Chapter 14
Pathology in Astroglia, Glutamate, and GABA in Major Depressive Disorder: Evidence from Studies of Human Postmortem Tissue

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Abstract Evidence will be reviewed for pathology in astroglial cells, and for glutamate and γ-aminobutyric acid (GABA) neurons, their receptors and transporters in human postmortem brain tissue from subjects diagnosed with major depressive disorder (MDD). These observations will be compared with similar endpoints in preclinical animal models of chronic stress. Repeated stressful experiences or stressful life events can be risk factors for the onset or relapse of depressive episodes. Thus, animal studies on the behavioral and biological responses to exposure to chronic stress may shed light on underlying pathological mechanisms relevant to findings in postmortem brain tissue from subjects that experienced depression. Moreover, dysfunction of astrocytes, glutamate, and GABA—vital components of the tripartite synapse—will be proposed as a major source of fundamental pathology in depression and related animal behavioral models. Finally, the role of glutamate-based drugs in treating depressive symptoms will be discussed. In summary, evidence from postmortem brain tissue in MDD and animal models related to depression supports the hypothesis that pathology in astrocytes, glutamate, and GABA systems may be fundamental to the pathophysiology of depression.

Abbreviations

AQP4	Aquaporin 4
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
BDNF	Brain derived neurotrophic factor
Ca^{+2}	Calcium
CA1	Ammoni horn region 1
CA3	Ammoni horn region 3
CNS	Central nervous system
EAAT1	Excitatory amino acid transporter-1
EAAT2	Excitatory amino acid transporter-2

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GABA	γ-aminobutyric acid
GAD	Glutamic acid decarboxylase
GC1	Mitochondrial glutamate carrier
GFAP	Glial fibrillary acidic protein
GLAST	Glutamate–aspartate transporter
GLT1	Glutamate transporter 1
GluR1	AMPA receptor subunit 1
GluR2	AMPA receptor subunit 2
GluR3	AMPA receptor subunit 3
GluR4	AMPA receptor subunit 4
GluR5	Kainate receptor subunit 5
GRINA	Glutamate receptor ionotropic NMDA-associated protein 1
IR	Immunoreactive
MDD	Major depressive disorder
mGluR5	Metabotropic glutamate receptor 5
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin
NeuN	Neuronal nuclei (neuron-specific nuclear protein)
NMDA	*N*-methyl-D-aspartate
NR1	NMDA receptor 1
NR2A	NMDA receptor 2A
NR2B	NMDA receptor 2B
NR2C	NMDA receptor 2C
PSD95	Postsynaptic density protein 95
SAP102	Synapse-associated protein 102
SSRI	Serotonin-selective reuptake inhibitor

14.1 Astrocyte Pathology in MDD

Cell counting studies in postmortem brain tissue revealed prominent glial pathology in MDD. Early studies examined the entire population of glial cells (astrocytes, oligodendrocytes and microglia) by using a routine stain for Nissl substance. The packing density or number of glial cells was decreased in subjects retrospectively diagnosed with MDD, as compared to nonpsychiatric control subjects (Ongür et al. 1998; Rajkowska et al. 1999; Cotter et al. 2001; Bowley et al. 2002; Torres-Platas et al. 2002; Cotter et al. 2002a; Gittins and Harrison 2011). Such changes were observed in fronto-limbic brain regions including the dorsolateral prefrontal cortex (Rajkowska et al. 1999; Torres-Platas et al. 2002; Cotter et al. 2002a), orbitofrontal cortex (Rajkowska et al. 1999), subgenual cortex (Ongür et al. 1998), anterior cingulate cortex (Cotter et al. 2001; Gittins and Harrison 2011) and amygdala (Bowley et al. 2002). However, in examining elderly subjects with MDD, Khundakar et al. (2011a, 2011b) noted no change in glial density in the orbitofrontal cortex or anterior cingulate cortex.

In addition to reductions in glial cell density and number in MDD, the average size of the nuclei of glial cells was also increased in the gray matter of dorsolateral prefrontal cortex (Rajkowska et al. 1999). However, one study in the dorsolateral prefrontal cortex reported no change in the size of glial nuclei in MDD (Cotter et al. 2002a). A detailed analysis of astrocytes stained with the Golgi method reported hypertrophy of astrocytic cell bodies and processes in the white matter of the anterior cingulate cortex in depressed subjects dying by suicide (Torres-Platas et al. 2011). These authors interpret astrocytic hypertrophy as a reflection of local inflammation in support of the neuroinflammatory theory of depression (Maes et al. 2009).

