Molecular Psychiatry (2020) 25:2251–2274
https://doi.org/10.1038/s41380-019-0639-2

EXPERT REVIEW

Neurobiology of BDNF in fear memory, sensitivity to stress, and stress-related disorders

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Received: 20 March 2019 / Revised: 1 December 2019 / Accepted: 12 December 2019 / Published online: 3 January 2020

Abstract

Brain-derived neurotrophic factor (BDNF) is widely accepted for its involvement in resilience and antidepressant drug action, is a common genetic locus of risk for mental illnesses, and remains one of the most prominently studied molecules within psychiatry. Stress, which arguably remains the "lowest *common* denominator" risk factor for several mental illnesses, targets BDNF in disease-implicated brain regions and circuits. Altered stress-related responses have also been observed in animal models of BDNF deficiency in vivo, and BDNF is a common downstream intermediary for environmental factors that potentiate anxiety- and depressive-like behavior. However, BDNF's broad functionality has manifested a heterogeneous literature; likely reflecting that BDNF plays a hitherto under-recognized multifactorial role as both a regulator and target of stress hormone signaling within the brain. The role of BDNF in vulnerability to stress and stress-related disorders, such as posttraumatic stress disorder (PTSD), is a prominent example where inconsistent effects have emerged across numerous models, labs, and disciplines. In the current review we provide a contemporary update on the neurobiology of BDNF including new data from the behavioral neuroscience and neuropsychiatry literature on fear memory consolidation and extinction, stress, and PTSD. First we present an overview of recent advances in knowledge on the role of BDNF within the fear circuitry, as well as address mounting evidence whereby stress hormones interact with endogenous BDNF-TrkB signaling to alter brain homeostasis. Glucocorticoid signaling also acutely recruits BDNF to *enhance* the expression of fear memory. We then include observations that the functional common BDNF Val66Met polymorphism modulates stress susceptibility as well as stress-related and stress-inducible neuropsychiatric endophenotypes in both man and mouse. We conclude by proposing a BDNF stress–sensitivity hypothesis, which posits that disruption of endogenous BDNF activity by common factors (such as the BDNF Val66Met variant) potentiates sensitivity to stress and, by extension, vulnerability to stress-inducible illnesses. Thus, BDNF may induce plasticity to deleteriously promote the encoding of fear and trauma but, conversely, also enable adaptive plasticity during extinction learning to suppress PTSD-like fear responses. Ergo regulators of BDNF availability, such as the Val66Met polymorphism, may orchestrate sensitivity to stress, trauma, and risk of stress-induced disorders such as PTSD. Given an increasing interest in personalized psychiatry and clinically complex cases, this model provides a framework from which to experimentally disentangle the causal actions of BDNF in stress responses, which likely interact to potentiate, produce, and impair treatment of, stress-related psychiatric disorders.

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Introduction

Neuropsychiatric disorders are heterogeneous in their clinical manifestations and pathogenesis, and arise from multiple genetic, biological, and environmental risk factors that combine to produce convergent clinical manifestations despite otherwise divergent biological ontogeny. Despite such disparate origins, it is highly likely that risk factors to these disorders converge upon common pathways to procure similar disease outcomes. Therefore, identifying and understanding common risk factors and mechanisms of mental illnesses is fundamental to understanding disease origins as well as next-generation therapeutics. Stress is one such "lowest common denominator" that promotes vulnerability to, or exacerbates the symptoms of, almost all mental illnesses. Therefore, stress is one example of a common risk factor with defined signaling pathways that appears highly relevant to understanding mental disorders. However, stress is particularly relevant to disorders such as posttraumatic stress disorder (PTSD), given that significant stress exposure (e.g., trauma) is a requisite etiological factor for its onset [1]. Yet, the relationship between stress and stress-related psychiatric disorders is not clear-cut and there are many molecular mechanisms involved in the *adaptation* to stress, as well as a variety of putative genetic intermediaries that gate *vulnerability* to stress that remain to be defined.

Here we provide a focused analysis of emergent themes in the brain-derived neurotrophic factor (BDNF) literature, with a focus on the role of BDNF in the fear circuitry. We describe mechanisms for how glucocorticoids regulate BDNF-TrkB dynamics and signaling, and review evidence that the BDNF Val66Met polymorphism may mediate sensitivity to stress. This includes analysis at both clinical, behavioral, cellular, circuit, and molecular levels, sex specificity, and with a specific focus on known interactions with glucocorticoid stress hormones within the context of PTSD.

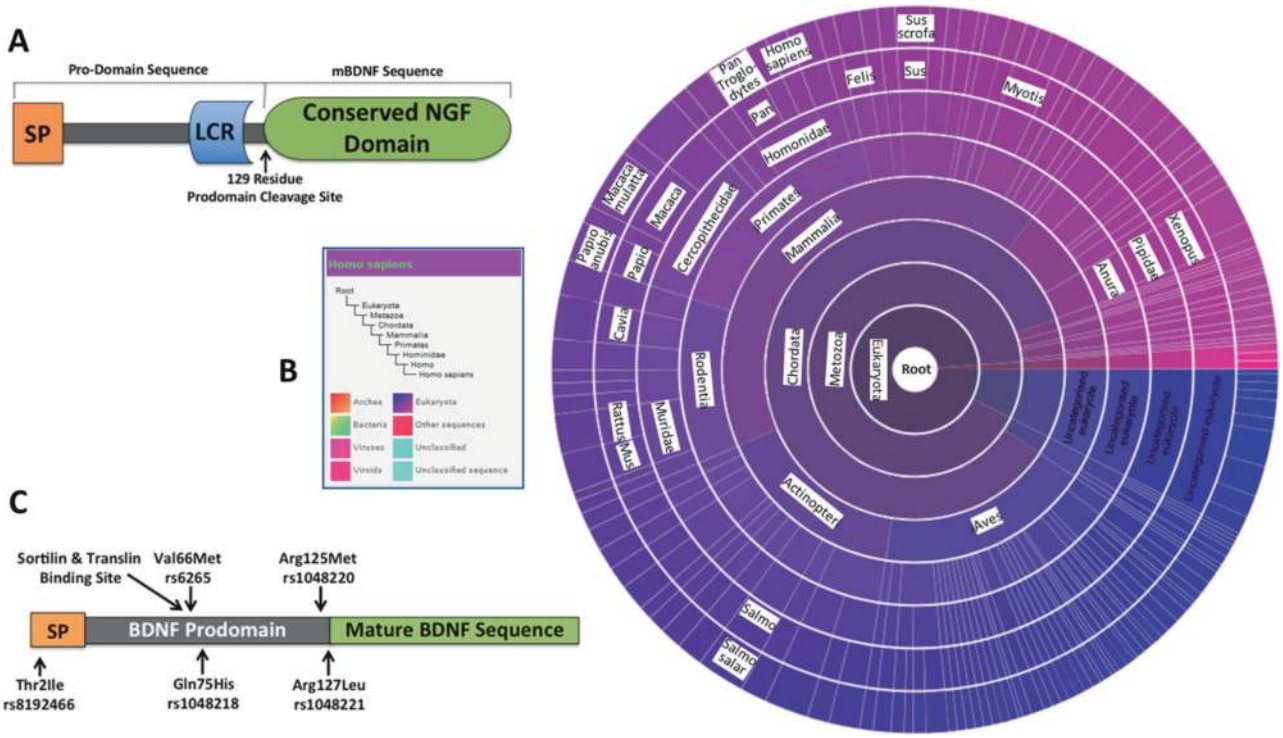

Fig. 1 The domain structure of BDNF, conservation of the NGF domain across species, and common BDNF coding polymorphisms in humans. a The BDNF peptide is comprised of a predicted signal peptide (amino acids 1–18; SP in figure), a low complexity region (amino acids 100–111; LCR in figure) and, following a pro- to mature cleavage site terminating at amino acid residue 129, a NGF family domain (amino acids 133–246). **b** This NGF domain represents almost the complete sequence of the mature BDNF amino acid sequence excepting approximately four amino acid residues, highlighting the functional segregation of mBDNF from proBDNF. The NGF domain is conserved across 142 eukaryote species in the Protein Family (PFAM) database [182]. Each line in the outer ring of this sunburst graph (taxa labeled) represents a conserved NGF domain in a species with available sequence data. These data and visualizations suggest that the BDNF amino acid sequence must maintain functional compartmentalization. **c** Most defined "coding" SNPs in the BDNF gene occur within the BDNF prodomain sequence, and not within the mature sequence. As the mBDNF sequence is comprised almost entirely of a NGF domain, modifications to this region may result in deleterious consequences leading to poor survival or deficient intergenerational transmission. The location of the $BDNF^{Val66Met}$ polymorphism coincides at a residue which sortilin (for BDNF protein) and translin (for BDNF mRNA) likely interact with. Coding polymorphism data adapted from UniProt [183].

Advances in the neurobiology of BDNF

A primer of biologically active BDNF peptides

Growth factors are molecules involved in maintaining the development, differentiation, and plasticity of neurons within the brain. Growth factors have thus become an integral component to many neurodevelopmental hypotheses of psychiatric disorders [2, 3], largely due to their diverse roles in maintaining normal brain development and adult plasticity. The principle growth factors expressed within the brain are a group of four proteins referred to as neurotrophins, comprising nerve growth factor (NGF), BDNF, neurotrophin-3 (NT-3), and NT-4 [3]. There is considerable overlap in the amino acid sequences of these molecules, estimated to be ~50%, with each containing a signal peptide and a prodomain that can undergo glycosylation within the N-terminus [4]. The evolutionary conservation of NGF protein domain within neurotrophins across organisms and within mammals (Fig. 1) highlights their fundamental importance in normal neural development and function. That said, each of these molecules binds with high affinity to specific cognate receptors, namely NGF to tyrosine-related kinase (Trk)A, BDNF and NT-4 to TrkB and NT-3 to TrkC (for more extensive discussion, see [3]). Much of the research implicating a role for trophic factors in psychiatric disorders has focused on BDNF, as this molecule is differentially expressed across the brain according to a developmental trajectory (see Fig. 2), is highly responsive to stress (see below), and is implicated as critically involved as a molecular intermediary of many therapeutics, especially antidepressants [5]. Furthermore, genetic association studies have provided evidence that several single-nucleotide polymorphisms (SNPs) within the *BDNF* gene may be involved in risk of psychiatric disorders.

The BDNF peptide was first discovered in 1982 [6], and is a highly conserved chain of 247 amino acids. The first BDNF peptide assembled following translation from mRNA to protein is a 32–35 kDa isoform termed pre-proBDNF, which is rapidly cleaved within the endoplasmic reticulum to yield to a 28–32 kDa isoform known as proBDNF. The proBDNF isoform can be secreted as a biologically functional peptide, although at lower quantities than its mature counterpart [7], and binds to a p75 NTR-Sortilin receptor complex. Alternatively, proBDNF can be cleaved to yield the mBDNF 14 kDa isoform best known for its trophic actions at the TrkB receptor [3, 8]. Until recently, it was believed that the cleaved BDNF prodomain met a proteolytic end. However, there is emergent evidence that the cleaved prodomain alters neuronal physiology [9] and is able to bind to the sortilin-related SorCS2 receptor [10] to initiate a number of biological effects that are independent of both proBDNF and mBDNF (see below for further discussion). The high conservation of the BDNF coding sequence as well as the observation that the mBDNF peptide sequence is almost exclusively comprised of an NGF signaling domain highlights a (1) likely conserved core set of functions for mBDNF in the brain across species (Fig. 1) and (2) possible sensitivity to genetic variation [3].

