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Markers of Glutamate Synaptic Transmission and Plasticity are Increased in the Anterior Cingulate Cortex in Bipolar Disorder

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Abstract

Background—Cortical glutamate levels are elevated in bipolar disorder, but the interpretation of this increase is unclear, since glutamate has metabolic as well as neurotransmitter roles. We investigated this by measuring vesicular glutamate transporter 1 (VGluT1) expression, which reflects activity at glutamate synapses. We also measured netrin-G1 and -G2 mRNAs since these genes are involved in the formation and plasticity of glutamatergic connections.

Methods—Using qPCR we quantified transcripts for VGluT1, netrin-G1 (isoforms G1c, G1d and G1f), and netrin-G2, in the anterior cingulate cortex from subjects with bipolar disorder (n=34), schizophrenia (n=35), and healthy controls (n=35).

Results—VGluT1, netrin-G2, and netrin-G1d and G1f, were increased in bipolar disorder but not in schizophrenia. Netrin-G1c did not differ between groups. Netrin-G1c and G1f expression showed left-right asymmetries. VGluT1 mRNA correlated with brain weight.

Conclusions—Increased VGluT1 expression is supportive of elevated glutamate neurotransmission in the anterior cingulate cortex in bipolar disorder. The netrin-G1 and netrin-G2 findings suggest there may be an underlying difference in the plasticity of the affected circuitry.

Keywords

vesicular glutamate transporter; netrins; mRNA; gene expression; laminets; schizophrenia

The anterior cingulate cortex (ACC) is involved in diverse cognitive and emotional processes and their integration (1-3) and it is integral to the structural and functional pathophysiology of mood disorders (4-7) and schizophrenia (8-11).

In bipolar disorder, levels of glutamate, both in the ACC and some other cortical areas, are increased, in all phases of the illness (12). The evidence comes from magnetic resonance spectroscopy (13-16) and post mortem (17,18) measurements. However, glutamate is not only a transmitter but involved in various biochemical pathways and processes (19), and together with the difficulty separating glutamate from other molecular species in the spectroscopic signal (15), means that the robustness and interpretation of the finding of elevated glutamate in bipolar disorder is still unclear. The vesicular glutamate transporter VGluT1 is responsible for loading glutamate into synaptic vesicles in most cortical excitatory neurons (20,21). Moreover, its expression regulates, and indexes, this process and impacts on quantal size and synaptic glutamate release (22-24). Here we have quantified VGluT1 mRNA as a probe to investigate whether glutamate transmission is altered in the ACC in bipolar disorder, or in schizophrenia.

We also measured the expression of netrin-G1 and netrin-G2 (also called laminin-1 and laminin-2), which are axon guidance and cell adhesion molecules that interact with post-synaptic NGL receptors (25) and are involved in the formation and maintenance of synaptic connections, primarily glutamatergic ones (26-30). Hence netrin-G expression provides an indication as to whether aberrant plasticity of ACC pathways in bipolar disorder might contribute to the putative disturbance in glutamate transmission. Moreover, although their distribution in human ACC has not been reported, in other cortical regions netrin-G1 and netrin-G2 are present in largely distinct neuron populations (27,28,31). Thus, their measurement in combination can provide information as to the localisation of any such alterations. Finally, netrin-G1 and -G2 have been reported to be associated with schizophrenia (32,33) and with Parkinson's disease (30), and so we also examined whether their expression was related to a risk SNP within each gene.

Methods and Materials

Demographic details

Frozen brain tissue from the supragenual part of the ACC (Brodmann area 24) was provided by the Stanley Medical Research Institute (SMRI) from 104 subjects (the Stanley Array Collection; Table 1, with additional demographic information in Supplementary Tables 1 and 2). Diagnoses were made using DSM-IV criteria. All experiments were conducted blind to diagnostic group and other demographic information. The data have been deposited with the SMRI.

Quantitative reverse transcriptase-PCR

RNA was extracted using Tri Reagent (Sigma Aldrich, Poole, UK) and standard methods, and the RNA integrity number (RIN) measured using an Agilent Bioanalyzer 2100 and RNA 6000 Nano kit (Agilent Technologies, Wokingham, UK). RNA was reverse transcribed as described (34). Netrin-G1 is expressed as multiple isoforms that are developmentally regulated and may be functionally distinct (28,35). Several of the isoforms are detectable in other regions of human brain (31,32). In pilot studies, three were reliably detectable in ACC, and selected for the quantitative study: netrin-G1c (missing exons 6-9), G1d (missing exons 8 and 9), and G1f (which lacks exons 6-10 but includes a retained intron 5). Netrin-G1f differs from the other isoforms by being truncated such that it does not contain a glycosyl phosphatidylinositol (GPI) lipid anchor. Quantitative RT-PCR for Netrin-G1c and -G1d was performed using isoform-specific minor groove binding probes as described (31). For Netrin-G1f, qPCR was performed using powerSYBR Green (Applied Biosystems, Warrington, U.K.) and 0.1 μ M of each primer, the forward primer spanning exons 4 and 5 with the reverse primer within the retained intron (forward, bases 1783-1805, reverse, bases 1968-1987 of NM_ AB023193). Netrin-G2 and VGluT1 transcripts were detected using pre-designed Taqman assays (Applied Biosystems): Netrin-G2: Hs00287286_m1; VGluT1: Hs01574210_g1). Four housekeeping gene transcripts were also measured using Taqman assays: β -2-microglobulin (B2M: Hs99999907_m1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH: Hs99999905_m1), glucuronidase beta (GUSB: Hs99999908_m1) and transferrin receptor (TFRC: Hs00951094_m1). Each qPCR reaction contained 10ng cDNA and were performed in triplicate for each transcript. Samples were quantified using a standard curve and SDS v2.2.2 software with an Applied Biosystems 7900HT qPCR system.