Of the three types of glial cells in the CNS, astrocytes have been implicated most often as a source of glial pathology in MDD (reviewed in Rajkowska and Stockmeier 2013). This astrocytic pathology may be directly responsible for alterations in glutamate noted in MDD as astrocytes are active in the clearance and metabolism of glutamate at the tripartite glutamatergic synapse (discussed in detail below). Astrocytes have been localized in postmortem brain tissue by antibodies to glial fibrillary acidic protein (GFAP), gap junctions proteins such as connexin 30 and 43, the aquaporin-4 (AQP4) water channel and glutamatergic markers including the excitatory amino acid transporters 1 and 2 (EAAT1 and EAAT2), and the enzyme glutamine synthetase. As outlined below, each of these markers related to astrocytes is affected in postmortem tissues from subjects with depression.

GFAP is the principle component of cytoskeletal intermediate filaments and is strongly expressed in the CNS by mature and reactive astrocyte cells (Jacque et al. 1978; Middeldorp and Hol 2011). The expression of GFAP in depression has been quantified in gray matter by measuring the density of GFAP-immunoreactive (IR) astrocytes or so-called area fraction, the area covered by GFAP-IR cell bodies and processes. There was a significant decrease in the density of GFAP-IR astrocytes and GFAP area fraction in gray matter of the dorsolateral prefrontal cortex in younger depressed subjects (<60 years' old), as compared to age-matched nonpsychiatric control subjects (Miguel-Hidalgo et al. 2000). In addition, GFAP-IR area fraction was significantly decreased in the gray matter of the orbitofrontal cortex in a mixture of younger and older subjects with MDD (Miguel-Hidalgo et al. 2010). In contrast, older subjects with late-onset depression showed increases in GFAP-IR area fraction and cell density in the gray matter of dorsolateral prefrontal cortex (Miguel-Hidalgo et al. 2000; Davis et al. 2002), suggesting a compensatory response to neuronal damage reported in older subjects with MDD (Rajkowska et al. 2005). Thus, there appears to be a unique pattern of astrocyte pathology in cortical gray matter in younger versus older subjects with depression (Rajkowska and Miguel-Hidalgo 2007; Khundakar and Thomas 2009; Paradise et al. 2012).

Expression of GFAP protein and mRNA has also been examined in MDD. As determined by Western blotting, levels of GFAP protein were decreased in gray matter from the dorsolateral prefrontal and orbitofrontal cortex in MDD (Si et al. 2004; Miguel-Hidalgo et al. 2010). GFAP mRNA was also under-expressed in MDD in the anterior cingulate (Webster et al. 2005) and orbitofrontal cortex (Newton and Rajkowska, unpublished observations). There is a consistent under-expression of GFAP markers in MDD, whether measuring immunohistochemical cell density or area fraction, protein levels or mRNA expression.

Astrocytes are also altered in depression in limbic brain regions and related structures. A reduced density of GFAP-IR astrocytes was found in amygdala of subjects with MDD (Altshuler et al. 2010). In a semiquantitative study, Müller et al. (2001) detected a significant decrease in GFAP-IR astrocytes in the CA1 and CA2 subregions of the hippocampus in depression. A similar decrease in GFAP-IR astrocytes was noted in subjects that had been treated with steroids, suggesting that elevated glucocorticoid hormones acting at glucocorticoid receptors on astrocytes may have contributed to the reduction in GFAP expression in astrocytes (Müller et al. 2001; Wang et al. 2013). In a three-dimensional quantitative study, a significant reduction in the density of GFAP-IR astrocytes was recently observed in the hilus of the hippocampus in subjects with MDD not treated with antidepressant medications (Stockmeier et al. 2010). Bernard et al. (2011) noted a significant decrease in the expression of the mRNA for GFAP in the locus coeruleus in MDD while Chandley et al. (2013) isolated astrocytes from sections of this nucleus and noted a decrease in expression of GFAP mRNA and protein in this subpopulation of glial cells in MDD. In summary, reductions in the density and area fraction of GFAP-IR astrocytes and in the levels of GFAP protein and mRNA reveal dysfunctional astrocytes in MDD in fronto-limbic cortical regions.

