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Different changes in cortical Tumour Necrosis Factor α -related pathways in schizophrenia and mood disorders.

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Abstract

The growing body of evidence implicating tumour necrosis factor α (TNF α) in the pathophysiology of psychiatric disorders led us to measure levels of that protein in the cortex of subjects with major depressive disorders (MDD). Having reported an increase (458%) in the levels of the transmembrane (tmTNF α), but not the soluble (sTNF α), form of the protein in Brodmann's area 46, but not 24, from people with disorder we decided to examine additional components of TNF α -related pathways in the same regions from people with MDD and extend our studies to the same cortical regions of people with schizophrenia (Sz) and bipolar disorders (BD). Using postmortem tissue, Western blots and quantitative polymerase chain reaction we have now shown there is a significant increase (305%) in tmTNF α in Brodmann's area 24, but not 46, from subjects with BD and that levels of the protein were not altered in Sz. Levels of sTNF α were not altered in BD or Sz. In addition we have shown that levels of TNF receptor 1 (TNFR1) mRNA are increased in BA 24 (53%) and BA 46 (82%) from people with Sz whereas levels of TNFR2 mRNA was decreased in BA 46 from people with mood disorders (MDD = -51%; BD = -67%). Levels of proteins frequently used as surrogate markers of neuronal, astrocytic and microglia numbers as well as levels of the pro-inflammatory marker (interleukin 1 β) were not changed in the cortex of people with mood disorders. Our data suggest there are differential changes in TNF α -related markers in the cortex of people with MDD, BD and Sz that may not be related to classical inflammation and may cause changes in different TNF α -related signaling pathways.

Keywords

Tumour necrosis factor α cortex schizophrenia bipolar disorder
major depressive disorders

Introduction

A growing body of evidence implicates inflammatory-related pathways in the pathophysiology of psychiatric disorders¹. Importantly, most of these data comes from the study of cytokines in blood with one of the most consistent findings being that levels of tumour necrosis factor α (TNF α) are increased in people with psychiatric disorders²⁻²¹. Despite this evidence suggesting a role for TNF α in the pathophysiology of psychiatric disorders no studies on that protein had been performed in the central nervous system (CNS) of individuals with these disorders. This led us to measure levels of TNF α in the cortex of subjects with major depressive disorders (MDD)²². We commenced our studies in MDD because this was the disorder where increased peripheral TNF α was most consistently reported. We measured levels of TNF α in the dorsolateral prefrontal cortex (Brodmann's area 46 (BA46)) and anterior cingulate (BA24). We chose to make our measurements in BA46 because it is important in maintaining cognitive function²³ and BA 24 because of its involvement in the control of mood²⁴; behaviors that have both been shown to be aberrant in MDD²⁵. Our data showed there was a 458% increase in the levels of the transmembrane (tmTNF α), but not soluble (sTNF α), form of TNF α in BA46, but not BA24, from subjects with MDD²².

Much is known about the processing of TNF α , allowing interpretation of our data in MDD. Human TNF α is encoded by a single gene on Chromosome 6 (6p21.3) and TNF α is initially synthesized as a monomeric type-2 transmembrane protein that forms a homotrimer, known as tmTNF α ²⁶. tmTNF α has a long leader sequence that forms an intracellular domain of 30 amino-acids, a transmembrane domain of 26 amino-acids and a 20 amino-acid extracellular

domain²⁷. tmTNF α can undergo cleavage by a number of metalloproteases, the predominant being TNF α converting enzyme (ADAM metalloproteinase domain 17: ADAM17), that gives rise to sTNF α ²⁶ which exists in the extracellular milieu and can have paracrine effects. The complexity of tmTNF α signaling is highlighted by the fact that ADAM17 and other metalloproteases can cleave both TNFR1 and TNFR2 to give soluble forms of both receptors²⁸. This means that TNF α signaling can occur by three mechanisms which are i) sTNF activating TNFR1 or TNFR2 giving forward signaling, ii) sTNFR1 or sTNFR2 activating tmTNF giving reverse signaling or iii) tmTNF interacting with TNFR1 or TNFR2 to activate both forward and reverse signaling¹. The physiological potencies of these interactions are governed by the fact that sTNF binds with high affinity to TNFR1 whilst tmTNF α binds with higher affinity to TNFR2²⁸. These data suggest that sTNF predominantly signals via TNFR1 whilst tmTNF and sTNFR2 interactions would be more potent in activating reverse signaling. Finally, interactions between tmTNF α and TNFR2 are likely to be the most potent combination in activating forward + reverse signaling.