Eccentricities in BDNF expression

A core characteristic of neurotrophins is their relative scarcity but potency. While many studies discuss the ubiquity of BDNF within the mammalian brain, this is a misnomer by designation. Specifically, expression levels of BDNF can be identified across many brain regions (Fig. 2) and numerous neural cell types, but total protein expression relative to tissue weight remains low. A published estimate is that 1 µg of BDNF is expressed per 1.5 kg of brain tissue [6]. Most detectable BDNF within the adult brain is also likely to be the mature isoform, with in vitro estimations suggesting that proBDNF accounts for as little as 10.8% of total BDNF expression [11]. It should be noted that the expression ratio of proBDNF:mBDNF is largely dependent on developmental stage (Fig. 2); in early postnatal development proBDNF is expressed more than mBDNF but by adolescence and adulthood an increase in the expression of cleavage factors results in comparably little proBDNF but more abundant mBDNF [12]. This developmental shift in pro- to mBDNF expression across adolescence is interesting for numerous reasons. First, it co-occurs with a major developmental window of risk for mental illness. Second, this developmental window is commonly defined by substantial chronic life stress which is different between the sexes [13]. The *BDNF* gene contains a putative estrogen-response element [14], making BDNF expression sensitive to fluctuations in estrogen availability [13] and thus estrus cycle stage [15]. As females are often reported to be more vulnerable to numerous stress-inducible mental illnesses, particularly during adolescence and young-adulthood, the timing of this biological shift in BDNF processing is of particular note and potential importance.

A further eccentricity in BDNF expression is that not all brain regions uniformly express BDNF (Fig. 2), predicting BDNF-TrkB signaling in a region-specific manner. For instance, the transport of BDNF between local and distal brain regions has been described between the cortex and striatum [16] as well as hippocampus and cortex [17]. It is also likely that the habenula, central nucleus of the amygdala, bed nucleus of the stria terminalis, lateral septum, and spinal cord may acquire at least some BDNF protein from axonal afferent projections [18]. Thus, sex (and, by extension, estrous cycle), developmental timing, and brain regions must be considered when discussing the

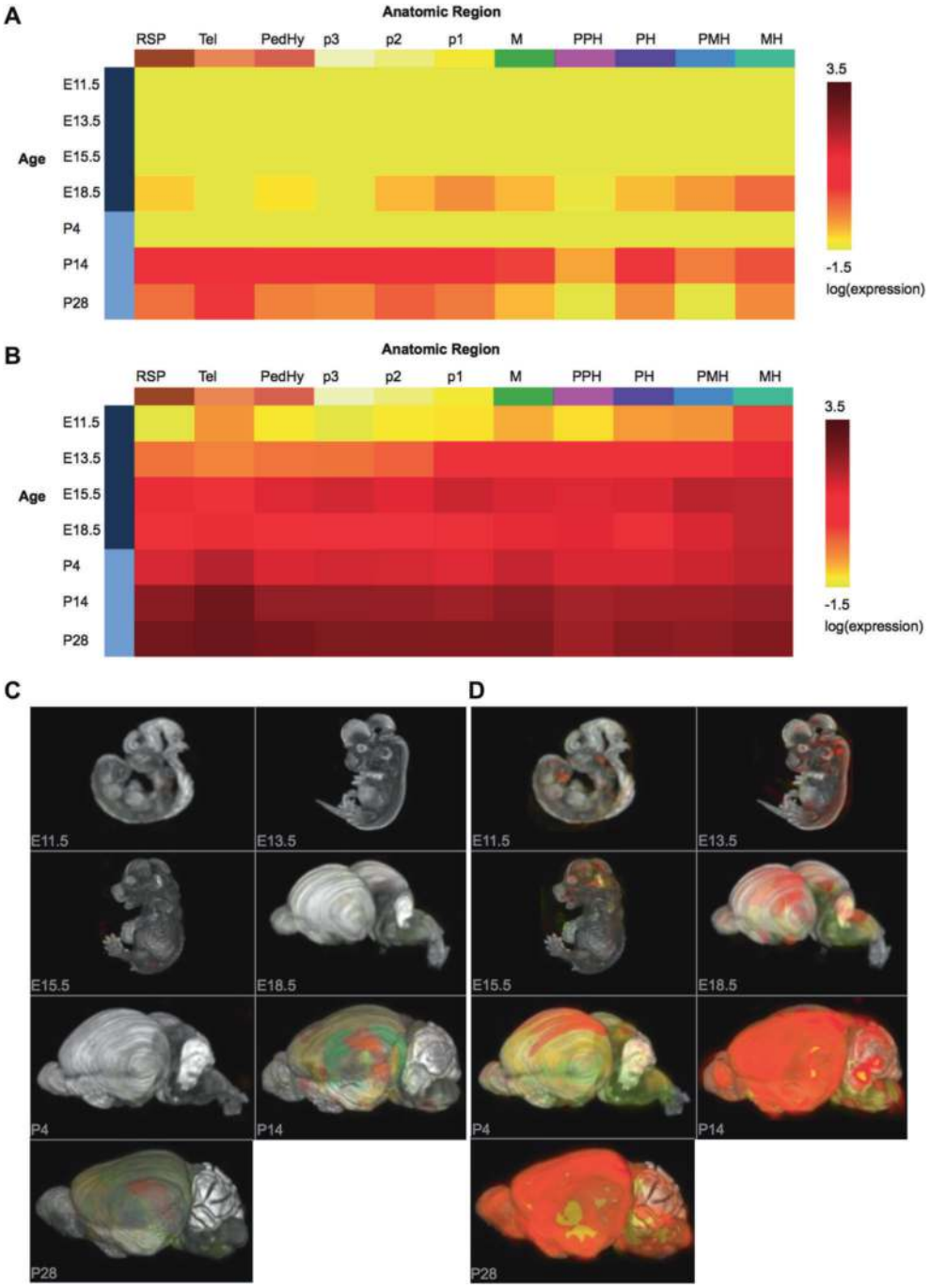

Fig. 2 The early developmental expression profile of BDNF (*Bdnf*) and TrkB (NTRK2) mRNA expression in the mouse brain. In situ hybridization (ISH) of BDNF mRNA expression in the developing murine brain. As discussed in text, expression of BDNF is modulated by an array of both endogenous innate and exogenous environmental factors and BDNF shows fluctuating expression depending upon developmental stage. Allen Institute ISH data have been collapsed and collated in both heatmaps (**a**, **b**) and in anatomical 3D reconstructions of serial sections (**c**, **d**) to showcase brain-wide *Bdnf* and *NTRK2* expression from embryonic day 11.5 (E11.5; onset of cortical development) to postnatal day 28 (P28; adolescence). **a** These visualizations reveal that *Bdnf* expression substantially increases during postnatal brain development from P4 to P14 and that this expression profile is refined by P28. **b** Conversely, mRNA expression of BDNF's cognate receptor *NTRK2* precedes major developmental changes in BDNF expression, and this mRNA expression profile increases dramatically during postnatal brain development across the entirety of the brain. BDNF within the adult brain is relatively ubiquitous but its fast turnover and potent activities result in constant peptide expression in the subpicomolar range [184]. TrkB expression thus becomes saturated to capture all available BDNF, as well as likely other neurotrophic agents that exhibit binding affinity. In addition, TrkB may also elicit BDNF-independent effects via interaction with other effectors, which may also contribute to this saturated expression profile. Data were adapted and reproduced from the Allen Institute, as consistent with their republication policy [185]. Image credit: Allen Institute.

expression and functionality of BDNF. This is particularly important to emphasize within the context of disease where clinical and/or peripheral measurements of BDNF (e.g., blood fractions, cerebrospinal fluid, etc.) are often taken to represent "brain BDNF" which is not uniformly patterned between regions.

BDNF genetic variation and coding polymorphisms

While the BDNF protein is highly conserved between species, with the coding sequence of the BDNF gene largely being spared of evolutionary drift (Fig. 1b), the human *BDNF* gene has been shown to contain a rather astounding number of gene variants, with over 40,000 variant entries currently stored in the Database of SNPs [19]. Most common *BDNF* SNPs reside within noncoding regions of the *BDNF* gene, and the functionality of almost all these SNPs thus remains unknown. To the best of the authors' knowledge, the only exception is the intronic rs12291063 variant, which disrupts hypothalamic BDNF expression in humans and risk of obesity [20]. Even so, the pool of *common* (typically >1% frequency) coding SNPs remains relatively small. These major SNPs are depicted in Fig. 1c. Because the reported variants fall within the BDNF prodomain, they may affect how BDNF is cleaved, trafficked, sorted, and released, and may induce a proapoptotic shift in BDNF function by reducing the availability of mBDNF while concomitantly increasing proBDNF availability [3]. However, the functionality of these coding SNPs remains largely unknown, with the exception of the widely studied $BDNF^{Val66Met}$ variant.

The $BDNF^{Val66Met}$ SNP

The $BDNF^{Val66Met}$ variant (rs6265) is one of the most widely studied gene variants within the psychiatric neuroscience literature [2]. This polymorphism involves a single-nucleotide substitution of guanine → adenine at position 196 within the BDNF coding region, resulting in a nonsynonymous amino acid replacement of valine → methionine within codon 66 of the BDNF prodomain. Based on this, in both humans and knockin rodent models, a Val/Val genotype represents a "wildtype" genotype while a Val/Met and Met/Met genotype reflects heterozygosity and homozygosity, respectively. The $BDNF^{Val66Met}$ polymorphism is a relatively common variant, however estimations of frequency vary between ethnicities. Amongst Caucasians the frequency of the $BDNF^{66Met}$ allele is ~30%, however amongst Asians this frequency increases amongst certain subethnic groups to reach 72% [21]. This is important to note, as susceptibility to the functional effects of this gene variant may be dependent upon genetic background [2]. Indeed, it was suggested as early as 2006 [22] that Caucasians may be more vulnerable to the $BDNF^{Val66Met}$ polymorphism than Asians, and speculation that population stratification is likely to gate the effects of this polymorphism on behavior and disease has continued to grow over the past decade [2].

