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Inflammation-related transcripts define "high" and "low" subgroups of individuals with schizophrenia and bipolar disorder in the midbrain

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ABSTRACT

Dopamine dysregulation in schizophrenia may be associated with midbrain inflammation. Previously, we found elevated levels of pro-inflammatory cytokine mRNAs in the post-mortem midbrain of people with schizophrenia (46%) but not from unaffected controls (0%) using a brain cohort from Sydney, Australia. Here, we measured cytokine mRNAs and proteins in the midbrain in the Stanley Medical Research Institute (SMRI) array cohort (N = 105). We tested if the proportions of individuals with schizophrenia and with high inflammation can be replicated, and if individuals with bipolar disorder with elevated midbrain cytokines can be identified. mRNA levels of 7 immune transcripts from post-mortem midbrain tissue were measured via RT-PCR and two-step recursive clustering analysis was performed using 4 immune transcripts to define "high and low" inflammatory subgroups. The clustering predictors used were identical to our earlier midbrain study, and included: *IL1B, IL6, TNF,* and *SERPINA3* mRNA levels. 46% of schizophrenia cases (16/35 SCZ), 6% of controls (2/33 CTRL), and 29% of bipolar disorder cases (10/35 BPD) were identified as belonging to the high inflammation (HI) subgroups [χ^2 (2) = 13.54, $p < 0.001$]. When comparing inflammatory subgroups, all four mRNAs were significantly increased in SCZ-HI and BPD-HI compared to low inflammation controls (CTRL-LI) ($p < 0.05$). Additionally, protein levels of IL-1β, IL-6, and IL-18 were elevated in SCZ-HI and BPD-HI compared to all other low inflammatory subgroups (all $p < 0.05$). Surprisingly, TNF-α protein levels were unchanged according to subgroups. In conclusion, we determined that almost half of the individuals with schizophrenia were defined as having high inflammation in the midbrain, replicating our previous findings. Further, we detected close to one-third of those with bipolar disorder to be classified as having high inflammation. Elevations in some pro-inflammatory cytokine mRNAs (IL-1β and IL-6) were also found at the protein level, whereas *TNF* mRNA and protein levels were not concordant.

1. Introduction

Dopamine dysregulation is one of the most consistent features of schizophrenia pathophysiology and has been the focus of therapeutic intervention for the past 70 years (Ban, 2007; Howes and Kapur, 2009). More recently, inflammation in the brains of some patients has been detected and may contribute to the development of disease, whether independent of dopamine dysfunction or by interfering with normal dopamine transmission (Purves-Tyson et al., 2021; Comer et al., 2020; Purves-Tyson, 2020). Multiple studies have reported upregulated immune-related mRNAs and proteins (Fillman et al., 2013; Fillman et al., 2014; Saetre, 2007; Arion et al., 2007; Volk et al., 2015; Pandey et al., 2018; Lanz et al., 2019; Dean et al., 2013), an increased parenchymal leukocytes (Purves-Tyson, 2020; Schlaaff et al., 2020; Cai et al.,

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2020; Weissleder et al., 2021) and reactive astrogliosis (Catts et al., 2014; Wierzba-Bobrowicz, 2005; Feresten et al., 2013; Martins-de-Souza et al., 2009) in the brains of people with schizophrenia relative to age-matched, unaffected controls. Though many studies have focused on immune/inflammation markers in the prefrontal cortex, the degree of inflammation in the ventral midbrain of patients – where most of the dopamine-producing cells are located – appears to be even higher than that in the cortex (Purves-Tyson et al., 2021). Dopamine cell bodies give rise to widespread dopaminergic projections, and inflammatory signaling surrounding dopamine neurons has been shown to modulate their development and activity (Ling et al., 1998; Zalcman et al., 1994; Dunn, 2006; Stojakovic et al., 2017). Thus, the hypothesis that aberrant immune activity in the midbrain may be a primary cause of dopamine dysfunction and associated psychiatric symptoms (namely psychosis) (Tost et al., 2010) in schizophrenia is reasonable. However, an important next step would be to confirm the extent of midbrain inflammation in schizophrenia with an independent cohort of brains.

We have previously shown that approximately half of people with chronic schizophrenia have elevated cytokine transcripts in the midbrain (Purves-Tyson et al., 2021); a proportion that is slightly higher but largely consistent with what has been reported in the frontal cortex (~40%) in two independent cohorts (Fillman et al., 2013; Fillman et al., 2014; Zhang et al., 2016). Interestingly, while these cortical studies also found comparable levels of inflammation in a minority of unaffected control cases (~10–25%), we found no evidence of midbrain inflammation in any of the control cases studied previously (Purves-Tyson et al., 2021). This suggests that some degree of cortical inflammation can arise from a variety of conditions (such as normal aging) (Erraji-Benchekroun et al., 2005) and this may not always result in psychiatric symptoms, while inflammation specifically in the midbrain may be unique to those with the disease and may be more linked with psychosis. Inflammation in schizophrenia could be in a relapsing remitting course instead of being static, which may be comparable to multiple sclerosis (MS) where acute inflammation is the focus of research, and which can occur in different brain areas over time leading to comprehensive clinical manifestation (Steinman, 2014). Thus, the classification of individuals with schizophrenia into subgroups based on inflammatory marker expression and focusing on those with elevated inflammation may provide a more powerful approach to investigating the neuropathology in schizophrenia than just diagnosis alone.

Bipolar disorder is another psychiatric illness where some patients can have psychotic symptoms, and though distinct from schizophrenia in many ways, individuals with bipolar disorder do share neuropathological features with schizophrenia patients (Gandal, 2018; Hayashi et al., 2012; Fung et al., 2014). Many post-mortem studies have demonstrated increased immune activity in the frontal cortex of bipolar patients that also appears most evident in a subset of patients (Fillman et al., 2014; Roman et al., 2021; Kim et al., 2016; Thomas et al., 2004; Pacifico and Davis, 2017). However, no study has assessed inflammation in the midbrain in bipolar disorder nor attempted to relate levels of inflammatory markers in the brain to symptomatology. Thus, to lend support to the hypothesis that midbrain inflammation is linked to psychosis, we tested if elevations in pro-inflammatory cytokine mRNAs characterized a subset of individuals with schizophrenia or with bipolar disorder in an independent post-mortem cohort.

To identify high inflammation versus low inflammatory subgroups within each diagnostic group, we performed a two-step recursive cluster analysis on transcript levels of the same four mRNAs (*SERPINA3, IL6, IL1B* and *TNF)* previously used to define subgroups in a separate post-mortem midbrain cohort of schizophrenia and control cases (Purves-Tyson et al., 2021). We then determined the proportion of each group falling in the two inflammatory categories and then assessed whether individuals with bipolar disorder who were prescribed antipsychotic medication during their lifetime were more likely to have midbrain inflammation than those not prescribed antipsychotics. The results may indicate a relationship between midbrain inflammation and psychotic symptoms and/or an effect of antipsychotic medication on midbrain inflammatory levels. We also measured additional inflammatory cytokines including IL8, IL1A, and IL18 for the following reasons. Firstly, we found CXCL8/IL8 was elevated in schizophrenia in the DLPFC, and it was one of the most important predictors in the inflammatory clustering analysis in DLPFC and OFC (Fillman et al., 2013; Zhang et al., 2016). Secondly, IL1A, IL1B and IL18 are the three best-characterized cytokines that belong to the IL-1 superfamily (Tapia et al., 2019) Thirdly, IL-1 and IL18 activities could be related to the inflammasome and they are co-regulated in human cortical development (Sager et al., 2022) (unpublished observations). Microglia inflammasome activation and IL-1 and IL-18 cleavage contribute to neuroinflammation and neurodegeneration in diseases, including: acute brain injury, Alzheimer's disease, Parkinson's disease, CNS autoimmunity post-infectious neuropathology, schizophrenia and febrile convulsion (Kawabori and Yenari, 2015; Goldmann and Prinz, 2013; Eyo et al., 2017; Shemer and Jung, 2015). Therefore, we chose these cytokines based on their contribution to neuroinflammation in schizophrenia in other brain regions or their contribution to neuroinflammation in other brain diseases.

