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REVIEW

Neuromodulators, stress and plasticity: a role for endocannabinoid signalling

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ABSTRACT

Any unanticipated threat to survival triggers an immediate sequence of events in the brain that culminate in a coordinated neural, endocrine and behavioural response. There is increasing evidence that stress itself modifies neural circuits. In other words, neural stress circuits learn from stress. This self-teaching is surprising as one might expect these essential circuits to be hard-wired. Our recent findings, however, indicate that repeated homotypic stress in rats causes functional changes in neural circuitry in the hypothalamus. In particular, we focus on signalling via endocannabinoids and describe plasticity in this system that impacts fast retrograde signalling at synapses on to the stress command neurons in the brain. Interestingly, this plasticity appears to be limited to early adolescence, hinting at unique modes of control of neural circuits by stress during different developmental stages.

KEY WORDS: Hypothalamus, Neuromodulation, Neurobiology

Introduction

In all organisms, maintaining a stable internal environment and responding to external threats is vital for survival. This requires the ability to sense, transduce and respond effectively to real and perceived stress. In mammals, multiple brain nuclei are activated in response to both physiological and psychological challenges. When faced with a challenge to internal homeostasis or an unanticipated, direct threat to survival, this 'stress circuitry' rapidly sets in motion a cascade of neural, neuroendocrine and behavioural responses. Mounting an appropriate physiological response to such challenges requires that the brain interprets, learns from and then remembers the appropriate stimuli. This series of tasks is accomplished by groups of interconnected neural networks that incorporate information from sensory systems, process this information in higher brain centres then engage downstream effectors to coordinate generalized behavioural and physiological adaptation to change. In the short-term, these adaptations are beneficial as they allow the organism to respond to the threat. They also promote learning, which results in more refined responses to subsequent challenges. When launched inappropriately, however – for example, in the absence of stress – they can have deleterious effects on mental and physical health (McEwen and Gianaros, 2011; Stetler and Miller, 2011).

At the organismal level, stress is a complicated response to study as there are a myriad of genetic, environmental and social contributions that influence how the body is designed to respond to challenges (Russo et al., 2012). In addition, the network of mediators that influence neural and neuroendocrine responses function in a non-linear fashion (McEwen, 2006). In an effort to better understand how the neuroendocrine response to stress is initiated and modified, we have focused exclusively on synapses that provide direct input to parvocellular neurosecretory cells (PNCs) in the paraventricular nucleus of the hypothalamus (PVN). These cells are the apex of the hypothalamic–pituitary–adrenal (HPA) axis, which drives the neuroendocrine response stress. We will focus here on neural and synaptic mechanisms and the rules that govern stress-induced changes in synaptic function that have been investigated in laboratory animals. More specifically, we will provide descriptions of changes to synaptic organization in the hypothalamus in rodent models of stress. In some respects, the occurrence of synaptic changes in the core stress centre seems curious, but recent findings from our lab and others indicate that there is a unique window when this area in particular is exquisitely sensitive to stress, during the young adolescent period (postnatal day, PND 21–30) of development in rodents. This susceptibility results in responses to repeated stress in adolescents that are distinct from those observed in adult animals. For example, while adult rats typically habituate to repeated homotypic stressors, adolescents exhibit a sensitization in response to repeated exposure to the same stressor (Romeo et al., 2006). These differences suggest unique forms of information storage, or plasticity, at different developmental stages in rodents. In order to study changes in synaptic function and plasticity, we commonly use 30–60 min restraint stress. This is a widely used paradigm which, in our hands, reliably engages the HPA and causes an increase in circulating corticosteroids (Hewitt et al., 2009).