Our data is significant in that it suggests that tmTNF α is altered in the cortex of subjects with MDD, rather than sTNF α which is the form of TNF α measured in blood. Given the role of tmTNF α it would seem reasonable to postulate that increased levels of this protein would be affecting levels of tmTNF α signaling in BA46, but not BA24, from people with MDD. The notion that changes in tmTNF α are involved in the pathophysiology of MDD is given credence by the fact that tmTNF α is a pharmacological site²⁹ and drugs that target tmTNF have antidepressant properties^{30,31}. Thus, following the concept that a good understanding of the underlying pathophysiology of a psychiatric disease will aid in developing better therapeutic agents to treat psychiatric disorders³², we decided to determine whether other critical

components of the TNF-related pathways were altered in BA46 and BA24 and whether such changes extended to the same CNS regions in bipolar disorder (BD) and schizophrenia (Sz). Moreover, the end point of chronic inflammatory processes in the CNS would be an increase in cell death³³ following an increase in microglia / macrophage activation³⁴. Thus, we decided to measure levels of well-established protein markers that have been shown to vary with changes in pre-³⁵ and post-³⁶ synaptic density (synaptophysin and PSD95 respectively), astrocyte number (GFAP)³⁷ and microglia / macrophage number (CD11b)³⁸ in the cortex of mood disorders to begin to determine if changes in TNF α -related pathways were reflecting a pro-inflammatory activity. Following on this theme we also measured levels of interleukin 1 β (IL1B) in the cortex of subjects with mood disorders as this protein and TNF α are increased in unison as part of increased classical pro-inflammatory activity³⁹.

Materials / Subjects and Methods

Tissue Collection

Permission to collect human CNS tissue was obtained from the Ethics Committee of the Victorian Institute of Forensic Medicine and was collected after obtaining permission from the closest surviving next of kin. Permission to complete specific studies was obtained from the Mental Health Research and Ethics Committee of Melbourne Health. All human tissue was sourced through the Victorian Brain Bank Network, Mental Health Research Institute, Parkville, Australia.

CNS left hemispheres were collected from people who had been seen alive up to 5 hr. prior to being found dead and where cadavers had been refrigerated within 5 hr. of discovery; refrigeration of cadavers would act to slow CNS autolysis⁴⁰ and thus reduce the impact of PMI. For each case, post-mortem interval (PMI) was calculated as the time between witnessed death and autopsy or the midpoint between the subject being found and being last seen alive and autopsy. To further optimize CNS preservation, each hemisphere was processed in a standardised manner to ensure tissue was frozen to -70°C within 30 min of autopsy⁴¹. To gain a measure of autolytic damage, CNS pH was measured as described previously⁴² as this is now recognised as a giving a better indication as to the quality of tissue preservation, which is often not related simply to PMI⁴³. RNA integrity numbers (RINs) were obtained to provide an assessment of mRNA integrity⁴³.

Following tissue collection and processing an extensive case history review was completed, including a pharmacological audit. On completion of the case history review a consensus DSM-IV based diagnosis was agreed by two senior psychiatrists and the psychologist completing the case history review^{44,45}. The duration of illness (DOI) in years was also calculated. In addition, the final recorded antipsychotic, anticholinergic, antidepressant and benzodiazepine drug doses were converted to standardised units according to principals outlined previously⁴⁶.

Diagnostic Cohorts

Studies were completed using tissue from 10 people with MDD, 10 people with BD, 19 people with Sz and 30 age and sex matched control subjects (Summary: Table 1; Full details:

Supplementary Table 1). Due to issues relating to tissue availability studies were completed using three control groups; the one for people with Sz consisting of 20 subjects (Cont. Group A: Table 1 and Supplementary Table 1). For the initial studies of TNF-related measures, results were compared to a sub-group of 10 individuals from Group A (Cont. Group B). Due to shortage of tissue in some controls the studies on cellular markers were completed on a new cohort of 10 control subjects Cont. Group C matched by age / sex to both Cont. Group B (Table 1) and the psychiatric cohorts.

Western blot analyses

Levels of sTNF α and tmTNF α were measured by Western blot analyses as described previously²² and the levels of other proteins were measured using methodology based on the approaches we have used extensively for other proteins⁴⁷⁻⁴⁹, but optimized for the measurement of each protein of interest (Supplementary Table 2). Importantly, the person completing the analyses was blind to diagnoses but the coding of the samples ensured that a case and their matched control were on the same gel. Two images were captured (light mode for the MW standards and chemiluminescent for the immunogenic bands) and merged to allow the estimation of the molecular weight of each antigenic band. The densities of the immunogenic bands were measured on the chemiluminescent image and expressed as a ratio of the corresponding immunogenic band in the internal control to accommodate gel-to-gel variation. As we have shown previously^{48,49}, this approach also allows comparisons of levels of proteins within and across CNS regions.

