

## Title

Progressive Subcortical Volume Loss in Treatment-Resistant Schizophrenia Patients After Commencing Clozapine Treatment

## Authors

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1 *Abstract*

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The association of antipsychotic medication with abnormal brain morphometry in schizophrenia remains uncertain. This study investigated subcortical morphometric changes 6 months after switching treatment to clozapine in patients with treatment-resistant schizophrenia compared with healthy volunteers, and the relationships between longitudinal volume changes and clinical variables. 1.5T MRI images were acquired at baseline before commencing clozapine and again after 6 months of treatment for 33 patients with treatment resistant schizophrenia and 31 controls, and processed using the longitudinal pipeline of Freesurfer v.5.3.0. Two-way repeated MANCOVA was used to assess group differences in subcortical volumes over time and partial correlations to determine association with clinical variables. Whereas no significant subcortical volume differences were found between patients and controls at baseline( $F(8,52)=1.79$ ;  $p= 0.101$ ), there was a significant interaction between time, group and structure( $F(7,143)=52.54$ ,  $p<0.001$ ). Corrected *post-hoc* analyses demonstrated that patients had significant enlargement of lateral ventricles ( $F(1,59)=48.89$ ;  $p<0.001$ ) and reduction of thalamus ( $F(1,59)=34.85$ ;  $p<0.001$ ), caudate ( $F(1,59)=59.35$ ;  $p<0.001$ ), putamen ( $F(1,59)=87.20$ ;  $p<0.001$ ) and hippocampus ( $F(1,59)=14.49$ ;  $p<0.001$ ) volumes. Thalamus and putamen volume reduction was associated with improvement in PANSS ( $r=0.42$ ;  $p=0.021$ ,  $r=0.39$ ;  $p=0.033$ ), SANS ( $r=0.36$ ;  $p=0.049$ ,  $r=0.40$ ;  $p=0.027$ ) and GAF ( $r=-0.39$ ;  $p=0.038$ ,  $r=-0.42$ ;  $p=0.024$ ) scores. Reduced thalamic volume over time was associated with increased serum clozapine level at follow-up ( $r=-0.44$ ;  $p=0.010$ ). Patients with treatment-resistant schizophrenia display progressive subcortical volume deficits after switching to clozapine despite experiencing symptomatic improvement. Thalamo-striatal progressive volumetric deficit associated with symptomatic improvement after clozapine exposure may reflect an adaptive response related to improved outcome rather than a harmful process.

## 33 1. Introduction

34

35 Approximately 30% of patients with schizophrenia meet criteria to be considered treatment-  
36 resistant[1,2], usually defined as the failure to respond to at least two adequate trials of  
37 antipsychotic medication[3]. Clozapine has an established superior clinical effect to control  
38 symptoms in treatment-resistant patients, with 60-70% having a positive response[4,5].  
39 Patients treated with clozapine also often experience troublesome side effects including  
40 significant weight gain and lipid abnormalities[6], which notably have been associated with  
41 improvement in symptomatology[7,8]. Cross-sectional MRI studies of patients with treatment  
42 resistant schizophrenia (TRS) receiving clozapine and other antipsychotic medications have  
43 reported a range of brain abnormalities compared with controls, including reduced global  
44 grey matter[9,10], predominantly in frontal and temporal regions[11–13], and volumetric  
45 reduction of the amygdala and hippocampus[12,13].

46 The association of antipsychotic medication use with progressive brain deficits has  
47 been explored in longitudinal studies of schizophrenia[14,15]. These studies mostly use an  
48 observational rather than randomised design approach and thus cannot fully account for  
49 illness or service-related factors which influence clinician and patient medication choice. In a  
50 meta-analysis of longitudinal MRI studies based on 1155 patients with schizophrenia and 911  
51 healthy controls, Vita and colleagues[15] reported reduced cortical grey matter volume over  
52 time in patients which was related to cumulative exposure and mean daily dose of  
53 antipsychotic medications. Patients treated with first-generation antipsychotic (FGA)  
54 medications compared to second-generation antipsychotics (SGA) displayed more  
55 progressive grey matter loss, which correlated with higher mean daily antipsychotic dose.  
56 Likewise, van Haren and colleagues'[16] 5-year longitudinal study reported an association  
57 between higher cumulative dose of FGA over time and more marked cortical thinning, while  
58 higher dose of SGA in contrast was associated with less cortical thinning. However, patients  
59 who received clozapine treatment during the interscan interval showed more pronounced  
60 superior temporal cortical thinning compared with those not treated with clozapine. In  
61 contrast, in another analysis of this cohort, higher cumulative dose of clozapine during the  
62 interscan interval was related to attenuated loss of grey matter in the left superior frontal  
63 gyrus[17].

64           Longitudinal subcortical neuroimaging studies specifically of treatment-resistant  
65 clozapine-naïve patients are sparse, with small numbers of participants or without a matched  
66 control group. An early study of subcortical structures by Chakos and colleagues[18] based on  
67 15 patients, and without a control group, reported a 10% decrease in caudate volume after  
68 55 weeks, when switched from treatment with typical antipsychotic medications to clozapine.  
69 In contrast, patients who stayed on typical antipsychotic medications displayed an 8%  
70 enlargement in the caudate. In another study of 26 patients by Scheepers and colleagues[19]  
71 volume reduction of caudate nucleus was identified after 24 weeks of treatment with  
72 clozapine. There was no neuroanatomical correlation with clinical response. In the same  
73 cohort, after 52 weeks of treatment, reduced volume of the left caudate was greater in  
74 patients who responded to treatment compared to non-responders[20]. Another small study  
75 with 8 patients and 8 controls reported reduced caudate volume after 2 years of treatment  
76 with clozapine, with analogous results for the putamen, which was not statistically  
77 significant[21]. Thus, these early studies consistently indicate that switching patients from  
78 FGA medication to clozapine is associated with a decrease of caudate volume over time, and  
79 has generally been interpreted as a correction by clozapine of caudate hypertrophy induced  
80 by FGA medication due to their potent dopamine blockade and the high concentration of  
81 dopamine receptors in the caudate[22]. However, nowadays most patients are already taking  
82 SGA medications prior to clozapine commencement and it remains unclear whether switching  
83 to clozapine in such circumstances would have a similar effect on the basal ganglia.  
84 Furthermore, other subcortical structures such as the hippocampus and thalamus have not  
85 been investigated in longitudinal studies of switching to clozapine.

