Stress as a one-armed bandit: Differential effects of stress paradigms on the morphology, neurochemistry and behavior in the rodent amygdala

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1. Introduction

Neuroplasticity may be defined as the ability of the central nervous system (CNS) to respond to changes in the internal and external environment and it is well established that some stimuli have the ability to facilitate or impair neuroplasticity depending on the pre-existing milieu. A classic example of a stimulus that can both facilitate and impair neuroplasticity is stress. Indeed, the ability of CNS to respond to acute stress is often dependent upon the prior stress history of the individual. While responses to acute stress are often viewed as adaptive in nature, stress reactivity in subjects with prior chronic stress experiences are often linked to neuropsychiatric disorders, including major depressive disorder, post-traumatic stress disorder (PTSD) and anxiety. In rodent studies, chronic stress exposure produces structural and functional alterations in the hippocampus and medial prefrontal cortex that are consistent across different types of stress paradigms. Conversely, the amygdala appears to exhibit differential structural and functional responses to stress that are dependent on a variety of factors, including the type of stressor performed and the duration of the stress paradigm. This is most evident in output measures including morphological analysis of amygdala neurons, measurement of glutamatergic tone in amygdalar subdivisions and the analysis of amygdala-centric behaviors. Accordingly, this review will provide an overview of the effects of stress on the structural and functional plasticity of the rodent amygdala, especially in relation to the differential effects of repeated or chronic stress paradigms on dendritic architecture, neurochemistry of the glutamatergic system and behavior.
critical epicenters for neuropsychiatric disorders. Closer examination of these studies suggests that different stress paradigms generally produce similar effects on neuroplasticity endpoint measures in the hippocampus and mPFC. However, the amygdala appears to exhibit differential responses to stress that are dependent on the type of stressor performed and the duration of the stress paradigm. This includes analysis of differential effects of stress on amygdalar neuroanatomy, neurochemistry and amygdalar-dependent behaviors. In view of these observations, the aim of the current review is to provide a comprehensive analysis of the differential effects of stress on structural and functional plasticity of the rodent amygdala with a focus on glutamatergic systems, and provide some potential implications for neuropsychiatric disease states in humans.

2. Stress-dependent effects on amygdalar morphology

2.1. Brief overview of amygdalar anatomy

The amygdala consists of multiple nuclei, each with unique cytoarchitectonic, chemoarchitectonic and connectional characteristics. Although an exhaustive description of the functions and connections of each of these nuclei is beyond the scope of this review, in broad terms the amygdalar nuclei can be grouped into two major macrostructures. First is the corticobasolateral nuclei, whose cell types resemble those of the cerebral cortex, with glutamatergic, calcium-calmodulin dependent (CaM) kinase-positive principal neurons (McDonald, 1996). The activity of these neurons is coordinated, synchronized, and otherwise modulated by a variety of different inhibitory, GABAAergic-interneurons, which can be classified according to their expression of various calcium-binding proteins as well as by morphological and connectional features. The second major amygdalar macrostructure is the centromedial complex, whose cell types resemble those of the striatopallidal region. Our studies on the anatomical and neurochemical correlates of stress in the amygdala have focused on the basolateral complex (BLA) and central nucleus (CeA). In addition to serving as representative nuclei of the corticobasolateral and centromedial macrostructures, respectively, the BLA and CeA form the major input and output systems of the amygdala and thus are likely to be sites of stress-related changes in amygdalar function. For example, the BLA receives sensory information from various cortical and thalamic regions, and in turn sends projections to other amygdalar nuclei, as well as to several extrinsic structures such as the hippocampus, prefrontal cortex, basal forebrain, hypothalamus, and brainstem (Jolkkonen et al., 2002; McDonald and Culberson, 1986; McDonald et al., 1999 Pikkarainen and Pitkanen, 2001; Sarter and Markowitsch, 1984). This vast connectivity allows the BLA to play a critical role in pairing conditioned stimuli (CS) and unconditioned stimuli (US) during learned fear (Sigurdsson et al., 2007), as well as mediate and influence multiple behavioral responses. The CeA, on the other hand, receives information from multiple extrinsic sources such as the thalamus, hypothalamus, midbrain, brainstem, ventral tegmental area, insular cortex, dorsal raphe, entorhinal cortex and perirhinal cortex (McDonald et al., 1999; McDonald, 1998; Sun and Cassell, 1993; Sun et al., 1994). While many of these projections are reciprocal, a significant proportion of CeA efferents (particularly those from its medial subdivision) target hypothalamic and brainstem centers (Cassell et al., 1999; LeDoux et al., 1988; Veenema et al., 1984). The CeA also has robust interconnections with the principal extramygdaloid component of the “extended amygdala” (Cassell et al., 1999), the bed nucleus of the stria terminalis, which is also heavily implicated in behavioral and endocrine responses to threats or other stressors (Fox et al., 2015; Walker et al., 2009). Collectively, this pattern of connectivity allows the amygdala to play a central role in coordinating autonomic, endocrine and behavioral responses to conditioned and unconditioned sensory cues with emotional valence. Thus, the BLA receives polymodal sensory information from the cortex and thalamus and conveys this information directly or indirectly (e.g. via the intercalated nuclei) to the CeA, which mediates amygdalar influence over autonomic, endocrine and behavioral functions via projections to the hypothalamus and brainstem.

2.2. Stress paradigms and amygdalar morphology

Because of the central role of the amygdala in stress responses (Herman and Cullinan, 1997; Van de Kar and Blair, 1999), this region may contribute directly to some of the specific behavioral deficits induced by stress, and may also be the site of initiation of changes in other brain regions, including the hippocampus and mPFC. In other words, the amygdala region may play a central role in integrating the well-established connection between stress and psychopathology. Stress influences the structure and function of the amygdala and clinical investigations suggest that neuroanatomical changes in the amygdala precede those that are observed in the hippocampus of patients with mood disorders, in particular depressive illness patients (McEwen, 2003). Pre-clinical studies also indicate that stress can alter the structural and functional plasticity of the rodent amygdala. In this regard, seminal observations by Chattarji and coworkers determined that a 10 day immobilization stress paradigm elicited hypertrophy of pyramidal neurons in the rat BLA. Specifically, chronic immobilization stress (CIS) increased total dendritic length and number of branch points of BLA pyramidal neurons when compared to non-stressed control rats (Vyas et al., 2002). These results represent an interesting and important departure from prior neuroanatomical analyses, which have demonstrated that exposure to repeated stress elicits atrophy of principal neurons in the rat hippocampus (Conrad et al., 1999; Watanabe et al., 1992; Magarinos et al., 1997; McLaughlin et al., 2007) and mPFC (Cook and Wellman, 2004; Holmes and Wellman, 2009; Radley et al., 2004; Liston et al., 2006). Similarly, while stress-induced dendritic atrophy is a reversible process in the hippocampus (Luine et al., 1994) and mPFC (Radley et al., 2005), subsequent studies from the Chattarji group determined dendritic hypertrophy elicited by CIS may represent a more permanent morphological alteration (Vyas et al., 2004). CIS also increases spine density in BLA pyramidal neurons, an effect not observed immediately following a single acute immobilization stress session. However, increases in spine density in the absence of dendritic changes were observed 10 days following exposure to a single immobilization stress session (Mitra et al., 2005a). Another important observation from these studies was that unlike the morphological changes induced by CIS, chronic unpredictable stress (CUS) did not affect the morphology of BLA pyramidal neurons but did induce dendritic atrophy of bipolar neurons in the rat BLA (Vyas et al., 2002). As will be detailed below, these initial observations suggested that morphological changes in the rat BLA may be stressor and cell-type specific. More importantly, the outcomes from different stress models provide important insight into the heterogeneous findings from clinical studies that have examined amygdalar volumes in patients with major depressive illness.

2.3. Heterogeneity of stress-induced morphological changes in the rodent amygdala

The studies described above from Chattarji and coworkers served as the genesis for subsequent studies that examined the
effects of stress paradigms of different duration and type. Many of these studies determined that repeated stress paradigms elicited hypertrophy of BLA pyramidal neurons. For example, similar to the 21-day paradigm, an immobilization stress paradigm spanning 21 days also produced dendritic hypertrophy of BLA pyramidal neurons, as well as increases in spine density that were accompanied by anxiety-like behaviors (Vyas et al., 2006). Similar to observations in rats, 21 days of restraint stress (Hill et al., 2013) or 10 days of immobilization stress (Qin et al., 2011) resulted in an increase in total dendritic length and increased spine density of BLA pyramidal neurons in wild-type mice, morphological alterations that were associated with anxiety-like behaviors (Qin et al., 2011). More discrete analysis of dendritic morphology within subregions of the BLA determined that intermittent restraint stress induced dendritic hypertrophy in the basal nucleus, but did not impact the dendritic length of pyramidal neurons in the lateral nucleus (Padival et al., 2013). Interestingly, this stress paradigm increased spine density in both subregions of the BLA. Prolonged isolation stress beginning on postnatal day (PND) 28 also increased the complexity of BLA pyramidal neurons without affecting total dendritic length (Wang et al., 2012). In addition to these repeated and chronic stress paradigms, analysis of dendritic morphology following exposure to a single prolonged stress paradigm followed by a 7 day recovery period identified increases in total dendritic length and branch points of BLA pyramidal neurons in the absence of changes in these parameters in the CeA (Cui et al., 2008). These results supported previous findings demonstrating that stress did not impact CeA dendritic morphology (Vyas et al., 2003). Collectively, the results from these studies indicate that a number of different stress paradigms elicits dendritic hypertrophy and increases in spine density of BLA pyramidal neurons that are accompanied by anxiety-like behaviors (see Table 1).