RNA purification and first-strand cDNA synthesis

Total RNA was isolated from 100 mg frozen cortical samples with 1.0 ml TRIzol[®] reagent (Invitrogen, Australia) for human tissue and QIAGEN RNA extraction kit for mouse tissue, according to the manufacturer's instructions. Following TRIzol extraction, the RNA was treated with 0.1 U/ μ L DNase in 1X DNase buffer (Invitrogen, Australia) to remove genomic DNA contamination, extracted using phenol (pH 4.2): chloroform extraction and ethanol precipitated. cDNA was synthesized as described previously⁵⁰.

Real-time PCR assay

cDNA was used as a template for real-time PCR performed with SYBR green detection using a Bio-Rad iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories). Reactions were performed in triplicate as previously described⁴⁹. Relative quantities were calculated from Ct values using the amplification efficiency for each gene. Efficiencies of each primer set were established across four 10-fold serial dilutions of amplified target sequence, spanning the Ct range of the target gene in the cDNA. Levels of mRNA for each gene of interest were expressed as the geometric mean of three reference genes⁵¹. Genevestigator ([www.genevestigator .com](http://www.genevestigator.com)) was used to initially select five stably expressed reference genes with similar expression levels to the target genes. mRNA levels of the five most stably expressed genes identified were measured in five subjects with BPD, five subjects with MDD, five subjects with schizophrenia and five controls using qPCR to assess the stability of reference gene expression within and between diagnosis. Expression level stability was then analysed using geNorm (Biogazelle, Zwijnaarde,

Belgium). The three most stably expressed genes, SKP1A ($M = 0.054$), NOL9 ($M = 0.54$) and TFB1M ($M = 0.057$), were selected as reference genes for further analysis, where the gene stability measures (M) < 1.5 were deemed suitable for use as reference genes⁵². Primers were designed using Beacon design software (Premier Biosoft International, Palo Alto, CA, USA: Supplementary Table 3) and amplicons were confirmed by sequencing prior to experimentation.

Statistical Analyses

Donor demographic, drug and CNS collection data were compared between cohorts using either one-way ANOVA followed by a post hoc Bonferroni test comparing data across cohorts (multiple cohorts) or student t-tests (2 cohorts). The sex ratios in each cohort were compared using a Fisher's exact test.

All experimental data was analysed using the D'Agostino & Pearson omnibus normality test to determine the distribution of data as this is the best way of determining data distribution in small cohorts⁵³. If data was normally distributed, outlying data was first identified using Grubb's test⁵⁴ (maximal allowable 1 per measurement) and any outlying data point was removed from further analyses. For our protein data, the use of the same IC across regions made results between regions comparable. Thus, normally distributed data was analysed with two-way ANOVA with CNS region and diagnoses as variants followed by a post hoc Dunnet's comparison to control data to identify any source of variance. As our qPCR data could not be compared across regions a one-way ANOVA followed by a post hoc Dunnet's comparison to control data was used to identify sources of variance within each region. Where the distribution of data in different

diagnostic cohorts showed mixed variance data was analysed using the General Linear Model with Bonferroni *post hoc* testing as this is a robust approach to analysing such data⁵⁵. Where data was shown to be uniformly non-parametric the appropriate non-parametric analyses was used.

Relationships between demographic, brain collection and pharmacological data from each donor and experimental data were integrated using a linear regression assuming a straight line fit. Where strong significant relationships⁵⁶ were revealed comparison of experimental data across diagnoses was re-assessed using an analysis of covariance with the identified variables as cofactors.

Results and Discussion

The complete analyses of variation in protein levels between cortical regions is given in Supplementary Data 1.

Analyses of patient demographics and tissue collection parameters

There were no significant variation in mean age, sex ratio, PMI, CNS pH, or RIN between subjects with Sz and their controls (Table 1: Cont. Group A). For studies on TNF-related measures involving Cont. Cohort B, there was no significant variation in mean age, gender ratio PMI or RIN across the diagnostic cohorts but there was significant variation in CNS pH (Table 1). This increased variation in CNS pH was due to higher values for MDD compared to controls ($p < 0.05$). For studies on cellular markers and IL-1 β , using Cont. Cohort C, there was no

significant variation in mean age, sex ratio, PMI or RIN between diagnostic cohorts but there was significant variation in CNS pH (Table 1). This increased variation in CNS pH was again due to higher values for MDD compared to controls ($p < 0.05$).