86           Given the importance of identifying factors predicting response to clozapine, the  
87 association of clinical response with baseline alterations in subcortical structures has also  
88 been studied, with conflicting results. In a randomised controlled trial by Arango and  
89 colleagues[23], whereas larger right prefrontal cortex predicted improvement in SANS scores  
90 compared with haloperidol treated patients, there was no such association between clinical  
91 symptom change and caudate or hippocampal volume at baseline. Smaller hippocampal  
92 volume compared to healthy controls at baseline predicted improvement in disorganised  
93 symptoms over time in a longitudinal study by Molina and colleagues[24]. In another  
94 longitudinal study, decreased left caudate volume over time was related to a significant  
95 improvements in positive and general symptoms, but not negative symptoms[20].

96           We have previously investigated cortical anatomy in a sample of patients before and  
97 after switching to clozapine in comparison to healthy volunteers[25], and demonstrated on-  
98 going cortical thinning in TRS patients over a 6 month period, in particular for younger  
99 patients. The present study, using a unique sample of treatment-resistant clozapine-naïve  
100 schizophrenia patients, offers a novel opportunity to comprehensively investigate whether  
101 subcortical structures demonstrate progressive neuroanatomical changes after 6 months of  
102 clozapine treatment and whether any such changes are related to clinical variables including  
103 treatment response and amount of clozapine taken.

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126        2. Method

127        *2.1 Participants*

128        As previously reported[25] 39 patients with treatment-resistant schizophrenia (TRS) prior to  
129        clozapine initiation and 40 healthy volunteers (HC) were initially recruited for the baseline  
130        assessment. At the follow-up, 33 patients, after 6 months of treatment with clozapine and a  
131        total of 31 healthy controls, matched for sex and age, were successfully re-recruited, scanned  
132        and assessed (Table 1). Patients were included if aged 18-60 years and clinically due to switch  
133        to clozapine because of treatment resistance. Patients and controls were excluded from the  
134        study if they had a previous trial of clozapine treatment, a learning disability, history of  
135        neurological illness, history of head injury which resulted in loss of consciousness for over 5  
136        minutes, treatment with oral steroid in the three months prior to participation, history of  
137        comorbid alcohol/ substance dependency as defined by the DSM-IV criteria or any  
138        contraindication to MRI scanning. Exclusion criteria for controls also included a current or past  
139        axis I mental disorder or any psychotic disorder in a first-degree relative. The study was  
140        approved by the Clinical Research Ethics Committee, Galway University Hospitals. Fully  
141        informed written consent was obtained for all participants.

142        *2.2 Clinical assessment*

143        All patients were diagnosed using the Diagnostic and Statistical Manual for Mental Disorders  
144        4th Edition text revision (DSM-IV-TR) (American Psychiatric Association, 2000). Treatment  
145        resistance was defined as the failure to respond to at least two adequate trials of  
146        antipsychotic medications, including at least one atypical antipsychotic drug, with a prolonged  
147        period of moderate to severe positive and/or negative symptoms[26]. The severity of positive  
148        and negative symptoms was assessed at both time points using the Positive and Negative  
149        Syndrome Scale (PANSS)[27], the Scale for the Assessment of Positive Symptoms (SAPS)[28]  
150        and the Scale for the Assessment of Negative Symptoms (SANS)[29]. Social, occupational and  
151        psychological functioning was assessed using a Global Assessment of Functioning Score[30].  
152        We used the symptomatic remission criteria of Andreasen[31] with the exclusion of the  
153        maintenance over 6-month observation period[32]. Remission at the 6 month follow-up  
154        assessment was therefore defined as having scores of mild or less (item scores of  $\leq 2$  using the  
155        0-6 range) on all eight of the following PANSS items: delusions (P1), conceptual

156 disorganisation (P2), hallucinatory behaviour (P3), blunted affect (N1), social withdrawal (N4),  
157 lack of spontaneity (N6), mannerisms / posturing (G5), unusual thought content (G9).

### 158 *2.3 MRI data acquisition*

159 MRI images were acquired for all participants at baseline and after 6 months at University  
160 Hospital Galway in a 1.5 Tesla Siemens Magnetom Symphony scanner (Erlangen, Germany)  
161 equipped with a 4-channel head coil. A magnetisation prepared rapid gradient echo  
162 (MPRAGE) sequence was acquired to generate high resolution volumetric T1-weighted  
163 images, with the following parameters: repetition time (TR): 1140 ms, echo time (TE): 4.38  
164 ms, inversion time (TI): 600 ms, flip angle: 15°, matrix size: 256x256, interpolated to 512 x  
165 512, slice thickness: 0.9 mm and in-pixel resolution: 0.45 mm<sup>2</sup>