Genotyping

Genotyping methods, and the rationale for selection of the SNPs (netrin-G2: rs1105684; netrin-G1: rs1373336), were as reported (31). Briefly, the SNPs were chosen on the basis that each tags a haplotype that has been reported to be associated with schizophrenia (and, in

the case of rs1105684, also shows allelic association), and they also have a reasonable minor allele frequency (32,33).

Data analysis

Data for each target transcript were normalised to the geometric mean of the four housekeeping gene transcripts, none of which differed between diagnostic groups (all $P > 0.35$). Two samples were excluded due to amplification failure. All data (both raw and normalised) were normally distributed (Kolmogorov-Smirnov one-sample test). Correlations with continuous variables (age, brain pH, RIN, autopsy delay, freezer storage time, brain weight) were determined using Pearson coefficient ($p < 0.05$). Any variable significant for that dataset was included as a covariate in the comparisons between diagnostic groups. Pearson coefficients were also used to assess whether any of the mRNAs were correlated with lifetime antipsychotic exposure. Secondary analyses were carried out for effects of genotype, hemisphere, sex, and other demographic factors (*e.g.* alcohol and recreational drug use, medication, bipolar subtype) using unpaired t-tests and one-way ANOVA as appropriate.

In situ hybridization for netrin-G1 and netrin-G2 mRNAs

To see whether netrin-G1 and -G2 are differentially expressed in the ACC as predicted, we carried out in situ hybridization using ^{35}S -labelled oligonucleotides in frozen tissue sections of ACC from 6 control subjects (31). The netrin-G1 oligonucleotide targeted a region within exon 10, common to all known transcripts except Netrin-G1f.

Results

VGluT1 expression

VGluT1 mRNA showed several correlations with demographic factors (Table 2), including fresh brain weight (Fig. 1A) and freezer storage time (Fig. 1B). The correlation with brain weight ($R = 0.233$, $p = 0.018$) was robust, in that it was also observed separately in the controls ($R = 0.381$, $p = 0.024$) and in bipolar disorder ($R = 0.376$, $p = 0.034$), though not in the schizophrenia group ($R = 0.057$), and the correlation survived partialling for age, sex and diagnosis ($r = 0.218$, $df = 97$, $p = 0.030$). The correlation with storage time was seen in all three diagnostic groups (controls: $R = +0.459$, $p = 0.006$; bipolar disorder: $R = +0.329$, $p = 0.066$; schizophrenia: $R = 0.454$, $p = 0.006$). VGluT1 mRNA was not affected by sex or hemisphere.

There was a significant effect of diagnosis on VGluT1 mRNA, made using RIN, freezer storage time, and brain weight used as covariates (Fig. 1C; $F_{2,96} = 7.16$, $p = 0.001$). VGluT1 mRNA was increased in bipolar disorder compared to controls ($p = 0.008$) and compared to schizophrenia ($p < 0.001$), with no difference between controls and schizophrenia subjects ($p = 0.35$). The result was robust to exclusion of any of the covariates. There were no interactions of diagnosis with sex, or hemisphere.

Within the bipolar disorder group, VGluT1 mRNA did not correlate with total inpatient time, age at onset, or duration of illness, nor did it differ between bipolar I and II subjects. Neither did the schizophrenia subjects show any correlations with these variables, but within the diagnostic group there was a significant inverse correlation between VGluT1 mRNA and age ($R = -0.509$, $p = 0.002$).

Netrin-G2 expression

Netrin-G2 mRNA correlated inversely with RIN but showed no other demographic correlations (Table 2). Neither did netrin-G2 mRNA vary according to sex or hemisphere. Covarying for RIN, netrin-G2 mRNA differed between diagnostic groups (Fig. 2A;

$F_{2,98}=3.85$, $p=0.025$), being increased in bipolar disorder compared to controls ($p=0.049$) and compared to schizophrenia ($p=0.009$). Schizophrenia subjects were not different from controls ($p=0.49$). There were no correlations of netrin-G2 mRNA with inpatient time, age at onset, or duration of illness, nor a difference between bipolar I and II disorder subjects.

