Effects of Emotional Stress on Astrocytes and Their Implications in Stress-Related Disorders

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Abstract

Stress is a major risk factor in the etiology of several psychiatric diseases, such as anxiety disorders and depression. On the other hand, a growing body of evidence has demonstrated that astrocytes play a pivotal role in the normal functioning of the nervous system. Hence, understanding the effects of stress on astrocytes is crucial for a better comprehension of stress-related mental disorders. Here, we describe the evidence showing astrocyte changes induced by stress in animals and how this plasticity could operate to induce behavioral sequelae. In addition, human data linking astrocytes with psychiatric disorders related to stress are also discussed. Altogether, the data indicate that both chronic and acute stressors are capable of changing the morphology and function of astrocytes in the brain areas that are known to play a critical role in emotional processing, such as the prefrontal cortex, hippocampus, and amygdala. Furthermore, different lines of evidence suggest that astrocyte plasticity may contribute to the behavioral consequences of stress.

Keywords

Astrocytes • Chronic stress • Acute stress • Plasticity • Anxiety • Depression

Abbreviations

AQP4	Aquaporin 4
ATP	Adenosine triphosphate
CUS	Chronic unpredictable stress
Cx43	Connexin 43
FGF2	Astrocytic fibroblast growth factor
GABA	Gamma aminobutyric acid
GFAP	Glial fibrillary acidic protein

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P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_10

GLAST	Glutamate aspartate transporter, also known as excitatory amino acid transporter 1 (EAAT1)
GLT-1	Glutamate transporter-1, also known as excitatory amino acid transporter 2 (EAAT2)
GS	Glutamine synthetase
IP3	Inositol triphosphate receptors
S100β	Calcium-binding protein β

Introduction

Stress is a relevant issue in neuroscience and has been the subject of intense research over many decades. A wide body of evidence has shown that the neurotransmitters, neuromodulators, and hormones released during exposure to stress reshape the brain in the long term. For instance, acute and chronic stress alter the morphology of neurons, leading to changes in spine density and dendritic length and complexity [1–3]. These changes induced by stress can help to explain the development of pathologic endophenotypes. For example, stress promotes an increase of dendritic spines in the amygdala (which is a brain region of particular interest for emotional processing) associated with increased anxiety-like behavior [4]. Hence, prior exposure to stress makes the amygdala more responsive, producing the emotional hyper-reactivity that is a hallmark of anxiety disorders [5, 6].

Surprisingly, even though across different brain tissues and different species the ratio of glia to neurons is approximately one [7–8], most of the research on the neurobiology of stress has focused exclusively on neurons. This bias was presumably generated by the misconception that glial cells were merely supportive cells. This obsolete view has been completely revised since the birth of the tripartite synapse more than two decades ago [9]. However, the study of the effect of stress on astrocytes and other types of glial cells is still in its infancy [10–12].

Here, we describe briefly the anatomy and function of astrocytes, which lays the groundwork for understanding the multiple ways that make astrocytes an obvious candidate for the alterations in brain functioning that may underlie stress-related pathologies. Then, we summarize the current evidence of the astrocyte alterations induced by stress from studies that used classical protocols to induce stress in mature animals. Then we explore the possible functional implications of astrocyte alterations induced by stress and the mechanisms that could be altered in the tripartite synapse. Finally, we bridge those findings in animals with the literature on humans with a focus on depression, since this is the pathology related to stress that has received more attention.

The Tripartite Synapses Concept

The two main subtypes of astrocytes are the protoplasmic and fibrous ones. Protoplasmic astrocytes are found throughout all gray matter and exhibit several stem branches that give rise to many finely branching processes. In contrast, fibrous astrocytes are found in white matter and exhibit many long, fiberlike processes. The fine processes of protoplasmic astrocytes envelop synapses, whereas the processes of fibrous astrocytes contact nodes of Ranvier, with both types of astrocytes forming gap junctions between the distal processes of neighboring astrocytes [13, 14]. From studies performed on rodent hippocampus and cortex, many finely branching processes from a single astrocyte are estimated to contact several hundred dendrites from multiple neurons and to envelop 100,000 or more synapses [15, 16]. The main stem processes of astrocytes, which have glial fibrillary acidic protein (GFAP) as their main constituent, represent around 15% of the total astrocyte volume. These processes ramify progressively to finally generate a dense matrix of thin elaborate terminal processes that associate with neuropil elements and in particular with the synapses. These fine astrocytic processes account for 70–80% of the astrocytic plasma membrane and are devoid of GFAP. It is important to point out that even perisynaptic processes are found in all brain regions, although the proportion of synapses having these

and the level of synaptic coverage vary significantly between areas and within the same area [17].