### 166 *2.4 MRI processing*

167 Volumetric T1-weighted images used in the analyses were intensity inhomogeneity corrected  
168 using non-parametric, non-uniform intensity normalisation (N3)[33] as previously  
169 reported[25,34]. Eight subcortical regions-of-interest: lateral ventricle, thalamus,  
170 hippocampus, caudate, putamen, globus pallidus, amygdala and nucleus accumbens, were  
171 bilaterally segmented using the longitudinal pipeline of Freesurfer v.5.3.0[35,36]. Specifically,  
172 this technique is based on an unbiased within-subject anatomical template[35], created using  
173 a robust and inverse consistent registration method[37], is able to overcome the limitations  
174 of longitudinal processing methods. It reduces the risk of underestimating change, giving an  
175 unbiased estimation of the neuroanatomical structure volume over time, removing  
176 asymmetry-induced processing bias and avoiding over-regularization or temporal  
177 smoothness constraints[35]. This technique has also sufficient sensitivity and reliability for  
178 small sample sizes[35]. The several steps of the processing pipeline to obtain the output have  
179 previously been described in detail[38]. Intracranial volume (ICV), is computed by dividing a  
180 predetermined constant with the factor by which the input magnetic resonance (MR) images  
181 are scaled in size to align to the MNI305 head atlas[39–41]. At each time point, quality check  
182 of the segmentation output was performed, which involves a visual inspection at each of the  
183 analysis stages, to verify that the segmentation was anatomically accurate and  
184 computationally successful[42]. Six images failed the quality check and required manual  
185 editing using control points to fix intensity normalization[43]. Following quality check and

186 manual editing, no images were excluded. Subsequently subcortical volumes were bilaterally  
187 extracted and summed together to obtain one measure for each ROI.

## 188 *2.5 Statistical analysis*

189 Statistical Package for the Social Sciences version (SPSS Inc., v23, IBM, New York, USA) was  
190 used to carry out all analyses. The Shapiro-Wilks Test was used to test for normal distribution  
191 of demographics, clinical, neuroanatomical and anthropomorphic variables, with outliers  
192 defined as greater or less than 3 standard deviations from the mean. Age, gender and time  
193 between scanning were compared between groups using either a T-test, Chi-square or Mann-  
194 Whitney U Test. Differences between baseline and follow-up on clinical variables and  
195 anthropomorphic measurements were tested using the Wilcoxon Signed Ranks and Paired-  
196 Sample T-test. An initial one-way Multivariate analysis of covariance (MANCOVA) was  
197 performed to evaluate differences between groups at baseline on the eight subcortical  
198 structures, covarying for age, sex and ICV. *Post-hoc* analyses were performed to assess  
199 differences at baseline on the 8 subcortical structures between controls and patients  
200 previously treated with atypical and/or typical medications. Thereafter two-way repeated  
201 MANCOVA was used to assess the course of changes in volume of subcortical structures over  
202 time between groups, covarying for age, sex and ICV. The group-by-age interaction was used  
203 to determine the effect of age on anatomical change between groups over time. *Post-hoc*  
204 analysis, corrected for multiple comparison (Bonferroni,  $\alpha= 0.006$ ) was carried out to clarify  
205 which regions were significantly changing over time. An additional one-way MANCOVA and  
206 subsequently a two-way repeated MANCOVA was performed to assess differences between  
207 clozapine responders and non-responders at baseline and over time on subcortical structures,  
208 covarying for age, sex and ICV. Partial correlations were carried out controlling for the  
209 potential influence of age, sex and ICV on the relationship between the subcortical brain  
210 regions which showed a significant change over time ( $\frac{Follow-up - Baseline}{Baseline} \times 100$ ) and  
211 change in PANSS, SANS, SAPS and GAF (*Follow-up-Baseline*)[10]. These correlations were  
212 hypothesis driven and not corrected for multiple comparison. Pearson correlation analyses  
213 were performed to explore the relationship between subcortical structures showing a  
214 significant change over time in TRS patients and the variables age, duration of illness, body  
215 mass index (BMI), daily dose and serum level of clozapine at follow-up.



## 216 3. Results

### 217 3.1 Clinical characteristics

218 Patient and control groups did not differ across age, sex, and time between scans (Table 1).  
219 Patients after treatment with clozapine displayed a substantial and statistically significant  
220 improvement in each symptom and function rating scale. At follow-up, patients also displayed  
221 a significant increase of weight, waist, body mass index, total cholesterol and triglycerides  
222 compared to baseline (Table 2). Twelve patients had previously been prescribed typical  
223 antipsychotic drugs and 5 were still taking FGA medications at the point of the baseline scan.  
224 At baseline before switching to clozapine, 21 patients were on monotherapy with one SGA  
225 medication (olanzapine=7, quetiapine=4, aripiprazole=4, amisulpiride=1, paliperidone=1,  
226 risperidone long acting injection=1), 10 patients were treated with two antipsychotic  
227 medications (olanzapine + another antipsychotic=7), with one patient treated with three and  
228 another patient treated with four antipsychotic medications. At follow-up 16 patients (48%)  
229 were in remission.

### 230 3.2 Differences between groups on subcortical regions at baseline and over time

231 There was no significant difference between TRS patients and controls at baseline (n=33 TRS;  
232 n=31 HC) when considering jointly the 8 subcortical structures and taking account of multiple  
233 comparisons ( $F(8,52)=1.79$ ;  $p=0.101$ , Table 3). We also assessed for differences in subcortical  
234 structures at baseline in the larger initially recruited sample (n=39 TRS; n=40 HC). Volumetric  
235 changes in structures such as hippocampus and lateral ventricles did not survive overall  
236 multiple comparison correction ( $F(8,66)=1.82$ ;  $p=0.088$ , Suppl. Table 1), but were in keeping  
237 with the effects sizes (circa 0.5) identified for such structures in larger case control samples  
238 of patients with schizophrenia[44]. However, a strong significant overall interaction between  
239 time, group and brain structure was demonstrated ( $F(7,143)=52.54$ ;  $p<0.001$ , Table 3). *Post-*  
240 *hoc* analyses, robustly corrected for multiple comparison (Bonferroni,  $\alpha=0.006$ ), revealed a  
241 significant volumetric increase in lateral ventricle ( $F(1,59)=48.89$ ;  $p<0.001$ , Figure 1A) and  
242 decrease in thalamus ( $F(1,59)=34.85$ ;  $p<0.001$ , Figure 1B), caudate ( $F(1,59)=59.35$ ;  $p<0.001$ ,  
243 Figure 1C), putamen ( $F(1,59)=87.20$ ;  $p<0.001$ , Figure 1D) and hippocampus ( $F(1,59)=14.49$ ;  
244  $p<0.001$ , Figure 1E) volumes for patients compared to healthy controls (Table 3). The relative  
245 consistency of the progressive volumetric changes in the patient cohort is apparent from the

246 individual level data points displayed in Supplementary Figure 1. There was no significant  
247 group-by-age interaction on the progression of the subcortical structures between patients  
248 and controls ( $F(84,112)= 1.13$ ;  $p=0.272$ ). *Post-hoc* analysis revealed no significant differences  
249 at baseline between controls and patients previously treated with atypical and/or typical  
250 medications when considering the 8 subcortical structures ( $F(8,16)=1.49$ ;  $p= 0.117$ ).

