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Altered Brain Tissue Volumes With Valproate Treatment for Schizophrenia

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ABSTRACT

Schizophrenia is a debilitating psychiatric condition that affects ≈0.5% of the global population. Some people with schizophrenia do not respond to first-line antipsychotics and are prescribed clozapine; this is termed treatment-resistant schizophrenia (TRS). When clozapine is ineffective for patients with TRS, and concomitant antipsychotics or mood stabilisers are required, this is termed ultra-TRS (UTRS). Patients with UTRS are often prescribed augmentation therapy with mood stabilisers such as valproate to relieve residual symptoms. However, to date, the relationship between valproate and brain structure in people with schizophrenia has not been well described. Using magnetic resonance imaging, we found significant decreases in whole brain and white matter volumes in participants taking valproate compared to those taking clozapine without valproate. Further analyses revealed hypertrophy of grey matter in the cerebellum. This research indicates that using valproate augmentation in people with schizophrenia may alter white matter volumes and fractional anisotropy (FA) and grey matter density in the cerebellum and brainstem when compared to those receiving clozapine.

1 | Introduction

Schizophrenia is a chronic and debilitating psychiatric illness with a lifetime prevalence of ≈0.5% (Simeone et al. 2015). It is expected that 20%–30% of people prescribed first-line medications for schizophrenia will have a suboptimal response. These patients subsequently meet the criteria for treatment-resistant schizophrenia (TRS). A diagnosis of TRS means that a patient has failed to respond adequately to at least two different first-line antipsychotics (FLRs), including a long-acting injectable antipsychotic. Their condition must also be at least moderately severe and have moderate functional impairment for at least 6 to 12 weeks, as measured by a standardised rating scale (Howes et al. 2017).

The gold-standard antipsychotic for TRS is clozapine with proven efficacy (Siskind et al. 2016). Clozapine is not used as a first-line treatment for schizophrenia due to the high frequency of significant adverse effects such as agranulocytosis, myocarditis, gastrointestinal hypomotility, and seizures (Iqbal et al. 2003; Wong and Delva 2007). Unfortunately, only 30%–50% of patients experience significant symptomatic improvements in response to clozapine (Fitton and Heel 1990; Nucifora et al. 2017). If patients do not experience a significant reduction in their symptoms after taking clozapine, they are then diagnosed with ultra-TRS (UTRS). Unfortunately, there is no standard treatment for UTRS, which has prompted clinicians to trial augmentation strategies either with or without clozapine, including other antipsychotics, antiepileptics and mood stabilisers (Roerig 2019).

The FDA does not officially categorise any drug as a mood stabiliser (Bauer and Mitchner 2004). However, antiepileptics such as lamotrigine and valproate are sometimes used to manage mood and as maintenance treatment in people with bipolar

disorder (BD) (Calabrese et al. 1999; Miranda et al. 2019). Valproate has also been used as an effective augmentation strategy to manage the mood variability associated with TRS (Siskind et al. 2018). Approximately 8.5% of people with first-episode schizophrenia are prescribed valproate within 3 years of receiving first inpatient diagnosis (Puranen et al. 2020). Consequently, investigating the effects of valproate on brain structure in those with schizophrenia may further inform clinicians who use this combination.

Evidence suggests that when valproate is used to treat BD it may decrease the regional brain volumes of the amygdala (Chang et al. 2005; Cazala et al. 2018), frontal cortex (Li et al. 2019; Rodríguez-Ramírez et al. 2021) and gyri (Tondelli et al. 2020). For a more detailed view of altered brain tissues with valproate treatment, see Table S1. There are also numerous shared clinical features between BD and schizophrenia, including a decrease in total brain volume and cortical thickness (Madre et al. 2020) and reductions in intracranial grey and white matter volumes that coincide with increasing lateral ventricular volume (De Peri et al. 2012). However, many of these differences are more pronounced in people with schizophrenia (De Peri et al. 2012; Madre et al. 2020). Furthermore, prior research suggests that both schizophrenia and long-term antipsychotic drug treatment are associated with decreased brain volumes (Cahn et al. 2002; Honea et al. 2005; Steen et al. 2006; Navari and Dazzan 2009; Moncrieff and Leo 2010; Ho et al. 2011; Haijma et al. 2013; van Erp et al. 2016). However, the interpretability of these findings is limited by methodological inconsistencies across studies. Specifically, many studies did not adequately control for confounding factors such as duration of illness, duration of untreated psychosis, and symptom severity. While Cahn et al. (2002) controlled for all three factors, Haijma et al. (2013), Ho et al. (2011), Moncrieff and Leo (2010) and van Erp et al. (2016) controlled for only one or two. Honea et al. (2005), Navari and Dazzan (2009) and Steen et al. (2006) did not control for any of these factors. Several of these citations are meta-analyses (Haijma, Honea, Moncrieff, Navari, & Steen et al.), which, except for Moncrieff and Leo (2010), did not explicitly address the influence of these confounding variables, even if the original studies included in the meta-analyses may have done so. However, if the underlying studies were sound, this may not have been necessary.

This study aimed to investigate the use of valproate on brain structure in patients with schizophrenia. It was hypothesised that this augmentation would decrease regional and total loss of grey matter, white matter and whole-brain volumes independent from symptom severity in contrast to patients taking clozapine without valproate and people responding to FLR medication.