A striking fact is that human protoplasmic astrocytes were found to be 2.6 times larger, and more complex (103 more primary processes), than rodent astrocytes. In addition, their larger diameter and more numerous processes imply that human protoplasmic astrocytes occupy a 16.5-fold greater volume than their mouse counterparts, and cover up to two million synapses [18]. In an interesting experiment, mice were engrafted with human glial progenitor cells, and upon maturation the recipient brains exhibited large numbers of human astrocytes. The engrafted human glia were coupled to host astroglia through gap junctions, yet retained the size and pleomorphism of hominid astroglia. Notably, the human glial chimeric mice showed enhanced learning and long-term potentiation, effects attributed to an increase in glia-released gliotransmitters [19].

The knowledge about the pivotal role that astrocytes play as fundamental units of the synaptic function began more than two decades ago [8, 20–22]. This revaluation started with the tripartite synapse concept, which incorporated the astrocytes as the third functional component of the synapse to the classic pre- and postsynaptic elements. This conceptual framework is based on the following aspects. First, astrocytes control the synaptic microenvironment through transporters, channels, and enzymes, with several of these highly or exclusively expressed by these glial cells. For instance, GLAST (glutamate aspartate transporter also known as EAAT1) and GLT-1 (glutamate transporter-1 also known as EAAT2), which remove extracellular glutamate. Furthermore, glutamine synthetase (GS), an enzyme that converts glutamate to glutamine is the precursor to synthetase glutamate and gamma aminobutyric acid (GABA). Second, astrocytes respond to the neurotransmitters released by neurons through membrane receptors, and in fact, most of the receptors present in neurons are also present in astrocytes. Third, they release substances termed "gliotransmitters", which in turn can affect neuronal activity. Specifically, they have been shown to be capable of releasing glutamate, D-serine, adenosine triphosphate (ATP), adenosine, GABA, tumor necrosis factor alpha, prostaglandins, atrial natriuretic peptide and brain-derived neurotropic factor, among other candidates.

Astrocytes communicate with each other through calcium waves, which are believed to represent for astrocytes what action potentials do for neurons [22]. These calcium signals are mainly due to the release of internal stores by activation of inositol triphosphate receptors (IP3), and the calcium waves propagate to neighboring astrocytes through gap junctions, where connexin 43 (Cx43) is an important constituent of the channels. These waves can be observed in vivo by two-photon microscopy after different sensory stimulations after 3–10 s, but even faster responses have also been reported. Calcium waves are implicated in gliotransmitter release and probably play an important role in other astrocyte functions. For instance, using whole-cell-path clamp combined with selective activation of astrocytes has shown that calcium waves can trigger an increase in the excitatory or inhibitory postsynaptic potential frequencies and, in more selected cases, also increase the amplitudes of excitatory potentials. These are transient effects that occur 20–60 s after stimulation [22, 23].

Emotional Stress and the Effects on Astrocyte Structure and Function

Stress is an adaptative physiological response that prepares the organism to face events that represent a physical and/or psychological threat. Hence, it is essential for survival and dealing with situations that require rapid "flight or fight" responses. However, when the stressors are overwhelming or are repeated over time they can eventually lead to pathology, especially when the predictability, control, and coping mechanisms are perceived as being insufficient to deal with the demands placed on them [24–26]. In fact, stress is considered to be one of the main risk factors for the development of psychiatric disorders such as anxiety-related disorders, depression and

drug addiction [27–30]. In general, brief and intensive aversive situations can provoke symptoms of anxiety [31, 32] while chronic mild stress tends to induce a more depression-related phenotype [33, 34].

Taking into account that morphological change in neurons is a hallmark of chronic stress effects with resulting increases, or reductions (depending on the brain structure) in both dendrite branches and spine density [1], it could be expected that astrocytes, which are in a close relationship at the synaptic level, could show structural plasticity. However, as mentioned above, the complexity of the astrocyte structures and the thickness of the perisynaptic processes have precluded an extensive morphological analysis of the intact brain after stress using the most common microscopy setups.

In-vitro evidence has clearly shown that astrocyte morphology is very dynamic, with highly motile astrocytic filopodia-like processes moving or growing over a time course of only a few minutes or even in seconds [17, 35]. Using organotypic hippocampal slices, a preparation that retains the three-dimensional architecture of astrocyte–synapse interactions, it has been demonstrated that astrocytes can rapidly extend and retract fine processes to engage or disengage postsynaptic dendritic spines [36]. Studies on intact brain also indicate that mature astrocytes are able to elongate or retract their perisynaptic processes and also to alter the whole shape of these cells [17, 37, 38]. One of the pioneering examples of astrocyte structural plasticity was shown in the paraventricular nucleus of the hypothalamus, which regulates the release of oxytocin, a hormone necessary for milk ejection from mammary glands. During lactation, astrocyte perisynaptic processes retract from the synapses, with the consequence (among other coordinated mechanisms) that astrocytes decrease the removal of glutamate from the synaptic cleft, thereby increasing the action of the neurotransmitter. At the time of weaning, the astrocytes then elongate again into the synapses and the oxytocin release returns to normal levels [39].