### 251 *3.3 Response to clozapine and subcortical changes at baseline and over time*

252 When investigating the baseline differences between those who remitted on clozapine  
253 treatment ( $n=16$ ) and non-responders ( $n=17$ ) for the 8 subcortical structures, no significant  
254 differences were revealed ( $F(8,21)=1.32$ ;  $p=0.286$ ). Likewise, there was no significant overall  
255 effect of time on subcortical brain structures between patients responding to clozapine  
256 compared to patients non-responders ( $F(7,20)=0.50$ ;  $p=0.834$ ).

### 257 *3.4 Correlation between neuroanatomy and clinical variables in treatment-resistant patients*

258 In TRS patients, when covarying for age, sex and ICV, volumetric reduction of thalamus and  
259 putamen over time were significantly associated with improvement in PANSS Total score  
260 ( $r=0.42$ ,  $p=0.021$ ;  $r=0.39$ ,  $p=0.033$ , respectively Figure 2A) and improvement in negative  
261 symptoms assessed with the SANS scale ( $r=0.36$ ,  $p=0.049$ ;  $r=0.40$ ,  $p=0.027$ , respectively Figure  
262 2B). Similarly, improvement in PANSS General score was significantly related to decreased  
263 volume in thalamus over time ( $r=0.39$ ;  $p=0.034$ ). Controlling for serum clozapine level at  
264 follow-up and duration of illness did not impact on the above findings, however improvement  
265 of GAF was additionally found to relate to reduced thalamic ( $r=-0.39$ ;  $p=0.038$ ) and putaminal  
266 ( $r=-0.42$ ;  $p=0.024$ ) volume (Figure 2C). Improvement in SAPS was associated with reduced  
267 putaminal volume ( $r=0.39$ ;  $p=0.035$ ), but this association weakened slightly and lost  
268 significance ( $r=0.31$ ;  $p= 0.102$ ) when removing one outlier who demonstrated a 76%  
269 improvement in positive symptoms. No other associations were found between change in  
270 other subcortical brain structures and clinical variables (Suppl. Table 2).

271

272

### 273 *3.5 Exploratory analyses between structures showing significant change over time in patients* 274 *and treatment-related factors.*

275 When exploring the association between changes over time in subcortical structures and  
276 treatment related factors in patients, including BMI change, and serum clozapine at follow-  
277 up, a significant association was identified between reduced volume of the thalamus over  
278 time and increased clozapine serum level at follow-up ( $r=-0.44$ ;  $p=0.010$ , Figure 2D), with this  
279 correlation strengthening ( $r=-0.49$ ;  $p=0.010$ ) when controlling for change in clinical symptoms  
280 (PANSS, SAPS, SANS) and functioning (GAF).

281

#### 282 **4. Discussion**

283 To the best of our knowledge, this is the largest sample to date to examine the effects of  
284 switching to clozapine on subcortical regions in a relatively clinically homogenous sample of  
285 TRS patients using a longitudinal semi-automated subject-specific approach (Freesurfer  
286 v.5.3.0)[36]. In this longitudinal study, minor subcortical differences were detected between  
287 patients and controls at baseline, which failed to survive multiple comparisons correction.  
288 However, we identified substantial progressive volumetric reduction of the thalamus,  
289 hippocampus, caudate, putamen and enlargement of lateral ventricles over a 6-month period  
290 in patients compared to controls. Reduced caudate volume over time has been consistently  
291 reported in the majority of studies of patients switched from typical antipsychotic  
292 medications to clozapine[18–21] and has been interpreted as reversal of previous  
293 enlargement due to excessive dopamine blockade. Consistent with this, longitudinal studies  
294 demonstrate basal ganglia enlargement when taking typical medications was reversed by  
295 switching to atypical antipsychotic medications[45,46]. Reduction of thalamic volume over  
296 time was also reported in a 5-year longitudinal voxel-based morphometry study[17].  
297 However, no association has previously been reported between cumulative doses of  
298 clozapine and subcortical deficits. The hippocampal progressive reduction identified in this  
299 cohort on switching to clozapine has not been previously reported, but notably the direction  
300 of change is in keeping with the other subcortical structures. The lateral ventricle enlargement  
301 over time could be interpreted as ventricular expansion as a result of the significant reduction  
302 of surrounding subcortical regions[47]. The degree of volumetric change in this cohort after 6  
303 months in regions such as the hippocampus and lateral ventricles is comparable to the rate  
304 of change detected in previous longitudinal studies over longer time periods [48,49]. The high  
305 density of dopamine D2 receptors[50] in basal ganglia and other structures such as thalamus

306 and hippocampus, renders them major targets to which dopaminergic pathways project[51].  
307 In a preclinical study, Guma and colleagues[52], presented evidence that D2 receptors play a  
308 significant role in mediating antipsychotic induced structural changes, whereby volumetric  
309 reduction in cortical areas, hippocampus and thalamus, was induced by genetic deletion of  
310 D2 receptors.

311 Our study did not detect any difference in subcortical structures between those who  
312 achieved clinical remission with clozapine treatment and non-responders, either at baseline  
313 or over time, consistent with some previous studies[9,19]. In one longitudinal study of  
314 patients (which did not include a control sample), responders showed a significant reduction  
315 in left caudate volume after 24 weeks of clozapine treatment[20].