While stratifying schizophrenia and bipolar disorder patients using immune-related mRNAs in the brain is useful in identifying those with increased neuroinflammatory signaling, it is not currently known whether these transcriptional changes are associated with higher levels of inflammatory proteins in the brain. Levels of cytokine mRNAs and proteins are not always correlated (Shebl et al., 2010; Du et al., 2014). Demonstrating a concurrent increase in cytokine proteins in the brains of patients with elevated cytokine mRNAs would support the hypothesis that these transcriptional changes are contributing to the underlying pathophysiology and are likely to have downstream effects on brain homeostasis and neurotransmission. Thus, in addition to measuring pro-inflammatory mRNAs in the midbrain of schizophrenia, bipolar disorder and control cases, we also measured IL-1β, IL-6, IL-18 and TNF-α proteins to determine if they were changed in the diagnosis and inflammatory groups, and we determined the degree to which changes in transcription were associated with changes in protein levels.

We expected to find evidence of midbrain inflammation at the transcriptional level in ~50% of individuals with schizophrenia, 0% of controls, and an intermediate level of individuals with bipolar disorder as previously reported in the cortex (Fillman et al., 2014). We further predicted that patients with bipolar disorder and elevated pro-inflammatory mRNAs would be more likely to have been prescribed antipsychotic medication and therefore, more likely to have experienced psychotic symptoms prior to their death than those not taking antipsychotics. Lastly, we hypothesized that individuals with increased inflammatory transcripts in the midbrain would also have increased inflammatory proteins in the midbrain across diagnosis.

2. Methods

2.1. Tissue collection and cohort demographics

All human post-mortem tissue experiments were conducted with the approval of the University of New South Wales Human Research Ethics Committee (HREC #17826). Fresh frozen human midbrain tissue from 35 schizophrenia cases, 35 bipolar disorder cases, and 35 control cases was obtained from the Stanley Medical Research Institute Array Collection. A stereological series of 14 μm and 100 μm sections were cut in the coronal plane and neuroanatomically matched at the level of the oculomotor nerve (CN III) exiting fibers. A series of 14 μm sections, spaced 1 mm apart through the entire midbrain were thaw mounted on gelatin-coated glass slides and stained for tyrosine hydroxylase to identify the dopaminergic neurons and the level of CN III. Adjacent 100 μm sections were collected between wax paper and were micro-dissected with a scalpel over dry ice, bisecting the midbrain directly above the substantia nigra dorsal tier lateralis into dorsal and ventral midbrain sections. Gene expression and protein levels were assessed in tissue

homogenates from 2 × 100 μm ventral midbrain sections each.

Demographic details and clinical variables are shown in Table 1. Diagnostic groups were matched for age, postmortem interval (PMI) and RNA integrity number (RIN). Brain pH was significantly lower in the schizophrenia ($p = 0.02$) and bipolar disorder cases ($p = 0.008$) relative to the control cases. There was a significantly higher proportion of females in the bipolar disorder group compared to both control and schizophrenia groups (both $p < 0.05$, Table 1). The lifetime antipsychotic dose is reported as fluphenazine equivalent dose. All schizophrenia cases and 23 bipolar disorder cases received antipsychotic medication with a significantly higher lifetime dose in the schizophrenia cases (Table 1).

2.2. Quantitative real-time polymerase chain reaction

Total RNA was isolated from fresh-frozen post-mortem midbrain tissue using TRIzol Reagent according to the manufacturer's protocol (Invitrogen, Life Technologies Inc., Mulgrave, VIC, Australia). RNA concentration and purity were determined using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, USA) and RNA quality using an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The mean RNA Integrity Number (RIN) for all samples was 6.2. Complementary DNA (cDNA) was synthesized from 2 μg total RNA using the Superscript IV First-Strand Synthesis Kit and random hexamers according to the manufacturer's protocol (Invitrogen, Life Technologies).

Transcript levels were measured by quantitative real-time polymerase chain reaction (qPCR) with an ABI Prism 7900HT system (Applied Biosystems, Life Technologies) as previously reported (Weickert et al., 2010). Pre-designed Taqman gene expression assays (Applied Biosystems, Life Technologies) were used to measure transcript levels of three housekeeper genes (*ACTB*, Hs99999903_m1; *UBC*, Hs00824723_m1; *GAPDH* Hs99999905_m1) and seven inflammatory markers (*SERPINA3,* Hs00153674_m1; *IL6*, Hs00174131_m1; *IL1B*, Hs01555410_m1; *TNF* Hs99999043_m1; *IL1A*, Hs00174092_m1; *CXCL8*, Hs00174103_m1, *IL18*, Hs01038788_m1). Gene expression was quantified by the relative standard curve method using serial dilutions of cDNA pooled from all samples and Sequence Detector Software (V2.4, Applied Biosystems, Life Technologies). No template controls were included to check for non-specific amplification. A control reaction without reverse transcriptase was performed to verify that genomic DNA was not amplified. None of the three housekeeper genes or their geometric mean varied by diagnostic group (all $p > 0.05$; Supplementary Fig. S1). Mean duplicate transcript expression levels of inflammatory markers were normalized to the geometric mean of the housekeeper genes (Vandesompele, 2002).

Table 1
Demographic and clinical details of post-mortem midbrain cases from the Stanley Medical Research Institute Array Collection. Data are mean ± SD (range). *p < 0.05, ****p < 0.0001.

Demographic Variable	Control (n = 35)	Schizophrenia (n = 35)	Bipolar disorder (n = 35)	Statistics
Age (years)	44.1 (31–60)	42.6 (19–59)	45.3 (19–64)	F(2,102) = 0.78, p = 0.46
Post-mortem interval (h)	29.4 ± 12.9	31.4 ± 15.5	37.9 ± 18.3	H(2) = 3.70, p = 0.16
Brain pH	6.6 ± 0.3	6.5 ± 0.2	6.4 ± 0.3	H(2) = 8.23, p = 0.02*
RNA integrity number	6.1 ± 1.2	6.3 ± 1.2	6.1 ± 1.4	H(2) = 0.78, p = 0.68
Sex (Male/Female)	26/9	26/9	17/18	χ^2 (2) = 6.85, p = 0.04*
Manner of death (natural/suicide)	35/0	28/7	20/15	–
Duration of illness (years)	–	21.3 ± 10.2	20.1 ± 9.5	U = 532.0, p = 0.34
Lifetime antipsychotics (fluphenazine equiv., mg)	–	85004.3 ± 100335.3	10034.3 ± 22556.4	U = 150.0, p < 0.0001****
Ethnicity (Caucasian/Non-Caucasian)	35/0	34/1	33/2	–

2.3. Inflammatory cytokine multiplex assay

Protein was extracted from ~ 80 mg (from 2 × 100 um sections) of post-mortem midbrain tissue of substantia nigra using N-PER Neuronal Protein Extraction Reagent according to the manufacturer's protocol (N-PER, Thermo Scientific, USA). Protein concentration was determined by BCA protein assay (Pierce BCA Protein Assay Kit, Thermo Scientific, USA). The mean protein concentration for all the samples was 3.6 μg/μL (range 2.9–4.8 μg/μL). Cytokine protein IL-1β, IL-6, IL-18, and TNF-α concentrations were measured by Bio-Plex Pro Human Cytokine Assays according to the manufacturer's protocol (Bio-Rad Laboratories, Inc, USA) using the Luminex 200 Multiplexing Instrument System (Luminex Corporation, USA). SERPINA3 protein levels were not measured because this detection bead is unavailable in Bio-Plex Human Cytokine panels. The observed concentrations of all the samples were calculated based on the fluorescent intensity of the known standards (standard curve) from the Luminex assay kit. The average coefficient of variance of all the samples is +/-10.31%. The cytokine concentration was normalized to the protein concentration by using the cytokine concentration divided by the amount of protein assayed (pg of cytokine per mg of protein).

2.4. Statistical analysis and clustering analysis

Statistical analyses were conducted with IBM SPSS V26 (IBM, Armonk, NY, USA) with statistical significance set at $p < 0.05$. Data is expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) or Kruskal Wallis H-tests was used to test for differences in cohort demographics [age, brain pH, PMI, and RIN; Table 1]. Pearson's χ^2 test and a *post hoc* Z-test were used to analyze the association between sex and diagnosis. Mann-Whitney U-tests or Kruskal Wallis H-tests were used to determine group differences between schizophrenia and bipolar disorder cases for clinical variables (duration of illness and lifetime fluphenazine equivalent antipsychotic dose). Fisher's least significant differences (LSD) or Dunn's *post hoc* tests were used as appropriate.