The $BDNF^{Val66Met}$ variant was first identified in the late 1990s, but its functionality was only confirmed in 2003 in a now seminal report [23]. In this paper, the $BDNF^{Val66Met}$ polymorphism was shown to disrupt episodic memory in humans, a form of hippocampus-dependent memory function. Since then the effect of the $BDNF^{Val66Met}$ polymorphism on hippocampus-dependent memory function has been extensively studied and we refer interested readers to our prior reviews covering this literature [2, 24]. In addition to cognition, Egan et al. also examined the effects of the $BDNF^{Val66Met}$ polymorphism in vitro using a $BDNF^{66Val\text{-}GFP}$ or $BDNF^{66Met\text{-}GFP}$ knockin construct that was expressed in cultured hippocampal neurons via a viral delivery system [23]. This approach revealed that while the $BDNF^{Val66Met}$ polymorphism did not alter BDNF expression per se [23], there were discrete abnormalities in the intracellular localization of BDNF in cells transfected with the $BDNF^{66Met\text{-}GFP}$ construct. Specifically, in these cells, BDNF was found to accumulate in the perinuclear region of the cell soma, was sparsely observed within dendrites and did not colocalize with the secretory vesicle marker secretogranin II or the presynaptic marker synaptophysin [23]. Several years later, in 2005, it was discovered that the $BDNF^{66Met}$ substitution serendipitously co-occurs at a sortilin interaction region [25], resulting in deficient sorting of $BDNF^{66Met}$ to the activity-dependent release pathway [25]. Constitutive release of BDNF is not altered by the $BDNF^{66Met}$ substitution [23]. This phenotype was later replicated, using hippocampal–cortical neurons isolated from $BDNF^{Val66Met}$ mutant mice [22]. Likewise, the $BDNF^{66Met}$ substitution also disrupts the binding of translin, a molecule which controls BDNF mRNA trafficking, resulting in perturbed dendritic targeting of BDNF mRNA [26]. Thus, the principle molecular mechanism associated with the $BDNF^{Val66Met}$ polymorphism is the deficient activity-dependent release of BDNF, which consequently impacts the efficiency of BDNF-TrkB signaling.

The effect of the $BDNF^{Val66Met}$ polymorphism at the synapse, however, extends beyond reduced BDNF-TrkB signaling during activity-dependent processing. Prominently, a recent advancement in our understanding of BDNF functionality is that the cleaved BDNF prodomain elicits its own set of biological functions [27, 28] that are independent of both proBDNF and mBDNF. It has also been reported that the functionality of this isoform is dependent on whether the prodomain sequence contains the $BDNF^{66Met}$ substitution [10, 28]. While little is known of exact pathways or defined mechanisms, there is convincing

evidence that the prodomain is secreted following neuronal depolarization [27] (i.e., in an activity-dependent manner [10]), that it can bind to the SorCS2 receptor [10], and that this ligand modulates dendritic spine density via caspase-3 [27]. These functions are dependent on whether the BDNF prodomain contains the 66Met substitution [10]. The $BDNF^{66Met}$ prodomain is able to elicit neuronal growth cone retraction in vitro—an effect which requires the SorCS2 receptor [10]. In addition, the WT BDNF prodomain can recruit a synapse weakening pathway that the $BDNF^{66Met}$ prodomain appears unable to initiate [28]. Lastly, while the WT BDNF prodomain has been reported to facilitate long-term depression (LTD), the $BDNF^{66Met}$ substitution putatively inhibits hippocampal LTD [9]. This result is striking, as one may expect the 66Met-containing prodomain to also elicit LTD given a growth cone retraction phenotype. In addition, it was recently shown that the 66Met-containing BDNF prodomain also results in dendritic spine density decreases both in vitro and in vivo [29]. However, there have been some speculative mechanisms for why this differential LTD phenotype exists in spite of structural correlates of LTD being induced by the 66Met-containing prodomain. Namely, some intrinsic difference may exist in the ligand potential of this 66Met-containing peptide that inhibits its ability to initiate or inhibit certain pathways via SorCS2 or p75 neurotrophin receptor complexes—perhaps due to its disordered state and differences in folding as suggested by [9]. It will thus be important to replicate this differential effect on LTD, as well as further characterize electrophysiological mechanisms of the BDNF prodomain. Nonetheless, these data highlight that both the WT and $BDNF^{66Met}$ prodomain can elicit discrete functionality.

Sex-steroid hormones: relevance to BDNF?

As briefly eluded to in previous sections, there is considerable evidence of interaction between BDNF, stress-related phenotypes, and sex-steroid hormones—particularly female sex-steroid hormones. Androgens may regulate some aspects of BDNF function, however comparatively speaking, androgen-BDNF effects are not as well documented as those for female sex-steroid hormones [30]. In the sections below, we review evidence that female sex-steroid hormones are capable of exerting local brain effects, and that these steroid-induced brain effects converge upon classical functions associated with BDNF-TrkB signaling.

While the main source of systemic 17β-estradiol is clearly the ovaries, estradiol can also be synthesized locally in the brain from cholesterol [31]. Brain-local estradiol maintains numerous biological activities that are fundamental to both cell and circuit function including neuronal survival, synaptogenesis [31, 32], and synaptic plasticity [32]. Neuronally localized aromatase also functions to convert androgen precursors into estrogens in males [33], implicating that estrogen is locally produced between sexes within neurons in the brain. In recent work, it has been aptly stated that de novo derived estradiol in the hippocampus, rather than from the periphery [34], plays a fundamental role in the neuroprotective and plasticity-promoting effects of estrogens in this brain region (see discussion in [34]). Not surprisingly, estradiol has also been shown to regulate hippocampal synaptic plasticity [35, 36], and this fluctuates over the estrous cycles as estradiol levels endogenously change [36]. This indicates that estradiol holds intrinsic potential to endogenously regulate synaptic plasticity in the brain, and is a modulator of learning and memory.

Many effects of estradiol in the brain mimic classical functions of BDNF and, consequently, there are a great number of papers that have reported interplay between BDNF and sex-steroid hormones. Prominently, the inclusion of a putative estrogen-response element in the BDNF gene may allow transcriptional control of BDNF by estrogens [14]. In support of this, BDNF is decreased by OVX in cortical regions [37] and the hippocampal formation [37] in mice. Estradiol has also been variably reported to modulate expression of BDNF's cognate receptor, TrkB [38], phosphorylation of Akt—one of the major BDNF-TrkB signaling cascades, as well as phosphorylation of TrkB in the hippocampus [39]. Furthermore, phosphorylation of TrkB changes across the estrous cycle [40]. Most phosphorylated TrkB was localized to presynaptic terminals of neurons, indicating that BDNF-dependent effects of estradiol at the synapse may reflect retrograde signals [40] (for review on BDNF retrograde signaling, see [3]). Not surprisingly then, the effect of estradiol on hippocampal plasticity appears to be modulated by mitogen-activated protein kinase (MAPK) signaling [36]—another principal BDNF-TrkB signaling pathway. This MAPK-signaling effect is dependent upon NMDA receptor levels [36], a receptor whose subunit expression levels are not surprisingly also regulated by both BDNF [41] and estradiol [42] alike. Likewise, BDNF is a likely molecular intermediary which supports estradiol's effects on promotion of spine formation within the hippocampus [43].

It should be noted that competing hormonal factors may also affect BDNF function. For example, progesterone [44] can act as a glucocorticoid agonist [45], can regulate BDNF [46], can block estradiol's neuroprotective effects by downregulating estrogen receptor β [47], and can prevent BDNF induction by estradiol [48]. Thus, understanding the effects of such additional hormonal factors on BDNF function is a particular priority.

Lastly, it will be essential to resolve developmental effects of BDNF–estradiol interactions if sex differences are to be holistically defined. Just as stress is able to exert developmental effects by regulating neural development

during critical periods [49], interactions between estradiol and BDNF may also be developmentally important and contribute to differential adult phenotypes [13, 50, 51]. While a number of longitudinal studies in mice have been published in recent years which begin to shed light on developmental effects and interactions between sex-steroid hormones, BDNF, and the brain, much remains unknown. It is therefore important to consider the broader brain landscape, including cell diversity, brain regions, other competing steroid factors, and developmental staging, in future studies that examine BDNF's contribution to sex differences.

BDNF's contribution to sex differences via interneurons

An interesting relationship has emerged between sex-steroid hormone effects on interneurons as these many interneuron subtypes express estrogen receptors α and/or β [52–55]. Importantly, these receptors are also capable of regulating aspects of development [52]. Estradiol's promotion of spine formation is also mediated by modulating GABAergic neurotransmission [56], indicating that GABAergic interneurons are regulated by estradiol both developmentally and acutely in their function when mature. A specific interest has emerged in the literature for a subset of interneurons which express Parvalbumin (PV). Estradiol has a role in modulating PV interneuron development and density in the hippocampus [57] and cortex [58], and these effects are both developmentally modulated by BDNF and sex in these brain regions [57, 58]. In addition, estradiol has been shown to functionally modulate PV interneuron functionality such as production of high-frequency gamma oscillations in vivo [59]. Similar to estradiol, BDNF also modulates PV interneuron development [58], density [60], and gamma oscillations in the hippocampus [61]. This establishes PV interneurons as a BDNF- and estradiol-sensitive cell-type. In the scope of the current review, this is of interest as these cells have also been shown to be functionally important for fear-related behavior in numerous brain regions [62], and their regulation of high-frequency synchronous activity in the gamma band has become an electrophysiological signature for various aspects of fear behavior in mice [63] as well as fear extinction in humans [64]. Thus, local regulation of BDNF-TrkB signaling in PV interneurons by estradiol may be a substrate and potential mechanism for sex differences with respect to fear behavior. Indeed, in mouse models of disrupted BDNF-TrkB signaling in PV interneurons, sexually dimorphic behavioral differences have emerged [65, 66]. This is convergent with the contrasting effects of testosterone and estradiol on BDNF expression and tonic suppression/promotion of hippocampal synaptic plasticity (see discussion in [67]), suggesting that female sex-steroid hormones may be able to compensate for BDNF-TrkB loss in PV interneurons [65, 66]. BDNF's and estradiol's mutual regulation of PV interneurons, and the sex-specific vulnerability induced by disrupting BDNF-TrkB signaling in PV interneurons, represents a concise, relevant, and cell-specific mechanism by which BDNF may contribute to sex differences relevant to disorders of fear memory. With yet further work, a fundamental molecular description for how sex-steroid hormones regulate BDNF-TrkB signaling in PV inhibitory interneurons may yield a sex-specific molecular mechanism or cascade that may hold therapeutic potential in promoting fear extinction.

BDNF in the fear circuitry and risk of PTSD

A baseline role for BDNF in PTSD and the fear circuitry?

PTSD arises following exposure to a severe stressor such as a threat to one's health, witnessing harm, or being the victim of trauma, and is characterized by the presence of flashbacks, increased arousal, and the inability to effectively learn safety signals. PTSD is considered a disorder of "*nonrecovery*" from traumatic stress [68]. Unfortunately, little is known about the mechanisms which determine this response variation to trauma, as well as pretraumatic and posttraumatic risk factors which may synergistically alter risk of PTSD. For instance, most people experience significant traumatic stress at some instance in their life—an estimated 60% of men and 51.2% of women [69]—but only a small number of individuals appear to carry latent vulnerability which results in persistent post-traumatic stress. Thus, modulatory factors including genetic variants are likely to play a role in promoting risk and conversion to disease states in vulnerable individuals.

