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White Matter Microstructure in Bipolar Disorder Is Influenced by the Interaction between a Glutamate Transporter EAAT1 Gene Variant and Early Stress

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Abstract

Glutamate is the principal excitatory neurotransmitter in the central nervous system. In mature brains, it is critically involved in neuroplasticity and, at high levels, neurotoxicity. The concentrations of glutamate in the extracellular space are maintained at low physiological levels by molecular glutamate transporters (excitatory amino acid transporters-EAATs). Adverse childhood experiences (ACEs) are highly reported in bipolar disorder (BD) and interact with the glutamatergic system in the brain. The aim of the study is to investigate the effect of a glutamate transporter polymorphism *EAAT2*-181A > C (rs4354668) and exposure to ACE on white matter microstructure in patients with BD. We assessed 175 bipolar subjects using diffusion tensor imaging, Risky Families Questionnaire, and EEAT2 rs4354668 variants. We observed an interaction between ACE and rs4354668: carriers of the G allele showed lower axial diffusivity compared to T/T homozygotes when exposed to high stress and higher axial diffusivity than T/T when exposed to low stress. Since the mutant G allele has been associated with a reduced transcriptional activity and expression of the transporter protein, and early stress is associated with a reduced expression of the EAAT2, we could hypothesize that after exposure to high levels of ACE G/G homozygotes are more vulnerable to stress reporting the highest damage as a consequence of an excess of free glutamate.

Keywords Adverse childhood experiences · Glial glutamate transporter · EAAT2 · SLC1A2 · Bipolar disorder · TBSS

Introduction

Glutamate is the principal excitatory neurotransmitter in the central nervous system, and glutamatergic neurons are highly represented in the cerebral cortex and limbic regions of the brain. In brain development, glutamate affects neuronal migration, differentiation, and survival, whereas in mature brains, it is critically involved in neuroplasticity and when it is present at high levels, neurotoxicity. Accumulation of excess extracellular glutamate and subsequent overstimulation of glutamatergic receptors and recruitment of extrasynaptic receptors increases the production of reactive and excitotoxic oxygen/nitrogen species, which induce oxidative stress leading to neuronal death [1]. Recent studies also suggest that this same mechanism can lead to toxic injury of oligodendrocytes forming white matter's myelin sheaths and consequently to axon demyelination [2–4]. Indeed, oligodendrocytes have been shown to express AMPA and kainate receptors [5] in their soma and NMDA receptors in their processes [6–8].

Changes in white matter (WM) integrity are considered biomarkers of bipolar disorder (BD) [9]. Post-mortem studies showed a reduction in the numerical density of oligodendroglial cells in BD [10–13], and in vivo studies consistently documented changes in WM microstructure that have been associated with the genetic risk for BD [14, 15], impulsivity and suicide [16], cognitive performances [17, 18], response to treatment [19], and environmental stressors [20].

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Also, alterations in the glutamatergic system have been suggested to contribute to the pathophysiology of depression [21]. Indeed, elevated levels of glutamate have been reported in pediatric [22] and adult patients affected by BD [23, 24] [25, 26]. Furthermore, inhibition of glutamate release and reduction in glutamate levels in depressed BD patients parallel antidepressant response [27–29].

The inactivation of glutamate is handled by a series of molecular glutamate transporters (excitatory amino acid transporters-EAATs) which are membrane-bound pumps that closely resemble ion channels. These transporters regulate the concentration of glutamate in the extracellular space, maintaining it at low physiological levels in order to promote biological function without promoting toxicity [30]. Among the five human excitatory amino acid transporters that have been cloned, *EAAT2* is responsible for up to 95% of extracellular glutamate clearance [31] and is expressed primarily on astrocytes [32], but it is also expressed by axons [33]. Increased astrocytic *EAAT2* expression appears to afford greater neuroprotection under excitotoxic conditions [34, 35]. Impaired glutamate uptake by dysfunction or reduced expression of *EAAT2* can lead to phenomena of excitotoxicity that have been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis [1], and also schizophrenia [36–39]. Also, post-mortem studies observed a reduced expression of the membrane transporter EAAT2 in major depression [40].

The gene encoding EAAT2 in humans is SLC1A2 [solute carrier family 1 (glial high-affinity glutamate transporter), member 2], located on chromosome 11p13–12 [41]. A T-to-G (DNA forward strand) functional polymorphism (rs4354668) at −181 bp from the transcription start site of the *EAAT2* gene, which is commonly known as −181A > C, was described by Mallolas and colleagues [42]. This mutation results in less transporter expression with the G allele inducing a 30% reduction in promoter activity compared with the T allele. Post-mortem studies showed lower mRNA expression levels of *EAAT2* in the PFC [43] and locus coeruleus [44] of patients with major depressive disorder, as well as lower EAAT2 immunoreactivity in the orbitofrontal cortex of depressed individuals compared with controls [45]. Furthermore, in multiple sclerosis, patients with the mutant allele showed an increase of 53.9% in glutamate levels during relapse as compared to those with the wild type [46].

Among the possible factors leading to a long-lasting altered glutamate homeostasis in the brain, a prominent role of stress has been suggested. A review of this topic suggests that chronic stress may affect both glutamate release and clearance [47]. Accordingly, under stress, when elevated levels of glutamate are present, effects of altered function of glutamate transporters are likely to be most pronounced. Also, stress exerts a detrimental effect on WM microstructure in several fiber tracts contributing to the functional integrity of the brain in BD [48]. These effects of ACE likely associate with other detrimental influences on WM integrity observed in BD: increased genetic liability to familial BD has been associated with reduced WM integrity in patients and their unaffected relatives [14], and genetic factors affecting the severity of BD also influence WM integrity [49]. Gene*environment interactions, including ACE, could then play a major role in the observed relationship between BD and altered DTI measures of WM integrity. Following this line of reasoning, we investigated the effect of a polymorphism in the promoter region of EAAT2 gene on WM integrity in bipolar patients exposed to different levels of ACE.