TNF α -related measures

Levels of tmTNF α were increased (158%; $p < 0.05$) in BA 24, but not BA 46, from subjects with BD and were not different in either cortical regions from people with Sz (Figure 1). By contrast, levels of sTNF α did not vary significantly with diagnosis ($p = 0.38$; Table 2) in cortex from subjects with mood disorders. There was a trend to a significant variation in data with diagnosis ($p = 0.06$; Figure 2A) in people with Sz and this trend sTNF appeared to have some validity because a power analyses (<http://statpages.org/postpowr.html>) suggested significance would have been obtained if diagnostic cohorts had consisted of 22 individuals in both diagnostic cohorts rather than the 19 people with Sz and the 19 controls used in this study.

Levels of TNF α mRNA did not vary with diagnosis in either BA 24 or BA 46 from people with mood disorders (Table 2) or Sz (BA24: $F = 3.06$, d.f. = 1,38, $p = 0.09$; Sz = 0.77 ± 0.21 vs. control = 0.41 ± 0.04 ; BA 46: $F = 0.58$, d.f. = 1,38, $p = 0.45$; Sz = 0.41 ± 0.15 vs. controls = 0.42 ± 0.15). Levels of TNFR1 mRNA did not vary with diagnosis in cortex from subjects with mood disorders (Table 2). By contrast, level of TNFR1 mRNA was significantly increased in both BA 24 (53%; $p = 0.03$) and BA 46 (82%, $p < 0.001$) from people with Sz (Figure 2B). There was significant variation in levels of TNFR2 mRNA with diagnoses in BA 46, but not BA 24, from

people with mood disorders due to decreased levels of mRNA in the cortex of people with BD (79%; $p < 0.001$) and MDD (50%, $p < 0.001$)(Figure 3).

Measures of Cellular Markers and Inflammatory Pathways

Despite optimising Western blot conditions to detect IL1B we were unable to detect that protein in any cortical sample from people with mood disorders. By contrast, we were able to visualise IL1B in a Western blot using cortical tissue from a subjects with meningitis. We were able to detect the IL1B precursor protein, pro-IL1B⁵⁷, in all cases.

Levels of synaptophysin, PSD95, GFAP43, GFAP41, CD11b or pro-IL1B did not vary with diagnoses in cortex from people with mood disorders (Table 2).

Potential Confounding Factors

In the overall analyses (independent of diagnoses) there were no strong correlations between any experimental factor and any confounding factors. There were few strong significant correlations between experimental measurements and potential confounding factors within diagnosis (Supplementary Table 4). There were strong significant correlations between levels of tmTNF α in BA 24 and PMI in control subjects ($r^2 = 0.56$; $p < 0.01$), TNF α mRNA and age ($r^2 = 0.50$; $p < 0.05$) in BA46 from MDD, TNFR1 mRNA and age in BA 24 ($r^2 = 0.63$; $p < 0.01$) in MDD, TNFR2 mRNA and brain pH in BA 24 ($r^2 = 0.54$; $p = 0.02$) and BA 46 ($r^2 = 0.73$; $p < 0.01$) in

controls, synaptophysin with DOI in BA24 ($r^2 = 0.66$; $p = <0.01$) in bipolar disorder and brain pH ($r^2 = 0.52$; $p = 0.02$) in controls as well as GFAP41 with PMI in BA 46 from controls ($r^2 = 0.59$, $p < 0.01$). Original analyses of data were reassessed with each of these potential confounds as covariates.

A re-analysis of the $\text{tmTNF}\alpha$ and TNFR2 mRNA in mood disorders with PMI and brain pH as covariates showed significant variance remained with diagnoses ($F = 9.0$, d.f. = 1,1,36, $p = 0.005$ and $F = 8.2$, d.f. = 1,2,53, $p = 0.001$). In comparing TNF α mRNA and TNFR1 mRNA with age as a covariant, the effect of diagnoses remained non-significant for both measures ($F = 9.7$, 1,2,53, $p = 0.19$ and $F = 1.2$, 1,2,53, $p = 0.33$). Similarly, in the mood disorders, analyses of synaptophysin and GFAP41 with brain pH and PMI, respectively, as a covariant showed the effect of diagnoses remained non-significant ($F = 0.81$, 1,2,53, $p = 0.45$, $F = 0.23$, 1,2,53, $p = 0.79$). Finally, comparing levels of synaptophysin in mood disorders with DOI as a covariant showed there was still no significant variance with diagnoses ($F = 0.29$, d.f. = 1,1,34, $p = 0.58$). These data suggest that the impact of the potential confounds identified in this study are not of sufficient strength to alter the interpretation of the comparison of experimental data across diagnosis.