Other markers of astrocytes located on astrocytic endfeet include connexin 30, connexin 43, and AQP4, and are also involved in the pathology of depression. Connexin 30 and connexin 43 form gap junctions that allow communication between astrocytes (Giaume and Theis 2010). The expression of genes and proteins for connexin 30 and connexin 43 was reduced in dorsolateral prefrontal cortex and orbitofrontal cortex in MDD (Ernst et al. 2011; Miguel-Hidalgo et al. 2012). The consequences of decreased expression of connexin 30 and connexin 43 alter calcium wave propagation and may affect communication between astrocytes (Blomstrand et al. 1999). In another study, reduced coverage of blood vessels by AQP4, which is a water channel expressed in astrocytic endfeet, was observed in the orbitofrontal cortex in MDD (Rajkowska et al. 2013). Finally, a decrease in the expression of mRNA for AQP4 was identified in locus coeruleus in MDD (Bernard et al. 2011). These decreases in AQP4 in depression could affect many brain functions as AQP4, in addition to its role in water redistribution, also regulates cerebral blood flow (Paulson and Newman 1987; Koehler et al. 2009), glucose transport and metabolism (Kimelberg 2004), integrity of the blood–brain barrier (Nico et al. 2001; Meshorer et al. 2005), glutamate turnover (Zeng et al. 2007), and synaptic plasticity (Li et al. 2012).

14.2 Astrocyte Pathology in Animal Models of Stress and Depression

Studies in preclinical animal models provide evidence for the involvement of GFAP and astrocytes in stress and depression-related behaviors. Various types of stress cause reductions in measures of GFAP-IR astrocytes. For example, the stress of sep-

arating juveniles from their family diminished the density of GFAP-IR astrocytes in the rodent medial prefrontal cortex (Braun et al. 2009). The stress of chronic social defeat in tree shrews reduced the number and soma volume of GFAP-IR astrocytes in the hippocampus (Czéh et al. 2006) and social defeat stress decreased the level of GFAP protein in rat hippocampus (Araya-Callís et al. 2012). Early life stress also resulted in a reduced density of GFAP-IR astrocytes in adult rats in various prefrontal and frontal cortical regions, hippocampus, and the basolateral amygdala (Leventopoulos et al. 2007). Furthermore, chronic unpredictable stress significantly decreased expression of GFAP mRNA in rat medial prefrontal cortex (Banasr et al. 2010). Interestingly, infusion of L-α aminoadipic acid in rodent prefrontal cortex, thought to selectively lesion glial cells including GFAP-IR astrocytes but not neurons, induced depressive-like behaviors (Banasr and Duman 2008; Lee et al. 2013). Assuming specificity of the toxin for glia, these two lesion studies appear to support the hypothesis that the loss of glia contributes to the pathology of depression (Rajkowska and Miguel-Hidalgo 2007). There is also support for a correlation between GFAP-IR astrocytes and depressive-like behavior in Wistar-Kyoto rats, a strain of rats that is genetically predisposed to anxiety-like and depressive-like behavior (Will et al. 2003). Significant reductions in the density of GFAP-IR astrocytes but not NeuN-IR neurons were observed in the prefrontal cortex, anterior cingulate cortex, amygdala, and hippocampus in Wistar-Kyoto rats as compared to Spraque-Dawley rats serving as controls (Gosselin et al. 2009). Thus, specific astrocytic deficits in the expression of GFAP in cortico-limbic circuits are associated with depressive-like behavior.

Astrocytes have been suggested as a target for therapeutic interventions in depression (Czéh and Di Benedetto 2013; Sanacora and Banasr 2013). Several animal studies reveal an influence of different classes of antidepressant medications on astrocytes. For example, treatment with fluoxetine, a serotonin-selective reuptake inhibitor (SSRI), prevented the psychosocial stress-induced reduction in astrocyte number in the hippocampus (Czéh et al. 2006). Riluzole, a glutamate modulating drug, also prevented the chronic, unpredictable stress-induced reduction in the expression of GFAP mRNA in the rat prefrontal cortex (Banasr et al. 2010). The beneficial effects of the SSRI antidepressants such as citalopram and fluoxetine may involve their ability to induce calcium signals in astrocytes in the prefrontal cortex (Schipke et al. 2011). However, not all studies show reversibility of the number of astrocytes or GFAP levels by an antidepressant drug. For example, a 4-week treatment with citalopram, also an SSRI, did not restore the social defeat-induced reduction in GFAP protein in the rat hippocampus, although the behavior of the animals was normalized within this treatment period (Araya-Callís et al. 2012). Likewise, imipramine, a tricyclic antidepressant drug, could not reverse the effects of learned helplessness on hippocampal astrocytes (Iwata et al. 2011).