2 | Materials and Methods

2.1 | Participants

64 people diagnosed with schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders IV criteria (American Psychiatric Association, 2000) by their treating clinician were recruited either from a community mental health centre or a forensic psychiatric inpatient unit. Recruitment was conducted as part of a cross-sectional study seeking biomarkers of TRS (McIlwain 2013; Anderson et al. 2015). 22 mentally healthy control participants with no history of psychiatric or neurological disorders were recruited from the same geographic location. Patients with schizophrenia were separated into groups based on a good response to a FLR, responding well to clozapine (CLZ) or receiving an augmented therapy that included valproate (VAL). Participants in the VAL group had prior or current exposure to antipsychotics (Table 1), but at the time of recruitment none were undergoing electroconvulsive therapy.

All participants were aged between 18 and 45 years. Exclusion criteria included a history of any other Axis I disorder from the DSM-IV (i.e., any other clinical psychiatric disorder), significant physical disorders that were uncontrolled and may have impacted brain structure or function, active substance dependence and contraindications for MRI. 81 of the original participants met the inclusion criteria for this study, 75 were included in this analysis (Figure 1). Of the participants included in the analysis, 13 were female and were in similar proportion in each of the different analysis groups. The final number of participants in each analysis group was $n = 20$ for CON, $n = 17$ for FLR, $n = 25$ for CLZ and $n = 13$ for VAL.

The study was conducted at the University of Auckland, between March 2011 and July 2013 and was approved by the Northern × Regional Ethics Committee (Health and Disability Ethics Committee, New Zealand). Written informed consent was obtained from all participants.

2.2 | MRI Scanning Sequences

All participants underwent MRI using a Siemens 3T Skyra. A 32-channel head coil was used for all acquisitions except for 5 participants, where a 20-channel head coil was used (1 FLR, 3 CLZ, 1 VAL) due to a larger head size. Acquisition parameters for the T1-weighted MPRAGE structural scans were as follows: repetition time (TR) = 1900 ms; echo time (TE) = 2.39 ms; inversion time (TI) = 900 ms; flip angle 9°; one repetition; parallel imaging (GRAPPA) factor of 2; field-of-view (FOV) 230 × 230 mm; matrix = 256 × 256; resulting in 0.9 × 0.9 × 0.8 mm voxels. Three-dimensional gradient distortion correction was applied to images to correct nonlinear changes in the magnetic field that could lead to image warping.

Diffusion-weighted images were acquired using an echo planar imaging (EPI) sequence with the following parameters: TR 8900 ms, TE 95 ms, FOV 240 mm, 122 × 122 matrix, 2.0 mm slice thickness, isotropic voxel size $2.0 \times 2.0 \times 2.0\ mm^3$, and a GRAPPA factor of 2. 67 slices were acquired parallel to the anterior commissure-posterior commissure (AC-PC) line with diffusion-weighting factor $b = 1000 s/mm^2$ in 64 gradient directions. Eight scans without diffusion-weighting ($b = 0$) scans were acquired. Before assessing acquired scans via the image processing pipeline, scans were examined for artefacts, including subject motion, excess signal-to-noise and incomplete acquisitions.

2.3 | Data Analysis

Participant demographics were analysed using appropriate statistical tests based on variable type. Continuous variables (age, illness duration, duration of untreated psychosis, duration of relevant treatment and antipsychotic dosage) were analysed using one-way ANOVA, with post hoc Bonferroni-corrected t-tests applied whenever statistical significance was detected. The lone categorical variable, participant sex, was analysed using a chi-square test.

TABLE 1 | Demographic data of different schizophrenia treatment groups and controls.

Characteristic	Controls ($n = 20$)	First-line antipsychotics ($n = 17$)	Clozapine ($n = 25$)	Valproate ($n = 13$)	
Age	33.2 (8.4)	31.3 (7.5)	34.2 (7.9)	34.0 (8.2)	$F(3,74) = 0.51$, $p = 0.677$
Sex (M:F)	17:3	14:3	19:6	12:1	$\chi^2(3, N = 75) = 1.696$, $p = 0.638$
Illness duration (years)	—	9.8 (8.2)	12.5 (6.5)	10.3 (4.6)	$F(2,54) = 0.94$, $p = 0.397$
Duration of untreated psychosis (months)*	—	12.4 (15.1)	16.9 (22.2)	19.3 (21.8)	$F(2,51) = 0.433$, $p = 0.651$
Duration of relevant treatment (days)	—	1024.2 (1246.5)	1742.3 (1724.6)	672.9 (947.4)	$F(2,53) = 2.70$, $p = 0.077$
Current antipsychotic(s) used	—	Amisulpride ($n = 1$) aripiprazole ($n = 3$) olanzapine ($n = 8$) risperidone ($n = 5$)	Clozapine ($n = 15$) clozapine + amisulpride ($n = 3$) clozapine + aripiprazole ($n = 4$) clozapine + quetiapine ($n = 1$) clozapine + risperidone ($n = 2$)	Clozapine ($n = 5$) clozapine + amisulpride ($n = 1$) clozapine + quetiapine ($n = 1$) clozapine + risperidone ($n = 1$) olanzapine ($n = 1$) olanzapine + quetiapine ($n = 1$) quetiapine + aripiprazole ($n = 1$) risperidone ($n = 2$)	
Current antipsychotic medication(s) dosage (mg)	—	53.3 (116.3)	421.5 (192.0)	339.0 (280.2)	$F(2,54) = 18.01$, $\mathbf{p < 0.001}$ FLR < CLZ & VAL
Previous treatment(s)	—	Aripiprazole ($n = 2$) chlorpromazine ($n = 3$) clozapine ($n = 6$) haloperidol ($n = 2$) olanzapine ($n = 6$) quetiapine ($n = 5$) risperidone ($n = 7$) stelazine ($n = 1$)	Amisulpride ($n = 1$) aripiprazole ($n = 3$) chlorpromazine ($n = 4$) clozapine ($n = 1$) flupentixol ($n = 1$) flupentixol decanoate ($n = 1$) fluphenazine decanoate ($n = 1$) haloperidol ($n = 4$) olanzapine ($n = 16$) paroxetine ($n = 1$) quetiapine ($n = 5$) risperidone ($n = 16$) thioridazine ($n = 1$) venlafaxine ($n = 1$) ziprasidone ($n = 1$) zuclopenthixol ($n = 1$)	Amisulpride ($n = 1$) chlorpromazine ($n = 3$) clozapine ($n = 2$) flupentixol ($n = 3$) haloperidol ($n = 1$) olanzapine ($n = 9$) paroxetine ($n = 1$) quetiapine ($n = 2$) risperidone ($n = 9$) zuclopenthixol ($n = 1$)	

