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The association between antidepressant effect of SSRIs and astrocytes: Conceptual overview and meta-analysis of the literature

Beina Chen¹, Manman Zhang¹, Ming Ji¹, Wenliang Gong¹, Binjie Chen¹, Robert Zorec^{2,3}, Matjaž Stenovec^{2,3}, Alexei Verkhratsky^{4,5,6*} and Baoman Li^{1,6*}

¹Practical Teaching Centre, School of Forensic Medicine, China Medical University, Shenyang, People's Republic of China.

²Celica BIOMEDICAL, Tehnološki park 24, 1000 Ljubljana, Slovenia

³Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia

⁴Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK.

⁵Achucarro Center for Neuroscience, IKERBASQUE, 48011 Bilbao, Spain

⁶Department of Poison Analysis, School of Forensic Medicine, China Medical University, Shenyang, China.

* Corresponding authors:

Prof. Baoman Li, Ph.D., M.D.

Practical Teaching Center, School of Forensic Medicine, China Medical University

No. 77, Puhe Street, Shenbei District, Shenyang, P.R. China

Postcode: 110177

Phone: 86(24)31939437

Fax: 86(24) 83282772

Email: bmli@cmu.edu.cn

Prof. Alexei Verkhratsky, Ph.D., M.D.

Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK.

Email: Alexej.Verkhatsky@manchester.ac.uk

Abstract

Major depressive disorders (MDD) a worldwide psychiatric disease, is yet to be adequately controlled by therapies; while the mechanisms of action of antidepressants are yet to be fully characterised. In the last two decades, an increasing number of studies have demonstrated the role of astrocytes in the pathophysiology and therapy of MDD. Selective serotonin reuptake inhibitors (SSRIs) are the most widely used antidepressants. It is generally acknowledged that SSRIs increase serotonin levels in the central nervous system by inhibiting serotonin transporters, although the SSRIs action is not ideal;. The SSRIs antidepressant effect develops with considerable delay; their efficacy is low and frequent relapses are common. Neither cellular nor molecular pharmacological mechanisms of SSRIs are fully characterised; in particular their action on astrocytes remain underappreciated. In this paper we overview potential therapeutic mechanisms of SSRIs associated with astroglia and report the results of meta-analysis of studies dedicated to MDD, SSRIs and astrocytes. In particular we argue that fluoxetine, the representative SSRI, improves depressive-like behaviours in animals treated with chronic mild stress and reverses depression-associated decrease in astrocytic glial fibrillary acidic protein (GFAP) expression. In addition, fluoxetine upregulates astrocytic mRNA expression of 5-hydroxytryptamin/serotonin_{2B} receptors (5-HT_{2B}R). In summary, we infer that SSRIs exert their anti-depressant effect by regulating several molecular and signalling pathways in astrocytes.

Keywords: astrocyte; major depressive disorder; selective serotonin reuptake inhibitors; GFAP; 5-HT_{2B} receptors

Introduction

Major depressive disorder (MDD) is a widespread psychiatric disease characterised by depressed mood, diminished interests, impaired cognitive function, anhedonia and aberrant sleep [1]. The MDD is associated with substantial alterations in the structure, chemistry, and function of the brain [2]. Studies of depressed patients and preclinical investigations of animal models demonstrated that exposure to various environmental factors, including psychological and physical stress, contribute to pathogenesis of depression and are linked to the aberrant homeostasis in the central nervous system (CNS) [3-6]. Astrocytes are primary homeostatic cells of the CNS, which control multiple aspects of brain physiology at all levels of organisation from molecular to organ [7, 8]. Pathological changes in astrocytes are indispensable part of all neurological and neuropsychiatric disorders [9-12]. Loss of astroglial homeostatic capabilities including deficient metabolic support, impairment of brain microcirculation, aberrant ionostasis and pathological neurotransmission alone or in combination contribute to neuropathology and impaired cognitive functions [13, 14]. Expression of multiple receptors and transporters makes astrocytes potential targets for specific therapy; indeed, astroglial cells respond to many drugs employed to treat diseases of the CNS [15-17], although the underlying mechanisms need further clarification.

Treatment of MDD is based on the monoamine hypothesis, which, however popular, does not fully explain the disease pathophysiology, clinical presentation and mechanisms of therapy [18]. Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed drugs worldwide for the treatment of depressive symptoms of various aetiology [19]. An increase in extracellular concentration of serotonin by inhibition of the serotonin transporter (SERT) has been generally acknowledged as leading mechanism of action of SSRIs. In recent years, however, SSRI interactions with other molecules have been revealed thus extending our understanding of the pharmacotherapy of depression. In particular, SSRIs were found to act as agonists of 5-HT_{2B} serotonin receptors present in astrocytes [21]. Chronic treatment of astrocytes *in vitro* with fluoxetine increases expression and molecular editing of the 5-HT_{2B} receptor [11, 16]. Furthermore, treatment of cultured astrocytes or rodent models of depression with fluoxetine regulates the expression of several astrocytic proteins, such as S100B and aquaporin-4 (AQP4) that are related to synaptic plasticity and glymphatic clearance [23].