Hypothalamus as central mediator: HPA axis and corticotropin-releasing hormone neurons

The HPA axis is recruited rapidly in response to perceived homeostatic challenges. Setting the HPA axis in motion contributes to adaptive cognitive, behavioural and emotional responses mediated by hormonal, neurochemical and physiological changes. The first step in this recruitment is an increase in the activity of PNCs in the hypothalamic PVN. These neurons produce and secrete corticotropin-releasing hormone (CRH) along with other secretogogues including vasopressin (VP) into the hypophyseal portal circulation. Interestingly, PNCs also send axonal projections to autonomic targets in the brainstem and spinal cord (Ulrich-Lai and Herman, 2009; Swanson and Kuypers, 1980) hinting at a coordination of neuroendocrine and autonomic output at the level of the hypothalamus. CRH and VP are carried through the portal blood supply to the anterior pituitary where they stimulate the synthesis and secretion of adrenocorticotropic hormone (ACTH) into the general circulation. Upon reaching the adrenal cortex, ACTH promotes the secretion of steroid hormones including glucocorticoids (GCs) and mineralocorticoids (MCs) into the circulation (Fig. 1A). In rodents corticosterone (CORT), analogous to cortisol in humans, is the primary GC. CORT can readily cross the blood–brain barrier with concentrations in the brain peaking

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List of abbreviations

ACTH	adrenocorticotropic hormone
CB1R	cannabinoid type-1 receptor
CORT	corticosterone
CRH	corticotropin-releasing hormone
DSE	depolarization-induced suppression of excitation
DSI	depolarization-induced suppression of inhibition
eCB	endocannabinoid
eIPSC	evoked inhibitory postsynaptic current
GC	glucocorticoid
GPCR	G-protein-coupled receptor
GR	glucocorticoid receptor
HPA axis	hypothalamic–pituitary–adrenal axis
KCC2	potassium–chloride co-transporter
MC	mineralocorticoid
mGluR	metabotropic glutamate receptor
MR	mineralocorticoid receptor
NKCC1	sodium–potassium–chloride symporter
PNC	parvocellular neuroendocrine cell
PVN	paraventricular nucleus of the hypothalamus
sIPSC	spontaneous inhibitory postsynaptic current
STP	short-term potentiation
VP	vasopressin

~20 min following stress exposure and remaining elevated for up to 2 h (Nguyen et al., 2000; Droste et al., 2008).

Feedback: genomic versus non-genomic

An important aspect of the HPA axis is its ability to self-regulate. This requires CORT-mediated feedback inhibition (Keller-Wood and Dallman, 1984) at the level of the hippocampus (Jacobson and Sapolsky, 1991), limbic forebrain structures (Furay et al., 2008) and the hypothalamus (Weiser et al., 2011; Evanson et al., 2010) (Fig. 1B). CORT acts at glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs), both of which are highly expressed in the PVN as well as the hippocampus, medial prefrontal cortex, amygdala and other structures associated with HPA regulation (Aronsson et al., 1988). The negative feedback mediated by CORT can be broken down into delayed and rapid effects. The delayed effects require the binding of CORT to cytosolic GRs and MRs. Once activated, this complex binds to nuclear DNA sites altering gene expression to enhance or suppress the synthesis of stress-associated proteins. These genomic effects impact the biosynthesis of CRH and are controlled by afferent signals influencing transcriptional regulation of *Crh* and *Avp* (arginine vasopressin) genes (Watts, 2005). Recent work further suggests that catecholaminergic neurons are integral to the suppression of *Crh* expression by elevated CORT (Kaminski and Watts, 2012). Because of the nature of genomic actions, subsequent effects are typically exhibited no less than 30 min following receptor binding (Keller-Wood and Dallman, 1984). The cytosolic MRs and GRs that mediate this negative feedback have different affinities for endogenous CORT; the high affinity MR typically mediates basal CORT effects while the lower affinity GR is thought to be recruited during a stressful event, when free CORT concentrations are significantly elevated. Recent work, however, challenges some elements of this framework by demonstrating that activation of MRs in the hippocampus can mediate rapid effects of CORT (Karst et al., 2005). CORT was shown to enhance glutamate transmission by increasing release probability in the hippocampus, dependent on the MR rather than the GR as predicted. These rapid non-genomic effects provide another mechanism through which the brain can react to stress, and allow one hormone, CORT, to alter synaptic activity in various ways over a prolonged period.