Added to our earlier finding²², our current data suggests there are changes in TNF α -related pathways in mood disorders and Sz. We found increased TNFR1 mRNA in the cortex of Sz, a result that was not expected and does not appear to have been reported elsewhere. As the only clearly significant change in TNF α -related markers in the cortex of people with Sz our data suggests further studies on TNFR1 signaling in Sz would be worthwhile. Importantly, TNFR1

recruits a number of intracellular adaptor proteins to facilitate signaling and activation of TNFR1 signaling can have a number of disparate outcomes such as changes in gene expression and / or apoptosis⁵⁸. One of the proteins recruited by TNFR1, TNF receptor-associated factor 2, has been suggested to be important in the actions of DISC1⁵⁹ which is a gene implicated in changing the risk for developing Sz⁶⁰. These data show that TNF signaling through TNFR1 can affect multiple pathways, some of which seem to be particularly relevant to Sz, which suggests a better understanding of the changes in TNFR1-related pathways in the cortex of subjects with the disorder will add to our knowledge of its pathophysiology.

Our data suggests there are complex changes in TNF α -related pathways in the cortex of people with mood disorders. However, in addition to failing to identify changes in sTNF α in the cortex of people with mood disorders, IL1B levels were too low to detect and levels of pro-IL1B did not change with diagnoses. Importantly, sTNF and IL1B have been shown to increase with increases in pro-inflammatory activity^{39,61}. Similarly, our data showing that CD11b (surrogate microglia / macrophages markers) as well as GFAP41 and 43 (surrogate astrocytic markers) were not increased in the cortex of people with mood disorders is also not consistent with what would be expected in tissue in which there as ongoing pro-inflammatory processes^{61,62}. Hence our data argues that changes in TNF α -related pathways in mood disorders have not resulted from increased pro-inflammatory activity. This argument is further supported by the absence of change in markers of neuronal integrity (synaptophysin and PSD95: surrogate markers of neuronal connections) which would be expected to be decreased after chronic inflammation³³.

As with all early studies involving a low number of cases, caution needs to be applied to over-interpretation. However, the changes we report are large (Mood disorders: increase in tmTNF α BA24 BD = 158%, decrease in TNFR2 mRNA BA46 = -67% BD and -51% MDD) and it is therefore unlikely these are simply a function of low diagnostic cohorts especially as a retrospective power analyses showed that the adequate cohort sizes, given the differences in our data with diagnoses, would be 10 for tmTNF α and three for both measures of TNFR2.

In interpreting our data on TNF α -related pathways in the cortex of people with psychiatric disorders it is important that current data suggest that TNF α signaling would predominantly involve sTNF α -TNFR1 and tmTNF α -TNFR2 interactions²⁶. Our current and previous data²² would argue that alterations in tmTNF α -TNFR2 pathways are present in mood disorders whereas it is sTNF α -TNFR1 signaling pathways that are altered in Sz. This delineation is important because i) the TNF gene is predominantly expressed by astrocytes⁶³ and microglia⁶⁴ and hence tmTNF is on these cells, ii) TNFR2 is predominantly expressed by microglia and neurons²⁶ and iii) TNFR1 is thought to ubiquitously expressed by all cells^{26,65}. Therefore the changes in TNF-related pathways in mood disorders should have more restricted outcomes compared with those in Sz. In addition, paracrine signaling through sTNF α appears to be generally linked to pathological process whereas tmTNF signaling appears to be linked more to “therapeutic” responses⁵⁸. Hence, our data argues that changes in TNF α -related pathways in the cortex of people with mood disorders may be a response that is designed to oppose the pathophysiological process associated with MDD and BD, whereas the changes in Sz may be directly contributing to the pathophysiology of that disorder.

We are not aware of any previous studies that have focus on TNF α -related pathways in the cortex of subjects with psychiatric disorders, particularly in people with mood disorders. Our data from people with mood disorders showing increased levels of tmTNF in BA 24 from people with BD and BA 46 from people with MDD open up the possibility of increased reverse signaling^{1,58} by that protein in these cortical regions. However, the changes in TNFR2 mRNA in BA 46 from subjects with MDD and BD could be indicating a change in bi-directional signaling¹ in that region. This could certainly be the case in BA46 from people with MDD where levels of tmTNF and TNFR2 mRNA are both changed. Given that the dorsolateral prefrontal cortex (BA 46) is known to be critical in maintaining cognitive processes²³ it would not be unreasonable to posit that changes in TNF α -related pathways could be involved in the changes in cognition that are known to occur in MDD²⁵ and BD⁶⁶. Similarly, the anterior cingulate (BA 24) is important in controlling mood²⁴ and therefore changes in TNF α -related pathways could be involved in changes in mood that particularly affects people with BD⁶⁷. Such hypotheses gains support from the finding that transgenic mouse that over-expresses TNF α have learning deficits⁶⁸.