Systematic evaluation of randomised controlled trials is recognised as the most reliable strategy to learn about the efficacy of drugs. However, there are only a few systematic analyses of animal experiments; while the results obtained from animal experiments are difficult to verify in clinical trials. In addition, there are numerous bias factors in different animal experiments that may mislead translation. Therefore, we performed an in depth meta-analysis of the associations between SSRIs as antidepressants in depression rodent models and astrocytes.

Astrocytes in major depressive disorder (MDD)

Astrocytes are main homeostatic cells of the CNS that support the function of individual neurones, as well as nervous tissue and the brain and the spinal cord as an organ. Astrocytes are responsible for metabolic support, molecular homeostasis, synapse formation, nutrition, repair, isolation and as well as in maintaining the blood-brain barrier and glymphatic system [7, 24-27]. Analyses of the human post-mortem brain tissue obtained from patients with depressive disorders have found that the number of astrocytes and expression of GFAP are decreased in the hippocampus, frontal cortex and amygdala [28-31].

Growing evidence supports a key role of astrocytes in MDD [32]. The decrease in the density of astrocytes, expression of GFAP expression and shrinkage of GFAP morphological profiles are well documented in MDD patients [31-34]. These changes, reflecting astroglial impairments, have been observed in many regions of the brain including the dorsolateral prefrontal cortex [35, 36], orbitofrontal cortex [36], subgenual cortex [37], anterior cingulate cortex [38, 39], and amygdala [40]. Similarly, astrocytic density and expression of GFAP were both reduced in the grey matter of mice and rats with depressive-like phenotypes induced by exposure to various types of stress [41, 42]. At the same time animal models of depression were characterized with down-regulation of several *bona fide* astroglial markers including aquaporin 4, astroglia-specific connexins, astroglial plasmalemmal glutamate transporters and glutamine synthetase [43-45]. Treating animals with astroglia-targeting pharmacological agents, such as inhibitors of gap junctions or glutamate transporters, resulted in development of depressive-like behaviours and anhedonia [46, 47]. In adult rats, ablation of astrocytes by injection of L- α -aminoadipic acid, the gliotoxin, into the frontal cortex triggered depressive-like behaviours [48]. Thus, development of depression is associated with prevalence of astroglial atrophy and degeneration, with little signs of astroglial reactivity [30, 49]. Asthenic astrocytes reduce their coverage of synapses, which may substantially affect neurotransmission, in particular glutaminergic, GABA-ergic and monoaminergic, which in turn can contribute to the dysbalance of inhibition and excitation linked to a variety of psychiatric disorders, including depression, anxiety, and schizophrenia [4, 50].

One of the fundamental functions of astrocytes is coordinated supply of the brain tissues with a variety of growth factors expressed and secreted by astroglia [51]. In particular, astrocytes secrete brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGFR) and fibroblast growth factor 2 (FGF2) [52-54]. Of note, treatment with fluoxetine increases BDNF in astrocytes, however serotonin at 100 μ M is ineffective, thus indicating that effects of fluoxetine on BDNF levels are linked to SERT [55]. At the same time SSRIs up-regulate astrocytic synthesis of BDNF, which had anxiolytic effects in mice [56]. Astroglial secretion of BDNF promotes synaptic plasticity by enhancing the expression of synaptic vesicle proteins [57]. In hippocampus, BDNF-dependent the synaptic and structural plasticity is

involved in the pharmacological effects of fluoxetine to improve depressive-like behaviours [56].

Another important part of MDD pathogenesis is associated with neuroinflammation as well as with systemic inflammation [58, 59]. The chronic stress by itself affects the inflammatory pathways in the brain; in particular exposure to stress stimulates the synthesis and increases brain levels of pro-inflammatory cytokines, including tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 β (IL-1 β) and interferon- α (IFN- α) [60]. At the same time, the plasma concentration of pro-inflammatory cytokines is increased in patients with MDD [61]. Moreover, the neuroinflammation induced by post-traumatic stress disorders activates astrocytes by up-regulating the expression and release of CC-chemokine ligand 2 (CCL2), which promotes the extravasation and infiltration of monocytes into the cerebral parenchyma [62]. Similarly, increased astroglial expression of CCL2 was discovered in the cortex of post-mortem brain of the suicide patients with MDD [63]. In astrocytes, the release of ATP can stimulate the expression of CCL2 through P2X₇ purinergic receptors (P2X₇R) [64]. At the same time stimulation of CCL2 and P2X₇R can boost astroglial IL-1 β release and activate inflammasome both associated with depression-like behaviours [65], likewise interfering the sleep quality [66].

