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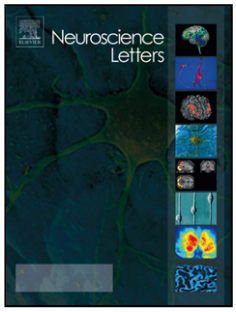

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Reduced interleukin 1A gene expression in the dorsolateral prefrontal cortex of individuals with PTSD and depression

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Highlights

- Alterations in inflammatory markers have been associated with PTSD and depression.
- These changes have not been extensively explored in human postmortem brain tissue.
- This study examined interleukin gene expression in postmortem dlPFC samples.
- IL1A gene expression was decreased in the dlPFC for PTSD and depressed cases.

Abstract

The inflammatory system has been implicated in the pathophysiology of a variety of psychiatric conditions. Individuals with PTSD, depression, and other fear- and anxiety-related disorders exhibit alterations in peripheral circulating inflammatory markers, suggesting dysregulation of the inflammatory system. The relationship between inflammation and PTSD has been investigated almost exclusively in the periphery, and has not been extensively explored in human postmortem brain tissue. Interleukins (ILs) represent a subtype of cytokines and are key signaling proteins in the immune and inflammatory systems. Based on prior research implicating IL signaling in PTSD and depression, we performed a preliminary investigation of IL gene expression in a region of the cortex involved in emotion regulation and PTSD, the dorsolateral prefrontal cortex (dlPFC), using tissue from the newly established VA National PTSD Brain Bank. Gene expression analyses were conducted on post-mortem tissue from the dlPFC from 50 donors: 13 controls, 12 PTSD cases, and 25 depressed cases. RNA was extracted from frozen dlPFC tissue, reverse transcribed to cDNA, and quantitative polymerase chain reaction (qPCR) was performed to assess gene expression of *IL1A, IL1B, IL6, IL8, IL10, IL13*, and *IL15*. We found a multiple-testing corrected significant decrease in *IL1A* expression in the dlPFC for PTSD and depression cases compared to controls (p<0.005) with age at death, sex, race and RNA integrity number (RIN) included as

covariates. To our knowledge this finding is the first demonstration of altered IL expression in brain tissue from deceased individuals with histories of PTSD and/or depression.

Keywords: PTSD; depression; prefrontal cortex; postmortem; interleukin; IL1A; gene expression; inflammation; neuroinflammation.

Introduction

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric disorder that occurs secondary to traumatic events. PTSD affects approximately 6.8% of the general population [1], with higher rates among combat veterans (23% of veterans of the recent conflicts in Iraq and Afghanistan; [2]) and individuals living in neighborhoods with high levels of violence (46.2% of individuals in inner city neighborhoods; [3]). Major depressive disorder (MDD) is highly comorbid with PTSD [1] with approximately 50% of newly diagnosed PTSD patients also meeting criteria for MDD [4].

Numerous studies have now shown that both PTSD and MDD are characterized by dysregulated levels of circulating inflammatory markers in the periphery [5-20]. A recent meta-analysis reported significant associations of greater circulating concentrations of the pro-inflammatory cytokines interleukin (IL) 1β (IL1B), IL6, tumor necrosis factor α (TNFa), and the acute phase reactant C-reactive protein (CRP), with trauma exposure [5]. A meta-analysis of 20 independent studies comparing individuals with PTSD to healthy controls found that peripheral levels of IL1B, IL6, TNFa, and IFNy were increased in PTSD [6]. Studies focused on individuals with major depression have found elevated peripheral cytokines including IL1B, IL6, TNFa, IFNy and CRP compared to control cases [9, 10, 21, 22]. These studies point towards an overall increased pro-inflammatory profile in MDD and PTSD, however, a number of studies have found either no differences, or evidence of reduced inflammatory markers [7, 16, 17, 23-28].

Previous gene expression studies in postmortem brain tissue have identified a number of differentially regulated targets in depressed versus control samples [29-36], including increased levels of pro- and anti-inflammatory cytokine gene expression in the prefrontal cortex (BA 10) of MDD cases [37]. To our knowledge, only three prior studies have evaluated gene expression in postmortem brain samples from individuals with PTSD, and all three focused specifically on alterations of genes related to glucocorticoid signaling [38-40]. Despite the abundance of evidence from the periphery, to our knowledge, no study has investigated inflammatory marker gene expression in postmortem brain tissue from PTSD and depression cases.

