Send Orders for Reprints to reprints@benthamscience.net

Current Neuropharmacology, **2014**, *12*, 365-379

Fluoxetine and all other SSRIs are 5-HT_{2B} Agonists - Importance for their Therapeutic Effects

Liang Peng*, Li Gu, Baoman Li and Leif Hertz

Laboratory of Metabolic Brain Diseases, Institute of Metabolic Disease Research and Drug Development, China Medical University, Shenyang, P. R. China

Abstract: Fluoxetine and other serotonin-specific re-uptake inhibitors (SSRIs) are generally thought to owe their therapeutic potency to inhibition of the serotonin transporter (SERT). However, research in our laboratory showed that it affects, with relatively high affinity the 5-HT_{2B} receptor in cultured astrocytes; this finding was confirmed by independent observations showing that fluoxetine loses its ability to elicit SSRI-like responses in behavioral assays in mice in which the 5-HT_{2B} receptor was knocked-out genetically or inhibited pharmacologically. All clinically used SSRIs are approximately equipotent towards 5-HT_{2B} receptors and exert their effect on cultured astrocytes at concentrations similar to those used clinically, a substantial difference from their effect on SERT. We have demonstrated up-regulation and editing of astrocytic genes for ADAR2, the kainate receptor GluK2, $cPLA_2$ and the 5-HT_{2B} receptor itself after chronic treatment of cultures, which do not express SERT and after treatment of mice (expressing SERT) for 2 weeks with fluoxetine, followed by isolation of astrocytic and neuronal cell fractionation. Affected genes were identical in both experimental paradigms. Fluoxetine treatment also altered Ca^{2+} homeostatic cascades, in a specific way that differs from that seen after treatment with the anti-bipolar drugs carbamazepine, lithium, or valproic acid. All changes occurred after a lag period similar to what is seen for fluoxetine's clinical effects, and some of the genes were altered in the opposite direction by mild chronic inescapable stress, known to cause anhedonia, a component of major depression. In the anhedonic mice these changes were reversed by treatment with SSRIs.

Keyword: Astrocytes, gene expression, 5-HT_{2A} receptor, 5-HT_{2B} receptor, SSRIs.

INTRODUCTION

It is generally assumed that fluoxetine and the other so-called 'serotonin-specific re-uptake inhibitors' (SSRIs) do not act on serotonin receptors at therapeutically relevant concentrations. At the time they were introduced clinically [1] it had been shown that SSRIs inhibited reuptake of serotonin (5-HT), but not of any other known transmitter, from the synaptic cleft in rat brain, decreased neuronal firing in the midbrain, and caused a rapid decrease in serotonin turnover [2]. Detailed studies showed very low affinity for any known receptor for serotonin or any other neurotransmitter [3,4]. The 5-HT_{2B} receptor was unknown at that time, because it was discovered in 1992 [5], and it was classified as the 5-HT_{2B} receptor only in 1994 [6]. Nevertheless, interaction of fluoxetine with a serotonin receptor on astrocytes had been shown already in 1979 by Hertz *et al*. [7], and the identity of this receptor as a 5-HT_{2B} receptor was demonstrated by Kong *et al*. in 2002 [8], after it erroneously (like many other effects on the 5-HT_{2B} receptor) had been identified as a 5-HT_{2C} receptor [9]. In contrast to all other serotonin (5-HT) receptors, the 5-HT_{2B} receptor has since been shown to have sufficiently high affinity for fluoxetine [10] to be activated by therapeutically relevant concentrations of these drugs (Table **1**).

SSRIs AS 5-HT_{2B} RECEPTOR AGONISTS

Subsequently it was shown that all clinically used SSRIs are not only specific 5-HT_{2B} receptor agonists [11], but also are virtually equipotent, in contrast to a large and important difference from the widespread difference in their potency as SERT inhibitors. Moreover, it was demonstrated that cultured astrocytes do not express the serotonin transporter, SERT [8]. Later Diaz *et al*. [12] found that effects of SSRIs seen after long-term treatment as well as the enhanced neurogenesis normally seen in fluoxetine-treated animals [13] can be prevented by genetic deletion or pharmacological inhibition of 5-HT_{2B} receptors; moreover stimulation of 5-HT_{2B} receptors by compounds that are not SSRIs induced SSRI-like responses in behavioral assays. They also showed expression of the 5-HT_{2B} receptor and SERT in raphe serotonergic neurons, and that SSRIs increased extracellular serotonin concentration in hippocampus. This increase was normally detectable after 20 min and peaked in 60 min after drug administration, and it was strongly reduced when 5-HT_{2B} receptors were deleted (Fig. **1**). These findings led to a conclusion that 5-HT_{2B} receptor expression on the raphe neurons is required for the therapeutic actions of SSRIs. Experiments by Launay *et al*. [14], performed on neuronal cultures from raphe nuclei (and serotonergic

*Address correspondence to this author at the Laboratory of Brain Metabolic Diseases, Institute of Metabolic Disease Research and Drug Development, China Medical University, No. 92 Beier Road, Heping District, Shenyang, P. R. China. Postal code: 110001; Tel: 86(24)23256666-5130; Fax: 86(24)23251769; E-mail: hkkid08@yahoo.com

1875-6190/14 $58.00+.00

Table 1. K_i values for 5-HT binding by selected SSRIs.

Receptor / Drug	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1D}$	5-HT$_{2A}$	5-HT$_{2B}$	5-HT$_{2C}$	5-HT$_3$
Fluoxetine	11,000	6,200	4,300	1,800	70*	270	19,000
Citalopram	16,600	EC$_{50}$>10,000	13,500	5,400		3,300	EC$_{50}$>10,000
Paroxetine	EC$_{50}$>10,000	25,000	4,900	10,200		17,400	2,600

*From Hertz *et al*. [10]. All other values from Wong *et al*. [4].

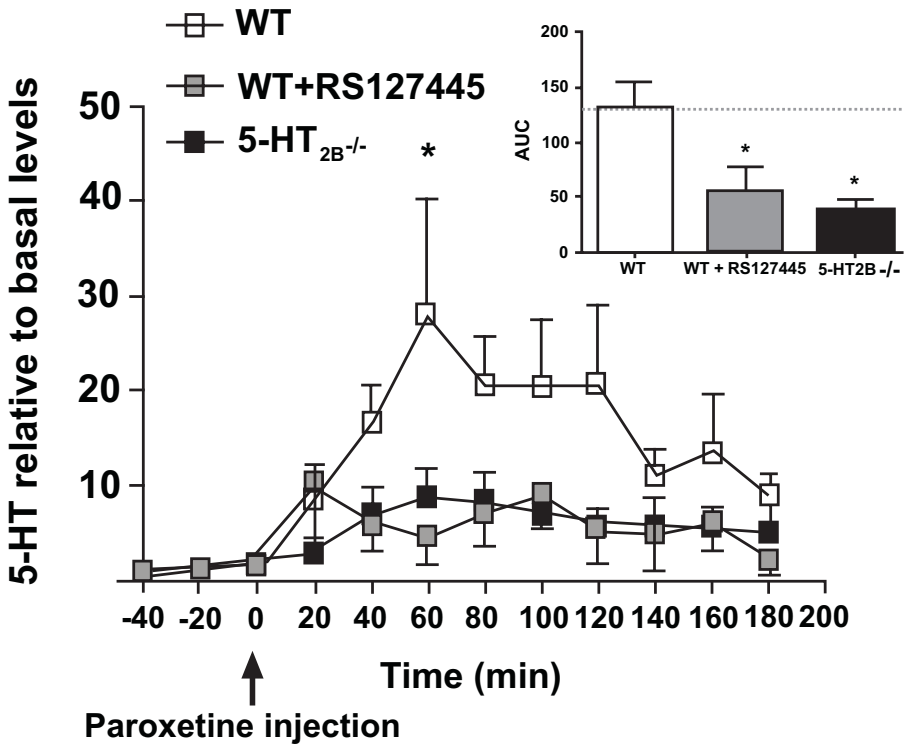

Fig. (1). Serotonin-selective reuptake inhibitor (SSRI)-induced increase in extracellular serotonin (5-HT) concentration in hippocampus of freely moving mice is greatly reduced when 5-HT$_{2B}$ receptors are absent. After 4 h of equilibration, the SSRI paroxetin (2 mg/kg, i.p.) was administered at time zero (0): hippocampal samples of extracellular fluid, obtained by microdialysis, were collected each 20 min for 3 h, and 5-HT concentrations were measured by high-pressure liquid chromatography. The peak observed at one hour after paroxetin injection was significantly higher in wild-type (WT) mice than in 5-HT$_{2B}^{-/-}$ mice or in WT mice pretreated with the 5-HT$_{2B}$ antagonist RS127445 15 min before the paroxetine injection. Inset represents the area under the curve (AUC) for each experimental group. Basal extracellular serotonin levels before the injection were identical under all three conditions. (From Diaz *et al*. [12]).

neurons induced from the 1C11 cell line) demonstrated that 5-HT$_{2B}$ receptor-PKC coupling promotes phosphorylations of SERT that control SERT activity. More specifically, it was shown that 5-HT$_{2B}$ receptor stimulation in the absence of added serotonin approximately doubled phosphorylation of SERT serotonin transport, a result very different from the generally accepted ability of fluoxetine to reduce SERT activity [14]. This pronounced difference may be explained by substantial differences between the immature and the mature brain [15, 16]. This age-dependent difference in fluoxetine effects is, however, in concordance with the finding by Sarkar *et al*. [17] that post-natal fluoxetine exposure results in the development of perturbed emotionality. Diaz *et al*. [12] also showed that fluoxetine was unable to elicit SSRI-like responses in behavioral assays in mice in which SERT was knocked out, but in these animals there is also a substantial depletion of serotonin [18].

5-HT$_{2B}$ receptors are present in many brain cells. Their expression was first identified on Purkinje cells by Choi and Maroteaux [19] and subsequently was demonstrated by mRNAs identification in freshly isolated fractions of neurons and astrocytes (Fig. **2**) from adult mice in which cell-specific markers were linked with differently fluorescent compounds that allowed cell separation [20] using fluorescence-activated cell sorting (FACS). Since the same primers were used for neurons and astrocytes, in Fig. **2** the heights of the columns provide direct information about the density of expression, which for all three 5-HT$_2$ receptor genes, but

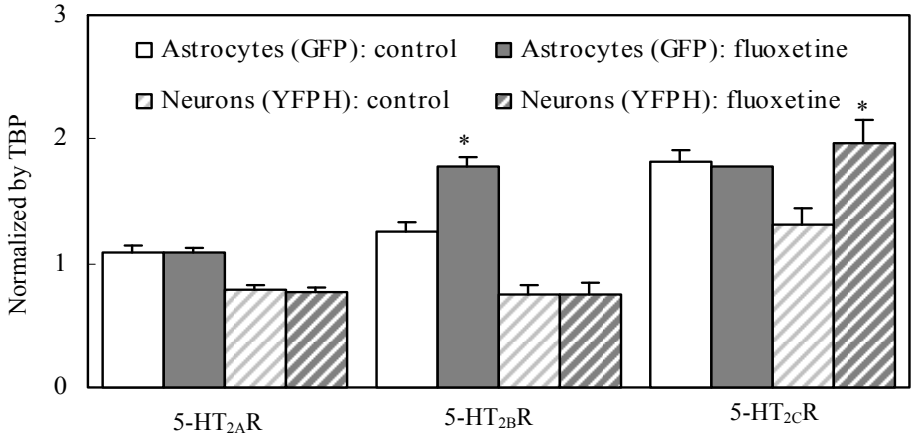

Fig. (2). mRNA expression measured by reverse transcription polymerase chain reaction (RT-PCR) of 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors in astrocytes and neurons isolated by fluorescence-activated cell sorting (FACS) from cerebral hemispheres from mice chronically treated with fluoxetine *in vivo*. Adult mice (FVB/NTg(GFAP-GFP)14Mes/J or B6. Cg-Tg(Thy1-YFPH)2Jrs/J) were either untreated controls or treated with fluoxetine (10 mg/kg/day) for 2 weeks. Results are means ± SEM of scanned blot ratios between 5-HT$_{2A}$, 5-HT$_{2B}$ or 5-HT$_{2C}$ receptors and TATA-binding protein (TBP) used as housekeeping gene. n = 3 (neurons) or 4 (astrocytes). *P<0.05 versus control group in astrocytes (5-HT$_{2B}$ receptor) or in neurons (5-HT$_{2C}$ receptor). (From Li *et al*. [20]).

especially the 5-HT$_{2B}$ receptor, is higher in astrocytes than in neurons.

