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ASTROCYTE PATHOLOGY IN MAJOR DEPRESSIVE DISORDER: INSIGHTS FROM HUMAN POSTMORTEM BRAIN TISSUE

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Abstract

The present paper reviews astrocyte pathology in major depressive disorder (MDD) and proposes that reductions in astrocytes and related markers are key features in the pathology of MDD. Astrocytes are the most numerous and versatile of all types of glial cells. They are crucial to the neuronal microenvironment by regulating glucose metabolism, neurotransmitter uptake (particularly for glutamate), synaptic development and maturation and the blood brain barrier. Pathology of astrocytes has been consistently noted in MDD as well as in rodent models of depressive-like behavior. This review summarizes evidence from human postmortem tissue showing alterations in the expression of protein and mRNA for astrocyte markers such as glial fibrillary acidic protein (GFAP), gap junction proteins (connexin 40 and 43), the water channel aquaporin-4 (AQP4), a calcium-binding protein S100B and glutamatergic markers including the excitatory amino acid transporters 1 and 2 (EAAT1, EAAT2) and glutamine synthetase. Moreover, preclinical studies are presented that demonstrate the involvement of GFAP and astrocytes in animal models of stress and depressive-like behavior and the influence of different classes of antidepressant medications on astrocytes. In light of the various astrocyte deficits noted in MDD, astrocytes may be novel targets for the action of antidepressant medications. Possible functional consequences of altered expression of astrocytic markers in MDD are also discussed. Finally, the unique pattern of cell pathology in MDD, characterized by prominent reductions in the density of astrocytes and in the expression of their markers without obvious neuronal loss, is contrasted with that found in other neuropsychiatric and neurodegenerative disorders.

Keywords

Glia; Fronto-limbic; Depression; Glutamate; Postmortem

What is Major Depressive Disorder?

Major depressive disorder (MDD) (also known as clinical depression or unipolar depression) is a chronic, recurrent and debilitating mental illness that affects the lives of millions of people worldwide. Depression is the 3rd leading contributor to the global burden of disease with the prevalence of depression among adults in the United States at 6.7 percent [1, 2]. MDD is characterized by core symptoms such as depressed mood, loss of interest or pleasure, changes in weight, changes in sleep, fatigue or loss of energy, feeling of worthlessness, concentration difficulties and thoughts of death or suicide [3]. Although the neurobiology of MDD has been intensely studied for several decades, its underlying

etiopathology is still not fully understood and only about half of individuals with depression respond to currently available treatments [4, 5]. Cellular and molecular abnormalities arising from genetic and environmental factors are believed to be critical in the pathology of depression [6]. A consistent observation of cell pathology in MDD has been reductions in glial cells, and astrocytes, in particular [7].

Introduction to Astrocytes

Of the three kinds of glial cells (i.e., astrocytes, oligodendrocytes, microglia) of the central nervous system (CNS), astrocytes or astroglia are the most numerous and versatile type of glial cells. Rather than serving as static or inert “brain glue” as formerly thought, astrocytes, outnumbering neurons by over fivefold, are now recognized as playing many active roles in the CNS [8-10]. The two major types of astrocytes, protoplasmic and fibrous, are distinguished by their morphology, biochemistry, development and location within CNS [11]. Protoplasmic astrocytes are found in the gray matter. Their numerous processes extend radially from the cell body which contains a spherical nucleus, and give rise to many smaller branches. Some of these processes contact blood vessels to form perivascular endfeet while others cover neuronal membranes. Fibrous astrocytes, in contrast to protoplasmic astrocytes, are present in the white matter. They have ovoid nuclei and fewer, longer and relatively thin processes with very few branches. Their cell bodies are often arranged in rows between the axon bundles and some of their processes reach the perinodal spaces of adjacent axons. Other processes from fibrous astrocytes contact blood vessels to also form perivascular endfeet [12]. Astrocytes are crucial to the neuronal microenvironment by regulating such things as glucose metabolism, neurotransmitter uptake (particularly glutamate), synaptic development and maturation and the blood brain barrier [13]. Studies revealing unexpected pathology in astrocytes in MDD and in rodent models of depressive-like behavior are outlined below. This pathology is unique and differs from reactive astrogliosis described in cases of brain injury, tumor or neurodegenerative disorders.