One control case with a diagnosis of the autoimmune disorder idiopathic thrombocytopenic purpura was excluded. An additional control case with asthma was identified as an outlier for 6 of the 7 inflammatory marker transcripts measured (>85%) and was excluded from all analyses. Group outliers for each gene and protein of interest were excluded if values were greater than two standard deviations from the group mean (0–4/group) after normalizing to the geometric mean of the housekeepers. Data were tested for normality using the Shapiro-Wilk test within each group and data that were non-normally distributed underwent $\log_{10}$ transformation. If normality was not achieved after transformation, non-normally distributed data were analyzed using non-parametric statistical tests.

Covariates were determined using Pearson's product-moment or Spearman's rank correlations between mRNA or protein levels with demographic variables (Supplementary Table S1). Brain pH was correlated with *CXCL8, IL6, SERPINA3, TNF* mRNA levels and IL-6 protein levels. IL18 mRNA and IL-18 and TNF-α protein levels were correlated with brain pH and RIN. IL-1β protein levels were correlated with PMI. Brain pH can be influenced by inflammation and vice versa (Kellum et al., 2004; Tyrtyshnaia et al., 2016), with research suggesting reduced brain pH is part of the disease process in psychiatric disorders (Hagihara, 2017); thus, brain pH was not used as a covariate in statistical analyses. Spearman's correlations were conducted between mRNA levels or protein levels with clinical variables in psychiatric disorder cases

(Supplementary Table S2). Group differences in mRNA or protein levels were determined using one-way ANOVA, ANCOVA (if a correlation was detected between mRNA levels and demographic variables) or Kruskal Wallis H-tests followed by Fisher's least significant differences (LSD) *post hoc* tests or Dunn's *post hoc* tests as appropriate. If distribution of an mRNA or protein of interest failed homogeneity of variance testing, Welch's one-way ANOVA and Games Howell *post hoc* tests were used.

To identify inflammatory subgroups, a two-step recursive cluster analysis (Chiu, et al., 2001; Bacher et al., 2004) using gene expression data was performed on all cases as previously described (Purves-Tyson et al., 2021; Fillman et al., 2013). The cluster analysis was conducted and identified four inflammatory markers (*SERPINA3, IL6, IL1B* and *TNF*) which were also used to define subgroups in our earlier study of a midbrain cohort (Purves-Tyson et al., 2021). Missing and outlier values were replaced using the SPSS-derived expectation maximization (EM) algorithm to retain as many cases as possible. Clustering was performed on 35 schizophrenia, 35 bipolar disorder, and 33 control cases. Overall model quality (on a scale of 0–1.0) needed to exceed 0.5 and individual predictor importance for each gene needed to exceed 0.4 (on a scale of 0–1.0). Pearson's χ^2 test and a *post hoc* Z-test were used to analyze the association between inflammatory subgroup and diagnosis. The high inflammatory control subgroup included only two cases and these cases are graphed for visualization but not included in statistical analyses of inflammatory subgroups.

3. Results

3.1. Increased SERPINA3 gene expression in the midbrain of schizophrenia and bipolar disorder cases

There was a significant effect of diagnosis on *SERPINA3* mRNA levels [Welch's ANOVA: F(2,60.87) = 8.47, p = 0.001; Fig. 1A]. *SERPINA3* mRNA levels were significantly increased in schizophrenia cases by ~193% relative to controls (p = 0.0001) and by ~48% in bipolar disorder cases relative to controls (p = 0.016). There was no significant effect of diagnosis on *TNF* [Welch's ANOVA: F(2,94) = 1.23, p = 0.30; Fig. 1B], *IL6* [H(2) = 2.38, p = 0.30; Fig. 1C], or *IL1B* [F(2,94) = 0.88, p = 0.42; Fig. 1D] mRNA levels. The additional inflammatory marker transcripts examined, but deemed not informative of cluster status, were also unchanged between diagnostic groups [*IL1A*, F(2,97) = 0.68, p = 0.51; *CXCL8*, H(2) = 0.67, p = 0.72; *IL18*, F(2,94) = 1.93, p = 0.15; Fig. 4].

3.2. Identification of high and low inflammatory subgroups defined by inflammatory marker gene expression in the midbrain

Two-step cluster analysis using all inflammatory marker gene expression yielded two inflammatory subgroups. The overall model quality was > 0.6 with four predictors contributing significantly to the model and the predictor importance (on a scale of 0–1.0, 1.0 being the most important) being 1.0 for *TNF,* 0.99 for *IL1B,* 0.75 for *IL6,* and 0.59 for *SERPINA3*. Cluster one (n = 75) had below-median inflammatory marker gene expression for all markers and was termed the "low inflammatory" subgroup. Cluster two (n = 28) had above-median inflammatory marker gene expression for all markers and was termed the "high inflammatory subgroup." Analysis showed 46% of schizophrenia cases (n = 16), 29% of bipolar disorder cases (n = 10), and 6% of control cases (n = 2) were in the high inflammatory subgroup (Fig. 2). A principal component analysis (PCA) plot including four inflammatory gene predictors of the cluster model showed the similarity in all the individuals defined as high. More controls were clustered in the low inflammatory group while the high inflammatory group mainly consisted of individuals with schizophrenia and bipolar disorder (Supplementary Fig. 3). We found a significantly higher proportion of schizophrenia and bipolar disorder cases in the high inflammatory group compared to

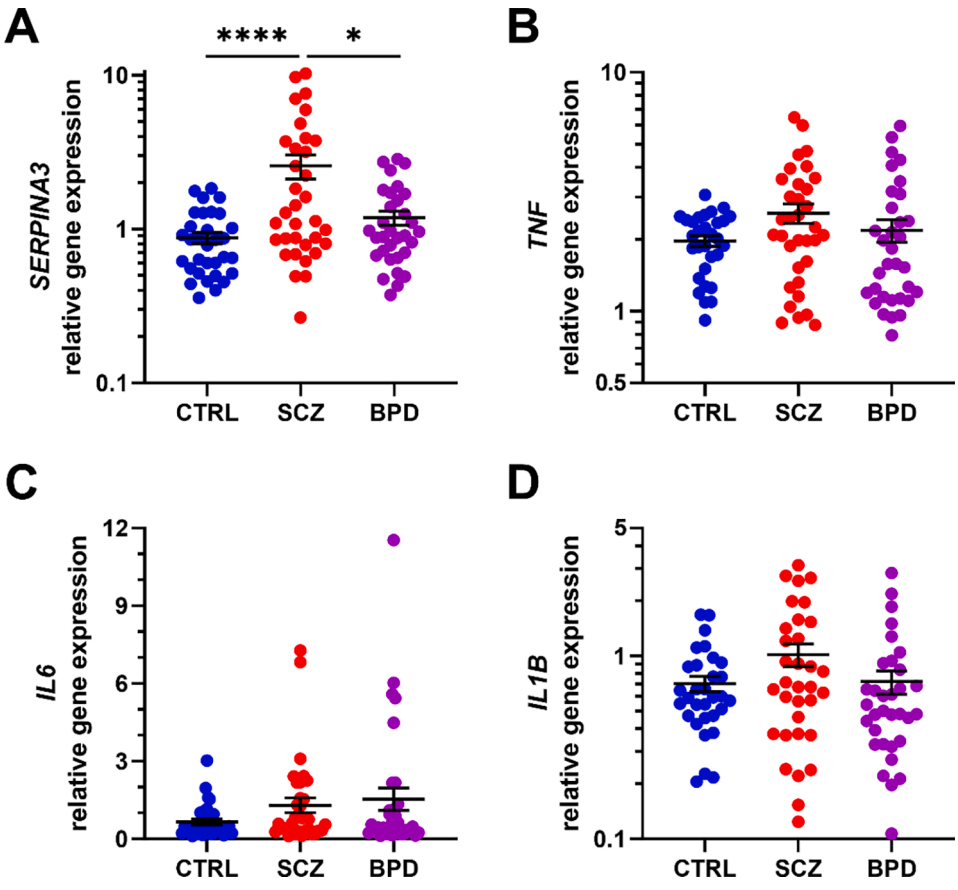

Fig. 1. Inflammatory marker gene expression in the post-mortem midbrain of schizophrenia, bipolar disorder, and control cases. (A) SERPINA3 mRNA levels were significantly increased in schizophrenia cases (p=0.0001) and bipolar disorder (p<0.05) relative to control cases. (B) TNF [$\log_{10}$ transformed], (C) IL6, and (D) IL1B mRNA levels [$\log_{10}$ transformed] did not significantly differ between diagnostic groups. Bar denotes mean ± SEM, *p ≤ 0.05, ****p ≤ 0.0001. CTRL, Control (blue); SCZ, schizophrenia (red); BPD, bipolar disorder (purple).