Similar to most anxiety disorders, little is known as to whether or how BDNF robustly modulates risk of PTSD [2]. The BDNF gene, in general, has not been widely studied within PTSD cases and most reports which have investigated the role of the $BDNF^{Val66Met}$ polymorphism within this disorder have only been published relatively recently [2]. The results of these studies are summarized in Table 1. While promising, the limited number of studies in this field as well as noted *Z*-score weightings of individual studies in a meta-analysis [70], make it unlikely that a single-*genomic* association exists between Val66Met and PTSD at this locus alone. Moreover, a range of other evidence suggests that these heterogeneously sampled and single-locus studies may not completely capture disease relevance. Specifically, there now exists overwhelming evidence that both BDNF at a basic mechanisms level, and the Val66Met substitution, are likely to modulate disease-predisposing endophenotypes of stress-related disorders including, but not limited to, PTSD. This includes

Table 1 Overview of $BDNF^{Val66Met}$ Polymorphism Association with PTSD.

Authors	Association with PTSD	Ethnicity and total sample
Lee et al. [186]	No association	Korean Control = 161 PTSD = 106
Zhang et al. [187]	No association	European-American Control = 250 PTSD = 96
Valente et al. [188]	No association	Brazilian Control = 335 PTSD = 99
Pivac et al. [189]	↑ Frequency of 66Met allele in PTSD patients with psychotic features	Caucasian War veteran = 576
Zhang et al. [71]	↑ Frequency of Met/Met genotype and 66Met allele in probable PTSD cases	Mostly Caucasian (73%) US Special Operations soldiers = 461
Felmingham et al. [72]	↓ Response to exposure therapy amongst PTSD cases carrying the 66Met allele	Caucasian PTSD = 55
Li et al. [190]	↓ PTSD severity in 66Val allele carriers 18 months following Wenchuan earthquake	Chinese Adolescents = 524
van den Heuvel et al. [191]	No association	Ethnicity unreported Road traffic accident survivors = 123
Lyoo et al. [192]	No association	South Korean Controls = 36 PTSD = 30
Dretsch et al. [193]	BDNF Val66Met genotype associated with positive screening for traumatic stress	Mixed-ethnicity veterans Pre-deployment = 231 Post-deployment = 458
Bruenig et al. [194]	↑ Protection amongst Val/Val in pooled meta-analysis	Veterans Controls = 106 PTSD = 151 Meta-analysis = 3,625
Pitts et al. [97]	↑ Symptom severity in Met/Met individuals ↑ Trauma-burden effect on severity greater in Met/Met ↓ Severity in Met/Met carriers who exercised	Euro-American veterans Cohort 1 = 1386 Replication cohort = 509
Tudor et al. [195]	No association of PTSD metabolic defects with Val66Met	Caucasian Combat veterans = 333
Guo et al. [196]	↑ Risk of PTSD in 66Met ↓ Cognitive function in 66Met with PTSD Low serum BDNF level was a contributor to cognitive deficits, while Val/Val genotype was a protective factor	Chinese Carcinoma patients with PTSD
Guo et al. [197]	No association ↑ Risk T allele carriers of BDNF C270T polymorphism	Chinese Controls = 150 Sporadic PTSD = 300

phenotypes such as fear behavior and vulnerability to stress. Notably, these phenotypes may only be subtle, and thus alter risk incrementally. Therefore, it will be important for human studies to adequately sample relevant populations to ensure appropriate power. It will be important to control for and stratify analyses for potential confounding effects of Val66Met phenotype which include factors such as ethnicity, disease status, diagnosis type, trauma type, and other genetic as well as environmental factors (see [2] for discussion). Interestingly, one meta-analysis evaluating the Val66Met variant as a single-variant risk factor for PTSD reported that "stress status" may mediate the relationship between Val66Met and disease risk at least amongst controls. This highlights that an endophenotype approach, which we define here as the parsing of clinical and behavioral phenotypes into discrete variables that can be evaluated in lieu of biological factors, may be useful for parsing gene–environment interactions with stress in PTSD. In humans, these include numerous important observations such as the probability of 66Met allele status in probable PTSD cases [71], circulating BDNF levels [71], disrupted extinction learning [22], and resistance to exposure therapy [72]. Therefore, these studies warrant both further investigation and new ideas if a role for growth factors, including BDNF, in PTSD pathophysiology is to be resolved.

Despite the small numbers in clinical studies and multivariate genetics likely involved in PTSD, there nonetheless remains strong support that BDNF functions within the fear response circuitry. In rodent models, fear conditioning is often employed to understand the mechanisms subserving the acquisition and expression of fear. Extinction learning is utilized as a model of exposure therapy to examine how effectively the fear response can be suppressed by new learning. The inability to retain successful extinction learning, leading to the spontaneous recovery of fear, is often studied as a model of fear persistence/recovery, which holds face validity for facets of anxiety disorders in humans [73]. These behaviors are often quantified by recording the freezing behavior of mice or rats to a conditioned stimulus (CS) associated with an unconditioned stimulus (US). A simple example often employed in the literature is using a tone (CS) that is paired with footshock (US), although other fear-potentiated behaviors (such as startle responses [74]) may also be substituted.

In the following sections we will discuss the fear literature that cumulatively identifies that BDNF plays a probable role in (1) the consolidation of fear memories, and (2) can induce and/or facilitate extinction learning and reconsolidation.

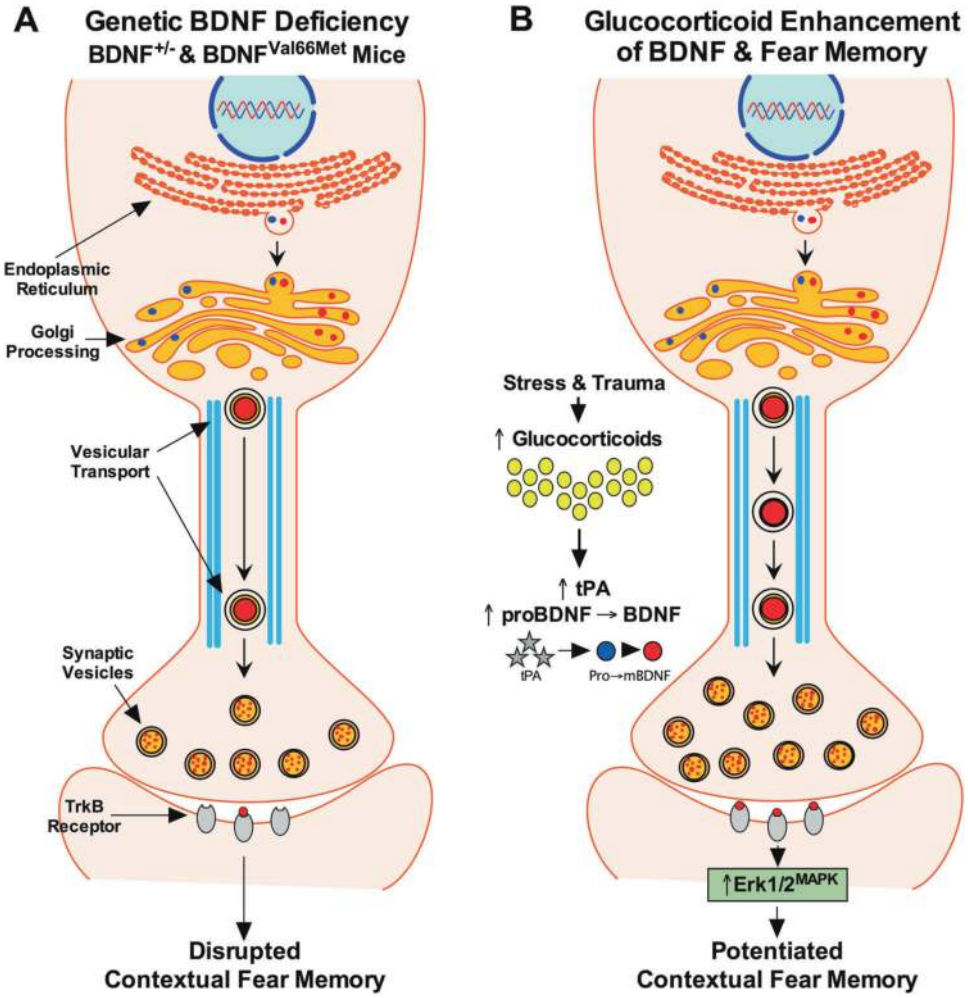

Fig. 3 Schematic of the interaction of BDNF depletion and glucocorticoid stress hormones on contextual fear. a Disrupted BDNF expression, as in several models of genetically induced BDNF deficiency, alters the expression (but not learning) of contextual fear. These studies imply that reduced BDNF expression disrupts BDNF-TrKB signaling. As $BDNF^{Val66Met}$ polymorphic mice mimic the contextual fear deficits of BDNF heterozygous knockout mice (see [22]), this phenotype is likely to arise at least partially from disrupted activity-dependent BDNF release and signaling. **b** Glucocorticoids enhance contextual fear memory (see mechanism derivation in [166]) by upregulating the conversion of proBDNF to mBDNF via tPA, which potentiates ERK1/2 MAPK signaling.

Role of BDNF in fear memory consolidation

Over the past decade, considerable progress has been made in understanding the role of BDNF in the fear circuitry. Both $BDNF^{+/-}$ mice and $BDNF^{Val66Met}$ transgenic mice exhibit deficient contextual, but not tone, fear memory deficits at baseline [22]. We have reported similar phenotypes in humanized $BDNF^{Val66Met}$ ($hBDNF^{Val66Met}$) transgenic mice [75], which are engineered to carry the Val66Met polymorphism as well as express humanized BDNF [76]. Cumulatively these studies confirm that major and ethologically relevant depletion of BDNF modifies contextual fear memory (see Figs. 3 and 4).

It should be noted that not all BDNF deficiency models have exhibited fear memory consolidation effects at baseline. For example, BDNF-KIV mice, a model of exon-specific disruption of BDNF, exhibit normal contextual and cued fear memory [77]. Moreover, while studies using genetically modified mice have implicated an important role for BDNF in maintaining normal fear memory, these studies did not resolve *how* BDNF acts within the fear circuitry to modify behavior. There is evidence that BDNF transcriptional activity plays an important role in the consolidation of contextual fear memory. For instance, following contextual fear conditioning, there is discrete hypomethylation of a CpG island at the end of *Bdnf* exon III within the hippocampus that emerges 30 min following conditioning but remains in this state for up to 24 h [78]. This phenomenon may play a role in the consolidation of fear engrams, as has been suggested for methylation at other *Bdnf* CpG sites [79]. Correspondingly, *Bdnf* transcripts from exon I and VI (and transcripts IV, VII, and IX in females—whom exhibit poor conditioned freezing behavior relative to males) are upregulated within the hippocampus for up to ~24 h following conditioning [78]. There is also a significant increase in *Bdnf* transcript IX expression within the hippocampus 2 h following conditioned context reexposure, which appears to be driven by a concomitant upregulation of exon IV splicing [79]. The time course of these phenotypes thus supports a specific role for BDNF in the consolidation of fear engrams [79]. If this is the case, disrupting this activity may provide a mechanism for early

intervention for acutely experienced and severe trauma, whereby the consolidation of emotionally salient memories may be able to be attenuated.