Methods

Patients and Data Collection

We studied 175 Caucasian subjects (110 females, 65 males) consecutively referred to our university hospital for BD type I (DSM-V—Diagnostic and Statistical Manual of Mental Disorders, 5th Edition [50]-criteria; SCID—Structured Clinical Interview for DSM-V clinical version). Exclusion criteria were other diagnoses on Axis I, mental retardation, history of epilepsy, and major medical and neurological disorders. Sixty patients (34%) took lithium in the previous 6 months. About 50% of patients were taking antidepressant drugs (SSRI 25%; SNRI 16%; tricyclics 8%), and 24% were taking antipsychotics.

Severity of ACE was rated on the Risky Families Questionnaire (RFQ) [51] after MRI scanning. The RFQ has been adapted from an instrument originally developed to assess the relation of family stress to mental and physical health outcomes in adulthood [52]. The instrument is aimed at rating the degree of harsh parenting with overt family conflict and deficient nurturing experienced by the children in their familial environment. Previous research validated this questionnaire against clinical interviewers; the dual assessment (questionnaire and interview) demonstrated high agreement and reliability [53]. This approach has been proven successful in detecting the structural and functional brain correlates of ACE in adult life [51, 54]. In agreement with previous studies [54–56], the participants were then divided into two subgroups, using median values as a discriminant between high and low scores of the RFQ.

All data were collected on the same day. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). After complete description of the study to the subjects, a written informed consent was obtained. The local ethical committee approved all the research activities.

Genotyping

DNA was extracted from whole blood by a manual extraction, using the "Illustra blood genomic Prep Midi Flow kit" (GE Healthcare, Milan, Italy). To identify the polymorphism EEAT2—181A > C T/G (DNA forward strand), a standard polymerase chain reaction (PCR) was carried with the following primers: 5′-GCC ACC TGT GCT TTG CTG-3′ and 5′-TGA TGT CAG CTC TCG ACG AA-3′. The PCR was carried out in a 10-μl volume containing 150 ng genomic DNA, 1 μl of 1× Hot Master Taq Buffer with Mg++ (Eppendorf), 0.1 μl of each primer [50 uM], 1 μl of deaza-dNTPs [10 mM], 0.5 μl of dimethyl sulfoxide (DMSO) solution (Sigma-Aldrich, Milan, Italy), and 0.1 μl of Hot Master Taq [5 U/ul] (Eppendorf). After an initial step of 5 min at 94 °C, 35 cycles of amplification (35 s at 94 °C, 35 s at 58 °C, 45 s at 70 °C) and a final extension step of 10 min at 70 °C were performed. An aliquot of PCR product was digested using Msp I (20 U/μl) (New England Biolabs, England, UK) and incubated at 37 °C for 8 h; fragments were separated in agarose gels. Depending on the presence of two or three restriction Msp I sites, either three fragments (allele T) or four fragments (allele G) were produced.

Brain Imaging Procedures and Data Analysis

Diffusion tensor imaging was performed on a 3.0 Tesla scanner (Gyroscan Intera, Philips, Netherlands) using SE Echo-planar imaging (EPI) and the following parameters: TR/TE = 8753.89/58 ms, FoV (mm) 231.43 (ap), 126.50 (fh), 240.00 (rl); acquisition matrix 2.14 × 2.71 × 2.31; 55 contiguous, 2.3-mm thick axial slices reconstructed with in-plane pixel size 1.88 × 1.87 mm; SENSE acceleration factor = 2; 1 b0 and 35 non-collinear directions of the diffusion gradients; b value = 900 s/mm^2. Fat saturation was performed to avoid chemical shift artifacts. On the same occasion and using the same magnet 22 Turbo Spin Echo (TSE), T2 axial slices (TR = 3000 ms; TE = 85 ms; flip angle = 90°; turbo factor 15; 5 mm thick, axial slices with a 512 × 512 matrix and a 230 × 230-mm^2 field of view), and T2w FLAIR (TR = 11,000 ms; TE = 120 ms; flip angle = 90°; turbo factor 21; 5 mm thick, axial slices with a 400 × 400 matrix and a 230 × 230-mm^2 field of view)were acquired to rule out brain lesions.

Image analyses and tensor calculations were done using the "Oxford Center for Functional Magnetic Resonance Imaging of the Brain Software Library" (FSL 5.0; www.fmrib.ox.ac.uk/fsl/index.html) [57, 58]. For a detailed description, see Supplementary data.

Voxelwise DTI analyses were performed using nonparametric permutation-based testing [59], corrected for FWE multiple comparisons, as implemented in Randomize in FSL with general linear models (GLM). Threshold-free cluster enhancement (TFCE) [60] was used to avoid defining arbitrary cluster-forming thresholds and smoothing levels. Voxelwise levels of significance, corrected for multiple comparisons, were then calculated with a standard permutation testing (10,000 permutations for each contrast) by building up the null distribution of the maximum TFCE scores. Corrected $p < 0.05$ in a minimum cluster size of $k = 100$ was considered significant.

First, an ANOVA was performed to test the interaction between ACE and rs4354668 on DTI measures of WM microstructure [Fractional anisotropy (FA), axial (AD), radial (RD), and mean diffusivity (MD)]. Post hoc *T* tests were then used to investigate the direction of the effect separately in the high ACE and low ACE groups in those WM tracts that emerged from the ANOVA. We accounted for the effects of nuisance covariates which could influence WM structure: age [61], sex [62], age at onset of the illness [63], and lithium treatment [49].

Results

Allelic frequencies were similar to those observed for the general population (*T* = 58.%; G = 42%) [42]. The distribution of *EAAT2* genotypes (T/T $n = 56$, T/G $n = 91$, G/G $n = 28$) was in Hardy–Weinberg equilibrium ($\chi 2 = 0.79$, $p = 0.37$). Clinical and demographic data are shown in Tables 1 and 2. No difference between high ACE and low ACE (Table 1) or between genotypic groups (Table 2) reached significance when investigating clinical variables.