In summary, models of chronic stress in experimental animals significantly diminish cortical and hippocampal astrocytes as measured by GFAP while lesions of cortical glia, including astrocytes, yield behavioral deficits comparable to those seen following chronic stress. The effects of chronic stress on GFAP-IR astrocytes can be reversed by chronic treatment with some, but not all, antidepressant medica-

tions. Thus, in light of astrocytic deficits noted in MDD and stress being a risk factor for depression, as well as astrocytic deficits in animal models of chronic stress, astrocytes may indeed be potential targets for the action of novel antidepressant medications.

14.3 Astrocyte Pathology and Glutamate Dysfunction in MDD

Astrocyte pathology described above could be related to dysfunction of the glutamate system, as reported in MDD. Astrocytes are a vital component of the tripartite glutamate synapse which consists of the (1) presynaptic neuronal terminal, (2) postsynaptic neuronal membrane, and (3) surrounding astrocyte processes (Araque et al. 1999; Nedergaard and Verkhratsky 2012). Synaptically associated astrocytes respond to neuronal activity by elevating their internal Ca^{2+} concentrations to trigger the release of glial transmitters which, in turn, regulate neuronal activity (Araque et al. 1999; Nedergaard and Verkhratsky 2012). Astrocytes also control the formation, maturation, function, and elimination of synapses through various secreted and contact-mediated signals (Clarke and Barres 2013). Moreover, astrocytes are actively involved in the uptake, metabolism, and recycling of glutamate. Levels of extracellular glutamate are regulated by removal of this neurotransmitter from the synaptic cleft via specialized transporters located on astrocytic processes (Anderson and Swanson 2000). In the human brain, these glutamate transporters include the EAAT1 and EAAT2, which in rodents are known as the glutamate–aspartate transporter (GLAST) and the glutamate transporter 1 (GLT1), respectively (Bezzi et al. 2004; Furuta et al. 2005). Glutamate internalized within astrocytes is subsequently converted to glutamine by the enzyme, glutamine synthetase (Toro et al. 2006). Glutamine then leaves astrocytes to be taken up by neurons where it can be converted into glutamate or GABA. Thus, astrocytes play a critical role in several aspects of glutamate neurotransmission.

Glutamate transporters and glutamine synthetase associated with astrocytes appear to be dysregulated in postmortem brain tissue from subjects with MDD. For example, reduced expression of mRNA for EAAT1, EAAT2, and glutamine synthetase was noted in the anterior cingulate and dorsolateral prefrontal cortex in subjects with MDD (Choudary et al. 2005). Expression of the mRNA for glutamine synthetase was also down-regulated in the dorsolateral prefrontal cortex, premotor cortex, and the amygdala of depressed suicide victims (Sequeira et al. 2009). Moreover, the expression of EAAT1, EAAT2, and glutamine synthetase protein was reduced in the orbitofrontal cortex in immunohistochemical and Western blotting studies of subjects with MDD (Miguel-Hidalgo et al. 2010). Finally, glutamate signaling and astrocyte-associated genes were under-expressed in locus coeruleus in MDD (Bernard et al. 2011; Chandley et al. 2013; Ordway et al. 2012), suggesting more global dysfunction of glutamate signaling and astrocyte pathology in MDD. Support for

disease-specific astroglial pathology in MDD comes from Bernard et al. (2011) demonstrating that these changes in glutamate-related gene expression do not occur in neurons. Other evidence supporting a role for dysregulated uptake of glutamate by astrocytes in depression comes from studies in rats where the pharmacological blockade of glutamate uptake into astrocytes in the amygdala (Lee et al. 2007), ventral tegmental area (Herberg and Rose 1990), or in the prefrontal cortex (John et al. 2012) is sufficient to decrease sucrose consumption, a behavioral marker thought to be related to anhedonia, a core symptom of depression. Finally, animal studies reveal that astrocytic GFAP plays a key role in the trafficking of glutamate transporters and protecting the brain against glutamate-mediated excitotoxicity (Hughes et al. 2004; Sullivan et al. 2007).

14.4 Glutamate Neurons and Receptors in Postmortem Tissues in MDD

Other studies of postmortem tissue reveal a link between neuronal pathology and glutamate dysfunction in MDD. Alterations in glutamatergic neurons' density, levels of their receptors, and other proteins involved in glutamate signaling are reported in MDD. Prominent reductions in the density of glutamatergic, pyramidal neurons were observed in the orbitofrontal cortex in elderly depressed subjects (Rajkowska et al. 2005).