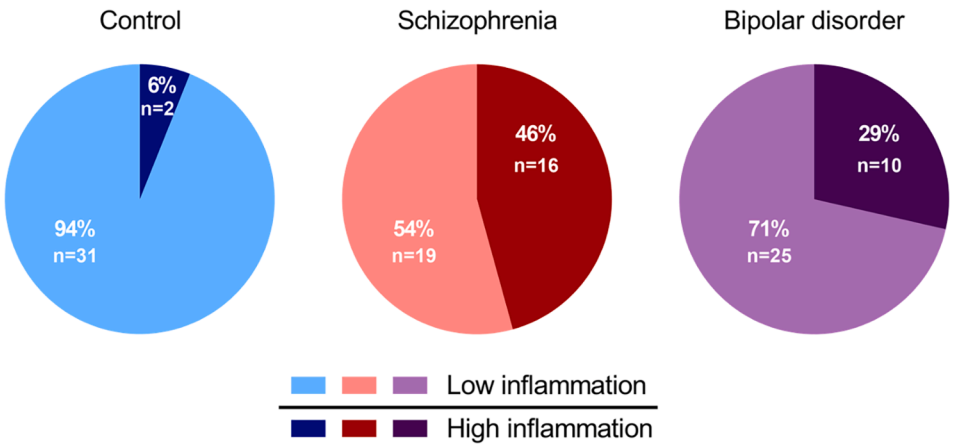

Fig. 2. Inflammatory subgroups were defined by cluster analysis of inflammatory marker transcript levels in the post-mortem midbrain of schizophrenia, bipolar disorder, and control cases. Two-step cluster analysis of inflammatory marker transcript levels classified 94% (31/33) of control, 54% (19/35) of schizophrenia, and 71% (25/35) of bipolar disorder cases as having a low inflammatory status (lighter colors) termed the "low inflammatory" subgroup. Whereas 6% (2/33) of control, 46% (16/35) of schizophrenia and 29% (10/35) of bipolar disorder cases were classified as being in high inflammatory status termed the "high inflammatory" subgroup (darker colors) [control (blue); schizophrenia (red); bipolar disorder (purple)].

control cases [χ^2 (2) = 13.54, p < 0.001; *post hoc* Z-tests schizophrenia and control p < 0.001, bipolar disorder and control p < 0.05].

No significant differences were found between inflammatory subgroups for age, PMI, RIN, or sex (Table 2). Brain pH [H(4) = 16.88, p = 0.002] was higher in the low inflammatory control subgroup relative to both low and high schizophrenia and bipolar disorder subgroups (p ≤ 0.056). There was a significant relationship between inflammation status and lifetime antipsychotic dose [H(3) = 18.21, p < 0.001]. As expected, both high and low inflammatory schizophrenia subgroups had significantly higher lifetime antipsychotic doses relative to both high and low inflammatory bipolar disorder subgroups (all p < 0.05). Schizophrenia cases had significantly higher lifetime antipsychotic doses compared to bipolar disorder cases overall (p < 0.0001). There was no significant difference in lifetime antipsychotic dose between low and high inflammatory bipolar disorder subgroups. Interestingly, all high inflammatory bipolar disorder cases (n = 10) had been prescribed antipsychotic medication prior to their death compared to ~ 54% (13 of 24) of low inflammatory bipolar disorder cases [χ^2 (2) = 6.42, p = 0.01].

3.3. *Inflammatory marker gene expression is increased in the midbrain of a subgroup of schizophrenia and bipolar disorder cases*

The transcript levels of all four genes that informed the cluster analysis significantly differed when assessed across inflammatory subgroups. *SERPINA3* mRNA levels [Welch's ANOVA: F(4,34.42) = 10.71, p < 0.0001; Fig. 3A] were increased in the high inflammatory schizophrenia (+222–377%, all p < 0.001) and bipolar disorder (+81–167%, all p < 0.01) subgroups compared to all low inflammatory subgroups. There was a trend towards an increase of 79% in *SERPINA3* mRNA levels in the high inflammatory schizophrenia subgroup compared to the high inflammatory bipolar disorder subgroup (p = 0.08). *IL1B* mRNA levels [Welch's ANOVA: F(4,33.34) = 9.64, p < 0.0001; Fig. 3B] were increased in the high inflammatory schizophrenia subgroup (+267–341%, all p < 0.001) and bipolar disorder subgroup (+45–108%, all p < 0.05) compared to all low inflammatory subgroups.

TNF mRNA levels [F(4,90) = 25.38, p < 0.0001; Fig. 3C] were increased in the high inflammatory schizophrenia (+78–131%, all p < 0.0001) and bipolar disorder (+155–235%, all p < 0.0001) subgroups compared to all low inflammatory subgroups. The low inflammatory control subgroup had higher *TNF* mRNA levels than both the low inflammatory schizophrenia (+26%, p = 0.03) and bipolar disorder (+31%, p = 0.005) subgroups. *TNF* mRNA levels were increased in the high inflammatory bipolar disorder subgroup compared to the high inflammatory schizophrenia subgroup at the level of statistical significance (+45%, p = 0.05).

IL6 mRNA levels [Welch's ANOVA: F(4,34.00) = 7.13, p < 0.0001; Fig. 3D] were increased in the high inflammatory schizophrenia (+216–294%, all p < 0.05) and bipolar disorder (+913–1165%, all p < 0.05) subgroups compared to all low inflammatory subgroups. The high inflammatory bipolar disorder subgroup had higher *IL6* mRNA levels than the high inflammatory schizophrenia subgroup (+221%, p = 0.03).

Table 2
Demographic and clinical details of post-mortem midbrain cases from the Stanley Medical Research Institute Array Collection classified by inflammatory subgroup. Data are mean ± SD (range). The high inflammatory control subgroup is not included in statistical analyses. $Statistical analysis performed only in psychiatric disorder cases. ** p < 0.01, ***p < 0.001.

Demographic Variable	Low Control (n = 31)	High Control (n = 2)	Low Schizophrenia (n = 19)	High Schizophrenia (n = 16)	Low Bipolar disorder (n = 25)	High Bipolar disorder (n = 10)	Statistics
Age (years)	45.3 ± 7.3 (32–60)	37.5 ± 9.2 (31–44)	41.8 ± 9.4 (19–54)	43.6 ± 7.4 (32–59)	46.1 ± 9.6 (29–63)	43.2 ± 13.0 (19–64)	F(4,96) = 0.78, p = 0.54
Post-mortem interval (h)	30.8 ± 12.6	10.5 ± 0.7	27.0 ± 14.0	36.7 ± 16.1	38.2 ± 18.4	37.1 ± 19.1	H(4) = 6.45, p = 0.17
Brain pH	6.6 ± 0.3	6.2 ± 0.05	6.4 ± 0.3	6.5 ± 0.2	6.5 ± 0.2	6.2 ± 0.3	H(4) = 16.88, p = 0.002**
RNA integrity number	6.0 ± 1.2	6.2 ± 2.5	6.4 ± 1.3	6.2 ± 1.1	6.4 ± 1.3	5.5 ± 1.6	H(4) = 4.7, p = 0.32
Sex (Male/Female)	24/7	1/1	14/5	12/4	12/13	5/5	χ^2(4) = 7.66, p = 0.11
Manner of death (natural/suicide)$	31/0	2/0	15/4	13/3	13/11 (n = 24)	6/4	χ^2(3) = 4.75, p = 0.19
Duration of illness (years)$	–	–	19.5 ± 10.3	23.4 ± 9.9	20.3 ± 8.7 (n = 24)	19.7 ± 12.1	H(3) = 1.52, p = 0.68
Lifetime antipsychotics (fluphenazine equiv., mg)$	–	–	77113.2 ± 111443.1	94375.0 ± 87993.8	5195.8 ± 8285.1 (n = 13)	22250.0 ± 38915.3	H(3) = 18.21, p < 0.001***
Ethnicity (Caucasian/Non-Caucasian)	31/0	2/0	19/0	15/1	24/1	9/1	–