While these studies above reported that stress paradigms elicit dendritic hypertrophy of BLA neurons, other studies have yielded different stress-induced morphological consequences. For example, adult mice exposed to a chronic restraint stress (CRS) paradigm exhibited dendritic atrophy of interneurons in the lateral and basolateral amygdala (Glibert-Juan et al., 2011). Recent studies from our group indicate that repeated restraint stress (i.e. 10 days; RRS) also produces atrophy of BLA pyramidal neurons (Grillo et al., 2015), while Wellman and coworkers reported that a single exposure to platform stress induced dendritic retraction (decreased branch points and total dendritic length) of BLA pyramidal neurons (Maroun et al., 2013). Such results suggest that while stress more consistently elicits dendritic atrophy in the hippocampus and mPFC irrespective of the duration or type of stress paradigm that is employed, the morphological consequences to stress in the rodent amygdala appear to be more heterogeneous; see Fig. 1.

It is interesting to note that although anxiety-like behaviors were observed in rats exposed to CUS, this stress paradigm did not modulate total dendritic length or spine density of BLA pyramidal neurons (Pego et al., 2008). As will be described below, these results suggest that stress-induced behavioral outcomes (i.e. anxiety-like behaviors) may not always reflect stress-induced morphological parameters in the rodent BLA. In support of this hypothesis, rats selected for a ‘well-adapted’ phenotype in response to predator stress exhibited dendritic atrophy (total dendritic length and dendritic extent) when compared to ‘mal-adapted’ rats and handled controls (Mitra et al., 2009). In addition, some studies suggest that stress-induced morphological changes are reversible. In this regard, increases in spine density and hypertrophy of BLA pyramidal neurons induced by fear (cue) conditioning were reversed with extinction training (Henrichs et al., 2013). Such results suggest that like the heterogeneity of stress-induced morphological changes, stress associated with different behavioral paradigms influences morphological plasticity in the rodent amygdala.

2.4. Age-dependent effects of stress on morphological parameters

Studies that have examined the effects of stress on BLA morphology at different developmental time points have also yielded disparate findings. For example, while maternal...
deprivation stress did not affect BLA pyramidal cell morphology in male and female rats (Krugers et al., 2012), juvenile male and female rats exhibited dendritic hypertrophy and increased anxiety-like behaviors in response to a chronic restraint stress paradigm (Eiland et al., 2012). A more recent study that examined the effects of chronic stress in juvenile and adult rats provides some insight into these disparate findings. In this regard, juvenile rats exposed to 5 weeks of instability stress (daily immobilization stress combined with changing of cage mates) elicited atrophy of BLA pyramidal neurons, decreases in spine density and impairments in fear-potentiated startle. Conversely, this same instability stress paradigm elicited dendritic hypertrophy, increases in spine density and enhanced fear-potentiated startle in adult male rats (Tsai et al., 2014). These results suggest that in addition to the factors described above (i.e. type of stress, duration of stress, cell-type examined), that age may also be an important factor that influences stress-induced morphological changes in the rodent amygdala.

### 2.5. Stress-induced morphological changes: role of corticosterone

While not entirely recapitulating the experience of stress, administration of stress levels of corticosterone (CORT) provides an approach to more selectively examine the role of CORT in stress-induced morphological changes in the rodent brain. Unlike the CORT-induced morphological changes observed in hippocampus (Wooley et al., 1980) and mPPC (Wellman, 2001), chronic exposure to stress levels of CORT does not modulate morphological parameters in BLA pyramidal neurons, including total dendritic length, spine density, neuronal density or amygdalar volume (Pego et al., 2011; Vouimba et al., 2004, 2006). However, studies by Mitra and Sapolsky determined that a single exposure to stress levels of CORT elicited dendritic hypertrophy when morphological analyses were preformed 12 days later (Mitra and Sapolsky, 2008). More recent studies replicated these findings and also demonstrated that acute CORT-induced hypertrophy of BLA pyramidal neurons was no longer evident 20 days post CORT treatment (Kim et al., 2014). Collectively, these studies once again demonstrate that the timing and/or duration of CORT exposure are important factors that determine/influence stress-induced morphological changes in the rodent BLA. Another important observation from these studies is that under certain conditions morphological changes in the rodent BLA are reversible. Such results suggest that like the heterogeneity of stress-induced morphological changes, experimental conditions and endocrine status may also influence whether stress-induced morphological plasticity is a reversible process in the rodent amygdala.

### 2.6. The neuropharmacology of stress-induced morphological changes in the rodent BLA

In spite of these potential stress-specific responses, one consistent observation is that pharmacological interventions used in the treatment of mood disorders effectively inhibit stress-induced morphological changes in the rat amygdala. In this regard, Young and coworkers reported that the mood-stabilizer lithium effectively prevented hypertrophy of BLA pyramidal neurons elicited by CRS (Johnson et al., 2009). More recently, Chattarji and coworkers reported that the antidepressant tianeptine also prevented CORT-induced dendritic hypertrophy of BLA pyramidal neurons (Pillai et al., 2012). This same study also reported that tianeptine modulated NMDA receptor-mediated currents in the lateral amygdala, thereby providing additional evidence to support the hypothesis that the mechanism of action of tianeptine involves normalization of glutamatergic tone in the rat amygdala (Pirroli et al., 2013; Reznikov et al., 2007; Vouimba et al., 2004, 2006).

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| Paradigm          | Duration | Species          | Sex   | Dendritic morphology                                                                 | Reference                  |
|-------------------|----------|------------------|-------|-------------------------------------------------------------------------------------|----------------------------|
| CIS               | 10 days  | Adult Wistar rats| Male  | Hypertrophy of pyramidal neurons                                                    | Vyas et al., 2002          |
| CUS               | 10 days  | Adult Wistar rats| Male  | Hypertrophy of bipolar neurons                                                      | Mitra and Sapolsky, 2008  |
| CIS               | 21 days  | Adult Wistar rats| Male  | Hypertrophy of pyramidal neurons                                                    | Vyas et al., 2006          |
| CIS               | 10 days  | Adult Wistar rats| Male  | Hypertrophy of pyramidal neurons; inhibited by tianeptine                           | Pillai et al., 2012        |
| Restraint         | 21 days  | Adult c57/Bl6 mice| Male  | Hypertrophy of pyramidal neurons                                                    | Hill et al., 2013          |
| CIS               | 10 days  | Adult c57/Bl6 mice| Male  | Hypertrophy of pyramidal neurons; inhibited by lithium                              | Qin et al., 2011           |
| Restraint         | 21 days  | Adult SD rats    | Male  | Hypertrophy of pyramidal neurons                                                    | Johnson et al., 2009       |
| Restraint         | Intermittent | Adult SD rats  | Male  | Hypertrophy of pyramidal neurons                                                   | Padival et al., 2013       |
| SPS + recovery    | 1 day    | Adult SD rats    | Male  | Hypertrophy of pyramidal neurons                                                   | Cui et al., 2008           |
| Isolation stress  | 8 weeks  | PND 28 Wistar rats| Male  | Increased complexity of BLA pyramidal neurons; no change in dendritic length        | Wang et al., 2012          |
| Cort Rx           | 1 day; 10 day delay | Adult Wistar rats| Male  | Hypertrophy of pyramidal neurons                                                   | Mitia and Sapolsky, 2008  |
| Cort Rx           | 1 day; 10 day delay | Adult SD rats  | Male  | Hypertrophy of pyramidal neurons                                                   | Kim et al., 2014           |
| SIS               | 5 weeks  | Adult SD rats    | Male  | Hypertrophy of pyramidal neurons                                                    | Tasi et al., 2014          |
| Restraint stress  | 21 days  | Juvenile SD rats | Male & female | Hypertrophy of pyramidal neurons                                                | Eiland et al., 2012       |
| Restraint stress  | 21 days  | Adult SD rats    | Male & female | Hypertrophy of pyramidal neurons                                                  | Eiland et al., 2012       |
| CIS               | 21 days  | GIN mice         | Male  | Atrophy of BLA interneurons                                                        | Gilbert-Juan et al., 2011 |
| Platform stress   | 1 day    | Adult SD rats    | Male  | Atrophy of BLA pyramidal neurons                                                   | Maroun, 2013              |
| SIS               | 5 weeks  | Adolescent SD rats| Male  | Atrophy of BLA pyramidal neurons                                                   | Tsai et al., 2014          |
| Restraint         | 10 days  | Adult SD rats    | Male  | Atrophy of BLA pyramidal neurons; inhibited by agomelatine                          | Grillo et al., 2015        |
| CIS               | 10 days  | Adult Wistar rats| Male  | No change in BLA morphology                                                        | Vyas et al., 2002          |
| CIS               | 28 days  | Adult Wistar rats| Male  | No change in BLA morphology                                                        | Pego et al., 2008          |
| Cort Rx           | 28 days  | Adult Wistar rats| Male  | No change in BLA morphology                                                        | Pego et al., 2008          |
| Cort Rx           | 21 days  | Adult SD rats    | Male  | No change in BLA morphology                                                        | Morales-Medina et al., 2009|
| Cort Rx           | 10 days  | Adult Wistar rats| Male  | No change in BLA morphology                                                        | Mitia and Sapolsky, 2008  |
| Maternal deprivation | 24 h    | Adult Wistar rats| Male & female | No change in BLA morphology                                                   | Krugers et al., 2012       |
| Predator stress   | 10 min   | Adult Long-Evans rats| Male & female | No change in BLA morphology                                                   | Adamac et al., 2012        |

Abbreviations. CIS: chronic immobilization stress. CUS: Chronic unpredictable stress. SPS: single prolonged stress. CORT Rx: corticosterone administration. SIS: social instability stress. SD: Sprague Dawley. PND: post-natal day.
McEwen et al., 2010). More recently, we reported that the antidepressant agomelatine, a melatonergic receptor agonist and 5HT2C receptor antagonist, effectively prevents repeated restraint stress-induced dendritic atrophy of BLA pyramidal neurons (Grillo et al., 2015).

