Published in final edited form as:

Neuropathol Appl Neurobiol. 2010 October ; 36(6): 498–504. doi:10.1111/j.1365-2990.2010.01098.x.

Human brain weight is correlated with expression of the 'housekeeping genes' beta-2-microglobulin (β 2M) and TATA-binding protein (TBP)

P.J. Harrison, L.M. Laatikainen, E.M. Tunbridge, and S. L. Eastwood

Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford OX3 7JX, United Kingdom

Abstract

Aims—Many variables affect mRNA measurements in post mortem human brain tissue. Brain weight has not hitherto been considered to be such a factor. This study investigated whether there is any relationship between brain weight and mRNA abundance.

Methods—We investigated quantitative real-time RT-PCR data for five 'housekeeping genes' using the 104 adult brains of the Stanley Microarray Consortium series. Eleven datasets were analysed, from cerebellum, hippocampus, and anterior cingulate cortex. We used a specified sequence of correlations, partial correlations, and multiple regression analyses.

Results—Brain weight correlated with the 'raw' (i.e. non-normalised) data for two mRNAs, 2-microglobulin (β 2M) and TATA-binding protein (TBP), measured in cerebellum and hippocampus respectively. In hippocampus, the geometric mean of three housekeeping gene transcripts also correlated with brain weight. The correlations were significant after adjusting for age, sex and other confounders, and the effect of brain weight was confirmed using multiple regression. No correlations with brain weight were seen in the anterior cingulate cortex, nor for the other mRNAs examined.

Conclusions—The findings were not anticipated; they need replication in another brain series, and a more systematic survey is indicated. In the interim, we suggest that quantitative gene expression studies in human brain should inspect for a potential influence of brain weight, especially since the affected transcripts are commonly used as reference genes for normalisation purposes in studies of neurological and psychiatric disorders. The relationship of brain weight with β 2M mRNA may reflect the roles of MHC class I genes in synapse formation and plasticity.

Keywords

Brain size; Gene expression; Messenger RNA; Schizophrenia, Real-time PCR

Quantitative measurements of mRNA abundance are frequently performed in human post mortem brain tissue to study psychiatric and neurological disorders. Such studies must consider a wide range of demographic and peri-mortem variables that are known to influence mRNA detection [1-10]. Brain weight is not usually considered to be a factor which might impact on gene expression. However, brain weight was recently shown to correlate with the normalised abundance of vesicular glutamate transporter VGLUT1 mRNA [11] and, in males, with methylation of the insulin-like growth factor 2 (*IGF2*) gene, an epigenetic process that regulates gene expression [11]. These observations led us to examine

in detail for relationships between brain weight and transcript abundance, in a large series of brains widely utilised for the study of schizophrenia and bipolar disorder.

Materials and Methods

The tissue came from the Stanley Medical Research Institute (SMRI) Microarray Consortium brains ('SMC series') which comprises brains from 104 adults: 35 controls, 35 with schizophrenia, 34 with bipolar disorder. For demographics see Table 1, also <http://www.stanleyresearch.org/dnn/Portals/0/Stanley/Array%20Collection%20Demographic%20Details%20Chart-Final.pdf>. The specimens were collected, with informed consent from next-of-kin, by participating medical examiners in the USA, and tissue from the SMC series has been made available free-of-charge to researchers worldwide. Fresh brain weight was recorded for each subject. We extracted RNA from blocks of cerebellar cortex and anterior cingulate cortex, and from sections of hippocampus (including parahippocampal cortex). The RNA was used for quantitative real-time RT-PCR (qPCR; see below). We focussed on housekeeping genes (HKG), since their expression should not be confounded by differences between the diagnostic groups which might obscure any relationship with brain weight.