Meta-analysis of the literature data

Fluoxetine as the representative SSRIs can regulate functions of astrocytes, although some details remain uncertain. For example, it is not confirmed whether fluoxetine affects the level of GFAP or the number of astrocytes in MDD; whether there is an astroglial target specifically regulated by fluoxetine; or whether the behavioural tests applied for the identification of depressive-like behaviours are consistent.

Study selection and quality assessment

A total of 359 articles were initially identified, and after removing duplicates and screening study titles and abstracts, 38 articles that matched inclusion criteria (see methods in supplementary materials) remained for full-text screen. Ten articles were included in the list for the meta-analysis [67-76]. The article selection process is shown on Fig. 1. Among the 10 included studies, eight used mice and two used rats. In addition, from these articles, eight experiments reported behavioural outcomes, including sucrose preference test, forced swimming test, and tail suspension test. Five studies reported the expression levels of GFAP and the number of astrocytes, and two studies reported the influence of fluoxetine on 5-HT₂ receptors. Detailed characteristics of the articles are shown in Table 1. Methodological quality of included studies, which were assessed using SYRCLE's risk of bias tool and CAMARADES checklist for study quality as described in [77] are shown in Table 2, indicating overall sufficient quality of the studies.

Data analysis

GFAP

The overall meta-analysis included 5 studies that evaluated GFAP expression. One of them [68] was excluded after heterogeneity analysis. The pooled result showed a significant difference between these control and depressed groups, indicating that GFAP expression decreased in depression-like rodents (four studies, pooled SMD = 4.49, 95% CI: 2.85 to 6.13; $p < 0.05$) (Fig. 2A). The pooled result also demonstrated that fluoxetine treatment significantly increased GFAP expression (four studies, pooled SMD = -2.66, 95% CI: -3.42 to -1.90; $p < 0.05$) (Fig. 2B).

Astrocytes numbers

Changes in the number of astrocytes were reported in 3 articles. A single paper reported the number of astrocytes in the local area of hippocampus which was significantly different from other studies, so it was not included in the calculation. The number of astrocytes in the brains of depressed mice was smaller when compared to the control group (two studies, pooled SMD = 8.78, 95% CI: 6.82 to 10.75; $p < 0.05$) (Fig. 3A). Treatment with fluoxetine significantly increased the number of astrocytes in mice models with depression (two studies, pooled SMD = -4.94, 95% CI: -7.22 to -2.66; $p < 0.05$) (Fig. 3B).

5-HT₂ receptor

Two articles reported changes in the levels of expression of 5-HT₂ receptors in the mouse brains. Combined results showed that, compared with the control group, the mRNA expression of 5-HT_{2B} receptors significantly increased in the fluoxetine-treated group (two studies, pooled MD = -0.53, 95% CI: -0.60 to -0.46; $p < 0.05$) (Fig. 4A). However, there was no significant difference in mRNA expression of 5-HT_{2C} receptors in mice (two studies, pooled MD = -0.05, 95% CI: -0.11 to 0.00; $p = 0.06$) (Fig. 4B).

Behavioural tests

Eight articles reported behavioural outcomes in mice. Sucrose preference was invariably increased after treatment with fluoxetine in depression rodent models (five studies, pooled SMD = -2.60; 95% CI: -4.07 to -1.41; $p < 0.05$) (Fig. 5A), although there was significant heterogeneity ($p < 0.05$; $I^2 = 80\%$). Therefore, subgroup analyses were performed. Five studies were divided into two subgroups according to their different recording measurement units of sucrose preference. One subgroup included three studies which reported sucrose preference by percentage of sucrose, and the other subgroup included two studies which reported relative sucrose intake in terms of g/kg. The results of subgroup analyses show a consistent trend of increased sucrose preference between the depressive rodent group and fluoxetine treatment group with low heterogeneity within each of subgroups. High heterogeneity existed between two subgroups, which may result from different recording methods used to quantify sucrose preference. The immobility times of forced swimming tests (five studies,

pooled MD = 56.32, 95-Cl: 39.83 to 72.81; $p < 0.05$) (Fig. 5B) and tail suspension tests (three studies, pooled MD = 73.24, 95% CI: 68.34 to 79.77; $p < 0.05$) (Fig. 5C) decreased after administration of fluoxetine. The pooled result of forced swimming tests similarly exhibited high level of heterogeneity.