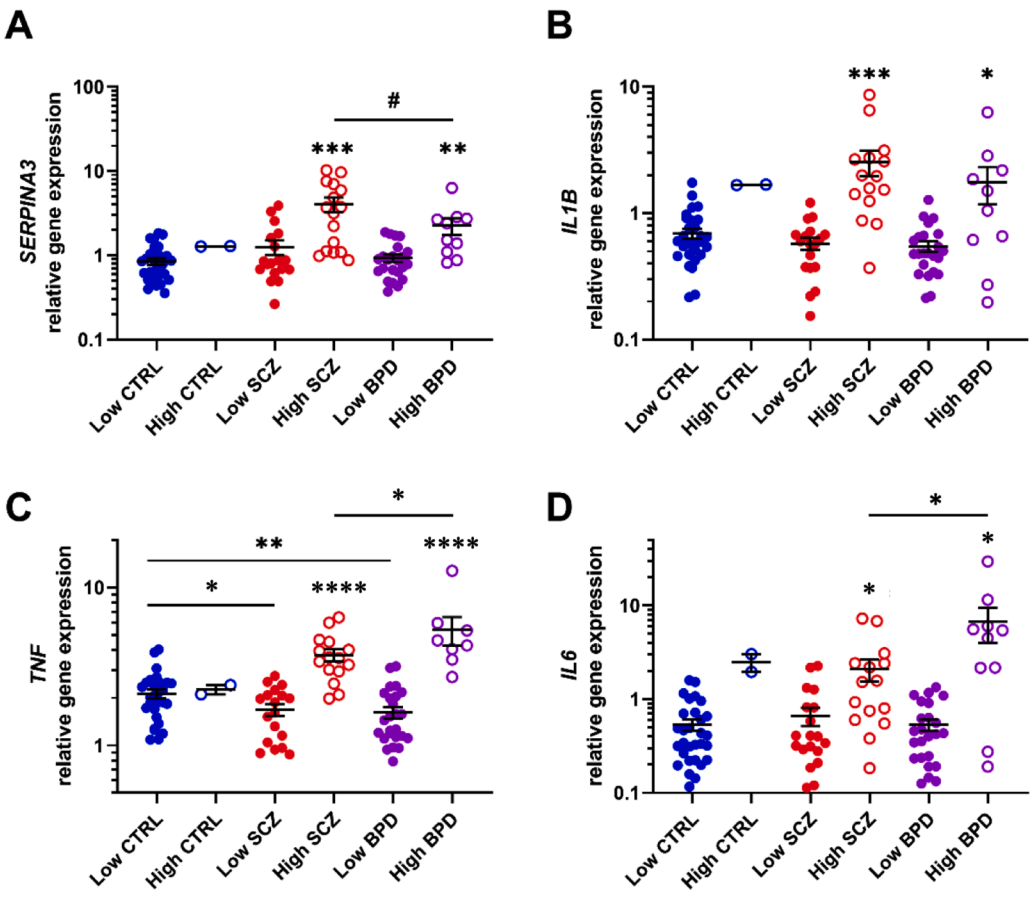

Fig. 3. Inflammatory marker transcript in the post-mortem midbrain of schizophrenia, bipolar disorder, and control cases after stratification into inflammatory subgroups. (A) *SERPINA3*, (B) *ILIB* (C) *TNF*, and (D) *IL6* mRNA levels were significantly increased in the high inflammatory schizophrenia and high inflammatory bipolar disorder subgroups relative to all low inflammatory subgroups. Note the log scale used on the Y-axis on all graphs. Bar denotes mean ± SEM, * or # denotes comparison with all low inflammatory subgroups unless the comparison is indicated by a line, #p≤0.10, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001 CTRL, control (blue); SCZ, schizophrenia (red); BPD, bipolar disorder (purple). Closed circles, low inflammatory subgroups; open circles, high inflammatory subgroups.

3.4. Gene expression of additional inflammatory markers not included in clustering analyses were increased in the midbrain of a subgroup of schizophrenia and bipolar cases

Of the additional inflammatory marker transcripts examined but not selected for cluster analysis, *IL1A* mRNA levels [Welch's ANOVA: F (4,35.90) = 4.523, p = 0.005; Fig. 4B] were significantly different when assessed across inflammatory subgroups. *IL1A* mRNA levels were increased in the high inflammatory schizophrenia subgroup compared to the low inflammatory schizophrenia (+57%, p = 0.002) and low inflammatory bipolar disorder (+60%, p = 0.002) subgroups, and trended towards an increase relative to the low inflammatory control subgroup (+27%, p = 0.057). There was no significant difference detected for *CXCL8* mRNA levels [H(4) = 5.33, p = 0.26], or *IL18* mRNA levels [F (4,92) = 1.63, p = 0.17; covariate: RIN] across inflammatory subgroups (Fig. 4).

3.5. Inflammatory cytokine protein levels are increased in the midbrain of a subgroup of schizophrenia and bipolar disorder cases

Similar to the lack of overall effect of diagnosis on cytokine mRNA levels, the protein levels of three inflammatory cytokines, 1) IL-1β [F (2,91) = 0.007, p = 0.99; covariates: RIN, PMI], 2) IL-6 [H(4) = 4.15, p = 0.13], and 3) TNF-α [F(2,95) = 2.25, p = 0.11; covariate: RIN] did not significantly differ across diagnostic groups (Supplementary Fig. S2). However, IL-18 [F(2,97) = 9.25, p = 0.00021; covariate: RIN] protein levels were significantly elevated in schizophrenia (+31%, p < 0.01) and bipolar disorder cases relative to control cases (+88%, p < 0.0001; Supplementary Fig. 2).

In contrast, the protein levels of IL-1β, IL-6, and IL-18 differed significantly across inflammatory subgroups. IL-1β protein levels [F (4,91) = 7.15, p < 0.0001; Fig. 5A] were elevated in high inflammatory schizophrenia subgroup compared to all low inflammatory subgroups (CTRL-LI: +194%, SCZ-LI: +338%, BPD-LI: +181%, all p < 0.01) and was also elevated in high inflammatory bipolar disorder subgroup compared to all low inflammatory subgroups (CTRL-LI: +291%, SCZ-LI: +485%, BPD-LI: +275%, all p < 0.012). IL-6 protein levels [Welch's ANOVA: F(4, 33.76) = 11.34, p = 6.10E-06] were elevated in high inflammatory schizophrenia (+181–338%, all p < 0.0001) and bipolar disorder subgroups (+275–483%, all p < 0.0001) compared to all low inflammatory subgroups (Fig. 5B). IL-18 protein differed across inflammatory subgroups [F(4,93) = 7.95, p < 0.0001; covariate: RIN; Fig. 5C] such that IL-18 levels were elevated in high inflammatory schizophrenia subgroup compared to the low inflammatory control subgroup (+106%, p < 0.001) and in high inflammatory bipolar disorder subgroup compared to all the low inflammatory subgroups (+170–397%, from p < 0.01 to p<0.00001) and compared to high inflammatory schizophrenia subgroup (+142%, p = 0.05). Further, IL-18 protein was significantly elevated in low inflammatory schizophrenia and low inflammatory bipolar disorder subgroups compared to the low inflammatory control subgroup (+33–84%, both p ≤ 0.05). TNF-α protein levels did not differ across inflammatory subgroups [F(4,92) = 1.31, p = 0.27; Fig. 5D], though the mean TNF-α protein level was reduced in the high inflammatory bipolar disorder subgroup compared to the low inflammatory control and high inflammatory schizophrenia subgroups (65–69%).