FLUOXETINE TREATMENT DOWN-REGULATES EXPRESSION OF 5-HT$_{1A}$ RECEPTORS

A putative explanation for the long time lag between the onset of therapy with SSRIs and the clinical effect was first suggested by Blier and de Montigny [21] after administration of another serotonin reuptake blocker, zimelidine (no longer on the market). Administration of this drug not only increased extracellular serotonin concentration, but also strongly reduced electrical activity of serotonergic neurons, and this inhibition was gradually reduced during continued drug treatment. The authors therefore concluded that administration of a serotonin reuptake inhibitor may not result in enhanced serotonergic activity until normal excitability has been restored and it may thus have no therapeutic effect until then. The reason for the normalization of excitability was found to be down-regulation of activity of 5-HT$_{1A}$ autoreceptors, which generally are homoautoreceptors, located proximally in the neurons [22]. Czachura and Rasmussen [23] examined the effects of administration of different fluoxetine doses on the recovery of activity of serotonergic neurons in the dorsal rat raphe nucleus during a 21-day exposure. They found that i) acute intravenous, subcutaneous and intraperiperitoneal administration of fluoxetine inhibited the activity of serotonergic neurons; ii) chronic administration of fluoxetine at clinically relevant doses caused recovery of the activity of the neurons to baseline levels over the course of 14–21 days independently of either plasma or cerebrospinal fluid levels of fluoxetine or norfluoxetine; and iii) a non-parallel shift in their dose-response curve to the 5-HT$_{1A}$ agonist 8-OH-DPAT occurred over the 21 days of treatment, indicating 5-HT$_{1A}$ receptor desensitization. Internalization of 5-HT$_{1A}$ autoreceptors was shown by immune electronmicroscopy not only after acute administration of its selective agonist 8-OH-DPAT but also after administration of fluoxetine [24]. This phenomenon, which the authors also demonstrated by imaging in humans, is specific for 5-HT$_{1A}$ receptors in the dorsal raphe nucleus. In agreement with the data in Table **1** it is not likely to be a direct fluoxetine effect on the 5-HT$_{1A}$ receptor. It becomes obvious approximately 15 min after fluoxetine administration. This is simultaneous with the onset of an increase in extracellular serotonin concentration, which then might act on the 5-HT$_{1A}$ receptor. This interpretation is consistent with a finding by Miguelez *et al*. [25] that fluoxetine dose-dependently decreases the firing rate of serotonergic neurons in the dorsal raphe nucleus and that the 5-HT$_{1A}$ antagonist WAY-100635 restores the firing rate to basal values. Whether or not inhibition of 5-HT$_{1A}$ receptors can accelerate the onset of clinical effect after beginning of the therapy with SSRI is un-resolved. Artigas *et al*. [26] found that inhibition of this receptor could decrease the lag period from administration of an SSRI till therapeutic effect. That this is not the case has, however, also repeatedly been reported [27, 28]. Also, in the study by Czachura and Rasmussen [23], firing recovery did not correlate with levels of fluoxetine or norfluoxetine in plasma or cerebrospinal fluid. This is in contrast to a report by Önder and Tural [28] of faster clinical effect of higher than lower doses of fluoxetine and may accordingly disagree with the hypothesis that 5-HT$_{1A}$ receptor desensitization and recovery of firing of 5-HT cells in the dorsal raphe nucleus is of importance in the delayed therapeutic onset of fluoxetine. Finally, prevailing models suggest that 5-HT autoreceptors become desensitized not only in response to antidepressant administration but also in response to stress, two seemingly opposite manipulations [22]. Thus, questions remain about the role of the autoreceptors in determination of the time when the response to SSRIs becomes manifest.

FURTHER STUDY IN CULTURED AND FRESHLY DISSOCIATED ASTROCYTES

Acute Effects

The demonstration that fluoxetine acutely stimulates the 5-HT$_{2B}$ receptor in astrocyte cultures [8] was followed by

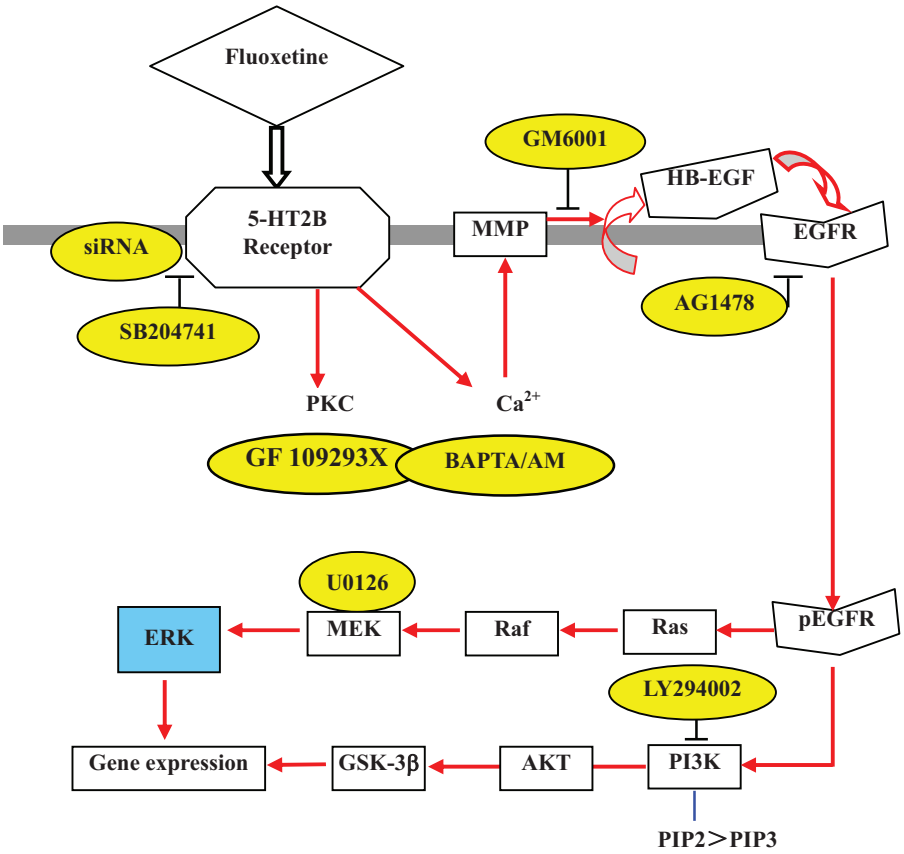

Fig. (3). Schematic illustration of pathways leading to stimulation of ERK and AKT phosphorylation by fluoxetine in astrocytes, established by use of specific inhibitors (see below) or siRNA during fluoxetine administration to cultured astrocytes. Fluoxetine binds to 5-HT_{2B} receptors. The activation of the receptors in turn induces an enhancement of protein kinase C (PKC) activity and of intracellular Ca^{2+} concentration by Ca^{2+} release from intracellular stores. The latter activates Zn-dependent metalloproteinases (MMPs) and leads to shedding of growth factor(s). The released epidermal growth factor receptor (EGFR) ligand stimulates phosphorylation of the EGFR. The downstream target of EGFR, extracellular-regulated kinase (ERK) (shown in blue) is phosphorylated *via* the Ras/Raf/MEK pathway, and AKT is phosphorylated *via* PI3K pathway. PIK3 is also known to catalyze the formation of PIP_3 from PIP_2. During chronic fluoxetine administration, siRNA against the 5-HT_{2B} receptor or inhibitors (shown in yellow) of this receptor (SB204741), of PKC (GF 109203X), of intracellular Ca^{2+} homeostasis (BAPTA/AM, an intracellular Ca^{2+} chelator), of Zn-dependent metalloproteinase's (GM6001), of the receptor-tyrosine kinase of the EGFR (AG1478), of ERK phosphorylation (U0126, a mitogen-activated kinase (MEK) inhibitor) or of the AKT pathway (LY294002, a PI3K inhibitor) were used to prevent changes in gene expression and editing. (From Hertz *et al*. [10]).

identification of the signal pathway that was activated in the cultured cells by Li *et al*. [29]. A slightly expanded version of this pathway is shown in Fig. **3**. It has previously been shown that fluoxetine acutely stimulates glycogenolysis, an effect that is secondary to an increase in $[Ca^{2+}]_i$ [9, 30]. Involvement of 5-HT_{2B} receptor-stimulated glycogenolysis has also been demonstrated during establishment of memory, where acute administration of serotonin can rescue long-term learning in a one trial aversive learning paradigm in day-old chickens under conditions when the aversive stimulus was otherwise too weak to establish more than transient long-term memory retention [31, 32]. Fluoxetine and paroxetine have a similar effect in this paradigm and are equipotent, indicating that the rescue was not due to inhibition of SERT (where different SSRIs have widely different potencies), and the rescuing effect was inhibited by an inhibitor of glycogenolysis [32]. In contrast to high concentrations of 5-HT itself, which also stimulate 5-HT_{1A} receptors and thereby can inhibit learning, fluoxetine and paroxetine at high levels have no inhibitory effect on learning [32]. Fluoxetine might also affect glycogen synthesis, since the AKT pathway (Fig. **3**) is stimulated leading to AKT phosphorylation (Fig. **4**). AKT phosporylation in turn stimulates GSK phosphorylation (Fig. **3**), making these *in vitro* findings consistent with demonstrations by Jope and coworkers [33-34] that administration of fluoxetine in brain cortex increases phosphorylation of GSK, and that serotonergic stimulation of GSK3 has mood effects.

Chronic Effects on 5-HT-Receptor and Related Proteins in Fluoxetine-Treated Animals and Cultures

Fig. **2** shows that only one astrocytic 5-HT_2 receptor, the 5-HT_{2B} receptor is up-regulated by 14 days of *in vivo* treatment with fluoxetine, as also indicated in Table **2**. This receptor is also up-regulated in whole brain [20]. The astrocytic 5-HT_{2A} and 5-HT_{2C} receptors are unaltered, but one neuronal 5-HT_2 receptor, the 5-HT_{2C} receptor, is also up-regulated in whole brain [20]. In addition the 5-HT_{2B}

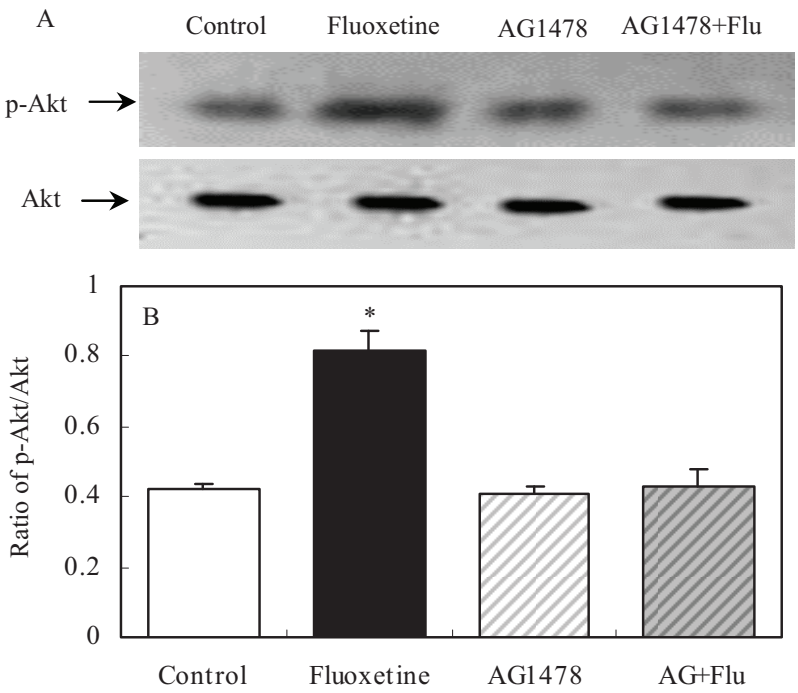

Fig. (4). Fluoxetine-induced AKT phosphorylation in cultured astrocytes. (A) Cells were incubated for 20 min in serum-free medium in the absence of any drug (Control) or in the presence of 10 μM fluoxetine. (A) Immunoblot from a representative experiment. Similar results were obtained from three independent experiments. (B) Average AKT phosphorylation was quantitated as ratios between p-AKT and AKT. SEM values are indicated by vertical bars. *Indicates statistically significant (P<0.05) difference from any other group. (Unpublished experiments by B. Li and L. Peng, using similar technique as for example in Li *et al*. [29]).

receptor sites are normally unedited in both astrocytes and neurons, but after 2 weeks of treatment up to one quarter of each of 8 different editing sited become edited, i.e., undergo shifts in base pair composition, as indicated in Table **2**. The importance of this is unknown, but for the 5-HT$_{2C}$ receptor editing can change G protein coupling [35]. Experiments in cultured astrocytes [36] have shown that upregulation of the 5-HT$_{2B}$ receptor itself in contrast with the changes in gene expression of ADAR2, cPLA$_2$ and GluK2 and in Ca^{2+} homeostasis (these all will be discussed below) occurs very slowly (Fig. **5**, **A**, **B**), but with the usual dependence on the fluoxetine concentration, i.e., an effect of 1 μM after 2 weeks. For comparison, the combined extracellular concentrations of fluoxetine and norfluoxetine in treated patients may reach up to 3 μM [37]. In contrast editing of the receptor (Fig. **5C**) was obvious after 3 days of treatment and thus precedes up-regulation. After 7 days the edited receptor no longer responded to serotonin with an increase in IP_3 turnover measured as described in the legend to the Fig. **5D**. To ascertain that this was a direct result of receptor editing, and not due to other effects by chronic fluoxetine administration, COS-7 cells were infected with receptor plasmids of either normal 5-HT$_{2B}$ receptors or receptors with 8 RNA sites RNA edited, and a similar inhibition was shown (Fig. **5E**). Thus an important result of chronic exposure to fluoxetine is to alter the normal response to serotonin.