Human Postmortem Studies

Histopathological studies of postmortem brain tissue reveal prominent glial pathology in MDD. Of the three types of CNS glia, astrocytes are most often implicated as a source of glial pathology in MDD. Several cell counting studies report decreases in the packing density or number of the general, Nissl-stained populations of glial cells in subjects diagnosed with MDD as compared to non-psychiatric control subjects [14-20]. Such changes were observed in fronto-limbic brain regions including the dorsolateral prefrontal cortex [15, 17, 19], orbitofrontal cortex [15], subgenual cortex [14] anterior cingulate cortex [16, 20] and amygdala [18]. However, Khundakar *et al.* [21, 22] noted no change in glial density in the orbitofrontal cortex or anterior cingulate cortex in elderly subjects with MDD.

In addition to alterations in glial cell density and number, the average size of nuclei of glial cells was increased in the gray matter of dorsolateral prefrontal cortex in MDD [15]. In contrast, one other study in the dorsolateral prefrontal cortex reported no change in glial size in MDD [17]. A recent detailed analysis of astrocytes stained with the Golgi method revealed hypertrophy of astrocytic cell bodies and processes in the white matter of the anterior cingulate cortex in depressed subjects dying by suicide [23]. The authors interpret astrocytic hypertrophy as a reflection of local inflammation in support of the neuroinflammatory theory of depression [24].

Markers of Astrocytes

Astrocytes have been localized in brain tissue by antibodies to a number of proteins. These astrocytic markers include glial fibrillary acidic protein (GFAP), gap junctions proteins such

as connexin 40 and 43, the water channel aquaporin-4 (AQP4), a calcium-binding protein S100B and the glutamatergic markers including the excitatory amino acid transporters 1 and 2 (EAAT1, EAAT2) and glutamine synthetase. Evidence is presented below from human postmortem studies demonstrating changes in each of these astrocytic markers in MDD.

GFAP

Glial fibrillary acidic protein (GFAP) is the principle component of cytoskeletal intermediate filaments that is strongly expressed in the CNS by mature and reactive astrocyte cells [25, 26]. Eight isoforms of GFAP have been identified to mark specific subpopulations of astrocytes in the human brain during development, aging and disease, however the function of the specific isoforms is not fully understood [26]. GFAP is expressed in astrocyte processes and cell bodies and it is thought this protein helps astrocytes to maintain mechanical strength and shape [27, 28]. Moreover, GFAP is involved in processes related to cell movement and structure and has been proposed to play a role in astrocyte-neuron communication [29, 30]. Antibodies to GFAP, an astrocyte-specific protein, can be used to immunohistochemically distinguish astrocytes from other types of glial cells [10]. However, antibodies to GFAP only identify about 15–20 percent of astrocytes expressed in the cortex of mature animals [26].

The expression of GFAP in gray matter has been quantified in depression by assessing GFAP-immunoreactive (IR) astrocyte density or the area covered by GFAP-IR cell bodies and processes, so called area fraction. 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 non-psychiatric control subjects [31]. In addition, GFAP-IR area fraction is significantly decreased in the gray matter of the orbitofrontal cortex in a mixture of younger and older subjects with MDD [32]. 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 [31, 33], which may reflect a compensatory reaction to neuronal damage reported in older subjects with MDD [34]. Thus, the pattern of astrocyte pathology in cortical gray matter is unique in younger vs. older subjects with depression [7, 35, 36]. In a semi-quantitative study, Muller *et al.* [37] 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 [37, 38]. In a 3-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.*, personal communication). The observations of pathological changes in GFAP in MDD in gray matter of dorsolateral prefrontal cortex and orbitofrontal cortex have been extended to white matter in other cortical regions. GFAP immunolabeling was decreased in MDD in white matter of the anterior cingulate cortex [39] and the orbitofrontal cortex (Rajkowska *et al.*, unpublished observations).