The more immediate effects, within seconds to minutes, of CORT-mediated non-genomic feedback, are not reliant on transcriptional regulation of specific genes, but rather are coordinated by CORT actions at membrane-bound GC receptors coupled to G-proteins (GPCRs). Non-genomic feedback effects include modulation of neuronal excitability and peptide release into the median eminence (Groeneweg et al., 2011). While the identity of a GC receptor that acts through a non-genomic mechanism remains elusive, there is compelling work showing clear examples of this non-genomic mechanism of fast feedback at glutamatergic synapses in the PVN (Di et al., 2003; Di et al., 2009; Wamsteeker et al., 2010). This fast feedback requires CORT-mediated recruitment of endocannabinoids (eCBs) in the PVN. In addition to negative feedback, CORT also acts in an adaptive fashion. The best example of this is seen in the demonstrations that intra-hypothalamic injection of CORT prior to restraint stress inhibits a stress-induced rise in CRH and ACTH (Weiser et al., 2011). This adaptive capacity may be important in preventing maladaptive secretion of CORT. Thus CORT promotes negative feedback and adaptation though a genomic mechanism to regulate transcription of target genes, as well as a non-genomic mechanism that influences receptors and ion channels. A more detailed review of genomic GC regulation of negative feedback can be found elsewhere (Watts, 2005; Groeneweg et al., 2011).

Glutamate and GABA primary inputs to PNCs

The ability of stress to induce CORT secretion is dependent on peptide release from CRH neurons. Vesicular release from these synaptic terminals is a function of membrane potential and action potential firing, which is tightly controlled by the integration of glutamatergic and GABAergic afferents onto PNCs (Watts, 2005; Decavel and Van Den Pol, 1990). These cells receive input from multiple sources and their ability to not just drive the HPA system but also adapt in response to different stimuli is intriguing, yet the mechanisms for these adaptations remain largely unresolved. Glutamatergic input to PNCs is fundamental for generating HPA responses and both ionotropic and metabotropic glutamate receptors are highly expressed in the PVN (Ziegler and Herman, 2000; Herman et al., 2000; Kocsis et al., 1998; Van Den Pol et al., 1994). Glutamate microinjections into the PVN induce CORT secretion, and application of the ionotropic glutamate receptor antagonist kynurenic acid or the selective NMDA glutamate receptor antagonist LY235959 suppresses stress-induced HPA activation and CORT release (Ziegler and Herman, 2000; Busnardo et al., 2013). These results support the necessary role of glutamate in generating GC stress responses. The activity of glutamatergic synapses, however, is labile in the face of stress. Work from our lab has shown that following acute stress, glutamate synapses can undergo a form of activity-dependent short-term potentiation (STP). This STP can be induced in response to many different stressors including psychological stressors such as restraint, physical stressors such as swim or ethologically relevant stressors such as predator odour (Kuzmiski et al., 2010). Mechanistically, STP requires the depression of NMDA receptor signalling in CRH neurons. This results in a loss of negative feedback at glutamate synapses from the postsynaptic cell and allows these synapses to release multiple vesicles of glutamate in response to bursts of afferent activity. The identity of this retrograde feedback signal remains unknown. This is a powerful and enduring form of plasticity, lasting up to a week following a single stress session. These observations open up a host of new questions that revolve around the advantages of coding such long-lasting changes after a single event. More efforts

