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Journal:	*Expert Review of Neurotherapeutics*
Manuscript ID	ERN-2015-0099.R1
Manuscript Type:	Reviews
Keywords:	Astrocyte, Major depression, mechanism of drug action, SSRI, neuropsychiatric diseases

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ERN 1511
REVIEW
US ENGLISH

Targeting astrocytes in major depression

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Running title: Therapeutic potential of astroglia in major depression

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Abstract

Astrocytes represent a highly heterogeneous population of neural cells primarily responsible for the homeostasis of the central nervous system. Astrocytes express multiple receptors for neurotransmitters, including the serotonin 5-HT_{2B} receptors and interact with neurones at the synapse. Astroglia contribute to neurological diseases through homeostatic response, neuroprotection and reactivity. In major depression, astrocytes show signs of degeneration and are decreased in numbers, which may lead to a misbalance in neurotransmission and aberrant synaptic connectivity. In this review we summarise astroglia-specific effects of major antidepressants and outline future strategies for astroglia-specific therapy in neuropsychiatric disorders.

Key words: astrocytes; major depression; mechanism of drug action; SSRI; neuropsychiatric diseases

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INTRODUCTION: ASTROCYTES MAINTAIN THE INTEGRITY OF THE BRAIN

The central nervous system (CNS) tissue is an intricate network of many cell types, which are represented by neurones, neuroglia, pericytes, muscle and endothelial cells of brain vessels, ependymal cells etc. The neuroglia conceptually are defined as a heterogeneous population of cells of neural and non-neural origin which provide for homeostasis and defence of the CNS [1, 2]. Neuroglia are sub-classified into astrocytes, oligodendrocytes, NG2 glia (all are of neuroepithelial, ectodermal origin, also known as macroglia) and microglial cells of mesodermal, myeloid descent [1, 2]. Astrocytes are highly heterogeneous glial cells, which populate the brain and spinal cord and are primarily responsible for homeostasis of the CNS [2].

Astrocytes significantly contribute to the brain metabolism (see [3] for comprehensive review on astroglial bioenergetics) and are central elements for potassium ion (K^+) buffering, neurotransmitter homeostasis, maintenance and regulation of synaptic transmission and CNS metabolism. Astrocytes are the only cells in the brain capable of synthesizing glutamate from glucose, and are essential for the supply of glutamatergic and GABA-ergic neurones with glutamine that is subsequently converted into glutamate and GABA [4]. In the glutamate-glutamine cycle, glutamate released from neurones is mainly taken up into astrocytes by Na^+-dependent transporters EAAT1⁄2 (excitatory amino acid transporters 1 and 2 - see [5]). In astrocytes glutamate (together with the *de novo* synthesized glutamate) is converted to glutamine, which is subsequently transported to neurones through the system of cell-specific glutamine transporters; in neuronal terminals glutamine serves as a precursor for glutamate and GABA [6] this cycles are known as glutamate- glutamine and GABA - glutamine shuttles. Astrocytes themselves are also capable of releasing glutamate via different mechanisms, including Ca^{2+}-regulated exocytosis and diffusion through membrane channels [7].

Astrocytes express a multitude of neurotransmitter receptors, including NMDA and non-NMDA glutamate receptors, purinoceptors, serotonin ($5\text{-}HT_2$) receptors and □- and □-adrenergic receptors [8], although expression of the receptors varies substantially between brain regions [9]. Stimulation of neurotransmitter receptors in astrocytes activates cytosolic and nuclear signalling pathways, altering cellular functions as well as gene expression. Many astroglial receptors are linked to an increase in free cytosolic calcium concentration ($[Ca^{2+}]_i$), which can activate secretion of numerous astroglia-derived signalling molecules that may in turn modulate neuronal activity. Astroglial Ca^{2+} signals can also propagate through astrocytic network as Ca^{2+} waves [10]. Astrocytes also utilise transient fluctuations in intracellular Na^+ concentration to regulate numerous plasmalemmal transporters [11]. Pharmacological agents interacting with neurotransmitter receptors can therefore influence neuronal-astroglial communication. Astrocytes are important source of the brain cytokine network that is involved in the pathogenesis of various psychoses [12]. Three astrocytic membrane proteins (the Na,K-ATPase, the sodium-potassium chloride co-transporter NKCC1 and inward rectifier $K_{ir}4.1$ channels) are involved in extracellular K^+ buffering. The Na,K-ATPase is the main K^+ transporter at $[K^+]_e$ of ~10 mM as has been shown by inhibition

