue; and the application of new fusion algorithms derived from bioengineering to complex, multivariable data.

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Competing interests statement
The authors declare no competing financial interests.

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