Review

Stress and anxiety: Structural plasticity and epigenetic regulation as a consequence of stress

Bruce S. McEwen [a,*], Lisa Eiland [a,b], Richard G. Hunter [a], Melinda M. Miller [a]

[a] Laboratory of Neuroendocrinology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
[b] Department of Pediatrics, Weill Cornell Medical College, 525 E. 68th Street, N-506, New York, NY 10065, USA

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ABSTRACT

The brain is the central organ of stress and adaptation to stress because it perceives and determines what is threatening, as well as the behavioral and physiological responses to the stressor. The adult, as well as developing brain, possess a remarkable ability to show reversible structural and functional plasticity in response to stressful and other experiences, including neuronal replacement, dendritic remodeling, and synapse turnover. This is particularly evident in the hippocampus, where all three types of structural plasticity have been recognized and investigated, using a combination of morphological, molecular, pharmacological, electrophysiological and behavioral approaches. The amygdala and the prefrontal cortex, brain regions involved in anxiety and fear, mood, cognitive function and behavioral control, also show structural plasticity. Acute and chronic stress cause an imbalance of neural circuitry subserving cognition, decision making, anxiety and mood that can increase or decrease expression of those behaviors and behavioral states. In the short term, such as for increased fearful vigilance and anxiety in a threatening environment, these changes may be adaptive; but, if the danger passes and the behavioral state persists along with the changes in neural circuitry, such maladaptation may need intervention with a combination of pharmacological and behavioral therapies, as is the case for chronic or mood anxiety disorders. We shall review cellular and molecular mechanisms, as well as recent work on individual differences in anxiety-like behavior and also developmental influences that bias how the brain responds to stressors. Finally, we suggest that such an approach needs to be extended to other brain areas that are also involved in anxiety and mood.

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1. Introduction

The brain is the central organ of stress and adaptation to stress because it perceives and determines what is threatening, as well as the behavioral and physiological responses to the stressor. The adult, as well as developing brain, possess a remarkable ability to adapt and change with stressful, and other experiences. Structural changes – neuronal replacement, dendritic remodeling, and synapse turnover – are a feature of the adult brain's response to the environment. Nowhere is this better illustrated in the mammalian brain than in the hippocampus, where all three types of structural plasticity have been recognized and investigated using a combination of morphological, molecular, pharmacological, electrophysiological and behavioral approaches. At the same time, new data on the amygdala and the prefrontal cortex, brain regions involved in anxiety and fear, mood, cognitive function and behavioral control, have demonstrated that the adult brain is indeed a malleable and adaptable structure capable of reversible structural plasticity. Steroid hormones play an important role, acting via both genomic and non-genomic mechanisms. In addition, other intracellular mediators and neurotransmitter systems participate in structural plasticity.

The theme of this review is that stress causes an imbalance of neural circuitry subserving cognition, decision making, anxiety and mood that can increase or decrease expression of those behaviors and behavioral states. In the short term, such as for increased fearful vigilance and anxiety in a threatening environment, these changes may be adaptive; but, if the danger passes and the behavioral state persists along with the changes in neural circuitry, such maladaptation may need intervention with a combination of pharmacological and behavioral therapies, as is the case for chronic or mood anxiety disorders.

* Corresponding author. Tel.: +1 212 327 8624.
E-mail address: mcewen@rockefeller.edu (B.S. McEwen).

Besides reviewing cellular and molecular mechanisms, we shall discuss recent work on individual differences in anxiety-like behavior in the animals that we and others study, as well as possible developmental influences that may underlie those differences and bias how the brain responds to stressors. Finally, we shall note that multiple brain regions are involved and that investigations on amygdala, prefrontal cortex and hippocampus that have been very productive, need to be extended to other brain areas that are also involved in anxiety and mood.

2. Structural plasticity in the hippocampus, amygdala and prefrontal cortex

2.1. Hippocampus

Stress hormones modulate function within the brain by changing the structure of neurons. The hippocampus is one of the most sensitive and malleable regions of the brain. Within the hippocampus, the input from the entorhinal cortex to the dentate gyrus is ramified by the connections between the dentate gyrus and the CA3 pyramidal neurons. One granule neuron innervates, on the average, 12 CA3 neurons, and each CA3 neuron innervates, on the average, 50 other CA3 neurons via axon collaterals, as well as 25 inhibitory cells via other axon collaterals. The net result is a 600-fold amplification of excitation, as well as a 300 fold amplification of inhibition, that provides some degree of control of the system (McEwen, 1999).

The circuitry of the dentate gyrus-CA3 system is believed to play a role in the memory of sequences of events, although long-term storage of memory occurs in other brain regions (Lisman and Otmakhova, 2001). But, because the dentate gyrus DG -CA3 system is so delicately balanced in its function and vulnerability to damage, there is also adaptive structural plasticity, in that new neurons continue to be produced in the dentate gyrus throughout adult life; and CA3 pyramidal cells undergo a reversible remodeling of their dendrites in conditions, such as hibernation and chronic stress, including a combination of food restriction and increased physical activity (McEwen, 2010). Whatever the physiological significance of these changes, be it protection (McEwen, 2007) or increased vulnerability to damage (Conrad, 2008), the hippocampus undergoes a number of adaptive changes in response to acute and chronic stress.