The ANOVA showed an interaction between the glutamate transporter polymorphism and ACE on AD in WM tracts encompassing inferior and superior longitudinal fasciculus and the inferior fronto-occipital fasciculus (Fig. 1 and Supplementary Table 1). Post hoc *T* tests (Fig. 2) showed that, in the high ACE subgroup, subjects with at least one G allele had lower AD compared to T/T subjects (GG < TT $p < 0.001$; GT < TT $p = 0.002$). In the low ACE subgroup, we observed the opposite pattern with subjects with at least a G allele

Table 1 Clinical and demographic characteristics of the sample divided according to adverse childhood experiences

	Adverse childhood experiences		
	High $n = 91$ Mean ± SD	Low $n = 84$ Mean ± SD	*p*
Age (years)	46.14 ± 11.11	46.69 ± 11.15	0.74
Education (years)	11.68 ± 4.29	11.26 ± 3.78	0.49
Onset (year)	30.33 ± 10.14	31.08 ± 10.73	0.63
Duration of illness (years)	15.81 ± 10.34	15.62 ± 10.76	0.90
Number of episodes	8.93 ± 9.04	7.91 ± 8.89	0.45

Table 2 Clinical and demographic characteristics of the sample as a whole and divided according to *SLA1A2 -181A > C* genotype

	Total sample	GG ($n = 28$)	EAAT2 GT ($n = 91$)	TT (n = 56)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	p
Age (years)	46.40 ± 11.10	44.64 ± 7.86	46.19 ± 11.29	47.62 ± 12.16	0.49
Education (years)	11.48 ± 4.05	12.5 ± 4.12	11.41 ± 3.94	11.09 ± .18	0.31
Onset (year)	30.69 ± 10.41	30.53 ± 6.67	30.28 ± 10.27	31.43 ± 12.13	0.81
Duration of illness (years)	15.72 ± 10.52	14.14 ± 9.07	15.91 ± 10.68	16.19 ± 11.01	0.68
Number of episodes	8.45 ± 8.96	9.61 ± 9.59	7.92 ± 8.74	8.74 ± 9.09	0.66
ACE	27.03 ± 9.05	28.32 ± 8.91	25.86 ± 8.95	28.30 ± 9.19	0.20

showing higher AD compared to T/T subjects (GG > TT $p = 0.007$; GT > TT $p < 0.001$). No direct association between EAAT2 genotypes and AD was observed.

Discussion

This is the first study to investigate the effect of a polymorphism in the promoter region of the SLC1A2 gene on WM microstructure in BD patients exposed to different levels of ACE. Here, we show that rs4354668 exerts a significant effect on WM integrity and that this effect is moderated by ACE. When exposed to low levels of ACE, carriers of the G allele reported significantly higher AD than T/T homozygotes whereas they showed lower AD when exposed to high ACE. The opposite pattern was observed in T/T homozygotes. This finding is in agreement with a previous study from our group reporting higher gray matter volume in carriers of the G allele exposed to low ACE compared to T/T homozygotes [55] and confirms the crucial role of glutamate for the integrity of brain structure.

Here, we showed that the interaction between ACE and rs4354668 influences AD. AD represents the diffusion of water parallel to the axonal fibers, reflecting the greater freedom of water to diffuse along the principal fiber axis rather than to travel across the surrounding myelin sheaths. Both myelin and axonal microstructure, including microtubules and neurofilaments [64], contribute to this anisotropy. Higher AD is associated with better functional connectivity among brain regions, and with network-related aspects of human brain function also including emotions [65], reasoning, and cognitive flexibility [66]. The WM tracts involved include fibers belonging to the neocortical association pairs and connecting the frontal lobes to more posterior regions of the brain. WM integrity of these tracts was consistently reported to be reduced in BD [14, 67–70].

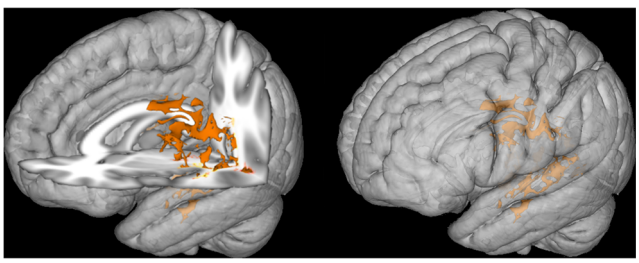

Fig. 1 3D reconstruction of white matter tracts where we observed a significant interaction between EAAT2-*181A > C* genotype and ACE on axial diffusivity. Voxel of significant effect surviving a significant threshold of $p < 0.05$ corrected for multiple comparison is shown on a 3D T1-weighted image

In humans, ACEs have been shown to exert a detrimental effect on WM microstructure in BD [48], while animal models suggest a stress-induced increase in glutamate levels in the synaptic cleft [71]. Normally, higher levels of glutamate induce increased expression of glutamate transporters [72]. However, in animal models, helpless animals expressed significantly lower levels of EAAT2 in the hippocampus and cortex compared to littermates that did not exhibit helpless behavior [73]. Also, a reduced expression of the membrane transporters SLC1A2 was reported in major depression while a trend has been observed in bipolar subjects [40]. EAAT2 expression was also found to be reduced in oligodendrocytes following enhanced exposure to tumor necrosis factor (TNF) [74] and oxidative stress [75]. Elevated biomarkers of cell-mediated immune activation and inflammation, in the absence of active somatic immune diseases, have been consistently reported in BD [76] irrespective of mood state [77], and the inflammation-related cytokines TNF-α, IL-8, IFN-γ, and IL-10 have been associated to reduced WM microstructure in BD [78] in the same tracts observed here.

All WM macroglial cells, including oligodendrocytes, express the EAAT2. This transporter contributes to maintaining basal levels of extracellular glutamate in the range of 1 to 2 mM and preventing over-activation of glutamate receptors under physiological conditions. Should the uptake of glutamate be inefficient, this could lead to an over-activation of AMPA and kainate receptors and causes oligodendrocyte death and myelin destruction [79]. Indeed, the direct inhibition of glutamate uptake in axonal tracts leads to oligodendroglial loss, massive demyelination, and severe axonal damage [80].