316 These results lead us to speculate on three reasons for the lack of significant baseline  
317 subcortical volume deficits in patients compared with controls in this cohort and the  
318 subsequent marked progressive volume loss over time after commencing clozapine. (i) Direct  
319 effects of clozapine treatment, (ii) withdrawal of prior treatment with other medications, or  
320 (iii) illness progression independent of medication use.

321 (i) This cohort of TRS patients may be a categorically different illness subtype with  
322 different underlying mechanisms and pathophysiology compared with D2 receptor antagonist  
323 responsive schizophrenia[53,54]. Lack of the striatal dopaminergic elevation in TRS, typical in  
324 schizophrenia could explain why treatment with dopamine antagonists are ineffective as they  
325 target the wrong processes[55]. Abnormal glutamatergic function, with higher glutamate +  
326 glutamine level concentrations have been reported in TRS compared to first-line  
327 responders[54,56]. Indeed it has been suggested that clozapine's efficacy might relate to its  
328 ability to attenuate glutamate release, as demonstrated in preclinical studies[57]. In our  
329 cohort the previous lack of symptomatic response to typical and atypical antipsychotic  
330 medications may have related to relative subcortical volume preservation compared with  
331 healthy controls. Hence, the subcortical volume loss after commencing clozapine treatment  
332 may directly have been related to clozapine efficacy[19]. Indeed, cross-sectional studies on  
333 neuroanatomy of TRS patients are usually on patients already receiving clozapine, and  
334 demonstrate reduction of cortical and subcortical volumes[9,12,13], as we see at the follow-  
335 up point in our study when patients are on clozapine treatment. It may also be that acutely  
336 symptomatic phase of illness is linked to increased neuroinflammation which has been

337 associated with increases in local blood flow, vascular permeability, microglia activation and  
338 extracellular volume[58] . In this scenario, successful treatment with clozapine might have  
339 resulted in an anti-inflammatory process[59] that reversed these inflammatory changes,  
340 resulting in subcortical volume reduction.

341 (ii) Prior exposure of this cohort to antipsychotic medications over the years might  
342 have ameliorated or corrected disease-related volume loss[15,16,44,60], which may explain  
343 our finding of only minor baseline volume differences. Interestingly unmedicated patients  
344 have been reported to display greater subcortical deficits, especially of the caudate and  
345 thalamus, compared to medicated patients[44,61]. On this interpretation, the progressive  
346 brain volume change of subcortical structures on switching to clozapine treatment might have  
347 been related to the withdrawal of other atypical antipsychotic medications. The  
348 neurobiological mechanism that underlies the progressive volumetric loss of subcortical  
349 structures is still unknown, however neural apoptosis, necrosis, synaptic pruning might play  
350 a role in producing volume deficits[62].

351 (iii) The progressive volume loss of subcortical structures in patients revealed by  
352 scanning over two time-points was not associated with pharmacotherapy, but rather to the  
353 underlying pathophysiology of this malignant form of schizophrenia illness and/or other  
354 illness-related factors which were not present in controls. However, this explanation seems  
355 unlikely since patients in our cohort have a mean illness duration of 13 years and only some  
356 were in the early stages of illness.

357 The progressive loss of volume in subcortical structures despite symptomatic and functional  
358 improvement suggests that volume loss as detected by neuroimaging in vivo in our cohort  
359 should not be necessarily interpreted as harmful to patients. Although cognitive impairment  
360 has been related to cortical thinning or volume reduction in schizophrenia[63–65], grey  
361 matter loss has been associated with greater response to atypical antipsychotics[66,67].  
362 Moreover, cortical thinning in first-episode schizophrenia patients on pharmacotherapy has  
363 been associated with physiological and cognitive improvement[68]. Consistent with this,  
364 progressive volumetric reduction of putamen and thalamus was significantly associated with  
365 better response to clozapine. This result was unaltered after controlling for the serum level  
366 of clozapine and duration of illness. Interestingly Scheepers and colleagues, reported an  
367 association between clinical improvement in positive and general symptoms and reduction of

368 left caudate volume, in TRS patients[20]. Molina and colleagues, in a 2 years randomised  
369 clinical trial of clozapine on 17 neuroleptic-naïve patients with schizophrenia and 11 controls,  
370 have shown that inferior frontal thinning, specifically, pars orbitalis, opercularis and  
371 triangularis, was positively associated with better clinical and cognitive response to clozapine  
372 [69].

373 We also found that patients who were exposed to higher amounts of clozapine displayed a  
374 greater reduction of thalamus volume, this association was further reinforced when  
375 controlling for clinical symptoms and functioning, suggesting a direct effect of clozapine on  
376 the volumetric change of the thalamus. Vita and colleagues' meta-analysis described a  
377 consistent finding where the greater the exposure to antipsychotics the greater the reduction  
378 in grey matter volume[15]. Two longitudinal studies have shown that the amount of exposure  
379 to antipsychotics predicted reduction of caudate and grey matter volumes[14] and the  
380 greater progressive brain reduction and ventricular enlargement were predicted by greater  
381 exposure to antipsychotic medication[70]. Although these studies have been interpreted as  
382 consistent with a toxic effect of antipsychotic medication on grey matter, generally patients  
383 were not randomised in these longitudinal studies and it is likely that patients with more  
384 severe illness were given larger amounts of medication. In our study other variables, such as  
385 age, duration of illness and daily dose of clozapine were not significant moderators of  
386 subcortical volume change over time, as previously reported[15].

387 A recent systematic review concludes that after 25 years of research it remains unclear which  
388 are the biological predictors of symptomatic response to clozapine [71]. Greater integrity and  
389 activity in prefrontal cortical areas associated with a good response to clozapine is the most  
390 consistent finding, however, studies have failed to find any accurate and reproducible  
391 neuroanatomical biomarker to inform clinical decision-making. Although our study identified  
392 a relationship between thalamo-striatal progression and clinical and functional improvement,  
393 we did not identify any baseline subcortical predictor of remission on clozapine.