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with ouabain (for review, see [13] and references therein). At higher $[K^+]_e$, NKCC1 plays the dominant role, as shown by inhibition with bumetanide, an inhibitor of the co-transporter NKCC1. The NKCC1, however, it is metabolically driven by ion gradients established by the Na,K-ATPase. The $K_{ir}4.1$ channels are differentially involved in K^+ buffering in various regions of the brain.

The importance of astrocytes in pathology is yet underappreciated, although astroglia contribute to the pathogenesis of most (if not all) neurological disorders [2, 10]. In this review we shall focus on the effects of drugs used in neuropsychiatry on astrocytes.

ASTROCYTES IN MAJOR DEPRESSION

Astroglial reaction to major depression (similarly to other psychiatric pathologies) does not involve astrogliosis and hypertrophy; rather the astrodegenerative signs prevail. The number of astrocytes, and expression of astrogliotic marker GFAP is significantly decreased in the brains of depressed patients (for review, see [14] and references therein). At the same expression of GFAP is increased in the brains of patients treated with antidepressants. Expression of mRNA for another astroglial marker, the calcium binding protein S100□ was found to be reduced in the ventral prefrontal cortex of depressed suicide victims. A large scale screening of thirty-six biological markers in thirty inbred mouse strains [15] showed that GFAP and S100□□□proteins are among four genes in mouse brains responding to chronic treatment with fluoxetine (the other two being glyoxylase 1 and histone deacetylase 5). Similarly, reduction in the number of astroglial GFAP-positive profiles and overall GFAP immunoreactivity were detected in several animal models of chronic stress (see [14] and references therein). In animal models selective ablation of astroglial cells (with L-□-aminoadipic acid, which poisons astrocytes) triggered depressive behavior (see [14] and references therein). Pharmacological inhibition of astroglial gap junction connectivity [16], or astroglial plasmalemmal glutamate transporters [17] resulted in anhedonia, one of the key symptoms of depression. All these findings indicate astrocytic abnormality in the MD.

Contemporary pathophysiology regards imbalance in neurotransmission and, in particular, aberrant glutamatergic neurotransmission as a primary mechanisms for major psychiatric disorders, including major depression [18, 19]. Astrocytes are fundamental elements of glutamatergic and GABAergic neurotransmission being the hubs for glutamate-glutamine and glutamine - GABA shuttles (see above and [20]). In the brains of MD patients, expression of astrocyte-specific glutamate transporters EAAT1/2 as well as of glutamine synthetase is reduced (for review, see [14]), indicating compromised astrocytic uptake glutamate and decreased glutamine production. An increase in glutamate release and a decrease of the glutamate uptake in the hippocampus were also detected following exposure to the chronic unpredictable stress [21]. Decreased glutamate uptake was also observed in the hippocampus, striatum and prefrontal cortex after completion of a learned helplessness paradigm [22]. Experiments in rats showed that blockade of astroglial glutamate uptake was sufficient to decrease sucrose consumption, which is indicative of anhedonia (see [14] and references therein). Furthermore, antidepressant effects of two glutamate modulators,

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ketamine and riluzole have been demonstrated in patients and in animal models ([23] and references therein).

Serotonin-specific reuptake inhibitors' (SSRIs)

The SSRIs fluoxetine, fluvoxamine, sertraline, paroxetine, and citalopram are, arguably, the most widely used antidepressants around the world. Increased of extracellular concentration of serotonin by inhibition of the serotonin transporter (SERT) has been regarded as SSRI's sole (and undisputed) mechanism of action, despite of the fact that the inhibition of the transporter is instant, whereas the clinical response requires weeks of treatment. Moreover, the relatively high affinity of 5-HT_{2B} receptor for fluoxetine (see [24] and references therein) was unknown when it was concluded that the SSRIs had no receptor-mediated effects.