The aim of this study was to perform a preliminary investigation of the gene expression of a subset of IL genes (*ILs*) chosen based on their published altered expression levels in the periphery in PTSD and depression cases [7-14, 16-20, 41]: *IL1A*, *IL1B*, *IL2*, *IL4*, *IL6*, *IL8*, *IL10*, *IL12B*, *IL13*, *IL15*, *IL17A*. Interleukins (ILs) represent a subtype of cytokines and are key signaling proteins in the immune and inflammatory systems; at baseline, ILs play an important role in responding to injury, infection, and disease, and they are also involved in cell signaling mechanisms between diverse cell types of the brain (neurons, astrocytes, microglia) as well as immune and non-immune tissue types [42].

We investigated gene expression of these *ILs* in the dorsolateral prefrontal cortex (dlPFC), specifically Brodmann Area (BA) 9/46, of individuals with histories of PTSD and/or depression, and of controls with no history of psychopathology. The PFC is significantly involved in the executive control of emotional and behavioral processes; neuroimaging work has shown impaired function of the PFC in individuals with PTSD [43-45], which is thought to contribute to impaired emotion regulation and the persistence of traumatic memories. We chose to focus on the dlPFC for several reasons. Specifically, the dlPFC plays a role in fear extinction [46], and other studies have proposed a role for it in the conscious regulation of emotion and recovery from PTSD [45, 47-49]. The dlPFC has extensive reciprocal connections to the ventral and medial prefrontal cortex regions, which directly project to the amygdala

and play important roles in PTSD and depression [50]. A longitudinal study found that greater dlPFC thickness in individuals with PTSD was associated with greater PTSD symptom reductions and increased recovery over time [51]. Additionally, abnormalities in the function and structure of the dlPFC has been implicated in clinical neuroimaging studies of PTSD [52-54]; for example, our group previously identified a locus in an oxidative stress related gene that moderated the association between PTSD and reduced thickness of this region, suggesting compromised neural integrity in the dlPFC of individuals with PTSD who carried a specific risk allele [52]. We hypothesized that pro-inflammatory *ILs* previously implicated in PTSD and depression would be similarly upregulated in cases with PTSD and depression relative to control samples. We investigated this in a sample of 50 postmortem brain tissue samples with PTSD (with or without depression), depressed cases without PTSD, and no-psychopathology controls.

Materials and Methods

Sample Procedures and Measures

Table 1 provides the clinical and demographic characteristics of the cohort. Tissue acquisition, processing, diagnostic assessment protocols, ethical considerations, governance and oversight of the VA National PTSD Brain Bank was described previously by Friedman et al. [55], and are described below.

Sample Acquisition, Clinical Assessment and Neuropathological Examination.

The 50 samples examined in this study were obtained from the Lieber Institute for Brain Development (LIBD) after informed consent from next-of-kin. In depth clinical characteristics of the LIBD PTSD collection were described in Mighdoll et al. [56]. Intake records, agonal state, autopsy, toxicology and medical record reviews were completed by qualified staff and accompanied the brain tissue from LIBD. Clinical assessments were based on a process that consisted of: a telephone interview with next-of-kin of that collected demographic, medical, social, and clinical histories (which included a history of combat or childhood-related trauma, as well as history of TBI/head trauma). In addition, toxicology

testing and a macroscopic and microscopic neuropathological examination were completed by a board-certified neuropathologist to screen for neuritic pathology or evidence of neurodegenerative disease.

Additional clinical assessment included a review of psychiatric record and/or additional family informant interviews, using the MINI International Neuropsychiatric Interview 6.0 and the PTSD checklist for DSM-5 (adapted for postmortem studies), as well as the LIBD Psychological Autopsy Interview [56]. All clinical and demographic data were consolidated into a psychiatric narrative summary that was reviewed by two independent board-certified psychiatrists to arrive at consensus for DSM-5 PTSD and depression versus control status (no history of psychopathology, psychiatric or neurological illness, or lifetime alcohol and substance use disorders). For PTSD diagnosis, each assessor provided a probability score for lifetime PTSD diagnosis on a 1-5 scale with 5 being “PTSD Definite” and 1 being “PTSD Unlikely.” PTSD diagnosis was based on a score greater than or equal to 3. A more in-depth overview of the diagnostic confidence determination is provided in Supplementary Table 2. Depression diagnosis was also made based on psychiatric chart record examination and family informant interviews, and was reviewed by two independent board-certified psychiatrists to arrive at consensus diagnosis.