Diaz *et al*. [12] showed that abrogation of the enhanced neurogenesis occurred in fluoxetine-treated animals after inactivation of 5-HT$_{2B}$ receptors, and a prominent feature in Fig. **3** is growth factor release transactivating the epidermal growth factor receptor (EGFR). For these reasons mRNA expression of EGFR was studied in freshly isolated neurons and astrocytes from both untreated control mice and fluoxetine-treated mice. Fig. **6** shows that its expression level was approximately 4 times higher in freshly isolated mouse brain astrocytes than in neurons obtained from the same brain. It was not increased after 14 days of *in vivo* treatment with fluoxetine (10 mg/kg per day ip.) in any of the two cell types. However, entry into neurons and especially astrocytes of nucleoside precursors for synthesis of DNA and RNA *via* the equilibrative nucleoside transporter ENT2 was increased during fluoxetine treatment as indicated by an elevation in its mRNA expression in both astrocytes and neurons – if anything most in astrocytes – after 14 day of fluoxetine treatment in the mouse [38]. Since participation of the 5-HT$_{1A}$ receptor so often has been anticipated in the process leading to manifestation of the therapeutic effect of SSRIs, and since this receptor is generally regarded as confined to the cell bodies of serotonergic neurons, its expression was also studied in the two cell types. Fig. **7** shows that 5-HT$_{1A}$ receptors are not confined to cell bodies of serotonergic neurons but also expressed in neurons from the cerebral hemispheres and even – although at lower density – in astrocytes. This is consistent with previous observations in astrocyte cultures and in striatal astrocytes [39-40]. Finally, the cell culture finding that SERT is absent in astrocytes was confirmed in freshly isolated astrocytes from the cerebral hemispheres. As can be seen in Fig. **8** this also applies to neurons from this location, although a minor expression cannot be excluded.

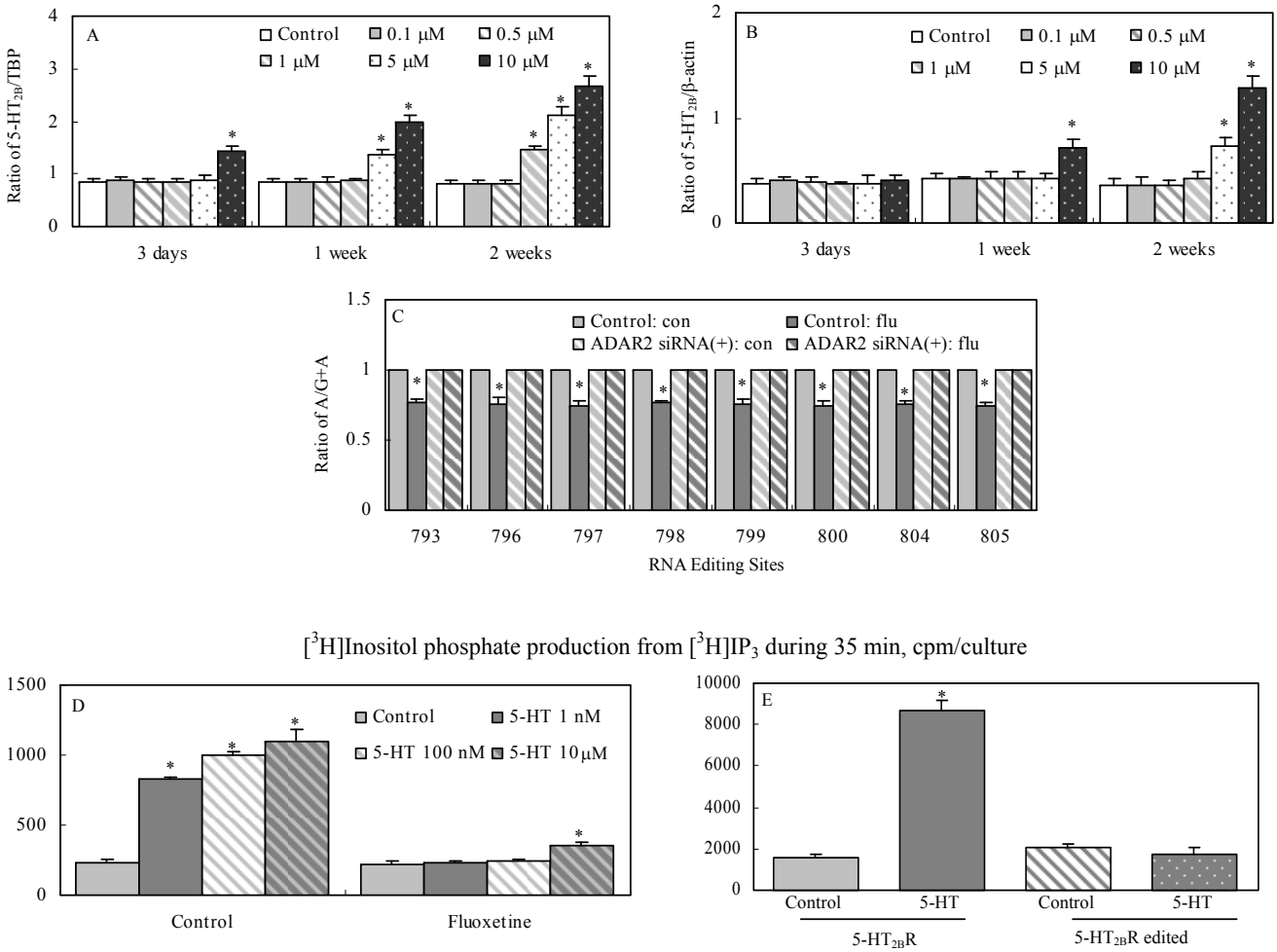

Fig. (5). (**A**, **B**) Time course for upregulation of 5-HT_{2B} receptor mRNA (**A**) and protein (**B**) during treatment of cultured mouse astrocytes with different concentrations of fluoxetine. (**C**) editing of 5-HT_{2B} receptor after 3 days of treatment with 10 μM fluoxetine. (**D**, **E**) Reduction of effect of 5-HT_{2B} receptor stimulation after downregulation of cultured astrocytes and transfected COS-7 cells with 10 μM fluoxetine for 7 days. Methodologies for C was as in Li *et al.* [46]. Response of the receptor to serotonin was measured as increase in the ability of serotonin to evoke release of ^{3}H-inositol phosphate (IP) from labeled IP_3 in cultured astrocytes (**D**) and in COS-7 cells infected with receptor plasmids of either normal 5-HT_{2B} receptors or receptors with 8 RNA sites RNA (**E**). (From Hertz *et al.* [36]).

Chronic Effects on other Receptors in Fluoxetine-Treated Animals and Cultures

The effects of SSRIs that are important in connection with major depression are the chronic effects, since the effect of clinical treatment takes several weeks to appear. The cells obtained by FACS directly from the brains of fluoxetine-treated or control animals are intact enough that gene expression can be reliably determined, but for many functional studies they are probably not sufficiently well preserved. Both types of studies are easily carried out in cultured astrocytes, but astrocyte cultures have often been used rather uncritically. Our cultures are different from most in (i) originating from neonatal mice, (ii) being very clean, because the cells have been filtered through dense meshes, and (iii) being treated with dibutyryl cyclic AMP from the third week to replace a noradrenergic signal they would have received, had they remained in the brain. Comparison of effects of chronic treatment with fluoxetine on up-regulation of selected genes showed in all studied cases similar effects in these cultures and in astrocytes freshly isolated from animals chronically treated with fluoxetine, 10 mg/kg per day, the only SSRI studied in astrocytes *in vivo* (Table **2**). They have also been similar to those found by other authors or ourselves in total brain of treated animals with the exception of gene expression of 5-HT_{2C}, $sPLA_2$ and GluK4, genes that were up-regulated in neurons of the treated animals and accordingly also in whole brain (reviewed by Li *et al.* [20]). Moreover, genes for *cfos* and *fosB* were up-regulated in both neurons and astrocytes (Table **2**).

ADARs

The up-regulation of ADAR2 by chronic treatment with fluoxetine is of special importance since editing of other genes depends on ADAR2 as shown for up-regulation of $cPLA_2$ [41]. ADARs constitute a family of adenosine deaminases, which catalyze deamination of adenosine to inosine in double-stranded regions of mRNAs. This changes the amino acids in the translated protein sequence, since

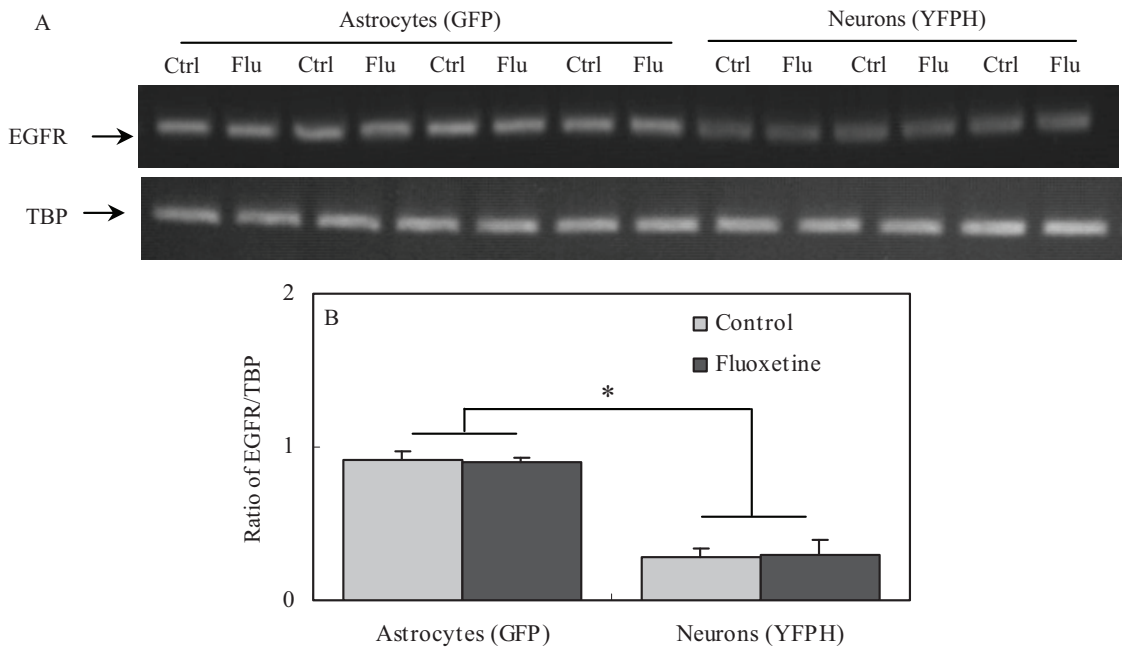

Fig. (6). mRNA expression measured by RT-PCR of epidermal growth factor receptor (EGFR) in astrocytes and neurons isolated by FACS from cerebral hemispheres *in vivo* from similar fluoxetine-treated mice as used in Fig. 2. (A) A representative experiment showing mRNAs for EGFR and for TBP, as a house-keeping gene, in control animals and the corresponding results in fluoxetine-treated animals. The sizes of the PCR products of EGFR is 305 bp and of TBP 236 bp. (B) Means ± SEM of scanned ratios between EGFR and TBP. n = 3 (neurons) or 4 (astrocytes). *P<0.05 between neuron group and astrocyte group. (Unpublished experiments by B. Li, L. Hertz and L. Peng, using similar technique as in Li *et al*. [20]).