Experiments on astrocytes *in vitro* revealed high levels of expression of 5-HT_{2B} receptors, together with 5-HT_{2A} and 5-HT_{2C} receptors as well as non-5-HT_2 receptors; the 5-HT_{2B} receptors being indispensable for SSRIs action (see [8, 24] and references therein). In addition, cultured astroglial cells express high levels of monoamine oxidase (MAO) A and B, allowing them to metabolise 5-HT. Both acute and chronic effects of SSRIs on astrocytes can be suppressed by specific inhibition of 5-HT_{2B} receptors, indicating that SSRIs act as agonists of astroglial 5-HT_{2B} receptors (see [24] and references therein); which conclusion has been independently confirmed [25].

Acute effects of SSRIs

5-HT_{2B} receptors

All astroglial 5-HT_2 receptors are $G_{q/11}$ protein-coupled; stimulation of these receptors with serotonin activates phospholipase C (PLC), thus generating diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($InsP_3$) by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2). The $InsP_3$ in turn activates $InsP_3$ receptors of the endoplasmic reticulum membrane which leads to an increase of free cytosolic calcium concentration ($[Ca^{2+}]_i$) ([5] and references therein). The 5-HT_{2B} receptor functionally expressed in astrocytes has much higher affinity to 5-HT than the 5-HT_{2A} and 5-HT_{2C} receptors [24]. Exposure to fluoxetine triggers an increase in $[Ca^{2+}]_i$ in cultured astrocytes [24], as well as in astrocytes in brain slices [26]. Cellular calcium signals have numerous targets that may include glycogenolysis, which in astrocytes is stimulated by treatment with fluoxetine ([24]).

ERK signalling cascade

Acute treatment with fluoxetine at concentrations 1 □M or higher stimulates $ERK_{1/2}$ phosphorylation [24]. This effect was abolished by broad spectrum 5-HT_2 receptors antagonist SB204741, although neither SB242084, a selective 5-HT_{2C} receptor antagonist, nor M100907, a selective 5-HT_{2A} receptor antagonist, had an effect; hence fluoxetine stimulation of $ERK_{1/2}$ phosphorylation is likely to be mediated through 5-HT_{2B} receptor ([24] and

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references therein). The stimulation of $ERK_{1/2}$ pathway was also abolished by deletion of the 5-HT_{2B} receptor with small interfering RNAs (siRNAs), further corroborating the above conclusion [24]. The fluoxetine-induced and 5-HT_{2B} receptor-mediated $ERK_{1/2}$ phosphorylation, as well as the effect of fluoxetine on EGFR phosphorylation, could be abolished by both AG1478, an inhibitor of the EGFR tyrosine kinase and by GM6001, a potent and broad-based inhibitor of Zn^{2+}-activated metalloproteinases, suggesting the role for EGF receptor transactivation ([24] and references therein). $ERK_{1/2}$ phosphorylated in response to fluoxetine can enter cell nuclei, thus regulating gene expression ([27] and references therein). The mRNA and protein expression of *cfos* and *fosB* is also induced in astrocytes treated with fluoxetine ([27] and references therein), contingent on $ERK_{1/2}$ phosphorylation.

Chronic effects of SSRIs

The Ca^{2+}-dependent phospholipase A_2

The Ca^{2+}-dependent phospholipase A_2 ($cPLA_2$ IVA) mobilises arachidonic acid, which is a precursor for a multiple physiologically active molecules, including prostaglandins and inflammatory agents [28]. Chronic treatment with fluoxetine led to an up-regulation of $cPLA_2$ in the brain *in vivo* [24], which is likely to be associated with astrocytes that predominantly express $cPLA_2$ in the grey matter [27]. Chronic treatment with fluoxetine increased expression of $cPLA_2$ mRNA and protein in mouse astrocytes in primary cultures in a time and concentration-dependent manner ([27] and references therein). Similarly chronic treatment of cultured astrocytes with other commonly used SSRIs, such as paroxetine, fluvoxamine, sertraline, and citalopram at 1 □M concentration up-regulates $cPLA_2$ [24]. Chronic treatment of mice with fluoxetine increased expression of $cPLA_2$-specific mRNA solely in astrocytes, as was demonstrated in experiments with fluorescence-activated sorting of neurones and astrocytes in transgenic animals, carrying astroglial (FVB/NTg(GFAP-GFP)14Mes/J) and neuronal (B6.Cg-Tg(Thy1-YFPH)2Jrs/J) markers [27].