Taken together these studies suggest that different types of stressors can induce various changes in morphological characteristics of BLA neurons that are reversible, and differ from those seen in other brain areas such as the hippocampus and prefrontal cortex. A challenge for the field is to determine how (or if) these changes in different brain areas are linked and if it is a change in the cortical-hippocampal-amygdalar circuit that underlies the behavioral shifts described below. Understanding the stress-related endocrine and neurotransmitter changes that drive the morphological changes in BLA, and how they differ from the mediators in hippocampus and prefrontal cortex, will require systematic and parametric studies.

The neural mechanisms that induce stress-related anatomical changes to revert to a normal state remain to be elucidated, although studies with various antidepressant agents continue to offer some intriguing clues. Such studies also implicate a critical role for the glutamatergic system in mediating stress-induced changes in the amygdala.

3. Glutamatergic correlates of stress in the amygdala

Glutamate is the primary fast-acting excitatory neurotransmitter in the mammalian brain, including the amygdalar complex. Thus, glutamate, along with its fast-acting inhibitory amino acid counterpart, GABA, mediates the basics of synaptic neurotransmission on which the numerous amygdalar neuromodulatory systems—including dopamine, serotonin, norepinephrine and numerous neuropeptides—exert their influence. Principal cells of the BLA are glutamatergic, bear morphological resemblance to cortical pyramidal neurons, and are immunohistochemically detectable by labeling for the enzyme CaM kinase (McDonald et al., 2002). BLA neurons also serve as a significant source of neuronal glutamate in other amygdalar nuclei, such as the CeA (Pitkanen et al., 1997), and extra-amygdalar nuclei that receive BLA inputs. Additional presumptive sources of neuronal glutamate in the amygdalar complex include afferents originating from the cortex, including prefrontal cortex and sensory areas, the thalamus and hypothalamus (LeDoux et al., 1990; McDonald et al., 1999; Turner and Herkenham, 1991). Stress has been shown to impact virtually all of these regions, and it is likely that stress-related increases in amygdalar glutamate reflect a summed neurochemical correlate of activity in these stress-responsive circuits. Finally, as in other brain regions, a substantial fraction of extracellular amygdalar glutamate is likely derived from non-vesicular and glial sources (Timmerman and Westerink, 1997). Nonetheless, even glutamate derived from non-exocytotically-mediated release may affect neurotransmission and alter behavioral correlates of glutamatergic function (Baker et al., 2003; 2002; Moran et al., 2003) and changes in extracellular glutamatergic tone are likely to play a role in the morphological, synaptic, and neuroendocrine changes associated with stress in the amygdala and other limbic regions (Gabrieli et al., 1995; Herman et al., 2003). Thus, regardless of the source, glutamatergic neurotransmission in the amygdala likely represents an important mediator of stress effects on this area and, in turn, may play a key role in facilitating amygdalar activation of the HPA axis (Gabrieli et al., 1995). The important role played by glutamate in both acute rapid neurotransmission, as well as processes related to long-term synaptic plasticity, suggests that the dynamics of extracellular glutamate may yield key insights into the mechanisms underlying the effects of stress on amygdalar function. We and others have investigated the role of amygdalar glutamate in stress responses using microdialysis and other approaches amenable to in vivo analysis in awake animals.

3.1. Acute stress effects on BLA glutamate

Several lines of evidence indicate that acute stress increases release of glutamate within the BLA. Using in vivo microdialysis in awake rats, we have demonstrated that a one-hour restraint stress in previously stress-naïve animals produces robust increases in BLA glutamate levels (Reagan et al., 2012; Reznikov et al., 2007). Several features of the BLA glutamate response to acute stress are worth noting: First, the response is rapid—peaking within the first 15 min stress collection—but transient, returning to baseline levels by the second 15 min interval of the restraint period. Secondly, while basal extracellular glutamate measured by microdialysis in the BLA appears largely axon depolarization-independent (Timmerman and Westerink, 1997), stress-elicited increases in glutamate are completely abolished by the voltage-gated sodium channel blocker, tetrodotoxin (TTX). Finally, of therapeutic significance, acute stress-elicited glutamate release in the BLA is prevented by acute pretreatment with some (e.g., tianeptine and agomelatine) but not all (e.g., fluoxetine) antidepressant drugs (Reagan et al., 2012; Reznikov et al., 2007). Indeed, acute fluoxetine increases glutamate efflux in the BLA in the pre-stress period and prolongs the duration of the stress-elicited glutamate release, which may provide a potential mechanism through which initiation of selective serotonin reuptake inhibitor (SSRI) treatment elicits anxiety in depressive illness patients (Goldstein and Goodnick, 1998).

The effects of acute stress on BLA glutamate do not appear to be specific to restraint stress as BLA glutamate is also increased by...
found that acute noise stress signiﬁcantly elevated glutamate release in the BLA of spontaneously hypertensive rats. Interestingly, this effect, which was TTX-sensitive, was not observed in Wistar-Kyoto rats even though the two strains did not differ in basal or veratridine-elicited glutamate release, suggesting a true species difference in stress sensitivity. Recent work in mouse models further supports the idea that glutamatergic neurotransmission not only reﬂects strain differences in stress responsiveness, but may actually mediate these differences (Mozhui et al., 2010).

3.2. Acute stress effects on CeA glutamate

Although fewer studies have investigated the effects of acute stress alone on glutamate in the CeA, we have shown that the initial response in this region roughly parallels that seen in the BLA. That is, rats experiencing their ﬁrst episode of acute stress exhibit a rapid, signiﬁcant and relatively short-lasting increase in CeA glutamate which is TTX-sensitive and prevented by the antidepressants tianeptine or agomelatine (Reagan et al., 2012; Reznikov et al., 2007).

While there have been few investigations on the acute effects of other (non-restraint) stressors on CeA glutamate, there is evidence that glutamate in this region is modulated by several neuromodulatory peptides which, themselves, are considered to play important roles in stress responses. For example, both corticotropin-releasing factor (CRF) (Beckerman et al., 2013) and endocannabinoids (Ramikie et al., 2014) have been hypothesized to regulate CeA glutamatergic transmission in the context of stress responses. Skorzewska and colleagues showed that i.c.v. CRF administration in animals exposed to novelty stress produced a robust increase (~200% relative to baseline) in CeA glutamate levels (Skrzewska et al., 2009). Bosch and colleagues (Bosch et al. 2007) showed an important role for oxytocin in differential regulation of CeA glutamate in rat dams selectively bred for high or low anxiety, whereas Ebner et al. (Ebner et al., 2005) demonstrated the importance of oxytocin in regulating swim stress-induced release of glutamate in the CeA. Finally, intravenous administration of the hypothalamic neuropeptide orexin A, which innervates the central amygdala and plays a crucial role in panic and stress responses (Johnson et al., 2012; Kuwaki, 2011; Berridge et al., 2010; Schmitt et al., 2012), has been shown by microdialysis to increase amygdala glutamate in a calcium-dependent manner (John et al., 2003).

One caveat with using in vivo microdialysis to measure extracellular glutamate responses in the CeA is the inability to differentiate the medial and lateral compartments of this nucleus in rodents due to the small size of these subnuclei and the relatively large diameter (approximately 400 μm) of conventional microdialysis probes. This is potentially important given the different patterns of connectivity—and putatively different functional roles in stress responses—of these divisions of the CeA (Pitkanen et al., 1997; Ciocchi et al., 2010). Nonetheless, the relatively few studies using static (e.g. c-Fos expression) or dynamic (e.g. electrophysiological recordings) approaches that allow for more discrete targeting fail to suggest qualitatively opposite responses of these regions to acute stress or other aversive stimuli. For example, c-Fos expression in both lateral and medial divisions of the CeA is elevated by renewal of conditioned fear or active threat responding (Knapka and Maren, 2009; Martinez et al., 2013). Thus, it is likely that the ability of acute stress to increase extracellular glutamate in the CeA as a whole holds signiﬁcant implications for behavioral and autonomic correlates of stress responses mediated by this nucleus.