Meta-analysis conclusion

According to the meta-analysis of articles which studied the effects of SSRIs on astrocytes in the rodent depression model the following can be concluded: (i) fluoxetine could recover depression-induced decrease in the expression of GFAP and in the number of astrocytes (Fig. 2 and Fig. 3); (ii) fluoxetine selectively increased the mRNA expression of 5-HT_{2B} receptors in astrocytes, but had no influence on the mRNA expression of 5-HT_{2C} receptors (Fig. 4); (iii) Through a high degree of inter-study heterogeneity existed in the pooled results of sucrose preference and forced swimming test, these heterogeneity was mainly due to the different measured patterns or various measurement units (Fig. 5).

SSRIs and astrocytes

The universally acknowledged mechanism of anti-depressant action of SSRIs is an inhibition of serotonin uptake, which increases the level of bioavailable 5-HT in the brain, thereby enhancing serotonergic neurotransmission [78]. The mammalian family of serotonin receptors family includes seven members (5-HT₁₋₇ receptors), out of which 5-HT_{1A-1F}, 5-HT_{2A-2C}, 5-HT₆, 5-HT₇ subunits are expressed in astrocytes [75, 79-82]. In experiments *in vitro*, stimulation of 5-HT_{1A} receptors was shown to increase the mRNA expression of Ca²⁺-binding protein S100B as well as its release from astrocytes [83]. At the same time higher level of serum S100B was measured in patients with MDD compared with healthy subjects [84, 85]. Transgenic mice overexpressing S100B show depressive-like behaviours, which can be alleviated by inhibitors of 5-HT₇ receptors [86]. An atypical antipsychotic drug olanzapine augments fluoxetine-induced increase of extracellular 5-HT levels and rescues depressant-like behaviours by inhibiting 5-HT₆ receptors through decreasing the firing frequency of γ -aminobutyric acid GABA-ergic neurones in dorsal raphe nucleus [87]. Trazodone, a triazolopyridine derivative, can exert neuroprotective roles by activating 5-HT_{1A} receptors, however it also inhibits 5-HT_{2A/2C} receptors in human astrocytes, and stimulates the ERK/AKT/c-Jun N-terminal kinases signalling pathway [88].

The 5-HT_{2B} receptors, similarly to the majority of other serotonin receptors, are Gq-coupled 7-transmembrane domains metabotropic receptors expressed in astrocytes [80]. The SSRIs were shown to selectively stimulate astroglial 5-HT_{2B} receptors, which effect is linked to anti-depressive potency of these drugs [89, 90]. In primary mouse cultured astrocytes acute treatment with fluoxetine (5 - 10 μ M, 20 to 40 min) induces the phosphorylation of extracellular regulated kinases 1 and 2 (ERK_{1/2}) [89]. Fluoxetine-induced ERK_{1/2} phosphorylation can be inhibited by the 5-HT₂ antagonist SB204741, but not by 5-HT_{2A} selective receptors antagonist M100901, or 5-HT_{2C}

selective receptors antagonist SB242084; inhibition of the expression of 5-HT_{2B} receptors by siRNA duplex chains abolishes the effect of fluoxetine on the activation of ERK_{1/2} [89]. This indicates that the effect of fluoxetine to increase ERK_{1/2} phosphorylation is mediated by 5-HT_{2B} receptor. Astroglial 5-HT_{2B} receptors are coupled with G_{q/11} protein [91]; activation of 5-HT_{2B} receptors stimulates phospholipase C (PLC), which produces diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (InsP₃) by hydrolysing phosphatidyl-inositol 4,5-bisphosphate (PIP₂). The InsP₃, in turn, evokes Ca²⁺ release from the endoplasmic reticulum thus generating Ca²⁺ signals, which form the basis of astroglial excitability [92, 93]. Astroglial Ca²⁺ signals, in particular, enhance the activity of Zn-dependent metalloproteinases by stimulating protein kinase C (PKC). Activated metalloproteinases can hydrolyse heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) and promote its shedding from the membrane [94]. The HB-EGF transactivates epidermal growth factor receptor (EGFR) [95], induces receptor tyrosine kinases phosphorylation, and ultimately regulates two signalling pathways: the MEK/ERK and PI3K/AKT [96]. The 5-HT_{2B} receptors-induced ERK_{1/2} phosphorylation can increase mRNA and protein expression level of *c-fos* [89, 97], with the latter regulating expression of other bioactive factors including Ca²⁺-dependent phospholipase A2 (cPLA2) and BDNF [98, 99].