Expression of GFAP protein and mRNA has also been examined in MDD. Levels of GFAP protein were decreased in gray matter from the dorsolateral prefrontal and orbitofrontal cortex in MDD [32, 40]. An earlier proteomic study reported decreases in four isoforms of GFAP in the frontal cortex of subjects with MDD [41]. In agreement with protein expression studies, GFAP mRNA was also under-expressed in the white matter of the anterior cingulate cortex in MDD [42]. We recently observed a decrease in the expression of mRNA for GFAP in white and gray matter of the orbitofrontal cortex in subjects with MDD (Newton and

Rajkowska, unpublished observations). 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. Moreover, reduced density of astrocytes may account, at least in part, for volumetric reductions reported by neuroimaging studies in the prefrontal cortex of depressed patients [43-47].

Subcortical brain regions also show a reduction in the expression of astrocyte-associated genes and protein. In locus coeruleus, limbic thalamic nuclei, putamen and the internal capsule from subjects with MDD, there was a reduction in glia-associated genes expression [48, 49]. Moreover, Chandley *et al.* [50] recently observed a reduction in expression of GFAP mRNA and protein and a decrease in the density of GFAP-IR astrocytes in the locus coeruleus in MDD. Other astrocyte-associated genes were also down-regulated in MDD in astrocytes that were laser-dissected from locus coeruleus [50, 51]. Finally, significant decreases in GFAP protein expression and GFAP-IR astrocyte density were noted in the cerebellum [52] and amygdala [53] in MDD. Thus, a number of subcortical brain areas in addition to fronto-limbic cortical regions reveal pathology of astrocytes in MDD.

Preclinical studies provide evidence for the involvement of GFAP and astrocytes in animal models of depressive-like behavior. Various types of stress cause reductions in measures of GFAP-IR astrocytes. For example, the stress of separating juveniles from their family diminished the density of GFAP-IR astrocytes in the rodent medial prefrontal cortex [54]. The stress of chronic social defeat reduced the number and soma volume of GFAP-IR astrocytes in the hippocampus of tree shrews [55] and decreased the level of GFAP protein in rat hippocampus [56]. 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 [57]. Furthermore, chronic mild stress significantly decreased levels of GFAP mRNA in rat medial prefrontal cortex [58]. Interestingly, infusion of L-aminoadipic acid in rodent prefrontal cortex, thought to selectively lesion glial cells including GFAP-IR astrocytes but not neurons, induced depressive-like behaviors [59, 60]. These two lesion studies appear to support the hypothesis that the loss of glia contributes to the pathology of depression [7], although this conclusion rests on the specificity of the toxin to glia. There is also correlative support for a role of GFAP-IR astrocytes in depressive-like behavior in Wistar-Kyoto rats, a strain of rats that is genetically predisposed to anxiety-like and depressive-like behavior [61]. 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 control Sprague-Dawley rats [62]. Thus, specific astrocytic deficits in the expression of GFAP in corticolimbic circuits are associated with depressive-like behavior.

Astrocytes have been suggested as a target for therapeutic interventions in depression. Several animal studies reveal an influence of different classes of antidepressant medications on astrocytes. Treatment with fluoxetine, a serotonin-selective reuptake inhibitor (SSRI), prevented the psychosocial stress-induced reduction in astrocyte number [55]. Riluzole, a glutamate modulating drug, also prevented the chronic, mild stress-induced reduction in the expression of GFAP mRNA in the rat prefrontal cortex [58]. The beneficial effects of the SSRI antidepressants citalopram and fluoxetine may involve their ability to induce calcium signals in astrocytes in the prefrontal cortex [63]. However, not all studies show reversibility of number of astrocytes or GFAP level by antidepressants. For example, a four-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 [56]. Likewise, imipramine, a tricyclic antidepressant drug, could not reverse the effects of learned helplessness on hippocampal astrocytes [64].

Other effective treatments for depression such as electroconvulsive therapy (ECT) and transcranial magnetic stimulation can also alter the expression of GFAP in animal models. Repeated treatment of rats with electroconvulsive shock (ECS) increased the expression of GFAP but not neuron specific enolase in limbic brain regions including the piriform cortex, amygdala and hippocampus [65]. Chronic ECS in rats also increases proliferation of astrocytes and changes the morphology of astrocytes and other types of CNS glia in limbic brain regions including hippocampus, piriform cortex and prefrontal cortex [66]. A single ECS or transcranial magnetic stimulation up-regulated mRNA for GFAP selectively in the dentate gyrus of the mouse hippocampus [67, 68]. Thus, ECT and transcranial magnetic stimulation therapy can modulate expression of the gene for GFAP and therefore may account for some features of astrocytic plasticity in the action of antidepressant treatments of depression.