3.3. Chronic stress effects on amygdalar glutamate

Studies utilizing in vivo neurochemical and behavioral approaches suggest that stress effects on extracellular glutamate in the amygdala are not just a neurotransmitter epiphenomenon, but may actually mediate functional consequences of chronic stress. As mentioned above, Mozhui and colleagues demonstrated an association between differences in trait- and stress-induced anxiety responses and measures of amygdalar glutamatergic neurotransmission in different strains of mice. Using in vivo microdialysis with elevated K+ to increase extracellular glutamate in rat amygdala (nucleus not speciﬁed), Fujikawa and colleagues showed that prolonged elevations in glutamate (~300% relative to baseline) increased markers of neuronal damage (Fujikawa et al., 1996). Consistent with the hypothesis that chronic elevations in extracellular glutamate in BLA can have deleterious effects, our own work suggests that repeated restraint-induced decreases in CeA glutamate may be the consequence of RRS-elicited atrophy in BLA pyramidal neurons (Grillo et al., 2015), which represent a signiﬁcant source of the neurotransmitter pool of glutamate in the CeA (Pitkanen et al., 1997). Thus, rats subjected to 10 days of RRS continued to show increased glutamate in the BLA in response to a subsequent acute stress challenge, although the magnitude of this increase tended to be smaller than in previously stress-naïve animals. However, these same animals showed marked reductions in extracellular glutamate, relative to animals experiencing their ﬁrst episode of restraint stress, in the CeA. Importantly, chronic treatment with the antidepressant drug agomelatine normalized both the enhanced glutamate response in the BLA and the decrease in extracellular glutamate seen with chronic stress in the CeA. These studies demonstrate a qualitatively different response in glutamate homeostasis in the CeA and BLA of repeatedly-stressed rats and—combined with our morphological and c-Fos expression data (Reznikov et al., 2008)—suggest that repeated stress-induced decreases in extracellular glutamate in the CeA may result from dendritic atrophy and decreased activation of BLA inputs to this region (See Fig. 2). Indeed, repeated prenatal stress has been shown to result in reductions in hippocampal glutamate release in rats examined as adults, and this effect was prevented by chronic antidepressant treatment with either agomelatine or ﬂuoxetine (Marrocco et al., 2014).

An additional mechanism which may underlie the effects of repeated stress on amygdalar glutamate includes alterations in glial and/or neuronal reuptake and vesicular transport. This hypothesis stems, in part, from clinical data showing alterations in in vivo measures of glutamate levels (Auer et al., 2000; Sanacora et al., 2003; Sanacora et al., 2004), as well as post-mortem decreases in glial cell density (Ongur et al., 1998; Rajkowska and Miguel-Hidalgo, 2007) in some limbic brain regions. In our preclinical RRS model, repeated stress is associated with both blunted initial glutamate release in response to an acute stressor as well as dramatic reductions in extracellular glutamate levels in the CeA during both the stress and post-stress periods (Piroli et al., 2012). These changes in extracellular glutamate were also associated with dramatic reductions in expression of the vesicular glutamate transporter-2 (vGlut2). Importantly, both the neurochemical and vesicular protein deﬁcits in markers of amygdalar glutamatergic...
neurotransmission in this study were prevented with daily administration of the antidepressant drug tianeptine (Piroli et al., 2013). Thus, restoration of capacity for glutamate release via increased vGlut2 expression may represent one mechanism by which some antidepressants restore glutamatergic neurotransmission and associated neuroplasticity in the amygdalar complex.

In summary, as shown in Table 2, the available data suggest that acute stress increases the neurotransmitter pool of extracellular glutamate in both central and basolateral divisions of the amygdala, but that these regions exhibit fundamentally different responses to repeated stress. Combined with morphological and neuronal activation studies, these data suggest the importance of glutamatergic neurotransmission in modulating the intra-amygdalar circuitry that contributes to behavioral and autonomic stress responses. These data further suggest the importance of amygdalar glutamate dynamics as a potential therapeutic neurochemical correlate of antidepressant treatment in animal models of stress. In the future, it will be interesting to directly examine the role of BLA inputs in repeated stress-induced changes in CeA neurochemistry, as well as to investigate how this phenomenon might be associated with a variety of other chronic stress paradigms. A challenge for the field will be to understand the relative role of glutamate release from glutamatergic inputs to specific amygdala regions, versus changes in extracellular glutamate levels induced by altered glial and/or neuronal reuptake or vesicular transport. Since these sources of extracellular glutamate flux will have distinct time courses, this could have interesting and complex effects on tripartite synapses. Stress related changes from different sources of glutamate might also be associated with specific types of stress and are likely to differ between acute and chronic stress paradigms.

4. Stress and amygdala-centric behaviors

Various stress paradigms have been used to examine anxiety and depression-like behaviors in rodent models, since many of these acute or chronic stress models are thought to model the

Fig. 2. Neurochemical and behavioral correlates of repeated restraint stress (RRS)-induced atrophy of pyramidal neurons in the rat basolateral amygdala. Unlike the effects of immobilization stress (Vyas et al., 2002), our previous studies indicate that RRS elicits atrophy of pyramidal neurons in the rat BLA when compared to non-stressed control rats (Control BLA pyramidal neuron shown in blue; BLA pyramidal neuron from RRS rat shown in red; [Grillo et al., 2015]). Since BLA pyramidal neurons extend their axons and axon collaterals to the central nucleus of the amygdala, a potential neurochemical consequence of RRS-induced atrophy of glutamatergic BLA neurons would be a decrease in glutamatergic tone in the central nucleus. In support of this hypothesis, unlike acute stress in a non-stress control (NSC) rat that elicits an increase in glutamate efflux in the central nucleus (Reznikov et al., 2007), rats with a prior RRS history exhibit decreases in glutamate efflux in the central nucleus in response to an acute stressor (Grillo et al., 2015; Piroli et al., 2013) Blue arrows: BLA glutamatergic projections to central nucleus in NSC rat; red arrow: reduced BLA glutamatergic projections to central nucleus in RRS rat. Ultimately, these neuroanatomical and neurochemical changes contribute to stress-induced changes in amygdalar-centric behaviors, as depicted by the ‘anxious’ rat wearing the red bowtie. See text for details.
4.1. Responses to novelty in the open field test

Rodent responses to novelty have been tested in a variety of paradigms including the open field test (OF), holeboard test, elevated plus maze (EPM), and light:dark box (LDB). Enhanced levels of anxiety in these tests include decrease time in the center portion of an open field, time or relative (percent) time in the open arms of the EPM, or time in the light portion of the LDB. Additional behaviors, such as rearing, grooming, approaches, and risk assessment behaviors, as well as activity measures, have been assessed during these tasks in some studies as well.

Overall, stress effects in the open field (OF) are variable, most likely related to the various factors noted above. For example, RRS has been shown to decrease center time in the OF, but is generally without effects in general locomotion, although the RRS in one of these studies was done in adolescent (21 day old) rats (Chiba et al., 2012; Negron-Oyarzo et al., 2014). In contrast, the number of line crossings was reduced after 21 days of restraint stress performed after the animals had been previously exposed to a conditioned cue (Conrad et al., 1999). Although acute immobilization stress decreased ambulation in the OF 24 h after the stress session, this effect was prevented by chronic immobilization stress (CIS) for 9 days prior to the OF test suggesting protection or habituation with repeated stress exposure (Pastor-Ciurana et al., 2014). This habituation postulate is supported by the reduction in struggling behaviors during CIS protocols (Pastor-Ciurana et al., 2014; Wood et al., 2008). In the same Pastor-Ciurana et al. (2014) study, CIS for 9 days also produced a decrease in OF ambulation, although other studies have failed to show changes in OF locomotion (distance) or center time after longer CIS protocols [28 days; (Ventura-Silva et al., 2013)]. This could be related to the duration of stress, or the inclusion of footshock in these CIS protocols. Indeed, 5 day paradigms of repeated footshock decreased locomotion in the OF for up to 10 days after the end of the stress (Pijlman and van Ree, 2002; Pijlman et al., 2002; Pijlman et al., 2003b). Chronic mild stress paradigms have also been shown to decrease central OF time (Hu et al., 2010). Predator-related stress (cat exposure) did not change locomotion in a related holeboard task (Adamec et al., 2012, 1999a, 1999b, 2005b; Blundell et al., 2005; Mitra et al., 2009), although one study demonstrated a decrease in center time following cat exposure (Adamec et al., 2012).