Of note, chronic and acute treatments with different concentrations of fluoxetine produce distinct pharmacological actions. In particular, fluoxetine shows biphasic regulatory effects on caveolin-1/PTEN/PI3K/AKT/GSK-3 β pathway in astrocytes [97]. Acute treatment with fluoxetine at low concentration (0.5 μ M -1 μ M) activates 5-HT_{2B} receptor, with subsequent stimulation of Src that triggers PI3K/AKT signalling pathway which induces phosphorylation of EGFR and AKT. This leads to a subsequent down-regulations of expression of *c-fos* and caveolin-1 (Cav-1) in primary cultured astrocytes [97]. Next, Cav-1 decreases membrane content of phosphatase and tensin homologue (PTEN) and induces the phosphorylation of PI3K and glycogen synthase kinase 3 β (GSK-3 β), which ultimately inhibits activity of the latter [97]. Astrocytes treatment with high concentrations of fluoxetine (5 μ M - 10 μ M) up-regulates *c-fos* through increasing phosphorylation of ERK_{1/2}, with subsequent increase in the expression of Cav-1 that activates the PTEN/PI3K/AKT/GSK-3 β pathway [97]. The GSK-3 β is one of the key kinases that participates in the regulation of glycogen phosphorylation. Impaired activity of GSK is related to MDD; it was reported that the phosphorylation of GSK-3 β is lower in the brain of MDD patients [99]. An increase in GSK-3 β activity has been found in prefrontal cortex and in platelets of depressed patients [100-102], while the gene expression of GSK-3 β is up-regulated in the frontal cortex, in the raphe, and in the hippocampus of rat model of depression [103].

Chronic treatment with fluoxetine increases the mRNA and protein expression of cPLA2 by stimulating 5-HT_{2B} receptor [98]. The up-regulation of cPLA2 is enzyme-specific since neither secretory PLA2 (sPLA2) nor intracellular PLA2

(iPLA2) respond to fluoxetine [98]. The majority of cPLA2 in the grey matter is located in astrocytes [104]; the cPLA2 is a Ca^{2+} -sensitive cytosolic enzyme that acts as an activator of the release of arachidonic acid (AA). The AA is linked with pathophysiology of depression through its effects on serotonin transports [105]. Chronic treatments with fluoxetine can mediate post-translational editing of some genes at different concentrations. Another relevant pathway is associated with the adenosine deaminase acting on RNA (ADAR) signalling cascade. The ADAR2 that catalyzes the conversion of adenosine to inosine is highly expressed in astrocytes [106]. Treatment of astrocytes for three days with fluoxetine at high concentration (10 μM) increases both mRNA and protein expression of ADAR2 by stimulating 5-HT_{2B} receptors. The up-regulated expression of ADAR2 alleviates the functions of kainate and 5-HT_{2B} receptors through RNA editing in cultured astrocytes [107, 108]. In contrast, chronic treatments with low concentration (1 μM) of fluoxetine, decreases the expression of ADAR2, thus increasing the activation of 5-HT_{2C} receptors through the suppressed RNA editing [109].

Astrocytes in MDD associated neurological disorders

Newly revealed antidepressant effects of fluoxetine linked to astrocytes have been extensively studied in the recent decade; these new findings may clarify the relationships between MDD and other neurological diseases, such as alcoholism, sleep disturbance and Alzheimer's disease. Chronic administration alcohol decreases the release of ATP from astrocytes by increasing the expression of ADAR2, while the latter can inhibit 5-HT_{2C} receptors by RNA editing; this in turn, results in the decreased 5-HT_{2C} receptor-mediated ATP release from astrocytes [109]. Diminished astroglial ATP secretion can be associated with the depressive-like behaviours [110]. Fluoxetine selectively stimulates astrocytic 5-HT_{2B} receptors and activates the downstream Src/EGFR/PI3K/AKT/*c-fos* signalling cascade thus decreasing the expression of ADAR2 upregulated by alcohol consumption [109]. Meanwhile, *in vivo*, fluoxetine improves the depressive- and anxiety-like behaviours caused by chronic alcohol administration; furthermore, fluoxetine can decrease the alcohol preference in mice models of chronic alcohol addiction [109].

Sleep disorders and chronic insomnia in particular, are strongly associated with MDD [99, 111], although the pathological mechanism underlying this association remains to be fully characterised. Our recent studies analysed possible mechanisms linking anti-depressant effects of fluoxetine to the depressive like behaviours induced by sleep deprivation [99, 112]. Sleep deprivation could trigger depressive-like behaviour by activating nucleotide-binding domain and leucine-rich repeat protein-3 (NLRP3) inflammasome and by decreasing the level of BDNF in astrocytes [99]. Astrocytic 5-HT_{2B} receptors seem to be the key molecule involved in sleep deprivation-induced depression and the mechanism of action of antidepressants [112, 113]. Firstly, chronic sleep deprivation induced the stimulation of NLRP3 inflammasome in astrocytes requires P2X₇ receptors mediated inhibition of signal transducer and activator of

transcription 3 (STAT3). Fluoxetine can alleviate activation of NLRP3 inflammations by inducing phosphorylation of ERK_{1/2} and AKT to re-activate STAT3 [99]. Fluoxetine also activates, by 5-HT_{2B} receptors mediated mechanism, the ERK_{1/2} and AKT cascades in astrocytes [107, 114]. However, sleep deprivation down-regulates the expression of astroglial 5-HT_{2B} receptors through activation of P2X₇ purinoceptors [113]. In contrast, leptin up-regulates astrocytic 5-HT_{2B} receptors by stimulating JAK2/STAT3 and augments the anti-depressive effects of fluoxetine [99].