Notes: All data given as mean (SD) except sex and antipsychotics used. Bolded p values are statistically significant at $p < 0.05$. *Time prior to first psychiatric contact based on treating physician's notes and self-report.

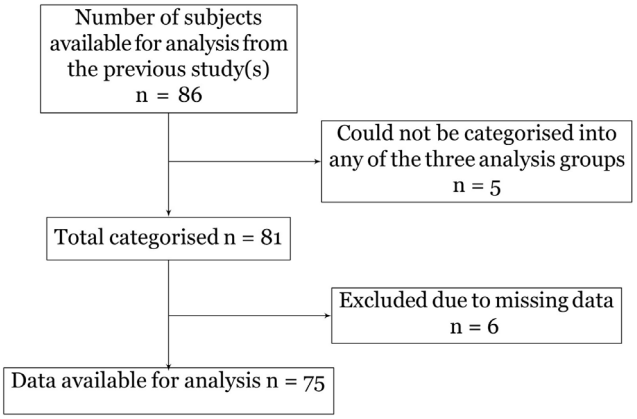

FIGURE 1 | A flowchart of participant inclusion and exclusion.

Structural MRI data were analysed using FSL version 6.0 (https://fsl.fmrib.ox.ac.uk/fsl/). All participants had optimal grey matter/white matter contrast and minimal artefacts. SIENAX was used to estimate the normalised tissue volumes of the whole brain, grey matter, white matter, peripheral grey matter (PGM) and ventricular cerebrospinal fluid (VCSF) (Smith et al. 2002). The SIENAX pipeline included brain tissue extraction using BET (Smith 2002), normalisation of this extraction by registration to the MNI152 standard space (Jenkinson and Smith 2001; Jenkinson et al. 2002), and creation of a standard brain image mask that excludes nonbrain tissues. Next, tissue-type segmentation with partial volume estimation was conducted (Zhang et al. 2001) to calculate the total volume of brain tissues normalised for head size (including separate estimates of volumes of grey matter, white matter, PGM and VCSF). The SIENAX results were analysed using a one-way between-subjects ANOVA to test for group-level significance within different tissue types, followed by post hoc t-tests to examine the degree of relevance between individual groups. The tissue types analysed for significant differences using SIENAX were grey matter, whole-brain volumes, PGM and VCSF.

Voxel-wise differences in grey matter volume between groups were analysed using FSL-VBM (Douaud et al. 2007). Structural images were brain-extracted, and the grey matter was segmented before being registered to MNI-152 2 mm standard space using nonlinear registration. The resulting images were averaged and flipped along the x-axis to create a left–right symmetric, study-specific template and modulated to correct for local expansion or contraction due to the nonlinear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Statistical analyses were performed to investigate voxel-wise differences in the grey matter density between groups using permutation-based nonparametric testing (5000 permutations) implemented using Randomise (FSL version 6.0). Threshold-free cluster enhancement (Smith and Nichols 2009) was used, and family-wise error-corrected values of $p < 0.05$ were taken to be significant. Results were analysed using a one-way between-subjects ANOVA to test for group-level significance across the brain, followed by post hoc t-tests to examine the degree of relevance between individual groups. The Harvard-Oxford cortical structural atlas was used to obtain the anatomical localisation of significant cluster peaks (Desikan et al. 2006).