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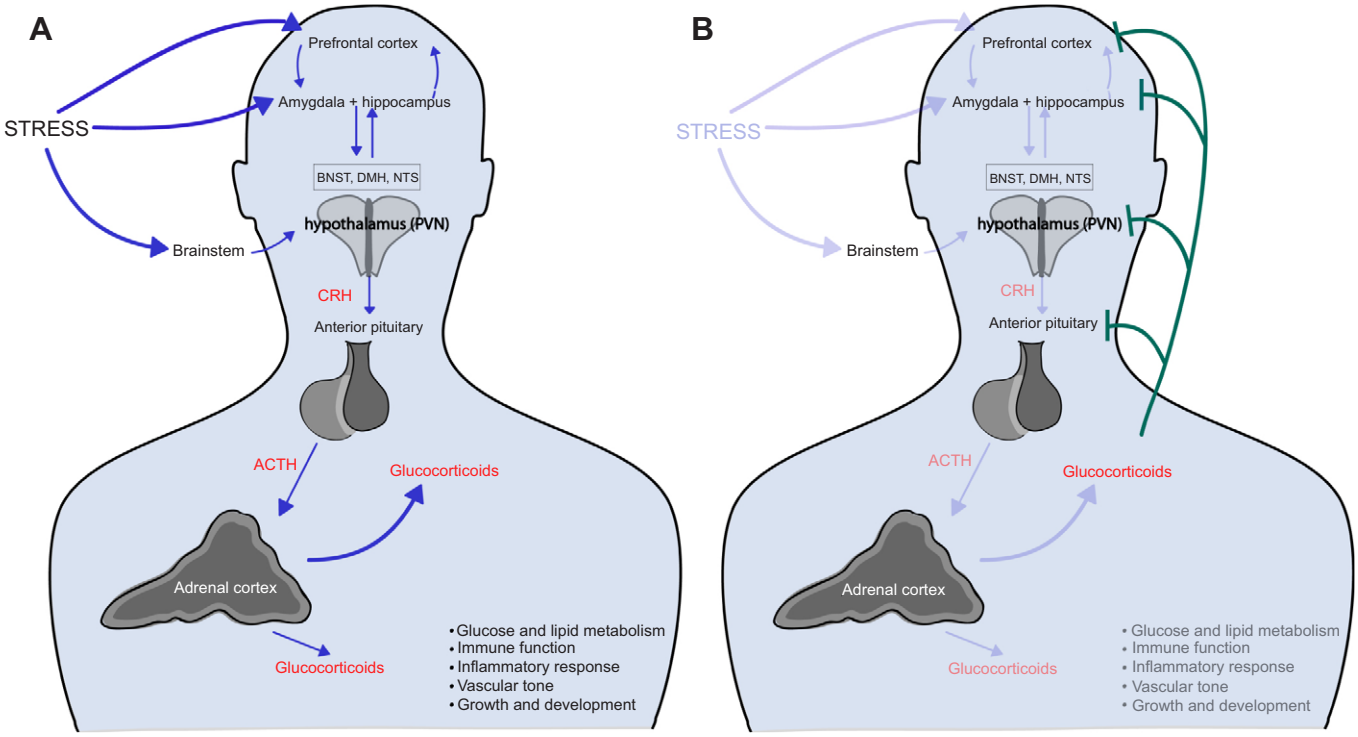

Fig. 1. Summary of the hypothalamic–pituitary–adrenal (HPA) axis stress response. (A) Multiple limbic brain areas are affected by stress and relay relevant information through GABAergic, glutamatergic and noradrenergic synaptic afferents, converging on parvocellular neuroendocrine cells (PNCs) in the paraventricular nucleus of the hypothalamus (PVN). Corticotropin-releasing hormone (CRH) is produced by PNCs and released at the median eminence into the portal circulation of the anterior pituitary. Adrenocorticotropic hormone (ACTH) is subsequently produced and released into the general circulation, stimulating the adrenal cortex to produce glucocorticoid hormones, which have numerous systemic effects. (B) Importantly, glucocorticoids (GCs) act in a negative feedback fashion (green lines) at multiple levels of HPA initiation to curtail activation. BNST, bed nucleus of the stria terminalis; DMH, dorsomedial hypothalamic nucleus; NTS, nucleus tractus solitarius.

are clearly needed in this area both to better tease apart the mechanisms and to provide more insight into the functional relevance of this effect.

The PVN receives significant input from higher brain regions and while the hippocampus, amygdala and prefrontal cortex have no significant direct connections with PNCs, they interact indirectly via multiple relay nuclei. The majority of these relay nuclei, including the bed nucleus of the stria terminalis, dorsomedial hypothalamus, local interneurons surrounding the PVN (peri-PVN), arcuate nucleus and medial pre-optic area, send GABAergic projections that richly innervate the medial parvocellular PVN (Cullinan et al., 1993; Roland and Sawchenko, 1993; Ulrich-Lai and Herman, 2009). In step with the inputs, PNCs densely express $GABA_A$ receptor subunits (Cullinan, 2000). Considering the large number of inhibitory inputs from higher brain regions, and that over 50% of inputs to PNCs are in fact GABA reactive, GABA should be a critical regulator of PNC output (deCavel et al., 1990; Miklós and Kovács, 2002). In the adolescent and adult CNS, GABA is the primary inhibitory neurotransmitter. The binding of GABA to the $GABA_A$ receptor results in a net influx of Cl^- down its electrochemical gradient. This is in contrast to the early stages of development, during which Cl^- moves out of the cell upon activation of $GABA_A$ receptors. This switch of GABA from excitatory to inhibitory during the course of development is driven by reciprocal changes in the expression of the sodium–potassium–chloride symporter NKCC1 and the potassium–chloride co-transporter KCC2. In prenatal and neonatal rodents, NKCC1 is highly expressed (Yamada et al., 2004; Ben-Ari et al., 2007). As the nervous system matures, NKCC1 expression wanes while KCC2 expression increases (Lee et al., 2005). This lowers intracellular Cl^- levels, and switches GABA from excitatory to inhibitory (Rivera et al., 1999). Consistent with GABA being inhibitory in the developed brain, intra-hypothalamic injection of the $GABA_A$ agonist muscimol prior to stress inhibits restraint-induced CORT release (Cullinan, 1998), while application of the $GABA_A$ antagonist bicuculline in naive (unstressed) animals initiates HPA responses (Cole and Sawchenko, 2002; Hewitt et al., 2009). Overall, there is ample evidence that GABA exerts an inhibitory tone on PNCs that must be reduced in order to mount an appropriate stress response. Equally important is the restoration of this inhibition to curtail the system and promote recovery following a stress experience.