Particularly important to our data is the finding that drugs that target tmTNF have mild antidepressant activity^{30,31}. Therefore it would seem likely that a better understanding of changes in tmTNF α and TNF α -related pathways in the CNS from subjects with mood disorders would cast light on why drugs that target tmTNF have antidepressant activity. More importantly, a better understanding of how TNF α -related pathways are affected in the CNS of people with mood disorders could suggest better targets in the pathway for more effective intervention which should lead to drugs of greater therapeutic efficacy and, given the problem of treatment resistant depression⁶⁹, therapeutic reach.

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Conflicts of Interest

The authors have no conflict of interest.

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Figure Legends

Figure 1: Levels (mean $\pm$ SEM) of tmTNF α in Brodmann's area 24 and 46 from people with bipolar disorders, schizophrenia and age / sex matched controls. In the case of bipolar disorders, levels of tmTNF α showed significant variation with diagnosis ($F = 6.51$, d.f. = 1,1,36, $p = 0.01$). Post hoc analyses showed that the variation in levels of tmTNF α with diagnosis was due to increased levels of the protein in BA 24, but not BA 46, from subjects with BD. For schizophrenia, levels of tmTNF α did not vary with diagnosis ($F = 0.00$, d.f. = 1,1,75, $p = 0.96$).

a: $p < 0.05$.

Figure 2A: Levels (mean $\pm$ SEM) of sTNF α in Brodmann's area 24 and 46 from people with schizophrenia and age / sex matched controls. There was significant variation in the levels of sTNF between regions ($F = 4.74$, d.f. = 1,1,75, $p = 0.03$) whilst variation with diagnosis approached significance ($F = 3.70$, d.f. = 1,1,75, $p = 0.06$) comparing Sz to controls.

2B: Levels (mean $\pm$ SEM) of sTNFR1 mRNA in Brodmann's area 24 and 46 from people with schizophrenia and age / sex matched controls. Level of TNFR1 mRNA was significantly increased in both BA 24 ($F = 5.12$, d.f. = 1,38, $p = 0.03$) and BA 46 ($F = 23.5$, d.f. = 1,38, $p < 0.001$) from people with Sz.

a: $p < 0.05$; e: $p < 0.0001$.

Figure 3: Levels (mean $\pm$ SEM) of sTNFR2 mRNA in Brodmann's area 24 and 46 from people with major depressive disorders, bipolar disorders, schizophrenia and age / sex matched controls. There was significant variation in levels of TNFR2 mRNA with diagnoses in BA 46 from

people with mood disorders ($F = 12.0$, d.f. = 2,29, $p = 0.00$) due to decreased levels of mRNA in the cortex of people with BD ($p < 0.001$) and MDD ($p < 0.001$). By contrast, levels of TNFR2 mRNA did not vary with diagnosis in BA 24 from subjects with mood disorders ($F = 0.43$, d.f. = 2,29, $p = 0.65$) or in either region from people with Sz (BA 24: $F = 0.51$, d.f. = 1,38, $p = 0.51$; BA 46: $F = 0.26$, d.f. = 2,29, $p = 0.60$).

d: $p < 0.001$; e: $p < 0.0001$.

Table 1: Summary of demographic, mode of death and brain collection and preservation data (mean $\pm$ SEM) relating to donors of tissue used in the study of tumor necrosis factor 1- α – related pathways in the pathophysiology of psychiatric disorders. Full details see Supplementary Table 1.