2.2. Replacement of neurons in dentate gyrus

One type of structural change that occurs in the hippocampus involves replacement of neurons (Altman and Das, 1965; Kaplan and Bell, 1984; Cameron and Gould, 1994). The subgranular layer of the dentate gyrus contains cells that have some properties of astrocytes, e.g. expression of glial fibrillary acidic protein which give rise to granule neurons (Seri et al., 2001). After BrdU administration to label DNA of dividing cells, these newly born cells appear as clusters in the inner part of the granule cell layer, where a substantial number of them will go on to differentiate into granule neurons within as little as 7 days. In the adult rat, up to as many as 5–9000 new neurons are born per day and survive with a half-life of 28 days (Cameron and McKay, 2001). There are many hormonal, neuro-chemical and behavioral modulators of neurogenesis and cell survival in the dentate gyrus, including estradiol, IGF-1, antide-pressants, glucocorticoids, voluntary exercise and hippocampal-dependent learning (Kempermann and Gage, 1999; McEwen, 2010; van Praag et al., 1999). With respect to stress, certain types of acute stress and many chronic stressors suppress neurogenesis or cell survival in the dentate gyrus, and the mediators of these

inhibitory effects include excitatory amino acids acting via NMDA receptors and endogenous opioids (McEwen, 2010).

2.3. Functional consequences

As to the functional consequences of structural remodeling in hippocampus, repeated stress can impair hippocampal-dependent behaviors in a manner that is reversible, along with dendritic shrinkage in the CA3 region, within days or weeks after the termination of the stressor. This supports the notion that the remodeling is not brain damage but a form of adaptive plasticity that may also protect the hippocampus from permanent excitotoxic damage (McEwen, 2010). Yet, it is important to note that chronic stress causes other changes in the brain besides dendritic remodeling in CA3, e.g., prolonged stress can diminish the size of the dentate gyrus (Pham et al., 2003) and also cause dentate gyrus dendritic remodeling (Sousa et al., 2000) and dentate gyrus long-term potentiation LTP (Pavlides et al., 2002). Moreover, 21d chronic restraint alters the ability of acute stress to affect hippo-campal functions, such as spatial memory, and here an increase in sensitivity to glucocorticoids appears to be involved and to mediate at least some of the behavioral changes (Conrad, 2006).

2.4. Prefrontal cortex and amygdala

Acute and repeated stress for 21days of CRS also causes functional and structural changes in other brain regions, such as the prefrontal cortex and amygdala (McEwen, 2010). CRS and chronic immobilization caused dendritic shortening in medial prefrontal cortex (McEwen, 2010), but produced dendritic growth in neurons in amygdala (Vyas et al., 2002), as well as in orbitofrontal cortex (Liston et al., 2006). Similarly, in the domain of substance abuse, different, and sometimes opposite, effects were seen on dendritic spine density in orbitofrontal cortex, medial prefrontal cortex and hippocampus CA1 (Crombag et al., 2005; Robinson and Kolb, 1997).

Behavioral correlates of CRS-induced remodeling in the prefrontal cortex include impairment in attention set shifting, possibly reflecting structural remodeling in the medial prefrontal cortex (Liston et al., 2006). Moreover, chronic restraint stress impairs extinction of a fear conditioning task (Miracle et al., 2006) and the prefrontal cortex is involved in extinction of fear conditioning (Santini et al., 2004).

Regarding the amygdala, chronic stress for 21 days or longer not only impairs hippocampal-dependent cognitive function, but it also enhances amygdala-dependent unlearned fear and fear conditioning (Conrad et al., 1999). Chronic stress also increases aggression between animals living in the same cage, and this is likely to reflect another aspect of hyperactivity of the amygdala (Wood et al., 2003). Moreover, chronic corticosterone treatment in the drinking water produces an anxiogenic effect that could be due to the glucocorticoid enhancement of CRF activity in the amygdala (Corodimas et al., 1994; Makino et al., 1995; McEwen, 2010).

3. Cellular and molecular mechanisms involved in stress-related structural and functional plasticity

3.1. Adrenal steroids

Because the hippocampus was the first higher brain center that was recognized as a target of stress hormones (McEwen et al., 1968), both the hippocampus and adrenal steroids have figured prominently in our understanding of how stress impacts brain structure and behavior. The hippocampus expresses both Type I mineralo-corticoid, MR and Type II glucocorticoid, GR receptors, and these receptors mediate a biphasic response to adrenal steroids in the CA1

region, although not in the dentate gyrus (Joels, 2006), which, nevertheless, shows a diminished excitability in the absence of adrenal steroids (Margineanu et al., 1994) along with debranching of dentate granule neuron dendrites (Gould et al., 1990). Other brain regions, such as the paraventricular nucleus, lacking in MR but having GR, show a monophasic response (Joels, 2006).

Regarding the biphasic effects of adrenal steroids, these have been seen for excitability of hippocampal neurons in terms of long-term potentiation and primed burst potentiation (Diamond et al., 1992; Pavlides et al., 1994, 1995), with parallel biphasic effects upon memory (Okuda et al., 2004; Pugh et al., 1997). As to mechanisms for the biphasic responses, the co-expression of MR and GR in the same neurons could give rise to heterodimer formation and a different genomic activation from that produced by either MR or GR homodimers (Joels, 2006). Moreover, in addition, deletion of the Type I MR receptor by genetic means has revealed that MR is involved (Karst et al., 2005) in corticosterone enhancement of extracellular levels of glutamate (Venero and Borrell, 1999).