Glutamatergic neurons and astrocytes directly control synaptic and extrasynaptic glutamate levels and release through integrative effects that target glutamate transporters, postsynaptic density proteins, ionotropic receptors (N-methyl-D-aspartate, NMDA;, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, AMPA; kainate) as well as metabotropic receptors. Recent studies in postmortem tissue implicate the NMDA class of glutamate receptors in the pathophysiology of MDD. Significant reductions in the protein expression of NMDA receptor subunits, NR2A and NR2B, and PSD-95 were observed in the anterior pole of prefrontal cortex from subjects with MDD as compared to psychiatrically normal control subjects (Feyissa et al. 2009). PSD-95 is linked to the NMDA receptor and plays a role in mediating trafficking and clustering of the receptor and related downstream signaling events. Reduced expression of NR2A transcript in the dorsolateral prefrontal cortex and reductions in expression of both NR2A and NR2B transcripts were noted in the perirhinal cortex in subjects with MDD (Beneyto et al. 2007; Beneyto and Meador-Woodruff 2008). In addition, there is a significant upregulation of genes coding for mitochondrial glutamate carrier (GC1) and the glutamate receptor ionotropic NMDA-associated protein 1 (GRINA) in the anterior pole of prefrontal cortex from subjects with MDD (Goswami et al. 2013). There are conflicting reports on whether expression of mRNA and/or proteins related to the NMDA receptor subunits are altered in the hippocampus in depression. A reduction in the expression of mRNA for the NR1 subunit of the NMDA receptor was noted in the dentate gyrus of the hip-

pocampus in depression (Law and Deakin 2001). In contrast, no change was noted in gene expression for several NMDA receptor subunits (including NR1) in either dentate gyrus or CA1 regions of the hippocampus in MDD (Duric et al. 2013). In the superior temporal cortex, while a decrease in radioligand binding to the glycine site of the NR1 subunit was observed in depression, the expression of the NR1 protein was not significantly different from control subjects (Nudmamud-Thanoi and Reynolds 2004). Furthermore, in MDD, protein expression of the NR1 subunit was also unchanged versus control subjects in the anterior pole of prefrontal cortex, amygdala, locus coeruleus, and cerebellum (Feyissa et al. 2009; Karolewicz et al. 2005; Karolewicz et al. 2009). Thus, NR2A and NR2B subunits, but not the NR1 subunit, appear to be consistently under-expressed in MDD.

Alterations in components of glutamate system in MDD are not restricted to limbic cortical regions (i.e., prefrontal cortex, hippocampus, temporal cortex) but are also found in the brainstem, striatum, and amygdala, regions that receive glutamatergic projections from the cerebral cortex. Increases in the expression of NR2C subunit were observed in the locus coeruleus and NR2A subunit in amygdala (Karolewicz et al. 2005; Karolewicz et al. 2009). There were significant changes in the expression of other glutamate signaling genes in the locus coeruleus in MDD (Bernard et al. 2011). Decreased expression of the mRNA transcript encoding the NMDA interacting postsynaptic density protein SAP 102 was detected in the striatum of depressed subjects (Kristiansen and Meador-Woodruff 2005). Thus, glutamate pathology in MDD affects limbic cortical regions and their subcortical projection areas. Taken together, the above studies provide evidence for pathology of the NMDA receptor in specific brain regions and support hypotheses that drugs altering NMDA receptor signaling may be effective in treating depression.

Fewer studies have been undertaken in depression on non-NMDA receptors such as the ionotropic AMPA and kainate receptors. Radioligand binding to the AMPA receptor was increased in the anterior cingulate cortex but not in the dorsolateral prefrontal cortex in MDD (Gibbons et al. 2012). In the same study, there was no significant depression-related change in radioligand binding to the kainate receptor in either prefrontal or cingulate cortex. However, mRNA expression of the GluR5 subunit of the kainate receptor was decreased in the prefrontal cortex in subjects with MDD (Knable et al. 2001). The expression of mRNA for subunits of the AMPA receptors (GluR1 and GluR3) was downregulated in both dentate gyrus and CA1 whereas mRNA for the GluR4 subunit was decreased only in dentate gyrus in MDD (Duric et al. 2013). Levels of GluR3 were significantly decreased in the dorsolateral prefrontal cortex in subjects with MDD (Beneyto and Meador-Woodruff 2006).

Finally, a reduction in radioligand binding to metabotropic glutamate receptor 5 (mGluR5) was reported by neuroimaging study in multiple brain regions including anterior prefrontal cortex in living depressed subjects (Deschwanden et al. 2011). There was a comparable reduction in protein level of this receptor in the same brain region in postmortem tissue from subjects with MDD (Deschwanden et al. 2011). Thus, reduced binding to mGluR5 receptors in MDD suggests reduced density of

functional receptors because of decreased levels of mGluR5 protein. Moreover, as the mGluR5 receptor is present on postsynaptic neurons and on glia, it may modulate extrasynaptic NMDA receptors (D'Ascenzo et al. 2007).