Following this line of reasoning, we could hypothesize that, in subjects exposed to high ACE, more efficient transporters are needed to face the reduced expression of glutamate

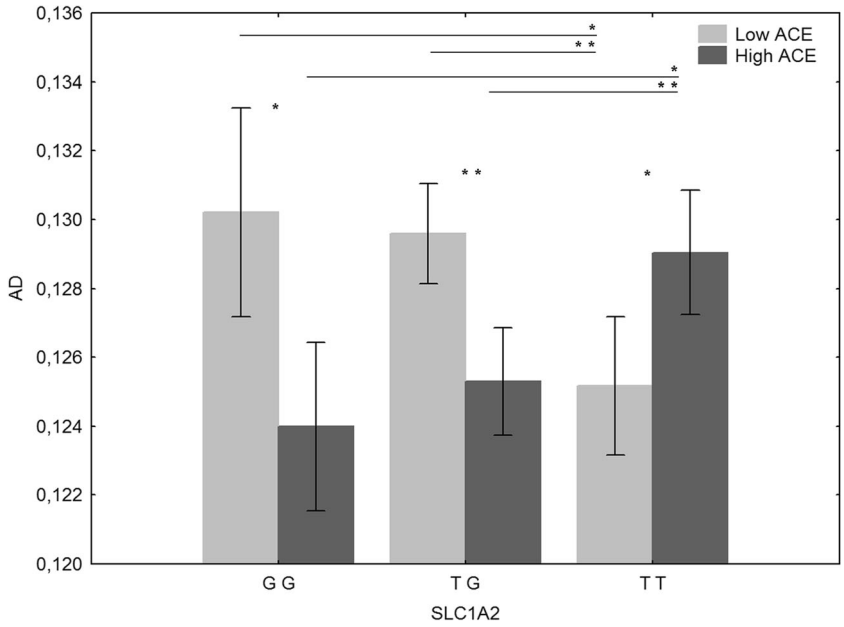

Fig. 2 Post hoc *T* tests showing direction of the effects of EAAT2-*181A > C* genotype and ACE on axial diffusivity. *$p < 0.005$; **$p < 0.001$

transporters. This could explain why subjects with the less efficient G allele showed lower AD compared to T/T subjects. Similarly, we could hypothesize that after exposure to higher levels of ACE, carriers of the G allele are more vulnerable to stress and report the highest damage to brain volume as a consequence of an excess of free glutamate [1]. This is also in line with a reduced response to clozapine in G allele subjects [81]. Interestingly, G allele carriers showed also higher reward dependence [81], making them more vulnerable to high ACE. This effect could not be specific to ACE but extend to other kinds of insults such as stroke, which caused the highest damage in G/G [42]. However, the higher dependency of AD on the environment in rs4354668*G carriers does not justify a deficit-oriented connotation of the rs4354668 variants, because the environment shapes the outcome of these fundamentally neutral common genetic factors: when facing low adversities, carriers of the rs4354668*G allele have a higher AD in respect to T/T homozygotes. A similar effect has been described for the serotonin promoter polymorphism 5-HTTLPR, which impacts stress sensitivity, emotion regulation, and social cognition [82]: while 5-HTTLPR*s carriers have a worse outcome of depression when exposed to ACE, with both emotional instability [83], and levels of neurotrophins [84] being proportional to the breadth of exposure to adverse events, the same *s carriers show better outcomes when ACE levels are low [85]. It can then be surmised that common genetic variants could reveal opposite biological and behavioral effects in patients with psychiatric conditions depending on their interaction with the environment [86].

Several studies have highlighted the importance of ACE in psychiatric disorders. The present study, in agreement with the current literature, confirms that the exposure to ACE may influence the biological effect of the genetic pool. Recent studies also suggest that early interventions may counteract the detrimental effects of ACE [87, 88]; therefore, considering the early family environment could be important for the development of personalized treatments. Indeed, patients exposed to ACE may benefit from treatments acting on brain structure like lithium salts which act on oligodendrocytes [89] and influence the lipid composition of myelin by inducing BDNF [90–92]. Accordingly, treatment with lithium salts was associated with higher AD in BD and was shown to counteract the effect of stress [49].

The major limitation of the study is the lack of information regarding previous psychopharmacological treatments, received by the patients. Recent studies showed that highly complex medication regimens are often required during naturalistic outpatient treatment of BD depression [93]. Further prospective studies will consider drug treatments and other factors (e.g., other genes, drugs, exposure to emotional and physical stressors) to assess the potential role of *SLA1A2* − 181A > C in bipolar illness. Other limitations also include issues such as generalizability, possible undetected past comorbidities, population stratification, lack of complete coverage of gene variability, and absence of placebo control. Moreover, genome-wide association studies and gene–gene and other gene–environment interactions were not taken into account.

In conclusion, we demonstrated an association between glutamate transporter polymorphism and early stress in influencing WM integrity in BD. Carriers of the rs4354668*G allele, which is associated with less transporter expression and a 30% reduction in promoter activity compared with the T allele, showed lower AD than T/T homozygote when exposed to higher ACE, while the opposite effect was observed in the low ACE group. A reduced efficiency in

the uptake of glutamate in subjects with a reduced number of transporters could cause a higher vulnerability to neurotoxic processes. Further studies, however, are needed to better understand the possible role of genetic polymorphisms regulating glutamate transporter activity in the maintenance of WM integrity and the moderator role of ACE.

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Compliance with Ethical Standards

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). After complete description of the study to the subjects, a written informed consent was obtained. The local ethical committee approved all the research activities.