Kainate receptors GluK2

Astrocytes express several types of ionotropic and metabotropic glutamate receptors [4]. Kainate GluK2 receptors were identified in astrocytes in hippocampus and in the spinal cord [27]. In astrocytes, glutamate-induced ERK phosphorylation is mediated by GluK2 receptors, as demonstrated by an inhibitory effect of NS102, a specific antagonist of GluK1 and GluK2, and siRNA-induced down-regulation of GluK2 expression ([27] and references therein). Fluoxetine abolishes glutamate-induced $ERK_{1/2}$ phosphorylation and astroglial Ca^{2+} signals [27], suggesting possible connection with GluK2 editing. Adenosine deaminases acting on RNA's (ADARs) constitute an enzyme family that catalyzes the deamination of adenosine to inosine in double-stranded regions of mRNAs. This changes the translated protein sequence, because inosine is perceived by the cells as guanosine [27]. Fluoxetine up-regulates ADAR2 mRNA and protein expression in cultured astrocytes as well as in astrocytes in the *in vivo* brain, which may affect GluK2 editing. Indeed, an increased editing of GluK2 was identified by amplification refractory mutation system (ARMS)-PCR (B. Li and L. Peng, unpublished

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results) and by DNA sequencing [29]. It involved three editing sites (I/V, Y/C and Q/R sites) of GluK2 in the intact brain *in vivo*, in cultured astrocytes and in freshly isolated astrocytes [29, 30].

5-HT_{2B} receptor

Up-regulation of expression and RNA editing of the 5-HT_{2B} receptor by chronic treatment with fluoxetine was found in cultured astrocytes (B. Li and L. Peng, unpublished results) and in astrocytes from fluoxetine-treated mice [30]**.** In cultured astrocytes these changes were inhibited by specific siRNA against the 5-HT_{2B} receptor. Fluoxetine treatment substantially increased editing at 8 sites in 5-HT_{2B} receptor mRNA between position 793 and position 805, encoding amino acids Ile-Lys-Lys-Pro-Ile (5-HT_{2B} (NM_008311.2)) [30].

Growth factors

Chronic treatment of primary cultured astrocytes with fluoxetine induced gene expression of brain derived nerve factor (BDNF) and its receptors; likewise this treatment increased expression of glial derived nerve factor (GDNF) and deiodinase 3 (D3) [31, 32]. The SSRIs fluoxetine and paroxetine, but not the tricyclic antidepressants (TCAs), desipramine or imipramine, increased expression of mRNA for BDNF, vascular endothelial growth factor (VEGF) and VGF in cultured astrocytes [33]. Incidentally, increased expression of BDNF by fluoxetine in hippocampal astrocytes may be linked to anxiolytic-like activities [34]. The concomitant induction of neurogenesis, however, is probably not related to the therapeutic effect [35].

Transient receptor potential canonical 1 (TRPC1) channels

Astroglial Ca^{2+} signalling, generally regarded as the substrate for glial excitability [10], is mainly generated by Ca^{2+} release from the ER, mediated by $InsP_3$ receptors ($InsP_3Rs$) or ryanodine receptors (RyRs), as well as by Ca^{2+} entry across the cell membrane through Ca^{2+}-permeable channels [10]. The release of Ca^{2+} from the ER is driven by intra-ER Ca^{2+} concentration, that in turn reflects a balance between ER Ca^{2+} release and ER Ca^{2+} refilling. Depletion of ER Ca^{2+} activates “capacitative” Ca^{2+} entry through store-operated channels (SOCEs). An essential component of SOCEs in astrocytes is the cationic channel TRPC1 [36]. Chronic treatment of astrocytes with fluoxetine reduces neurotransmitter-induced Ca^{2+} signalling [37], which, in part, reflects TRPC1 dysfunction. In contrast, the same treatment with fluoxetine increased depolarisation-induced Ca^{2+} entry due to an up-regulation of L-type Ca^{2+} channels [37]. Incidentally, SSRIs possess an anxiolytic capability, and the quintessential anxiolytic drugs, the benzodiazepines, can also increase depolarisation-induced (i.e. mediated by voltage-gated channels) Ca^{2+} signals ([24] and references therein).