Tissue Processing, RNA Extraction, Reverse Transcription to cDNA, and Real-Time qPCR

Tissue collection and processing for samples is described in detail in the Supplementary Materials. The coronal slab containing the dlPFC was taken at the level of the genu of the corpus callosum; dlPFC was dissected from Brodmann Area (BA) 9/46. 0.3-0.5 milligrams of tissue was used for RNA extraction. RNA extraction, RNA integrity number (RIN) determination, reverse transcription to cDNA and qPCR methods are described in detail in the Supplementary Materials. Gene expression was normalized to within sample 18S expression. 18S ribosomal RNA is a part of ribosomal RNA was used as an internal control for normalizing all gene mRNA levels in control and experimental samples. All samples were run in at least duplicate.

The following TaqMan gene expression primers (Thermo Fischer Scientific; Supplementary Table 3) were used for the qPCR gene expression analyses: *IL1A*, *IL1B*, *IL6*, *IL8*, *IL10*, *IL13*, *IL15*. *IL2*, *IL4*, *IL12B*, and. *IL2*, *IL4*, *IL12B*, *IL17A* were undetected in the majority of samples due to low or no expression in the human dlPFC, and were thus excluded from primary and follow-up analyses, yielding seven genes for analysis: *IL1A*, *IL1B*, *IL6*, *IL8*, *IL10*, *IL13*, and *IL15*.

Statistical Analysis

We normalized mRNA expression levels to within-sample *18s* expression by subtracting cycle threshold values for *18s* from the cycle threshold values for each gene of interest (ΔCT). Analyses were performed in the R statistical computing language. To examine associations between PTSD, depression, or control (no psychopathology) status and normalized gene expression values, we used a linear mixed effects ANOVA as implemented in the lme4 package [57]. In protected omnibus analyses, samples from subjects with PTSD were compared to samples with depression and to samples from control subjects with no known psychopathology (i.e., a 3-level variable). Diagnostic status and the covariates sex, age at death, self-reported race, and RIN were entered into the model as fixed effects, and subject ID was entered into the model as a random effect to account for non-independence of replicate qPCR assessments of the same sample. To obtain *p*-values for the overall model, we used a likelihood ratio test to compare the full model to a null model, which excluded psychopathology status. To address multiple testing across the seven genes, we applied a Bonferroni correction, which set the *p*-value threshold for significance to $p < .007$. Follow-up pair-wise comparisons were conducted when the omnibus analysis was significant after correction for multiple testing. To obtain between-group *p*-values, we used a Tukey's post-hoc test as implemented in the multcomp package [58].

For genes with significant corrected overall ANOVA p-values, follow-up targeted linear mixed effect ANOVA analyses were run to investigate the contribution of potential confounding variables including antidepressant use at time of death, body mass index (BMI), manner of death (suicide or

alcohol or drug related), postmortem interval (PMI), and possible history of traumatic brain injury (TBI) and/or concussion, all of which have previously been shown to be associated with both traumatic stress and mRNA expression (Supplementary Materials). For the purposes of graphing (Figure 1A), relative fold change was computed using the $2^{-\Delta\Delta CT}$ method [59].

Results

Clinical Demographic Characteristics

Phenotypic characteristics are shown in Table 1. There were no significant differences between PTSD, depression, and control samples in age at death, sex, race, RIN, PMI, BMI or suicide related cause of death.

Associations between PTSD, Depression, and Interleukin gene expression

After co-varying for age at death, sex, race and RIN, and correcting for multiple testing, only *IL1A* was significantly altered in the dlPFC of PTSD and depression cases (Figure 1; Supplementary Table 1). *IL1A* gene expression was significantly decreased in PTSD and depression cases relative to controls (Figure 1B; p=0.004); pairwise comparisons between groups showed a significant decrease in PTSD compared to controls (β=1.45, SE=.42, p=.0006), a significant decrease in depression cases compared to controls (β=.985, SE=.399, p=.014), and no significant difference between PTSD and depression cases (β=.466, SE=.393, p=.235).

Effects of covariates and potential confounds on IL1A gene expression

Covariate analyses were conducted as a follow-up to our initial results related to *IL1A.* In separate linear models adjusting for age at death, sex, race, and RIN, we found that RIN was associated with expression of *IL1A* (β=-.573, SE=.177, p=0.002); however, the effect of psychopathology remained significant. No other covariates were associated with *IL1A* expression (Supplementary Materials). In separate linear models none of the potential confounding variables (BMI, time of death antidepressant

use, suicide, alcohol, or drug-related cause of death, PMI, and possible history of TBI and/or concussion) were associated with *IL1A* gene expression (p>0.061), and their inclusion in the model did not alter the main pattern of results (Supplementary Materials). Covariate analyses for the other IL genes included in the study are described in the Supplementary Materials.