### Table 2

Effects of acute and repeated stressors on glutamate neurochemistry in the rat amygdala.

| Paradigm                          | Region | Glutamate neurochemistry            | Reference                          |
|-----------------------------------|--------|--------------------------------------|------------------------------------|
| Acute stress effects in BLA       | BLA    | Increased above baseline; inhibited  | Reznikov et al., 2007              |
|                                   |        | by tianeptine                        |                                    |
| Acute stress in naive rats        | BLA    | Increased above baseline; inhibited  | Reagan et al., 2012                |
|                                   |        | by agomelatine                       |                                    |
| Mixed stressor paradigms          | BLA    | Increased above baseline; inhibited  | Piroli et al., 2013                |
|                                   |        | by tianeptine                        |                                    |
| Acute stress in naive rats        | CeA    | Increased above baseline; inhibited  | Reznikov et al., 2007              |
|                                   |        | by agomelatine                       |                                    |
| CRF + novelty stress              | CeA    | Increased above baseline             | Szkretzewska et al., 2009          |
| Oxytocin in high- and low-anxiety dams | CeA | Increased basal in high-anxiety; oxytocin antagonist increased in low-anxiety | Bosch et al., 2007 |
| Oxytocin regulation of swim stress | CeA  | Swim stress increased above baseline; potentiated by oxytocin antagonist | Ehner et al., 2005 |
| Orexin A (hypocretin-1)           | CeA    | Increased above baseline             | John et al., 2003                  |
| Effects of repeated stress        |        |                                      |                                    |
| Repeated restraint stress + acute  | BLA    | No changed vis-à-vis baseline        | Pirol et al., 2013                 |
| stress                            |        |                                      |                                    |
| Repeated restraint stress + acute  | BLA    | No change vis-à-vis baseline         | Pirol et al., 2013                 |
| stress                            |        |                                      |                                    |
| Repeated restraint stress + acute  | CeA    | Decrease below baseline; inhibited    | Pirol et al., 2013                 |
| stress                            |        | by tianeptine                        |                                    |
| Repeated restraint stress + acute  | CeA    | Decrease below baseline; inhibited    | Pirol et al., 2013                 |
| stress                            |        | by agomelatine                       |                                    |

Abbreviations. BLA: basolateral nucleus of the amygdala. CeA: central nucleus of the amygdala. CRF: corticotrophin releasing factor.
immobilization followed by social instability for 21 days (Zoladz et al., 2008). The latter report suggested that the individual stressors in these paradigms were insufficient to induce changes in EPM behavior. The changes in EPM behaviors after CUS paradigms are more variable, with some investigators seeing decreases in open arm time following CUS (Bondi et al., 2008; Pego et al., 2008; Ventura-Silva et al., 2013), while others see no difference in EPM behaviors with this type of stress (Mitra et al., 2005b; Vyas et al., 2002).

Importantly, the effects of stress in these two tasks appear to be dependent upon both the sex and strain of the rodents. Unlike the changes observed in male rats, Mitra et al. reported that females fail dependent upon both the sex and strain of the rodents. Unlike the behaviors with this type of stress (Mitra et al., 2005b; Vyas et al., 2008; Ventura-Silva et al., 2013), while others see no difference in EPM behaviors with this type of stress (Mitra et al., 2005b; Vyas et al., 2002).

EPM behavior. The changes in EPM behaviors after CUS paradigms are somewhat confounded by baseline differences in these measures in the different mouse strains examined (Mozhui et al., 2010). In this regard, DBA/2J mice show decreased open arm time in the EPM and decreased time in the light compartment of the LDB after RRS, but C57BL/6J mice show an increase in these measures after stress (Masneuf et al., 2014; Mozhui et al., 2010). The increased time in the light compartment of the LDB in C57BL/6J mice after RRS was suggested to be a shift into a more active coping style (Masneuf et al., 2014).

4.3. Acoustic startle responses

Acoustic startle reflexes represent a conserved defensive reflex in response to a sudden, threatening stimulus. Since shifts in startle responses are seen in psychiatric disorders, including PTSD, many studies have examined stress-related changes in acoustic startle responses in rodent models. In general, different forms of repeated or traumatic stress paradigms sensitize the acoustic startle response, which is generally seen as an enhanced startle amplitude or delay in habituation of the acoustic startle response [for additional references see (Stam, 2007)]. Stressors that induce sensitized startle responses include repeated exposure to footshocks (Davis and Walker, 2013; Walker and Davis, 1997; Gewirtz et al., 1998), a single prolonged stress paradigm (Yamamoto et al., 2009), predator stress (Adamec et al., 2012; Blondell et al., 2005), predator stress coupled with immobilization and social instability (Zoladz et al., 2008), and CUS (Pego et al., 2008; Ventura-Silva et al., 2013). Although there are reports that baseline startle responses are decreased by repeated daily footshocks for five days (Pijlman et al., 2003a), this difference may be due to the time of startle testing following stress. Davis and Walker (2013) have reported that this shock-induced sensitization diminishes over time, so the decreased basal startle might have been due to a long (50 day) delay after stress prior to behavioral testing (Pijlman et al., 2003b). There are also some reports that predator stress does not enhance baseline startle responses (Mackenzie et al., 2010). Interestingly, repeated footshock stress has been shown to increase prepulse inhibition of acoustic startle [PPI (Pijlman et al., 2003a)], while neither psychological stress (observing other rats receiving footshock) or CUS altered PPI or fear potentiated startle responses (Pijlman et al., 2003a). Acoustic startle responses are also enhanced by testing in anxiogenic high light conditions (Davis and Walker, 2013; Walker and Davis, 1997).

4.4. Fear conditioning and extinction

In addition to stress- or fear-potentiated startle, studies have demonstrated stress effects on conditioning of other responses, including context or cue-conditioned freezing plus the extinction of this response during repeated cue presentation. There is considerable interest in assessing changes in fear conditioned responses, particularly extinction, given the relevance of such responses to disorders like PTSD. Several different stress paradigms either have been shown to enhance contextual- or cue-conditioned fear responses (primarily freezing) or reduce extinction of cue-conditioned fear. However, there are inconsistencies in the findings likely due to numerous factors including the stress paradigm, time after stress, and specifics of the cue conditioning and extinction protocols. Both RRS and CUS have been shown to enhance cue-conditioned freezing or contextual conditioning (Conrad et al., 1999; Farrell et al., 2013; Wood et al., 2008; Zhang and Rosenkranz, 2013; Farrell et al., 2010), although there are also reports that do not see changes in these responses (Baran et al., 2009; Miracle et al., 2006). Other stressors have also been seen to enhance context or cue conditioned freezing, such as prior exposure to multiple footshocks in another context (Rau et al., 2005) or a single prolonged stress exposure (Yamamoto et al., 2009). Further, conditioning of additional responses beyond freezing or startle is enhanced with other stressors such as footshock [inhibitory avoidance (Kooiendaal et al., 2002)] or predator exposure [locomotion in a conditioned context, (Mackenzie et al., 2010)].

Perhaps more uniformly, many stress paradigms demonstrate deficits in the recall after extinction, including RRS (Baran et al., 2009; Farrell et al., 2013; Zhang and Rosenkranz, 2013; Miracle et al., 2006; Farrell et al., 2010), single prolonged stress (Yamamoto et al., 2009), or an acute exposure to an elevated platform stress (Maroun et al., 2013), but not a single restraint stress (Zhang and Rosenkranz, 2013). Interestingly, these deficits in extinction recall display dependence on both age and sex (Zhang and Rosenkranz, 2013; Baran et al., 2009; Farrell et al., 2013). The ability of stress to produce deficits in extinction recall implicates effects of stress on prefrontal—amygdalar circuits underlying extinction learning, as PFC inputs to the amygdala play a crucial role in this phenomenon (Amano et al., 2010; Maroun, 2013). Indeed, the effects of chronic stress on the prefrontal cortex, which include dendritic atrophy, excitatory synapse loss, elevated extracellular glutamate and impaired plasticity of projections to the amygdala, are consistent with a powerful regulatory role of this structure in both BLA and CeA stress responses (Berretta, 2005; Maroun, 2006; Moghaddam, 2002; Radley and Morrison, 2005). Since increases in spine density and hypertrophy of BLA pyramidal neurons induced by cue conditioning are reversed with extinction training (Heinrichs et al., 2013), this might suggest stress modulates the ability of extinction training to induce morphological shifts in BLA neurons. Such results suggest that, like the heterogeneity of stress-induced morphological changes, behaviorally induced stress may also influence morphological plasticity in the rodent amygdala.

4.5. Amygdala glutamatergic processes mediating stress-induced behavioral changes

Several studies have implicated the glutamatergic system in the BLA in mediating the influences of stress on conditioned and unconditioned anxiety-related behaviors, although different glutamatergic receptors may influence different behavioral endpoints in these tasks. For example, NMDA NR2A (Grin2a) knockout mice fail to show the normal increase in time in the light compartment of the LDB after repeated stress observed in wildtype C57BL/6J mice, while mice lacking the AMPA receptor subunit (Gria1 null mutants) showed typical stress responses in this test (Mozhui et al., 2010). Further, injection of a kainate receptor agonist (APTA) into the BLA blocked the effect of RRS in C57BL/6J mice (Masneuf et al., 2014).
Systemic injections of NMDA antagonists (MK801, AP7, CPP) administered before predator stress (cat exposure) also dose dependently attenuated the reduced open arm time and risk assessment behaviors in the EPM, and enhanced startle responses, seen 7–9 days later in vehicle-treated groups (Adamec et al., 1999a, 1999b; Blundell et al., 2005). d-Cycloserine has also been shown to attenuate the stress-induced deficits in extinction recall (Yamamoto et al., 2009) implicating NMDA-mediated process in that endpoint as well. In addition, bilateral injections of MK801 into the BLA, or unilateral injections into the left BLA, blocked predator induced decreases in risk assessment but not open arm time in the EPM (Adamec et al., 1999b). NMDA antagonists in the right amygdala have greater effects on increases in acoustic startle responses induced by predator stress, suggesting that there is a lateralized control of these glutamatergic processes over stress effects. This intriguing study suggests that glutamatergic processes associated with stress in amygdala may exert very specific influences on distinct behavioral endpoints, even during the same task, and that there is a lateralized control of these glutamatergic processes over stress effects.