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395

396 *Strengths and limitations*

397 The main strength of this study is the longitudinal nature of a relatively large and homogenous  
398 sample of TRS patients. The careful segmentation of the subcortical structures using the  
399 longitudinal stream of Freesurfer based on an unbiased within-subject anatomical  
400 template[35] enabled increased anatomical sensitivity to better detect anatomical changes  
401 and relationships to clinical symptoms and functioning. A potential limitation of this study is  
402 the lack of a comparative group of schizophrenia patients treated with other antipsychotic  
403 medications, in order to disentangle disease effects from treatment effects. However, such a  
404 comparative group may represent a less malign subgroup of patients with schizophrenia who  
405 are not treatment resistant and consequently may have a different underlying  
406 pathophysiology/impact of antipsychotic medication on their neuroanatomy. Ultimately  
407 including MR imaging in longitudinal studies of schizophrenia where patients are randomised  
408 to different antipsychotic medications would be necessary to tease apart illness from  
409 treatment effects but only three such studies have been conducted to our  
410 knowledge[60,69,72] and none on patients with treatment resistance. In addition, to reduce  
411 multiple analyses we assessed only subcortical structures summed bilaterally and did not  
412 explore any lateralised effects.

#### 413 *Conclusion*

414 This study demonstrates that, despite the clinical and functional improvement of most  
415 patients with schizophrenia who are switched to clozapine, there is a counterintuitive  
416 progressive volume reduction in several subcortical structures over time. Furthermore,  
417 patients who have the greatest symptomatic improvement display the largest thalamo-  
418 striatal reductions, suggesting that volume reduction reflects an adaptive response associated  
419 with symptom improvement rather than a harmful process in these treatment resistant  
420 patients. Further longitudinal studies with larger sample size, randomised designs and  
421 multimodal imaging will be necessary to disentangle the potentially dynamic effects of  
422 neuroprogression and antipsychotic treatment on different brain structures in schizophrenia.

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### 433 **Contributors**

434 Author CMcD designed and revised the manuscript for intellectual content; DMC and BH  
435 supervised the general progress of the study; MA recruited and collected data; LH collected  
436 data; TNA developed protocols for MRI processing. GT processed all the MRI data, performed  
437 statistical analyses and wrote the manuscript. All authors edited or approved the final  
438 manuscript.

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664 **Figure Legends**

665 Figure 1

666 (A,B,C,D,E) Plots of subcortical structures that presented significant changes over time in  
667 treatment-resistant schizophrenia patients compared to healthy controls. Note: all values  
668 corrected for age, sex and ICV.

669 Figure 2

670 Association between percentage of volume change in thalamus and putamen and change in  
671 (A) PANSS Total score (B) SANS (C) GAF and (D) association between percentage of volume  
672 change in thalamus and level of serum of clozapine at follow-up.

673 Supplementary Figure 1

674 Illustration of the change in lateral ventricles, thalamus, caudate and putamen volume from  
675 baseline to follow-up for each patient and healthy control. Red bars represent the mean.

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696 Table 1. Characteristics of patients with treatment resistant-schizophrenia and controls.

	Patient group (n=33)	Control group (n=31)	Test statistic/p-value
Sex (m/f)	23/10	20/11	$\chi^2= 0.19; 0.660$
Age at onset (years)	22.8 ± 0.8		
Age at baseline (years)	36.4 ± 10.7	39.3 ± 10.6	t= 1.10; 0.274
Age range	(22-61)	(23-59)	
Time between baseline and follow-up MRI scans (months)	6.6 ± 1.7	7.4 ± 3.2	t= 1.21; 0.230
Illness duration before commencing clozapine (years)	13.6 ± 8.8		
Intracranial volume (mm <sup>3</sup> )	1610322.58 ± 29886.83	1591515.15 ± 27500.42	t= 0.46; 0.644

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699 Table 2. Clinical features of patient group at baseline and follow-up (n=33)

	Baseline (Mean ± SD)	Follow-up (Mean ± SD)	Test statistic/ p-value
<i>Clinical scales</i>			
PANSS positive score	14.1 ± 5.7	6.1 ± 5.0	*z= -4.98; < 0.001
PANSS negative score	16.2 ± 7.0	9.1 ± 7.1	*z= -4.51; < 0.001
PANSS general score	24.1 ± 8.9	11.7 ± 8.3	*z= -4.90; < 0.001
PANSS Total Score	54.3 ± 17.8	26.9 ± 17.6	t= 10.04; < 0.001
SANS	42.5 ± 20.7	27.8 ± 22.9	*z= -3.78; < 0.001
SAPS	28.0 ± 16.3	13.2 ± 11.0	*z= -4.45; < 0.001
Global assessment of functioning	46.8 ± 10.8	64.9 ± 14.1	t= 13.12; < 0.001
<i>Medications</i>			
Typical antipsychotics (n)	5	0	
Atypical antipsychotics (n)	33	2	
Clozapine (n)	0	33	
Serum level of clozapine at follow-up (ng/ml)		0.5 ± 0.1	
Daily dose of clozapine at follow-up (mg)		349.2 ± 17.8	
Daily dose of clozapine range (mg)		(200-625)	
<i>Anthropomorphic measurements</i>			
Weight (kg)	85.9 ± 15.4	90.1 ± 16.6	t=-3.31; 0.002
Waist circumference (cm)	97.8 ± 12.1	103.1 ± 13.4	t=-4.94; < 0.001
Body Mass Index	28.0 ± 4.9	29.3 ± 5.0	*z= -2.78; 0.005
Total Cholesterol (mmol/L)	4.8 ± 1.1	5.5 ± 0.8	t=-3.38; 0.003
Triglycerides (mmol/L)	1.8 ± 1.0	2.5 ± 1.4	*z= -2.62; 0.009