In cerebellum and anterior cingulate cortex, the mRNAs measured were 2-microglobulin (2M), glucuronidase beta (GUSB), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and transferrin receptor (TFRC), using Taqman assays as described previously [12]. In hippocampus, we measured GAPDH, TFRC, and TATA box binding protein (TBP) mRNAs, using SYBR Green assays. In brief, hippocampal RNA was extracted using Tri Reagent (Sigma Aldrich, Poole, United Kingdom). RNA integrity (RIN) was measured using an Agilent Bioanalyzer 2100 and RNA 6000 Nano kit (Agilent Technologies, Wokingham, U.K.). 500ng RNA was DNase treated for 30mins at 37°C and 10mins at 70°C, using 1 unit RNase-free DNase and 24 units RNasin ribonuclease inhibitor (both Promega, Southampton, U.K.) and reverse transcribed using 30ng oligo-dT, 200 units M-MLV reverse transcriptase, 24 units RNasin, 0.5mM of each dNTP and reaction buffer (Promega) at 42°C for 1h and 70 °C for 10min. qPCR was performed on 10ng cDNA using powerSYBR Green (Applied Biosystems, Warrington, U.K.) and 2 µmol/L of TBP or GAPDH primers or 3 µmol/L TFRC primers (sequences available on request), on an AB7900HT machine. Each qPCR reaction was performed in triplicate. The PCR cycle consisted of a initial denaturing step at 95 °C for 10min, followed by 40 cycles of 95 °C for 15s, 63 °C for 1min, 95 °C for 15s, and a melting curve. Given the purpose of the study, we did not normalise the data to the expression of other genes but used the raw mRNA data read off against a serial dilution calibration curve run for each experiment (Applied Biosystems SDS v2.2.2 software).

We took a sequential approach to analysis of the relationship between each mRNA and brain weight. First, the mRNAs were screened to identify those whose abundance showed a significant correlation (Spearman rho) with brain weight in the whole sample ($\rho = 0.02$) and in the 35 control subjects alone ($\rho = 0.05$). We required the correlation to be present in controls as well as in the whole sample, in case it was being driven by the diagnostic groups; we relaxed the significance level for the control group to reflect its smaller size. For only those mRNAs that met both criteria, we then examined in more detail the basis for, and robustness of, the correlation. Since adult brain weight declines with age and differs between men and women [13-16], and age and sex can also affect mRNA abundance [17-20] - we adjusted for these factors using partial correlations between the implicated mRNAs and brain weight. (The expected sex difference in brain weight is seen in the SMC series [men vs. women 1474±119g vs. 1339±118g, mean±S.D.; $t=5.54$, $p<0.001$], but there is no correlation between brain weight and age [$r=-0.038$, $p=0.70$], likely reflecting the absence of elderly [>64 years] subjects). We also assessed whether the transcript correlated with other variables known to affect mRNA detection in human brains, notably RIN [7,9,10,21],

also post mortem interval and freezer storage time; if any of these correlations were significant, we included them in partial correlations between the mRNA and brain weight. Finally, to complement these correlational methods, we ran multiple regressions for the mRNAs with brain weight, age, and sex, together with any other factor that influenced the mRNA in question, as independent variables. As well as running this sequence of analyses for each HKG, we did the same for the geometric mean of the HKGs run in each brain region, since this is an increasingly common normalisation strategy for qPCR studies [22,23].

Results

In cerebellum, 2M mRNA met the criterion of being significantly correlated with brain weight in the whole sample *and* in the controls alone (Table 2 and Fig. 1A). The correlation with brain weight remained significant after partialling for sex and age (Table 2). By stepwise multiple regression, 2M mRNA was associated with brain weight ($\beta = -0.293$, $t = 2.52$, $p = 0.010$) and age ($\beta = -0.230$, $t = 2.31$, $p = 0.023$) but not with RIN, sex or diagnosis, with brain weight explaining 10% of the variance in 2M mRNA (i.e. R^2 change $= 0.10$). Cerebellar TFRC mRNA correlated inversely with brain weight in the whole sample and in the controls; however, the result was driven by an outlier, and exclusion of this subject abolished the correlations (Table 2). There were no correlations between brain weight and cerebellar GUSB or GAPDH mRNAs, or with the geometric mean of the four cerebellar HKGs (Table 2).