References

1. Kim K, Lee SG, Kegelman TP, Su ZZ, Das SK, Dash R, Dasgupta S, Barral PM et al (2011) Role of excitatory amino acid transporter-2 (EAAT2) and glutamate in neurodegeneration: opportunities for developing novel therapeutics. J Cell Physiol 226:2484–2493. https://doi.org/10.1002/jcp.22609
2. Groom AJ, Smith T, Turski L (2003) Multiple sclerosis and glutamate. Ann N Y Acad Sci 993:229–275 discussion 287-228
3. Pitt D, Werner P, Raine CS (2000) Glutamate excitotoxicity in a model of multiple sclerosis. Nat Med 6(1):67–70. https://doi.org/10.1038/71555
4. Matute C, Alberdi E, Domercq M, Perez-Cerda F, Perez-Samartin A, Sanchez-Gomez MV (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 24(4):224–230
5. Matute C, Alberdi E, Domercq M, Sanchez-Gomez MV, Perez-Samartin A, Rodriguez-Antiguedad A, Perez-Cerda F (2007) Excitotoxic damage to white matter. J Anat 210(6):693–702. https://doi.org/10.1111/j.1469-7580.2007.00733.x
6. Baltan S, Besancon EF, Mbow B, Ye Z, Hamner MA, Ransom BR (2008) White matter vulnerability to ischemic injury increases with age because of enhanced excitotoxicity. J Neurosci 28(6):1479–1489. https://doi.org/10.1523/JNEUROSCI.5137-07.2008
7. Karadottir R, Cavelier P, Bergersen LH, Attwell D (2005) NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438(7071):1162–1166. https://doi.org/10.1038/nature04302
8. Salter MG, Fern R (2005) NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438(7071):1167–1171. https://doi.org/10.1038/nature04301
9. Saricicek A, Zorlu N, Yalin N, Hidiroglu C, Cavusoglu B, Ceylan D, Ada E, Tunca Z et al (2016) Abnormal white matter integrity as a structural endophenotype for bipolar disorder. Psychol Med 46(7):1547–1558. https://doi.org/10.1017/S0033291716000180
10. Uranova NA, Vostrikov VM, Orlovskaya DD, Rachmanova VI (2004) Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley neuropathology consortium. Schizophr Res 67(2–3):269–275
11. Manji HK, Moore GJ, Rajkowska G, Chen G (2000) Neuroplasticity and cellular resilience in mood disorders. Mol Psychiatry 5(6):578–593
12. Rajkowska G, Miguel-Hidalgo JJ (2007) Gliogenesis and glial pathology in depression. CNS Neurol Disord Drug Targets 6(3):219–233
13. Ongur D, Bechtholt AJ, Carlezon WA Jr, Cohen BM (2014) Glial abnormalities in mood disorders. Harv Rev Psychiatry 22(6):334–337
14. Chaddock CA, Barker GJ, Marshall N, Schulze K, Hall MH, Fern A, Walshe M, Bramon E et al (2009) White matter microstructural impairments and genetic liability to familial bipolar I disorder. Br J Psychiatry 194(6):527–534. https://doi.org/10.1192/bjp.bp.107.047498
15. Whalley HC, Sprooten E, Hackett S, Hall L, Blackwood DH, Glahn DC, Bastin M, Hall J et al (2013) Polygenic risk and white matter integrity in individuals at high risk of mood disorder. Biol Psychiatry 74:280–286. https://doi.org/10.1016/j.biopsych.2013.01.027
16. Matsuo K, Nielsen N, Nicoletti MA, Hatch JP, Monkul ES, Watanabe Y, Zunta-Soares GB, Nery FG et al (2010) Anterior genu corpus callosum and impulsivity in suicidal patients with bipolar disorder. Neurosci Lett 469(1):75–80. https://doi.org/10.1016/j.neulet.2009.11.047
17. Poletti S, Bollettini I, Mazza E, Locatelli C, Radaelli D, Vai B, Smeraldi E, Colombo C et al (2015) Cognitive performances associate with measures of white matter integrity in bipolar disorder. J Affect Disord 174:342–352. https://doi.org/10.1016/j.jad.2014.12.030
18. Oertel-Knochel V, Reinke B, Alves G, Jurcoane A, Wenzler S, Prvulovic D, Linden D, Knochel C (2014) Frontal white matter alterations are associated with executive cognitive function in euthymic bipolar patients. J Affect Disord 155:223–233. https://doi.org/10.1016/j.jad.2013.11.004
19. Bollettini I, Poletti S, Locatelli C, Vai B, Smeraldi E, Colombo C, Benedetti F (2015) Disruption of white matter integrity marks poor antidepressant response in bipolar disorder. J Affect Disord 174:233–240. https://doi.org/10.1016/j.jad.2014.11.010
20. Benedetti F, Bollettini I, Radaelli D, Poletti S, Locatelli C, Falini A, Smeraldi E, Colombo C (2014) Adverse childhood experiences influence white matter microstructure in patients with bipolar disorder. Psychol Med 44(14):3069–3082. https://doi.org/10.1017/S0033291714000506
21. Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, Epperson CN, Goddard A et al (2002) Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. Mol Psychiatry 7(Suppl 1):S71–S80. https://doi.org/10.1038/sj.mp.4001021
22. Castillo M, Kwock L, Courvoisie H, Hooper SR (2000) Proton MR spectroscopy in children with bipolar affective disorder: preliminary observations. AJNR Am J Neuroradiol 21(5):832–838
23. Cecil KM, DelBello MP, Morey R, Strakowski SM (2002) Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy. Bipolar Disord 4(6):357–365
24. Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF (2004) Brain metabolic alterations in medication-free patients with bipolar disorder. Arch Gen Psychiatry 61(5):450–458. https://doi.org/10.1001/archpsyc.61.5.45061/5/450
25. Altamura CA, Mauri MC, Ferrara A, Moro AR, D'Andrea G, Zamberlan F (1993) Plasma and platelet excitatory amino acids in psychiatric disorders. Am J Psychiatry 150(11):1731–1733. https://doi.org/10.1176/ajp.150.11.1731
26. Hoekstra R, Fekkes D, Loonen AJ, Pepplinkhuizen L, Tuinier S, Verhoeven WM (2006) Bipolar mania and plasma amino acids: increased levels of glycine. Eur Neuropsychopharmacol 16(1):71–77. https://doi.org/10.1016/j.euroneuro.2005.06.003