In summary, models of chronic stress in experimental animals significantly diminish cortical and hippocampal astrocytes as measured with 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 antidepressant medications. Upregulation in GFAP mRNA can also be induced by ECS or transcranial magnetic stimulation. Thus, in light of astrocyte deficits noted in MDD and stress being a risk factor for depression, astrocytes may be potential novel targets for the action of antidepressant medications [69].

Connexins

Connexin 30 and connexin 43 are two other proteins located on astrocytic endfeet that implicate these glial cells in the pathology of depression. Connexin 30 and connexin 43 are gap junction-forming membrane proteins that allow communication between astrocytes [70]. Decreases in the gene and protein expression of connexin 30 and connexin 43 have been observed in the dorsolateral prefrontal cortex of suicide completers, some of whom were diagnosed with MDD [71]. In the orbitofrontal cortex, a decrease in the expression of connexin 43 protein was noted in MDD (Miguel-Hidalgo, personal communication). In addition, down-regulation of genes for connexin 43 and 40 was reported in the locus coeruleus of subjects with MDD [49]. Moreover, rats exposed to chronic unpredictable stress exhibited depressive-like behavior and abnormal morphology and functions of gap junctions as well as a significant decrease in the expression of connexin 43 [72]. These stress-induced changes were reversed by chronic treatment with the antidepressant medications fluoxetine or duloxetine. In another study, connexin 43 was also up-regulated in rat prefrontal cortex after chronic exposure to fluoxetine [73]. Taken together, these animal studies support the involvement of connexin in depression and the therapeutic action of antidepressant medications. The consequences of decreased expression of connexin 30 and connexin 43 may alter calcium wave propagation and communication between astrocytes [64, 74]. Finally, mice lacking connexin 30 and connexin 43 had a weakened blood-brain barrier, in which astrocytes are a crucial component [75].

AQP4

A recent study has implicated another protein, aquaporin-4 (AQP4), in the pathophysiology of MDD [76]. AQP4 is located predominantly in astrocytic endfeet that are in contact with blood vessels [77, 78]. AQP4 is a water channel that regulates water and ion homeostasis in the brain and is an integral part of the neurovascular unit [79-81]. Reduced coverage of blood vessels by astrocytic endfeet that are immunopositive for AQP4 has been observed in the orbitofrontal cortex in subjects with MDD as compared to psychiatrically-normal control subjects [76]. In addition, a decrease in the expression of mRNA for AQP4 was identified in

locus coeruleus in MDD [49]. These decreases in AQP4 in MDD could affect many brain functions as AQP4, in addition to its role in water redistribution, also regulates cerebral blood flow [82, 83], glucose transport and metabolism [84], integrity of the blood brain barrier [85, 86], glutamate turnover [87] and synaptic plasticity [88]. Thus, AQP4 may serve as a marker of astrocytic pathology in MDD.

S100B

Another marker of astrocytes is the calcium binding protein S100B [89]. In the gray matter, this protein is predominantly expressed and secreted by astrocytes, while oligodendrocytes expressed S100B in white matter [90-92]. Levels of expression of S100B mRNA and protein, both intracellular and extracellular, may signify astrocyte reaction or death in several types of brain injury [93-96]. Altered expression of S100B may also be present in mood disorders. A transcript for S100B was down-regulated in the ventral prefrontal cortex of depressed suicide victims [97].

Damage to astrocytes causes leakage of S100B into the extracellular compartment and cerebrospinal fluid, continuing to the bloodstream [98]. *In vivo* support for damage to astrocytes in depression is provided by reports of elevated levels of S100B in the cerebrospinal fluid and serum during major depressive or manic episodes [98-103]. In contrast, six weeks of successful antidepressant treatment lowered serum level of S100B [103]. In the study by Schroeter *et al.* [103], both the astroglial marker S100B and neuron-specific enolase, a marker specific for neurons, were measured. Only S100B was elevated with no change noted in neuron-specific enolase in mood disorders. Thus, the *in vivo* studies on S100B support our original hypothesis based on postmortem brain tissue that depression is characterized by prominent glial and only subtle neuronal pathology [7].