Role of BDNF in fear memory extinction and reconsolidation

Not surprisingly, there is also an established role of BDNF in extinction learning [62, 80]. The $BDNF^{Met/Met}$ genotype has been shown to disrupt the extinction learning of mice [81]. In humans, the $BDNF^{66Met}$ allele has also been shown to disrupt extinction learning [82] and modulate fear memory reconsolidation [83], highlighting cross-species translation of 66Met extinction phenotypes published to date (see Fig. 4). Furthermore, hippocampus-specific deletion of BDNF also results in attenuated extinction learning in mice [84]. However, the underlying mechanisms and functionality of BDNF in fear extinction have been difficult to pinpoint. Peters et al. [17] reported that a single infusion of recombinant human BDNF to the infralimbic cortex following, but not prior to, conditioning was sufficient to induce the extinction of fear, with or without actual extinction training, in rats [17]. As the hippocampus projects to the infralimbic cortex [85], Peters et al. hypothesized that BDNF may be transported from the hippocampus to the infralimbic cortex to facilitate the extinction of fear. Indeed, infusion of BDNF to the hippocampus replicated the induction of fear extinction previously observed when administered to the infralimbic cortex, and could be blocked via simultaneous application of anti-BDNF antibody to this region [17]. In other work, BDNF expression has been observed to increase in the ventral hippocampus (VHP), but not in the infralimbic cortex, following extinction training; while infusion of recombinant BDNF within the VHP selectively increased the firing rate of single units within the infralimbic but not prelimbic (PL) cortex of rats [86]. As such, there appears to be a VHP → infralimbic circuit that governs the functionality of BDNF in the fear suppression circuitry, which is noteworthy given that the VHP expresses SorCS2 [29], at least during late adolescence, and has broad roles in emotionally salient memory and stress sensitivity [87].

Several other studies have also examined the role of BDNF in the extinction circuitry but have produced varying results. These studies raise important questions that require resolution given their potential to alter interpretations of phenotype specificity. For instance, BDNF-KIV mice, which have altered GABAergic inhibition within the medial prefrontal cortex (mPFC) [88] but normal fear conditioning behavior [77], also exhibit attenuated contextual extinction learning.

It has also been suggested that BDNF holds a functional role in the reconsolidation of fear engrams following extinction learning. In support of this, *Bdnf* mRNA expression within the basolateral amygdala (BLA) increases ~2 h following extinction learning [74], which is evidence for a role of BDNF in the transcriptional remodeling that occurs during the updating of new and old engrams [89]. Indeed, knockdown of BDNF-TrkB signaling within the BLA of mice results in disrupted reconsolidation between extinction training sessions which attenuates the "therapeutic benefit" of this fear-erasure strategy [74]. Likewise, it was recently shown that intrahippocampal infusion of anti-BDNF following extinction learning impairs reconsolidation and leads to the renewal of fear in rats [90]. Opposite to these data, it has also been reported that, when

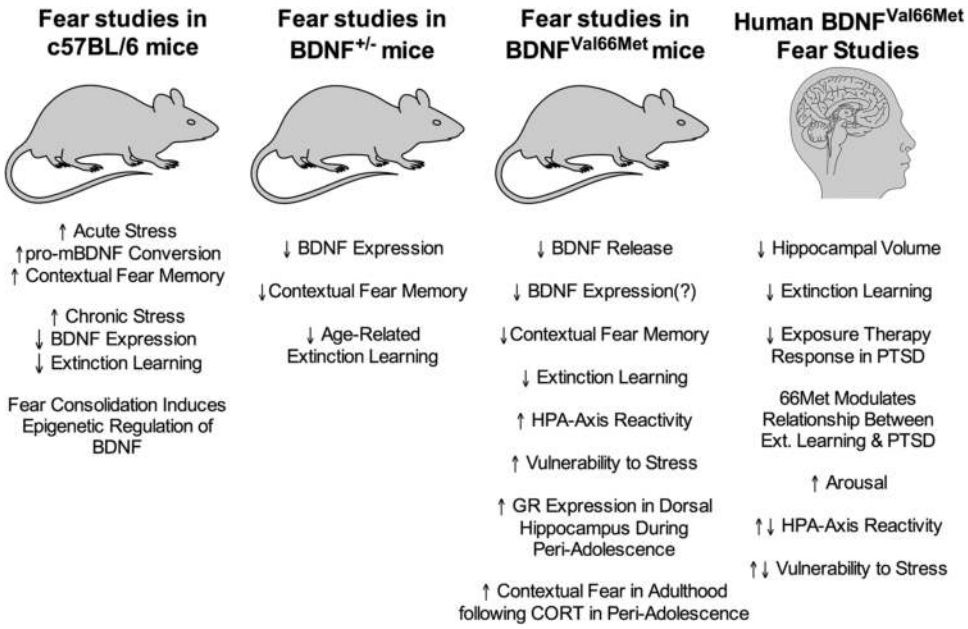

Fig. 4 Comparative overview of stress-related fear phenotypes in mice and humans. A schematic outlining major phenotypes, and phenotype compliance across models/species, regarding common fear and stress-related phenotypes reported in C57BL/6 mice, $BDNF^{+/-}$ mice, $BDNF^{Val66Met}$ mice, and human $BDNF^{Val66Met}$ carriers. This broad compliance in phenotype, and convergence upon altered stress-responses as well as fear behavior, highlights the functional interaction between BDNF and stress within the fear and emotionally salient memory machinery in both man and mouse.

infused prior to extinction learning, BDNF may impair extinction reconsolidation in rats [91]. It should be acknowledged that the reconsolidation of fear has not been widely studied within the scope of BDNF, and there also exist a number of studies that have failed to find an effect of various BDNF manipulations on extinction learning [74, 92]. Yet continued study on the role of BDNF within the fear circuitry is thus necessary.

Of additional interest, it has also been noted that the successful extinction of fear is associated with an increase in histone H4 acetylation surrounding the p4 promoter of the *Bdnf* gene within the mPFC of mice, resulting in the upregulation of the activity-related I and IV *Bdnf* transcripts [93]. To illustrate causality, the authors of this study used valproic acid—a potent histone deacetylase inhibitor that results in target genes being released from histone deacetylase-dependent transcriptional repression [94] and therefore commonly used to modulate transcriptional effects of histone acetylation in experiments. Correspondingly, valproic acid did indeed potentiate acetylation of histones around the *Bdnf* gene p4/p1 promoters as well as expression of *Bdnf* exon IV mRNA in the mPFC of mice [93]. Because these modifications are both biological signatures of fear extinction in the cortex, this treatment also enhanced the effect of weak extinction training in mice, leading the authors to suggest that valproic acid may be a useful adjunctive therapeutic to be combined with exposure therapies in disorders such as PTSD [93].

While BDNF depletion models exhibit defective contextual fear conditioning as well as disrupted fear extinction behavior and infusion of BDNF to the hippocampus [91] and infralimbic cortex [17] has been shown to induce the extinction of fear, in clinical cases, BDNF deficiencies are not so dramatic, and the most robust association has been with the $BDNF^{Val66Met}$ variant [3]. Addressing the mechanism by which this variant affects fear conditioning and extinction, a recent study focused on the effect of the BDNF prodomain in the fear circuitry, and whether there was a differential effect of the 66Met substitution within this protein product. First, the $SorCS2/p75^{NTR}$ receptor complex was found to be enriched predominantly in the ventral CA1 region of the hippocampus (CA1, VHP), providing a focal neuronal population that may be responsive to the BDNF prodomain [29]. Expression peaked at day 30 (denoted by the authors as "peri-adolescence") and had diminished by day 60 in mice. When administered to the CA1 region of the VHP of periadolescent mice, the 66Met prodomain induced dendritic spine loss in vivo [29]. In addition, VHP CA1 projections to the PL cortex are also disrupted by the 66Met prodomain [29]. Based on reciprocal connectivity between the VHP and cortical regions involved in fear extinction, the authors examined the effect of 66Met-containing BDNF prodomain in extinction learning. Indeed, administration of the 66Met prodomain to the CA1 region of the VHP disrupted fear extinction learning [29]. In addition, in adolescent $BDNF^{Met/Met}$ mice similar alterations in prefrontal connectivity are also observed as are extinction learning defects [29] and in vivo fiber photometry confirmed that these 66Met-containing prodomain responsive cells were extinction-responsive neurons [29]. This is not surprising because PL neurons are known agents of the broader fear extinction circuitry [95] and ventral CA1 projection neurons have a documented role in modulating fear behavior [96]. Thus, the periadolescent expression of $SorCS2/p75^{NTR}$ in the ventral CA1 during adolescence may constitute a developmentally transient critical period whereby disruption of BDNF, particularly by the Val66Met variant, alters development of the fear extinction machinery.

Linking BDNF's role in extinction learning to PTSD risk

As previously noted, a number of studies have found association of the 66Met allele with risk of PTSD development (see Table 1) and severity of PTSD symptoms [97]. The mechanisms linking PTSD risk with BDNF are now becoming clearer. First, and in addition to mouse phenotypes already discussed, 66Met phenotypes that occur in healthy humans are important clues. Prominently, this includes the 66Met-induced increase in sensitivity to early-life stress, which produces long-lasting changes in brain and arousal pathways in healthy Val66Met carriers [98]. This elevated sensitivity to early-life stress likely contributes to the extinction learning defects of 66Met carriers, which is evident with [99] or without [82] a PTSD diagnosis, as well as the perturbed response to exposure therapy in 66Met PTSD cases [72]. Biologically, the effect of the $BDNF^{66Met}$ variant on risk of PTSD likely arises from several potential mechanisms. The first is the classical mechanism of BDNF depletion effects, whereby the 66Met variant induces a chronic loss in activity-dependent BDNF secretion leading to decreased neurotrophic support during extinction-induced synaptic remodeling of fear memories. Evidence for this proposition come from $BDNF^{Met/Met}$ [81, 82], $BDNF^{+/-}$ [80], and hippocampus-restricted BDNF knockout mice [84], which all exhibit an inability to extinguish aversive memories. Similarly, healthy $BDNF^{66Met}$ human carriers [82] and $BDNF^{66Met}$ PTSD cases [99] also exhibit extinction learning defects. Thus, extinction learning defects emerge from genetically induced BDNF deficiency in both man and mouse [82]. Nascent studies have expanded upon these BDNF depletion effects on the fear extinction circuitry by now defining how BDNF levels may contribute to *circuit function* (see [17, 86]) and how the 66Met substitution specifically alters the biology of the

BDNF prodomain ligand to elicit extinction defects in a circuit-specific manner [29]. These circuit-level mechanisms provide important new insight into regional specificity of BDNF functionality in the extinction circuitry. As mentioned, BDNF may be transported from the VHP to the mPFC to induce risk and/or may alter VHP excitability to alter reciprocal projection partners [86]. Importantly, these underlying mechanisms may arise, or at least reach their prominence, during putative developmental critical periods during which these circuits develop [29]. Thus, these human and basic science studies substantiate a mechanistic role for BDNF in stress-related disease, and that this mechanistic role is both circuit-specific and developmentally sensitive.