As has been already mentioned, BDNF is implicated in the pathogenesis of the MDD [115]. Astrocytes are essential for regulating neuronal morphology through BDNF release [116], while metabolic disturbances or reduced expression of BDNF induced by chronic stress impair neuroplasticity [117]. Chronic sleep deprivation decreases protein expression of BDNF [99]. Fluoxetine alleviates depressive-like behaviours and reverses the decreased BDNF release induced by sleep deprivation by stimulating 5-HT_{2B} receptors [99]. In experiments *in vivo*, fluoxetine increased the expression of BDNF by stimulating 5-HT_{2B} receptors-mediated phosphorylation of ERK_{1/2} and *c-fos* [99]. Similarly, fluoxetine significantly up-regulates the phosphorylation of ERK and the mRNA expression level of BDNF in human astrocytes [88].

In addition, MDD is a high risk factor for the increased occurrence of Alzheimer's diseases (AD), and SSRIs can obviously improve the depressive symptoms and daily activities of AD patients [118, 119]. However, the potential pathogenetic link between MDD and AD is still unclear. Our recent studies reveal the potential pathological mechanism common to MDD and AD, associated with the dysfunction of glymphatic system [112, 120]. Chronic stress down-regulates AQP4 expression in astrocytes in cortex and hippocampus and leads to glymphatic dysfunction [23]. This dysfunction of glymphatic clearance affects brain microenvironment and may instigate neurotoxicity through suppressed removal of pathological proteins such as β -amyloid [121]. Glymphatic systems dysfunction caused by chronic unpredictable mild stress (CUMS) promotes accumulation of endogenous and exogenous β -amyloid in neurones, while fluoxetine improves the efficacy of the glymphatic pathway by increasing AQP4 expression [23]. In addition, AQP4 deficiency impairs learning and memory. These results may hint at the possible mechanism related to depression with cognitive dysfunction and indicate a potential therapeutic relevance of AQP4. Our recent study reports that iron overload can aggravate the depressive-like behaviours induced by CUMS and trigger the cognitive impairments, resulted from the over-dose of iron-induced deficiency of the glymphatic system [119]. Furthermore, there are some suggestions indicating that AQP4 is linked to the therapeutic effects of SSRIs in depression [121]. Genetic deletion of AQP4 abolishes the anxiolytic and anti-depressive effects of fluoxetine [121]. Similarly, fluoxetine requires AQP4 to modulate the morphological plasticity of astrocyte processes [122].

Conclusion

Here we overviewed the contribution of astrocytes to the pathological mechanism of MDD and the pharmacological mechanisms of SSRIs *in vivo* and *in vitro*. We presented the data showing the astrocyte-related effects of fluoxetine that restores astroglial density and GFAP which are both suppressed in animal models of depression. Fluoxetine selectively increases the mRNA and protein expression of 5-HT_{2B} receptors in astrocytes, but does not affect the expression of 5-HT_{2C} receptors. There is also experimental evidence suggesting the existence of astroglial mechanisms linking MDD with other neurological diseases, such as AD, sleep disorders and alcoholism. In summary, astroglial 5-HT_{2B} receptors play a key role in the pathophysiology of MDD and in the therapeutic effects of SSRIs. In addition, fluoxetine-dependent activation of astrocytic 5-HT_{2B} receptors transactivates EGFR and recruits MEK/ERK_{1/2} and PI3K/AKT signalling cascades, which regulate the expression and function of cPLA2, ADAR2, and AQP4. The sleep deprivation-induced selective downregulation of astroglial 5-HT_{2B} receptors and BDNF may contribute to pathological relationship between sleep disturbance and MDD. Furthermore, the pathophysiology of MDD is also the high risk factor for the occurrence of neurodegenerative diseases. The decrease of AQP4 and the circulation of glymphatic system reduced by chronic stress may create susceptible pathological micro-environments for the occurrence of AD.

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Figure legends

Figure 1. Study identification and selection progress in the meta-analysis. By searching PubMed and Embase, a total of 359 articles were initially identified. After screening title and abstract of studies and removing duplicates, 38 studies were remained for full-text review. Our study excluded 28 studies according to our inclusion criteria, ten articles were finally included in meta-analysis.