Generally, the aforementioned studies suggest pathology of various components of the glutamate system in depression. Alterations in NMDA, AMPA, kainate, and metabotropic glutamate receptors are found in several areas of postmortem brain tissue in MDD as compared to age- and gender-matched psychiatrically normal control subjects. Reduced levels of glial glutamate transporters and glutamine synthetase suggest enhanced synaptic and/or perhaps presynaptic concentrations of glutamate in MDD. A study of postmortem tissue supporting this hypothesis reported increased tissue levels of glutamate in the frontal cortex in subjects with MDD (Hashimoto et al. 2007). However, several neuroimaging studies of prefrontal and anterior cingulate cortex using magnetic resonance spectroscopy report a significant decrease in glutamate or glutamate/glutamine levels in depressed patients (Auer et al. 2000; Michael et al. 2003; Pfleiderer et al. 2003; Mirza et al. 2004; Hasler et al. 2007), while one study notes an increase in glutamate levels in the occipital cortex in depression (Sanacora et al. 2004). In spite of these discrepancies in whether glutamate levels increase or decrease, other clinical studies support the relevance of glutamate in depression.

There is a growing body of preclinical and clinical research implicating riluzole, an inhibitor of glutamate release, and ketamine, an antagonist of the NMDA receptor, as potent antidepressant medications (reviewed in Pilc et al. 2013). There are several reports that a single low dose of ketamine induces a rapid (within hours), long lasting (up to 7 days), and robust antidepressant effect in treatment-resistant patients with MDD (Berman et al. 2000; Zarate et al. 2006; Pilc et al. 2013). Potential mechanisms underlying the rapid action of ketamine are being identified. Li et al. (2010) reported that ketamine rapidly activated the mammalian target of rapamycin (mTOR) pathway, leading to increased signaling proteins at the synapse and increased number and function of new spine synapses in the prefrontal cortex of rat. Moreover, acute administration of ketamine in rats increased brain-derived neurotrophic factor (BDNF) and mTOR levels in the hippocampus during forced swimming (Yang et al. 2013). Interestingly, a recent study in postmortem prefrontal cortex on the expression of mTOR protein and its core downstream signaling targets reported a decrease in the expression of mTOR, p70S6K, eIF4B, and p-eIF4B proteins in subjects with MDD as compared to nonpsychiatric control subjects (Jernigan et al. 2011). Thus, a deficit in the initiation of mTOR-dependent protein expression may occur in depression and suggests an association between deficits in synaptic proteins and dysregulation of mTOR signaling in this disorder. Other components of the glutamate system also appear to be targets for antidepressant medications. For example, enhanced transmission through glutamatergic AMPA receptor may provide a common mechanism of antidepressant actions (reviewed by Sanacora et al. 2008).

14.5 Preclinical Studies on Stress and Glutamate

The pathology noted in the glutamate system in depression may be related to the effects of chronic stress. MDD is often preceded by exposure to chronic stress or stressful life events. There is evidence that both the onset of and relapse into depression are precipitated by severe repeated stressful experiences (Kessler 1997; Mazure et al. 2000; Kendler et al. 2001; Hammen 2005; Monroe et al. 2006; Pittenger and Duman 2008; Venzala et al. 2012).

Preclinical studies show that stress influences glutamate neurotransmission and metabolism and morphology of glutamate neurons. Consistent with studies in MDD, unpredictable chronic mild stress decreased expression of proteins for NR2A and NR2B subunits of NMDA receptor in the frontal cortex and hippocampus in rats (Feyissa et al. 2009; Lou et al. 2010). Repeated stress in young rats also significantly decreased expression of NMDA (NR1) and AMPA (GluR1) receptor subunits in pyramidal neurons of the prefrontal cortex and had a detrimental effect on cognitive processes dependent on this brain region (Yuen et al. 2012). Thus, glutamate receptors appear to be crucial neural substrates related to the effects of stress on synaptic plasticity and memory (Krugers et al. 2010; Yuen et al. 2012). No consensus has emerged on the effects of chronic mild stress on synaptic and vesicular levels of glutamate (reviewed in Hill et al. 2012). Chronic mild stress increased the expression of the glial glutamate transporter-2 and the vesicular glutamate transporter-1 protein and doubled the vesicular levels of glutamate in rat hippocampus (Raudensky and Yamamoto 2007; Garcia-Garcia et al. 2009). In contrast, reduced levels of mRNA for vesicular glutamate transporter-1 were noted in rat hippocampal subfield CA1 but not CA3 or dentate gyrus (Elizalde et al. 2010a). Within the frontal cortex, expression of both glial glutamate transporter-2 and the vesicular glutamate transporter-1 was not significantly changed by chronic stress in two studies (Garcia-Garcia et al. 2009; Banasr et al. 2010); however, a third study reported reduced levels of mRNA for vesicular glutamate transporter-1 (Elizalde et al. 2010a). Protein levels of the mGluR5 receptor were increased in the hippocampal CA1 subregion in rat in response to chronic mild stress but the receptor was decreased in the CA3 subregion and unchanged in the dentate gyrus (Wierońska et al. 2001). The above data reveal that stress influences glutamate receptors and transporters and these changes are region specific.