Interestingly, GABA actions on PNCs can be dramatically altered following stress. Although stress activates GABAergic PVN-projecting neurons (Campeau and Watson, 1997), which is expected if GABA is acting to diminish activation of the system, evidence suggests that GABA agonists applied after stress actually enhance stress-induced CORT release (Borycz et al., 1992; Sarkar et al., 2011). Work from our lab provided the first clue, at the cellular level, that GABA inhibition was compromised at the onset of stress. Specifically, we showed that acute stress in adolescent rats was accompanied by a decrease in signalling capacity at transmembrane K^+–Cl^- co-transporters in PVN neurons. This results in decreased Cl^- extrusion from neurons that manifests as a depolarizing shift in the reversal potential for Cl^- (Hewitt et al., 2009). The functional consequence is the loss of GABA signalling and, in some cases, even a GABA-mediated excitation (Hewitt et al., 2009; Sarkar et al.,

2011) as Cl^- now leaves the cell upon the opening of the anion-permeable $GABA_A$ receptor. Our results also suggest that this depolarizing shift may be necessary in order to overcome GABAergic inhibitory tone to establish a rapid stress response. Furthermore, although reports indicate that GABA synapses show a decrease in release probability following a single stress (Verkuyl et al., 2005), our recent work shows no change in basal presynaptic GABA function following stress (Wamsteeker Cusulin et al., 2013). Rather, we show that GABA synapses exhibit unique forms of metaplasticity that are gated by stress. There are two distinct forms of activity-dependent metaplasticity. Immediately following stress, GABA synapses undergo long-term potentiation. This requires a priming of the system by noradrenaline, activation of metabotropic glutamate receptors and the insertion of postsynaptic $GABA_A$ receptors (Inoue et al., 2013). The temporal window for potentiation is brief as within 90 min, circulating CORT causes an unmasking of an activity-dependent long-term depression. This depression requires the liberation of opioids from the postsynaptic cells and manifests as a decrease in the release of GABA from presynaptic terminals (Wamsteeker Cusulin et al., 2013).

eCBs and stress

The cannabinoid type-1 receptor (CB1R) is one of the most highly expressed GPCRs in the brain and there is clear anatomical evidence demonstrating the localization of CB1Rs on glutamatergic and GABAergic synapses in the PVN (Herkenham et al., 1990; Wittmann et al., 2007). The native ligands for the CB1Rs are the eCBs, of which there are two primary molecules: anandamide (*N*-arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG). Of the two, 2-AG is more highly concentrated in the brain (Stella et al., 1997), but both likely play important roles in regulating synaptic transmission. As eCBs are lipid molecules, they can easily cross biological membranes, but it is not yet clear how they traverse the aqueous synaptic cleft. These lipid-derived molecules are thought to be recruited in an ‘on-demand’ fashion and act as retrograde transmitters (Piomelli, 2003).