Diffusion-weighted data were used to calculate and analyse fractional anisotropy (FA) values using tract-based spatial statistics (TBSS) (Smith et al. 2006), part of FSL (Smith et al. 2004). First, brain data were extracted from the image using BET (Smith 2002), and FA images were created by fitting a tensor model to the raw diffusion data using FMRIB's Diffusion Toolbox (FDT). All subjects' FA data were then aligned into a shared space using the nonlinear registration tool FNIRT (Andersson et al. 2007a, 2007b), which uses a b-spline representation of the registration warp field (Rueckert et al. 1999). A mean FA image was then created and thinned at a threshold of 0.2 to exclude low anisotropic regions, resulting in a mean FA skeleton representing the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton, and the resulting data was fed into voxelwise cross-subject statistics. Statistical analyses to investigate voxel-wise differences across the FA skeleton between groups were performed using permutation-based nonparametric testing (5000 permutations) implemented using randomise (FSL version 6.0). Threshold-free cluster enhancement (Smith and Nichols 2009) was used, and family-wise error-corrected values of $p < 0.05$ were taken to be significant. Results were analysed using a one-way between-subjects ANOVA to test for group-level significance across the white matter skeleton, followed by post hoc t-tests to examine the degree of relevance between individual groups.

3 | Results

3.1 | Demographics

All participants with schizophrenia were taking atypical antipsychotics and were clinically stable for at least 6 weeks before the investigation to minimise the impact of any acute relapses and medication modifications. No significant differences were identified across the groups when examining age, sex, illness duration, duration of untreated psychosis or duration of relevant treatment ($p > 0.05$). At the time of recruitment, people in the FLR group took significantly lower antipsychotic daily doses compared to the treatment-resistant cohort (Table 1). However, symptom severity, as measured by positive and negative symptom scores, did not differ significantly between the FLR ($M = 60.65$, SD = 13.19), CLZ (M = 59.54, SD = 11.88) and VAL ($M = 67.92$, SD = 13.41) groups following a one-way ANOVA ($F(2,59) = 1.91$, $p = 0.15$). The average dose of sodium valproate for people in the VAL group was 893 mg (SD = 423.29), with individual doses ranging from 400 to 1600 mg (Table S2). Information was not collected on hydration status or metabolic problems, but there was no reason to suspect this would alter the findings, as all participants had access to water before the imaging procedure, and any metabolic issues in patients were treated according to best practice guidelines.

3.2 | SIENAX

Significant effects were present across all SIENAX comparisons performed (Figure 2). An overall effect was observed across the four groups concerning whole-brain volumes ($F(3, 71) = 12.39$, $p < 0.0001$), where post hoc tests revealed that the whole-brain volumes of the VAL group were significantly smaller compared to CON ($p < 0.0001$), FLR ($p < 0.0001$) and CLZ ($p < 0.05$). For grey matter, an overall effect was also observed across groups ($F(3,71) = 13.50$, $p < 0.0001$), with the grey matter volumes of the VAL group significantly smaller than CON ($p < 0.001$) and FLR ($p < 0.0001$). However, no significant differences were