In hippocampus, TBP mRNA (Table 2 and Fig. 1B) and GAPDH mRNA (Table 2) were both correlated with brain weight in the whole sample, and in the controls. TBP mRNA also correlated with RIN ($r = +0.380$, $p < 0.001$); the correlation between TBP mRNA and brain weight remained after adjusting for RIN ($r = +0.284$, d.f.96, $p = 0.005$), as it did after adjusting for age and sex (Table 2). For analysis by multiple regression, TBP mRNA values were square root transformed because the raw data were not normally distributed (one-sample Kolmogorov-Smirnov test, $z = 1.95$, $p < 0.001$). The transformed TBP mRNA data were associated with brain weight ($\beta = 0.294$, $t = 3.239$, $p = 0.002$), as well as with RIN ($\beta = 0.338$, $t = 3.73$, $p < 0.001$), with brain weight explaining 8% of the variance. In contrast, the correlation between brain weight and hippocampal GAPDH mRNA did not survive after partialling for RIN ($r = 0.069$, d.f.100, $p = 0.49$), age, or sex (Table 2), and thus it did not meet our criteria for robustness, and neither was it significant by multiple regression (data not shown). Hippocampal TFRC mRNA did not correlate with brain weight. The geometric mean of the three hippocampal HKG transcripts correlated significantly with brain weight in the whole sample, in the controls, and after partialling for age and sex (Table 2). It also correlated with RIN ($r = +0.364$, $p < 0.001$), with the brain weight correlation persisting after adjusting for RIN ($r = +0.240$, d.f.98, $p = 0.016$). The geometric mean data were non-Gaussian (one-sample Kolmogorov-Smirnov test, $z = 1.64$, $p = 0.009$) and hence were normalised by square root transformation; using the transformed data, the geometric mean of the HKG mRNAs was associated with brain weight ($\beta = 0.264$, $t = 2.91$, $p = 0.005$), and with RIN ($\beta = 0.339$, $t = 3.728$, $p < 0.001$), with brain weight explaining 7% of the variance.

No significant correlations with brain weight were seen for any of the 4 HKGs in the anterior cingulate cortex (Table 2). Neither did any of the mRNAs, in any region, correlate with the post mortem interval or freezer storage time, nor differ between males and females, nor differ between diagnostic groups (data not shown).

Discussion

We present evidence that, in a series of over 100 adult human brains, two individual mRNAs, *2M* and *TBP*, correlate with brain weight. The influence of brain weight is independent of other variables that are known to impact on mRNA abundance, with brain weight explaining a non-trivial proportion (up to 10%) of the variance in mRNA abundance between subjects. The results indicate that brain weight should be added to the list of pre- and post-mortem variables that can affect mRNA detection in human brain gene expression studies.

We have no convincing explanation for the unexpected finding of a relationship between brain weight and mRNA abundance. Indeed, until recently, we had had no suspicion that brain weight was a relevant factor, and had never examined for its potential influence despite working in the field for many years. However, two issues may be relevant. First, brain size as measured by MRI, and therefore presumably brain weight, is a quantitative trait with a substantial heritable component [24]. It is possible that the transcripts correlating with brain weight are either amongst the genes underlying this heritability [25-27], or are regulated by them. Second, the known functions of *2M* and *TBP* may shed some light on the basis for the correlations. *2M* encodes the invariant chain of the major histocompatibility complex (MHC) class I [28], and MHC class I molecules, including *2M*, are now known to be expressed in neurons [29] and to be involved in synaptogenesis and synaptic plasticity [30,31]. *TBP* is a member of the family of TATA-box transcription factors (see [32]), which might thus impact on brain weight via the regulation of many other genes; its functionality in the brain is highlighted by the fact that *TBP* mutations cause a form of spinocerebellar ataxia [33] and *TBP* expansions may influence the age of onset of schizophrenia [34]. Clearly, all these suggestions are speculative, and the biological basis for the correlations with brain weight – and the causality of the relationship – remains to be determined. These important caveats extend to a lack of an explanation for the absence of brain weight correlations in the anterior cingulate cortex, even for transcripts that did show a correlation in cerebellum or hippocampus.

The mRNAs studied here are so-called HKGs, and often used as reference genes for normalisation of qPCR data. The rationale is that their expression is largely invariant, and hence normalisation, either to a single HKG or to multiple HKGs, can overcome various sources of error in mRNA measurement, and facilitate interpretation [22,23]. The finding that HKG expression can be affected by brain weight complements many recent studies that reveal that HKG transcripts are altered by a range of physiological and pathophysiological factors [7,10,21,35-38]. Together, these cast doubt on any simple concept of a HKG, and suggest that their use for normalization purposes needs a critical reappraisal [39-41]. Moreover, in the hippocampus, the geometric mean of the three HKGs was also correlated with brain weight, indicating that this strategy [22] does not necessarily overcome the problem.