3.6. Correlation of inflammatory marker gene expression and protein levels with clinical variables and inflammatory comorbidities

3.6.1. Transcripts

In the patient cohort (schizophrenia and bipolar disorder combined), lifetime antipsychotic exposure (fluphenazine equivalent) was positively correlated with mRNA levels of *SERPINA3* (Spearman's ρ = 0.47,

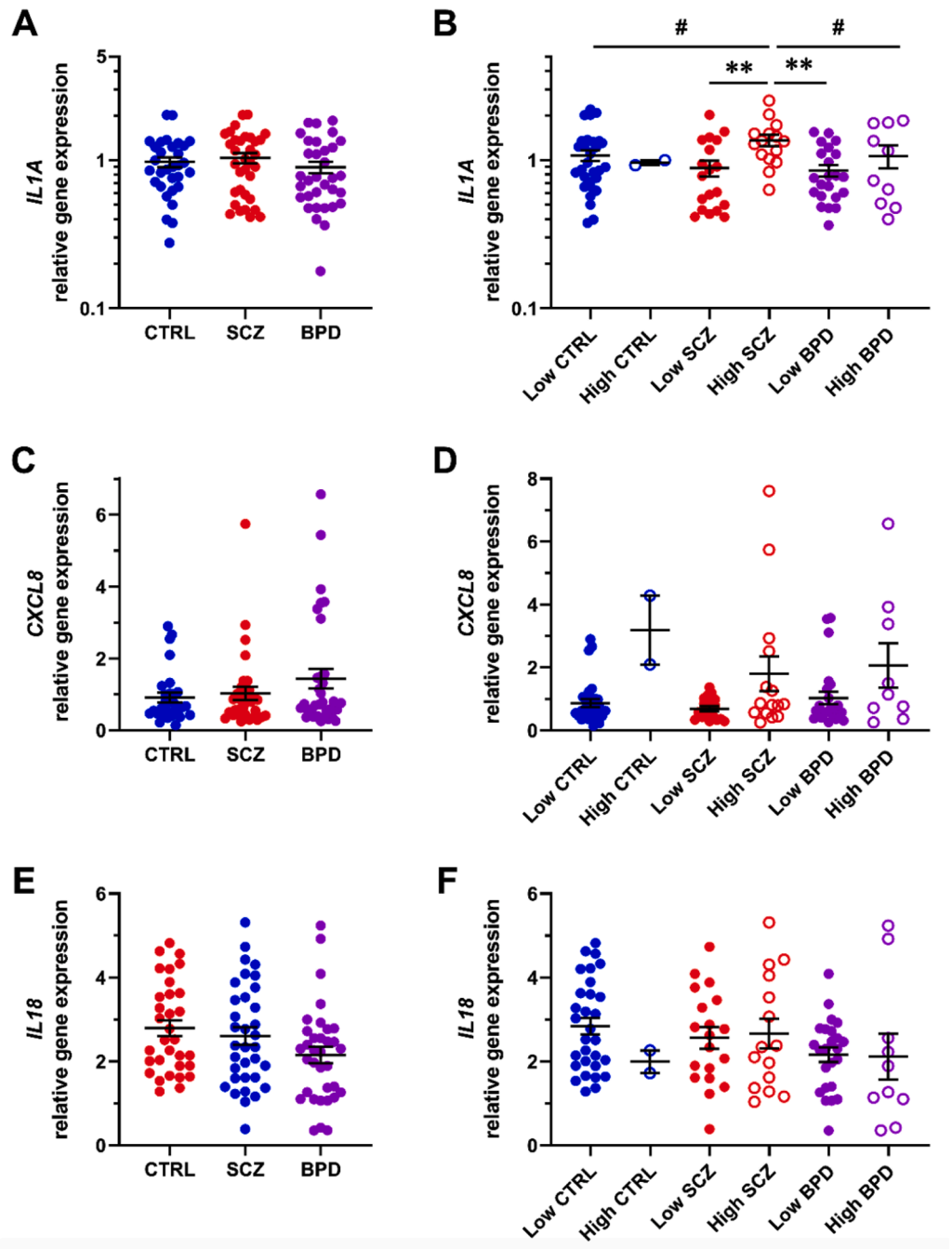

Fig. 4. Inflammatory marker transcript levels in the post-mortem midbrain by diagnosis and by inflammatory subgroup. Transcript levels of (A) *IL1A*, (C) *CXCL8*, and (E) *IL18* did not significantly differ by diagnostic group. When assessed by inflammatory subgroup, (B) *IL1A* mRNA levels were significantly increased in the high inflammatory schizophrenia subgroup relative to the low inflammatory schizophrenia and bipolar disorder subgroups (both $p<0.01$). (D) *CXCL8* and (F) *IL18* mRNA levels did not significantly differ between inflammatory subgroups. Bar denotes mean ± SEM, $^{\#}p \leq 0.10$, $^{**}p \leq 0.01$. CTRL, Control (blue); SCZ, schizophrenia (red); BPD, bipolar disorder (purple). Note only Panel A and B are on a log scale for the Y axis. Closed circles, low inflammatory subgroups; open circles, high inflammatory subgroups.

$p < 0.0001$), *IL1B* (Spearman's $\rho = 0.27$, $p = 0.023$) and *TNF* (Spearman's $\rho = 0.39$, $p = 0.001$) but not *IL6* (Spearman's $\rho = 0.20$, $p = 0.09$). Lifetime antipsychotic exposure was not correlated with mRNA levels of *CXCL8* or *IL18* (all Spearman's $\rho < 0.11$, all $p > 0.39$) and trended towards a positive correlation with *IL1A* mRNA (Spearman's $\rho = 0.23$, all $p = 0.059$). Age of onset (schizophrenia and bipolar disorder combined) was negatively correlated with mRNA levels of *IL6* (Spearman's $\rho = -0.26$, $p = 0.03$) and *SERPINA3* (Spearman's $\rho = -0.31$, $p = 0.01$), but none of the other transcript levels (all Spearman's $-0.212 < \rho < -0.027$, $p > 0.08$). Duration of illness was positively correlated with *SERPINA3* (Spearman's $\rho = 0.29$, $p = 0.016$), *TNF* (Spearman's $\rho = 0.29$, $p = 0.017$) and *IL1A* (Spearman's $\rho = 0.24$, $p = 0.045$) mRNAs, but not with any other inflammatory marker mRNA levels (Supplementary Table S2).

3.6.2. Proteins

Lifetime antipsychotic exposure was positively correlated with protein levels of IL-1β (Spearman's $\rho = 0.31$, $p = 0.01$) and IL-6 (Spearman's $\rho = 0.36$, $p = 0.003$), but not IL-18 (Spearman's $\rho = 0.18$, $p = 0.15$) or TNF-α (Spearman's $\rho = 0.018$, $p = 0.88$). Age of onset was negatively correlated with IL-6 protein levels (Spearman's $\rho = -0.24$, $p = 0.043$) but was not correlated with other cytokine protein levels (Supplementary Table S2).

3.6.3. Co-morbidities

BMI was not significantly correlated with any cytokine mRNA or protein levels (Supplementary Table 3). There were more individuals in schizophrenia and bipolar disorder who committed suicide, consumed more alcohol or drugs, and smoked more than controls (suicide status: $\chi^2(2) = 19.35$, $p = 6.30E\text{-}05$, alcohol use: $\chi^2(10) = 22.28$, $p = 0.01$, drug