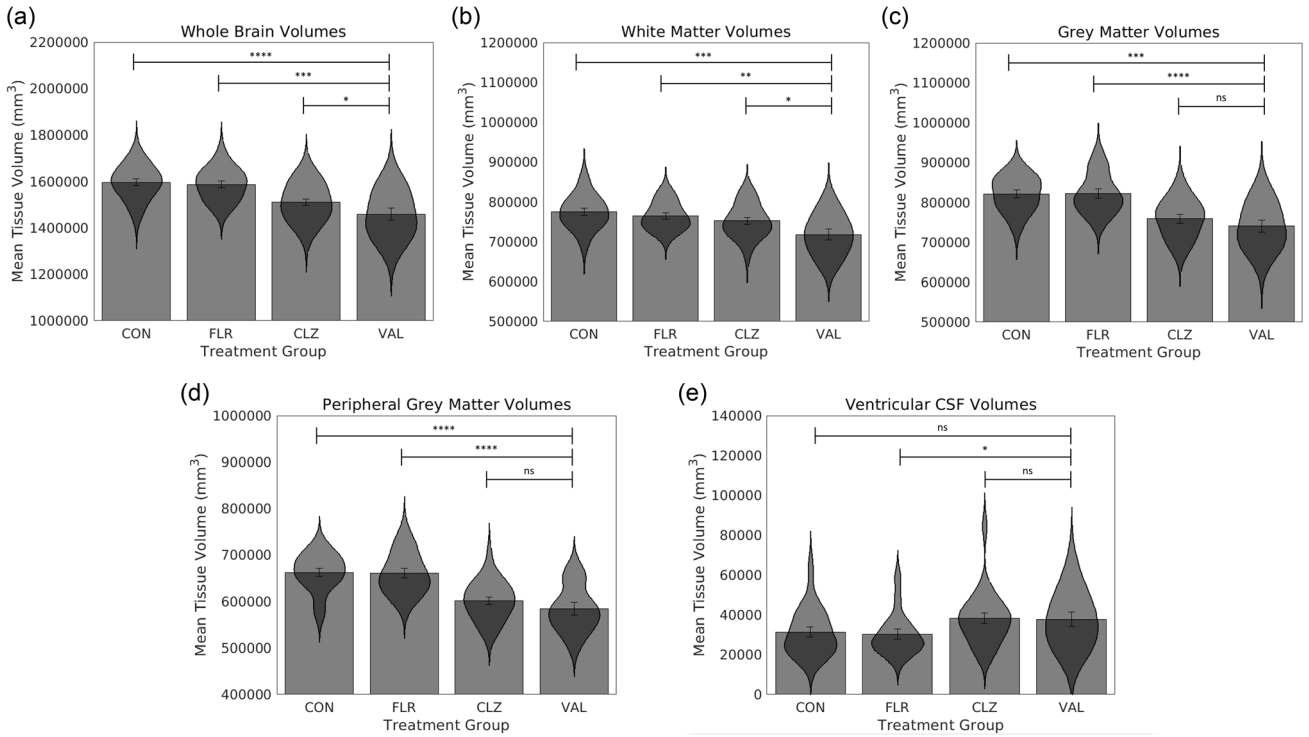

FIGURE 2 | Between-group comparisons of mean brain tissue volumes in controls (CON), first-line responders (FLR), those responding to clozapine (CLZ), and those augmented with valproate (VAL). (a) Whole-brain volumes, (b) white matter volumes, (c) grey volumes, (d) peripheral grey matter (PGM) volumes and (e) ventricular cerebrospinal fluid (VCSF) volumes. Results are expressed as means ± SEM. Statistical significance is denoted as **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, or ns for not significant. One-way ANOVA with post hoc t-tests was used for statistical analysis. For individual group means, see Table S3.

observed between VAL and CLZ concerning grey matter volumes ($p = 0.1418$). PGM volumes were also found to differ significantly across the four groups ($F(3,71) = 15.86$, $p < 0.0001$), where CON had larger PGM volumes than those of the VAL group ($p < 0.0001$), and the FLR group had substantially larger PGM volumes than the VAL group ($p < 0.0001$). No significant differences in size were found between CLZ and VAL ($p = 0.1248$).

Similarly, significant differences in white matter volumes were observed across the groups ($F(3,71) = 5.24$, $p = 0.0025$). CON had significantly larger white matter volumes compared to VAL subjects ($p < 0.001$), and FLR subjects also had substantially larger white matter volumes compared to VAL subjects ($p = 0.0025$). Significant differences were also found between the CLZ and VAL groups ($p = 0.0157$). Finally, no statistically significant differences in VCSF volumes were observed between the four groups according to a one-way ANOVA ($F(3,71) = 2.21$, $p = 0.0947$).

3.3 | Tract-Based Spatial Statistics

There were widespread significant decreases in FA between VAL and other groups (Figure 3). The differences were ubiquitous; no specific area had a noticeably more significant decrease in FA compared to any other location. There were no indications of any increases in FA across the contrasts. Furthermore, none of the other contrasts showed any increases or decreases in FA. The observed differences were specific to contrasts involving the VAL group, with no significant areas observed in the CON-FLR, CLZ-FLR and CON-CLZ contrasts.

3.4 | Voxel-Based Morphometry

Significant differences in regional grey matter densities were observed between each group using VBM (Figure 4). The between-subjects ANOVA yielded widespread areas of significant changes throughout the cortex, cerebellum and brainstem. Comparing localised grey matter differences between CON and those taking VAL demonstrated widespread atrophy throughout the cerebral cortex and hypertrophy in the subcortex and cerebellum. Significant changes were also observed in the frontal cortex and cingulate gyrus ($p < 0.0001$), where there was less grey matter in those taking VAL. Similarly, there were pockets of grey matter atrophy in those taking VAL in contrast to FLR. There was no hypertrophy in the cerebellum, but two areas showed increased grey matter in the midbrain and medulla oblongata. There were no significant differences in atrophy when comparing those taking CLZ in contrast to those taking VAL. However, the superior portion of the cerebellum in the right lobule VI and vermis VI shows hypertrophy ($p < 0.05$). There were no differences between CON and FLRs. However, when comparing the CON and CLZ groups, there were areas of increased grey matter in the frontal medial cortex extending into the paracingulate gyrus ($p < 0.01$) as well as the insular cortex and on bilateral hemispheres ($p < 0.05$). Increases were also detected in the lingual gyrus of the occipital lobe ($p < 0.05$). Similar differences were also found in the CLZ group compared to FLR's. Decreased grey matter density was identified in the frontal pole, superior frontal gyrus, the supramarginal gyrus of the right hemisphere, and the central opercular cortex of the left hemisphere ($p < 0.05$).