Most that we know about stress-induced morphological plasticity came from investigations that have used the gold standard marker of astrocyte GFAP detected by immunohistochemistry. GFAP is an intermediate filament protein present in the astrocyte cytoskeleton, but only expressed in the main processes; hence, it does not stain the perisynaptic processes that emanate from the principal astrocyte branches. In addition, changes in GFAP probably not only reflect a structural, but also a functional consequence for the astrocyte physiology, since this protein has been implicated in cell-to-cell communication, anchoring of proteins, and the reaction to brain insults [14]. For instance, cells lacking GFAP proteins do not develop perisynaptic processes with neurons [40], and have a reduction in the trafficking of the astrocytic glutamate transporter GLAST [41].

One of the pioneering studies that revealed astrocyte changes induced by stress was performed by Czéhet et al. [42]. In this study, adult male tree shrews were subjected to 5 weeks of psychosocial stress, and the number of cells (measured using stereological methods) showed a 25% reduction in the number of GFAP-positive cells in the hippocampus. Moreover, this work showed that the somatic volume of astrocytes was reduced by 25% in stressed animals. Even though GFAP is not a good marker for somas, since it is a protein exclusively present in the main processes, the changes reported are suggestive of an astrocyte process rearrangement. In support of this hypothesis, recent work carried out an extensive analysis of GFAP staining after chronic restraint described that stress induces a reduction in both the number and shortening of main processes [43].

In another seminal work performed by Banasr and coworkers [44], a chronic unpredictable stress (CUS) induced a 19% reduction in the number of GFAP-positive cells in the rat infralimbic cortex. These types of observations have been replicated and extended in a number of studies in rats, resulting in one of the most consistently reproducible results in the field [43, 45–49]. However, since GFAP is not present in all astrocytes, these studies are not conclusive. Another important limitation

of these pioneering works was the lack of measurement of the total cell number with other markers. Hence, it was unclear if there were fewer GFAP-positive cells because they had died or maybe had stopped producing GFAP at detectable levels for immunohistochemistry.

Subsequent findings by Gosselin and coworkers [50] represented an important step forward in this issue. This research analyzed some broader areas in Wistar Kyoto rats, which are more responsive to stressors and manifest more anxiety and depressive-like behavior compared to Sprague Dawley rats. In this model, GFAP-positive cells were again found to be reduced in hippocampus, prefrontal cortex, and amygdala, but not in the other cortical areas evaluated. However, when astrocytes were counted using calcium-binding protein β (s100β) marker which stains astrocyte somas, there were no differences observed, suggesting that the astrocytes were not degenerating, but instead that the expression of GFAP was being downregulated. In fact, when the protein level was assessed by western blot, GFAP levels were found to be decreased in the prefrontal cortex and amygdala. In addition, since there were no differences between rat strains in terms of the number of nuclei quantified with DAPI (which stain all cell types) or with the neuronal marker NeuN, then this strongly suggests that there was no loss of astrocytic cells (or neurons).

Unfortunately, we do not know if the differences reported in Wistar Kyoto rats in stress sensitivity are a cause or a consequence of astrocyte differences. However, similar results were obtained with the chronic restraint stress model, using a similar staining approach in the prefrontal cortex [43], suggesting that stress induce astrocyte plasticity. Regardless of the limitation that Nissl staining was used to identify astrocytes, Kassem et al. [51] also did not find any differences in the astrocyte number in CA1, amygdala, or retrosplenial cortex after chronic restraint. On the other hand, using the CUS model, a decrease in the GFAP level was reported in the hippocampus detected by western blot [45, 52], and also at the RNA level in the hippocampus [45, 49] and prefrontal cortex [49, 53], indicating that downregulation operates at the transcription level.

A quite different result has been reported by other authors who used the chronic restraint model, in which they found an increase of GFAP-positive cells and protein level in hippocampus [54, 55]. Using this model, but analyzing other areas, a GFAP downregulation in the periaqueductal and the raphe nucleus was reported [56, 57]. Thus, unlike the CUS and psychosocial stress paradigms, the chronic restraint model has produced more variability in the results of GFAP measurements, with differences in the predictability and controllability in those models probably accounting for the differences reported following stress exposure [25].