Netrin-G1c expression

Netrin-G1c mRNA correlated with age, RIN and pH (Table 2). In the whole sample, netrin-G1c expression was asymmetrical (Fig. 2B), being higher in the left (1.10 ± 0.05 , $n=50$) than right (0.84 ± 0.05 ; $n=52$) ACC ($t=3.47$, $p=0.001$). This effect was also seen in the control group ($p=0.023$) with similar trends in both disease groups. Hemisphere was thus included as a factor in the ANOVA. This confirmed the asymmetry ($F_{1,93}=7.67$, $p=0.007$), but there was no main effect of diagnosis on netrin-G1c mRNA ($F_{2,93}=1.93$, $p=0.15$), nor a diagnosis-by-hemisphere interaction ($F_{2,93}=2.19$, $p=0.12$). Netrin-G1c mRNA was unaffected by the other demographic and disease-related factors (not shown).

Netrin-G1d expression

Netrin-G1d mRNA was affected by RIN (Table 2). There was also a sex difference, with netrin-G1d higher in male than females controls (1.17 ± 0.08 vs. 0.82 ± 0.15 ; $t=2.07$, $p=0.046$), with a similar trend in the whole sample ($p=0.078$). There was no effect of hemisphere, or suicide.

Given the male-female difference, we included sex as a between-subjects factor in the ANOVA, which showed netrin-G1d mRNA affected by diagnosis (Fig. 2C; $F_{2,95}=5.78$, $p=0.004$), and by sex ($F_{1,95}=5.67$, $p=0.019$), with no diagnosis-by-sex interaction ($F_{2,95}=0.05$, $p=0.95$). Netrin-G1d mRNA was increased in bipolar disorder relative to controls ($p=0.007$) and to schizophrenia ($p=0.003$). Control and schizophrenia subjects were not different from each other ($p=0.80$). Netrin-G1d mRNA was not related to age at onset or duration of illness, in either schizophrenia or bipolar disorder groups (not shown).

Netrin-G1f expression

Netrin-G1f correlated with RIN and pH (Table 2). Netrin-G1f mRNA was more abundant in the left than right ACC (Fig. 2D; $t=2.32$, $p=0.023$) ACC, an asymmetry also significant in the control group ($t=2.69$, $p=0.011$). Netrin-G1f mRNA was not affected by sex, nor by the other demographic variables.

In an ANOVA with diagnosis and hemisphere as between-subjects factors, and RIN as covariate, netrin-G1f mRNA showed a main effect of hemisphere ($F_{1,94}=4.36$, $p=0.039$), no main effect of diagnosis ($F_{2,94}=0.61$, $p=0.54$), and a diagnosis-by-hemisphere interaction ($F_{2,93}=3.86$, $p=0.024$) (Fig. 2D). Exploring this interaction, separate ANOVAs showed an effect of diagnosis in the right ($F_{2,48}=3.31$, $p=0.045$) but not the left ($F_{2,45}=1.30$, $p=0.282$) ACC. In the right ACC, netrin-G1f mRNA was increased in bipolar disorder ($p=0.013$), with no alteration in schizophrenia; in the left ACC, there were no significant group differences.

Effects of other demographic variables on expression of the transcripts

A range of demographic and disorder- or treatment-related variables in addition to those mentioned above were known for the subjects (Table 1; Supplementary Tables 1 and 2), and here we summarise the results of exploratory analyses for their potential influence on the transcripts carried out using categorical or correlation tests as appropriate (see Methods).

None of the mRNAs were affected by a history of suicide, or smoking; nor did they correlate with lifetime antipsychotic exposure, nor did they differ according to whether the subject had or had not been prescribed lithium, sodium valproate, other mood stabilisers,

antidepressants, or anticholinergics (Supplementary Table 1; data not shown). Alcohol use history, rated on a five point scale, had no significant effect on any of the transcripts (Supplementary Table 2; data not shown). Recreational drug use, rated in the same way, did impact on VGluT1 mRNA abundance, as determined by one-way ANOVA ($F_{5,94}=3.10$, $p=0.012$; Supplementary Table 3). Although none of the post-hoc comparisons were significant, there was a trend for heavy current drug users ($n=11$) to have higher VGluT1 mRNA levels compared to non-users ($n=55$; $p=0.064$) or social users ($n=12$; $p=0.056$). Given that the heavy use category includes 6 subjects with bipolar disorder but no control subjects, the drug influence might confound the difference between these diagnostic groups (Fig. 1C). However, any such effect appears modest since a) 28 of the 34 bipolar subjects were not heavy drug users; b) when drug use is included as a between-subjects factor in the VGluT1 ANOVA, it has a trend main effect ($p=0.083$), but there is no diagnosis-by-drug use interaction ($p=0.79$), and the difference between groups remains significant ($p=0.001$); c) VGluT1 mRNA was not increased in the schizophrenia group even though it includes 6 heavy drug users; and c) within each drug use category, bipolar disorder subjects always had the highest mean VGluT1 mRNA level compared to schizophrenia and to controls.

Effects of genotype on netrin-G1 and netrin-G2 expression

There were no genotype effects, nor interactions of genotype with diagnosis, for any of the transcripts (Supplementary Table 4).