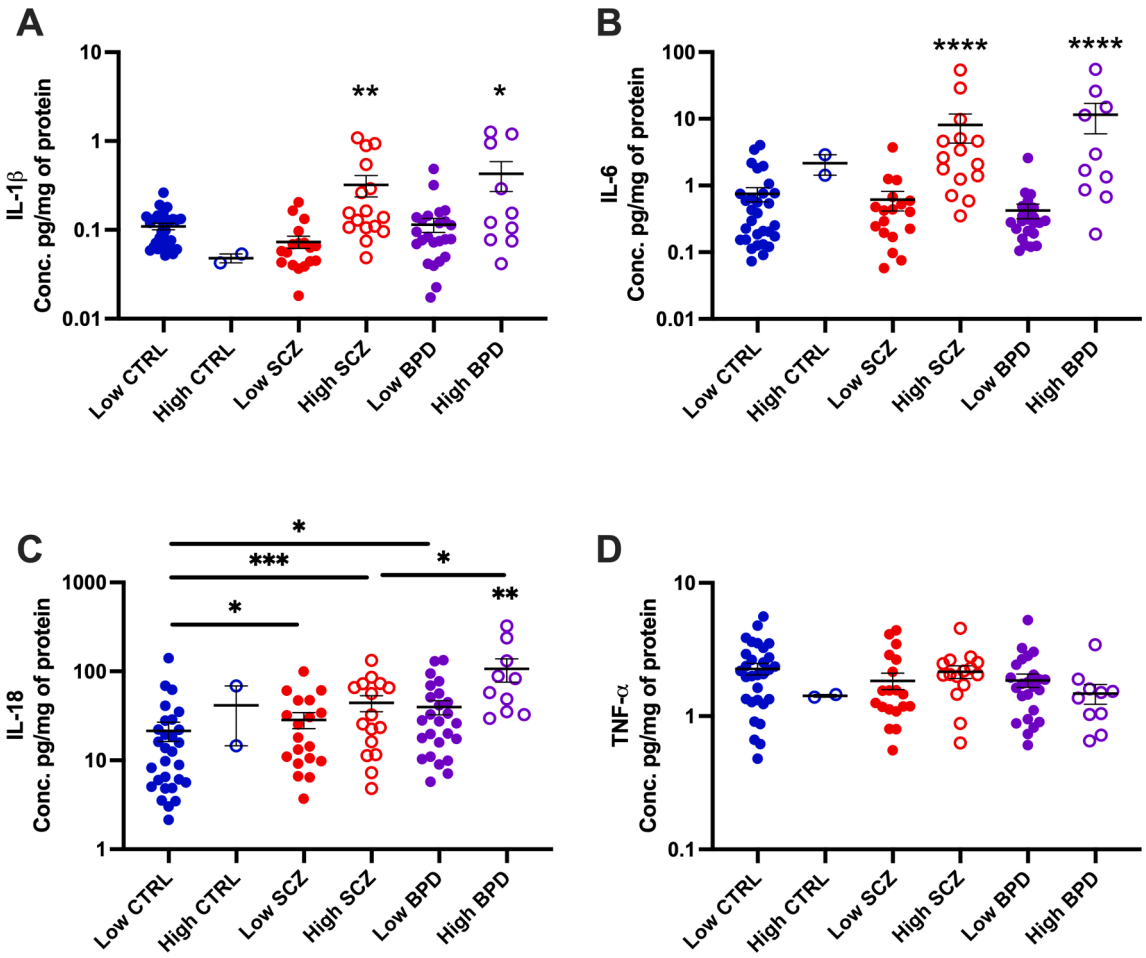

Fig. 5. Inflammatory cytokine protein levels in the post-mortem midbrain of schizophrenia, bipolar disorder and control cases across inflammatory subgroups. (A) IL-1β, (B) IL-6, and (C) IL-18 protein levels were significantly elevated in most high inflammation schizophrenia and high inflammation bipolar disorder subgroups relative to low inflammatory subgroups. (D) TNF-α did not show significant changes across all inflammatory subgroups. Note log scale on Y axis of all graphs. Bar denotes mean ± SEM, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. CTRL, Control (blue); SCZ, schizophrenia (red); BPD, bipolar disorder (purple). Closed circles, low inflammatory subgroups; open circles, high inflammatory subgroups.

use: $\chi^2(10) = 27.39$, p = 2.26E-03, smoking: $\chi^2(2) = 6.52$, p = 0.04) (Supplementary Fig. 4). There was no significant difference in terms of suicide status, lifetime alcohol or drug use, and smoking status between low and high inflammatory subgroups (suicide status: $\chi^2(1) = 0.27$, p = 0.60, alcohol use: $\chi^2(5) = 6.75$, p = 0.24, drug use: $\chi^2(5) = 2.95$, p = 0.71, smoking $\chi^2(2) = 0.20$, p = 0.65) (Supplementary Fig. 4).

4. Correlation of inflammatory marker gene expression with protein levels

The mRNA levels of cytokine *IL1B*, *IL6* and *IL18* were correlated with their protein levels, but TNF mRNA level was not correlated with TNF-α protein level (Spearman's ρ = -0.033, p = 0.742) (Table 3). The mRNA levels of IL1B and IL6 were positively correlated with IL-1β (Spearman's ρ = 0.234, p = 0.017) and IL-6 protein (Spearman's ρ = 0.730, p = 2.26E-18) levels respectively, while mRNA level of IL18 was negatively correlated with IL-18 protein level (Spearman's ρ = -0.516, p = 2.43E-8) (Table 3).

Table 3
Correlations of inflammatory marker gene expression with protein levels.

mRNA	Protein	rho	p value
IL1B	IL-1β	0.234	0.017
IL6	IL-6	0.730	2.26E-18
IL18	IL-18	−0.516	2.43E-8
TNF	TNF-α	−0.033	0.742

5. Discussion

Our findings confirm that almost half of the people with schizophrenia show increased inflammatory molecules – at both the transcript and protein level – in the midbrain, a region of prime importance to schizophrenia aetiology and pathology (Howes and Kapur, 2009). We also demonstrate that a substantial proportion (~1/3, 10/35) of people with bipolar disorder show similarly elevated levels of pro-inflammatory mRNAs in the midbrain. The current findings highlight the necessity for cohort stratification when analyzing inflammatory markers, since only 1 of the 7 pro-inflammatory transcripts measured differed by diagnosis alone, but 5/7 differed in high inflammatory schizophrenia and/or bipolar disorder subgroups relative to age-matched controls. Additionally, we found that lifetime antipsychotic exposure was positively correlated with 3 of the 4 mRNAs that distinguished 'high' from 'low' subgroups, which suggests a deleterious effect of these medications on midbrain homeostasis and/or a relationship between midbrain inflammation and psychiatric symptom severity that may necessitate the need for higher antipsychotic dose. Finally, transcriptional increases in IL1B and IL6 mRNAs in high inflammatory patient subgroups were also detected at the protein level, underscoring the biological impact of immune-related transcript changes in the midbrain of people with schizophrenia and bipolar disorder.

The finding that approximately 46% (16/35) of people with chronic schizophrenia show elevations in *SERPINA3*, *IL1B*, *IL6*, and *TNF* mRNAs in the midbrain relative to age-matched controls replicates our previous study in an independent post-mortem cohort using the same four transcripts to define 'high' and 'low' inflammatory subgroups (Purves-Tyson et al., 2021). Though we did not consider the high inflammatory control subgroup in subsequent analyses, this group contained only 2 individuals, which is also comparable to our previous finding of zero high inflammatory controls in a separate cohort (Purves-Tyson et al., 2021). As few control cases show elevations in these inflammatory transcripts, this might suggest a causal relationship between midbrain inflammation and psychiatric symptoms. Importantly, we also showed that individuals in the high inflammatory schizophrenia and high inflammatory bipolar subgroups had increased protein levels of IL-1β, IL-6 and IL-18 in the midbrain. Levels of cytokine mRNAs and proteins are not always correlated (Shebl et al., 2010; Du et al., 2014), which is likely due to the distinct regulatory control over cytokine mRNA and cytokine protein levels and the complex mechanisms of immunoregulation (Palanisamy et al., 2012). During inflammation, cytokine mRNA stability is increased and cytokine degradation is decreased to facilitate appropriate immune responses (Anderson, 2008). Thus, our finding that 2 of the elevated pro-inflammatory mRNAs were also elevated at the protein level in high inflammatory patients – and an additional cytokine IL-18 was not changed at the mRNA level but was increased at the protein level – supports a biologically relevant immune response occurring in the midbrain of at least some people with schizophrenia and bipolar disorder. However, since IL18 mRNA was negatively associated with IL-18 protein levels, this suggests that there may be some negative feedback occurring with the human brain. The biological activity of IL-18 is mostly regulated at the protein level but not at the transcription level (Puren et al., 1999). Indeed, when IL-18 protein is increased, IL18 mRNA is decreased in the process called counter-regulation as found in wound repair (Kämpfer et al., 1999) and in differentiating osteoblasts (Hori et al., 2006). Future studies of IL-18 regulation may help define the role of IL-18 signaling in schizophrenia and bipolar disorder. Interestingly, unchanged TNF-α protein levels were not consistent with the elevation in mRNA levels, which could relate to increased processing or proteolysis of TNF-α. There is evidence to suggest that proportions of the two forms of TNF-α, transmembrane (tmTNF-α) and soluble (sTNF-α), are differentially changed during inflammation and in psychiatric disorders with only tmTNF-α being elevated but not sTNF-α (Dean, 2013). Our measurement of TNF-α includes both forms of TNF-α which may not show any differences if the two forms change in different directions. The TNF-α signaling pathways involve multiple receptors including TNFR1, TNFR2 and the soluble forms of these receptors (sTNFR1, sTNFR2). It therefore seems that further studies into the different forms of TNF-α and TNF-α signaling targets may help to better define alterations in TNF-α protein in the midbrain of schizophrenia.