Glutamate Markers

We extensively review studies of depression which are specifically related to glutamate markers located on or in astrocytes. While astrocytes have transporters and receptors for other neurotransmitters, the role of astrocytes in glutamate neurotransmission has been studied much more extensively in depression using postmortem brain tissue than the role of astrocytes in other neurotransmitter systems. Thus, our review is focused on the role of astrocytes in glutamate neurotransmission in depression.

Astrocytes are actively involved in the uptake, metabolism and recycling of glutamate. Extracellular levels of glutamate are regulated by removal of this neurotransmitter from the synaptic cleft via specialized transporters located on astrocytic processes [104]. In human brain these glutamate transporters include the excitatory amino acid transporter-1 and -2 (EAAT1 and EAAT2) which in rodents are known as glutamate-aspartate transporter (GLAST) and glutamate transporter 1 (GLT1), respectively [105, 106]. The internalized glutamate is subsequently converted within astrocytes into glutamine by the enzyme, glutamine synthetase [107]. Glutamine leaves astrocytes to be taken up by neurons where it can be reconverted into glutamate or GABA. Thus, astrocytes play a critical role in several aspects of glutamate neurotransmission.

Glutamate transporters and glutamine synthetase associated with astrocytes may serve as markers of astrocyte function and these markers 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 postmortem brain samples from subjects with MDD [108]. 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 [109]. Moreover, the expression of EAAT1, EAAT2 and glutamine synthetase protein was also reduced in the orbitofrontal cortex in immunohistochemical and Western blotting studies of subjects with MDD [32]. Finally, glutamate signaling and astrocyte-associated genes were under-expressed in locus coeruleus in MDD [49, 50, 51], 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.* [49] demonstrating that these changes in glutamate-related gene expression do not occur in neurons. Other evidence supporting a role for dysregulated astrocytic glutamate uptake in depression comes from rat studies where the pharmacological blockade of glutamate uptake into astrocytes in the amygdala [110], ventral tegmental area [111] or in the prefrontal cortex [112] 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 [113, 114].

Neuroimaging studies reveal alterations in glutamate in MDD that may be related to cellular changes detected in postmortem brain tissue. Levels of glutamate and glutamine or combined glutamate/glutamine (Glx) are significantly decreased in living subjects with MDD, as determined in plasma [115] and several brain regions including prefrontal [116, 117] and anterior cingulate cortex [118-120], and the combined region of amygdala plus anterior hippocampus [121, 122]. However, one imaging study and one study in postmortem brain tissue reported increases in glutamate levels in the occipital cortex and frontal cortex, respectively, in MDD [123, 124]. These differing reports of glutamate levels in depression may be due to variations in the brain region examined, relative involvement of the hypothalamic-pituitary-adrenal axis, and the age of the subject and chronicity of depression.

The sequence of events relating glial pathology with changes in glutamate levels is not clearly understood. Based on studies outlined above, one hypothesis suggests that elevated levels of glucocorticoids, which significantly decrease expression of mRNA for GFAP [125], reduce the density of astrocytes early in the course of depression [7]. As a result, extracellular glutamate accumulates due to fewer glutamate transporters EAAT1 and EAAT2 located on astrocytes. These initially high levels of extracellular glutamate may also be toxic to GABA neurons [126, 127]. Diminished astrocytic uptake of glutamate, and its reduced subsequent conversion to glutamine and eventually to glutamate, may be related to reduced density of glutamatergic pyramidal neurons in elderly depressed subjects [34] and be responsible for depleted cortical levels of glutamate as depression progresses.

Functions of Astrocytes and Consequences of Their Pathology in MDD

In addition to astrocyte functions described above, astrocytes are involved in other higher brain functions. For example, astrocytes actively control neuronal activity and synaptic neurotransmission. They are an active component of the tripartite synapse, including 1) the presynaptic terminal, 2) the postsynaptic neuronal membrane and 3) the surrounding astrocyte [128, 129]. Within the synapse, astrocytes respond to neuronal activity by elevating their internal Ca^{2+} concentration that triggers the release of glial transmitters which, in turn, regulate neuronal activity [128, 129]. Astrocytes are also crucial in promoting the development and modeling of synapses [130-133]. Correspondingly, the density of synapses and expression of several synapse-related genes were significantly decreased in the dorsolateral prefrontal cortex in the same depressed subjects in which astrocyte density was decreased [31, 134].