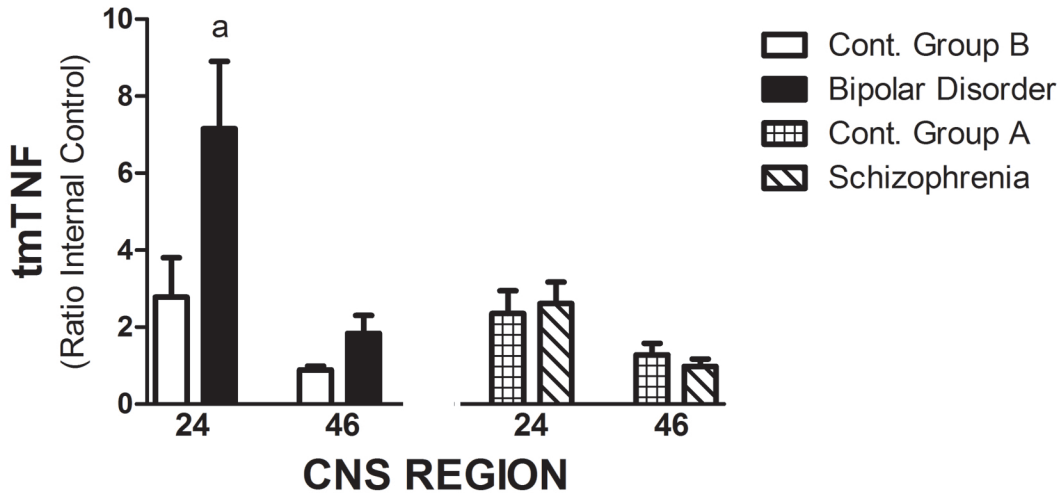
Diagnosis	Age yr.	Sex M / F	PMI hr.	pH	RIN BA24	RIN BA 46	DOI yr.	Suicide* Yes / No
Major Depressive Disorders	61 $\pm$ 5.4	6 / 4	39 $\pm$ 5.0	6.58 $\pm$ 0.06	7.23 $\pm$ 0.24	6.98 $\pm$ 0.23	15 $\pm$ 2.4	8 / 2
Bipolar Disorder	60 $\pm$ 4.0	6 / 4	31 $\pm$ 4.5	6.35 $\pm$ 0.06	6.87 $\pm$ 0.34	6.69 $\pm$ 0.57	19 $\pm$ 4.2	4 / 6
Schizophrenia	48 $\pm$ 4.4	15 / 4	41 $\pm$ 3.6	6.33 $\pm$ 0.04	7.31 $\pm$ 0.23	7.10 $\pm$ 0.24	21 $\pm$ 3.8	8 / 11
Cont. Group A	47 $\pm$ 4.1	16 / 4	45 $\pm$ 3.0	6.36 $\pm$ 0.04	7.40 $\pm$ 0.20	7.39 $\pm$ 0.18		
Cont. Group B	56 $\pm$ 4.8	7 / 3	48 $\pm$ 4.7	6.37 $\pm$ 0.05	7.63 $\pm$ 0.21	7.40 $\pm$ 0.18		
Cont. Group C	63 $\pm$ 4.1	6 / 4	48 $\pm$ 5.4	6.32 $\pm$ 0.08	6.40 $\pm$ 0.42	6.51 $\pm$ 0.36		
Comparison Parameters for Group B and Group C	p = 0.32	0.99	0.97	0.60	0.02	0.04		
Comparison Group A to Sz	p = 0.88	0.99	0.43	0.64	0.77	0.31		
Comparison Group B to Mood Disorders	F = 0.34 d.f. = 2,27 p = 0.72		3.20 2,27 0.06	5.69 2,27 < 0.01	2.03 2,27 0.15	0.94 2,27 0.40		
Comparison Group C to Mood Disorders	F = 0.09 d.f. = 2,27 p = 0.91	1.00	2.80 2,27 0.08	4.83 2,27 0.02	1.52 2,27 0.24	0.34 2,27 0.72		

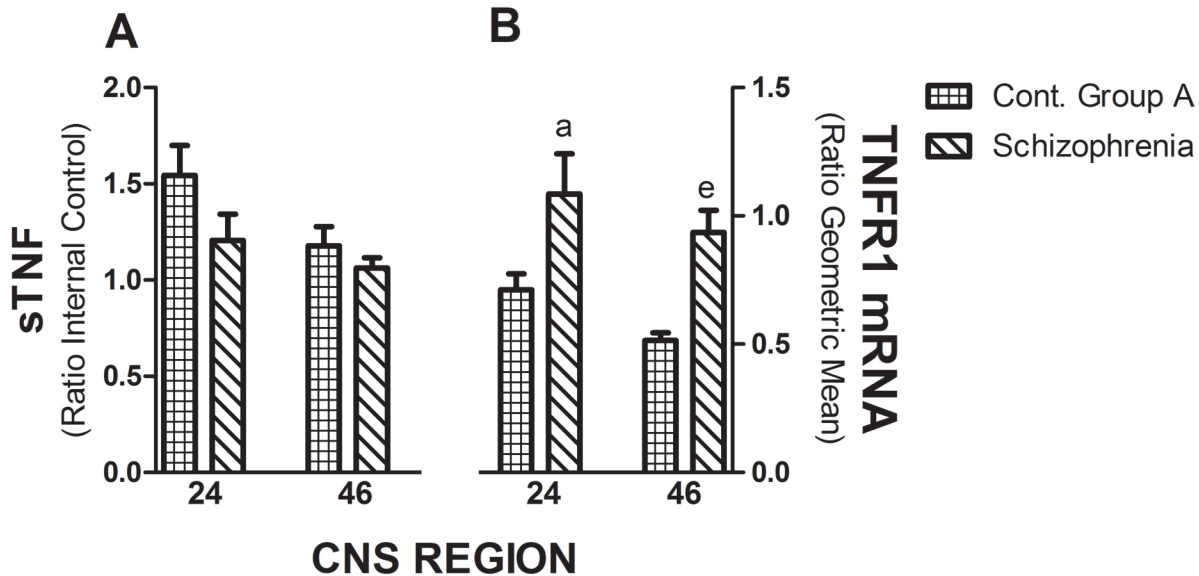
Abbreviations: BA = Brodmann's area, DOI = duration of illness, PMI = postmortem interval, RIN = RNA integrity number.