Figure 2. Meta-analysis on the expression of GFAP in rodent built with or without depression model and the effects of fluoxetine on rodent depressive models. The zero vertical line represents no difference between two groups. The area of each green point represents its contribution to meta-analysis. Horizontal lines indicate 95% confidence intervals of the estimate. The black square point represents pooled effect of all included studies in forest plot.

A: Forest plot showing GFAP expression in healthy and depressed rodents. Points located at the positive side indicate reduced expression of GFAP.

B: Forest plot showing GFAP expression in depressed rodents treated with fluoxetine. Points located at the negative side indicate increase in expression of GFAP. P value less than 0.05 denotes statistical significance.

Figure 3. Meta-analysis of astrocyte numbers of depressive rodent models with or without fluoxetine treatment. The solid vertical line represents pooled effect size is zero. The area of each green point represents its contribution to meta-analysis. Horizontal lines indicate 95% confidence intervals of the estimate.

Figure 4. Forest plot with all included studies of 5-HT₂ receptors of rodent depressive models treated with or without fluoxetine. The solid vertical line of zero represents no difference between two groups. The area of each green point represents its contribution to meta-analysis. Horizontal lines indicate 95% confidence intervals of the estimate. The black square point represents pooled effect of all included studies in forest plot.

A: Forest plot of the mRNA expression of 5-HT_{2B} receptors. Points located at the negative side indicate increased mRNA expression of 5-HT_{2B} receptors in depressive-like rodent treated with fluoxetine.

B: Forest plot of the mRNA expression of 5-HT_{2C} receptors. Points located at the negative side indicate increased mRNA expression of 5-HT_{2C} receptors in rodent depressive model treated with fluoxetine, while points at the positive side indicate decreased mRNA expression of 5-HT_{2C} receptors in depressive model treated with fluoxetine. P value less than 0.05 denotes statistical significance.

Figure 5. Meta-analysis on behavioural test of depressive rodent models with or without fluoxetine treatment.

A: Forest plot of sucrose preference.

B: Forest plot of forced swimming test.

C: Forest plot of tail suspension test. The solid vertical line represents pooled effect size is zero. The area of each green point represents its contribution to meta-analysis. Horizontal lines indicate 95% confidence intervals of the estimate.

Figure 6. Astroglial 5-HT_{2B} receptors and pathogenesis of depression. Astrocytic 5-HT_{2B} receptors play a key role in the pathological mechanisms of sleep deprivation-induced depressive-like behaviours and contribute to action of SSRI antidepressants. Chronic sleep deprivation decreases BDNF levels and instigates activation of NLRP3 inflammasome, which both contribute to the development of depressive-like behaviours. Sleep deprivation suppresses activation of AKT and STAT3, while indirectly increasing the activity of GSK-3 β , which activates of NLRP3 inflammasome. Administration of fluoxetine can increase the expression of BDNF by stimulating ERK_{1/2}-*cfos* signalling pathway in astrocytes. In addition, fluoxetine can also increase the phosphorylation of STAT3 by stimulating ERK_{1/2} or AKT, to suppress the activation of NLRP3 inflammasome. Furthermore, leptin augments the anti-depressant effects of fluoxetine by increasing the expression of 5-HT_{2B} receptors through activation of JAK2-STAT3 cascade. Chronic sleep deprivation selectively decreases the expression of astroglial 5-HT_{2B} receptors through P2X₇ receptors-mediated inhibition of AKT phosphorylation, which again converge into depressive-like behaviour.