Astrocytes also modulate glucose metabolism, as well as neuronal activity and synaptic density. Astrocytic endfeet enveloping cerebral microvasculature are enriched with glucose

transporters. Glucose, the main energy substrate for neurons and glia, is taken up by the astrocytic glucose transporter. In astrocytes, glucose undergoes glycolysis and oxidative phosphorylation and this is believed to provide the observed signal in functional magnetic resonance imaging [135, 136]. In positron emission tomography (PET), the uptake of radioactive ^{18}F -deoxyglucose by astrocytes is associated with neuronal activity [137]. A well-replicated finding in the PET literature is that of reduced glucose metabolism in prefrontal cortex in MDD (reviewed in [138]). Astrocytes are also able to control blood flow by inducing local vasoconstriction or vasodilation [139-141]. Thus, defective or missing astrocytes, as reported in studies of postmortem brain tissue from subjects with MDD, may significantly contribute to the changes in cerebral blood flow in depressed patients as noted with PET and functional magnetic resonance imaging (fMRI).

Astrocytes regulate synaptic and intracellular levels of neurotransmitters via transporters located on their processes. In addition to the glutamate transporters described above, astrocytes also have transporters for GABA [142-144], serotonin [145, 146], dopamine [147, 148], norepinephrine [147-149] and histamine [150] and they contain the enzyme monoamine oxidase (MAO) [151], which further regulates the concentrations of the monoamine neurotransmitters. Abnormally elevated levels of MAO-A have been reported in patients with MDD [152-154]. In postmortem prefrontal cortex from subjects with MDD, an elevated expression of MAO-A protein is coupled with a reduction in expression of R1, a novel protein that represses expression of MAO-A [155]. Thus, astrocytes may be a significant cellular substrate for the above mentioned pathology of MAO in depression. Astrocytes express nearly all of the receptors and ion channels found in neurons [156-165], although no specific pathologies of these astrocytic receptors have been linked to depression.

Astrocytes also play a role in inflammatory and neurodegenerative processes in the brain. Reactive astrocytes, together with microglia and oligodendrocytes, are both source and target of various inflammatory cytokines such as interleukins and tumor necrosis factor. These cytokines are expressed under pathological conditions and involved in the regulation of neuroinflammation, immune processes and tissue repair [166]. Peripheral cytokines may serve as biomarkers of MDD and the response to antidepressant treatment, and their entry into the brain may induce production of cytokines by astrocytes [167]. For example, in MDD, pro-inflammatory markers in plasma such as C-reactive protein (CRP), interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) were increased in depressed patients as compared to control subjects [168-171]. Protein and mRNA levels of IL-1, IL-6 and TNF- α in prefrontal cortex were also significantly increased in teenage suicide victims with a mood and/or psychoactive substance use disorder [172]. Treatment with antidepressant medications (SSRIs) reduced plasma levels of inflammatory cytokines in patients with MDD [168, 173-177].

Astrocytes, in addition to microglia, also respond to neuronal injury. The astrocytic reaction to injury involves increasing their number, size and expression of GFAP [178, 179]. However, in direct contrast, astrocytic pathology in depression is characterized by a *decrease* in astrocyte density and expression of GFAP. Thus, MDD is viewed primarily as a disease of disrupted neuroplasticity and cellular resilience rather than cell loss [180, 181]. Evidence for impaired neuroplasticity in depression is provided by studies using postmortem brain tissue and by preclinical models reporting a reduction in protein and mRNA expression of neurotrophic factors and their receptors such as brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 2 (FGF2) [182-190]. Conversely, repeated treatment with antidepressant medication up-regulates expression of BDNF and FGF2 factors in humans and rodents [183, 191-193]. The reduction in neurotrophic factors in depression may be occurring in astrocytes, in addition to neurons, as astrocytes also secrete

neurotrophic factors [194]. Cultured cortical astrocytes treated with SSRIs showed an increase in expression of BDNF and other neurotrophic factors [195]. Thus, astrocytic pathology may significantly contribute to the disrupted neuroplasticity of depression.