*Death by suicide was taken from the Coroner's verdict.

Table 2: Levels (mean $\pm$ SEM) of soluble tumor necrosis factor (sTNF α), synaptophysin, postsynaptic density protein 95, glial fibrillary acidic protein 43 and 41, CD11b and the IL1B precursor (pro-ILB), as well as mRNA for TNF and tumor necrosis factor receptors 1 and 2 (TNFR1 and 2), in Brodmann's area 24 and 46 from the left hemisphere of subjects with major depressive disorder (MDD) and bipolar disorder (BPD) and age / sex matched controls that did not differ significantly with diagnoses.

	sTNF		TNF mRNA		TNFR1 mRNA	
	BA 24	BA 46	BA 24	BA 46	BA 24	BA 46
Cont. Group A						
MDD	1.31 $\pm$ 0.17	1.27 $\pm$ 0.15	0.38 $\pm$ 0.05	0.32 $\pm$ 0.07	0.58 $\pm$ 0.06	0.45 $\pm$ 0.02
BPD	1.49 $\pm$ 0.26	1.44 $\pm$ 0.17	0.19 $\pm$ 0.06	0.62 $\pm$ 0.14	1.07 $\pm$ 0.23	0.69 $\pm$ 0.17
F	0.80		0.44		2.26	
d.f.	1,1,36		2,29		2,29	
p	p = 0.38		0.65		0.12	
Cont. Group B	Synaptophysin		PSD95		GFAP 43	
	BA 24	BA 46	BA 24	BA 46	BA 24	BA 46
	1.51 $\pm$ 0.08	1.33 $\pm$ 0.11	1.24 $\pm$ 0.23	1.31 $\pm$ 0.22	1.06 $\pm$ 0.16	0.64 $\pm$ 0.12
	1.50 $\pm$ 0.11	1.67 $\pm$ 0.09	1.30 $\pm$ 0.25	1.63 $\pm$ 0.22	0.99 $\pm$ 0.10	0.63 $\pm$ 0.11
MDD						
BPD	1.30 $\pm$ 0.11	1.69 $\pm$ 0.22	1.29 $\pm$ 0.29	1.65 $\pm$ 0.29	0.91 $\pm$ 0.19	0.63 $\pm$ 0.11
F	0.82		0.37		0.18	
d.f.	1,2,54		1,2,54		1,2,54	
p	0.44		0.68		0.84	
Cont. Group B	GFAP 41		CD11b		Pro-IL1B	
	BA 24	BA 46	BA 24	BA 46	BA 24	BA 46
	1.06 $\pm$ 0.10	0.84 $\pm$ 1.07	0.84 $\pm$ 0.15	0.70 $\pm$ 0.17	0.21 $\pm$ 0.07	0.46 $\pm$ 0.11
	1.18 $\pm$ 0.10	0.70 $\pm$ 0.11	0.61 $\pm$ 0.12	0.57 $\pm$ 0.10	0.29 $\pm$ 0.13	0.56 $\pm$ 0.10
MDD						
BPD	1.01 $\pm$ 0.16	1.35 $\pm$ 0.62	0.78 $\pm$ 0.11	0.46 $\pm$ 0.12	0.17 $\pm$ 0.05	0.55 $\pm$ 0.12
F	0.48		1.13		0.42	
d.f.	1,2,54		1,2,54		1,2,54	
p	0.62		0.33		0.66	





TNFR2 mRNA

(Ratio Geometric Mean)

1.5
1.0
0.5
0.0

24

46

CNS REGION



Cont. Group B



Bipolar Disorder



Major Depressive Disorder



Cont. Group A



Schizophrenia

24

46

e

d