Laminar distribution of netrin-G1 and netrin-G2 mRNAs

Netrin-G1 and G2 mRNAs had a differential expression profile within the ACC (Fig. 3). Netrin-G2 mRNA was abundant in layer V/VI, with a punctuate signal in these laminae indicative of dense expression in specific, presumably pyramidal, neurons (Fig. 3A). Netrin-G1 mRNA was expressed at considerably lower level, with signal concentrated over superficial laminae, with little or no signal above background in deeper laminae (Fig. 3B). These findings are similar to those reported in the temporal lobe (31).

Discussion

VGluT1 is expressed in glutamatergic neurons (20,21,36,37), and its expression reflects quantal size and the release of glutamate into the synapse (22-24). As such, our finding of increased VGluT1 expression in bipolar disorder (Fig. 1C) argues that the prior spectroscopic (12-16) and post-mortem (17,18) data showing elevated glutamate concentrations are indicative of increased glutamate neurotransmission, rather than, or in addition to, alterations in glutamate metabolism or cycling. If glutamate transmission is indeed elevated in bipolar disorder, it supports the rationale for anti-glutamatergic therapies (38,39).

The other main finding of the present study is an increased expression in bipolar disorder of netrin-G2, netrin-G1d and, in the right ACC, netrin-G1f (Fig. 2). Given the roles of netrins in the formation and plasticity of excitatory pathways (26,27,29,40), the finding may contribute to the inferred alteration in glutamate transmission in bipolar disorder, or at least be a marker of a shift in the developmental or plasticity profile of these pathways. The fact that netrin-G1c mRNA was not changed makes it unlikely that the increases in the other transcripts simply reflect an increased synaptic or neuronal density, especially since other presynaptic markers and cell counts are either unchanged, or are reduced, in the ACC in bipolar disorder (4,6,7,41,42). Further study is needed to ascertain which glutamatergic ACC circuits are affected in bipolar disorder by the changes reported here. The fact that netrin-G1 and -G2 are expressed by largely separate neuronal populations (Fig. 3;

[27,28,31,40]), yet both are increased in bipolar disorder, suggests that the consequences of their increased expression are likely to be relatively widespread within the ACC.

The VGluT1 and netrin-G mRNA increases in bipolar disorder appear to be unique to the ACC, based upon the cortical regions studied to date. That is, VGluT1 mRNA in the same bipolar disorder subjects is unaltered in the cerebellum (unpublished observations), and is unaltered or decreased within temporal lobe areas in a separate series (43). Similarly, we previously found netrin-G1 and -G2 mRNAs to be either unaltered or decreased in the medial and inferior temporal cortex in the current bipolar disorder group (31). These findings together support the view that the ACC may be a region of particular relevance to the pathophysiology of bipolar disorder (4-7, 44, 45).

In schizophrenia, we found no alterations in VGluT1 expression, in line with recent findings of unchanged cortical glutamate levels (15,17), and with a prior study in the temporal lobe (43). In contrast, increased ACC VGluT1 mRNA was seen in a series of elderly subjects with schizophrenia (mean age ~80 years; [37]), whereas VGluT1 mRNA was decreased in the hippocampus and dorsolateral prefrontal cortex in another sample (mean age 55 years; [46]). These findings suggest age-related as well as anatomical influences on VGluT1 expression in schizophrenia.

The human ACC is structurally and functionally asymmetric (47-50), and lateralised differences in the ACC in bipolar disorder have been reported (44,51,52). Recent data are beginning to identify genes involved in development of brain asymmetries (53), but to our knowledge, netrin-G1c and netrin-G1f are the first transcripts found to be asymmetrically expressed in the adult human ACC. For netrin-G1f, there was also a right-lateralised increase in bipolar disorder. As noted, unlike other netrin-G1 isoforms, netrin-G1f lacks the GPI lipid anchor domain sequence, and it is thus predicted to be released from the membrane and to act as a soluble molecule. Experimental data are needed to substantiate this prediction, and to investigate the role that netrin-G1 isoforms may play in the formation or maintenance of normal or bipolar disorder-related ACC asymmetries.

Our study has several limitations. First, we did not measure the encoded proteins, and so it is possible that the transcript alterations are not reflected in protein abundance (37). However, interpretation of a complementary study using anti-VGluT1 or anti-netrin-G antibodies would be problematic, since there is an imperfect concordance between location of each mRNA and its protein product. That is, some of the VGluT1 and netrin-G expressed in ACC neurons, as measured here, will be distributed to axons and synaptic terminals in other brain regions (via efferent projecting neurons); equally, some VGluT1 and netrin-G protein in the ACC will have arisen from neurons located outwith the ACC (via afferent projections). For discussion of this issue, see refs. 37 and 54. Second, there was some suggestion that VGluT1 expression was related to substance misuse history, and further study is needed to ascertain the extent to which this or other co-morbidities, or clinical variables (e.g. antidepressant medication or suicide [43]), may affect findings in bipolar disorder. Third, Table 2 revealed two unexpected correlations with perimortem variables: VGluT1 mRNA correlated positively with freezer storage time, and netrin-G2 mRNA correlated inversely (rather than positively) with RIN. Note that the data represent each target transcript normalised to the geometric mean of the four housekeeping genes, as is usual in qPCR studies. The storage time correlation arises because VGluT1 mRNA shows a weak positive correlation, whereas the housekeeping genes show a weak inverse one; for RIN, VGluT1 mRNA is positively correlated, but less so than the HKGs, resulting in an inverse correlation for the normalised data. Neither of the correlations is significant for the 'raw' mRNA data (not shown). These issues together highlight some of the complexities affecting the analysis and interpretation

of post mortem gene expression studies, and the ongoing need for large and well characterised brain collections (55-57).