In contrast to unconditioned stress effects in novelty based tasks, sensitized startle responses appear to be dependent on BLA glutamatergic processes mediated via AMPA receptors. Studies by Davis and colleagues demonstrated that glutamatergic processes in the amygdala are important for fear potentiated startle responses (Walker et al., 2002; Walker and Davis, 2000), and that microinjections of the AMPA antagonist NBQX into the BLA before the startle test blocked both shock (fear) potentiated startle and light enhanced startle responses (Davis and Walker, 2013; Walker and Davis, 1997). Studies also demonstrate that AMPA antagonists or lesions of the CeA block fear-potentiated startle, while AMPA antagonists or lesions on the bed nucleus of the stria terminalis (BNST) attenuate light-enhanced startle responses (Davis and Walker, 2013; Walker and Davis, 1997; Ventura-Silva et al., 2013). Overall, these studies implicate BLA glutamatergic processes in mediating stress effects in both conditioned and unconditioned anxiety-related responses, although there are distinct roles for NMDA, AMPA and kainate receptors in modulating specific behavioral endpoints. Moreover, these glutamatergic mechanisms in response to stress may be lateralized, which is not unlike the hemispheric changes seen in imaging studies of patients with conditions such as PTSD or other anxiety disorders. Lateralized effects could also explain the inconsistency between studies examining stress-related responses, morphological endpoints and the glutamatergic system. Thus, although it is clear that stressors create potentially long-lasting changes in behavior that involve glutamatergic mechanisms, it remains unknown if specific glutamatergic receptors mediate distinct behavioral endpoints and if these mechanisms differ between types of stress. In addition, since some types of stressors, such as predator stress, require a sensitization period before the behavioral shifts emerge, understanding how acute glutamatergic changes in the BLA drive long-term modifications in behavior represents a challenging scientific question, since it might be dependent on type of stress, timing, and sex or strain of the subjects. For example, it appears that chronic stress depresses glutamate release but ultimately sensitizes many amygdala-related behavioral endpoints. Finally, understanding how morphological changes in the BLA underlie behavioral shifts might require a more careful assessment of the lateralized effects of stress in this region.

5. Antidepressant drugs, stress and the amygdala

If glutamatergic neurotransmission is adversely affected by stress, drugs that restore appropriate glutamatergic tone may prevent or reverse the morphological changes in the CNS and would thereby be helpful in treating mood and anxiety disorders. Indeed, clinical research has shown alterations in levels, clearance and metabolism of glutamate in mood and anxiety disorders. Interestingly, these changes are consistent with volumetric changes in brain areas enriched in glutamate neurons (Gorman and Docherty, 2010). For these reasons, there is a growing interest in the development of drugs that directly target the glutamatergic system. The main advantages of this pharmacological approach are the rapid onset of the therapeutic effects, the avoidance of the typical side effects of monoaminergic antidepressants, as well as providing an alternative strategy in treatment-resistant patients. In this regard, it is well described that a single administration of non-specific NMDAR antagonists, such as ketamine, produces rapid antidepressant effects in clinical and preclinical settings (Berman et al., 2000; Autry et al., 2011; Sanacora et al., 2008). However, the mechanisms by which NMDAR blockers produce antidepressant effects remain to be fully elucidated. Ketamine increases the expression of synaptic proteins and the number of excitatory spines in PFC, suggesting that behavioral effects may be a consequence of changes in synaptic plasticity and synaptogenesis (Li et al., 2010, 2011). More recently, Liu et al. showed that ketamine restores the strength of granule cell PFC inputs from BLA, leading to the hypothesis that ketamine would ameliorate the disproportional negative influence of the amygdala in chronic stress and major depression (Liu et al., 2015). Taken together these recent studies suggest that ketamine exerts its rapid and prolonged antidepressant effect acting on the inputs that reach to the mPFC from BLA rather than acting directly on the BLA. There is a great interest in developing specific compounds that act on NMDAR subunits to avoid the adverse effects of ketamine. In this regard, Ro25–6981, a GluN2B selective antagonist, elicits anti-depressant-like behavior, although these effects were not sustained as long as those of ketamine (Maeng et al., 2008). Additional studies showed that GluN2B antagonist microfusion into the mPFC, but not the BLA, reversed stress-related behavioral changes (Kiselycznyk et al., 2015). Beyond ionotropic glutamate receptors, the metabotropic glutamate receptor subtype 7 (mGlu7) has been identified as a key regulator of brain emotion circuits; specific blocking of mGlu7 has anti-stress, anti-depressant and anxiolytic-like effects in rodent behavior (Gee et al., 2014). More critically, these pharmacological studies support the notion that glutamatergic projections between PFC and amygdala are important mediators of stress-induced behavioral changes and suggest that these glutamatergic projections provide a novel target for reversing the effects of chronic stress.

Monoaminergic-based antidepressants also interfere with the glutamate system, modifying the protein expression and the phosphorylation of different glutamate receptor subunits [for more details, see (Musazzi et al., 2013)]. Although the anxiolytic mechanism of the specific serotonin reuptake inhibitors (SSRIs) is unclear, these drugs are widely used for the treatment of anxiety disorders. Local administration of citalopram into the BLA decreases freezing behavior induced by conditioned fear stress, and serotonin levels are simultaneously increased, suggesting that increases of extracellular serotonin levels in the BLA may be related to the anxiolytic effects of SSRIs (Kitaichi et al., 2014). Chronic unpredicted mild stress (CUMS) produces considerable remodeling of type 1 synapses accompanied by changed expression of several synaptic associated proteins in BLA. Chronic administration of another SSRI (escitalopram) decreased the length of the active zone of synapses that was increased by CUMS. In addition, escitalopram was able to restore spinophilin levels that were decreased in BLA after CUMS (Li et al., 2015).

Taken together, while the existing literature shows an important body of reports that studied the effects of antidepressants upon
amygdala-dependent behavior, fewer reports have focused on the electrophysiology that underlies these behaviors and even fewer manuscripts investigated the structural synaptic plasticity of the amygdala after antidepressant treatment (Johnson et al., 2009; Grillo et al., 2015; Pillai et al., 2012). More systematic studies including behavior, electrophysiology, molecular mechanism and morphological analysis, will help to elucidate the drug mechanisms and assist in linking the remodeling of the glutamatergic neurons with the neurochemistry and the behavior. Understanding the mechanism of action will provide a pharmacological basis for the use of the antidepressant drugs, and subunit selective glutamatergic compounds, to treat amygdala-dependent behavior disorders. Going beyond empirical use will provide a more effective and appropriate use of the drugs used to treat the variety of disorders organized under the general term of “anxiety disorders”.

6. Conclusions

In recent years it has become clear that the amygdala represents a site of substantial neuropsychological changes in the context of stress responses. The nature of these neuropsychological changes appears to differ from those observed in other stress-sensitive brain regions such as the PFC and hippocampus. The identification and further mechanistic characterization of the factors that drive amygdalar neuropsychological plasticity will assuredly contribute to our understanding of the role of this brain structure in multiple stress- and anxiety-related disorders. Importantly, it may also lead to the development of novel tools from the fields of neuroimaging, neurogenetics and neuropharmacology that will aid the discovery of novel predictive biomarkers, prevention strategies, and drug-based treatments for these disorders. The full realization of these possibilities hinges on several unanswered questions going forward. For example, regarding the morphological alterations seen in the amygdala as a whole or in selected amygdalar neuronal populations—what is the precise time course of these morphological changes? How does this time course, and the accompanying variations in amygdalar nuclei or neuronal subpopulations, differ from other stress-responsive brains regions or across different stress paradigms? To what extent are these changes reversible? Or, for that matter, which of these changes are adaptive as opposed to facilitating cognitive, behavioral and affective dysfunction? The answers to such questions will require the field to adopt strategies to conduct parametric studies that include analysis of different brain areas with attention to potential hemispheric differences, different types of stressors, and different behavioral endpoints in conditioned and unconditioned tests. Most critically such studies will need to include a time course of changes both after a single stress and at time points during and after chronic stress exposures. A potentially very exciting and understudied area is the role of sex and sex hormones in these responses. The few studies that have examined sex differences have not shown differences in morphological changes, but demonstrate significant sex effects on behavioral endpoints and possible glutamatergic mechanisms. This might be due to fundamental differences between the sexes in baseline responses in glutamate tone and/or behavioral responses in these tests, so such studies will be a challenging prospect as the field moves forward.

The neurotransmitter correlates of stress-elicited neuropsychological changes in the amygdala may also represent new opportunities for mechanistic descriptions of these changes as well as drug-based therapeutic targets. Glutamate-based therapeutics have received much interest in the past decade in the context of several neuro-psychiatric disorders, and it appears this neurotransmitter system plays a crucial role in stress effects on the amygdala. Characterizing the role of glutamate—either as a secondary mediator of responses to drugs that act on other neurotransmitter systems, or as a primary target of drugs that directly target glutamate receptors or other molecular aspects of glutamatergic transmission—will be important for understanding the mechanism of existing drugs and the development of novel therapeutics. Understanding the nature of the relationship between stress-related glutamatergic neurotransmission and morphological alterations in the amygdala will also be necessary to develop new treatments that are informed by both of these levels of analysis. Finally, a better understanding of how precortical stress paradigms model and recapitulate the amygdalar neuroplastic changes seen in human anxiety- and affective disorders will aid the translational impact of animal-based models.