Another marker of astrocytes that has been studied is s100β. This is a protein that acts as a calcium sensor, which when activated, interacts with several other proteins and thus affects broad cellular functions. Moreover, it is secreted and induces cellular activities by acting in autocrine, paracrine, and endocrine manners [58]. As mentioned above, although the number of astrocytes expressing this protein does not change after stress, the level of s100β has been reported to be increased in the prefrontal cortex [43] and the hippocampus after CUS [47; however, see 59]. This implies that calcium waves may be altered by stress, but as far as we are aware there are no publications that have measured calcium waves after stress protocols.

As mentioned above, an important aspect of astrocytes is that they are highly interconnected through gap junctions which are the substrate for calcium-wave propagation. Accordingly, some authors have explored whether the substrate for this communication is disrupted after CUS, and found that the intra–infralimbic diffusion of a permeable dye, which was preferentially spreading among astrocytes through gap junctions, was notably decreased after CUS. Furthermore, alterations in astrocyte gap junctions were confirmed at electron microscopy level, and were associated with downregulation of Cx43 [48].

A more direct functional measurement of astrocytes after CUS was performed based on

infusion of (2-13C) acetate, which has been shown to be preferentially metabolized in astrocytes. The findings of this experiment showed that after stress, the animals had a reduction in the marked glutamate, glutamine, and GABA, indicating a slowing in the astrocyte metabolism [53]. In the same series of experiments, other proteins that are involved in glutamatergic transmission and preferentially expressed by astrocytes, such as GLT-1, GLAST, or GS, were found to be unchanged in the prefrontal cortex after CUS, at least at the mARN level [53].

All the above results were performed in chronic stress paradigms; hence, an important question not answered in those works is how much stress is necessary to observe changes in astrocytes. However, a couple of studies have explored astrocyte proteins after acute stressors that give a partial answer to this question. There was no change in GFAP immunostaining in the hippocampus from rats that were restrained for 2 h with the additional stress of being submerged in water [60]. However, when a presumably "stronger stressor" was used (the combination of restrain, forced swimming test, and ether exposure in an acute sequential session), there was a reduction of hippocampal GFAP expression in the hippocampus [61]. On the other hand, investigations that observed a downregulation of GFAP in the periaqueductal area and raphe nucleus in the chronic restraint model did not observe these changes during a shorter stress session (3 day/6 h compared to the standard 21 day/6 h), suggesting that changes in GFAP in these areas require exposure to chronic stress [56, 57].

In a predator paradigm in which rats were exposed for 5 min to the sight and smell of a cat, the s100β content was enhanced in cerebrospinal fluid, but not in the hippocampus or cerebral cortex, 1 h after the stressful experience [62]. A similar result was found in the restraint model [63], suggesting that s100β is rapidly released from astrocytes after acute stress. Other experiments have further shown an increase in the number of astrocytes expressing the inflammatory protein interleukin 1β in the hippocampus, hypothalamus, amygdala, and periaqueductal gray [60]. In addition, 3 h of restraint induced an increase of astrocytic fibroblast growth factor (FGF2) which was associated with an enhancement in hippocampal neurogenesis, suggesting a beneficial effect of astrocyte release FGF2 induced by stress [64]. In lateral/basolateral amygdala samples from rats subjected to 15 footshocks over a 93-min period, many astrocyte-enriched genes were either upregulated or downregulated in the stressed animals, and these seemed to be long lasting changes since measurements were taken 22 days after stress [65]. For example, an upregulation of GLAST and downregulation of serine racemase, which synthetizes the gliotransmitter D-serine, were detected in stressed animals.

Taken together, these data indicate that acute stress is able to induce changes in astrocytes, which suggests that these cells are rapidly sensing and responding to hormones and/or neurotransmitter released during the stress response. Furthermore, those changes could be long-lasting and more pronounced after chronic stress. However, a not-answered issue in most publications cited above, either after acute or chronic paradigms, is whether these astrocyte changes are reversible after a time of recovery. This is important, since more permanent changes are most probably related to the physiopathological changes that underlie long-lasting maladaptive behavioral effects of stress, such as anxiety and depression.

The Role That Stress-Induced Astrocyte Plasticity May Be Playing in the Behavioral Sequelae of Stress

A long tradition in neuroscience research has shown that stress can induce depressive and anxiety-like behavior in animals [24, 66]. For instance, the CUS model induces anhedonic-like effects, operationally defined as a decrement in sucrose consumption, and also hopelessness measured by a forced swimming test and active avoidance paradigms [53, 67]. On the other hand, as described in this review, there is extensive evidence that chronic and acute stress are capable of inducing changes in astrocyte morphology or

functionality, which are presumed to be deleterious for brain functioning and eventually form a part of the physiopathology of stress-related disorders. Therefore, an important question arises: does stress-induced astrocyte plasticity play any role in the behavioral sequelae induced by stress?