A novel and potentially interesting correlation was that between VGluT1 mRNA and brain weight, (Fig. 1A and Table 2). A correlation with brain weight was also seen for netrin G1f mRNA in the control group ($R=+0.576$, $p<0.001$) and as a trend in the whole sample ($p=0.069$). The relationship between VGluT1 expression and brain weight was robust, in that it survived partialling for age and sex, both of which impact on brain weight (58,59), and, in a multiple regression model including these factors and diagnosis, brain weight accounted for about 8% of the variance in VGluT1 mRNA ($\beta=0.277$, $p=0.003$). Speculatively, the finding may hint at a relationship between activity in ACC glutamatergic circuits and brain growth, and that VGluT1 might be one gene contributing to the heritability of brain size (60,61). It also indicates a hitherto neglected factor that may contribute to the individual variability seen in brain gene expression studies.

Finally, the majority of positive findings reported in the two SMRI brain series are observed in both schizophrenia and bipolar disorder (45,62,63). As such, the robust diagnostic specificity of the current results is relatively unusual, and may thus provide clues as to pathophysiological processes that differentiate the disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci.* 2000; 4:215–222. [PubMed: 10827444]
2. Paus T. Primate anterior cingulate cortex: where motor control, drive and cognition interface. *Nat Rev Neurosci.* 2001; 2:417–424. [PubMed: 11389475]
3. Vogt BA. Pain and emotion interactions in subregions of the cingulate gyrus. *Nat Rev Neurosci.* 2005; 6:533–544. [PubMed: 15995724]
4. Harrison PJ. The neuropathology of primary mood disorder. *Brain.* 2002; 125:1428–1449. [PubMed: 12076995]
5. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biol Psychiatry.* 2003; 54:515–528. [PubMed: 12946880]
6. Fountoulakis KN, Giannakopoulos P, Kovari E, Bouras C. Assessing the role of cingulate cortex in bipolar disorder: Neuropathological, structural and functional imaging data. *Brain Res Rev.* 2008; 59:9–21. [PubMed: 18539335]
7. Price JL, Drevets WC. Neurocircuitry of mood disorders. *Neuropsychopharmacol Rev.* 2009 AOP 19 August 2009. doi:10.1038/npp2009.104.
8. Benes FM. Neurobiological investigations in cingulate cortex of schizophrenic brain. *Schizophr Bull.* 1993; 19:537–549. [PubMed: 8235456]