Our study has three main limitations. First, it utilised datasets for HKG mRNAs that had already been measured as part of our research into schizophrenia and mood disorders; the choice of HKGs was, to an extent, arbitrary, reflecting their widespread use in the field and our prior experience with them. As such, this study was an opportunistic one, not an unbiased (systematic or random) survey of the transcriptome; we therefore cannot estimate meaningfully the proportion of mRNAs that may be related to brain weight, nor identify whether the affected mRNAs share any common features (e.g. regulatory motifs) or encode proteins participating in particular pathways, nor can we explore the apparent regional specificity of the relationships. Parenthetically, using the same statistical approach as adopted here, we observed no significant correlations between brain weight and the ‘raw’

(i.e. non-normalised) expression of eight ‘target’ mRNAs we have measured in this brain series (data not shown). This might relate to the fact that the target transcripts were all selected because of their hypothesized involvement in psychosis; indeed, several of them do differ between diagnostic groups [11,42,43]. Hence, disease-related factors may contribute to their abundance, whereas HKGs are chosen for precisely the opposite reason, perhaps rendering an effect of brain weight upon their abundance easier to identify. The second limitation is that we have not formally corrected for multiple testing (*viz.*, we assessed 11 mRNA datasets). It is debatable whether any correction for multiple testing in this instance is required, and if so, what the correction would be. We would argue that the multiple and sequential criteria we adopted to define significance, and the α values set (see Methods), render false positive results unlikely. The final limitation is that all the results here come from one brain series, which is also the same one that produced the pilot observations that led us to do the study [11,12]; hence, a comparable analysis is needed in another brain series of equal or greater size to establish to what extent these findings can be generalised. Such replication would also help address the possibility that there exists an unknown factor which correlates with brain weight in this brain series and which explains the current findings.

In summary, the abundance of two transcripts, both widely used as HKGs, correlates with human brain weight, independent of age, sex, RIN, and other known potential confounders. The causality, interpretation, and replicability, of these correlations remains to be determined. In the meantime, it would be prudent to add brain weight to the list of variables to be taken into account for quantitative mRNA studies in human brains, particularly when designing and implementing normalisation strategies.

Acknowledgments

Work supported by grants from the Medical Research Council and Stanley Medical Research Institute to PJH and SLE. LL holds an MRC studentship. EMT is supported by a Royal Society Research Fellowship. Brain tissue kindly provided by the Stanley Medical Research Institute, courtesy of Drs Michael B Knable, E Fuller Torrey, Maree J Webster, Serge Weis and Robert H Yolken.

References

1. Harrison PJ, Procter AW, Barton AJL, Lowe SL, Najlerahim A, Bertolucci PHF, Bowen DM, Pearson RCA. Terminal coma affects messenger RNA detection in post mortem human temporal cortex. *Mol Brain Res.* 1991; 9:161–164. [PubMed: 1673215]
2. Barton AJL, Pearson RCA, Najlerahim A, Harrison PJ. Pre- and postmortem influences on brain RNA. *J Neurochem.* 1993; 61:1–11. [PubMed: 7685811]
3. 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]
4. Kingsbury AE, Foster OJF, Nisbet AP, Cairns N, Bray L, Eve DJ, Lees AJ, Marsden CD. Tissue pH as an indicator of mRNA preservation in human post- mortem brain. *Mol Brain Res.* 1995; 28:311–318. [PubMed: 7723629]
5. Preece P, Cairns NJ. Quantifying mRNA in postmortem human brain: influence of gender, age at death, postmortem interval, brain pH, agonal state and inter-lobe mRNA variance. *Mol Brain Res.* 2003; 118:60–71. [PubMed: 14559355]
6. Li JZ, Vawter MP, Walsh DM, Tomita H, Evans SJ, Choudary PV, Lopez JF, Avelar A, Shokoohi V, Chung T, Mesarwi O, Jones EG, Watson SJ, Akil H, Bunney WE Jr. Myers RM. Systematic changes in gene expression in postmortem human brains associated with tissue pH and terminal medical conditions. *Hum Mol Genet.* 2004; 13:609–616. [PubMed: 14734628]
7. Lipska BK, Deep-Soboslay A, Weickert CS, Hyde TM, Martin CE, Herman MM, Kleinman JE. Critical factors in gene expression in postmortem human brain: Focus on studies in schizophrenia. *Biol Psychiatry.* 2006; 60:650–658. [PubMed: 16997002]