Discussion

We examined gene expression of a subset of ILs in the left dlPFC of individuals with PTSD (with and without depression), depression, and no-psychopathology controls. The results of our preliminary investigation suggest significant decreases in gene expression of *IL1A* in PTSD and depression cases relative to controls. No other ILs survived multiple testing correction across the seven genes, though nominally significant pairwise comparison results (i.e., $p < .05$) were obtained for *IL8*, *IL13* and *IL15* (Supplementary Table 1).

IL1A is a potent pro-inflammatory cytokine belonging to the IL1 family [60, 61], which also includes IL1B. Much attention has been paid to understanding the role of IL1B in PTSD and depression, based on the repeated finding of increased IL1B levels in the periphery [5, 6]. IL1A and IL1B share many features including that they both bind the receptor IL1R1 and trigger identical biological responses, however, IL1A possesses a number of unique characteristics distinguishing it from IL1B [60]: both the preform and the cleaved version of IL1A are biologically active [62], IL1A exerts its effects as both a secreted and membrane-bound cytokine [63], and IL1A has a higher affinity for IL1R1 compared to IL1B [64].

A number of studies have found significant increases in IL1A levels in the periphery in individuals with PTSD or panic disorder (PD) compared to healthy controls, and significant increases in individuals with MDD [13]. Gene set analysis from a microarray data set of postmortem prefrontal cortex (BA 10) samples from individuals with MDD suggested up-regulation of inflammatory cytokines, including IL1A [37]. In contrast to these studies, our findings showed a significant decrease in expression of *IL1A* in the

brain. There are several possible explanations for this difference. First, chronic, as opposed to acute, peripheral immune activation associated with the chronic stress of PTSD and depression may lead to compensatory increases in anti-inflammatory processes and other mechanisms [13, 37]. Indeed, prior research has found that central inflammatory signaling may be down regulated in response to severe peripheral inflammation [65]. In cell culture models, the synthetic glucocorticoid dexamethasone has been found to decrease levels of *IL1A* and *IL1B* mRNA [66], and in mouse models, mice subjected to chronic psychosocial stress exhibited down regulation of *Il1b* mRNA in the hippocampus, along with decreased *TNFα* mRNA levels in the striatum and hippocampus [67]. Prior work has also shown that some anti-inflammatory cytokines are increased in PTSD and depression (e.g. Hoge et al. [13] found increases in the anti-inflammatory molecules IL-4 and IL-10 in PTSD and PD, and Shelton et al. [37] showed increases in the anti-inflammatory cytokine IL-10 in MDD). Taken together these reports emphasize the complex mechanisms of action of cytokines in the immune response, and the impact of the presence of stress hormones and severe and chronic inflammation. Additional research will be essential to understand the complex role of IL1A in the inflammatory response.

Several limitations should be considered when interpreting these results. Peripheral blood samples were not available for any of the investigated cases, making it impossible to compare our observations in the brain to the periphery in this cohort. The extracted RNA used for gene expression analyses contained a heterogeneous mixture of cell types that includes neurons, astrocytes, microglia, and oligodendrocytes, each with a unique expression pattern. Locally resident immune cell types including microglia and astrocytes are known to mount innate immune responses in the brain, which may be neurotoxic or neuroprotective [68-71]; future work will need to distinguish whether specific cell types differentially express *IL1A*. Although our results suggest altered expression of *IL1A*, we did not investigate levels of the resulting gene products or downstream signaling cascades. This investigation was limited to the left dlPFC; whether the observed decrease in *IL1A* expression extends to other brain

regions is unknown. Future work examining gene expression changes in additional regions that are known to play a role in PTSD and depression, such as the amygdala, hippocampus, insula, anterior cingulate cortex (ACC), and medial regions of the PFC will be critical. As nearly all PTSD cases were also diagnosed with comorbid depression (Table 1), we were unable to examine diagnostically "clean" cases of this disorder, though comorbidity with depression is highly prevalent in PTSD cohorts. Thus, we cannot rule out the possibility that results may be influenced by depression. Finally, the small sample size, and the lack of a replication sample highlight the provisional nature of our findings, and the need for further evaluation in future cohorts.

These limitations are offset by the strengths of the study, which include being the first to investigate IL gene expression in postmortem tissue of individuals with PTSD and/or depression. These findings contribute to a growing literature demonstrating a dysregulated inflammatory state in individuals with PTSD and depression and provide evidence for decreased expression of *IL1A* in the dlPFC of individuals with PTSD and/or depression compared to control cases with no psychopathology. Future work will be essential to validate these findings in larger samples, and investigate the upstream factors contributing to altered *IL1A* expression, along with the downstream effects on gene products and signaling cascades. Doing so will further elucidate inflammatory markers as potential targets for therapeutic intervention and/or prognostic value.