9. Harrison PJ. The neuropathology of schizophrenia - A critical review of the data and their interpretation. *Brain*. 1999; 122:593–624. [PubMed: 10219775]
10. Tamminga CA, Vogel M, Cao XM, Lahti AC, Holcomb HH. The limbic cortex in schizophrenia: focus on the anterior cingulate. *Brain Res Rev*. 2000; 31:364–370. [PubMed: 10719164]
11. Fornito A, Yucel M, Dean B, Wood SJ, Pantelis C. Anatomical abnormalities of the anterior cingulate cortex in schizophrenia: Bridging the gap between neuroimaging and neuropathology. *Schizophr Bull*. 2009; 35:973–993. [PubMed: 18436528]
12. Yildiz-Yesiloglu A, Akerst DP. Neurochemical alterations of the brain in bipolar disorder and their implications for pathophysiology: A systematic review of the in vivo proton magnetic resonance spectroscopy findings. *Prog Neuropsychopharmacol Biol Psychiatry*. 2006; 30:969–995. [PubMed: 16677749]
13. Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo K, et al. Brain metabolic alterations in medication-free patients with bipolar disorder. *Arch Gen Psychiatry*. 2004; 61:450–458. [PubMed: 15123489]
14. Frye MA, Watzl J, Banakar S, O'Neill J, Mintz J, Davanzo P, et al. Increased anterior cingulate/medial prefrontal cortical glutamate and creatine in bipolar depression. *Neuropsychopharmacology*. 2007; 32:2490–2499. [PubMed: 17429412]
15. Öngür D, Jensen JE, Prescot AP, Stork C, Lundy M, Cohen BM, et al. Abnormal glutamatergic neurotransmission and neuronal-glial interactions in acute mania. *Biol Psychiatry*. 2008; 64:718–726. [PubMed: 18602089]
16. Colla M, Schubert F, Bubner M, Heidenreich JO, Bajbouj M, Seifert F, et al. Glutamate as a spectroscopic marker of hippocampal structural plasticity is elevated in long-term euthymic bipolar patients on chronic lithium therapy and correlates inversely with diurnal cortisol. *Mol Psychiatry*. 2009; 14:696–704. [PubMed: 18347601]
17. Hashimoto K, Sawa A, Iyo M. Increased levels of glutamate in brains from patients with mood disorders. *Biol Psychiatry*. 2007; 62:1310–1316. [PubMed: 17574216]
18. Lan MJ, McLoughlin GA, Griffin JL, Tsang TM, Huang JTJ, Yuan P, et al. Metabonomic analysis identifies molecular changes associated with the pathophysiology and drug treatment of bipolar disorder. *Mol Psychiatry*. 2009; 14:269–279. [PubMed: 18256615]
19. Nedergaard M, Takano T, Hansen AJ. Beyond the role of glutamate as a neurotransmitter. *Nat Rev Neurosci*. 2002; 3:748–755. [PubMed: 12209123]
20. Bellocchio EE, Hu HL, Pohorille A, Chan J, Pickel VM, Edwards RH. The localization of the brain-specific inorganic phosphate transporter suggests a specific presynaptic role in glutamatergic transmission. *J Neurosci*. 1998; 18:8648–8659. [PubMed: 9786972]
21. Fremeau RT Jr, Troyer MD, Pahner I, Nygaard GO, Tran CH, Reimer RJ, et al. The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron*. 2001; 31:247–260. [PubMed: 11502256]
22. Wojcik SM, Rhee JS, Herzog E, Sigler A, Jahn R, Takamori S, et al. An essential role for vesicular glutamate transporter 1 (VGLUT1) in postnatal development and control of quantal size. *Proc Natl Acad Sci USA*. 2004; 101:7158–7163. [PubMed: 15103023]
23. Wilson NR, Kang JS, Hueske EV, Leung T, Varoqui H, Murnick JG, et al. Presynaptic regulation of quantal size by the vesicular glutamate transporter VGLUT1. *J Neurosci*. 2005; 25:6221–6234. [PubMed: 15987952]
24. Daniels RW, Collins CA, Chen K, Gelfand MV, Featherstone DE, DiAntonio A. A single vesicular glutamate transporter is sufficient to fill a synaptic vesicle. *Neuron*. 2006; 49:11–16. [PubMed: 16387635]
25. Woo J, Kwon S-K, Kim E. The NGL family of leucine-rich repeat-containing synaptic adhesion molecules. *Mol Cell Neurosci*. 2009; 42:1–10. [PubMed: 19467332]
26. Nakashiba T, Ikeda T, Nishimura S, Tashiro K, Honjo T, Culotti JG, et al. Netrin-G1: a novel glycosyl phosphatidylinositol-linked mammalian netrin that is functionally divergent from classical netrins. *J Neurosci*. 2000; 20:6540–6550. [PubMed: 10964959]
27. Nakashiba T, Nishimura S, Ikeda T, Itohara S. Complementary expression and neurite outgrowth activity of netrin-G subfamily members. *Mech Dev*. 2002; 111:47–60. [PubMed: 11804778]