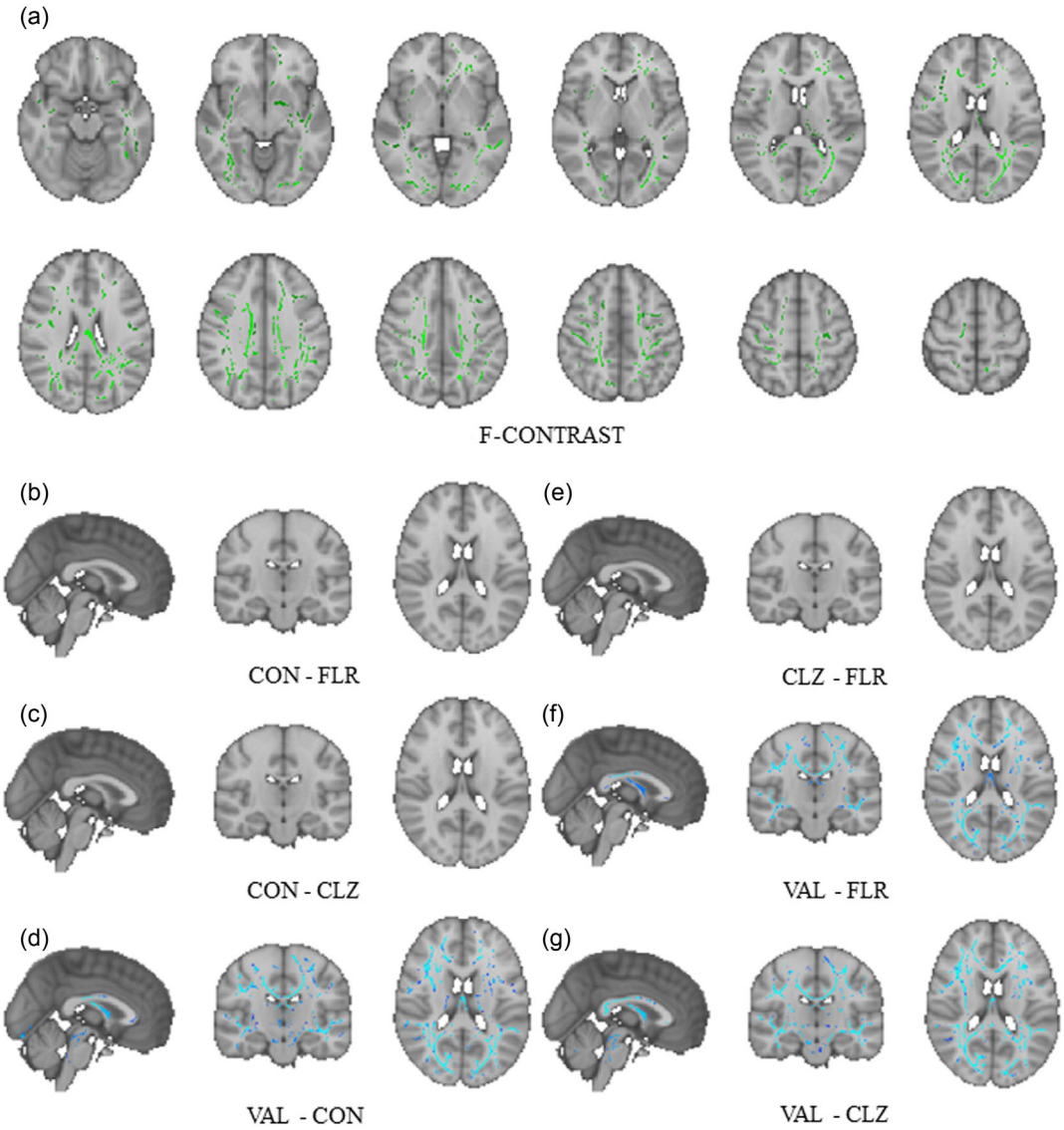

FIGURE 3 | Between group comparisons of regional significant fractional anisotropy changes in controls (CON), first-line responders (FLR), those responding to clozapine (CLZ) and those augmented with valproate (VAL). (a) Single factor ANOVA across all analysis groups. Green areas indicate zones of statistically significant differences. (b–g) Between-group t-test comparisons of fractional anisotropy values. Highlights indicate areas of significance, red-yellow indicates area(s) of increased volume and blue-light blue indicates area(s) of decreased volume ($p < 0.05$).

4 | Discussion

This preliminary research investigated differences in brain structure across three different levels of treatment for schizophrenia. All participants (except for controls) had a confirmed diagnosis of schizophrenia, were prescribed a stable dose of antipsychotics, and underwent a medication review to establish their past response to antipsychotics and confirm group allocations. Positive and Negative Syndrome Scale (PANSS) interviews were conducted to determine the severity of symptoms, and it was determined that each group displayed similarly low levels of residual symptoms.

Here, we observed structural brain differences between healthy controls, people responding to FLR medication, people taking clozapine for schizophrenia and people taking an augmentation therapy using valproate. Overall, the results of the SIENAX analysis revealed a tendency for reduced average brain tissue volumes from controls to FLR to CLZ to VAL (Figure 2). However, this study's main comparison of interest was between CLZ and VAL, where only white matter demonstrated a significant difference in average volume. These differences were further evident in the TBSS analysis, which revealed widespread decreases in white matter FA in each group in comparison to CON. These decreases are consistent with previous research in people taking valproate as treatment for epilepsy (Pardoe et al. 2013; Umlauf et al. 2023). This study has extended these findings by making similar observations in people taking valproate augmentation compared to controls, FLR and those with TRS.