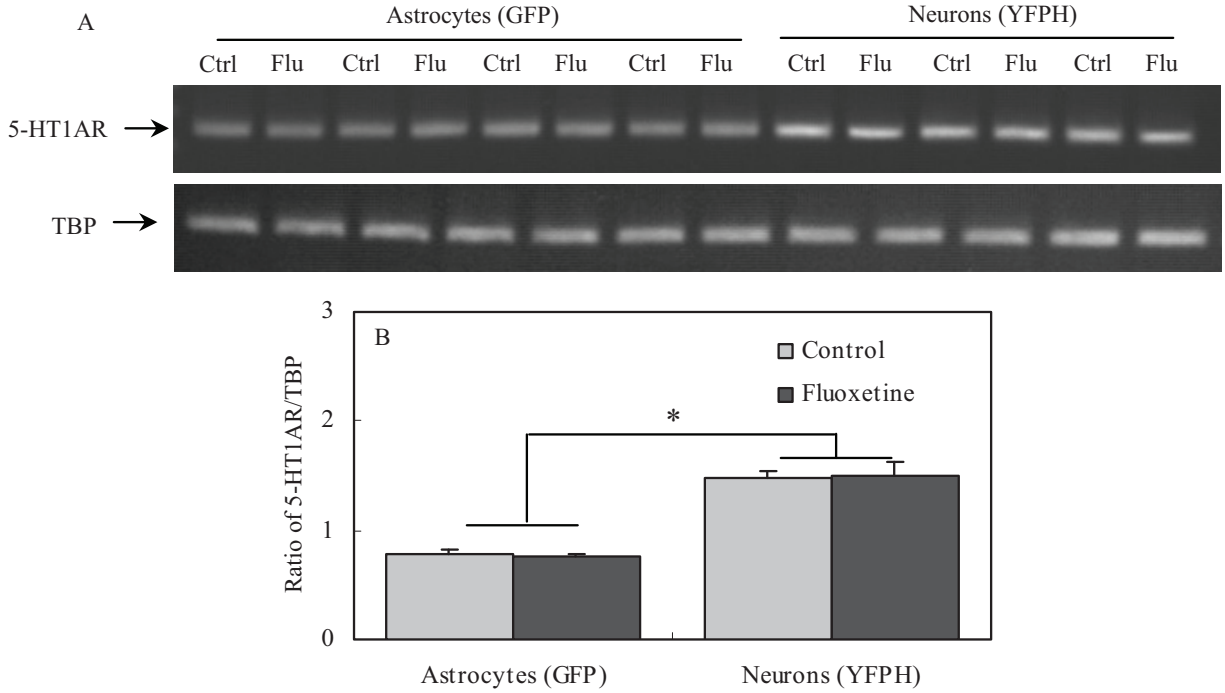

Fig. (7). mRNA expression measured by RT-PCR of 5-HT$_{1A}$ receptor in astrocytes and neurons isolated by FACS from cerebral hemispheres *in vivo* from similar fluoxetine-treated mice as used in Fig. **2**. (A) A representative experiment showing mRNAs for 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) and for TBP, as a house-keeping gene in control animals and the corresponding results in fluoxetine-treated animals. The sizes of the PCR products of the 5-HT$_{1A}$ receptor is 401 bp and of TBP 236 bp. (B) Means ± SEM of scanned ratios between 5-HT$_{1A}$ receptor and TBP. n = 3 (neurons) or 4 (astrocytes). *P<0.05 between neuron group and astrocyte group. (Unpublished experiments by B. Li, L. Hertz and L. Peng, using similar technique as in Li *et al*. [20]).

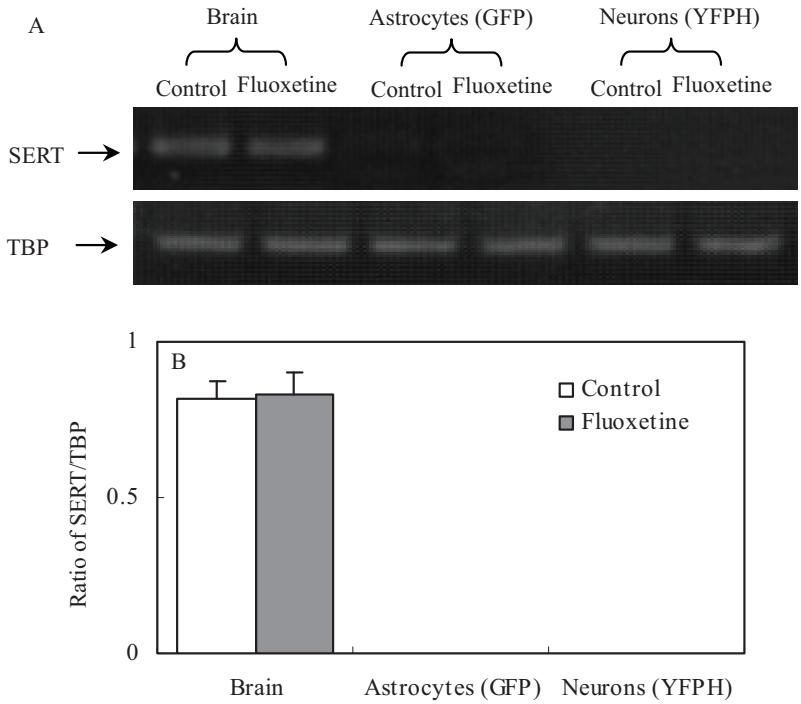

Fig. (8). mRNA expression measured by RT-PCR of the serotonin transporter SERT in brain and in astrocytes and neurons isolated by FACS from cerebral hemispheres *in vivo* treated with fluoxetine. The whole brains were from CD1 mice. Astrocytes and neurons were from adult mice (FVB/NTg(GFAP-GFP)14Mes/J or B6.Cg–Tg(Thy1-YFPH)2Jrs/J), which were either untreated controls or treated with fluoxetine (10 mg/kg/day) for 2 weeks. (A) Representative experiments showing mRNAs for SERT and for TBP, as a house-keeping gene in control animals and the corresponding results in fluoxetine-treated animals. The sizes of the PCR products of SERT is 200 bp and that of TBP 236 bp. (B) Means ± SEM of scanned ratios between SERT and TBP n = 5 (brain), n = 3 (neurons) or 4 (astrocytes). (Unpublished experiments by B. Li, L. Hertz and L. Peng, using similar technique as in Li *et al*. [20]).

inosine is perceived by the cells as guanosine [42]. The ADAR family comprises ADAR1, ADAR2 and ADAR3 [43], which all are expressed in the brain [44]. In the brain ADAR2 is expressed in hippocampal pyramidal neurons and cerebellar Purkinje cells and Bergmann glial cells, but ADAR 1 and 3 are sparsely expressed [45]. ADAR2 up-regulation in fluoxetine-treated mice is subtype- and astrocyte-specific. In cultured astrocytes mRNA and protein expression of ADAR2 almost doubled within 3 days which can be prevented by treatment with 5-HT_{2B} receptor siRNA [46]. However $sPLA_2$ is up-regulated by fluoxetine in neurons and in whole brain (Table **2**).

Calcium-Dependent Phospholipase 2 ($cPLA_2$)

This enzyme in the brain *in vivo* is strongly expressed in astrocytes [47-49]. Its activation releases the unsaturated fatty acid arachidonic acid from the *sn*-2 position of membrane-bound phospholipid substrate in neural preparations [50-53], including glioma cells [54]. Arachidonic acid strongly stimulates glucose metabolism in cultured astrocytes [55]. So does treatment with 10 µM fluoxetine for 24 h, which might have sufficed to induce an increase in $cPLA_2$ [56]. In contrast, acute exposure of astrocyte cultures to fluoxetine has no similar effect (L. Peng and L. Hertz, unpublished experiments). Arachidonic acid also stimulates glycogenolysis [57, 58].

Rapoport and coworkers [59-61] showed that chronic administration of fluoxetine leads to stimulation of $cPLA_2$ activity and enhanced mRNA and protein expression of its gene in rat brain. Neither of the two other phospholipases A_2 (secretory PLA_2 [$sPLA_2$] and intracellular PLA_2 [$iPLA_2$]) was similarly affected. Li *et al*. [41] confirmed a slow and selective up-regulation of mRNA and protein expression of $cPLA_{2a}$, the major isoform of $cPLA_2$, in mouse astrocytes in primary cultures during chronic exposure to 1 or 10 µM fluoxetine. The up-regulation was prevented by the 5-HT_{2B} antagonist SB 204741, the metalloproteinase inhibitor GM6001 and the inhibitor of EGF receptor tyrosine phosphorylation AG1478 and by U0126, the inhibitor of $ERK_{1/2}$ phosphorylation, all inhibitors of the signaling pathway shown in Fig. **3** for fluoxetine. Thus, up-regulation of mRNA and protein of $cPLA_2$ were inhibited by the same drugs that acutely inhibit $ERK_{1/2}$ phosphorylation and by inhibition of the phosphorylation itself. As shown in Table **2**, up-regulation, specifically of $cPLA_{2a}$, has been confirmed in freshly dissociated astrocytes isolated by FACS after 2 weeks treatment of rats with fluoxetine, whereas no corresponding effect was found in neurons [20]. Accordingly, the enhanced $cPLA_2$ activity demonstrated in whole brain after chronic fluoxetine treatment [60] is likely to selectively occur in astrocytes. However, as already mentioned, $sPLA_2$ is up-regulated by fluoxetine in neurons and in whole brain [20].

Stimulation of glucose metabolism may be important in the pharmacological treatment of depressive illness. In patients suffering from unipolar depression brain glucose

Table 2. Comparison between effects on gene expression (mRNA) and editing of chronic treatment with the SSRI fluoxetine in cultured mouse astrocytes and in astrocytes freshly isolated from drug-treated mice using fluorescence-activated cell sorting FACS.

Gene	FACS Astrocytes	Cultured Astrocytes
5-HT$_{2B}$ receptor expression	up	up
5-HT$_{2B}$ editing	up	up
5-HT$_{2c}$ receptor expression	unchanged	unchanged
ADAR2	up	up
sPLA$_2$	unaltered	unaltered
Ca$_v$1.2	up	up
cfos expression	up	up
fosB expression	up	up
GluK2 expression	up	up
GluK2 editing	up	up
GluK4 expression	unaltered	unaltered

All data except those for Ca$_v$1.2 are from Li *et al*. [20] and those for Ca$_v$1.2 from Du *et al*. [83].

metabolism is reduced in many regions, primarily in the fronto-temporal parts [62-65], with a correlation between the degree of hypometabolism and severity of the illness [66], and normalization following treatment with an SSRI [67-69]. Sublette *et al*. [70] showed that arachidonic acid may play a role in determining rates of cerebral glucose metabolism. This could be seen from a rectilinear correlation in depressed patients between plasma concentration of arachidonic acid and rate of cerebral glucose utilization in a region affected metabolically. That major depression may even be related to an astrocyte-specific energy failure was suggested by Hundal [71]. Arachidonic acid metabolites, including prostaglandins, exert additional beneficial effects important for the amelioration of depression and are not only important for induction of inflammation [72]. Thus, prostaglandin synthesis inhibitors in doses used to treat pain may cause fear, agitation, and affective liability [73, 74], and one euthymic bipolar patient repeatedly developed a depression during such exposure [75]. This should in no way be taken as a suggestion that inflammatory events do not contribute to major depression, since such a contribution is well known, as described by Müller [76]. However, the lack of up-regulation of cPLA$_2$ in neurons by fluoxetine suggests that inflammatory effects on neurons may not be enhanced by fluoxetine. Whatever the reasons are, genetic associations are found between cPLA$_2$ and major depression [77, 78].

The Calcium Channel Gene Ca$_v$1.2 and Ca^{2+} Homeostasis

Free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in astrocytes is low in resting cells (~100 nM, compared to 1-2 mM in the extracellular fluid), but $[Ca^{2+}]_i$ increases are a necessary and essential component of all astrocytic activities (glycogenolysis, release of transmitter ATP, formation of glutamate and activities of many transmitters [36, 58, 79]. Increases in $[Ca^{2+}]_i$ can also spread between astrocytes as Ca^{2+} waves [80]. Inside the cell Ca^{2+} can be accumulated into and released from intracellular organelles (endoplasmic reticulum or ER, mitochondria). Ca^{2+} transport across the cell membrane is therefore of utmost importance for astrocytic functions. In contrast to the ability of fluoxetine [9] (and many transmitters) acutely to cause an increase in $[Ca^{2+}]_i$ in astrocytes, chronic treatment with fluoxetine rapidly abolishes or reduces transmitter and fluoxetine-induced $[Ca^{2+}]_i$ increase [46]. However, a corresponding increase in astrocytic $[Ca^{2+}]_i$ by elevation of extracellular K^+ concentrations above 15 mM [81] is *not* reduced, but increased, by chronic treatment with fluoxetine [82-83]. The reason for this is a fluoxetine-mediated up-regulation of the L-channel gene Cav1.2 [83], shown both in cultured cells and in astrocytes freshly obtained from fluoxetine-treated animals (Table **2**). This overcompensates for a down-regulation of the store-operated (or capacitative) Ca^{2+} entry (SOCE) occurring *via* store-operated channels, SOCS [82, 84] as shown by the increase in astrocytic $[Ca^{2+}]_i$ [83]. In contrast, the Ca$_v$1.3 gene, which plays a smaller role in astrocytes than Ca$_v$1.2, is unaffected by treatment of mice with fluoxetine for 2 weeks [83]. SOCS are very important for regulation of intracellular Ca^{2+}, especially for the levels in the ER, which controls the amount of Ca^{2+} released by activation of inositoltrisphosphate ($InsP_3$) receptors ($InsP_3R$) or ryanodine receptors (RyR). The 'transient receptor potential channel' (TRPC) protein TRPC1 is a major component of SOCS [84-86]. In cells in which TRPC1 had been knocked down by treatment with antisense oligonucleotides TRPC1 antibody the capacitative Ca^{2+} uptake is greatly reduced [82, 87]. The same occurs after short-lasting chronic treatment with fluoxetine and many other drugs (see below) and reduces or abrogates the ability of transmitters to increase astrocytic $[Ca^{2+}]_i$ [82]. In conclusion, chronic treatment with

SSRIs inhibits the ability of transmitters but, on account of the up-regulation of $Ca_V1.2$, not that of elevated K^+ concentrations to increase astrocytic $[Ca^{2+}]_i$ and thus glycogenolysis [79]. K^+-mediated stimulation of glycogenolysis has been shown both in intact brain tissue [88] and in cultured cells [89] to increase with the magnitude of the K^+ elevation. The effect on K^+ stimulation by fluoxetine is opposite to that seen after chronic treatment with any of the 3 anti-bipolar drugs lithium, carbamazepine or valproic acid, which inhibits both transmitter- and K^+-mediated Ca^{2+} uptake [81].