27. Benedetti F, Calabrese G, Bernasconi A, Cadioli M, Colombo C, Dallaspezia S, Falini A, Radaelli D et al (2009) Spectroscopic correlates of antidepressant response to sleep deprivation and light therapy: a 3.0 Tesla study of bipolar depression. Psychiatry Res 173(3):238–242. https://doi.org/10.1016/j.pscychresns.2008.08.004
28. Singh J, Zarate CA, Jr., Krystal AD (2004) Case report: successful riluzole augmentation therapy in treatment-resistant bipolar depression following the development of rash with lamotrigine. Psychopharmacology 173 (1–2):227–228. doi:https://doi.org/10.1007/s00213-003-1756-8
29. Zarate CA, Jr., Quiroz JA, Singh JB, Denicoff KD, De Jesus G, Luckenbaugh DA, Charney DS, Manji HK (2005) An open-label trial of the glutamate-modulating agent riluzole in combination with lithium for the treatment of bipolar depression. Biol Psychiatry 57 (4):430–432. doi: https://doi.org/10.1016/j.biopsych.2004.11.023
30. Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65(1):1–105
31. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA et al (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16(3):675–686
32. Rao JS, Kellom M, Reese EA, Rapoport SI, Kim HW (2012) Dysregulated glutamate and dopamine transporters in postmortem frontal cortex from bipolar and schizophrenic patients. J Affect Disord 136(1–2):63–71. https://doi.org/10.1016/j.jad.2011.08.017
33. Li S, Mealing GA, Morley P, Stys PK (1999) Novel injury mechanism in anoxia and trauma of spinal cord white matter: glutamate release via reverse Na+-dependent glutamate transport. J Neurosci 19(14):RC16
34. Rosenberg PA, Aizenman E (1989) Hundred-fold increase in neuronal vulnerability to glutamate toxicity in astrocyte-poor cultures of rat cerebral cortex. Neurosci Lett 103(2):162–168
35. Weller ML, Stone IM, Goss A, Rau T, Rova C, Poulsen DJ (2008) Selective overexpression of excitatory amino acid transporter 2 (EAAT2) in astrocytes enhances neuroprotection from moderate but not severe hypoxia-ischemia. Neuroscience 155(4):1204–1211. https://doi.org/10.1016/j.neuroscience.2008.05.059
36. Smith RE, Haroutunian V, Davis KL, Meador-Woodruff JH (2001) Expression of excitatory amino acid transporter transcripts in the thalamus of subjects with schizophrenia. Am J Psychiatry 158(9):1393–1399
37. Ohnuma T, Tessler S, Arai H, Faull RL, McKenna PJ, Emson PC (2000) Gene expression of metabotropic glutamate receptor 5 and excitatory amino acid transporter 2 in the schizophrenic hippocampus. Brain Res Mol Brain Res 85(1–2):24–31
38. Bauer D, Haroutunian V, Meador-Woodruff JH, McCullumsmith RE (2010) Abnormal glycosylation of EAAT1 and EAAT2 in prefrontal cortex of elderly patients with schizophrenia. Schizophr Res 117(1):92–98. https://doi.org/10.1016/j.schres.2009.07.025
39. Ohnuma T, Augood SJ, Arai H, McKenna PJ, Emson PC (1998) Expression of the human excitatory amino acid transporter 2 and metabotropic glutamate receptors 3 and 5 in the prefrontal cortex from normal individuals and patients with schizophrenia. Brain Res Mol Brain Res 56(1–2):207–217
40. Medina A, Burke S, Thompson RC, Bunney W, Jr., Myers RM, Schatzberg A, Akil H, Watson SJ (2013) Glutamate transporters: a key piece in the glutamate puzzle of major depressive disorder. J Psychiatr Res 47 (9):1150–1156. https://doi.org/10.1016/j.jpsychires.2013.04.007
41. Meyer T, Ludolph AC, Morkel M, Hagemeier C, Speer A (1997) Genomic organization of the human excitatory amino acid transporter gene GLT-1. Neuroreport 8(3):775–777
42. Mallolas J, Hurtado O, Castellanos M, Blanco M, Sobrino T, Serena J, Vivancos J, Castillo J et al (2006) A polymorphism in the EAAT2 promoter is associated with higher glutamate concentrations and higher frequency of progressing stroke. J Exp Med 203(3):711–717. https://doi.org/10.1084/jem.20051979
43. Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP, Myers RM, Bunney WE, Jr., Akil H, Watson SJ, Jones EG (2005) Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. Proc Natl Acad Sci U S A 102 (43):15653–15658. doi:https://doi.org/10.1073/pnas.0507901102
44. Bernard R, Kerman IA, Thompson RC, Jones EG, Bunney WE, Barchas JD, Schatzberg AF, Myers RM et al (2011) Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. Mol Psychiatry 16(6):634–646. https://doi.org/10.1038/mp.2010.44
45. Miguel-Hidalgo JJ, Waltzer R, Whittom AA, Austin MC, Rajkowska G, Stockmeier CA (2010) Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. J Affect Disord 127(1–3):230–240. https://doi.org/10.1016/j.jad.2010.06.003
46. Pampliega O, Domercq M, Villoslada P, Sepulcre J, Rodriguez-Antiguedad A, Matute C (2008) Association of an EAAT2 polymorphism with higher glutamate concentration in relapsing multiple sclerosis. J Neuroimmunol 195(1–2):194–198. https://doi.org/10.1016/j.jneuroim.2008.01.011
47. Popoli M, Yan Z, McEwen BS, Sanacora G (2012) The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci 13(1):22–37. https://doi.org/10.1038/nrn3138
48. Benedetti F, Bollettini I, Radaelli D, Poletti S, Locatelli C, Falini A, Smeraldi E, Colombo C (2014) Adverse childhood experiences influence white matter microstructure in patients with bipolar disorder. Psychol Med 44(14):3069–3082. https://doi.org/10.1017/S0033291714000506
49. Benedetti F, Bollettini I, Barberi I, Radaelli D, Poletti S, Locatelli C, Pirovano A, Lorenzi C et al (2013) Lithium and GSK3-beta promoter gene variants influence white matter microstructure in bipolar disorder. Neuropsychopharmacology 38(2):313–327. https://doi.org/10.1038/npp.2012.172
50. American Psychiatric Association (2012) Diagnostic and statistical manual of mental disorders, 5th edn. American Psychiatric Association, Washington DC
51. Taylor SE, Eisenberger NI, Saxbe D, Lehman BJ, Lieberman MD (2006) Neural responses to emotional stimuli are associated with childhood family stress. Biol Psychiatry 60(3):296–301. https://doi.org/10.1016/j.biopsych.2005.09.027
52. Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, Koss MP, Marks JS (1998) Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study Am J Prev Med 14(4):245–258
53. Taylor SE, Lerner JS, Sage RM, Lehman BJ, Seeman TE (2004) Early environment, emotions, responses to stress, and health. J Pers 72(6):1365–1393. https://doi.org/10.1111/j.1467-6494.2004.00300.x
54. Benedetti F, Radaelli D, Poletti S, Falini A, Cavallaro R, Dallaspezia S, Riccaboni R, Scotti G et al (2011) Emotional reactivity in chronic schizophrenia: structural and functional brain correlates and the influence of adverse childhood experiences. Psychol Med 41(3):509–519. https://doi.org/10.1017/S0033291710001108
55. Poletti S, Locatelli C, Radaelli D, Lorenzi C, Smeraldi E, Colombo C, Benedetti F (2014) Effect of early stress on hippocampal gray matter is influenced by a functional polymorphism in EAAT2 in bipolar disorder. Prog Neuro-Psychopharmacol Biol Psychiatry 51C:146–152. https://doi.org/10.1016/j.pnpbp.2014.01.021
56. Poletti S, Vai B, Smeraldi E, Cavallaro R, Colombo C, Benedetti F (2015) Adverse childhood experiences influence the detrimental effect of bipolar disorder and schizophrenia on cortico-limbic grey matter volumes. J Affect Disord 189:290–297. https://doi.org/10.1016/j.jad.2015.09.049

57. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M et al (2004) Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 23(Suppl 1):S208–S219. https://doi.org/10.1016/j.neuroimage.2004.07.051
58. Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, Beckmann C, Jenkinson M et al (2009) Bayesian analysis of neuroimaging data in FSL. NeuroImage 45(1 Suppl):S173–S186. https://doi.org/10.1016/j.neuroimage.2008.10.055
59. Nichols TE, Holmes AP (2002) Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 15(1):1–25. https://doi.org/10.1002/hbm.1058 [pii]
60. Smith SM, Nichols TE (2009) Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. NeuroImage 44(1):83–98. https://doi.org/10.1016/j.neuroimage.2008.03.061
61. Kochunov P, Thompson PM, Lancaster JL, Bartzokis G, Smith S, Coyle T, Royall DR, Laird A et al (2007) Relationship between white matter fractional anisotropy and other indices of cerebral health in normal aging: tract-based spatial statistics study of aging. NeuroImage 35(2):478–487. https://doi.org/10.1016/j.neuroimage.2006.12.021
62. Herting MM, Maxwell EC, Irvine C, Nagel BJ (2011) The impact of sex, puberty, and hormones on white matter microstructure in adolescents. Cereb Cortex 22:1979–1992. https://doi.org/10.1093/cercor/bhr246
63. Lu LH, Zhou XJ, Fitzgerald J, Keedy SK, Reilly JL, Passarotti AM, Sweeney JA, Pavuluri M (2012) Microstructural abnormalities of white matter differentiate pediatric and adult-onset bipolar disorder. Bipolar Disord 14(6):597–606. https://doi.org/10.1111/j.1399-5618.2012.01045.x
64. Kinoshita Y, Ohnishi A, Kohshi K, Yokota A (1999) Apparent diffusion coefficient on rat brain and nerves intoxicated with methylmercury. Environ Res 80(4):348–354. https://doi.org/10.1006/enrs.1998.3935
65. Baur V, Hanggi J, Langer N, Jancke L (2013) Resting-state functional and structural connectivity within an insula-amygdala route specifically index state and trait anxiety. Biol Psychiatry 73(1):85–92. https://doi.org/10.1016/j.biopsych.2012.06.003
66. Borghesani PR, Madhyastha TM, Aylward EH, Reiter MA, Swarny BR, Warner Schaie K, Willis SL (2013) The association between higher order abilities, processing speed, and age are variably mediated by white matter integrity during typical aging. Neuropsychologia 51:1435–1444. https://doi.org/10.1016/j.neuropsychologia.2013.03.005
67. Benedetti F, Yeh PH, Bellani M, Radaelli D, Nicoletti MA, Poletti S, Falini A, Dallaspezia S et al (2011) Disruption of white matter integrity in bipolar depression as a possible structural marker of illness. Biol Psychiatry 69(4):309–317. https://doi.org/10.1016/j.biopsych.2010.07.028
68. Versace A, Almeida JR, Quevedo K, Thompson WK, Terwilliger RA, Hassel S, Kupfer DJ, Phillips ML (2010) Right orbitofrontal corticolimbic and left corticocortical white matter connectivity differentiate bipolar and unipolar depression. Biol Psychiatry 68(6):560–567. https://doi.org/10.1016/j.biopsych.2010.04.036
69. Zanetti MV, Jackowski MP, Versace A, Almeida JR, Hassel S, Duran FL, Busatto GF, Kupfer DJ et al (2009) State-dependent microstructural white matter changes in bipolar I depression. Eur Arch Psychiatry Clin Neurosci 259(6):316–328. https://doi.org/10.1007/s00406-009-0002-8
70. Lu LH, Zhou XJ, Keedy SK, Reilly JL, Sweeney JA (2011) White matter microstructure in untreated first episode bipolar disorder with psychosis: comparison with schizophrenia. Bipolar Disord 13(7–8):604–613. https://doi.org/10.1111/j.1399-5618.2011.00958.x
71. Martisova E, Solas M, Horrillo I, Ortega JE, Meana JJ, Tordera RM, Ramirez MJ (2012) Long lasting effects of early-life stress on glutamatergic/GABAergic circuitry in the rat hippocampus. Neuropharmacology 62(5–6):1944–1953. https://doi.org/10.1016/j.neuropharm.2011.12.019
72. Liang J, Takeuchi H, Doi Y, Kawanokuchi J, Sonobe Y, Jin S, Yawata I, Li H et al (2008) Excitatory amino acid transporter expression by astrocytes is neuroprotective against microglial excitotoxicity. Brain Res 1210:11–19. https://doi.org/10.1016/j.brainres.2008.03.012
73. Zink M, Vollmayr B, Gebicke-Haerter PJ, Henn FA (2010) Reduced expression of glutamate transporters vGluT1, EAAT2 and EAAT4 in learned helpless rats, an animal model of depression. Neuropharmacology 58(2):465–473. https://doi.org/10.1016/j.neuropharm.2009.09.005
74. Pitt D, Nagelmeier IE, Wilson HC, Raine CS (2003) Glutamate uptake by oligodendrocytes: implications for excitotoxicity in multiple sclerosis. Neurology 61(8):1113–1120
75. Domercq M, Sanchez-Gomez MV, Sherwin C, Etxebarria E, Fern R, Matute C (2007) System xc- and glutamate transporter inhibition mediates microglial toxicity to oligodendrocytes. J Immunol 178(10):6549–6556
76. Anderson G, Maes M (2015) Bipolar disorder: role of immune-inflammatory cytokines, oxidative and nitrosative stress and tryptophan catabolites. Curr Psychiatry Rep 17(2):541. https://doi.org/10.1007/s11920-014-0541-1
77. Luo Y, He H, Zhang M, Huang X, Fan N (2016) Altered serum levels of TNF-alpha, IL-6 and IL-18 in manic, depressive, mixed state of bipolar disorder patients. Psychiatry Res 244:19–23. https://doi.org/10.1016/j.psychres.2016.07.027
78. Benedetti F, Poletti S, Hoogenboezem TA, Mazza E, Ambree O, de Wit H, Wijkhuijs AJ, Locatelli C et al (2016) Inflammatory cytokines influence measures of white matter integrity in bipolar disorder. J Affect Disord 202:1–9. https://doi.org/10.1016/j.jad.2016.05.047
79. Matute C (2011) Glutamate and ATP signalling in white matter pathology. J Anat 219(1):53–64. https://doi.org/10.1111/j.1469-7580.2010.01339.x
80. Domercq M, Etxebarria E, Perez-Samartin A, Matute C (2005) Excitotoxic oligodendrocyte death and axonal damage induced by glutamate transporter inhibition. Glia 52(1):36–46. https://doi.org/10.1002/glia.20221
81. Taylor DL, Tiwari AK, Lieberman JA, Potkin SG, Meltzer HY, Knight J, Remington G, Muller DJ et al (2017) Pharmacogenetic analysis of functional glutamate system gene variants and clinical response to clozapine. Mol Neuropsychiatry 2(4):185–197. https://doi.org/10.1159/000449224
82. Canli T, Lesch KP (2007) Long story short: the serotonin transporter in emotion regulation and social cognition. Nat Neurosci 10(9):1103–1109. https://doi.org/10.1038/nn1964
83. Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE (2010) Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am J Psychiatry 167(5):509–527. https://doi.org/10.1176/appi.ajp.2010.09101452
84. Benedetti F, Ambree O, Locatelli C, Lorenzi C, Poletti S, Colombo C, Arolt V (2017) The effect of childhood trauma on serum BDNF in bipolar depression is modulated by the serotonin promoter genotype. Neurosci Lett 656:177–181. https://doi.org/10.1016/j.neulet.2017.07.043
85. Benedetti F, Riccaboni R, Poletti S, Radaelli D, Locatelli C, Lorenzi C, Pirovano A, Smeraldi E et al (2014) The serotonin transporter genotype modulates the relationship between early stress and adult suicidality in bipolar disorder. Bipolar Disord 16(8):857–866. https://doi.org/10.1111/bdi.12250