While there was no difference in average grey matter volume in the VAL group, they did show localised increases in grey matter density in the brainstem and cerebellum compared to other groups. This could be due to potential compensatory mechanisms within the brain. These mechanisms may attempt to maintain normal function by counteracting the symptoms of schizophrenia or the side effects of any medications (Lewis and González-Burgos 2008; Paulzen et al. 2014). While there can be no direct evidence of this taking place, such compensatory mechanisms

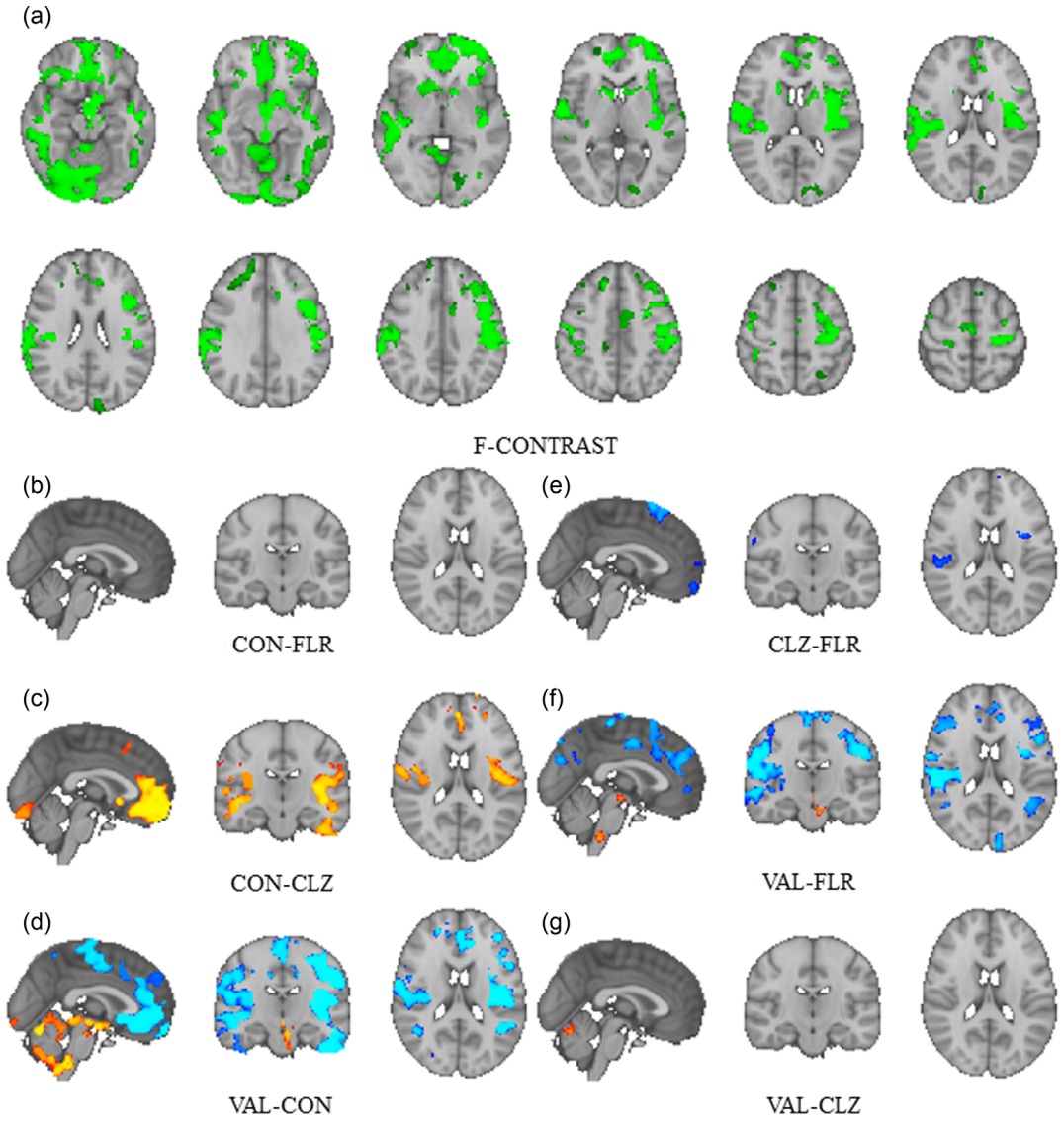

FIGURE 4 | Between-group comparisons of localised grey matter densities in controls (CON), first-line responders (FLR), those responding to clozapine (CLZ) and those augmented with valproate (VAL). (a) Single factor ANOVA across all analysis groups. Green areas indicate zones of statistically significant differences. (b–g) Between-group t-test comparisons of local grey matter volumes. Highlighted areas indicate areas of significance, red-yellow indicates area(s) of increased volume and blue-light blue indicates area(s) of decreased volume ($p < 0.05$; family-wide error corrected for multiple comparisons).

have been proposed to explain idiopathic grey matter hypertrophy observed in people with amblyopia (Lu et al. 2020), blindness (Voss et al. 2014), depression (Bansal et al. 2018), epilepsy (Zubal et al. 2025) and schizophrenia (Li et al. 2023; Palaniyappan 2023; Wang et al. 2024). Imaging studies in schizophrenia have identified altered cerebello-cerebral connectivity, sometimes interpreted as compensatory recruitment of cerebellar circuits (Guo et al. 2015), and consistent involvement of cerebellar subregions that are strongly integrated with cortical networks that support cognition (Li et al. 2022). Additionally, valproate can activate neurotrophic signalling pathways and has been associated with volumetric increases in cortical and subcortical regions (Hao et al. 2004; Nakamura et al. 2007). Taken together, the localised increases in cerebellar grey matter density in the VAL group might reflect a combination of medication-linked trophic effects and compensatory neuroplasticity. Longitudinal structural and connectivity analyses are needed to further investigate this potential mechanism.