Several of the studies presented above using stress chronic models have also shown that antidepressant drugs, e.g., fluoxetine and clomipramine, which normalize stress-induced behavioral changes, prevented stress-induced astrocyte changes [42, 45, 48]. This strongly suggests that astrocytes are involved in the behavioral consequences of stress. The question about their sufficiency, however, is not simple to address, but the use of gliotoxins and transgenic animals has indicated that astrocytes may indeed play a causal role in the long-lasting effects induced by stressful experiences. One of the first experimental findings supporting this proposal came from experiments performed by Banasr and coworkers [44]. By applying L-alpha-aminoadipic acid microinjections into the rat prefrontal cortex, which selectively decreased the number of GFAP positive cells by 23% (but not neurons), anhedonia and hopelessness were induced in the short term. This type of experiment has been subsequently replicated and extended using other gliotoxins [48, 68–70]. Moreover, gliotoxin-induced depressive behavior was prevented by systemic antidepressant drugs [68].

Transgenic mice with an alteration in the nitric oxide synthetase 2 (which is predominantly expressed in glial cells) produce high levels of nitric oxide in astrocytes. This astrocytic alteration render the animals more susceptible to acute stress, as evidenced by higher anxiety-like behavior, increased acoustic startle responses, and higher plasma corticosterone levels compared to wild-type mice after predator scent exposure [71]. Another mousee which had a reduction in the ATP secreted from astrocytes showed a depressive phenotype, which was similar to the one observed after chronic stress paradigms that also decreased the release of ATP [67], Furthermore, in another transgenic mouse line, the release of ATP from astrocytes was increased after injection of a specific ligand, which induced antidepressant-like effects in the forced swimming test and in the chronic social defeat stress model [67]. Thus, selective alterations of the astrocyte machinery were sufficient to either trigger stress-like effects or give protection from the behavioral consequences of stress. However, these findings have been challenged by a recent paper which did not find any behavioral alteration in several emotional and cognitive tasks in similar transgenic mice that were knockout for astrocytic IP3R2, which is a critical receptor for triggering calcium wave signals [72].

Current advances in more selective and less invasive ways of activating or silencing astrocyte activity, such as optogenetic and designer receptors exclusively activated by designer drugs [73], will be critical for understanding how astrocytes contribute to the emergence of the behavioral aberrations associated to stress exposure.

Pathophysiological Changes in the Tripartite Synapse That Could Underlie Behavioral Sequelae of Stress

As mentioned before, the retraction of astrocytes from synapses in the hypothalamus has been shown to be critical to increase the glutamate effects as a result of a reduction in the removal of this transmitter, which is mainly taken up by astrocyte transporters [39]. In the same direction, the retraction of astrocytes (Bergmann glia) in cerebellar cortex enhances the excitatory postsynaptic current amplitude of Purkinje cells [38]. In hippocampus also, a mutation that makes astrocytes to retract from the synapses facilitates glutamate spillover and increases the NMDA currents in pyramidal neurons after burst stimulation [74]. On the other hand, acute and chronic stress has been associated with increases in glutamate release/content in the synaptic cleft, and excitotoxicity has been claimed as an important mechanism to produce cellular effects that underlie morphological and behavioral disturbances induced by stress [75]. Hence, the reduction in astrocyte processes induced by stress

could be a mechanism by which enhancement in excitability or even excitotoxicity is produced or increased. In fact, administration of GLT-1 blocker in PFC induced anhedonia-like behavior in rats [70]; and systemic injection of rulizole, a drug that facilitates glial cell glutamate uptake and decreases presynaptic release, prevented both the behavioral and astrocyte sequelae of chronic stress [53].

Another way in which stress-induced astrocyte alterations could affect behavior is through modulation of the GABAergic system. GABAergic synapses play a pivotal role in both anxiety disorders and emotional disturbance induced by stress [5, 76]. On the other hand, recent findings suggest that astrocytes release GABA and regulate GABA extrasynaptic content, which in turn is responsible for tonic GABA-A receptor-mediated currents [77]. Interestingly, chronic stress exposure induced a loss of tonic (but not phasic) inhibition in amygdala, an effect blocked by glucocorticoid synthesis inhibitor and mimicked by corticosterone [78]. Moreover, a study performed in slices from thalamus has shown that astrocytes also release a peptide that mediates a benzodiazepine-mimicking effect, and treatment with a gliotoxin reduced the effective inhibitory charge of GABA-A mediated spontaneous inhibitory postsynaptic currents [79]. Thus, the retraction of astrocytes from synapses or impairment in their function after chronic stress could account for the loss of tonic inhibition and consequent excitability of amygdala which is the hallmark of anxiety disorders such as posttraumatic stress disorder [27].