8. Stan AD, Ghose S, Gao XM, Roberts RC, Lewis-Amezcu K, Hatanpaa KJ, Tamminga CA. Human postmortem tissue: What quality markers matter? *Brain Res.* 2006; 1123:1–11. [PubMed: 17045977]
9. Webster MJ. Tissue preparation and banking. *Prog Brain Res.* 2006; 158:3–14. [PubMed: 17027689]
10. Durrenberger PF, Fernando S, Kashefi SN, Ferrer I, Hauw JJ, Seilhean D, Smith C, Walker R, Al-Sarraj S, Troakes C, Palkovits M, Kasztner M, Huitinga I, Arzberger T, Dexter DT, Kretschmar H, Reynolds R. Effects of antemortem and postmortem variables on human brain mRNA quality: A BrainNet Europe study. *J Neuropathol Exp Neurol.* 2010; 69:70–81. [PubMed: 20010301]
11. Eastwood SL, Harrison PJ. Markers of glutamate synaptic transmission and plasticity are increased in the anterior cingulate cortex in bipolar disorder. *Biol Psychiatry.* 2010; 67:1010–1016. [PubMed: 20079890]
12. Pidsley R, Dempster EL, Mill J. Brain weight in males is correlated with DNA methylation at IGF2. *Mol Psychiatry.* Dec 15.2009 advance online publication. doi:10.1038/mp.2009.138.
13. Dekaban AS. Changes in brain weights during the span of human life: relation of brain weights to body heights and body weights. *Ann Neurol.* 1978; 4:345–356. [PubMed: 727739]
14. Haug H. Macroscopic and microscopic morphometry of the human brain and cortex. A survey in the light of new results. *Brain Pathol.* 1984; 1:123–149.
15. Skulderud K. Variations in the size of the human brain. *Acta Neurol Scand.* 1985; 71(suppl. 102): 1–94.
16. Harrison PJ, Freemantle N, Geddes JR. Meta-analysis of brain weight in schizophrenia. *Schizophr Res.* 2003; 64:25–34. [PubMed: 14511798]
17. Burnet PWJ, Eastwood SL, Harrison PJ. Detection and quantitation of 5-HT_{1A} and 5-HT_{2A} receptor mRNAs in human hippocampus using a reverse transcriptase- polymerase chain reaction (RT-PCR) technique and their correlation with binding site densities and age. *Neurosci Lett.* 1994; 178:85–89. [PubMed: 7529388]
18. Vawter MP, Evans S, Choudary P, Tomita H, Meador-Woodruff J, Molnar M, Li J, Lopez JF, Myers R, Cox D, Watson SJ, Akil H, Jones EG, Bunney WE. Gender-specific gene expression in post-mortem human brain: Localization to sex chromosomes. *Neuropsychopharmacology.* 2004; 29:373–384. [PubMed: 14583743]
19. Erraji-Benchekroun L, Underwood MD, Arango V, Galfalvy H, Pavlidis P, Smyrniotopoulos P, Mann JJ, Sibille E. Molecular aging in human prefrontal cortex is selective and continuous throughout adult life. *Biol Psychiatry.* 2005; 57:549–558. [PubMed: 15737671]
20. Berchtold NC, Cribbs DH, Coleman PD, Rogers J, Head E, Kim R, Beach T, Miller C, Troncoso J, Trojanowski JQ, Zielke HR, Cotman CW. Gene expression changes in the course of normal brain aging are sexually dimorphic. *Proc Natl Acad Sci USA.* 2008; 105:15605–15610. [PubMed: 18832152]
21. Weickert CS, Sheedy D, Rothmond DA, Dedova I, Fung S, Garrick T, Wong J, Harding AJ, Sivagnanasundaram S, Hunt C, Duncan C, Sundqvist N, Tsai S-Y, Anand J, Draganic D, Harper C. Selection of reference gene expression in a schizophrenia brain cohort. *Aus NZ J Psychiatry.* 2010; 44:59–70.
22. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 2002; 3:0034.
23. VanGuilder HD, Vrana KE, Freeman WM. Twenty-five years of quantitative PCR for gene expression analysis. *BioTechniques.* 2008; 44:619–626. [PubMed: 18474036]
24. Peper JS, Brouwer RM, Boomsma DI, Kahn RS, Poll HEH. Genetic influences on human brain structure: A review of brain imaging studies in twins. *Hum Brain Mapping.* 2007; 28:464–473.
25. Bond J, Woods CG. Cytoskeletal genes regulating brain size. *Curr Opin Neurobiol.* 2006; 18:95–101.
26. Tang BL. Molecular genetic determinants of human brain size. *Biochem Biophys Res Commun.* 2006; 345:911–916. [PubMed: 16716254]
27. Rimol LM, Agartz I, Djurovic S, Brown AA, Roddey JC, Kahler AK, Mattingsdal M, Athanasiu L, Joyner AH, Schork NJ, Halgren E, Sundet K, Melle I, Dale AM, Andreassen OA. Sex-dependent