The pathology of glutamate systems in depression and chronic stress appears to involve several levels of neuronal morphology. Exposure to chronic unpredictable stress results in a reduction in the length and branching of apical dendrites of glutamate pyramidal neurons in layer V and decreases the number of synapses on these neurons in rat medial prefrontal cortex (Li et al. 2011; Duman & Aghajanian 2012). These observations may parallel findings from human postmortem studies in depression showing a reduction in glutamate, pyramidal neurons density in layer V of prefrontal cortex and smaller sizes of neurons in this and other prefrontal layers (Rajkowska et al.1999; Cotter et al. 2001; Rajkowska et al. 2005). The decreased

number of synapses observed in prefrontal cortex of stressed rats is consistent with the recent study of postmortem tissue showing significant decreases in the number of synapses and expression of synapse-related genes in the prefrontal cortex from subjects with MDD (Kang et al. 2012). The expression of several synapse- and glutamate-related genes was also decreased in the dentate gyrus and CA1 regions of hippocampus in MDD (Duric et al. 2013). This synaptic pathology may be related in part to the pathology of astrocytes in MDD since astrocytes control the formation, maturation, function, and elimination of synapses in the brain (Clarke and Barres 2013). In sum, the above findings clearly point to the pathology of glutamate synapses in MDD.

14.6 GABA Dysfunction in Postmortem Tissues in MDD

While neuronal pathology in MDD appears to be less prominent than glial pathology, several studies of postmortem tissue show reductions in the packing density and/or size of a general (Nissl-stained) population of cortical neurons (Rajkowska et al.1999; Cotter et al. 2001; Cotter et al. 2002a; Rajkowska et al. 2005). The most prominent neuronal changes in MDD have been observed in superficial layers of the prefrontal cortex (Rajkowska et al. 1999). Interestingly, these cortical layers are highly populated by GABA neurons. GABA dysfunction in MDD has been suggested by neuroimaging studies showing decreased levels of GABA in occipital and dorsolateral prefrontal cortex (Sanacora et al. 2004; Hasler et al. 2007). Also, some studies of postmortem tissue clearly demonstrate 30–50% reductions in the density of a subpopulation of GABA neurons, calbindin-IR neurons, in MDD. These decreases, noted only for calbindin- and not parvalbumin-IR GABA neurons, were observed in upper cortical layers (II and upper III) in the dorsolateral prefrontal cortex and in occipital cortex (Rajkowska et al. 2007; Maciag et al. 2010). In both of these studies, reductions in the soma size of calbindin-IR neurons were also noted in MDD. Thus, the studies in postmortem tissue support neuroimaging observations of alterations in GABA neurotransmission in the same brain regions. However, one study of postmortem tissue, examining all three populations of GABA neurons IR for calcium binding proteins, noted no changes in these neurons in the anterior cingulate cortex in MDD (Cotter et al. 2002b). The differences between studies showing alterations in GABA neurons (Rajkowska et al. 2007; Maciag et al. 2010) and that of Cotter et al. (2002b) may be explained by differences in hemispheres and brain regions studied as well as clinical features of the patient cohorts.

A reduction in the density and size of GABA neurons in dorsolateral prefrontal cortex in depression suggests that the synthesis of GABA may also be affected in that region. Glutamic acid decarboxylase (GAD), the enzyme that converts glutamate to GABA, exists as two isoforms, GAD-65 kDa and GAD-67 kDa, which are encoded by two distinct genes (Erlander et al. 1991; Kaufman et al. 1991). There was a significant decrease in the expression of GAD-67, but not GAD-65, in the

dorsolateral prefrontal cortex of many of the same depressed subjects used for the calbindin studies (Rajkowska et al. 2007; Karolewicz et al. 2010). The decrease in GAD-67 was only noted in depressed subjects in which antidepressant drugs were absent from postmortem blood. In contrast, subjects with an antidepressant drug in postmortem blood showed no change in protein levels of GAD-67 in comparison to psychiatrically normal control subjects. Antidepressant drugs may either promote synthesis of GAD-67 or prevent the depression-related decrease in GAD-67.