The substantia nigra is the primary location of dopamine synthesizing cells and is important for the control of movement, learning and motivation (Zhang et al., 2017) and given the relative efficacy of dopamine-targeting therapies in schizophrenia (specifically in the treatment of positive symptoms of schizophrenia), has often been assumed to be a locus of neuronal dysfunction in patients (Sonnenschein et al., 2020). Indeed, we find molecular evidence for midbrain dopamine dysregulation consistent with excess subcortical dopamine (including abnormal expression of factors influencing dopamine reception, transport and reuptake) and several others have found clinical evidence for increased dopamine synthesis capacity in the midbrain of patients (Purves-Tyson et al., 2017; Howes et al., 2012; Fusar-Poli and Meyer-Lindenberg, 2013; Howes et al., 2013). Increased striatal dopamine synthesis can even be found in some individuals in the schizophrenia prodrome (Howes et al., 2009), suggesting excess dopamine availability subcortically precedes the onset of psychosis. Inflammation can interfere with dopamine neurotransmission in many ways, including disrupted dopamine synthesis, packaging and release and increased reuptake, which purportedly contributes to many sickness-related behavioral and cognitive changes in rodents (Felger and Miller, 2012). In Parkinson's disease, which is a disease that involves dopaminergic neuron degeneration, cytokines from activated microglia may be neuroprotective during the initial stage but become neurotoxic with the progression of the disease (Sawada et al., 2006). Thus, it is plausible that symptom severity in schizophrenia and bipolar patients with midbrain inflammation may be linked to increased pro-inflammatory signaling in this region, which subsequently alters dopamine-dependent behavior. Markers of reactive astrocytes and reactive microglia are elevated in the midbrain of the high inflammatory subgroup of individuals with schizophrenia (Purves-Tyson et al., 2021), implicating glia as a putative cellular source of pro-inflammatory signals. Additionally, we have recently found evidence to suggest a higher number of macrophages in the midbrain of high inflammatory schizophrenia patients relative to low inflammatory patients and controls (Purves-Tyson, 2020). When we compared midbrain with the other brain regions, we determined the midbrain can be considered as an "inflammatory center" for the following reasons: 1) the change in inflammatory cytokines was of a greater magnitude in the midbrain as compared to the dorsolateral prefrontal (Fillman et al., 2013) and orbital frontal cortex (Zhang et al., 2016); 2) the percentage of cases falling into the high inflammation category was slightly higher in the midbrain compared to other regions (46% in the midbrain compared to 39% in other regions) and more discriminatory as compared to controls (overall 3% in the midbrain compared to 10% in other regions); 3) the midbrain is the only brain region that we clearly saw microglia increase in our previous studies (microglial markers appear suppressed in the prefrontal cortex (Zhu et al., 2022) and subependymal zone (SEZ) (Weissleder et al., 2021; North et al., 2021). Taken together, these findings point to the local production of immune mediators in the midbrain by both brain-resident and possibly blood-borne cells in schizophrenia and suggest that immune activation in this region contributes to symptomatology in patients.

It is also important to consider that patient exposure to antipsychotic medication may account for the higher occurrence of midbrain inflammation in people with schizophrenia and bipolar disorder compared to controls. We found higher lifetime antipsychotic exposure was associated with higher levels of *SERPINA3*, *IL1B* and *TNF* mRNAs and IL-1β and IL-6 protein levels in the midbrain of schizophrenia and bipolar disorder patients. We also found that bipolar disorder patients who had been prescribed antipsychotic medication during their lifetime were more likely to have midbrain inflammation than those who had never been prescribed antipsychotics. Antipsychotic drugs are actually reported to have anti-inflammatory effects on white blood cells and to reduce cytokine levels in the blood of people with schizophrenia (Al-Amin et al., 2013; Marcinowicz et al., 2021), though we and others have found positive correlations between different measures of antipsychotic exposure (mean daily dose and lifetime cumulative dose) and neuroinflammatory markers in the brain (Purves-Tyson et al., 2021; Murphy et al., 2020). This may point to divergent effects of antipsychotics on immune activity in the periphery versus the brain and is partially supported by *in vitro* findings of increased microglial and astrocytic reactivity upon treatment with antipsychotics (Bobermin et al., 2018; Quincozes-Santos et al., 2010; Cotel et al., 2015), though some antipsychotic drugs may induce stronger pro-inflammatory responses in glia than others (Bobermin et al., 2018; Quincozes-Santos et al., 2010) and may actually inhibit microglia-mediated inflammation (Zheng et al., 2008; Zhu et al., 2014). Taken together, it seems possible that antipsychotic drugs may play a role in causing midbrain inflammation. Alternatively, it may be that midbrain inflammation necessitates the use of higher doses of antipsychotics due to deleterious effects on dopamine-dependent brain function. Indeed, if antipsychotics were a major cause of midbrain inflammation, we might expect them to worsen symptoms of schizophrenia rather than alleviate them. The positive association between inflammatory markers and antipsychotic exposure,

therefore, could reflect a 'dose-response' relationship between increased inflammation and greater psychotic symptom severity that consequently requires higher doses of medication (rather than a causal effect of antipsychotics on midbrain inflammation). Moreover, midbrain inflammatory gene mRNA and proteins were sometimes positively correlated with duration of illness, suggesting midbrain inflammation may worsen with time in both schizophrenia and bipolar disorder. However, midbrain inflammation may occur early in the course of illness regardless of the duration, and higher levels of IL6 and SERPINA3 mRNAs were associated with an earlier age of onset suggesting that they may be related to clinical features. In terms of inflammatory comorbidities, inflammation was not related to BMI, suicide status, lifetime alcohol or drug use, smoking status, and diagnosis seems to have a greater association with these comorbidities compared to the possibility that these comorbidities are the sole reason for midbrain inflammation.

While we have confirmed the evidence that a subset of people with schizophrenia and bipolar disorder have elevated levels of inflammation in the midbrain, our study has several limitations. First, we did not measure the protein levels of all the immune transcripts, especially SERPINA3, and mRNA and protein levels of TNF do not correspond. Future studies need to determine how these changes in cytokine mRNA and protein relate to other features of immune activity. For example, in this study, we do not know how cytokine receptors may also be changed. Another limitation is that we do not know in what cells these increases in inflammation-related cytokines and SERPINA3 are occurring, or which cells may be targeted by inflammation. As for all post-mortem studies, the impact of antemortem effects and possible confounding factors cannot be excluded. Given that some of the inflammatory marker mRNA or protein levels correlated with lifetime antipsychotics in our study, we cannot rule out that some of these changes may be a direct result of antipsychotics and further studies of the impact of chronic antipsychotics on brain cytokines are needed, particularly in the mammalian midbrain.

Overall, our findings support that ~ 50% (16/35) of people with schizophrenia and ~ 30% (10/35) of people with bipolar disorder have increased levels of pro-inflammatory mediators at both the mRNA and protein levels in the midbrain. This increase in cytokines and the astroglial reactivity marker, SERPINA3 (Murphy et al., 2020), plausibly has downstream detrimental effects on the function of dopaminergic neurons and, in turn, on dopamine-dependent brain functions. Since inflammation of the midbrain appears to be more robust and more specific than inflammation in the cortex of patients with schizophrenia or bipolar disorder (Fillman et al., 2013; Fillman et al., 2014), targeting presumptive glial immune activation and/or immune cell infiltration in the midbrain specifically may be an important goal for the treatment of psychotic illnesses.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2022.06.012.

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