Across all three comparisons, there was consistently an increase in tissue volume found within the cerebellum, an important centre for motor control. The observed change in the grey matter density of the cerebellum may represent an adaptive attempt to mitigate stereotyped movements associated with schizophrenia (Morrens et al. 2006). However, only minor cerebellar increases were apparent between the VAL and CLZ groups. While other studies have shown medication-associated hypertrophy in both the cortical and subcortical structures of people with schizophrenia compared to people who were not medicated (Chua et al. 2009; Gur et al. 1998), to our knowledge this is the first demonstration of hypertrophy of the cerebellum and brainstem in people taking valproate.

Furthermore, results from VBM analysis were generally consistent with expectations of atrophy in the cerebral cortex in the VAL group compared to CON and FLRs (Pardoe et al. 2013; Anderson et al. 2015; Li et al. 2019; Rodríguez-Ramírez et al. 2021; Shin et al. 2023; Umlauf et al. 2023). This is consistent with previous findings that have associated valproate with grey matter

atrophy in people with BD (Rodríguez-Ramírez et al. 2021). However, while the current findings further support the idea that valproate therapy may be associated with cortical grey matter loss generally, there was no significant atrophy when comparing the VAL and CLZ groups. Therefore, the underlying disease pathology may have a larger effect on grey matter volume than valproate use.

The structural brain differences observed here between VAL and CLZ groups may be because of valproate usage for schizophrenia. For this cohort, there were no outliers in the usage of valproate; 8 out of 13 participants were prescribed it within the recommended range of 1000–2000 mg per day (Medsafe 2023), and the five remaining participants had dosages of either 400 or 500 mg per day (Table S2). This means that any potential effects attributed to valproate could not be attributed to an unusually high dosage. However, a causative effect between valproate and brain changes cannot be made here, as this study is cross-sectional. Interestingly, longitudinal research has reported that the use of valproate over a 12-month period in people with mild to moderate Alzheimer's disease had an accelerated loss of brain tissue with a potentially more significant loss in cognitive function (Fleisher et al. 2011). Although this could be explained by the sedative properties of valproate (Wang et al. 2016). Given that schizophrenia, BD and Alzheimer's disease are all accompanied by brain tissue loss (Hulshoff Pol et al. 2002; Rimol et al. 2012; Scheltens et al. 2021), valproate augmentation may accelerate brain atrophy due to an unknown mechanism. However, this may not be the case, as other research has reported valproate promoting neurogenesis (Hao et al. 2004).

As mentioned previously, the combination of valproate and clozapine tends to be reserved for UTRS (Scruth 2018; Wang et al. 2016). Other researchers have shown that people with TRS have reduced GM volumes in the prefrontal cortex and other areas compared to those who are typically FLRs (Molina et al. 2008) and alterations in white matter microstructure associated with an increased likelihood of TRS (Kochunov et al. 2019; McNabb et al. 2020). Therefore, an increasing severity of grey matter and white matter reductions in the study groups could be representative of the growing severity of schizophrenia. Although, no difference in PANSS scores were found between the CLZ and VAL groups. If the observed results are attributed to disease severity, it is not to the extent that it results in a measurable difference in symptoms. It could also be the case that there is a difference in disease severity between groups, but that this difference is not reflected in the PANSS scores because the medications in the VAL group are working as intended to minimise residual symptoms. Alternatively, these findings might represent differences in disease duration and duration of untreated psychosis, which differ slightly but not significantly across groups (Table 1), as those who were eventually treated with valproate may have taken longer to have their symptoms be brought under control. An extended time without proper treatment may have led to greater tissue changes if these can be attributed to the disease.

Limitations are present in the current study. The current study is cross-sectional, and longitudinal study data before and after commencing valproate augmentation would allow for calculation of the rate of tissue loss and better allow for inferences to be drawn about causal relationships between structural brain changes, treatment resistance, and the effects of valproate augmentation. Due to the relatively small number of participants in each group, these should be interpreted as preliminary findings. As is typical in this patient population, diverse medication history precluded calculation of lifetime chlorpromazine equivalents. This introduces another level of uncertainty. Although treatment adherence is a concern for patients in the community, the majority of participants in the TRS and UTRS groups were inpatients in a forensic unit where they received prescribed medication and continuous care. We have confidence that these participants were treatment adherent.

5 | Conclusion

While numerous studies have investigated how valproate might induce changes in brain morphology in those with BD, this is the first study to examine its role in schizophrenia. These findings suggest that using valproate augmentation correlates with an overall decrease in white matter volume and FA and an increase in grey matter in the cerebellum compared to those taking clozapine alone. Further research is required to determine if valproate augmentation accelerates brain structural changes in those with schizophrenia or if it indicates a difference in the presentation of schizophrenia underpinned by differences in brain morphology, perhaps even a different disorder for which these findings may be a biomarker.

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Conflicts of Interest

Meghan Mcilwain is an employee of MSD New Zealand and engaged in this research as a private individual, not as a company affiliate. As such the principles, ideas and perspectives provided are the authors' own and not those of MSD New Zealand.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Table S1**: Prior Studies Investigating the Effects of Valproate on Brain Morphology. **Supporting Table S2**: Participant Valproate Dosage and Associated PANSS. **Supporting Table S3**: Summary of SIENAX Results Showing Mean Tissue Volumes for Each Analysis Group.