It is well documented that eCBs play a significant role in many different brain processes including motor learning, pain and synaptic plasticity (Lévénés et al., 1998; Walker et al., 1999; Yoshida et al., 2002; Zhu and Lovinger, 2007). Accordingly, cells in almost every brain region produce eCBs. eCB production is set in motion by postsynaptic depolarization, which stimulates an increase in intracellular Ca^{2+} (Wilson and Nicoll, 2001; Kreitzer and Regehr, 2001a; Ohno-Shosaku et al., 2001; Llano et al., 1991) or GPCR activation (Ohno-Shosaku et al., 2005), including group I metabotropic glutamate receptors (mGluRs) (Varma et al., 2001). Following production, eCBs diffuse retrogradely across the synaptic cleft to bind to presynaptic CB1Rs. The activation of CB1Rs decreases the production of cAMP and adenylate cyclase at both GABA and glutamate nerve terminals, resulting in a decrease in the release of neurotransmitter (Piomelli, 2003) (Fig. 2A). This postsynaptic depolarization causing a transient suppression of GABA or glutamate release is termed depolarization-induced suppression of inhibition (DSI) (Pitler and Alger, 1992) or excitation (DSE), respectively (Kodirov et al., 2010; Kreitzer and Regehr, 2001b). This method is used to measure retrograde eCB production and signalling, and has been shown to occur in core regions implicated in the stress response, including the hippocampus (Pitler and Alger, 1992), amygdala (Kodirov et al., 2010), prefrontal cortex (Yoshino et al., 2011) and hypothalamus (Wamsteeker et al., 2010).

Interestingly, induction of DSI in one cell can inhibit nearby synapses as well (Wilson and Nicoll, 2001). Application of the group I mGluR agonist DHPG causes a comparable suppression of evoked inhibitory postsynaptic currents (eIPSCs), through enhanced production of eCBs, confirmed by occlusion with the CB1R agonist WIN55,212-2 (WIN) (Neu et al., 2007; Maejima et al., 2001). The inability of the Ca^{2+} chelator BAPTA to block these effects of DHPG confirms that group I mGluR activation is a Ca^{2+}-independent mechanism of eCB production (Maejima et al., 2005). Activation of the presynaptic CB1R leads to inhibition of neurotransmitter release through suppression of voltage-sensitive Ca^{2+} channels (Twitchell et al., 1997; Guo and Ikeda, 2004) along with inhibition of adenylate cyclase activity (Bonhaus et al., 1998) and enhanced conduction through inwardly rectifying K^+ channels (Mackie et al., 1995; Kirby et al., 2000). The role of voltage-sensitive calcium channels is confirmed by occlusion of CB1R agonist actions by the application of an N-type Ca^{2+} channel blocker (Wilson et al., 2001). This eCB-induced depression of eIPSCs is accompanied by an inhibition of spontaneous IPSC (sIPSC) frequency, and in some cases sIPSC amplitude (Pitler and Alger, 1992; Wamsteeker et al., 2010; Wilson and Nicoll, 2001). This reduction in sIPSC frequency appears to be directly proportionate to eIPSC inhibition (J. I. Wamsteeker, personal communication). This effect requires Ca^{2+} influx into the postsynaptic cell and is independent of changes to postsynaptic K^+ conductance (Pitler and Alger, 1992; Wilson and Nicoll, 2001). These data further support that postsynaptic depolarization exerts its inhibitory effect on synaptic transmission through a presynaptic reduction of release probability.

Work from our lab and others has demonstrated the complex role of the eCB system in the stress response. eCBs regulate the HPA axis but, in turn, are influenced by CORT. In fact there is evidence that the eCB system controls even basal HPA activity, as application of the CB1R antagonist SR141716 leads to an increase in circulating CORT levels in rodents (Patel et al., 2004). Patel and colleagues have also shown that enhancing eCB signalling dampens stress-induced CORT secretion, which is to be expected if eCBs are acting at CRH neurons to dampen neurotransmitter release. Likewise, blockade of the CB1R causes potentiation of CORT release following activation of the HPA axis by a stressor or GC agonist (Evanson et al., 2010), consistent with a release of inhibition. However, there is growing evidence that stimulation of the HPA axis and subsequent CORT release directly influences eCB-mediated retrograde signalling, with acute and chronic stressors facilitating or inhibiting different eCB-mediated outcomes in adolescent rodents (Wamsteeker et al., 2010; Crosby and Bains, 2012). Following acute stress or GC administration, eCB synthesis in PNCs is triggered by activation of GC receptors, which acts to dampen acute HPA axis hyperactivity (Di et al., 2003; Hill et al., 2010). The presumed pathway for fast negative-feedback regulation of the HPA involves activation of the membrane-associated GC receptor, leading to production of eCBs through G-protein-coupled signalling (Di et al., 2003). This eCB-mediated effect on HPA output is modest. One reason for this may be that GC-mediated eCB production is limited to glutamate synapses. This specificity is surprising given that GABA synapses in PVN are sensitive to exogenous CB1R ligands and exhibit DSI (Wamsteeker et al., 2010). Further investigation is needed to better understand the synapse specificity of the CORT–eCB interactions.