The Immediate Early Genes *cfos* and *fosB*

As shown in Table **2** these two genes are also up-regulated by chronic treatment with fluoxetine, and this happens in both neurons and astrocytes. In astrocytes the up-regulation depends on normal operation of the signaling pathway shown in Fig. **3** [29]. The mechanism for up-regulation in neurons is unknown. It may or may not be secondary to the effect in astrocytes and could be secondary to growth factor release as shown in Fig. **9** for a different tissue [90].

The Kainate Receptors GluK2 and GluK4 and other Glutamate Receptors or Transporters

The role of glutamate in major depression and its drug treatment has repeatedly been discussed [10, 20, 91-92], but the precise roles of different receptors are far from resolved. However, astrocytes are essential for all aspects of glutamate homeostasis in brain (synthesis, uptake and oxidation) as reviewed by Hertz and Zielke [93] and Schousboe *et al*. [94]. Many aspects of glutamatergic transmission including astrocyte-specific glutamate transporters are affected in depressive illness [95-96]. Correlations between drug effects on major depression and on glutamatergic activities have attracted special interest in connection with the rapid but short-lasting therapeutic effects of ketamine and riluzole in depressed patients [95]. Recently the effects of riluzole and of ketamine have also been reviewed by Murrough and colleagues [97, 98]. The paper by Lapidus *et al*. [97] mainly discusses neuronal effects, although disregarding the GluK4 receptor but mentioning mGluRs. However, as seen from Table **2**, GluK4 becomes up-regulated in neurons after chronic fluoxetine treatment [20], whereas at least mGluR5 is virtually unaffected by fluoxetine treatment [36]. It is also often assumed that increase in $[Ca^{2+}]_i$ in astrocytes is mediated by the metabotropic glutamate receptor mGluR5, a response which however is present only in the immature brain [15]. Murrough *et al*. [98] notes very interesting correlations between ketamine, major depression and cognitive function. This is reminiscent of the finding that stimulation of 5-HT_{2B} receptor activity is important not only for the mechanism of action of SSRIs but also during learning [32].

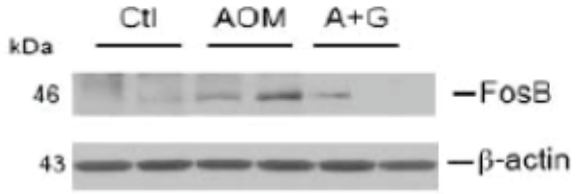

Fig. (9). An inhibitor of the epidermal growth factor receptor, EGFR (gefitinib) inhibits EGFR signal to FosB in tumors induced by azoxymethane. Colonic mucosa from control rats (Ctl) and colonic tumors from azoxymethane-treated rats (AOM), or tumors from azoxymethane-treated rats fed gefitinib (A+G) were homogenized and FosB and β-actin, as a housekeeping gene, determined by Western blots. A representative blot out of 4-6 is shown for each. (Modified from Dougherty *et al*. [90]).

The kainate receptor GluK2 can operate not only in an inotropic, but also in a metabotropic mode [99]. It is up-regulated and edited in astrocytes both in culture and in the brains of mice treated with fluoxetine for 14 days [20, 46]. The genome-encoded GluK2 mRNA can be edited at 3 sites, the I/V site, the Y/C site, and the Q/R site. The editing was increased at all three sites by chronic treatment with fluoxetine [46]. Fluoxetine-mediated changes in GluK2 editing are consistent with a previous observation by the Barbon group, but they found editing of the Q/R site in intact brain to be slightly decreased by chronic fluoxetine treatment in a brain preparation including white matter [100]. Inclusion of subunits containing the edited R form of the Q/R site often lowers Ca^{2+} permeability [101], and in fluoxetine-treated cultures a normally occurring increase in free cytosolic Ca^{2+} concentration to 300-400% of control value in response to 100 μM glutamate was abolished by the fluoxetine treatment [46]. This increase is evoked by the GluK2 receptor operating in its metabotropic manner [10]. mRNA expression analysis has demonstrated that the human GluK2c splice variant in brain is mainly expressed in non-neuronal cells and barely expressed in neurons [102]. Knock-out of the GluK2 receptor in mice results in less anxious or more risk-taking type behavior and less manifestation of despair [103]. Obsessive-compulsive disorder is also genetically linked to abnormalities in *Grik2*, the gene coding for GluK2 [104, 105]. GluK4 is up-regulated in neurons after chronic treatment with fluoxetine [20].

Gene Changes in Models of Major Depression

Since most experiments on SSRI action are carried out in normal animals or in cultures from normal animals it is important that some at least partial models of major depression exist. Chronic stress can induce anhedonia, the inability to experience pleasure from activities usually found enjoyable. Short-lasting exposure of experimental animals to moderately stressful experiences have been used to create anhedonia as a model of major depression, but anhedonia occurs only in a fraction of the stressed mice. However, mice displaying or not displaying anhedonia can be clearly separated into two distinct groups according to their preference of a sucrose solution over water, making this an even better test. Anhedonia is, however, only *one* of the components of major depression, and yet it causes changes opposite to those evoked by chronic exposure to fluoxetine in several of the genes shown in Table **2**. This is illustrated in Fig. **10** and includes a down-regulation of the 5-HT_{2B} receptor in astrocytes but not in neurons, but there is no effect on 5-HT_{2C} expression in either neurons or astrocytes

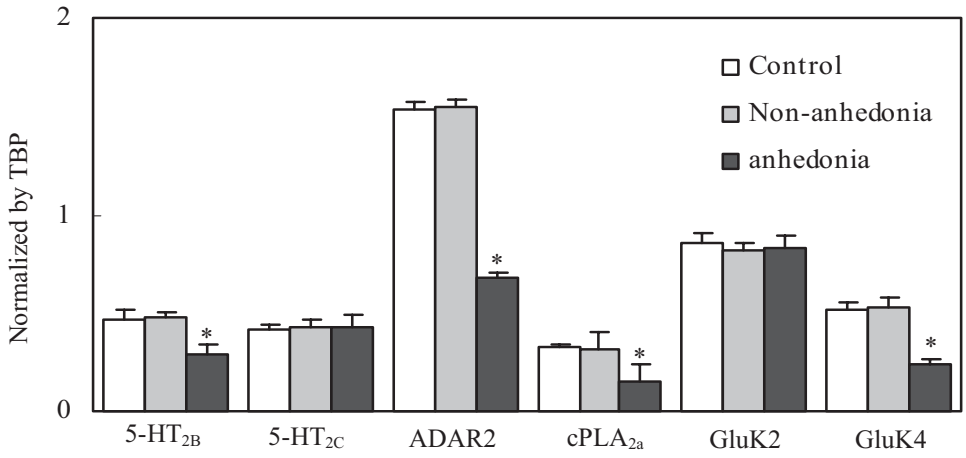

Fig. (10). Effects of stress-induced anhedonia on gene expression in brain *in vivo*. mRNA expression (measured by RT-PCR) for 5-HT$_{2B}$ receptor, 5-HT$_{2C}$ receptor, ADAR2, cPLA$_{2a}$, GluK2, GluK4 and TBP were determined from 22 animals studied. Based on history and sucrose consumption the animals had been identified as controls (no stress), stressed, non-anhedonic animals (sucrose-preferring), and stressed anhedonic animals (identical consumption of sucrose and water). Mean ± SEM of scanned experimental-gene/TBP ratio in each group, with n = 10, n = 5 and n = 7. Note down-regulation only in those animals that developed anhedonia. *P<0.05: statistically significant difference from other groups. (From Li *et al*. [20]).

[20]. These changes occurred only in the mice that became anhedonic, but were not shown by mice, which had suffered the same stresses but still preferred a sucrose solution over water, i.e., had not become anhedonic. Moreover, some gene expression changes expected from those established in the cultured and freshly isolated cells during drug treatment did not occur. This included the already mentioned absence of any effect on neuronal 5-HT$_{2C}$ receptor density in anhedonic mice and GluK2 in astrocytes, and cfos and fosB were not studied. However, it should be remembered that anhedonia is only one component of major depression. Fluoxetine can reverse both the anhedonia and some gene expression changes (B. Li and L. Peng, unpublished results).

Another model of major depression is defeat-induced social avoidance, which is also seen only in susceptible mice. Non-defeated control mice treated with fluoxetine for 20 days revealed no alterations in social behavior, but exhibited an accumulation of ΔFosB, the product of fosB, in nucleus accumbens. Fluoxetine treatment of susceptible mice reversed their social avoidance and enhanced ΔFosB levels and induction of the AMPA glutamate receptor subunit GluA2 (GluR2) [106].

The gene for this receptor is expressed in both neurons and astrocytes, although perhaps at greatest density in neurons [107]. Mice susceptible to chronic social defeat showed a significant decrease in GluA2 levels compared to controls, while resilient mice showed increased GluA2 levels. The induction of GluA2 seen in resilient mice appears to reflect a direct effect of ΔFosB on the GluA2 gene, and chronic fluoxetine treatment of non-defeated mice increased GluA2. In a later study from the same group [108] different responses were found between different areas, but no determination of cell type was made. Ten days of exposure to stressful events also down-regulates Grin2C, the gene of the subunit 2C of the NMDA receptor, as well as Gabdr, the GABA$_A$ delta subunit receptor [109].

CONCLUDING REMARKS

Combination of our own findings with those of Diaz *et al*. [12] leaves no doubt that SSRIs act *via* the 5-HT$_{2B}$ receptor. However their interpretation of the mechanism differs widely from ours. In principle both could be operating, since the enhanced release of 5-HT envisaged by Diaz *et al*. [12] as the cause of the therapeutic effect also will act on astrocytic 5-HT$_{2B}$ receptors. That a direct, SERT-independent stimulation of this receptor is evoked in astrocytes by SSRIs is shown by the cell culture experiments and its *in vivo* relevance is proven by the similar effects on gene up-regulation and editing in the cultures (which express no SERT) and in astrocytes from fluoxetine-treated animals (expressing SERT in at least some neurons). The neurogenic effect by fluoxetine emphasized by Diaz *et al*. [12] is also consistent with the demonstrated signaling pathway for fluoxetine in astrocytes. Although this effect probably is not significant for the therapeutic effects of SSRIs [110], the release of growth factor may be important for the demonstrated gene effects of these drugs on neurons in intact animals. The claim by Diaz *et al*. [12] that fluoxetine cannot elicit SSRI-like responses in behavioral assays in mice in which SERT is knocked out, is unconvincing on account of the concomitant severe depletion of serotonin in the SERT$^{-/-}$ mice [18]. Evidence for SERT-independent effects of fluoxetine has also been shown by Pinna and Rasmusson [111]. This opens the possibility that SSRI-mediated inhibition may be an epiphenomenon rather than the mechanism by which they exert their therapeutic action.

5-HT$_{2B}$ receptors are involved in multiple biological functions besides brain activity, including cardiovascular function. There is a growing concern regarding adverse drug reactions, specifically cardiac valvulopathy associated with 5-HT$_{2B}$ agonists [112], as first seen in association with the anti-obesity drug fenfluramine [113]. The reason that similar side effects are not seen with SSRIs may be the high protein

binding of most of these drugs. However, there are other cardiovascular concerns, suggesting that 5-HT_{2B} receptors may be involved in apoptotic events associated with cardiac remodeling during increased adrenergic stimulation [114]. Jaffré *et al*. showed an interaction between autocrine stimulation of AT_1 receptors by angiotensin II and 5-HT_{2B} receptors in cardiac fibroblasts during β -adrenergic–dependent hypertrophic responses. There is also evidence that 5-HT_{2B} receptors may play a role in the development of DOCA-salt hypertension [115]. These effects may be the reason for a greatly increased mortality following acute coronary occlusion if the patients are treated with an SSRI, especially if the treatment is started after the coronary attack [116]. Paroxetine and possibly fluoxetine use in early pregnancy is also associated with a small increased risk for cardiovascular malformations, perinatal respiratory distress and persistent pulmonary hypertension [117]. Thus, as with most other drugs, it is important to remember that they have actions at more organs than the selected therapeutic target and that these actions in some cases can be adverse.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by Grants No. 31171036 to LP from the National Natural Science Foundation of China.