Another nerve growth factor implicated in depression and the mechanism of action of antidepressant medications is a glial cell line-derived neurotrophic factor (GDNF). Several studies reveal that this protein was under-expressed in the peripheral blood of depressed patients [196-198], while GDNF levels in hippocampus were significantly decreased in the rodent model of chronic unpredictable stress [199]. In rodents, the chronic administration of electroconvulsive shock but not antidepressant medications increased levels of the GDNF receptors (GFRalpha-1 and GFRalpha-2) in hippocampus, while repeated clomipramine treatment reversed the chronic stress-induced decrease in hippocampal GDNF [199, 200]. In subjects with MDD, serum levels of GDNF were significantly increased by chronic treatment with antidepressant medications or in responders to electroconvulsive therapy [196, 201]. One study has examined GDNF directly in postmortem brain tissue in a small number of subjects with recurrent or reactive depression [202]. Several cortico-limbic regions were examined, but there was a paradoxical increase in the expression of GDNF protein in only the parietal cortex. Additional studies need to be performed on GDNF and its receptors in postmortem human brain of subjects experiencing recurrent depression or major depressive disorder to determine whether brain levels correspond to plasma levels of GDNF in depression.

Unique Pattern of Glial and Neuronal Cell Pathology in MDD

As described above, MDD is a disorder with a prominent pathology of astroglia. However, the pattern of astrocyte pathology in MDD is very different from that observed in several neurological and neurodegenerative disorders such as CNS injury, brain tumors, CNS inflammation, stroke, epilepsy, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease or Alzheimer's disease. In these disorders, reactive astrogliosis, glial scar formation and neuronal degeneration take place [10]. In contrast to neurodegenerative disorders where there is astrogliosis and an increased expression of GFAP, in MDD there is no astrogliosis, the expression of GFAP and other markers of astrocytes is decreased and no prominent neuronal pathology is observed. Thus, the neuropathology of depression is significantly different from that observed in brain injury or neurodegeneration.

There are several lines of evidence pointing to the preservation of neurons in MDD. Most cell counting studies in postmortem brain tissue did not find reductions in neuronal density or total number of neurons in subjects with MDD [14, 16, 17, 21, 22, 203-205]. Rather, smaller sizes of neuronal cell bodies or reductions in dendritic branching are reported [15-17, 19, 203, 206, 207] suggesting neuronal atrophy rather than neuronal loss in MDD. In addition, *in vivo* studies measuring brain metabolite N-acetylaspartate (NAA), a putative marker of neurons revealed no changes in the concentration of this marker in the frontal lobes and basal ganglia in depressed patients further suggesting no neuronal loss in depression [208]. In another study measuring serum levels of neuron-specific enolase, a specific marker of neurons in the CNS, there were no changes in patients with MDD compared to control patients [103, 209]. In the same study of depressed patients, there was an increase in levels of serum S100B, a marker of astrocytes. The expression of mRNA for the neuronal markers neurofilament and enolase2 was not significantly changed in the locus coeruleus of subjects with MDD, whereas mRNA for glial markers (glial glutamate transporters and glutamine synthetase) were significantly reduced as compared to control subjects [49]. Interestingly, a neuron-specific toxin injected into the rodent prefrontal cortex caused no change in behavior while ablation of glia with a glial-specific toxin led to depressive-like behaviors [59]. In summary, cellular pathology in MDD appears to be

selective for glia without obvious neuronal loss. It is noteworthy that such pathology appears to apply mostly to younger and middle age subjects with MDD (<60 years-of-age) [31, 35, 40]. In older subjects with MDD (>60 years-of-age), neuronal pathology consisted of prominent reductions in the density of pyramidal glutamatergic neurons in the orbitofrontal cortex [34, 35]. In contrast, astrocytes density and GFAP levels are unaltered in elderly depressed compared to age-matched control subjects [31, 33, 40]. In light of such age-related cell pathology in MDD, we hypothesized that glial (and astrocytic, in particular) reductions take place early in depression and neuronal pathology occurs later in the progression of the disease [7]. Neuronal pathology may be induced by excitotoxicity due to an excess of extracellular glutamate building up in synaptic cleft due to reduced numbers of astrocytes and astrocytic glutamate transporters. Later in the course of depression, astrocytes may react to neuronal pathology by maintaining their density with age in the face of decreased neuronal density. Support for this hypothesis comes from our observation of no significant reductions in astrocyte density or expression of GFAP in elderly subjects with depression (for further details on this hypothesis see [7]).