28. Yin Y, Minoer JH, Sanes JR. Laminets: laminin- and netrin-related genes expressed in distinct neuronal subsets. *Mol Cell Neurosci.* 2002; 19:344–358. [PubMed: 11906208]
29. Kim S, Burette A, Chung HS, Kwon S-K, Woo J, Lee HW, et al. NGL family PSD-95-interacting adhesion molecules regulate excitatory synapse formation. *Nat Neurosci.* 2006; 9:1294–1301. [PubMed: 16980967]
30. Lin L, Lesnick TG, Maraganore DM, Isacson O. Axon guidance and synaptic maintenance: preclinical markers for neurodegenerative disease and therapeutics. *Trends Neurosci.* 2009; 32:142–149. [PubMed: 19162339]
31. Eastwood SL, Harrison PJ. Decreased mRNA expression of netrin-G1 and netrin-G2 in the temporal lobe in schizophrenia and bipolar disorder. *Neuropsychopharmacology.* 2008; 33:933–945. [PubMed: 17507910]
32. Aoki-Suzuki M, Yamada K, Meerabux J, Iwayama-Shigeno Y, Ohba H, Iwamoto K, et al. A family-based association study and gene expression analyses of netrin-G1 and-G2 genes in schizophrenia. *Biol Psychiatry.* 2005; 57:382–393. [PubMed: 15705354]
33. Ohtsuki T, Horiuchi Y, Koga M, Ishiguro H, Inada T, Iwata N, et al. Association of polymorphisms in the haplotype block spanning the alternatively spliced exons of the NTNG1 gene at 1p13.3 with schizophrenia in Japanese populations. *Neurosci Lett.* 2008; 435:194–197. [PubMed: 18384956]
34. Bristow GC, Lane TA, Walker M, Chen L, Sei Y, Hyde TM, et al. Expression of Kinase Interacting with Stathmin (KIS, UMHK1) in human brain and lymphoblasts: effects of schizophrenia and genotype. *Brain Research.* 2009 doi 10.1016/j.brainres.2009.08.090.
35. Meerabux JMA, Ohba H, Fukasawa M, Suto Y, Aoki-Suzuki M, Nakashiba T, et al. Human netrin-G1 isoforms show evidence of differential expression. *Genomics.* 2005; 86:112–116. [PubMed: 15901489]
36. Alonso-Nanclares L, DeFelipe J. Vesicular glutamate transporter 1 immunostaining in the normal and epileptic human cerebral cortex. *Neuroscience.* 2005; 134:59–68. [PubMed: 15961236]
37. Oni-Orisan A, Kristiansen LV, Haroutunian V, Ruff JHMW, McCullumsmith RE. Altered vesicular glutamate transporter expression in the anterior cingulate cortex in schizophrenia. *Biol Psychiatry.* 2008; 63:766–775. [PubMed: 18155679]
38. Sanacora G, Zarate CA, Krystal JH, Manji HK. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nat Rev Drug Disc.* 2008; 7:426–437.
39. Martinowich K, Schloesser RJ, Manji HK. Bipolar disorder: from genes to behavior pathways. *J Clin Invest.* 2009; 119:726–736. [PubMed: 19339764]
40. Nishimura-Akiyoshi S, Niimi K, Nakashiba T, Itohara S. Axonal netrin-Gs transneuronally determine lamina-specific subdendritic segments. *Proc Natl Acad Sci USA.* 2007; 104:14801–14807. [PubMed: 17785411]
41. Eastwood SL, Harrison PJ. Synaptic pathology in the anterior cingulate cortex in schizophrenia and mood disorders. A review and a Western blot study of synaptophysin, GAP-43 and the complexins. *Brain Res Bull.* 2001; 55:569–578. [PubMed: 11576753]
42. Todtenkopf MS, Vincent SL, Benes FM. A cross-study meta-analysis and three-dimensional comparison of cell counting in the anterior cingulate cortex of schizophrenic and bipolar brain. *Schizophr Res.* 2005; 73:79–89. [PubMed: 15567080]
43. Uezato A, Meador-Woodruff JH, McCullumsmith RE. Vesicular glutamate transporter mRNA expression in the medial temporal lobe in major depressive disorder, bipolar disorder, and schizophrenia. *Bipolar Disord.* 2009; 11:711–725. [PubMed: 19839996]
44. Drevets WC, Price JL, Simpson JR, Todd RD, Reich T, Vannier M, et al. Subgenual prefrontal cortex abnormalities in mood disorders. *Nature.* 1997; 386:824–827. [PubMed: 9126739]
45. Knable MB, Barci BM, Bartko JJ, Webster MJ, Torrey EF. Abnormalities of the cingulate gyrus in bipolar disorder and other severe psychiatric illness: postmortem findings from the Stanley Foundation Neuropathology Consortium and literature review. *Clin Neurosci Res.* 2002; 2:171–181.
46. Eastwood SL, Harrison PJ. Decreased expression of vesicular glutamate transporter 1 and complexin II mRNAs in schizophrenia: further evidence for a synaptic pathology affecting glutamate neurons. *Schizophr Res.* 2005; 73:159–172. [PubMed: 15653259]

47. Paus T, Tomaiuolo F, Otaky N, MacDonald D, Petrides M, Atlas J, et al. Human cingulate and paracingulate sulci: Pattern, variability, asymmetry, and probabilistic map. *Cereb Cortex*. 1996; 6:207–214. [PubMed: 8670651]
48. Stephan KE, Marshall JC, Friston KJ, Rowe JB, Ritzl A, Zilles K, et al. Lateralized cognitive processes and lateralized task control in the human brain. *Science*. 2003; 301:384–386. [PubMed: 12869765]
49. Gittins R, Harrison PJ. A quantitative morphometric study of the human anterior cingulate cortex. *Brain Res*. 2004; 1013:212–222. [PubMed: 15193531]
50. Yan H, Zuo XN, Wang DY, Wang J, Zhu CZ, Milham MP, et al. Hemispheric asymmetry in cognitive division of anterior cingulate cortex: A resting-state functional connectivity study. *Neuroimage*. 2009; 47:1579–1589. [PubMed: 19501172]
51. Cotter D, MacKay D, Landau S, Kerwin R, Everall I. Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. *Arch Gen Psychiatry*. 2001; 58:545–553. [PubMed: 11386983]
52. Versace A, Aleida JRC, Hassel S, Walsh ND, Novelli M, Klein CR, et al. Elevated left and reduced right orbitomedial prefrontal fractional anisotropy in adults with bipolar disorder revealed by tract-based spatial statistics. *Arch Gen Psychiatry*. 2008; 65:1041–1052. [PubMed: 18762590]
53. Sun T, Walsh CA. Molecular approaches to brain asymmetry and handedness. *Nat Rev Neurosci*. 2006; 7:55–662.
54. Eastwood SL, Cairns NJ, Harrison PJ. Synaptophysin gene expression in schizophrenia - Investigation of synaptic pathology in the cerebral cortex. *Br J Psychiatry*. 2000; 176:236–242. [PubMed: 10755070]
55. Harrison PJ, Heath PR, Eastwood SL, Burnet PWJ, McDonald B, Pearson RCA. The relative importance of premortem acidosis and postmortem interval for human brain gene expression studies: Selective mRNA vulnerability and comparison with their encoded proteins. *Neurosci Lett*. 1995; 200:151–154. [PubMed: 9064599]
56. Lewis DA. The human brain revisited: Opportunities and challenges in postmortem studies of psychiatric disorders. *Neuropsychopharmacology*. 2002; 26:143–154. [PubMed: 11790510]
57. Lipska BK, Deep-Soboslay A, Weickert CS, Hyde TM, Martin CE, Herman MM, et al. Critical factors in gene expression in postmortem human brain: Focus on studies in schizophrenia. *Biol Psychiatry*. 2006; 60:650–658. [PubMed: 16997002]
58. Skullerud K. Variations in the size of the human brain. *Acta Neurol Scand*. 1985; 71(suppl. 102): 7–90.
59. Harrison PJ, Freemantle N, Geddes JR. Meta-analysis of brain weight in schizophrenia. *Schizophr Res*. 2003; 64:25–34. [PubMed: 14511798]
60. Bartley AJ, Jones DW, Weinberger DR. Genetic variability of human brain size and cortical gyral patterns. *Brain*. 1997; 120:257–269. [PubMed: 9117373]
61. Baaré WFC, Pol HE, Hulshoff, Boomsma DI, Posthuma D, de Geus EJC, Schnack HG, et al. Quantitative genetic modelling of variation in human brain morphology. *Cereb Cortex*. 2001; 11:816–824. [PubMed: 11532887]
62. Tkachev D, Mimmack M, Ryan MM, Wayland M, Freeman T, Jones PB, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet*. 2003; 362:798–805. [PubMed: 13678875]
63. Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB. Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biol Psychiatry*. 2005; 57:252–260. [PubMed: 15691526]