Astroglial markers, astroglial morphology and density

Chronic treatment with fluoxetine increased the density of S100□-positive cells and cells

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double labeled with BrdU and receptor for advanced glycation end products (RAGE), the latter being S100□□receptor, suggesting some contribution of astrocyte-mediated neurogenesis [38]□ An enhanced *de novo* generation of both astrocytes and neurones is also corroborated by an increase in nucleoside transporter expression in both cells types after fluoxetine treatment of mice [39, 40]. Increase of serum S100□□concentration was also observed in patients treated with antidepressants [41]. Nevertheless, neither serum S100□ nor BDNF levels reflect their levels in the brain [42], and changes in these proteins in patients with neuropsychiatric diseases should be interpreted with caution. In hippocampal astrocytes in culture and slices, the secretion of S100□□induced by fluoxetine□ depended on PKA, but not on serotonin [43]. Aquaporin-4 (AQP4) water channels are selectively expressed in astrocytes in the CNS and are concentrated in astrocytic endfeet that plaster blood vessels [44]. Expression of AQP4 is decreased in MD patients [45, 46], and there are some indications that genetic deletion of AQP4 removes antidepressant effect of fluoxetine in chronic mild stress model [47].

The number of astrocytes as wells as their somata volume were decreased in hippocampi of adult tree shrews exposed to chronic stress; treatment with fluoxetine prevented cell loss but not the shrinkage of somata [48]. Repetitive citalopram treatment suppressed kainate-induced reactive astrogliosis and inhibited neurogenesis in hippocampus [49]. Reactive astrocytes are source of and target for inflammatory cytokines. In cultured astrocytes sertraline significantly increased production of an anti-inflammatory cytokine, IL-10, and suppressed production of tumour necrosis factor-□ (TNF-□) in response to lipopolysaccharide (LPS) [50]. Connexin 43 (Cx 43) channels located in astrocytic processes underlie intercellular communication in astroglial syncytia. Chronic unpredictable stress decreased expression of Cx43 and inhibited diffusion of connexin-permeable dye [16]. This stress-induced decrease of gap junction connectivity was prevented by fluoxetine as well as by serotonin-norepinephrine reuptake inhibitor duloxetine [16].

Serotonin and/or norepinephrine reuptake inhibitors

Amitriptyline, clomipramine, imipramine, desipramine, nortriptyline belong to tricyclic antidepressants (TCAs). Most of them are supposed to selectively block noradrenaline uptake although desipramine has also some inhibitory effect on serotonin transport. Maprotiline is tetracyclic antidepressant, which suppresses reuptake of both serotonin and norephinephrine, but is less effective against serotonin reuptake. Venlafaxine is a structurally novel inhibitor of serotonin and norepinephrine reuptake.

Growth factors

Amitriptyline, fluoxetine, clomipramine, mianserin and paroxetine increase synthesis and release of glial cell-derived neurotrophic factor (GDNF) in C6 astrocytoma cell line [51]. Of note, mianserin, being not a reuptake inhibitor, increases norepinephrine release by blocking inhibitory □2 adrenergic receptor. In cultured astrocytes amitriptyline stimulated mRNA expression of fibroblast growth factor-2 (FGF-2), BDNF, VEGF and GDNF [52]. The effect of

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amitriptyline, as well as imipramine on GDNF expression was mediated by MAPK/ERK signal pathway [53, 54]. In contrast to SSRIs, amitriptyline-induced ERK phosphorylation was mediated by FGF receptor [54]. Imipramine up-regulated BDNF expression in astrocytes [55, 56], this effect being depended on PKA and/or ERK signal pathways [56].