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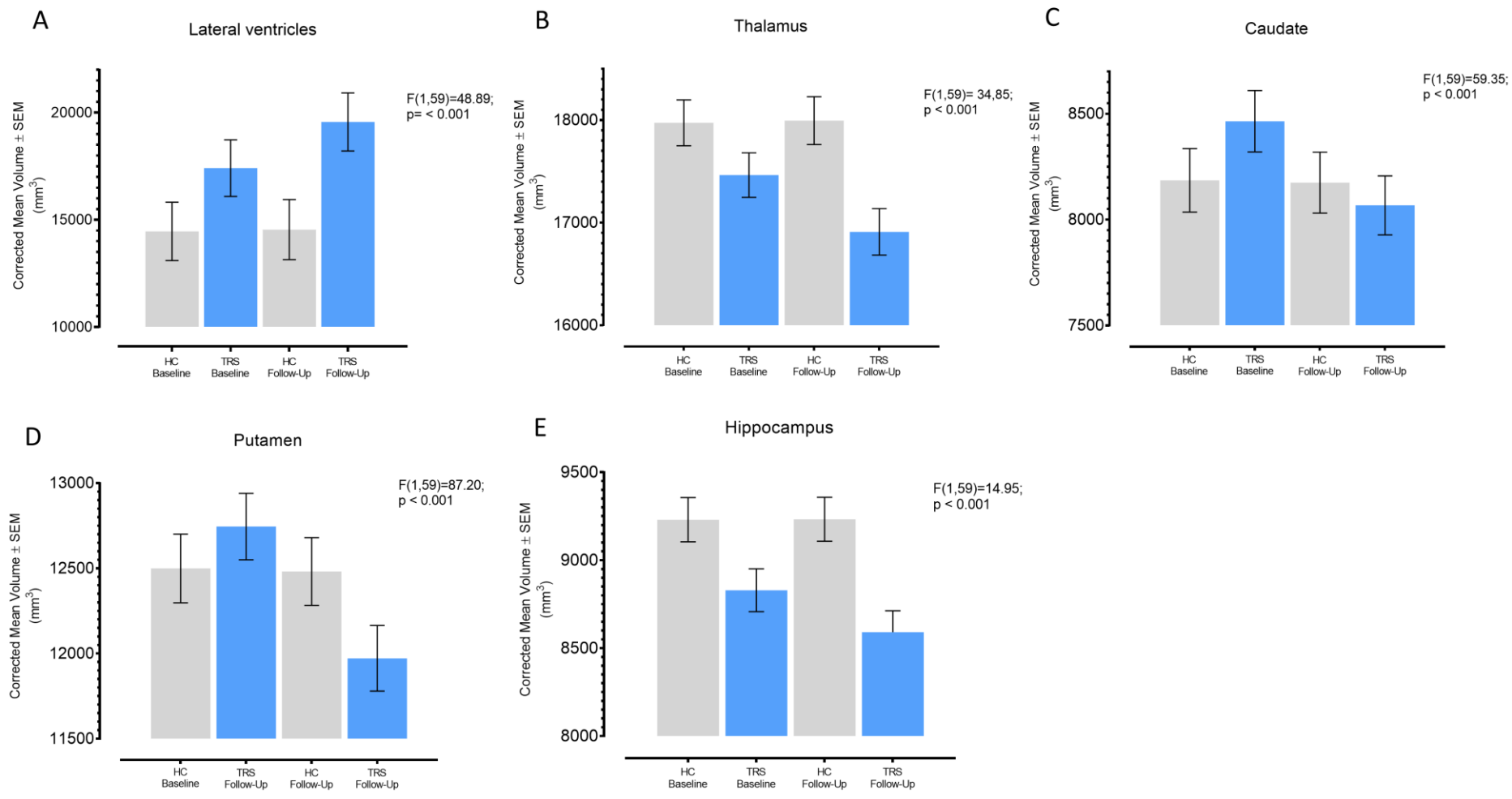
Note: \*= variable non-normal distributed; PANSS: Positive and negative Syndrome scale; SANS: The Scale for the Assessment of Negative Symptoms; SAPS: The Scale for the Assessment of Positive Symptoms. PANSS 0-6 scale was used. Twelve patients were prescribed typical antipsychotic drugs at some stage of their illness