Another important non-genomic action of glucocorticoids is the rapid stimulation of endocannabinoid release (Hill et al., 2010a; Tasker et al., 2006), which is involved not only in HPA regulation (Hill et al., 2010b), but also in negative regulation of glutamate, GABA and acetylcholine release (Hill and McEwen, 2010).

Finally, although much of the work on MR and GR has been done on rat and mouse brains, it is important to note that the rhesus monkey hippocampus has a predominance of MR and relatively less GR compared to rodent species (Sanchez et al., 2000). This finding may have important implications for the effects of adrenal steroids on learning and vulnerability to stress and excitotoxicity in primates and humans, as well as age-related changes, as discussed earlier.

Exploration of the underlying mechanisms for structural remodeling of dendrites and synapses reveals that it is not adrenal size or presumed amount of physiological stress per se that determines dendritic remodeling, but a complex set of other factors that modulate neuronal structure (McEwen, 1999). Indeed, in species of mammals that hibernate, dendritic remodeling is a reversible process and occurs within hours of the onset of hibernation in European hamsters and ground squirrels, and it is also reversible within hours of wakening of the animals from torpor (Arendt et al., 2003; Magarinos et al., 2006; Popov and Bocharova, 1992). This implies that reorganization of the cytoskeleton is taking place rapidly and reversibly and that changes in dendrite length and branching are not "damage" but a form of structural plasticity.

3.2. Importance of glutamate for remodeling in hippocampus

Adrenal steroids are important mediators of remodeling of hippocampal neurons during repeated stress, and exogenous adrenal steroids can also cause remodeling in the absence of an external stressor (Magarinos et al., 1999; Sousa et al., 2000). However, effects of chronic stress on dendritic remodeling are blocked by blocking NMDA receptors, as well as blocking adrenal steroid synthesis (Magarinos and McEwen, 1995). A recent report indicates that NMDA receptors and glutamate are also involved in stress-induced shortening of dendrites in medial prefrontal cortex (Martin and Wellman, 2011).

Further evidence for the importance of glutamate is the stress-induced elevation of extracellular glutamate levels, leading to induction of glial glutamate transporters, as well as increased activation of the nuclear transcription factor, phosphoCREB (Reagan et al., 2004; Wood et al., 2004). Excitatory amino acids released by the mossy fiber pathway play a key role in the remodeling of the CA3 region of the hippocampus, and regulation of glutamate release by adrenal steroids may play an important role (McEwen,

1999). Moreover, 21d of CRS leads to depletion of clear vesicles from mossy fiber terminals and increased expression of presynaptic proteins involved in vesicle release (Grillo et al., 2005; Magarinos et al., 1997). Taken together with the fact that vesicles which remain in the mossy fiber terminal are near active synaptic zones and that there are more mitochondria in the terminals of stressed rats, this suggests that CRS increases the release of glutamate (Magarinos et al., 1997).

3.3. Maintaining the balance between adaptation and excitotoxicity

Glucocorticoids also synergize with excitatory amino acids to promote excitotoxic damage and impairment of energy generation through inhibition of glucose uptake and energy metabolism appears to be one major mechanism (Sapolsky, 1992). As another example of an inverted U shaped action of adrenal steroids, recent evidence indicates that glucocorticoid receptors translocate to mitochondria and, at physiological levels, reduce potentially damaging oxidative stress, whereas, at high levels, this mechanism fails after some hours and there is increased excitotoxicity (Du et al., 2009). Therefore, mitochondria and their sensitivity to glucocorticoids (Roosevelt et al., 1973) play an important role in maintaining the balance between adaptation and excitotoxicity.

In being able to protect and promote adaptation such as reversible stress-induced structural plasticity, on the one hand, and yet contribute to damage, on the other, glucocorticoids oppose some actions of stress and mediate others. For example, chronic stress induced induction of glutamate transporter Glt 1 in hippocampus (Reagan et al., 2004) is biphasically modulated by glucocorticoids (Autry et al., 2006), and kainate (KA1) receptor up-regulation by chronic stress is also biphasically modulated by glucocorticoids. See Table 1. At the same time, chronic stress induction of cocaine-amphetamine-regulated transcript (CART) in the hippocampus is mediated by glucocorticoids (Hunter et al., 2007). In hippocampus, the up-regulation of CART is associated with a form of resistance of anxiety-generating effects of stress (Miller, M., Hunter, R., McEwen, B., unpublished). The subtlety and complexity of adrenal steroid actions that is revealed by these and others (Joels, 2006; Joels et al., 2006), are reminiscent of their role in modulation of the immune system (Sapolsky et al., 2000), including apparent pro- as well as anti-inflammatory actions (Munhoz et al., 2010).

3.4. Other mediators of hippocampal structural plasticity

Besides glutamate, the role of adrenal steroids in the hippocampus involves other interactions with neurochemical systems, including serotonin, endogenous opioids, calcium currents, and GABA-benzodiazepine receptors (McEwen, 1999; McEwen and Chattarji, 2004). Moreover, and beyond the scope of this article, there are other molecular players in the stress-induced remodeling

Table 1
Glucocorticoid actions mediate or biphasically modulate actions of chronic stress.