Figure 1. VGlut1 mRNA abundance correlates with (A) fresh brain weight and (B) freezer storage time. C: VGlut1 mRNA increased in bipolar disorder (BIP, squares) but not schizophrenia (SCZ, inverted triangles) compared to controls (CON, triangles). See Table 2 and text for analyses.

Figure 2.

Expression of netrin-G1 and -G2 mRNAs in ACC in bipolar disorder and schizophrenia using qPCR. All data shown are normalised to the geometric mean of the four housekeeping transcripts. A: Netrin-G2 mRNA is increased in bipolar disorder but not schizophrenia. B: Netrin-G1c mRNA shows no diagnostic differences, but has higher expression in the left ACC compared to the right ACC. C: Netrin-G1d mRNA is increased in bipolar disorder but not schizophrenia. D: Netrin-G1f mRNA shows a normal left>right asymmetry, and a right-lateralised increase in bipolar disorder. Symbols as in Fig. 1.

Figure 3.

A: Distribution of netrin-G2 mRNA in human ACC, showing concentration in deep laminae (V/VI) compared to superficial laminae (II/III) and to white matter (wm). B: Distribution of netrin-G1 mRNA in human ACC, showing a lower signal, but with relative enhancement in superficial laminae (II/III).

Table 1
Demographic details of the subjects studied.

	Controls	Schizophrenia ^a	Bipolar disorder ^b
Number	35	35	34
Gender (M:F)	26:9	26:9	16:18
Age (years)	44.2 ± 7.6	42.6 ± 8.5	45.4 ± 10.7
Side of brain (L:R)	16:19	17:18	19:15
Brain pH	6.61 ± 0.27	6.47 ± 0.24	6.43 ± 0.30
RNA integrity number (RIN)	4.72 ± 1.56	5.07 ± 1.52	4.89 ± 1.82
Autopsy delay (hours)	29.4 ± 12.9	31.4 ± 15.5	37.9 ± 18.6
Storage time (months)	81 ± 18	87 ± 23	93 ± 26
Brain weight (g)	1444 ± 148	1442 ± 107	1395 ± 142
Onset of illness (years)	-	21.3 ± 6.1	25.3 ± 9.2
Duration of illness (years)	-	21.3 ± 10.1	20.1 ± 9.6
Total inpatient time (years)	-	1.23 ± 2.25	0.53 ± 1.39
Lifetime fluphenazine equivalents (g)	-	85.0 ± 100.3	10.2 ± 23.2

Values are mean ± S.D.

^aSubtypes: undifferentiated (n=26), paranoid (n=8), disorganised (n=1).

^bSubtypes: bipolar I (n=21), bipolar II or NOS (n=6), not known (n=7).

Table 2

Correlations of the transcripts with demographic variables.

	Age	RIN	Brain pH	Autopsy delay	Storage time	Brain weight
VGluT1	-.118	+.234 ^{***a}	+.162	+.170	+.435 ^{***a}	+.233 ^{***a}
Netrin-G2	-.102	-.347 ^{***a}	-.178	+.177	+.021	+.049 ^a
Netrin-G1c	-.202 [*]	+.389 ^{***}	+.200 ^a	+.092	-.057	+.056
Netrin-G1d	-.109	+.409 ^{***a}	+.174 ^a	+.007	+.093	+.118
Netrin-G1f	+.051	+.290 ^{***}	+.227 ^a	-.008	-.176	+.182 ^a

Values are Pearson coefficients.

^aCorrelation also significant (p<0.05) in control group considered separately.

* p<0.05

**

p<0.02

p<0.01

p<0.001