Stress as a common denominator in PTSD and BDNF function

A primer of stress as a conceptual framework

When these behavioral neuroscience experiments are amalgamated, it becomes clear that BDNF has a complex role within the fear circuitry at baseline. Unfortunately, only a very small number of studies have considered how these baseline effects of BDNF in the fear circuitry compound upon exposure to additional disease factors, namely stress. Stress-inducible disorders such as PTSD are not "baseline" disorders that develop per their own volition but are *induced* disorders that emerge posttrauma in those with latent or acquired vulnerability. The experience of, or second-hand exposure to, traumatic stress is therefore a prerequisite factor common to all PTSD cases. Therefore, it becomes particularly pertinent to examine the functional modulation of fear behavior in mice with altered BDNF signaling following controlled stress manipulations, i.e., a "two-hit" approach.

To promote brevity, here we take a broad-strokes approach by defining stress as the physiological adaptation to adverse or organismal-challenging events that triggers engagement of the HPA axis. This is a purposefully biologically reductionist definition that provides a framework from which to deconvolve the complex interaction of BDNF in vulnerability to stress and stress-inducible disease.

A primer of glucocorticoids as a pathophysiological factor in PTSD

The HPA axis governs circulating concentrations of glucocorticoid stress hormones. It is believed that mineralocorticoid receptor (MR) binding is saturated by basal stress hormone concentrations, and that stress-initiated mechanisms predominantly occur via elevations in circulating CORT that target glucocorticoid receptors (GRs). GRs can exert their actions via dimerization-dependent binding to glucocorticoid response elements within the genome, or indirectly via monomer-mediated interactions with transcription factors. Both GR and MR are expressed rather diffusely across the brain, but are highly expressed within the hippocampus where both receptors play a probable role in maintaining HPA axis homeostasis. It is interesting to note that GR expression is higher in the dorsal hippocampus relative to the VHP in both rat [100] and mouse [101], and that stress has been shown to remodel the hippocampus transcriptome, proteome, and epigenome differentially across its longitudinal axis [102].

In PTSD, glucocorticoid stress hormones are a known regulator of fear memory consolidation and subsequent extinction learning. A commonly accepted framework is that PTSD may be mediated by alterations in glucocorticoid feedback. Indeed, impaired glucocorticoid feedback is a central mechanism associated with extinction learning [103]. Similarly, in PTSD cases, elevated plasma corticotrophin-releasing hormone levels [104] have been observed (albeit variably [105], discussed in [106]). Increased cortisol suppression is also often observed amongst PTSD cohorts in dexamethasone challenges indicating negative feedback inhibition of the HPA axis [106, 107]. Evidence for alterations in dexamethasone challenge responses has been produced in numerous PTSD and trauma-specific samples including in child abuse [108], sexual-specific abuse [109], natural disaster [110], and holocaust survivor [111] cohorts. In support of this, a recent paper found that nonremitting PTSD only arose in individuals whom exhibited low cortisol during their posttrauma emergency room admission, which appeared related to a history of childhood trauma [112]. This suggests that glucocorticoid feedback risk may be influenced, or even induced, by earlier stress or trauma exposure leading some individuals vulnerable to subsequent trauma exposure. Another interesting observation reflects the transgenerational inheritance of glucocorticoid dysfunction. These studies have reported that parental PTSD may be associated with lower offspring cortisol levels [113] and offspring responses to dexamethasone challenge [114] which may mechanistically underlie increased offspring risk for PTSD [115]. It seems likely that cross-generational stress phenotypes may be nongenetically inherited via targeted gene methylation [116], alterations in long-noncoding RNAs in sperm [117] or other germline changes [118] all of which influence PTSD risk.

Importantly, PTSD-related HPA axis dysregulation is different from other disorders (e.g., major depression, see [106]). Thus, feedback inhibition and glucocorticoid sensitivity may be an innate and specific component of PTSD pathophysiology [109]. It has been suggested that this alteration in tonic glucocorticoid responses may encode risk

for PTSD by influencing the consolidation or retrieval of trauma [106]. Specifically, during trauma exposure, alterations in glucocorticoid sensitivity may result in a failure to initiate adaptive and/or restraining mechanisms in the brain (e.g., BDNF, as we previously proposed [75] and revisit here). This may result in deleteriously elevated or prolonged activity of memory consolidation factors or pathways, resulting in a maladaptive potentiation of memory of trauma and thus elevated risk of PTSD. Evidence for glucocorticoid sensitivity as a pathway of PTSD risk is further supported by studies which have found that glucocorticoid administration putatively reduces risk of PTSD [119] via modulating memory for traumatic events [120–123]. Thus, these HPA axis changes represent a series of replicable PTSD phenotypes in humans that define a potential pathway of risk to disease.

Of note, the effects described above do not rule out that other stress-related risk factors that influence the extinction circuitry may also produce other (or overlapping) mechanisms of risk leading to PTSD. It is therefore of considerable note that many of these molecules are genetic risk factors for stress-related disorders, including PTSD. A prominent example is the FKBP51 protein, which plays a critical role in determining GR sensitivity to CORT during an on-going stress response [124]. Within cells, chaperones such as FKBP51 and HSP-90 [125, 126], regulate the cytosolic activity of GR at baseline. During stress FKBP51 is displaced by FKBP52 which initiates the translocation of CORT-bound GR to the nucleus [125]. Interestingly, the activation of GRs results in the rapid upregulation of FKBP51 [127], which in turn decreases the binding affinity of unbound GR to CORT [124] and thus physiological responsiveness to stress.

The induction of FKBP51 expression by activated GRs [127] ensures availability of this factor in stress-sensitive hypothalamic neurons, and thus creates "ultra-short" feedback loops to regulate glucocorticoid levels and their activity in the brain [124]. Thus FKBP5 has been termed a potential "*molecular amplifier of the stress response*" [128], leading to its emergence as a potential PTSD risk factor. A number of studies have now reported association of various FKBP5 polymorphisms with PTSD cases in humans with a history of trauma [129, 130] evidencing a gene–environment pathway of risk. Similarly, consistent with a role in GR sensitivity, FKBP5 expression was predicted by cortisol and PTSD severity in a cohort of survivors of the terrorist attack on the World Trade buildings [131]. In addition, and consistent with the transgenerational inheritance of glucocorticoid abnormalities, FKBP5 is also a prominent gene-target of PTSD-related methylation [132, 133]. In humans, FKBP5 polymorphisms were associated with abnormalities in extinction trajectory, while in mice the pro-extinguishing qualities of dexamethasone were associated with FKBP5 expression [134]. The pro-extinguishing effects of dexamethasone may be related to methylation-related changes in FKBP5 expression in the amygdala that subsequently influence glucocorticoid receptor sensitivity [135]. This implicates that several intermediaries of stress hormone function hold the potential to modify vulnerability to stress, including those that coordinate the downstream actions of stress hormones such as BDNF.

Stress hormone regulation of BDNF-TrkB signaling

Stress exposure induces many remodeling events within the brain [136], and these events are likely to at least partially involve the actions of BDNF. For instance, stress has long been known to decrease BDNF expression [137–140]. Importantly, chronic CORT exposure (commonly used as a reductionist model of chronic stress) also results in decreased BDNF expression [141], implicating that the effect of stress on BDNF expression is under specific control of glucocorticoid stress hormones. This effect of CORT on BDNF expression is also long-lasting [142], implicating that glucocorticoids may have long-term effects on BDNF concentrations within the brain leading to a chronic decrease in BDNF-derived neurotrophic support (not withstanding compensation, see following paragraph). If glucocorticoids exert a robust effect on BDNF it may be expected that this neurotrophin also plays an equally important role in modulating the stress-response axis. Indeed, there is emergent evidence for such a role of BDNF in the hypothalamus. Specifically, BDNF has been shown to modulate CRH expression in the paraventricular nucleus of the hypothalamus [143]. Deletion of GR in this region increases both BDNF and CRH expression, and results in HPA axis dysregulation [143]. Likewise, BDNF overexpression in the paraventricular nucleus also results in increased CRH expression [143]. It therefore appears that GR modulates BDNF expression in the paraventricular nucleus of the hypothalamus, and that BDNF correspondingly regulates CRH expression in this region [143]. Interestingly, while restraint stress classically downregulates BDNF expression in the hippocampus, within the hypothalamus BDNF expression is actually *increased* by restraint stress in rats [144]. This observation is thus consistent with the idea that BDNF is likely to have a non-classical yet functional role in the hypothalamus that extends beyond energy homeostasis [145].

However, the effects of stress on BDNF are more elaborate than just modulating the availability of this molecule. Specifically, there is rather convincing evidence that glucocorticoids also modulate the activity of BDNF's cognate receptor, TrkB. First, TrkB and GR colocalize in both the cortex as well as hippocampus, suggesting an intertwined

role for these receptors [146]. In support of this view, in vitro BDNF is able to phosphorylate GR [146], while glucocorticoids have been shown to increase phosphorylation of TrkB via a genomic effect of GRs [147]. Importantly, this latter effect is independent of a potential increase in neurotrophin expression, affirming that this glucocorticoid activation of TrkB is independent of neurotrophin signaling [147]. As an in vivo corollary, we have also observed long-term recruitment of the $TrkB^{Y515}$ pathway in adult mice following chronic CORT exposure in periadolescence, which persists for at least 6 weeks following termination of exogenous glucocorticoids [148]. Interestingly, similar increases in TrkB phosphorylation also exist in $BDNF^{+/-}$ mice despite a loss of 50% of BDNF [149]. These data therefore imply that compensatory mechanisms likely occur following the loss of BDNF, whether it be by stress or other means, but that glucocorticoids are also likely to have a directed role in coordinating this activity in the stress-exposed brain. Central to this idea is that in addition to dimerization-dependent transcriptional regulation of BDNF/TrkB by GRs, GRs must also directly interact with TrkB to play a role in the efficacy of BDNF-TrkB signaling. Specifically, both dexamethasone and CORT exposure suppress BDNF-stimulated PLCγ signaling via TrkB, resulting in the disrupted release of glutamate [150]. Knockdown of GR via siRNA recapitulates this phenotype, whereas overexpression of GR protects against the disrupted recruitment of PLCγ induced by glucocorticoids [150]. Likewise, glucocorticoids have also been shown to repress BDNF signaling via ERK by modulating the interaction of TrkB with proteins involved in pathway recruitment [151], resulting in a direct effect of glucocorticoids on BDNF-initiated ERK activity.

These studies therefore evidenced that stress hormones and GR may be capable of functional "cross-talk" [152] with TrkB that modifies signaling events that are independent of the classical dimerization-dependent (i.e., genomic) effects of GR. Importantly, while glucocorticoids activated neurotrophins receptors, they did not phosphorylate other growth factor-related receptors. Because these phenotypes were independent of neurotrophins, some yet unknown mechanism likely regulates TrkB activity induced by glucocorticoids. A mechanistic clue is that the glucocorticoid-induced activation of neurotrophin receptors required the AF1 genomic-transactivation domain, which facilitates DNA-binding, in a mechanistic assay [147]. This strongly suggests that the activation of Trk neurotrophin receptors by glucocorticoids is accomplished specifically via a DNA-binding mechanism rather than glucocorticoids binding to an extracellular receptor.