Mechanisms That Could Underlie Stress-Induced Astrocyte Plasticity

An important issue in this context is whether astrocytes can express receptors for stress-related hormones and neurotransmitters (e.g., glucocorticoids, norepinephrine) that allow them to directly respond to stress chemical mediators. Immunohistochemical studies have shown that beta receptors are extensively present in the lateral amygdala astrocytes [80] and alpha receptors in the prefrontal cortex [81]. It has long been established in astrocyte cultures that they show a morphological change (called stellation) in response to adrenergic beta receptor stimulation [37]. On the other hand, it is known that glucocorticoid and mineralocorticoid receptors are widely expressed in astrocytes and other glial cells [82, 83]. Recent postmortem studies in human tissue revealed the presence of glucocorticoid receptors in amygdala [84], hippocampus, and cortex [85]. Interestingly, experiments in vitro have demonstrated that corticosterone, at stress-relevant concentrations of 0.1–1 μM [86], induces an increase in the velocity of calcium waves and in gliotransmitter release [87]. Taken together, these findings indicate that astrocytes can directly sense and possibly change their morphology or functionality in response to chemicals released during the stress response.

Interestingly, corticosterone administration to rats (5 days or 4 months) caused a reduction in GFAP content in hippocampus and cortex [88] which, as after chronic stress, operates at the transcription level [89]. Norepinephrine is also able to modulate astrocyte activity. Using 2-photon microscopy in mice that express a Ca2+ indicator in astrocytes, it was shown that alpha adrenoceptor antagonists inhibited the activation of astrocyte networks that are triggered by the arousal associated to locomotion [90]. This effect seems to be specific for norepinephrine, since it was abolished by chemical depletion of norepinephrine but not by antagonists of serotonergic, muscarinic, metabotropic glutamate, or cannabinoid receptors [90]. In the same direction, when the locus coeruleus output was triggered by an air-puff startle response, it produced astrocyte calcium waves in prefrontal cortex that were suppressed by cortical administration of alpha adrenergic receptor antagonists or chemical depletion of norepinephrine [91]. Another way that norepinephrine could affect astrocytes is through phosphorylation of GFAP [92], which is believed to regulate the structural plasticity of glial filaments [93].

The molecular cascades that are triggered by stress in astrocytes and how they orchestrate the stress-induced astrocyte plasticity is essentially unknown, but clearly the astrocytes possess the machinery to sense and response to norepinephrine and glucocorticoids and probably other stress-released mediators.

Evidence of Astrocyte Alterations in Human Psychiatric Disorders Associated with Stress

Research related to this topic is strongly limited by the lack of non-invasive techniques that allow discriminating cell types in the intact brain. As a result, the only direct way of visualizing astrocytes in human brain is through postmortem studies. As far as we know, the principal psychiatric illness strongly associated to stress that has been studied in humans and focused on glial cells is depression. Related to this, several cell-counting studies have reported decreases in the packing density or number of the Nissl-stained populations of glial cells in subjects diagnosed with major depression, compared to non-psychiatric controls. These types of changes have been observed in fronto-limbic brain regions, including the dorsolateral prefrontal cortex, orbitofrontal cortex, subgenual cortex, anterior cingulate cortex and amygdala [94]. Another approach has been to study astrocyte morphology using the Golgi staining method, which allows the identification of scattered cells, permitting a 3D reconstruction of the whole individual cell. This technique was applied by Torres-Plata et al. [95] in the anterior cingulate cortex from suicidal depressive patients, and compared to matched control samples. These authors found an increase in the volume of the cell body and the number and length of the fibrous astrocyte processes located in the white matter adjacent to the anterior cingulate cortex, but not in the cortex itself.

Immunohistochemistry with antibodies against astrocyte specific proteins applied to postmortem tissue enables a more direct assessment of the astrocyte contribution to glial alterations in depressive subjects. Müller and coworkers [96] observed a reduction of GFAP immunoreactivity in the hippocampal areas CA1 and CA2, with the caveat that an observational criterion was used. A lower level of coverage of GFAP staining as well as a reduction in the number of GFAP-positive cells in the dorsolateral prefrontal cortex were found in young depressed patients, but not in older patients [97]. Similar findings were obtained in the orbitofrontal cortex using the western blot technique and fraction area of immunostaining [98]. In another study which used the s100β marker instead, a decrease in the number of astrocytes was also found in depressed and bipolar patients compared to matched controls [99]. By studying the amygdala postmortem tissue belonging to different psychiatric patients, another investigation found a reduction in GFAP-positive astrocytes, but only in depressive disorder [100; however, see 101]. In contrast, glucocorticoid receptors in amygdala astrocytes were increased in depressive patients compared to healthy controls or bipolar disorder patients [84]. This may be a compensatory response to high levels of glucocorticoids usually associated to depression.