Cocaine amphetamine related transcript (CART) mRNA and protein in dentate gyrus. Function: Modulation of stress effects on anxiety CORT mediates stress-induced increase in CART (Hunter et al., 2007)
KA1 receptor mRNA in dentate gyrus. Function: Promote glutamate release and actions CORT biphasically modulates stress-induced increase in KA1 (Hunter et al., 2009a)
Glutamate transporter (Glt 1) mRNA and protein in CA1-3 Function: Reuptake of glutamate after release CORT biphasically modulates stress-induced increase in Glt1 (Autry et al., 2006)

of dendrites, which include extracellular molecules of the NCAM family, including PSA-NCAM; a transmembrane glycoprotein, M6a; corticotrophin releasing factor; tissue plasminogen activator (tPA), which is an extracellular protease and signaling molecule; and brain-derived neurotrophic factor, BDNF (McEwen, 2010).

## 3.5. Mechanisms of structural plasticity in amygdala and prefrontal cortex

We know less about stress-related structural plasticity in the amygdala and prefrontal cortex. Yet, both amygdala and prefrontal cortex express adrenal steroid receptors and there is evidence that adrenal steroids play a role in structural plasticity, along with a role for tPA, CRF and BDNF (McEwen, 2010). BDNF plays a particularly interesting role in amygdala plasticity, since over-expression of BDNF, without any applied stressor, enhances anxiety in an elevated plus maze and increases spine density on basolateral amygdala neurons and this occludes any further effect of immobilization stress on both anxiety and spine density (Govindarajan et al., 2006). As noted, NMDA receptors and glutamate are also involved in stress-induced shortening of dendrites in medial prefrontal cortex (Martin and Wellman, 2011) and, thus far, their role in structural plasticity in the amygdala is unclear.

3.6. Key role of BDNF

BDNF is particularly interesting because it appears to be a facilitator of plasticity directed by other cellular signals, as suggested originally by work in vitro (Horch and Katz, 2002; Horch et al., 1999). BDNF +/− mice show a less branched dendritic tree and do not show a further reduction of CA3 dendrite length with chronic stress, whereas wild-type mice show reduced dendritic branching after chronic stress (Magarinos et al., 2011) (Fig. 1). At the same time, over-expression of BNDF prevents stress-induced reductions of dendritic branching in the CA3 hippocampus and results in anti-depressant-like effects in a Porsolt forced-swim task (Govindarajan et al., 2006). Activation of neuronal activity by stress triggers increased BDNF synthesis to replace depletion of BDNF caused by stress (Marmigere et al., 2003). Yet, BDNF and corticosteroids appear to oppose each other − with BDNF reversing reduced excitability in hippocampal neurons induced by stress levels of corticosterone (Hansson et al., 2006). The occurrence of and timing of opposing effects of stress and glucocorticoids on BDNF may be the reason that chronic stress has been reported to deplete BDNF levels in some studies (Smith et al., 1995) but not in others (Isgor et al., 2004; Kuroda and McEwen, 1998).

At the same time, glucocorticoids appear to activate trk B signaling in a ligand-independent manner that has neuroprotective implications (Jeanneteau et al., 2008), although this effect is transient and accommodates to chronic glucocorticoid exposure (Numakawa et al., 2009). The authors of this study propose that TrkB-GR interaction plays a critical role in the BDNF-stimulated PLC-g pathway, which is required for glutamate release, and the decrease in TrkB-GR interaction caused by chronic exposure to glucocorticoids results in the suppression of BDNF-mediated neurotransmitter release via a glutamate transporter (Numakawa
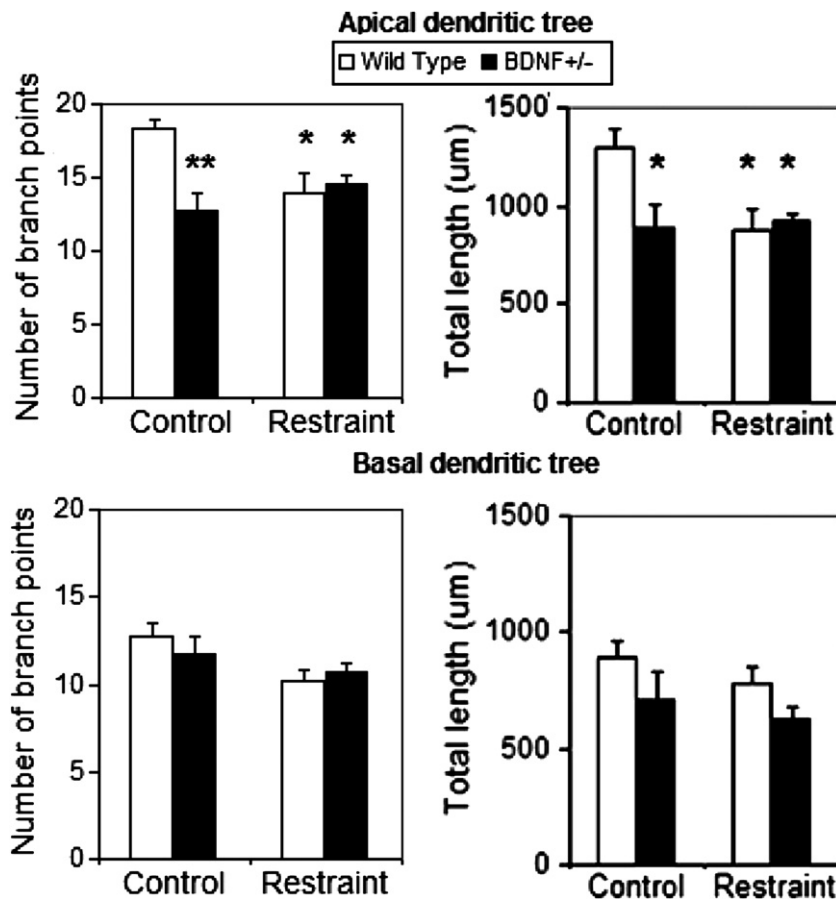
Fig. 1. Effect of chronic restraint stress on the number of apical and basal (upper and lower panel on left) dendritic branch points and total dendritic length (upper and lower panel on right) of CA3 pyramidal neurons from wild type and haploinsufficient BDNF (BDNFBl/6) mice. ** and *$P < 0.01$ and $P < 0.05$ respectively, compared with control wild types. One-way ANOVA, Tukey post hoc test. Bars represent means 1 SEM. From Magarinos et al. (2011).