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Fig_6

Table 1 Main characteristics of included studies in the meta-analysis

Study	Animal	Experiment model	Intervention	Biochemistry Outcomes	Behavior tests
Shu 2019	C57/BL6J (male,18-25g), n=15	CMS	Fluoxetine (10mg/kg, 4 weeks)	GFAP, LC3	SPT, FST, TST
Zhao 2018	C57BL/6J (male,18-22g), n=10	CORT (20mg/kg; i.p.;3 weeks or 5 weeks)	Fluoxetine (10mg/kg, 5 weeks, orally)	GFAP, S100B	EPM, FST, TST
Liu 2019	C57BL/6J (male, aged 10 weeks), n=20	CUMS, 21 days	Fluoxetine (2.6mg/kg, 21 days, orally)	GFAP, EAAT1, EAAT2, NeuN	OFT, SPT, FST
Sha 2018	SD rats (male, aged 7 weeks,160-200g), n=8	Chronic restraint stress combined with solitary raising, 28days	Fluoxetine (1.8mg/kg, 28 days, orally)	GFAP, IL-10	OFT, SPT
Francis 2017	Swiss mice (female,30-40g, aged 45-50 days), n=14	CORT (20mg/kg; p.o.; 21 days)	Fluoxetine (10mg/kg, 21days, p.o.)	GFAP, Serum corticosterone	SPT, FST, TST, OFT
Chen 2017	C57BL/6J (male, 18-20g), n=10	CORT (20mg/kg; s.c.; 5 weeks)	Fluoxetine (10mg/kg, 5 weeks, i.g.)	GFAP	FST, TST
Nastra 2016	Swiss albino mice (male, 30-40g), n=10	CUMS, 4 weeks	Fluoxetine (20mg/kg, 4 weeks, i.g.)	GFAP, BDNF, GR, Caspase-3, Serum corticosterone, Testosterone levels	EPM, OFT, FST
Pan 2014	Wistar Rats (male,180-220g), n=6-8	CUMS, 12 weeks	Fluoxetine (10mg/kg, 6 weeks, orally)	IL-1β, NF-κB, IKKα, IKKb, NLRP3, ASC, Caspase-1, CD11b, Iba1, GFAP, Glutamate, Glutamine	SPT
Li 2012	FVB/NTg(GFAP-GFP)14Mes/J or B6.Cg-Tg(Thy1-YFPH)2Jrs/J mice (male and female, 20-25g)	CMS, 14 days	Fluoxetine (10mg/kg, 14 days, i.p.)	5-HT _{2A} R, 5-HT _{2B} R, 5-HT _{2C} R, cPLA ₂ a, sPLA ₂ , iPLA ₂ , ADAR1, ADAR1, GluK2, GluK3, GluK4, GluK5, c-fos, fos-B	SPT
Dong 2015	FVB/NTg(GFAP-GFP)14Mes/J or B6.Cg-Tg(Thy1-YFPH)2Jrs/J mice (male and female, 20-25g)	CMS, 14 days	Fluoxetine (10mg/kg, 14 days, i.p.)	5-HT _{2B} R, 5-HT _{2C} R, cPLA ₂ a, ADAR2, GluK2, GluK4	SPT

CMS, chronic mild stress; CUMS, Chronic unpredictable mild stress; SPT, Sucrose preference; FST, Forced swimming test; TST, Tail suspension test; OFT, Openfield test; EPM, Elevated pluz maze.

Table 2. Methodological Quality Assessment

Study	Shu 2019	Zhao 2018	Liu 2019	Sha 2018	Francis 2017	Chen 2017	Nasra 2016	Pan 2014	Li 2012	Dong 2015
<i>Research question specified and clear?</i>	1	1	1	1	1	1	1	1	1	1
<i>Method of allocation to treatment group: i.e. Animals randomized across groups?</i>	1	1	1	1	1	1	1	1	1	1
<i>Sample-size calculation before start of experiment</i>	0	0	0	0	0	0	0	0	0	0
<i>Concealment of allocation?</i>	0	0	0	0	0	0	0	0	0	0
<i>Group characteristics clearly described?*</i>	0	0	0	0	0	0	0	0	0	0
<i>Correct control group used?</i>	1	1	1	1	1	1	1	1	1	1
<i>Body temperature controlled within 3°C variation?</i>	0	0	0	0	0	0	0	0	0	0
<i>Number of animals per group clear?</i>	1	1	1	1	0	1	1	1	1	1
<i>Age, sex and weight of the animal</i>	1	1	1	1	1	1	1	1	1	1
<i>Complete outcome data?</i>	1	1	1	1	1	1	1	1	1	1
<i>Description of how the disease or intervention is defined in the animal</i>	1	1	1	1	1	1	1	1	1	1
<i>Time schedule (Day and time of intervention within experiment)</i>	1	1	0	0	1	1	1	1	0	1
<i>Dose and/or frequency of intervention</i>	1	1	1	1	1	1	1	1	1	1
<i>Method and time of sampling (blood, urine, etc.)</i>	0	1	0	0	1	1	0	1	1	0
<i>Excluded animals (numbers and reasons why they were excluded)</i>	0	0	0	0	0	0	0	0	0	0
<i>Total numbers of animals included in the statistical analyses</i>	0	0	0	1	0	1	0	0	0	1
<i>Description of compliance to national regulatory principles</i>	1	1	1	1	1	1	1	1	1	1
<i>statement of potential conflicts of interest</i>	1	1	1	0	1	1	0	1	1	0
<i>Total score</i>	11	12	10	10	11	13	10	12	11	11
<i>Maximal possible score</i>	18	18	18	18	18	18	18	18	18	18
<i>Quality (%)</i>	61.1	66.7	55.6	55.6	61.1	72.2	55.6	66.7	61.1	61.1

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