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References

Adamec, R., Blundell, J., Burton, P., 2005a. Role of NMDA receptors in the lateralized potentiation of amygdala afferent and efferent neural transmission produced by predator stress. Physiol. Behav. 86, 75–91.
Adamec, R., Hebert, M., Blundell, J., Mervis, R.F., 2012. Dendritic morphology of amygdala and hippocampal neurons in more and less predator stress responsive rats and more and less spontaneously anxious handled controls. Behav. Brain Res. 226, 133–146.
Adamec, R.E., Blundell, J., Burton, P., 2005b. Neural circuit changes mediating lasting brain and behavioral response to predator stress. Neurosci. Biobehav. Rev. 29, 1225–1241.
Adamec, R.E., Burton, P., Shallow, T., Budgelj, J., 1999a. NMDA receptors mediate lasting increases in anxiety-like behavior produced by the stress of predator exposure—implications for anxiety associated with posttraumatic stress disorder. Physiol. Behav. 65, 723–737.
Adamec, R.E., Burton, P., Shallow, T., Budgelj, J., 1999b. Unilateral block of NMDA receptors in the amygdala prevents predator stress-induced lasting increases in anxiety-like behavior and unconditioned startle—effective hemisphere depends on the behavior. Physiol. Behav. 65, 739–751.
Amano, T., Ual, C.T., Pare, D., 2010. Synaptic correlates of fear extinction in the amygdala. Nat. Neurosci. 13, 489–494.
Auer, D.P., Putz, B., Kraft, E., Lipinski, B., Schill, J., Holsboer, F., 2000. Reduced glutamate in the anterior cingulate cortex in depression: an in vivo proton magnetic resonance spectroscopy study. Biol. Psychiatry. 47, 305–313.
Avery, A.E., Adachi, M., Nooyeva, E., Na, E.S., Loe, M.F., Cheng, P.F., Kavalali, E.T., Monteggia, L.M., 2011. NMDA receptor blockade at rest triggers rapid behavioral antidepressant responses. Nature 475, 91–95.
Baker, D.A., McFarland, K., Lake, R.W., Shen, H., Tang, X.C., Toda, S., Kalivas, P.W., 2003. Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. Nat. Neurosci. 6, 743–749.
Baker, D.A., Xi, Z.X., Shen, H., Swanson, C.J., Kalivas, P.W., 2002. The origin and neuronal function of in vivo nonsynaptic glutamate. J. Neurosci. 22, 9134–9141.
Baran, S.E., Armstrong, C.E., Niren, D.C., Hanna, J.J., Conrad, C.D., 2009. Chronic stress and sex differences on the recall of fear conditioning and extinction. Neurobiol. Learn. Mem. 91, 323–332.
Beckerman, M.A., Van Kempen, T.A., Justice, N.J., Milner, T.A., Glass, M.J., 2013. Corticosterone-releasing factor in the mouse central nucleus of the amygdala: ultrastructural distribution in NMDA-NR1 receptor subunit expressing neurons as well as projection neurons to the bed nucleus of the stria terminals. Exp. Neurol. 239, 120–132.
Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry. 47, 351–354.
Berretta, S., 2005. Cortico-amygdaloid circuits: role in the conditioned stress response. Stress 8, 221–232.
Berridge, C.W., Espana, R.A., Vittos, N.M., 2010. Hypocretin/orexin in arousal and stress. Brain Res. 1314, 91–102.
Blundell, J., Adamec, R., Burton, P., 2005. Role of NMDA receptors in the syndrome of behavioral changes produced by predator stress. Physiol. Behav. 86, 233–243.
Bondi, C.O., Rodriguez, G., Gould, G.G., Frazer, A., Morilak, D.A., 2008. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. Neuropsychopharmacology 33, 320–331.
Bosch, O.J., Sartori, S.B., Singewald, N., Neumann, I.D., 2007. Extracellular amino acid levels in the paraventricular nucleus and the central amygdala in high- and low-anxiety dams rats during maternal aggression: regulation by oxytocin.
role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biol. Psychiatr. 63, 349–352.
Magistretti, A.M., Virollet, J.A., McEwen, B.S., 1997. Chronic stress alters synaptic terminal structure in hippocampus. Proc. Natl. Acad. Sci. U. S. A. 94, 14002–14008.
Marou, M., 2006. Stress reverses plasticity in the pathway projecting from the ventromedial prefrontal cortex to the basolateral amygdala. Eur. J. Neurosci. 24, 2917–2922.
Marou, M., 2013. Medial prefrontal cortex: multiple roles in fear and extinction. Neuroscientist 19, 370–383.
Marou, M., Ioannides, A., Bergman, K.L., Kavushansky, A., Holmes, A., Wellman, C.L., 2013. Fear extinction deficits following acute stress associate with increased spine density and dendritic retraction in basolateral amygdaloid neurons. Eur. J. Neurosci. 38, 2611–2620.
Marou, M., Gysynov, G., Gatta, E., Gabriel, C., Mocaer, E., Di Prisco, S., Merega, E., Pittaluga, A., Nicolotti, F., Maccari, S., Morley-Fletcher, S., Mairees, J., 2014. The effects of antidepressant treatment in prenatally stressed rats support the glutamatergic hypothesis of stress-related disorders. J. Neurosci. 34, 2015–2024.
Martinez, R.C., Gupta, N., Lazaro-Munoz, G., Sears, R.M., Kim, S., Moscarello, J.M., LeDoux, J.E., Cain, C.K., 2013. Active vs. reactive threat responding is associated with differential ε-Fos expression in specific regions of amygdala and prefrontal cortex. Learn. Mem. 20, 446–452.
Masneuf, S., Lowery-Gionta, E., Colacicco, G., Pleil, K.E., Li, C., Crowley, N., Flynn, S., Marou, M., Ioannides, P.J., Bergman, K.L., Kavushansky, A., Holmes, A., Marrocco, J., Reynaert, M.L., Gatta, E., Gabriel, C., Mocaer, E., Di Prisco, S., Merega, E., Gabriel-Citré, M.A., Blume, S.R., Rosenkranz, J.A., 2013. Repeated restraint stress exerts different effect on structure of neurons in the lateral and basal nuclei of the amygdala. Neuroscience 240, 230–242.
Pastor-Curana, J., Babasa, C., Ortega-Sanchez, J.A., Sanchis-Ollo, M., Gabriel, C., Salazar, M., Ginesta, M., Belda, X., Daviu, N., Nadal, R., Armanio, A., 2014. Prior exposure to repeated immobilization or chronic unpredictable stress protects from some negative sequelae of an acute immobilization. Behav. Brain Res. 255, 729–739.
Pego, J.M., Morgado, P., Pinto, L.G., Cerqueira, J.J., Almeida, O.F., Sousa, N., 2008. Dissociation of the morphological correlates of stress-induced anxiety and fear. Eur. J. Neurosci. 27, 1503–1516.
Pijman, F.T., Herremans, A.H., van de, K.J., Kruis, C.G., van, J.E., 2003a. Behavioural changes after different stress paradigms: prepulse inhibition increased after physical, but not emotional stress. Eur. Neuropsychopharmacol. 13, 369–380.
Pijman, F.T., van, J.E., 2002. Physical but not emotional stress induces a delay in behavioural coping responses in rats. Brain Res. 136, 365–373.
Pijman, F.T., Wolterink, C., van, J.E., 2002. Cueing unavoidable physical but not emotional stress increases long-term behavioural effects in rats. Behav. Brain Res. 138, 393–401.
Pijman, F.T., Wolterink, C., van, J.E., 2003b. Physical and emotional stress have differential effects on preference for saccharine and open field behaviour in rats. Behav. Brain Res. 138, 125–135.
Pikkarainen, M., Pitkanen, A., 2001. Projections from the lateral, basal and accessory basal nuclei of the amygdala to the perihinal and prorhinal cortices in rat. Cereb. Cortex 11, 1064–1082.
Pilla, A.G., Anilkumar, S., Chattarji, S., 2012. The same antidepressant elicits contrasting patterns of synaptic changes in the amygdala vs hippocampus. Neuropsychopharmacology 37, 2702–2711.
Pirlo, G.G., Reznikov, L.R., Grillo, C.A., Hagar, J.M., Fadel, J.R., Reagan, L.P., 2013. Tianeptine modulates amygdalar glutamate neurochemistry and synaptic protein in rats subjected to repeated stress. Exp. Neurol. 241, 184–193.
Pitkanen, A., Savander, V., LeDoux, J.E., 1997. Organization of intra-amygdaloid circuitry in the rat: an emerging framework for understanding of function in the amygdala. Trends Neurosci. 20, 515–523.
Qin, M., Xia, Z., Huang, T., Smith, C.B., 2011. Effects of chronic immobilisation stress on anxiety-like behavior and basolateral amygdala morphology in Fmr1 knockout mice. Neuroscience 194, 282–290.
Radley, J.J., Morrison, J.H., 2005. Repeated stress and structural plasticity in the brain. Ageing Res. Rev. 4, 271–287.
Radley, J.J., Rocher, A.B., Janssen, W.G., Hof, P.R., McEwen, B.S., Morrison, J.H., 2005. Reversibility of apical dendritic retraction in the rat medial prefrontal cortex following repeated stress. Exp. Neurol. 196, 199–203.
Radley, J.J., Sisti, H.M., Hao, J., Rocher, A.B., McCall, T., Hof, P.R., McEwen, B.S., Morrison, J.H., 2004. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. Neuroscience 125, 218–226.
Rajkowska, G., Migiel-Hidalgo, J.J., 2007. Cognition and glial pathophysiology in depression. CNS. Neurol. Disord. Drug Targets 6, 219–233.
Ramík, T.S., Nyírás, R., Bluet, R.J., Gameble-George, J.C., Hartley, N.D., Mackie, K.W., Glowacki, M., Katouzian, H., 2014. Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. Neuuron 81, 1111–1125.
Rau, V., DeCola, J.P., Faseliov, M.S., 2005. Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder. Neurosci. Biobehav. Rev. 29, 1207–1223.
Reagan, L.P., Hendry, R.M., Reznikov, L.R., Pirolo, G.G., Wood, G.E., McEwen, B.S., Gray, C.A., 2007. Tianeptine increases brain-derived neurotrophic factor expression in the rat amygdala. Eur. J. Pharmacol. 565, 68–75.
Reagan, L.P., Reznikov, L.R., Evans, A.N., Gabriel, C., Mocaer, E., Fadel, J.R., 2012. The antidepressant agomelatine inhibits stress-mediated changes in amino acid neurotransmission in the rat hippocampus and amygdala. Brain Res. 1466, 242–250.
Reznikov, L.R., Grillo, C.A., Pirolo, G.G., Pasumarthi, R.K., Reagan, L.P., 2007. Amygdalar glutamate neurochemistry and synaptic plasticity in the amygdala following acute and repeated stress. J. Comp. Neurol. 508, 458–472.
Reznikov, L.R., Reagan, L.P., Fadel, J.R., 2009. Effects of acute and repeated restraint stress on GABA eflux in the rat basolateral and central amygdala. Brain Res. 1256, 61–68.
Roozendaal, B., Brunson, K.L., Holloway, B.L., McQuagh, J.L., Baram, T.Z., 2002. Incremental stress releases corticosterone-releasing hormone in the basolateral amygdala in regulating memory consolidation. Proc. Natl. Acad. Sci. U. S. A. 99, 13908–13913.
Roozendaal, B., McEwen, B.S., Chattarji, S., 2009. Stress, memory and the amygdala. Nat. Rev. Neurosci. 10, 423–433.
Sanacora, G., Guerguieva, R., Epperson, C.N., Wu, Y.T., Appel, M., Rothman, D.L., Krystal, J.H., Mason, G.F., 2004. Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. Arch. Gen. Psychiatry. 61, 705–713.