Similarly, there is evidence that BDNF may modulate the classical genomic/transcriptional activities of GR. Evidence supporting this position comes from a study where BDNF was observed to have a direct effect upon the glucocorticoid transcriptome [146], suggesting that the interaction between GR and TrkB is likely to have downstream effects on transcriptional activity. In this study, BDNF was found to remodel the gene profile of GR expressing cells when both TrkB and GR were activated in tandem relative to when each receptor was activated in isolation of the other [146]. Importantly, these differentially expressed genes appear to maintain at least partially disparate gene ontologies, and are not merely the sum of BDNF-responsive and GR-responsive genes (although, as expected, substantial overlap exists). This raises the idea that the regulation of BDNF-TrkB signaling by stress hormones may reflect the modulation of a unique gene-regulatory network that regulates behavioral and disease-related vulnerability to stress. Cumulatively, these data suggest that BDNF and glucocorticoid activities are calibrated [153, 154] and that physiological changes in either system may in turn influence the other—especially when intrinsically disrupted by other gene variants or environmental insults such as trauma.

Does the $BDNF^{Val66Met}$ polymorphism alter vulnerability to stress?

Given mounting evidence for an interaction between the activity of GR and BDNF-TrkB signaling, it seems plausible that loss-of-function *BDNF* variants such as the $BDNF^{Val66Met}$ polymorphism may alter vulnerability to stress. There is evidence from both human and transgenic mouse studies that the $BDNF^{66Met}$ substitution may alter HPA axis physiology as well as response to stress. Several animal models of the BDNFVal66Met polymorphism have now been generated, including $BDNF^{Val66Met}$ (or, $BDNF^{Val68Met}$) mice and rats, as well as a distinctly different humanized $BDNF^{Val66Met}$ ($hBDNF^{Val66Met}$) mouse line that expresses a humanized BDNF transcript via endogenous mouse promoters. In transgenic animal models the same nomenclature is used as it is for humans (with a Val/Val genotype representing the "wildtype control", Val/Met representing heterozygosity, and Met/Met representing homozygosity). All of these rodent models are knockin constructs that involve substitution of the closest murine Val/Met sequence (i.e., Val68Met), or translocation of a human sequence into the corresponding mouse gene (i.e., $hBDNF^{Val66Met}$ mice). Importantly, $BDNF^{Val66Met}$ rodent models and human carriers appear to similarly recapitulate core Val66Met effects, such as impaired hippocampal function. Due to their earlier generation and availability, nonhumanized $BDNF^{Val66Met}$ mice remain the most common preclinical model utilized within the literature. In these mice, stress exposure has been shown to result in a range of behavioral and physiological changes. Specifically, following 7 days of restraint stress, $BDNF^{Val/Met}$ mice have

decreased sucrose preference, increased immobility on the forced swim test, and increased anxiety-related behavior on the elevated-plus maze [155]. These stress-related behavioral phenotypes were also accompanied by increased expression of CRH within whole hypothalamus lysate, as well as increased plasma CORT and ACTH concentrations in $BDNF^{Val/Met}$ mice relative to WT mice [155]. Unfortunately, $BDNF^{Met/Met}$ homozygote mice were not independently sampled in this study [155], and as such it is impossible to conclude whether each of these phenotypes would follow a gene-dosage effect [2]—especially given several recent studies which have reported $BDNF^{Val/Met}$ heterozygote-specific differences in behavior [156]. The authors speculate that these heterozygote-specific phenotypes may reflect partial vulnerability devoid of complete compensation, resulting in the potential for unique phenotypes in all three $BDNF^{Val/Met}$ genotypes in mice and humans. Nonetheless, these data confirm that, at least in transgenic mice with their controlled laboratory stressors, behavioral assessments, and physiological measurements, the $BDNF^{66Met}$ substitution induces a likely intrinsic vulnerability to stress.

An effect of various stressors has also been reported in human carriers of the $BDNF^{Val66Met}$ polymorphism. Specifically, the $BDNF^{Val66Met}$ polymorphism has been shown to predict exposure to stress and stressful life events [157, 158]. Specifically, healthy $BDNF^{66Met}$ allele carriers exposed to early-life stress have smaller hippocampal and amygdala volumes as well as deficient working memory [98], with a structural equation model predicting that early-life stress increases neuroticism and depression in $BDNF^{66Met}$ allele carriers by elevating arousal [98]. Similarly, childhood abuse selectively increases psychosis-like symptomology in healthy $BDNF^{66Met}$ allele carriers, whilst having no reported nor specific effect upon $BDNF^{Val/Val}$ carriers [159]. However, relative to what has been reported in mice, data on HPA axis reactivity in human carriers of the $BDNF^{66Met}$ allele have been commonly reported but also inconsistent in direction. One recent report found that the $BDNF^{66Met}$ allele was associated with an enhanced cortisol response in both children as well as young adults in response to a laboratory stress paradigm [160]. Likewise, in children born prematurely, neonatal pain-related stress and the $BDNF^{66Met}$ allele interact to lower (resting) hair cortisol but increased cortisol reactivity in response to a laboratory challenge [161]. However, in adult samples, the $BDNF^{Val/Val}$ wildtype genotype has been associated with a greater cortisol response than that observed in $BDNF^{66Met}$ carriers following completion of a laboratory stressor [162, 163]. Promisingly, despite varying in direction, these studies nonetheless imply that the HPA axis is modulated by BDNF in humans. Therefore, inconsistencies in direction or effect may be caused by yet further biological intermediaries. An example of this is that the stress reactivity of $BDNF^{Val66Met}$ carriers is defined by sex—with females tending to show an opposite response to that described previously, with the $BDNF^{66Met}$ allele being associated with greater cortisol [163]. This interaction thus highlights the need to explore a potential modulatory role of biological sex on HPA axis physiology [163], or high–low estrogen phases [164, 165], to tease apart the mechanisms which may subserve a role of this gene variant in vulnerability to stress. These data implicate that the $BDNF^{Val66Met}$ variant may modify susceptibility to stress in humans like in mice, although yet further physiological assessments are required to better define this phenotype.

A glucocorticoid-induced BDNF mechanism that enhances trauma consolidation?

In our prior fear extinction section, we highlighted how gene and peptide variants as well as intraregional circuits have now been defined which unveil BDNF's mechanistic role in extinction learning. However, one critical gap in this literature remains. Specifically, while these mechanistic and circuit-identification studies shed light on how risk for PTSD may intrinsically manifest, they do not consider the acute and chronic remodeling induced by the influx of glucocorticoids during severe stress responses (e.g., trauma exposure). Glucocorticoids are known to acutely enhance fear memory formation and BDNF is a likely mechanistic substrate of this effect. Specifically, for some time, the enhancement of fear memory by glucocorticoids had been known to activate MAPK signaling [166]. In an elegant and mechanistic study by Revest et al. [166], it was shown that during contextual fear formation, stress-induced glucocorticoids induce proBDNF and tPA expression, which results in the rapid proteolytic conversion of pro → mBDNF which subsequently phosphorylates TrkB and its downstream effector MAPK [166]. Thus, BDNF-TrkB signaling is the mechanistic substrate responsible for the enhancement of contextual fear memory by glucocorticoid stress hormones [166]. Another notable observation is the long-lasting effect that glucocorticoid stress hormones exert in potentiating the fear memory of $hBDNF^{Val66Met}$ mice [75]. Specifically, while $hBDNF^{Met/Met}$ mice exhibit deficient fear expression at baseline, following chronic glucocorticoid treatment they exhibit dramatically enhanced fear memory that is consistent with (if not better than) $hBDNF^{Val/Val}$ control mice [75]. This coincided with a transient increase in glucocorticoid receptor levels in the hippocampus of $hBDNF^{Met/Met}$ mice [75]. In addition, following chronic glucocorticoid treatment, there is prolonged phosphorylation of TrkB at the $tyrosine^{515}$ residue, which activates the MAPK pathway [167], across the corticohippocampal axis

[148]. Thus, the elevated glucocorticoid sensitivity of hBDNF$^{Met/Met}$ mice may reflect a deleterious gain-of-function mechanism, whereby the BDNF$^{Met/Met}$ genotype may improve emotionally salient formation of stressful and/or traumatic events due to an elevated intrinsic sensitivity to stressful life events including trauma [75]. Indeed, clinical correlates of this proposed 66Met-induced potentiation of stress-related fear memory are exemplified in humans. This includes observations that the 66Met allele increases consolidation of emotional memories in healthy carriers, and that the 66Met allele increases the effect of trauma burden on PTSD symptom severity in patients [97]. In lieu of these mechanisms, phenotypes, and human data (see vulnerability to stress section above), there is sufficient evidence that BDNF orchestrates both acute and long-lasting stress hormone effects on the consolidation of emotionally salient memories including memory of fear. Future studies should determine what effect stress hormones have on the BDNF-dependent fear extinction mechanisms reported earlier in this paper, as this is likely to lead to yet fruitful excursions in defining additional BDNF mechanisms related to PTSD.

Along with BDNF's role in fear extinction mechanisms related to PTSD, these studies are a complement to clinical findings that link BDNF with PTSD. Importantly, they provide new context to clinical data, such as putative BDNF overexpression in PTSD cases [71]. In addition, because the 66Met allele is carried with greater frequency in likely PTSD cases [71], disrupts extinction learning in man and mouse [22], and confers resistance to exposure therapy in PTSD [72], it is possible that BDNF's role in the glucocorticoid-induced enhancement of fear memory [75] may represent a deleterious gain-of-function that enhances consolidation of events with negative emotional valence (e.g., trauma). Indeed, this is consistent with the effects of glucocorticoids in PTSD pathophysiology and its therapeutic uses in trauma cases. Given that the 66Met allele disrupts extinction learning and exposure therapy in cases, the requirement of BDNF in glucocorticoid-induced enhancement of fear provides additional context to how BDNF and the Val66Met polymorphism may modulate vulnerability to stress and memory of trauma, and explains why some but not all 66Met carriers may experience increased risk or conversion to disease states. Importantly, the baseline effects of the 66Met allele, such as altered synaptic physiology (see [168]), the induction of novel actions by the 66Met prodomain [27, 28, 169], and the many hippocampal and behavioral defects (see [22, 75, 148] for mice, and [2] for detailed review in humans) induced by this polymorphism, are likely to feedforward and act in concert with the additional effects and remodeling induced by stress hormones in Val66Met carriers [75].

A BDNF stress–sensitivity hypothesis for mechanistic disease modeling

Our work, as well as the work of many other groups, has converged upon the idea that BDNF is a likely arbitrator of vulnerability to stress and limbic function. However, we propose that the role of BDNF in vulnerability to stress and stress-related disorders is not likely to be as simple and dichotomous as "↓BDNF = ↓resilience".