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Disclosures

All authors report no financial or other conflicts of interest in relationship to the contents of this article.

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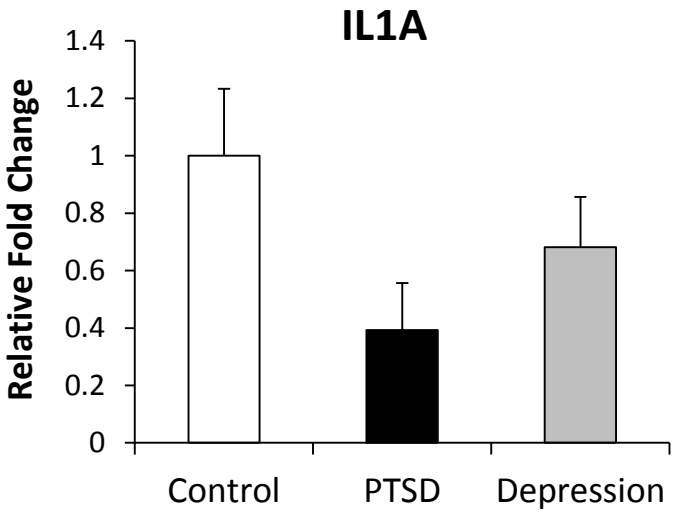
Figure 1: Association between PTSD, depression, and *IL1A* gene expression in the dlPFC.

A



B

	IL1A		
Overall Model	β	SE	p*
Age	.006	.015	.701
Sex	-.007	.457	.988
Race	-.709	.359	.052
RIN	-.627	.170	**<.001**
PTSD	1.45	.420	**.004**[a]
Depression	.985	.399	
Pairwise Comparisons	β	SE	p
PTSD vs. Control	1.45	.420	**.0006**
Depression vs. Control	.985	.399	**.014**
PTSD vs. Depression	.466	.393	.235

Figure 1. A) Relative fold change was computed using the $2^{-\Delta\Delta CT}$ method (Livak KJ, Schmittgen, 2001). Error bars were computed by taking the standard error of the CT values normalized using the $2^{-\Delta\Delta CT}$ method. B) Overall models used a linear mixed effects ANOVA to examine associations between PTSD, Depression, or Control status and normalized gene expression values. Bolded values indicate significance at the $p<.05$ level. SE = standard error. RIN = RNA integrity number. PTSD = posttraumatic stress disorder. Pairwise comparison *p*-values were computed using Tukey's post-hoc test. *P-values computed from likelihood ratio test comparing the full model to a null model that excludes psychopathology (PTSD or depression) status. [a]Comparison was significant after a Bonferroni correction for 7 genes examined ($p < 0.007$).

Table 1 *Clinical and demographic characteristics of sample cohort*

Clinical Demographic Characteristics	Control	PTSD	Depression
No. of Subjects	13	12	25
Age, years; Mean (SD)	49.54 (11.81)	44.82 (10.07)	42.51 (10.09)
Sex; Female/Male	2/11	2/10	3/22
Race (self-report); CAUC/AA	9/4	8/4	21/4
Military Service; # cases (%)	2 (15.3%)	9 (75%)	5 (20%)
PMI, hours; Mean (SD)	29 (6.9)	30 (8.9)	29 (6.5)
RIN score; Mean (SD)	7.88 (1.26)	7.98 (1.00)	8.51 (0.661)
BMI; Mean (SD)	32.9 (10.7)	28.4 (5.52)	30.3 (10.4)
Co-morbid Depression; # cases (%)	0 (0%)	10 (83.3%)	-
Time of death antidepressant usage*; Y/N	1/12	6/6	5/19
Suicide; # cases (%)	0 (0%)	3 (25%)	6 (24%)
Drug-related cause of death; # cases (%)	0 (0%)	6 (50%)	11 (44%)
Alcohol-related cause of death; # cases (%)	0 (0%)	2 (16.6%)	4 (16%)
Possible h/o TBI and/or concussion; # cases (%)	1 (7.69%)	3 (25%)	1 (4%)

Note. SD = standard deviation; CAUC = Caucasian; AA = African-American; PMI = post-mortem interval; h/o = history of; TBI = traumatic brain injury; for sex, male was coded as zero and female was coded as one; for race (self-report), Caucasian was coded as zero and African-American was coded as one.

*One sample was not tested for time-of-death antidepressant usage. Percent of cases was calculated from n=24.