et al., 2009). Thus, as is the case for glucocorticoid effects KA1 receptors and glutamate transporters (Table 1), the glucocorticoid actions on the BDNF-trkB system modulate this system and have a self-limiting (homeostatic) quality.

The role of BDNF as a facilitator of plasticity has led to expansion of the utility of BDNF in plasticity related to treatment of disease. Besides depression, where reduced BDNF may be a key feature of the depressive state and elevation of BDNF by diverse treatments ranging from antidepressant drugs, such as fluoxetine to regular physical activity (Duman and Monteggia, 2006), there are other potential applications, such as the recently reported ability of fluoxetine to enhance recovery from stroke (Chollet et al., 2011) and the previously reported effects of fluoxetine to ameliorate effects of monocular deprivation in the visual systems by enhancing visual stimulation-induced plasticity (Vetencourt et al., 2008). However, a key aspect of this new view (Castren and Rantamaki, 2010) is that the drug is opening a "window of opportunity" that may be capitalized by a positive behavioral intervention, e.g., behavioral therapy in the case of depression or the intensive therapy to promote neuroplasticity to counteract the effects of a stroke. In this connection, it is important to note that successful behavioral therapy, which is tailored to individual needs, can produce volumetric changes in both prefrontal cortex in the case of chronic fatigue (de Lange et al., 2008), and in amygdala in the case of chronic anxiety (Holzel et al., 2009). This implies at least 3 important lessons: i. that antidepressant drugs should not be given without such a positive intervention; ii. that there may be a limited window of opportunity, beyond which continued drug treatment may be ineffective; and iii. that negative experiences during the window may even make matters worse (Castren and Rantamaki, 2010). In this connection, it should be noted that BDNF also has the ability to promote pathophysiology, as in seizures (Heinrich et al., 2011).

4. Acute vs chronic stress effects

Responses to acute and chronic stress in both the amygdala and the prefrontal cortex present challenges to our understanding of the cellular and molecular mechanisms. In the amygdala, while chronic stress causes dendrites in the basolateral amygdala to increase in length along with increased spine density on dendrites (Vyas et al., 2002), a single acute stress to a naïve rat causes increased spine density without increased dendritic branching or length after a 10d interval (Mitra et al., 2005). The former increase in dendritic length after chronic stress can be mimicked by a single, acute injection of a large dose of glucocorticoids (Mitra and Sapolsky, 2008). Yet, in relation to the effect of the single traumatic stressor, glucocorticoid presence before the traumatic stressor prevents the delayed increase in dendritic spines (Rao, McEwen, Chattarji, unpublished). This raises, again, the important issue that both glucocorticoid dose and timing are important for the outcome (Joels, 2006; Joels et al., 2006). Since actions of adrenal steroids can directly or indirectly affect gene expression through direct interactions with response elements or indirect signaling via second messenger pathways, the regulation of gene expression may provide some clues. So far the hippocampus has begun to provide some insights.

## 5. Epigenetic involvement in alterations due to stress

Adrenal steroids bind to MR and GR and end up in the cell nucleus (McEwen and Plapinger, 1970), and thus the regulation of gene expression is a key aspect of their action, referred to now under the rubric of "epigenetic" influences. The subject of epigenetic influences on the function of the nervous system has been the subject of much recent research (Jiang et al., 2008). Epigenetics are of interest with regard to disorders, such as anxiety and depression, because epigenetic change is a means to explain how life events, like stress, cause persistent changes in the brain and in behavior. Epigenetics also offers a possible explanation of how individuals sharing the same or similar genes might show large differences in disease susceptibility and resilience.

The term "epigenetics" encompasses a number of mechanisms by which biological information is transmitted above the level of the genetic code. Broadly speaking, this definition includes everything from DNA methylation to the transmission of culture and language, so we might more strictly define epigenetics as molecular events, other than the genetic code itself, which influence gene transcription over time. These include, DNA methylation, a variety of non-coding RNA mechanisms, and covalent modifications of histones. Histone modifications and DNA methylation have been the most thoroughly examined with regard to stress and the hippocampus. Histone proteins are subject to a variety of modifications at an ever growing number of residues. These modifications include acetylation, methylation (which has three valences) and phosphorylation, as well as a number of others (Gardner et al., 2011; Jiang et al., 2008).