In the case of the Val66Met polymorphism specifically, we believe that the literature discussed in this review supports other mechanisms being involved—especially for stress-related disorders involving emotionally salient fear memory such as PTSD. Based on the evidence linking BDNF, TrkB signaling, and the Val66Met polymorphism to the actions of glucocorticoid stress hormones, we propose a "*BDNF stress–sensitivity*" hypothesis which implicates that ethologically relevant BDNF disruption (e.g., by the Val66Met variant) induces an intrinsically arising and self-perpetuating sensitivity to stress. Over time, as allostatic load is accumulated, long-term changes in behavior may manifest (vulnerability to stress) that may correspondingly elevate potential risk for conversion to stress-induced disorders (Fig. 5).

This hypothesis accepts that the Val66Met polymorphism induces baseline deficiencies in brain function and behavior, but posits that following stress these baseline deficiencies feedforward and act in concert with glucocorticoids to produce *yet further* and more severe deleterious outcomes that may predispose Val66Met carriers to stress-related mental illness. This hypothesis therefore integrates data that evidences a marriage in function between glucocorticoids and BDNF-TrkB signaling, but specifically suggests that this interplay results in biological epistasis that leads to disease vulnerability.

In support of our proposed hypothesis (see Fig. 5), it is important to explicate that the foundation of this model is the bidirectional interaction of BDNF and glucocorticoid signaling across the brain. Glucocorticoids dynamically control BDNF-TrkB signaling pathways [170] and BDNF expression in a region-specific manner (e.g., [145] vs [137]) that can produce targeted outcomes inter- and intraregionally. For instance, while chronic glucocorticoid saturation can induce spatial memory defects by negatively regulating BDNF levels [171], glucocorticoid stress hormones can also acutely enhance BDNF-TrkB signaling to potentiate stress-related fear behavior/emotionally salient memory formation [75, 166]. Thus, glucocorticoids can both disrupt one domain of memory while enhancing another in a BDNF-dependent manner based on the length, severity, and chronicity of stress exposure. This enables glucocorticoids to putatively calibrate specific cells and circuits leading to discrete behavioral alterations. Although

rarely investigated, similar interactions in other brain regions are likely to occur but remain ill-defined. However, one noteworthy exception that is pertinent to our proposed model is BDNF's regulation of CRH in the paraventricular nucleus of the hypothalamus [143], which is of note given that CRH functions to initiate the stress-response axis. In our proposed model endogenous disruption of BDNF (e.g., by Val66Met) may disrupt homeostasis of the HPA-brain axis, as evidenced in both man and mouse, and could lead to increased glucocorticoid sensitivity and thus vulnerability to stress-related disease. Indeed, Val66Met genotype predicts self-reported stress in humans [157, 158], HPA axis reactivity (see above section), PTSD-relevant fear behaviors [22, 75, 82], as well as risk of PTSD and PTSD symptom severity (see [2] and studies in Table 1). Therefore, the BDNF stress–sensitivity model proposed here (see Fig. 5) integrates these phenotypes, and provides a framework from which to study BDNF's role in the fear circuitry, endophenotypes of PTSD, and potentially other stress-related brain disorders.

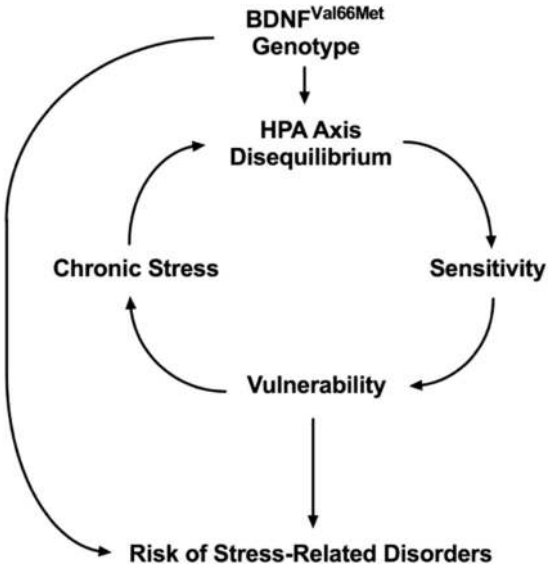

Fig. 5 Schematic of the BDNF stress–sensitivity hypothesis proposed here per the preexisting and conjoint BDNF and stress literature. Convergent themes and lines of evidence within the literature support that ecologically valid disruption of BDNF (e.g., the Val66Met polymorphism) alters aspects of HPA axis reactivity, sensitivity to stress, and vulnerability to stress-related pathology which includes but is not limited to PTSD. These phenotypes follow the linearity defined in the broader neurobiology of stress; namely, HPA axis disequilibrium is an established marker of stress sensitivity, which intrinsically leads to increased vulnerability to stress and related psychopathology in vulnerable individuals. As the BDNF^{Val66Met} variant has also been associated with frequency of stressful life event exposure/perceived stress, this would imply that the Val66Met polymorphism induces a stress–sensitivity feedback loop. This model is supported by emergent evidence for a role of BDNF within the hippocampus and hypothalamus, BDNF's role in the treatment (e.g., antidepressants) of stress-induced illnesses (e.g., PTSD, reactive depression etc.), as well as the association of the BDNF^{Val66Met} polymorphism with numerous stress-inducible biological, behavioral, and disorder-related phenotypes such as glucocorticoid-enhanced fear memory and deficient extinction learning.

Bringing BDNF from bench to bedside?

Throughout this paper, a number of emergent themes have been discussed that often times hold some theoretical potential within the clinic. Briefly, this includes (but is not limited to) concepts such as the Val66Met polymorphism as a genetic marker of PTSD risk, BDNF as a potential therapeutic for trauma exposure, other potential adjunctive therapeutics which target BDNF (e.g., valproic acid during extinction training/exposure therapy), and epigenetic modifications of BDNF as a PTSD biomarker. The theme is that BDNF may be a clinically useful tool, either as a therapeutic or adjunctive treatment.

However, what have not been discussed thus far are the significant and substantial challenges in executing many of these ideas. First, most investigations which have assessed BDNF's role in fear memory (as an endophenotype of anxiety disorders and PTSD) have comprised global genetic modifications that cannot rule out effects on normal development. While variants, such as the Val66Met polymorphism are intrinsic, studies on this variant have revealed that its induced depletion of BDNF may not be very dramatic [148]. Yet, in spite of this, it too is capable of developmental effects in both mice [29] and humans [172]. Promisingly, BDNF^{Val66Met} mice and hBDNF^{Val66Met} mice both recapitulate critical baseline phenotypes that are also present in humans (for review, see [2]), making these models the most translational of all BDNF genetic models to date. However, its persistent effects on brain development highlight that BDNFs role in brain development remains poorly defined.

A second limitation when interpreting basic studies should be the degree to which these basic science studies may be nonphysiological. This is particularly true for direct-infusion studies. Namely, because BDNF is expressed per a developmental trajectory and in low local concentrations (Fig. 2), the brain is hard wired to widely express neurotrophin receptors to capture any endogenously available BDNF. This ratio of BDNF:TrkB expression promotes fast-acting effects of BDNF at the synapse [3]. Thus altering levels in vivo by acutely saturating BDNF levels via central infusion to the brain is a mechanism-focused approach that is not easily transferred to drug design, development, and delivery within the clinic. Thus, while many studies propose BDNF supplementation strategies as potential therapeutics, this is fraught with consequences. This is important to emphasize because BDNF expression is tightly regulated both within- and between cell types, and merely raising

BDNF levels at a brain-wide level could yield many deleterious consequences (e.g., seizure risk [173] or alterations in metabolic functions [174]). BDNF is also blood–brain barrier impregnable [175] and oral delivery is perturbed by the effect of digestive enzymes on BDNF's biological half-life (<1 min) as well as poor parenchymal absorption (reviewed in [176]). This yields BDNF delivery into the human brain as a significant challenge in-and-of itself. While small-molecule BDNF mimetics have been developed [177], they too hold the same risks described above regarding safety and off-target effects. Naturally derived TrkB agonizts (e.g., 7,8-dihydroxyflavone) remain a potential therapeutic option [176] but they too require more stringent testing and analysis for both efficacy and safety. As such, BDNF itself is unlikely to be a major therapeutic anytime soon, unless next-generation drugs that use delivery systems to target BDNF in a cell-specific manner are developed, shown to be implementable, and proven to be safe. Similar critiques can also be raised regarding restricted- and conditional-genetic systems, which may hold theoretical promise when used to manipulate BDNF levels in vivo in a region- and cell-specific manner. However, such genetic models are not likely to be clinically implementable anytime soon due to similar off-target effects (albeit at a genetic level) as well as other ethical considerations. Indeed, the nascent but already storied CRISPR-Cas9 literature confirms that genetic therapies, at least for brain disorders, are unlikely to become clinically normative therapeutic options in the near future.

These points present substantial limitations on implementing BDNF-centric therapeutic options within the clinic. One exception to this is translational, safe, and putatively efficacious, namely environmental factors that interact with BDNF expression such as exercise. Exercise is well-known to upregulate hippocampal BDNF levels [178] and to regulate its effects on plasticity [179], and has been shown to rescue behavioral deficits in a rat model of PTSD [180]. Promisingly, in one of the larger genetic association studies reviewed here, acute exercise was shown to mitigate PTSD symptom severity amongst BDNF 66Met carriers [97]. Likewise, in a sample of PTSD patients and healthy controls, exercise was shown to be associated with lower methylation at three BDNF gene CpG islands [181]. This suggests that, pending large-scale randomized controlled trials in PTSD cohorts, interventions that promote exercise may be an ethical, low-risk, low-cost, and translational method to promote BDNF neuroprotection within the brain.

Concluding remarks

In closing, BDNF maintains an integral but complex role in vulnerability to stress and stress-related disorders. While BDNF's involvement in this phenomenon is not a new development, progress within these fields have been hindered by a now decades-old dogma that dichotomously associates BDNF levels with pathological states. While such models have proven useful in directing research, it is also clear that they do not extrapolate to most cases of stress-inducible illnesses nor do they reflect recent advances in the literature over the past decade. The framework provided here therefore reflects a more nascent state of the conjoint BDNF and stress literature, as well as provides a revised and more specific framework from which to resolve a role for BDNF in vulnerability to stress and stress-related disorders such as PTSD. Thus, here we promote and embrace the idea of biological complexity, acknowledging that discordant results between the population-genetic and behavioral neuroscience fields likely reflects BDNF being part of a functional gene-regulatory network that may be differentially engaged by response to stress, recruited during stress-related memory formation, and physiologically compromised in stress-related brain disorders. Variability within this stress-responsive gene-regulatory network, both at the BDNF genetic locus and at other relevant loci, are thus likely to result in altered disease susceptibility which likely potentiates risk via sensitivity to gene–environment interactions. Thus, without acknowledging complex gene–environment interactions and the numerous sophisticated actions of BDNF within the brain, stress-related illnesses may continue to face a stymied translational pipeline and thus arrested development of novel treatment interventions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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