In contrast to acute stress, a repetitive, homotypic stress paradigm induces a loss of activity-dependent eCB signalling at both glutamate and GABA synapses in young adolescent (PND 21–30) rats. This presents as a progressive reduction in DSE and DSI, respectively, over the course of multiple restraint stress exposures

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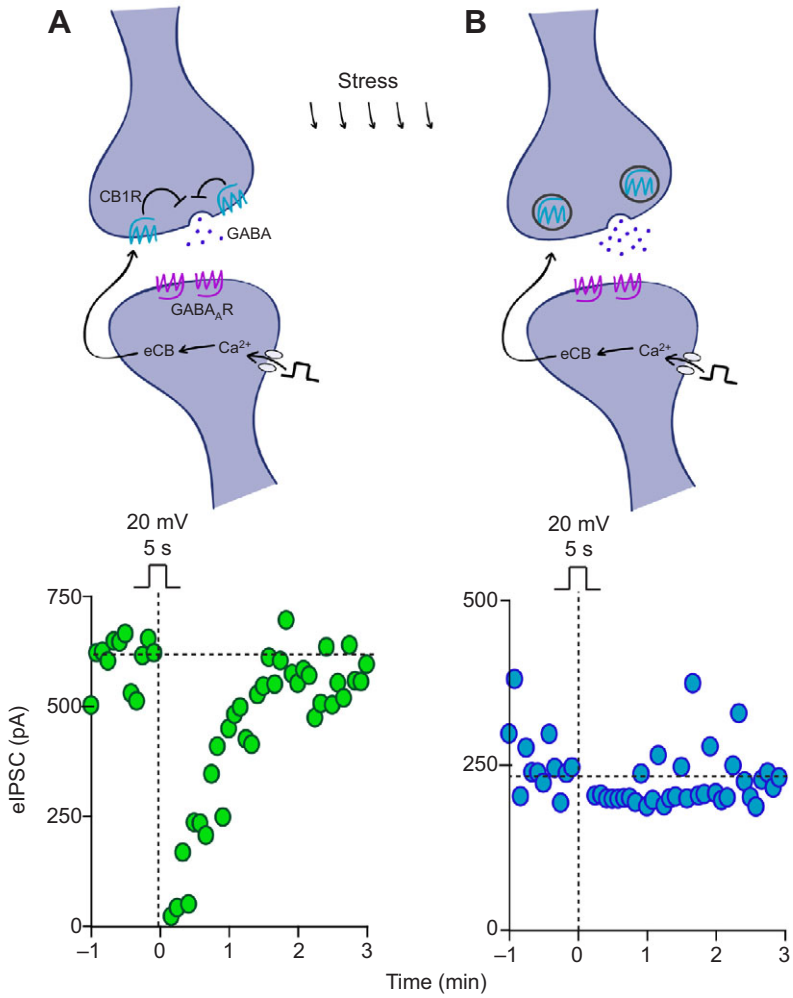

Fig. 2. Effect of a repetitive stress paradigm on endocannabinoid (eCB) signalling at GABA synapses in the PVN. (A) Normal function of activity-dependent eCB signalling. Postsynaptic depolarization (20 mV, 5 s) causes an influx of calcium, and subsequent eCB production. eCBs act retrogradely on cannabinoid type-1 receptor (CB1R)-containing PNCs to inhibit GABA release in naive animals. $GABA_AR$, $GABA_A$ receptor. (B) Five days of repetitive homotypic stress causes functional downregulation of presynaptic CB1Rs, resulting in diminished depolarization-induced suppression of inhibition (DSI) expression.