- association of common variants of microcephaly genes with brain structure. *Proc Natl Acad Sci USA*. 2010; 107:384–388. [PubMed: 20080800]
28. Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R. Beta-2-microglobulin deficient mice lack CD4-8+ cytolytic T-cells. *Nature*. 1990; 344:742–746. [PubMed: 2139497]
 29. Corriveau RA, Huh GS, Shatz CJ. Regulation of class I MHC gene expression in the developing and mature CNS by neural activity. *Neuron*. 1998; 21:505–520. [PubMed: 9768838]
 30. Boulanger LM. Immune proteins in brain development and synaptic plasticity. *Neuron*. 2009; 64:93–109. [PubMed: 19840552]
 31. Shatz CJ. MHC Class I: An unexpected role in neuronal plasticity. *Neuron*. 2009; 64:40–45. [PubMed: 19840547]
 32. van Roon-Mom WMC, Reid SJ, Faull RLM, Snell RG. TATA-Binding protein in neurodegenerative disease. *Neuroscience*. 2005; 133:863–872. [PubMed: 15916858]
 33. Nakamura K, Jeong SY, Uchihara T, Anno M, Nagashima K, Nagashima T, Ikeda Si, Tsuji S, Kanazawa I. SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. *Hum Mol Genet*. 2001; 10:1441–1448. [PubMed: 11448935]
 34. Ohi K, Hashimoto R, Yasuda Y, Kiribayashi M, Iike N, Yoshida T, Azechi M, Ikezawa K, Takahashi H, Morihara T, Ishii R, Tagami S, Iwase M, Okochi M, Kamino K, Kazui H, Tanaka T, Kudo T, Takeda M. TATA Box-Binding Protein gene is associated with risk for schizophrenia, age at onset and prefrontal function. *Genes Brain Behavior*. 2009; 8:473–480.
 35. Iwamoto K, Bundo M, Ueda J, Kato T. Expression of ribosomal subunit genes increased coordinately with postmortem interval in human brain. *Mol Psychiatry*. 2006; 11:1067–1069. [PubMed: 17130884]
 36. Johansson S, Fuchs A, Okvist A, Karimi M, Harper C, Garrick T, Sheedy D, Hurd Y, Bakalkin G, Ekstrom TJ. Validation of endogenous controls for quantitative gene expression analysis: Application on brain cortices of human chronic alcoholics. *Brain Res*. 2007; 1132:20–28. [PubMed: 17188656]
 37. Tanic N, Perovic M, Mladenovic A, Ruzdijic S, Kanazir S. Effects of aging, dietary restriction and glucocorticoid treatment on housekeeping gene expression in rat cortex and hippocampus - Evaluation by real time RT-PCR. *J Mol Neurosci*. 2007; 32:38–46. [PubMed: 17873286]
 38. Derks NM, Muller M, Gaszner B, Tilburg-Ouwens DTWM, Roubos EW, Kozicz LT. Housekeeping genes revisited: Different expressions depending on gender, brain area and stressor. *Neuroscience*. 2008; 156:305–309. [PubMed: 18722514]
 39. Huggett J, Dheda K, Bustin S, Zumla A. Real-time RT-PCR normalisation; strategies and considerations. *Genes Immunity*. 2005; 6:279–284. [PubMed: 15815687]
 40. Silberstein G, Baruch K, Navon R. Detection of stable reference genes for real-time PCR analysis in schizophrenia and bipolar disorder. *Anal Biochem*. 2009; 391:91–97. [PubMed: 19464249]
 41. Tunbridge EM, Eastwood SL, Harrison PJ. Changed relative to what? Housekeeping genes and normalization strategies in human brain gene expression studies. in review.
 42. Burnet PWJ, Eastwood SL, Bristow GC, Godlewska BR, Sikka P, Walker M, Harrison PJ. D-amino acid oxidase activity and expression are increased in schizophrenia. *Mol Psychiatry*. 2008; 13:658–660. [PubMed: 18560437]
 43. 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]