14.7 Preclinical Data on Stress and GABA

The pathology described in the GABA system in depression may be related to effect of chronic stress, which is considered a risk factor for depression. Some animal studies suggest that chronic mild stress and chronic unpredictable stress have a significant effect on the GABA system (reviewed in Hill et al. 2012). For example, the content of GABA is consistently decreased in the hippocampus and frontal cortex following chronic mild stress in the rat (Gronli et al. 2007; Garcia-Garcia et al. 2009; Elizalde et al. 2010b). In contrast, chronic mild stress has highly inconsistent effects on mRNA and protein expression of the GAD-65 and GAD-67 isoforms of the GABA synthetic enzyme in various limbic brain regions. Expression of mRNA for GAD-65 was decreased by this stress and chronic unpredictable stress in the bed nucleus of stria terminalis and preoptic area of the hypothalamus whereas expression of GAD-67 mRNA was decreased in rat prefrontal cortex (Herman and Larson 2001; Lepack et al. 2013). Reduced level of GAD-65 protein has been observed in the ventral hippocampus and frontal cortex following chronic mild stress (Garcia-Garcia et al. 2009; Elizalde et al. 2010b). In contrast, there was a report of increased expression of GAD-65 mRNA and GAD-67 mRNA in the hypothalamus, the bed nucleus of the stria terminalis and the hippocampus following chronic stress (Bowers et al. 1998), whereas, others report that expression of these two markers was unchanged in the same brain regions and in the amygdala, septum, and frontal cortex (Herman and Larson 2001; Herman et al. 2003). Additional studies are necessary to clarify the impact of chronic stress on measures of GAD.

Studies on the influence of chronic mild stress and chronic unpredictable stress on the density of GABA neurons reveal a more consistent effect. In these models of chronic stress, the density of calbindin-IR GABA neurons was decreased in the prefrontal cortex and hippocampus in two studies, whereas the density of parvalbumin-IR GABA neurons was unchanged in these brain regions (Herman and Larson 2001; Nowak et al. 2010; Zadrozna et al. 2011; Lepack et al. 2013). Thus, these studies in a rodent model of chronic stress closely correspond to studies in human postmortem tissue showing a decrease in calbindin-IR GABA neurons but not in parvalbumin-IR GABA neurons in the prefrontal cortex in MDD (Rajkowska et al. 2007). Decreased expression of GAD-67 protein but not GAD-65 was also observed in the same prefrontal cortical region in MDD (Karolewicz et al. 2010).

In summary, chronic stress, neuroimaging studies of depressed patients, and studies of postmortem tissue from depressed subjects show consistent decreases in GABA levels and the density of GABA IR neurons. These reports strongly support a hypothesis of GABA pathology in depression.

14.8 Conclusions

Studies of human postmortem tissue reveal prominent astrocyte pathology in fronto-limbic brain regions in MDD. The mechanisms regulating astrocyte pathology in depression are being explored in preclinical studies which show, in many cases, similar pathology of GFAP and astrocytes in animal models of stress and depressive-like behavior. Astrocyte pathology in MDD appears to be linked to the dysfunction of glutamate and GABA systems as astrocytes are vital components of glutamatergic tripartite synapses. Reductions in the expression of glutamate transporters and enzymes, exclusively found in astrocytes, are detected in studies of postmortem brain tissue from subjects with MDD. Other components of the tripartite synapse, such as postsynaptic glutamate receptors, and glutamate and GABA neurons, are also altered in brain tissue from subjects with MDD. These studies in humans are paralleled by studies in animal models related to depression that show dysregulation of similar components of glutamate and GABA systems as well as astrocytes after exposure to chronic mild and/or chronic unpredictable stress. Moreover, reductions in the density of prefrontal cortical synapses and in the expression of synapse-related genes have been reported in MDD and in animals experiencing chronic stress. This synaptic pathology may be related, in part, to the pathology of astrocytes in MDD since astrocytes control the formation, maturation, function, and elimination of synapses in the brain. Finally, numerous studies implicate glutamate-based drugs as antidepressant in the treatment of depression. Taken together these data suggest that the glutamate synapse is an important substrate in the pathology of MDD. The observations that chronic stress and depression exhibit many similar pathologies in astrocytes and glutamate and GABA support mechanistic studies to identify potential novel targets for new avenues in the treatment of depression.

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