REFERENCES

[1] Wong, D.T.; Perry, K.W.; Bymaster, F.P. Case history: the discovery of fluoxetine hydrochloride (Prozac). *Nat. Rev. Drug Discov.*, **2005**, *4*, 764-774. DOI: 10.1016/j.ajo.2005.02.011

[2] Fuller, R.W.; Wong, D.T. Inhibition of serotonin reuptake. *Fed. Proc.*, **1977**, *36*, 2154-2158. PubMed ID: 326579

[3] Wong, D.T.; Bymaster, F.P.; Reid, L.R.; Threlkeld, P.G. Fluoxetine and two other serotonin uptake inhibitors without affinity for neuronal receptors. *Biochem. Pharmacol.*, **1983**, *32*, 1287-1293. DOI: 10.1016/0006-2952(83)90284-8

[4] Wong, D.T.; Bymaster, F.P. Development of antidepressant drugs. Fluoxetine (Prozac) and other selective serotonin uptake inhibitors. *Adv. Exp. Med. Biol.,* **1995**, *363*, 77-95. **PubMed ID:** 7618533

[5] Foguet, M.; Nguyen, H.; Le, H.; Lübbert, H. Structure of the mouse 5-HT_{1C}, 5-HT_2 and stomach fundus serotonin receptor genes. Neuroreport, 1992, 3, 345-348. http://dx.doi.org/10.1097/00001756-199204000-00014

[6] Schmuck, K.; Ullmer, C.; Engels, P.; Lübbert, H. Cloning and functional characterization of the human 5-HT_{2B} serotonin receptor. *FEBS Lett.*, **1994**, *342*, 85-90. http://dx.doi.org/10.1016/0014-5793(94)80590-3

[7] Hertz, L.; Baldwin, F.; Schousboe, A. Serotonin receptors on astrocytes in primary cultures: effects of methysergide and fluoxetine. *Can. J. Physiol. Pharmacol.*, **1979**, *57*, 223-226. http://dx.doi.org/10.1139/y79-034

[8] Kong, E.K.; Peng, L.; Chen, Y.; Yu, A.CH; Hertz, L. Up-regulation of 5-HT_{2B} receptor density and receptor-mediated glycogenolysis in mouse astrocytes by long-term fluoxetine administration. *Neurochem. Res.*, **2002**, *27*, 113-120. http://dx.doi.org/10.1023/a:1014862808126

[9] Chen, Y.; Peng, L.; Zhang, X.; Stolzenburg, J.U.; Hertz, L. Further evidence that fluoxetine interacts with a 5-HT_{2C} receptor in glial cells. *Brain Res. Bull.*, **1995**, 38, 153-159. http://dx.doi.org/10.1016/0361-9230(95)00082-p

[10] Hertz, L.; Li, B.; Song, D.; Ren, J.; Dong, L.; Chen, Y.; Peng, L. Astrocytes as a 5-HT_{2B}-mediated, SERT-independent SSRI target, slowly altering depression-associated genes and functions. *Curr. Signal. Transduct. Ther.*, **2012**, *7*, 65-80. http://dx.doi.org/10.2174/1574362799278154

[11] Zhang, S.; Li, B.; Lovatt, D.; Xu, J.; Song, D.; Goldman, S.A.; Nedergaard, M.; Hertz, L.; Peng, L. 5-HT_{2B} receptors are expressed on astrocytes from brain and in culture and are a chronic target for all five conventional 'serotonin-specific reuptake inhibitors'. *Neuron Glia. Biol.*, **2010**, *6,* 113-125. http://dx.doi.org/10.1017/s1740925x10000141

[12] Diaz, S.L.; Doly, S.; Narboux-Nême, N.; Fernández, S.; Mazot, P.; Banas, S.M.; Boutourlinsky, K.; Moutkine, I.; Belmer, A.; Roumier, A.; Maroteaux, L. 5-HT(2B) receptors are required for serotonin-selective antidepressant actions. *Mol. Psychiatry*, **2012**, *17*, 154-163. http://dx.doi.org/10.1038/mp.2011.159

[13] Manev, H.; Uz, T.; Manev, R. Glia as a putative target for antidepressant treatments. *J. Affect Disord.*, **2003**, *75*, 59-64. http://dx.doi.org/10.1016/s0165-0327(02)00044-7

[14] Launay, J.M.; Schneider, B.; Loric, S.; Da Prada, M.; Kellermann, O. Serotonin transport and serotonin transporter-mediated antidepressant recognition are controlled by 5-HT_{2B} receptor signaling in serotonergic neuronal cells. *FASEB J.* **2006**, *20*, 1843-1854. http://dx.doi.org/10.1096/fj.06-5724com

[15] Sun, W.; McConnell, E.; Pare, J.F.; Xu, Q.; Chen, M.; Peng, W.; Lovatt, D.; Han, X.; Smith, Y.; Nedergaard, M. Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. *Science*, **2013**, *339*, 197-200. http://dx.doi.org/10.1126/science.1226740

[16] Hertz, L. The Glutamate-Glutamine (GABA) Cycle: Importance of Late Postnatal Development and Potential Reciprocal Interactions between Biosynthesis and Degradation. *Front. Endocrinol (Lausanne),* **2013a**, *4,* 59. http://dx.doi.org/10.3389/fendo.2013.00059

[17] Sarkar, A.; Chachra, P.; Vaidya, V.A. Postnatal Fluoxetine-Evoked Anxiety Is Prevented by Concomitant 5-HT(2A/C) Receptor Blockade and Mimicked by Postnatal 5-HT(2A/C) Receptor Stimulation. *Biol. Psychiatry,* **2014**, in press.

[18] Bengel, D.; Murphy, D.L.; Andrews, A.M.; Wichems, C.H.; Feltner, D.; Heils, A.; Mössner, R.; Westphal, H.; Lesch, K.P. Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ("Ecstasy") in serotonin transporter-deficient mice. *Mol. Pharmacol.,* **1998**, *53,* 649-655. PMID 9547354

[19] Choi, D.S.; Maroteaux, L. Immunohistochemical localisation of the serotonin 5-HT_{2B} receptor in mouse gut, cardiovascular system, and brain. *FEBS Lett.*, **1996**, 391, 45-51. http://dx.doi.org/10.1016/0014-5793(96)00695-3

[20] Li, B.; Dong, L.; Wang, B.; Cai, L.; Jiang, N.; Peng, L. Cell type-specific gene expression and editing responses to chronic fluoxetine treatment in the *in vivo* mouse brain and their relevance for stress-induced anhedonia. *Neurochem. Res.*, **2012**, *37*, 2480-2495. http://dx.doi.org/10.1007/s11064-012-0814-1

[21] Blier, P.; De Montigny, C. Electrophysiological investigations on the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. *J. Neurosci.*, **1983**, *3*, 1270-1278. **PubMed ID:** 6304261

[22] McDevitt, R.A.; Neumaier, J.F. Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *J. Chem. Neuroanat.*, **2011**, *41,* 234-246. http://dx.doi.org/10.1016/j.jchemneu.2011.05.001

[23] Czachura, J.F.; Rasmussen, K. Effects of acute and chronic administration of fluoxetine on the activity of serotonergic neurons in the dorsal raphe nucleus of the rat. *Naunyn Schmiedebergs Arch. Pharmacol.*, **2000**, *362,* 266-275. http://dx.doi.org/10.1007/s002100000290

[24] Descarries, L.; Riad, M. Effects of the antidepressant fluoxetine on the subcellular localization of 5-HT_{1A} receptors and SERT. *Philos. Trans. R Soc. Lond. B Biol. Sci.*, **2012**, *367*, 2416-2425. http://dx.doi.org/10.1098/rstb.2011.0361

[25] Miguelez, C.; Grandoso, L.; Ugedo, L. Locus coeruleus and dorsal raphe neuron activity and response to acute antidepressant administration in a rat model of Parkinson's disease. *Int. J. Neuropsychopharmacol.*, **2011**, *14,* 187-200. http://dx.doi.org/10.1017/s146114571000043x

[26] Artigas, F.; Romero, L.; de Montigny, C.; Blier, P. Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. *Trends Neurosci.,* **1996**, *19,* 378-383. http://dx.doi.org/10.1016/s0166-2236(96)10037-0

[27] Berman, R.M.; Anand, A.; Cappiello, A.; Miller, H.L., Hu, X.S., Oren, D.A., Charney, D.S. The use of pindolol with fluoxetine in the treatment of major depression: final results from a double-blind, placebo-controlled trial. *Biol. Psychiatry*, **1999**, *45,* 1170-1177. http://dx.doi.org/10.1016/s0006-3223(98)00383-7

[28] Önder, E.; Tural, U. Faster response in depressive patients treated with fluoxetine alone than in combination with buspirone. *J. Affect Disord.*, **2003**, *76*, 223-227. http://dx.doi.org/10.1016/s0165-0327(02)00090-3

[29] Li, B.; Zhang, S.; Zhang, H.; Nu, W.; Cai, L.; Hertz, L.; Peng, L.. Fluoxetine-mediated 5-HT$_{2B}$ receptor stimulation in astrocytes causes EGF receptor transactivation and ERK phosphorylation. *Psychopharmacology (Berl)*, **2008**, *201*, 443-458. http://dx.doi.org/10.1007/s00213-008-1306-5

[30] Zhang, X.; Peng, L.; Chen, Y.; Hertz, L. Stimulation of glycogenolysis in astrocytes by fluoxetine, an antidepressant acting like 5-HT. *Neuroreport,***1993**, *4*, 1235-1238. http://dx.doi.org/10.1097/00001756-199309000-00006

[31] Gibbs, M.E.; Hutchinson, D.; Hertz, L. Astrocytic involvement in learning and memory consolidation. *Neurosci. Biobehav. Rev.*, **2008**, *32*, 927-944. http://dx.doi.org/10.1016/j.neubiorev.2008.02.001

[32] Gibbs, M.E.; Hertz, L. Serotonin mediation of early memory formation *via* 5-HT$_{2B}$ receptor-iduced glycogenolysis in the day-old chick. *Front. Pharmacol.,* **2014**, *5,* 54. http://dx.doi.org/10.3389/fphar.2014.00054

[33] Li, X.; Zhu, W.; Roh, M.S.; Friedman, A.B.; Rosborough, K.; Jope, R.S. *In vivo* regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. *Neuropsychopharmacology,* **2004**, *29,* 1426-1431. http://dx.doi.org/10.1038/sj.npp.1300439

[34] Polter, A.M.; Yang, S.; Jope, R.S.; Li, X. Functional significance of glycogen synthase kinase-3 regulation by serotonin. *Cell Signal.*, **2012**, *24*, 265-271. http://dx.doi.org/10.1016/j.cellsig.2011.09.009

[35] Price, R.D.; Weiner, D.M.; Chang, M.S.; Sanders-Bush, E. RNA editing of the human serotonin 5-HT$_{2C}$ receptor alters receptor-mediated activation of G13 protein. *J. Biol. Chem.*, **2001**, *276,* 44663-44668. http://dx.doi.org/10.1074/jbc.m106745200

[36] Hertz, L.; Song, D.; Li, B.; Du, T.; Xu, J.; Gu, L.; Chen, Y.; Peng, L. Signal transduction in astrocytes during chronic or acute treatment with drugs (SSRIs; anti-bipolar drugs; GABA-ergic drugs; benzodiazepines) ameliorating mood disorders. *J. Signal. Transduc.*, **2014**, *2014,* 593934.

[37] Sawyer, E.K.; Howell, L.L. Pharmacokinetics of fluoxetine in rhesus macaques following multiple routes of administration. *Pharmacology*, **2011**, *88*, 44-49. http://dx.doi.org/10.1159/000329417

[38] Li, B.; Gu, L.; Hertz, L.; Peng, L. Expression of nucleoside transporter in freshly isolated neurons and astrocytes from mouse brain. *Neurochem. Res.*, **2013**, *38*, 2351-2358. http://dx.doi.org/10.1007/s11064-013-1146-5

[39] Eriksen, J.L.; Gillespie, R.; Druse, M.J. Effects of ethanol and 5-HT$_{1A}$ agonists on astroglial S100B. *Brain Res. Dev. Brain Res.*, **2002**, *139*, 97-105. http://dx.doi.org/10.1016/s0165-3806(02)00510-2

[40] Miyazaki, I.; Asanuma, M.; Murakami, S.; Takeshima, M.; Torigoe, N.; Kitamura, Y.; Miyoshi, K. Targeting 5-HT(1A) receptors in astrocytes to protect dopaminergic neurons in Parkinsonian models. *Neurobiol. Dis.*, **2013**, *59*, 244-256. http://dx.doi.org/10.1016/j.nbd.2013.08.003

[41] Li, B.; Zhang, S.; Li, M.; Hertz, L.; Peng, L. Chronic treatment of astrocytes with therapeutically relevant fluoxetine concentrations enhances cPLA$_2$ expression secondary to 5-HT$_{2B}$-induced, transactivation-mediated ERK$_{1/2}$ phosphorylation. *Psychopharmacology (Berl)*, **2009**, *207*, 1-12. http://dx.doi.org/10.1007/s00213-009-1631-3

[42] Bass, B.L. RNA editing by adenosine deaminases that act on RNA. *Annu. Rev. Biochem.*, **2002**, *71*, 817-846. http://dx.doi.org/10.1146/annurev.biochem.71.110601.135501

[43] Valente, L.; Nishikura, K. ADAR gene family and A-to-I RNA editing: diverse roles in posttranscriptional gene regulation. *Prog. Nucleic Acid Res. Mol. Biol.*, **2005**, 79, 299-338. http://dx.doi.org/10.1016/s0079-6603(04)79006-6