Other measurements that have been applied to human postmortem tissue include in-situ hybridization and quantitative real-time PCR, which allow the detection and quantification of the mRNA present in brain sections and dissected dissolved tissue, respectively. Using this approach in the locus coeruleus [102], it was found that several transcripts for astrocyte proteins were altered in major depression but not in bipolar disorder. Specifically a reduction of GLT-1, GLAST, GS, GFAP, s100β, AQP4 (aquaporin 4, a water channel), Cx43, and connexin 30 (another gap-junction protein) was observed. A decrease in the expression of GLT-1, GLAST, and GS mRNAs has also been described in the anterior cingulate and dorsolateral prefrontal cortices [103]. Correspondingly, some of these transcripts have been also found to be reduced at the protein expression level in other areas. For instance, Cx43 [104], AQP4 [105], GLT-1, and GLAST [98] were decreased in the orbitofrontal cortex of depressive patients. Glutamate astrocytic transporters were also reduced in the amygdala of alcoholic individuals [106] which, according to these authors, could increase amygdala activity and the expression of associative memories and anxiety which underlie continued drug-seeking and chronic relapse.

Since s100β is secreted into the blood stream, this protein makes it possible to perform serum measurements in living patients, making it possible to investigate alterations of this astrocyte-related protein in different illnesses. Several studies have used this approach in psychiatric

populations, and a meta-analysis has been performed by Schroeter and coworkers [107] indicating that serum levels of s100β are consistently elevated during acute episodes of depression, with an increase with respect to control of 2.57 ± 0.70 (mean ± SD) fold. It is important to note that this increase is not specific to depression, as bipolar patients have also revealed increases in serum 100β. On the other hand, as s100β is also expressed in oligodendrocytes and some other body cells, then the respective contribution of these cells to the blood concentration is uncertain.

A big limitation in almost all human studies with depressive patients cited above is that most of the subjects were under antidepressant or other kind of psychopharmacology treatment that could affect the astrocyte measurement performed. However, some of these studies took account of this issue and made statistical comparison between persons under treatment vs. no medicated patients. For instance, in the study of Miguel-Hidalgo et al. [98] when subjects with depression that had antidepressant medication detected in the postmortem toxicology screening were compared to those without antidepressant, no differences in GLT-1, GLAST, GS, or GFAP levels were detected. Similarly, no medication effects were observed by Gos et al. [99], Rajkowska et al. [105], Wang et al. [84], and Miguel-Hidalgo et al. [104]. Interestingly, studies involving serum s100β measurement before and after successful treatment with antidepressive drugs indicated that s100β levels (which are larger than in control subjects) were reduced after treatment [107]. Even though the effect was small, this meta-analysis found a significant positive correlation between clinical treatment effects and serological treatment effects of s100β, suggesting that antidepressant could act to reduce s100β release from glia.

Investigations in non-psychiatric populations presumed to be exposed to robust stressors have also suggested that stress affect astrocytes. In this sense, serum s100β measurements taken 2 days after cardiac surgery along with Spielberger's anxiety inventory performed in cardiologic patients indicated that individuals with elevated s100β had higher levels of state anxiety and trait anxiety [108]. In the same way, s100β, as well as serum cortisol, were significantly increased in soldiers during combat training compared to at rest period, being concomitant to greater stress, anxiety, and depression levels assessed by psychological questionnaires [109].

Taken together, the human data from depressive patients showed a reduction of astrocyte markers in the dorsolateral prefrontal cortex, orbitofrontal cortex, hippocampal CA1 and CA2, and amygdala. Based on the results obtained in animals, it is possible to speculate that these changes might be caused by the effects of being exposed to chronic stress. However, while the animal data indicate that stress induced a downregulation of astrocyte markers without inducing "astrodegeneration", human studies have revealed a reduction in the number of the glial population stained with Nissl or s100β, suggesting that they degenerate or that the proliferation was reduced. Undoubtedly, depression is a multifactorial disease that is not only dependent on stress, and as referred above, there is evidence that reduction of GFAP could be an early manifestation that "disappears" at more advanced stages of the illness. On the other hand, studies on individuals that underwent a significant stress exposure, revealed clear changes in the astrocyte-related proteins 100β, which could be detected even at the blood level, suggesting a strong involvement of astrocytes in response to stress. Clearly, additional human studies are still necessary to fully understand the impact of stress on astrocytes functioning and the neurobiological and behavioral consequences.