Potassium buffering

Regulation of ion homeostasis is one of the most fundamental astroglial function [57]. Astroglial K^+ buffering uptake is mediated, in part, by the Na^+,K^+-ATPase [58]. Accumulated K^+ is subsequently spatially redistributed through astroglial syncytium and released distantly by an inwardly rectifying K_{ir} 4.1 channel [58, 59]. The K_{ir}4.1 channel-mediated K^+ release also decreases the post-stimulatory undershoot [60]. Both tricyclic antidepressants (TCAs) and SSRIs inhibit K_{ir}4.1 channels [61-63]. This might alter astrocytic/neuronal interactions in K^+ homeostasis and neuronal excitability.

Anti-inflammatory effects

Cytokines exert both pro- and anti-inflammatory effects [64], and can also have other vital functions [28]. Interleukin (IL)-1 and tumour necrosis factor-□ (TNF-□) are pro-inflammatory cytokines. TCAs, clomipramine and imipramine significantly decreased the production of nitric oxide and TNF-□ in cultured astrocytes [65]. Imipramine also reduced TNF-□□induced inflammatory response [66], and amitriptyline and nortriptyline inhibited the release of IL-1□ and TNF-□ in mixed glial cultures [67]. In a drug screening with GFAP as the indicator of astrogliosis, chronic treatment of mice with clomipramine for three weeks decreased GFAP expression by nearly 50% [68]. In contrast to SSRIs, in an *in vitro* model of ischemia (combined glucose and oxygen deprivation) nortriptyline decreased $cPLA_2$ expression and $cPLA_2$-mediated arachidonic acid release in astrocytes [69], which may reduce production of several proinflammatory compounds.

Other astroglial markers

Chronic treatment with desipramine decreased the density of □1 and □2 adrenergic receptors in astrocytes in brain *in vivo*. A decrease of binding of [^{3}H]-CGP 12177, a □-receptor antagonist after 14 days of treatment with reboxetine alone or in combination with sertraline was observed in the olfactory bulb-ectomy rat model of depression and in control animals [70]. In prefrontal cortex of MD patients glucose metabolism was reduced [71]. In cultured astrocytes amitriptyline, clomipramine, mianserine but not imipramine increased ATP content [72]. It was also observed that ATP levels in the brain were lower in mice susceptible to chronic social defeat and administration of ATP reversed depressive-like behaviours, probably through P2X receptors [73], although this latter study is in need of confirmation. The P11 gene has been shown to influence 5-HT transmission and its expression is reduced in animal model of depression and in post-mortem brain tissue of MD patients. The decrease of P11 gene expression is associated with higher DNA methylation of its promoter region and can be reversed by escitalopram, a SSRI [74]. In cultured astrocytes, amitriptyline,

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imipramine and paroxetine decreased the expression of histone methyltransferase G9a, a stimulator of DNA methyltransferase 1, and therefore decreased enzyme activity [75].

Astroglial effects of glutamatergic modulators

Ketamine

Ketamine is a non-competitive antagonist of NMDA receptor, used primarily for the induction and maintenance of general anaesthesia, usually in combination with a sedative. Ketamine at sub-anaesthetic dose is effective in treating depression in patients with MD and bipolar disorder [18, 23]. It acts rapidly (within 2 hours) and its antidepressant effects persist for 1-2 weeks [18]. Ketamine induces a rapid and transient increase of glutamate release, which, in turn, stimulates presynaptic group II metabotropic glutamate receptors (mGluRs). The activation of mGluRs induces a long-lasting decrease of glutamate release that possibly compensates the impaired astrocytic glutamate clearance in MD [18].