The stress sensitive hippocampus (McEwen, 1999) expresses the enzymatic machinery of epigenetic change at levels as high or higher than other regions of the brain, as an afternoon spent on the Allan Brain Atlas will show. DNA methylation and histone H3 phospho-acetylation have been associated with hippocampal memory formation and the latter modification appears to be responsive to both stress and exercise (Collins et al., 2009; Miller and Sweatt, 2007; Reul et al., 2009) and our own work (see below) has shown that histone methylation in the hippocampus is also dynamically responsive to stress. It is significant that the hippocampus is the site of one of the only examples of epigenetic action in the strict, heritable, sense; that is, the demonstration that differences in early life environment result in lasting, transgenerational changes in behavior, mediated in part by changes in epigenetic histone marks and DNA methylation of the 1−7 promoter of the GR in the hippocampus (Szyf et al., 2005). The consequence of these modifications is a down, or up-regulation in the expression of the GR that persists across generations and produces significant differences in stress responsiveness and affective behaviors (Weaver et al., 2004). Significantly, epigenetic changes in the DNA methylation status of both the GR and BDNF genes have since been shown to be associated with a history of childhood abuse in humans (McGowan et al., 2009; Roth et al., 2009).

Recent work in our laboratory has demonstrated that acute stress produces rapid decreases in histone H3 lysine 27 trimethylation (a mark associated with transcriptional silencing and facultative heterochromatin) and equally rapid increases in levels of the heterochromatic H3 lysine 9 tri-methyl (H3K9me3) mark (associated with constitutive heterochromatin) (Hunter et al., 2009b). See Fig. 2. These effects attenuate over the course of 21 days of chronic stress, though the attenuation is partially blocked, in the case of H3K9, by co-administration of fluoxetine (Hunter et al., 2009b). These results were surprising due to the magnitude and rapidity of the changes observed: less than two hours after the onset of stress H3K9me3 levels had doubled and the change persisted for at least 24 h. Until recently, methyl marks, particularly constitutive heterochromatic marks like H3K9me3, were thought to be stable after cell fate was determined (Kubicek and Jenuwein, 2004). Similar observations have been made in the nucleus accumbens after cocaine administration (Maze et al., 2010). This change suggests that heterochromatin formation is much more dynamic than previously thought, and may play a role in the response of the brain to stress. More recent work suggests that this

Dentate Gyrus H3K9me3 Immunoreactivty
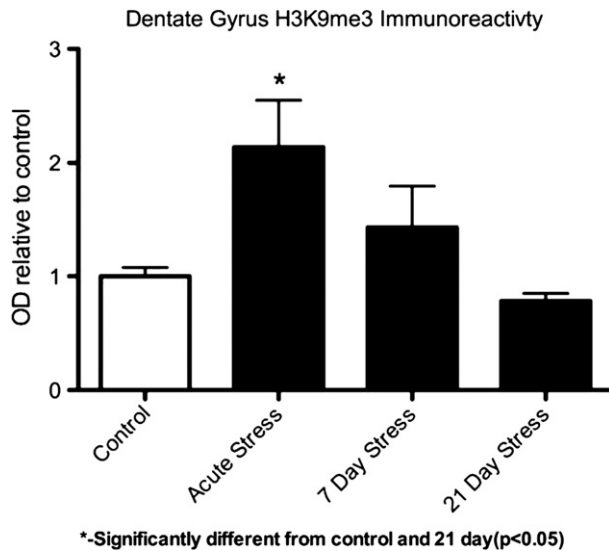
Fig. 2. Changes in dentate gyrus histone H3K9 trimethylation after acute immobilization stress as well as 7 and 21 day repeated immobilization stress. Acute stress induces a 2-fold increase in H3K9me3 levels. Adapted from Hunter et al. (2009b).

may constitute a genomic stress response, aimed at the protection of the genomic and transcriptomic stability required for the function of long lived, post-mitotic neurons via the control of "junk" transposable DNA elements (Hunter et al. unpublished data).

6. Individual differences in anxiety and their possible implications and origins

Whereas the amygdala and hippocampus have each provided challenging new information about the role of multiple mediators in acute and chronic stress effects upon neuronal structure and function, the prefrontal cortex has provided its own challenges when it comes to individual differences in anxiety related behavior. It is clear that the quite homogenous populations of laboratory rats show individual differences in anxiety related behaviors that can also influence lifespan, with more anxious rats having shorter lifespans (Cavigelli and McClintock, 2003; Cavigelli et al., 2006).

There is evidence pointing to a genetic component to these individual differences, as a number of rodent strains have been developed by crossbreeding individuals with particular anxiety phenotypes, such as novelty seeking high and low responders (Kabbaj et al., 2000), high and low fear conditioners (Bush et al., 2007), and the Fischer and Lewis rat strains. Behavioral differences in these strains correspond with chemical, electrophysiological and structural differences in brain regions involved in the stress circuit.

Rats that were chosen based on their anxiety and locomotor response to novelty (Dellu et al., 1996), have differences affecting the entire brain. High responders to novelty (i.e. those that have high locomotion and low freezing behavior in a novel environment) have increased expression of dopamine in the nucleus accumbens and decreased expression in the mPFC compared to low responders, as well as lower serotonergic expression overall (Piazza et al., 1991). High responders also have a larger and longer lasting dopamine response to stress, which is dependent on corticosterone release (Rouge-Pont et al., 1998). Yet high responders also have lower baseline and glucocorticoid receptor expression in the hippocampus, which appears to directly modulate novelty-induced exploratory behavior (Kabbaj et al., 2000). Furthermore, rats selected on the basis of their susceptibility or resistance to social

defeat show differences in HPA reactivity in which differences in CRF actions are implicated (Wood et al., 2010).