Conclusions and Remarks

Stress effects on neuron morphology and function have been the subject of numerous investigations, which has been crucial for a better understanding of the mechanisms through which stress induces deleterious effects on brain functioning and on behavior. As shown in this review, different approaches in animals and humans have indicated that astrocytes are also an important target of stress, with both chronic and acute stressors being able to alter the morphology or the expression of several astrocyte

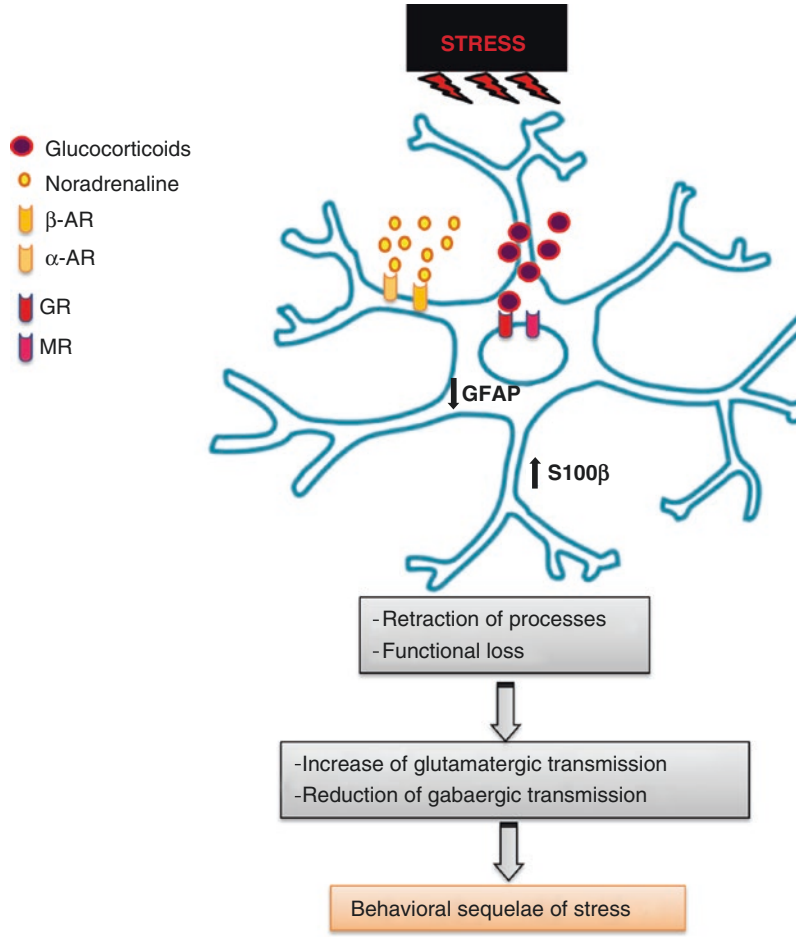

Fig. 10.1 Model of stress effects on astrocytes. Stress hormones (e.g., glucocorticoids) and neurotransmitters (e.g., norepinephrine) released during the stress response activate the receptors located in astrocytes and initiate intracellular cascades (including a decrease of GFAP levels and increase in S100b release) that ultimately produce changes in the morphology/physiology of astrocytes, which alters the normal functioning of tripartite synapses in a pathophysiological direction that is known to drive behavioral sequelae of stress, such as increases of glutamate transmission and/or reduction of GABAergic transmission

specific proteins in brain areas that are known to play a critical role in emotional processing, such as the prefrontal cortex, hippocampus, and amygdala. Furthermore, different lines of evidence have suggested that these changes may underlie the behavioral consequences of stress. First, astrocyte cellular effects induced by stress were prevented by the administration of drugs that averted the behavioral sequelae of stress. Second, astrocyte-specific toxins induced similar behaviors to those observed after stress exposure. Third, astrocyte-specific alterations in transgenic mice were able to emulate stress effects. Human data from psychiatric populations also support the notion that astrocytes are affected in mental disorders, with there being a remarkable agreement indicating that astrocyte-specific proteins are decreased in major depression, an illness strongly associated to stress. All together, the data suggest that stress hormones (e.g., glucocorticoids) and neurotransmitters (e.g., norepinephrine) through their receptors located in astrocytes directly induce intracellular cascades that ultimately introduce changes in the morphology/physiology of astrocytes, which alters the normal functioning of tripartite synapses in a pathophysiological direction that is known to drive behavioral sequelae of stress, such as increases of glutamate transmission and/or reduction of GABAergic transmission (see Fig. 10.1).

Acknowledgments This research was supported by grants from MinCyT-Cordoba, SECYT-UNC, CONICET, and Agencia Nacional de Promoción Científica y Tecnológica–FONCYT (Argentina) to Victor A Molina and SECYT-UNC, CONICET, and Agencia Nacional de Promoción Científica y Tecnológica–FONCYT (Argentina) to Gaston Calfa.

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