(Fig. 2). This effect requires the activation of the genomic GC receptor as it is prevented by intraperitoneal injections of the GC receptor antagonist RU486 prior to stress (Wamsteeker et al., 2010). Furthermore, this loss of eCB signalling is due to compromised function of the CB1R and not to an inability of this system to produce eCBs. This is based on the observation that the exogenous ligand of the CB1R, WIN, fails to reduce synaptic release in repeatedly stressed animals (Wamsteeker et al., 2010). Comparable studies in the striatum show chronic stress-induced reduction in CB1 receptor function at GABAergic synapses, which are similarly dependent on the genomic GC receptor (Rossi et al., 2008). Although it appears that CORT actions via GC receptors are necessary for diminished eCB signalling in the PVN following repeated stress, a clear mechanism for CB1R downregulation has yet to be elucidated. In the hippocampus, chronic GC administration reduces CB1R density (Hill et al., 2008); however, findings indicate that this impaired retrograde signalling in the PVN is unlikely to be caused by agonist-induced CB1 receptor downregulation as extended CORT incubation does not alter DSI expression (Wamsteeker et al., 2010). A more probable explanation involves CORT action on intracellular GC receptors inducing a genomic signalling cascade to ultimately alter CB1R transcription or some constituent of the receptor that allows it to be functionally responsive.

Ongoing synaptic activity is necessary for appropriate functioning of CB1Rs, and the efficacy of eCB-mediated inhibition of glutamate and GABA release is largely modulated by both presynaptic and postsynaptic activity. Postsynaptic activity level, experimentally controlled by the duration and magnitude of the depolarizing step used to elicit DSI, greatly influences eCB production and release (Wamsteeker et al., 2010). Studies suggest that CB1R activation enhances specific K^+ conductances to reduce neurotransmitter release (Kirby et al., 2000) while the K^+ channel blocker 4-AP abolishes DSI through a presynaptic mechanism (Varma et al., 2002). 4-AP increases the magnitude and duration of the terminal depolarization, increasing Ca^{2+} influx and enhancing eIPSC amplitude. Subsequently, the DSI-associated reduction in Ca^{2+} influx is probably overwhelmed by a saturation of the release process. The necessity of synaptic activity for CB1R function is further supported by evidence that presynaptic activity state plays a role in the regulation of eCB inhibition of GABA release. Increasing presynaptic firing rates in the hippocampus can recover inhibition of GABA release induced by a CB1R agonist, in an N-type Ca^{2+} channel-dependent fashion (Földy et al., 2006). These findings that CB1Rs are subject to modulation by synaptic activity might provide insights into how CB1Rs recover after stress.

Over a period of days following the final stressor, this retrograde system passively recovers to pre-stress activity levels in a time-dependent manner (Wamsteeker et al., 2010). Further, evidence has been put forward for the ability of exercise to re-set CB1R sensitivity in the striatum following stress, accompanied by resistance to measured behavioural consequences of stress (De

Chiara et al., 2010). eCBs clearly play a fundamental role in regulating basal and acute stress-induced HPA inhibition at synapses in the PVN; however, repetitive stress impairs their ability to influence neurotransmitter signalling at PNCs. It will be interesting to determine whether eCB signalling can be rapidly recovered in the PVN and the conditions under which this may occur.

The loss of CB1R signalling following repeated stress is not a generalizable phenomenon as it is not evident in the CA1 region of the hippocampus, an area thought to play an important role in regulating stress responses. Furthermore, we failed to observe any changes to eCB signalling in adult animals subjected to the same repeated stress protocol. This intriguing observation suggests a unique sensitivity of this system to stress in the adolescent and suggests that it might serve as a useful entry point for better understanding the physiology and plasticity of stress responses during development.

There are multiple neuromodulators that serve important roles in sculpting the brain's response to stress. Here we have focused on the eCBs, and specifically on their roles in the paraventricular nucleus of the hypothalamus in rodents. These fascinating molecules are rapidly induced, highly labile, yet ubiquitous in the brain. In addition, numerous lines of work now show eCB signalling itself is plastic and understanding the key steps that regulate eCB signalling may provide new insights into stress neurobiology.

Competing interests
The authors declare no competing financial interests.

Author contributions
L.S. conducted the experiments presented in Fig. 2. The authors contributed equally in the writing of the manuscript.

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