Specifically in the prefrontal cortex, rats bred for high anxiety also showed lower baseline activity and reduced tissue oxygenation, which corresponded to reduced post-stress mPFC activity compared to those bred for low anxiety (Kalisch et al., 2004). Furthermore, high anxiety rats demonstrated impaired extinction learning and lower c-fos expression after extinction in both the mPFC and lateral amygdala (Muigg et al., 2008). This is consistent with what has been found in our lab in the general rodent populations. Large cohorts of Sprague–Dawley and the more anxious Lewis strain were screened for baseline anxiety behavior. Those individuals that fell one standard deviation above and below the mean were grouped into high anxiety and low anxiety groups. In both strains, individuals that exhibited the highest amount of basal anxiety-like behavior in the open field had shortest apical dendrites in layer II/III pyramidal neurons of the prefrontal cortex compared to their low anxiety counterparts (M. Miller, J.H. Morrison, B. McEwen, unpublished).

Individual variability in basal anxiety and locomotor behaviors has also been shown to correlate with serotonin levels (Antoniou et al., 2008; Schwarting et al., 1998), corticosteroid receptor concentrations (Herrero et al., 2006), CART peptide expression (Miller et al., unpublished), and cytokine expression (Pawlak et al., 2003). A better understanding of how these different systems interact to create behavioral variation may help to elucidate why some individuals develop anxiety and mood disorders while others appear to be resistant.

7. Developmental effects on stress responsiveness and behavior

A contributing factor to individual differences in anxiety-related behaviors may be early life experiences that effect development of key brain structures. The quality and quantity of maternal care determines anxiety profiles via epigenetic mechanisms (Meaney and Szyf, 2005) that can be transmitted across generations (Francis et al., 1999). The consistency of maternal care is also important and exposure to novelty with good maternal care also improves cognitive and social development (Akers et al., 2008; Tang et al., 2006). Prenatal stress increases anxiety-like behaviors, but "postnatal handling" which elicits good maternal care can reverse those effects (Wakschlak and Weinstock, 1990). However, prenatal stress, without good postnatal maternal care, causes abnormalities in hippocampal development and behavior (Maccari and Morley-Fletcher, 2007).

Maternal separation, the separation of a litter of pups from the dam for 3 h daily during the first two weeks of life (Plotsky and Meaney, 1993), has long lasting effects on neural circuitry, cognition and behavior. Specifically, in adult rats exposed to maternal separation (MS rats), we have demonstrated baseline, as well as, stress-induced alterations in neuroanatomy, cognition, and emotionality (Eiland and McEwen). Given the importance of the hippocampus in stress responsiveness, we have focused on early life stress associated changes in hippocampal anatomy and hippocampal associated functions.

In regard to neuroanatomy, adult MS rats exhibit a baseline deficiency in apical dendritic length in CA3 region pyramidal neurons. This deficiency is evident distal to the cell soma, at distances of 390–480 μm. Further, this deficiency in apical dendritic length seems to impact the capacity for stress-induced dendritic remodeling. Specifically, MS rats exposed to 3 weeks of chronic restraint stress (CRS) do not exhibit significant apical dendritic remodeling, whereas normally reared rats exhibit significant shortening of apical dendrites with chronic stress exposure. A similar deficiency in CA3 apical dendritic length that is

also coupled with failed stress-induced dendritic remodeling is seen in brain derived neurotrophic factor (BDNF) haploinsufficient mice (Magarinos et al., 2011). Perhaps this similarity between MS rats and BDNF haploinsufficient mice stems from the reduced hippocampal levels of BDNF mature protein and mRNA exhibited in adult MS rats (Aisa et al., 2009; Lippmann et al., 2007). Future investigations examining the contribution of BDNF and other factors to the deficiency of CA3 apical dendritic material in MS rats, as well as the potential functional correlates of this deficiency, are needed. Such investigations may help clarify whether stress-induced dendritic remodeling is a protective mechanism or a process that increases vulnerability of pyramidal neurons.

Coincident with neuroanatomical changes in the MS rat are alterations in cognition and emotionality. Adult MS rats exhibit deficiency in hippocampal dependent spatial memory (Aisa et al., 2009, 2007; Cannizzaro et al., 2007; Huot et al., 2002; Uysal et al., 2005). We specifically used the object placement task to demonstrate longstanding baseline impairment of spatial memory in adult MS rats. Of note, we have demonstrated that this baseline impairment in spatial memory emerges early in development, as prepubescent MS rats (postnatal day 30) exhibit significant impairment (Eiland, unpublished data).