86. Homberg JR, Lesch KP (2011) Looking on the bright side of serotonin transporter gene variation. Biol Psychiatry 69(6):513–519. https://doi.org/10.1016/j.biopsych.2010.09.024
87. Gosselin RD, O'Connor RM, Tramullas M, Julio-Pieper M, Dinan TG, Cryan JF (2010) Riluzole normalizes early-life stress-induced visceral hypersensitivity in rats: role of spinal glutamate reuptake mechanisms. Gastroenterology 138(7):2418–2425. https://doi.org/10.1053/j.gastro.2010.03.003
88. O'Connor RM, Moloney RD, Glennon J, Vlachou S, Cryan JF (2015) Enhancing glutamatergic transmission during adolescence reverses early-life stress-induced deficits in the rewarding effects of cocaine in rats. Neuropharmacology 99:168–176. https://doi.org/10.1016/j.neuropharm.2015.07.012
89. Azim K, Butt AM (2011) GSK3beta negatively regulates oligodendrocyte differentiation and myelination in vivo. Glia 59(4):540–553. https://doi.org/10.1002/glia.21122
90. Su H, Zhang W, Guo J, Guo A, Yuan Q, Wu W (2009) Lithium enhances the neuronal differentiation of neural progenitor cells in vitro and after transplantation into the avulsed ventral horn of adult rats through the secretion of brain-derived neurotrophic factor. J Neurochem 108(6):1385–1398. https://doi.org/10.1111/j.1471-4159.2009.05902.x
91. Leyhe T, Eschweiler GW, Stransky E, Gasser T, Annas P, Basun H, Laske C (2009) Increase of BDNF serum concentration in lithium treated patients with early Alzheimer's disease. J Alzheimers Dis 16(3):649–656. https://doi.org/10.3233/JAD-2009-1004
92. de Sousa RT, van de Bilt MT, Diniz BS, Ladeira RB, Portela LV, Souza DO, Forlenza OV, Gattaz WF et al (2011) Lithium increases plasma brain-derived neurotrophic factor in acute bipolar mania: a preliminary 4-week study. Neurosci Lett 494(1):54–56. https://doi.org/10.1016/j.neulet.2011.02.054
93. Post RM, Altshuler LL, Frye MA, Suppes T, Keck PE Jr, McElroy SL, Leverich GS, Luckenbaugh DA et al (2010) Complexity of pharmacologic treatment required for sustained improvement in outpatients with bipolar disorder. J Clin Psychiatry 71(9):1176–1186; quiz 1252-1173. https://doi.org/10.4088/JCP.08m04811yel