[44] Kawahara, Y.; Ito, K.; Sun, H.; Ito, M.; Kanazawa, I.; Kwak, S. Regulation of glutamate receptor RNA editing and ADAR mRNA expression in developing human normal and Down's syndrome brains. *Brain Res. Dev. Brain Res.*, **2004**, *148*, 151-155. http://dx.doi.org/10.1016/j.devbrainres.2003.11.008

[45] Köhr, G.; Melcher, T.; Seeburg, P.H. Candidate editases for GluR channels in single neurons of rat hippocampus and cerebellum. *Neuropharmacology*, **1998**, *37*, 1411-1477. http://dx.doi.org/10.1016/s0028-3908(98)00149-x

[46] Li, B.; Zhang, S.; Zhang, H.; Hertz, L.; Peng, L. Fluoxetine affects GluK2 editing, glutamate-evoked Ca(2+) influx and extracellular signal-regulated kinase phosphorylation in mouse astrocytes. *J. Psychiatry Neurosci.*, **2011**, *36*, 322-338. http://dx.doi.org/10.1503/jpn.100094

[47] Lautens, L.L.; Chiou, X.G.; Sharp, J.D.; Young, W.S. 3rd.; Sprague, D.L.; Ross, L.S.; Felder, C.C. Cytosolic phospholipase A2 (cPLA$_2$) distribution in murine brain and functional studies indicate that cPLA$_2$ does not participate in muscarinic receptor-mediated signaling in neurons. *Brain Res.*, **1998**, *809*, 18-30. http://dx.doi.org/10.1016/s0006-8993(98)00806-3

[48] Balboa, M.A.; Balsinde., J. Involvement of calcium-independent phospholipase A2 in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol Chem.*, **2002**, *27*, 40384-40389. http://dx.doi.org/10.1074/jbc.m206155200

[49] Sun, G.Y.; Xu, J.; Jensen, M.D.; Simonyi, A. Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. *J. Lipid Res.,* **2004**, *45*, 205-213. DOI: 10.1194/jlr.R300016-JLR200

[50] Felder, C.C.; Kanterman, R.Y.; Ma, A.L.; Axelrod, J. Serotonin stimulates phospholipase A2 and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis. *Proc. Natl. Acad. Sci. U S A*, **1990**, 87, 2187-2191. http://dx.doi.org/10.1073/pnas.87.6.2187

[51] Stout, B.D.; Clarke, W.P.; Berg, K.A. Rapid desensitization of the serotonin(2C) receptor system: effector pathway and agonist dependence. *J. Pharmacol. Exp. Ther.*, **2002**, *302*, 957-962. http://dx.doi.org/10.1124/jpet.302.3.957

[52] Qu, Y.; Chang, L.; Klaff, J.; Seemann, R.; Rapoport, S.I. Imaging brain phospholipase A2-mediated signal transduction in response to acute fluoxetine administration in unanesthetized rats. *Neuropsychopharmacology*, **2003**, *28*, 1219-1226. http://dx.doi.org/10.1038/sj.npp.1300177

[53] Rapoport, S.I. Brain arachidonic and docosahexaenoic acid cascades are selectively altered by drugs, diet and disease. *Prostaglandins Leuk. EssentFatty Acids,* **2008**, *79*, 153-156. http://dx.doi.org/10.1016/j.plefa.2008.09.010

[54] Garcia, M.C.; Kim, H.Y. Mobilization of arachidonate and docosahexaenoate by stimulation of the 5-HT$_{2A}$ receptor in rat C6 glioma cells. *Brain Res.*, **1997**, *768*, 43-48. http://dx.doi.org/10.1016/s0006-8993(97)00583-0

[55] Yu, N.; Martin, J.L.; Stella, N.; Magistretti, P.J. Arachidonic acid stimulates glucose uptake in cerebral cortical astrocytes. *Proc. Natl. Acad. Sci. U. S. A.*, **1993**, *90*, 4042-4046. http://dx.doi.org/10.1073/pnas.90.9.4042

[56] Allaman, I.; Fiumelli, H.; Magistretti, P.J.; Martin, J.L. Fluoxetine regulates the expression of neurotrophic/growth factors and glucose metabolism in astrocytes. *Psychopharmacology (Berl),* **2011**, *216*, 75-84. http://dx.doi.org/10.1007/s00213-011-2190-y

[57] Sorg, O.; Pellerin, L.; Stolz, M.; Beggah, S.; Magistretti, P.J. Adenosine triphosphate and arachidonic acid stimulate glycogenolysis in primary cultures of mouse cerebral cortical astrocytes. *Neurosci. Lett.*, **1995**, *188*, 109-112. http://dx.doi.org/10.1016/0304-3940(95)11410-x

[58] Hertz, L.; Xu, J.; Peng, L. Glycogenolysis and purinergic signaling glutamate and ATP at interface of metabolism and signaling in the brain. In: *Advances of Neurobiology*. Parpura, V., Schousboe, A., Verkhratsky, A. Eds.; Springer. **2014**, in press.

[59] Qu, Y.; Chang, L.; Klaff, J.; Seemann, R.; Greenstein, D.; Rapoport, S.I. Chronic fluoxetine upregulates arachidonic acid incorporation into the brain of unanesthetized rats. *Eur. Neuropsychopharmacol.*, **2006**, *16*, 561-571. http://dx.doi.org/10.1016/j.euroneuro.2006.01.008

[60] Rao, J.S.; Ertley, R.N.; Lee, H.J.; Rapoport, S.I.; Bazinet, R.P. Chronic fluoxetine upregulates activity, protein and mRNA levels of cytosolic phospholipase A2 in rat frontal cortex.

Pharmacogenom. J., **2006**, *6,* 413-420. http://dx.doi.org/10.1038/sj.tpj.6500391

[61] Lee, H.J.; Rao, J.S.; Ertley, R.N.; Chang, L.; Rapoport, S.I.; Bazinet, R.P. Chronic fluoxetine increases cytosolic phospholipase A(2) activity and arachidonic acid turnover in brain phospholipids of the unanesthetized rat. *Psychopharmacology (Berl)*, **2007**, *190*, 103-115. http://dx.doi.org/10.1007/s00213-006-0582-1

[62] Little, J.T.; Ketter, T.A.; Kimbrell, T.A.; Danielson, A.; Benson, B.; Willis, M.W.; Post, R.M. Venlafaxine or bupropion responders but not nonresponders show baseline prefrontal and paralimbic hypometabolism compared with controls. *Psychopharmacol. Bull.*, **1996**, *32*, 629-635. PubMed ID: 8993084

[63] Little, J.T.; Ketter, T.A.; Kimbrell, T.A.; Dunn, R.T.; Benson, B.E.; Willis, M.W.; Luckenbaugh, D.A.; Post, R.M. Bupropion and venlafaxine responders differ in pretreatment regional cerebral metabolism in unipolar depression. *Biol. Psychiatry*, **2005**, *57*, 220-228. http://dx.doi.org/10.1016/j.biopsych.2004.10.033

[64] Videbech, P. PET measurements of brain glucose metabolism and blood flow in major depressive disorder: a critical review. *Acta Psychiatr. Scand.*, **2000**, *101,* 11-20. http://dx.doi.org/10.1034/j.1600-0447.2000.101001011.x

[65] Rasgon, N.L; Kenna, H.A; Geist, C.; Small, G.; Silverman, D. Cerebral metabolic patterns in untreated postmenopausal women with major depressive disorder. *Psychiatry Res.*, **2008**, *164*, 77-80. http://dx.doi.org/10.1016/j.pscychresns.2007.12.006

[66] Kimbrell, T.A.; Ketter, T.A.; George, M.S.; Little, J.T.; Benson, B.E.; Willis, M.W.; Herscovitch, P.; Post, R.M. Regional cerebral glucose utilization in patients with a range of severities of unipolar depression. *Biol. Psychiatry*, **2002**, *51*, 237-252. http://dx.doi.org/10.1016/s0006-3223(01)01216-1

[67] Buchsbaum, M.S.; Wu, J.; Siegel, B.V.; Hackett, E.; Trenary, M.; Abel, L.; Reynolds, C. Effect of sertraline on regional metabolic rate in patients with affective disorder. *Biol. Psychiatry*, **1997**, *41*, 15-22. http://dx.doi.org/10.1016/s0006-3223(96)00097-2

[68] Mayberg, H.S.; Brannan, S.K.; Tekell, J.L.; Silva, J.A.; Mahurin, R.K.; McGinnis, S.; Jerabek, P.A. Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. *Biol. Psychiatry*, **2000**, *48*, 830-843. http://dx.doi.org/10.1016/s0006-3223(00)01036-2

[69] Kennedy, S.H.; Evans, K.R.; Krüger, S.; Mayberg, H.S.; Meyer, J.H.; McCann, S.; Arifuzzman, A.I.; Houle, S.; Vaccarino, F.J. Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression. *Am. J. Psychiatry*, **2001**, *158,* 899-905. http://dx.doi.org/10.1176/appi.ajp.158.987.899

[70] Sublette, M.E.; Milak, M.S.; Hibbeln, J.R.; Freed, P.J.; Oquendo, M.A.; Malone, K.M.; Parsey, R.V.; Mann, J.J. Plasma polyunsaturated fatty acids and regional cerebral glucose metabolism in major depression. *Prostaglandins Leukot. Essent. Fatty. Acids*, **2009**, *80,* 57-64. http://dx.doi.org/10.1016/j.plefa.2008.11.004

[71] Hundal, Ø. Major depressive disorder viewed as a dysfunction in astroglial bioenergetics. *Med. Hypotheses,* **2007**, *68*, 370-377. http://dx.doi.org/10.1016/j.mehy.2006.06.050

[72] Hertz, L.; Song, D.; Li, B.; Yan, E.; Peng, L. Importance of 'inflammatory molecules', but not necessarily of inflammation, in the pathophysiology of bipolar disorder and in the mechanisms of action of anti-bipolar drugs. *NPBR,* **2013**, *19,* 174-179. http://dx.doi.org/10.1016/j.npbr.2013.09.004

[73] Tharumaratnam, D.; Bashford, S.; Khan, S.A. Indomethacin induced psychosis. *Postgrad. Med. J.*, **2000**, *76*, 736-737. http://dx.doi.org/10.1136/pmj.76.901.736

[74] Clunie, M.; Crone, L.A.; Klassen, L.; Yip, R. Psychiatric side effects of indomethacin in parturients. *Can. J. Anaesth.*, **2003**, *50*, 586-568. http://dx.doi.org/10.1007/bf03018645

[75] Jiang, H.K.; Chang, D.M. Non-steroidal anti-inflammatory drugs with adverse psychiatric reactions: five case reports. *Clin. Rheumatol.*, **1999**, *18*, 339-345. http://dx.doi.org/10.1007/s100670050114

[76] Müller, N. Immunology of major depression. *Neuroimmunomodulation*, **2014**, *21*, 123-130.

[77] Pae, C.U.; Yu, H.S.; Kim, J.J.; Lee, C.U.; Lee, S.J.; Lee, K.U.; Jun, T.Y.; Paik, I.H.; Serretti, A.; Lee, C. BanI polymorphism of the cytosolic phospholipase A2 gene and mood disorders in the Korean population. *Neuropsychobiology*, **2004**, *49*, 185-188. http://dx.doi.org/10.1159/000077364

[78] Su, K.P.; Huang, S.Y.; Peng, C.Y.; Lai, H.C.; Huang, C.L.; Chen, Y.C.; Aitchison, K.J.; Pariante, C.M. Phospholipase A2 and cyclooxygenase 2 genes influence the risk of interferon-alpha-induced depression by regulating polyunsaturated fatty acids levels. *Biol. Psychiatry*, **2010**, *67,* 550-557. http://dx.doi.org/10.1016/j.biopsych.2009.11.005

[79] Hertz, L; Xu, J; Song, D; Du, T; Li, B; Yan, E; Peng, L. Astrocytic Glycogenolysis: Mechanisms and Functions. *Metabolic Brain Dis*., **2014**, in press. DOI: 10.1007/s11011-014-9536-1

[80] Verkhratsky, A.; Rodriguez, J.J.; Parpura, V. Calcium signalling in astroglia. *Mol. Cell Endocrinol.,* **2012**, *353*, 45-56. http://dx.doi.org/10.1016/j.mce.2011.08.039

[81] Yan, E.; Li, B.; Gu, L.; Hertz, L.; Peng L. Mechanisms for L-channel-mediated increase in [Ca(2+)](i) and its reduction by anti-bipolar drugs in cultured astrocytes combined with its mRNA expression in freshly isolated cells support the importance of astrocytic L-channels. *Cell Calcium*, **2013**, *54*, 335-342. http://dx.doi.org/10.1016/j.ceca.2013.08.002

[82] Li, B.; Dong, L.; Fu, H.; Wang, B.; Hertz, L.; Peng, L. Effects of chronic treatment with fluoxetine on receptor-stimulated increase of $[Ca^{2+}]_i$ in astrocytes mimic those of acute inhibition of TRPC1 channel activity. *Cell Calcium*, **2011**, *50*, 42-53. http://dx.doi.org/10.1016/j.ceca.2011.05.001

[83] Du, T.; Liang, C.; Li, B.; Hertz, L.; Peng, L. Chronic fluoxetine administration increases expression of the L-channel gene $Ca_v1.2$ in astrocytes from the brain of treated mice and in culture and augments K^+-induced increase in $[Ca^{2+}]_i$. *Cell Calcium*, **2014**, *55*, 166-174. http://dx.doi.org/10.1016/j.ceca.2014.01.002

[84] Verkhratsky, A.; Parpura, V. Store-operated calcium entry in neuroglia. *Neurosci. Bull.*, **2014**, *30,* 125-133. http://dx.doi.org/10.1007/s12264-013-1343-x

[85] Harteneck, C.; Plant, T.D.; Schultz, G. From worm to man: three subfamilies of TRP channels. *Trends Neurosci.*, **2000**, *23*, 159-166. http://dx.doi.org/10.1016/s0166-2236(99)01532-5

[86] Verkhratsky, A.; Reyes, R.C.; Parpura, V. TRP Channels Coordinate Ion Signalling in Astroglia. *Rev. Physiol. Biochem. Pharmacol.*, **2014**, *166,* 1-22. doi: 10.1007/112_2013_15.