Sanacora, G., Rothman, D.L., Mason, G., Krystal, J.H., 2003. Clinical studies implementing glutamatergic neurotransmission in mood disorders. Ann. N. Y. Acad. Sci. 1003, 292–308.

Sanacora, G., Zarate, C.A., Krystal, J.H., Manji, H.K., 2008. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. Nat. Rev. Drug Discov. 7, 426–437.

Sarter, M., Markowitsch, H.J., 1984. Collateral innervation of the medial and lateral prefrontal cortex by amygdaloid, thalamic, and brain-stem neurons. J. Comp. Neurol. 224, 445–460.

Schmitt, O., Usunoff, K.G., Lazarov, N.E., Itzev, D.E., Eipert, P., Rolfs, A., Wree, A., 2012. Orexinergic innervation of the extended amygdala and basal ganglia in the rat. Brain Struct. Funct. 217, 233–256.

Sigurdsson, T., Doyere, V., Cain, C.K., LeDoux, J.E., 2007. Long-term potentiation in the amygdala: a cellular mechanism of fear learning and memory. Neuroscience 52, 215–227.

Singewald, N., Kouvelas, D., Mostafa, A., Sinner, C., Philippu, A., 2000. Release of glutamate and GABA in the amygdala of conscious rats by acute stress and baroreceptor activation: differences between SHR and WKY rats. Brain Res. 864, 138–141.

Skorzewska, A., Bidzinski, A., Hamed, A., Lehner, M., Turzynska, D., Sobolewska, A., Szyndler, J., Maciejak, P., Wislowska-Stanek, A., Plaznik, A., 2009. The effect of CRF and alpha-helical CRF(9-41) on rat fear responses and amino acids release in the central nucleus of the amygdala. Neuropharm 57, 148–156.

Small, S.A., Schoel, S.A., Buxton, R.B., Witter, M.P., Barnes, C.A., 2011. A pathophysiological framework of hippocampal dysfunction in ageing and disease. Nat. Rev. Neurosci. 12, 585–601.

Stam, R. 2007. PTSD and stress sensitisation: a tale of brain and body part 2: animal models. Neurosci. Biobehav. Rev. 31, 558–584.

Sun, N., Cassell, M.D., 1993. Intrinsic GABAergic neurons in the rat central extended amygdala. J. Comp. Neurol. 340, 43–64.

Sun, N., Yi, H., Cassell, M.D., 1994. Evidence for a GABAergic interface between cortical afferents and brainstem projection neurons in the rat central extended amygdala. J. Comp. Neurol. 340, 43–64.

Timmerman, M., Westerink, B.H., 1997. Brain microdialysis of GABA and glutamate: what does it signify? Synapse 27, 242–261.

Tsai, S.F., Huang, T.Y., Chang, C.Y., Hsu, Y.C., Chen, S.J., Yu, L., Kuo, Y.M., Jen, C.J., 2014. Social instability stress differentially affects amygudal neuron adaptations and memory performance in adolescent and adult rats. Front. Behav. Neurosci. 8, 27.

Turner, B.H., Herkenham, M., 1991. Thalamoamygdaloid projections in the rat: a test of the amygdala’s role in sensory processing. J. Comp. Neurol. 313, 295–325.

Van de Kar, L.D., Appel, M., Rothman, D.L., Mason, G., Krystal, J.H., 2004. Forebrain pathways mediating stress-induced anxiety behavior. Front. Behav. Neurosci. 7, 32.

Voss, M.W., Vivar, C., Kramer, A.F., van Praag, H., 2013. Bridging animal and human models of exercise-induced brain plasticity. Trends Cogn. Sci. 17, 525–544.

Vouimba, R.M., Munoz, C., Diamond, D.M., 2006. Differential effects of predator stress and the antidepressant tianeptine on physiological plasticity in the hippocampus and basolateral amygdala. Stress 9, 29–40.

Vouimba, R.M., Yaniv, D., Diamond, D., Richter-Levin, C., 2004. Effects of inescapable stress on LTP in the amygdala versus the dentate gyrus of freely behaving rats. Eur. J. Neurosci. 19, 1887–1894.

Vyas, A., Bernal, S., Chattarji, S., 2003. Effects of chronic stress on dendritic arborization in the central and extended amygdala. Brain Res. 965, 290–294.

Vyas, A., Chattarji, S., 2004. Modulation of different states of anxiety-like behavior by chronic stress. Behav. Neurosci. 118, 1450–1454.

Vyas, A., JadHAV, S., Chattarji, S, 2006. Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. Neuroscience 143, 387–393.

Vyas, A., Mitra, R., Shankaranarayana Rao, B.S., Chattarji, S., 2002. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J. Neurosci. 22, 6810–6818.

Vyas, A., Pillai, A.G., Chattarji, S., 2004. Recovery after chronic stress fails to reverse amygdaloid neuronal hypertrophy and enhanced anxiety-like behavior. Neuroscience 128, 667–673.

Walker, D.L., Davis, M., 1997. Double dissociation between the involvement of the bed nucleus of the stria terminals and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. J. Neurosci. 17, 9375–9383.

Walker, D.L., Davis, M., 2005. Involvement of NMDA receptors within the amygdala in short- versus long-term memory for fear conditioning as assessed with fear-potentiated startle. Behav. Neurosci. 114, 1019–1033.

Walker, D.L., Miles, L.A., Davis, M., 2009. Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 1291–1308.

Walker, D.L., Resiler, K.J., Lu, K.T., Davis, M., 2002. Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. J. Neurosci. 22, 2343–2351.

Walker, D.L., Hu, U.O., Ko, M.C., Liao, C.C., Lee, L.J., 2012. Differential neuronal changes in medial prefrontal cortex, basolateral amygdala and nucleus accumbens after postweaning social isolation. Brain Struct. Funct. 217, 337–351.

Watanabe, Y., Gould, E., McEwen, B.S., 1992. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res. 588, 341–345.

Wong, C.J., 2001. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. J. Neurobiol. 49, 245–253.

Wood, G.E., Norris, E.H., Waters, E., Stoldt, J.T., McEwen, B.S., 2008. Chronic immobilization stress alters aspects of emotionality and associative learning in the rat. Behav. Neurosci. 122, 282–292.

Wooley, C.S., Gould, E., McEwen, B.S., 1990. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res. 521, 225–231.

Yamamoto, S., Morinobu, S., Takei, S., Fuchikami, M., Matsuki, A., Yamawaki, S., Woolley, C.S., Gould, E., McEwen, B.S., 1990. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res. 521, 225–231.

Yamamoto, S., Morinobu, S., Takei, S., Fuchikami, M., Matsuki, A., Yamawaki, S., Woolley, C.S., Gould, E., McEwen, B.S., 1990. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res. 521, 225–231.

Yamamoto, S., Morinobu, S., Takei, S., Fuchikami, M., Matsuki, A., Yamawaki, S., Woolley, C.S., Gould, E., McEwen, B.S., 1990. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res. 521, 225–231.