Riluzole

Riluzole is the only FDA approved drug for amyotrophic lateral sclerosis (ALS). Riluzole inhibits glutamate release from cultured neurones and brain slices probably by blocking voltage-dependent sodium channels on glutamatergic nerve terminals. Riluzole has antidepressant effect, but no evidence indicates that it acts faster than existing drugs ([23] and references therein). Riluzole increased GLT-1 gene expression and extracellular glutamate clearance in cultured astrocytes and in the *in vivo* brain [23, 76, 77]. Riluzole also prevented loss of GLT-1 induced by growth factor withdrawal in primary cultures of striatal astrocytes [78]. In addition, riluzole has neuroprotective properties. In cultured mouse astrocytes riluzole up-regulated expression of mRNA and protein of nerve growth factor (NGF), BDNF and GDNF [79]. In C6 cell lines, riluzole activated FGF receptor and its associated intracellular MEK/ERK/CREB signalling pathway, and increased gene expression of GDNF [80].

EXPERT COMMENTARY

Astroglia have emerged as a potential therapeutic target in psychiatric diseases only very recently, and fundamental knowledge about interactions of anti-psychotic drugs with astroglia are rather limited. Many of the findings discussed above originate from astrocytes in primary cultures, which approach allows for dissection of the basic mechanisms underlying drug action on astrocytes, especially when these compounds are activating or interacting with transporters or receptors. At the same time astrocytes *in vitro* do not faithfully replicate properties of astroglia in the *in vivo* brain, and more experiments on animal disease models are needed.

None of the antidepressants have been designed with astrocytes as target in mind. The main mechanism of drug actions in the brain may, however, be mediated by astrocytes (with subsequent neuronal consequences) and could be very different from current concepts, e.g.

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that SSRIs exert their effects mainly via SERT. Moreover, drug effects on astrocytes in cultures and/or astrocytes in brain from healthy animals should also be tested in proper model of the disease in question to establish their relevance. We have found that fluoxetine up-regulates 5-HT_{2B} receptor, $cPLA_2$, ADAR2 and GluK2 in astrocytes, but only the former three are decreased in brain of stress-induced anhedonia animals. However, anhedonia is only one component of major depressive illness and other components should also be investigated.

The investigation of drug effects on astrocytes in normal and pathological conditions will hopefully accelerate new drug development, but this is often a slow process due to adherence to existing dogma by research community and drug companies. This applies not only to the major depression discussed here but also to other neuropsychiatric and neurodegenerative disorders such as for example Alzheimer's disease, where advances in useful drug therapy have been minimal for decades.

FIVE YEAR VIEW

Astroglia are primary homeostatic and defencive elements of the CNS; and astrocytes contribute to all forms of neuropathologies. The data accumulated in recent decade clearly demonstrated specific changes in astroglia in neuropsychiatric diseases. Precise understanding of pathologically relevant mechanisms associated with astroglia in a context of psychiatric disorders including major depression are the major immediate challenge. Erecting the foundations of astrogliopathology is further frustrated by the absence of proper animal models of human diseases, which is particularly important for neuropsychiatric conditions. So far experimental access to human astroglia is limited to post-mortem tissues. Developments in the *in vivo* brain imaging, in combination with an emergence of novel astroglia-specific vital markers, as well as wider employment of stem cells derived from humans affected by diseases may offer an avenue for deeper understanding of astroglial pathology. This in turn will provide theoretical background for developing new, astroglial specific therapeutic strategies. Investigations of drug effects on astrocytes in normal and pathological conditions will hopefully accelerate new drug development, but this might be a slow process due to adherence to existing dogma by pharmaceutical companies.

KEY ISSUES

Astrocytes are highly heterogeneous population of specialised neural cells responsible for homeostasis and defence of the central nervous system.

Astrocytes undergo morphological and functional atrophy in major depression which may contribute to neurotransmission displace and hence to pathological progression.

Antidepressant drugs affect astroglial biochemistry and physiology, which may represent a part of the therapeutic action.

Specific targeting of astroglia may be regarded as a novel strategy in developing

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antidepressant drugs.

Financial and competing interests disclosure

This study was supported by Grant No. 31171036 to L Peng from the National Natural Science Foundation of China. A Verkhratsky was supported in part by the grant (agreement from August 27 2013 № 02.B.49.21.0003) between The Ministry of Education and Science of the Russian Federation and Lobachevsky State University of Nizhny Novgorod and by the grant of the Russian Scientific Foundation №14-15-00633. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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