[87] Malarkey, E.B.; Ni, Y.; Parpura, V. Ca^{2+} entry through TRPC1 channels contributes to intracellular Ca^{2+} dynamics and consequent glutamate release from rat astrocytes. *Glia,* **2008**, *56,* 821-835. http://dx.doi.org/10.1002/glia.20656

[88] Hof, P.R.; Pascale, E.; Magistretti, P.J. K^+ at concentrations reached in the extracellular space during neuronal activity promotes a Ca^{2+}-dependent glycogen hydrolysis in mouse cerebral cortex. *J. Neurosci.,* **1988**, *8,* 1922-1928. PubMed ID: 3385482

[89] Xu, J.; Song, D.; Xue, Z.; Gu, L.; Hertz, L.; Peng, L. Requirement of glycogenolysis for uptake of increased extracellular K^+ in astrocytes: potential implications for K^+ homeostasis and glycogen usage in brain. *Neurochem. Res.,* **2013**, *38*, 472-485. http://dx.doi.org/10.1007/s11064-012-0938-3

[90] Dougherty, U.; Sehdev, A.; Cerda, S.; Mustafi, R.; Little, N.; Yuan, W.; Jagadeeswaran, S.; Chumsangsri, A.; Delgado, J.; Tretiakova, M.; Joseph, L.; Hart, J.; Cohen, E.E.; Aluri, L.; Fichera, A.; Bissonnette, M. Epidermal growth factor receptor controls flat dysplastic aberrant crypt foci development and colon cancer progression in the rat azoxymethane model. *Clin. Cancer Res.*, **2008**, *14*, 2253-2262. http://dx.doi.org/10.1158/1078-0432.ccr-07-4926

[91] Sanacora, G.; Treccani, G.; Popoli, M. Towards a glutamate hypothesis of depression An emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*, **2012**, 62, 63-77. http://dx.doi.org/10.1016/j.neuropharm.2011.07.036

[92] Peng, L.; Li, B.; Du, T.; Wang, F.; Hertz, L. Does conventional anti-bipolar and antidepressant drug therapy reduce NMDA-mediated neuronal excitation by downregulating astrocytic GluK2 function? *Pharmacol. Biochem. Behav.*, **2012**, *100*, 712-725. http://dx.doi.org/10.1016/j.pbb.2011.03.021

[93] Hertz, L., Zielke, H.R. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci.,* **2004**, *27*, 735-743. http://dx.doi.org/10.1016/j.tins.2004.10.008

[94] Schousboe, A.; Bak, L.K.; Waagepetersen,, H.S. Astrocytic Control of Biosynthesis and Turnover of the Neurotransmitters Glutamate and GABA. *Front. Endocrinol (Lausanne),* **2013**, *4*, 102. DOI: 10.3389/fendo.2013.00102

[95] Sanacora, G.; Banasr, M. From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. *Biol. Psychiatry,* **2013**, *73*, 1172-1179. http://dx.doi.org/10.1016/j.biopsych.2013.03.032

[96] Niciu. M.J.; Henter, I.D.; Sanacora, G.; Zarate, C.A Jr. Glial abnormalities in substance use disorders and depression: Does shared glutamatergic dysfunction contribute to comorbidity? *World J. Biol. Psychiatry,* **2014**,*15*, 2-16. http://dx.doi.org/10.3109/15622975.2013.829585

[97] Lapidus, K.A.; Soleimani, L.; Murrough, J.W. Novel glutamatergic drugs for the treatment of mood disorders. *Neuropsychiatr. Dis. Treat.*, **2013**, *9,* 1101-1112. DOI: 10.2147/NDT.S36689

[98] Murrough, J.W.; Perez, A.M.; Pillemer, S.; Stern, J.; Parides, M.K.; aan, het, Rot, M.; Collins, K.A.; Mathew, S.J.; Charney, D.S.; Iosifescu, D.V. Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biol. Psychiatry*, **2013**, *74*, 250-256. http://dx.doi.org/10.1016/j.biopsych.2012.06.022

[99] Melyan, Z.; Lancaster, B.; Wheal, H.V. Metabotropic regulation of intrinsic excitability by synaptic activation of kainate receptors. *J. Neurosci.,* **2004**, *24*, 4530-4534. http://dx.doi.org/10.1523/jneurosci.5356-03.2004

[100] Barbon, A.; Popoli, M.; La Via, L.; Moraschi, S.; Vallini, I.; Tardito, D.; Tiraboschi, E.; Musazzi, L.; Giambelli, R.; Gennarelli, M.; Racagni, G.; Barlati, S. Regulation of editing and expression of glutamate alpha-amino-propionic-acid (AMPA)/kainate receptors by antidepressant drugs. *Biol. Psychiatry,* **2006**, 59, 713-720. http://dx.doi.org/10.1016/j.biopsych.2005.10.018

[101] Egebjerg, J.; Heinemann, S.F. Ca^{2+} permeability of unedited and edited versions of the kainate selective glutamate receptor GluR6. *Proc. Natl. Acad. Sci. U. S. A.*, **1993**, *90,* 755-759. http://dx.doi.org/10.1073/pnas.90.2.755

[102] Barbon, A.; Gervasoni, A.; LaVia, L.; Orlandi, C.; Jaskolski, F.; Perrais, D.; Barlati, S. Human GluR6c, a functional splicing variants of GluR6, is mainly expressed in non-nervous cells. *Neurosci. Lett.*, **2008**, *434*, 77-82. http://dx.doi.org/10.1016/j.neulet.2008.01.049

[103] Shaltiel, G.; Maeng, S.; Malkesman, O.; Pearson, B.; Schloesser, R.J.; Tragon, T.; Rogawski, M.; Gasior, M.; Luckenbaugh, D.; Chen, G.; Manji, H.K. Evidence for the involvement of the kainate receptor subunit GluR6 (GRIK2) in mediating behavioral displays related to behavioral symptoms of mania. *Mol. Psychiatry*, **2008**, *13,* 858-872. http://dx.doi.org/10.1038/mp.2008.20

[104] Delorme, R.; Krebs, M.O.; Chabane, N.; Roy, I.; Millet, B.; Mouren-Simeoni, M.C.; Maier, W.; Bourgeron, T.; Leboyer, M. Frequency and transmission of glutamate receptors GRIK2 and GRIK3 polymorphisms in patients with obsessive compulsive disorder. *Neuroreport*, **2004**, *15*, 699-702. http://dx.doi.org/10.1097/00001756-200403220-00025

[105] Sampaio, A.S.; Fagerness, J.; Crane, J.; Leboyer, M.; Delorme, R.; Pauls, D.L.; Stewart, S.E. Association between polymorphisms in GRIK2 gene and obsessive-compulsive disorder: a family-based study. *CNS Neurosci. Ther.*, **2011**, *17*, 141-147. DOI: 10.1111/j.1755-5949.2009.00130.x

[106] Vialou, V.; Robison, A.J.; Laplant, Q.C.; Covington, H.E. 3rd.; Dietz, D.M.; Ohnishi, Y.N.; Mouzon, E.; Rush, A.J. 3rd.; Watts, E.L.; Wallace, D.L.; Iñiguez, S.D.; Ohnishi, Y.H.; Steiner, M.A.; Warren, B.L.; Krishnan, V.; Bolaños, C.A.; Neve, R.L.; Ghose, S.; Berton, O.; Tamminga, C.A.; Nestler, E.J. DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat. Neurosci.*, **2010**, *13*, 745-752. http://dx.doi.org/10.1038/nn.2551

[107] Cahoy, J.D.; Emery, B.; Kaushal, A.; Foo, L.C.; Zamanian, J.L.; Christopherson, K.S.; Xing, Y.; Lubischer, J.L.; Krieg, P.A.; Krupenko, S.A.; Thompson, W.J.; Barres, B.A. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.*, **2008**, *28,* 264-278. http://dx.doi.org/10.1523/jneurosci.4178-07.2008

[108] Lobo, M.K.; Zaman, S.; Damez-Werno, D.M.; Koo, J.W.; Bagot, R.C.; DiNieri, J.A.; Nugent, A.; Finkel, E.; Chaudhury, D.; Chandra, R.; Riberio, E.; Rabkin, J.; Mouzon, E.; Cachope, R.; Cheer, J.F.; Han, M.H.; Dietz, D.M.; Self, D.W.; Hurd, Y.L.; Vialou, V.; Nestler, E.J. ΔFosB induction in striatal medium spiny neuron subtypes in response to chronic pharmacological, emotional, and optogenetic stimuli. *J. Neurosci.*, **2013**, *33*, 18381-18395. http://dx.doi.org/10.1523/jneurosci.1875-13.2013

[109] Warren, B.L.; Vialou, V.F.; Iñiguez, S.D.; Alcantara, L.F.; Wright, K.N.; Feng, J.; Kennedy, P.J.; Laplant, Q.; Shen, L.; Nestler, E.J.; Bolaños-Guzmán, C.A. Neurobiological sequelae of witnessing stressful events in adult mice. *Biol. Psychiatry.* **2013**, *73*, 7-14. http://dx.doi.org/10.1016/j.biopsych.2012.06.006

[110] Vollmayr, B.; Mahlstedt, M.M.; Henn, F.A. Neurogenesis and depression: what animal models tell us about the link. *Eur. Arch. Psychiatry Clin. Neurosci.*, **2007**, *257*, 300-303. http://dx.doi.org/10.1007/s00406-007-0734-2

[111] Pinna, G., Rasmusson, A.M. Up-regulation of neurosteroid biosynthesis as a pharmacological strategy to improve behavioural deficits in a putative mouse model of post-traumatic stress disorder. *J. Neuroendocrinol.,* **2012**, *24*, 102-116. http://dx.doi.org/10.1111/j.1365-2826.2011.02234.x

[112] Reid, T.E.; Kumar, K.; Wang, X.S. Predictive in silico studies of human 5-hydroxytryptamine receptor subtype 2B (5-HT$_{2B}$) and valvular heart disease. *Curr. Top Med. Chem.*, **2013**, *13*, 1353-1362. http://dx.doi.org/10.2174/15680266113139990039

[113] Rothman, R.B.; Baumann, M.H.; Savage, J.E.; Rauser, L.; McBride, A.; Hufeisen, S.J.; Roth, B.L. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*, **2000**, *102*, 2836-2841. http://dx.doi.org/10.1161/01.cir.102.23.2836

[114] Bai, C.F.; Liu, J.C.; Zhao, R.; Cao, W.; Liu, S.B.; Zhang, X.N.; Guo, H.J.; Yang, Q.; Yi, D.H.; Zhao, M.G. Role of 5-HT$_{2B}$ receptors in cardiomyocyte apoptosis in noradrenaline-induced cardiomyopathy in rats. *Clin. Exp. Pharmacol. Physiol.*, **2010**, *37*, e145-e151. DOI: 10.1111/j.1440-1681.2010.05388.x

[115] Banes, A.K; Watts, S.W. Arterial expression of 5-HT$_{2B}$ and 5-HT$_{1B}$ receptors during development of DOCA-salt hypertension. *BMC Pharmacol*, **2003**, *3*, 12. http://dx.doi.org/10.1186/1471-2210-3-12

[116] Rieckmann, N.; Kronish, I.M.; Shapiro, P.A.; Whang, W.; Davidson, K.W. Serotonin reuptake inhibitor use, depression, and long-term outcomes after an acute coronary syndrome: a prospective cohort study. *JAMA Intern. Med.*, **2013**, *173*, 1150-1151. DOI: 10.1001/jamainternmed.2013.910

[117] Ellfolk, M.; Malm, H. Risks associated with in utero and lactation exposure to selective serotonin reuptake inhibitors (SSRIs). *Reprod. Toxicol*, **2010**, *30*, 249-260. http://dx.doi.org/10.1016/j.reprotox.2010.04.015

Received: May 28, 2014 Revised: June 24, 2014 Accepted: June 30, 2014