Astrocyte Pathology in Other Neuropsychiatric Disorders

Although glial pathology has been noted in fronto-limbic cortex in several psychiatric disorders, the specific features of that pathology are unique in each disorder. In postmortem brain tissue of subjects diagnosed with MDD, a large number of markers of astrocytes are consistently altered in fronto-limbic cortical areas and related subcortical regions. Astrocytic proteins such as GFAP, AQP4, connexins, glutamate transporters (EAAT1 and EAAT2) and glutamine synthetase are all decreased in MDD.

In bipolar disorder, however, studies of postmortem brain tissue reveal mixed results with respect to GFAP and glutamine synthetase. One study reported a decrease in the optical density of GFAP-IR across all layers of the orbitofrontal cortex in bipolar disorder [210]. In contrast, another study found no significant changes in the density of astrocytes immunoreactive for GFAP in the gray or white matter of the subgenual cingulate cortex [211], while the expression of mRNA for GFAP was decreased in the adjacent dorsal portion of the anterior cingulate cortex in subjects with bipolar disorder [42]. However, expression of glutamine synthetase, mostly localized to astrocytes, was not significantly changed in the dorsolateral and orbitofrontal cortex in bipolar subjects [210] while the expression of mRNA and protein for glutamine synthetase was significantly decreased in these two regions in MDD [32,108].

In schizophrenia, relatively few studies have examined astrocytes and have yielded inconsistent results again as with bipolar disorder. The density of astrocytes immunoreactive for GFAP was not significantly changed in schizophrenia in few cortical and subcortical regions [212-214], while other studies noted a significant decrease in the density of these cells or mRNA expression for GFAP in the dorsolateral prefrontal, orbitofrontal and subgenual cingulate cortex [42, 210, 211, 215]. In contrast, Toro *et al.* [210] reported a significant increase in the GFAP-IR in dorsolateral prefrontal cortex in schizophrenia. In contrast to MDD, immunoreactivity for glutamine synthetase was not significantly affected in dorsolateral prefrontal or orbitofrontal cortex in schizophrenia [32, 210].

Alcohol dependence is another psychiatric disorder, whether comorbid with MDD or not, that is characterized by reductions in GFAP immunoreactivity and density of GFAP-IR astrocytes [216, 217]. However, in contrast to reductions in MDD, immunoreactivity for glutamatergic markers in astrocytes (glutamine synthetase, EAAT1 and EAAT2) was not significantly affected in orbitofrontal cortex in alcohol dependence [32].

The pattern of astrocyte cell pathology differs between neurological and depression disorders. In neurological disorders there is a loss of neurons with an accompanying proliferation of astroglia. On the other hand, in MDD there is prominent reduction in astroglial density. Thus, MDD should not be considered as a neurodegenerative disorder but rather a disorder of disrupted neuroplasticity and cellular resilience.

Concluding Remarks

Astrocyte pathology in MDD is well documented by a number of quantitative studies on postmortem fronto-limbic brain regions. There are consistent reductions in the density of astrocyte cell bodies immunoreactive for GFAP protein in MDD. Proteins expressed by astrocytes, such as GFAP, AQP4, connexins, glutamate transporters (EAAT1 and EAAT2) and glutamine synthetase are also decreased in MDD. Although the extent of astrocytic pathology in MDD has been examined for several years, accessible molecular targets to mitigate their obvious dysfunction have not been identified. The induction of depressive-like behaviors in rodents by lesions to astrocytes in prefrontal cortex may be evidence that astrocytic pathology in humans is crucial in the development of depression. Astrocyte pathology similar to that observed in MDD was noted in rodent models of chronic stress and depressive-like behaviors and repeated treatment with antidepressant medications reversed the morphological and behavioral changes observed after exposure to chronic stress. Thus, astrocytes may be a therapeutic target for novel and more effective antidepressant treatments. Further studies on the reciprocal communication between astrocytes and neurons are needed to better understand the effects of astrocyte pathology on neurons in MDD.

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