In regard to emotionality, we have demonstrated that early life stress exposure potentiates stress-induced anxiety. Using the elevated plus maze and open field, we do not find baseline differences between normally reared and MS rats. When MS rats are exposed to chronic restraint stress and given a 24-hour period of recovery, MS rats in the elevated plus maze exhibit significantly decreased open arm entry in comparison to normally reared CRS-exposed rats (Eiland and McEwen, in press). See Fig. 3. There is a significant interaction between early life stress and subsequent adult chronic stress in producing anxiety-like behavior. Similarly, MS rats that are exposed to an acute stress, such as forced swim demonstrate altered emotionality. During forced swim, MS rats demonstrate depressive-like behavior, as they exhibit significantly decreased latency to immobility in comparison to normally reared rats. Together these early life stress induced alterations in emotionality observed in the rat seem to parallel the increased vulnerability to depression and anxiety disorders observed in individuals with a history of early life adversity (Heim et al., 2008). Early life stress seems to program longstanding changes in hippocampal anatomy and plasticity that may in turn serve to modulate cognition and emotionality across the lifespan.
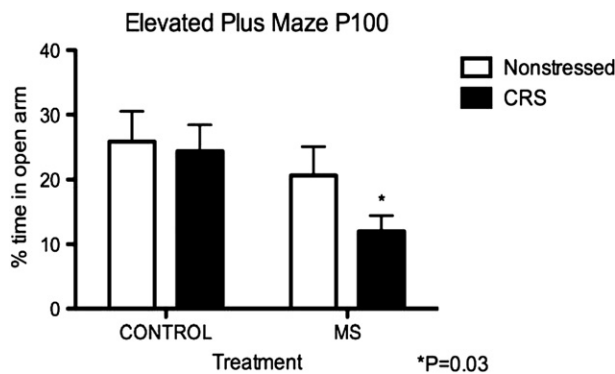
Fig. 3. Duration of time spent in open arms of elevated plus maze 24 h after termination of CRS. There was a significant effect of rearing on the percent time spent in the open arms of the EPM. MS-CRS spent significantly less time in the open arms than all other groups. NMS rats spent 25.98 ± 4.64%, NMS-CRS 24.44 ± 4.05%, MS 22.38 ± 4.58%, and MS-CRS 12.03 ± 2.40%. Two-way ANOVA rearing effect F1, 42 = 4.83, *P ≤ 0.03, n = 11–14 for all groups. From Eiland and McEwen (in press).

8. Interactions among brain regions

Although there has been a major emphasis on brain regions such as the hippocampus, amygdala and, more recently, the prefrontal cortex, that have led to many new insights into brain involvement in stress and adaptation to stress, as well as pathophysiology, it is important to further understand the network of interactions in the whole brain. The prefrontal cortex, amygdala and hippocampus are interconnected and influence each other via direct and indirect neural activity (Petrovich et al., 2001; Ghashghaei and Barbas, 2002; McDonald, 1987; McDonald et al., 1996), working together with the nucleus accumbens (Muschamp et al., 2011) with regard to fear and hedonic behavior and with the periaqueductal gray region of the midbrain with respect to autonomic regulation, pain sensitivity and anxiety (Huber et al., 2005; Panksepp et al., 1997; Panksepp and Watt, 2011). For example, the periaqueductal gray is a target of anxiolytic actions of benzodiazepines and works with the amygdala in mediating fear and anxiety (Graeff et al., 1993). Moreover, inactivation of the amygdala blocks stress-induced impairment of hippocampal LTP and spatial memory (Kim et al., 2005) and stimulation of basolateral amygdala enhances dentate gyrus field potentials (Ikegaya et al., 1995), while stimulation of medial prefrontal cortex decreases responsiveness of central amygdala output neurons (Quirk et al., 2003). The processing of emotional memories with contextual information requires amygdala – hippocampal interactions (Phillips and LeDoux, 1992; Richardson et al., 2004), whereas the prefrontal cortex, with its powerful influence on amygdala activity (Quirk et al., 2003), plays an important role in fear extinction (Milad and Quirk, 2002; Morgan and LeDoux, 1995). Because of these interactions, future studies need to address their possible role in the morphological and functional changes produced by single and repeated stress.

9. Conclusion: stress and brain plasticity over the life course

The brain responds to experiences with structural, as well as functional plasticity, and hormones play a role, along with neurotransmitters and other mediators via a complex collaborative network. Multiple brain regions are involved, including the amygdala, hippocampus, prefrontal cortex and nucleus accumbens; this list of brain regions needs to be expanded to include other parts of the brain, such as the periaqueductal gray matter and other components of the "separation distress circuit" (Northoff and Panksepp, 2008).

Mediators of the plastic changes in the brain include glucocorticoids, excitatory and inhibitory neurotransmitters, neurotrophic factors and inter-cellular signaling molecules, including endocannabinoids. These mediators operate in a non-linear network in which there are biphasic actions of each mediator system and reciprocal interactions that have self-limiting (homeostatic) features that are also subject to disruption that may lead to pathophysiology (McEwen, 1998, 2006).

Stressful experiences early in life can have long-lasting effects on brain development and the capacity to respond to later stressful challenges. Because of the central role of the brain in systemic physiology through its regulation of neuroendocrine, autonomic, metabolic and immune system function, adverse experiences in early life can have far-reaching effects for adult systemic and behavioral pathophysiology (Anda et al., 2010). Individual differences that are evident in human beings and also in relatively homogeneous rodent populations in the laboratory (Cavigelli and McClintock, 2003; Cavigelli et al., 2006) are manifestations of early life experiences and genetic and epigenetic factors that are just beginning to be recognized.

These individual differences, along with the capacity of the brain for structural and functional plasticity, argue for a different way of understanding and using pharmacological agents in treatment of disorders as diverse as depression and stroke, namely, where necessary, to enhance the capacity for plasticity in combination with individualized therapies that recognize each individual's unique needs (Kirkengen, 2010), including the differences between men and women (McEwen and Lasley, 2005).

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