Figure 1. Relationships between brain weight and (A) 2M mRNA in cerebellum and (B) TBP mRNA in hippocampus. Open circles: control subjects. Filled squares: other subjects. Solid line: regression line for whole sample. Dashed line: regression line for control group.

Table 1
Demographic details of SMC brain series

	Whole series (n=104)	Controls (n=35)	Schizophrenia (n=35)	Bipolar disorder (n=34)
Sex (M:F)	68:36	26:9	26:9	16:18
Age (years)	44.0±9.0	44.2±7.6	42.6±8.5	45.4±10.7
Fresh brain weight (g)	1427±134	1444±148	1442±107	1395±142
Side of brain studied (L:R)	52:52	16:19	17:18	19:15
Brain pH	6.50±0.28	6.61±0.27	6.47±0.24	6.43±0.30
RIN for cerebellar mRNA	6.26±1.73	6.15±2.02	6.43±1.45	6.21±1.72
RIN for hippocampal mRNA	4.80±1.27	5.03±1.13	4.68±1.36	4.68±1.33
RIN for cingulate mRNA	4.81±1.42	4.72±1.56	5.07±1.52	4.89±1.82
Autopsy delay (hours)	32.8±16.1	29.4±12.9	31.4±15.5	37.9±18.6
Storage time (months)	86.6±23.2	81±18	87±23	93±26

Values are mean±S.D.

RIN: RNA integrity number; higher numbers indicate more intact RNA.

Table 2
Correlations of housekeeping gene mRNAs with brain weight

	Spearman correlation with brain weight		Partial correlation with brain weight	
	In whole sample	In controls	Controlling for age	Controlling for sex
<i>Cerebellum</i>				
2M	+0.241 **	+0.354 *	+0.258 **	+0.276 ***
GAPDH	+0.126	ND	ND	ND
GUSB	+0.061	ND	ND	ND
TFRC ^a	-0.295 ***	-0.385 *	ND	ND
Geometric mean	+0.204 *	ND	ND	ND
<i>Hippocampus</i>				
GAPDH	+0.274 ***	+0.392 *	+0.115	+0.090
TBP	+0.320 ***	+0.481 **	+0.324 ***	+0.293 **
TFRC	+0.163	ND	ND	ND
Geometric mean	+0.306 **	+0.423 **	+0.284 **	+0.271 **
<i>Anterior cingulate cortex</i>				
2M	-0.019	ND	ND	ND
GAPDH	+0.132	ND	ND	ND
GUSB	-0.036	ND	ND	ND
TFRC	-0.004	ND	ND	ND
Geometric mean	+0.055	ND	ND	ND

*
p<0.05

**
p<0.02

p<0.01. ND: not determined, since one or both Spearman correlations failed to meet criteria for significance (see text).

^a Inspection of data showed correlation was driven by one control subject, who had a TFRC mRNA value >3.S.D. above mean; omission of the subject abolished the correlations (both $r<0.1$, $p>0.6$).