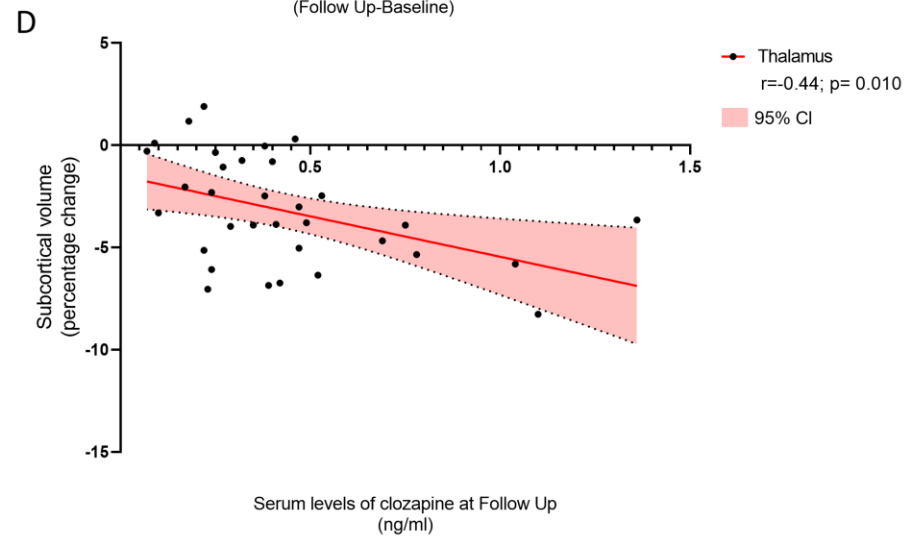
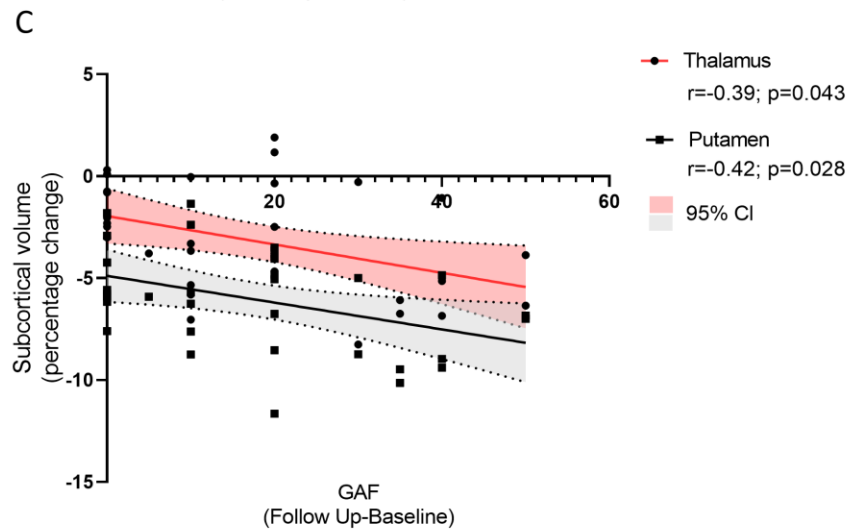
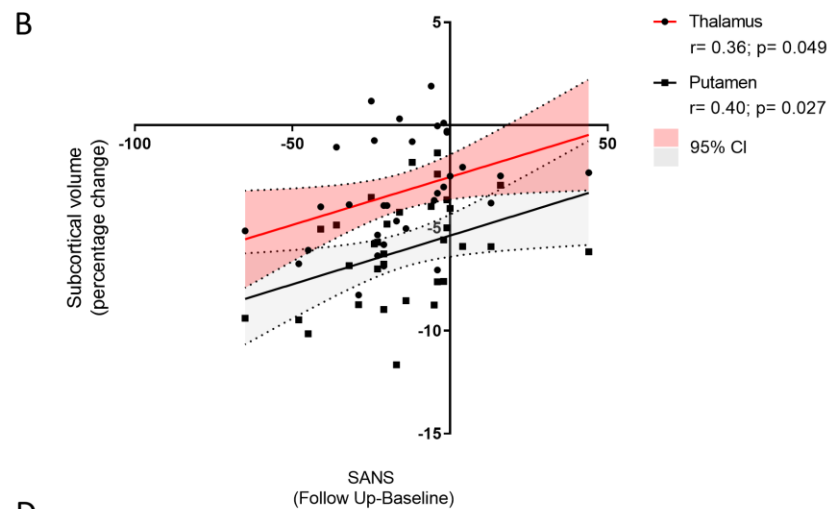
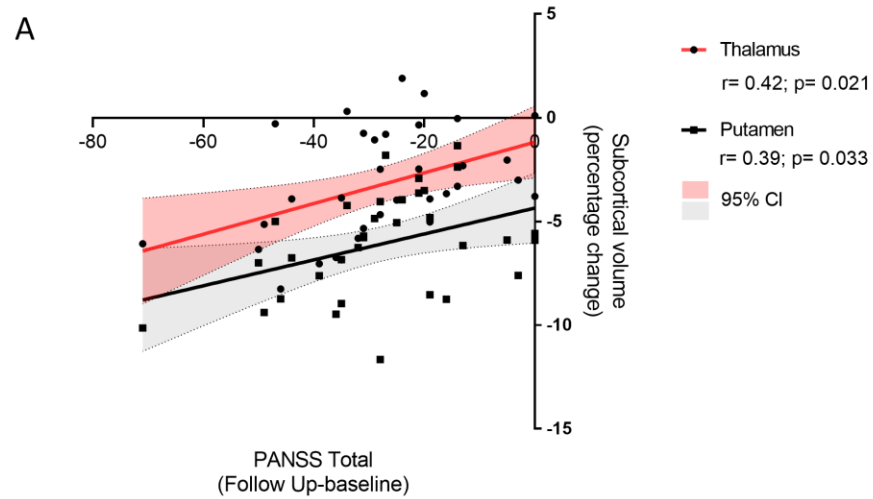
Table 3. Uncorrected means (SD) in mm<sup>3</sup> for each subcortical structure at baseline and follow-up, and results of statistical comparisons.

STRUCTURES	SCHIZOPHRENIA (n=33)	HEALTHY CONTROL (n=31)	GLM Baseline	SCHIZOPHRENIA (N=33)	HEALTHY CONTROL (N=31)	GLM Follow-Up	GLM Group*Time* Structure	Mean Vol. Diff. Over time (mm <sup>3</sup> ) [95% C.I.]	% Volume Difference Over Time (SD)		
	BASELINE	BASELINE	F (8,52) = 1.79, p= 0.101	FOLLOW-UP	FOLLOW-UP	F (8,52) = 3.11; p= <b>0.006</b>	F (7,41) = 52.54; p> <b>0.001</b>	TRS	HC	TRS compare d to HC	
	Means ± SD	Means ± SD	p	Means ± SD	Means ± SD	p	p				
Lateral Ventricle	16647.05 ± 9189.84	15272.53 ± 8836.20	0.128	18750.23 ± 9524.54	15413.12 ± 8927.10	<b>0.013</b>	> <b>0.001</b>	1962.58 [1351.80, 2573.36]	14.96 (11.63)	1.01 (3.58)	13.95 (11.34)
Thalamus	17443.74 ± 2078.97	17995.40 ± 2234.72	0.111	16883.71 ± 2065.36	18023.49 ± 2262.84	<b>0.002</b>	> <b>0.001</b>	-588.13 [-774.19, -402.06]	-3.21 (2.63)	0.15 (0.15)	-3.36 (3.22)
Hippocampus	8832.06 ± 773.80	9226.80 ± 830.35	0.027	8596.37 ± 773.36	9226.94 ± 846.99	<b>0.001</b>	> <b>0.001</b>	-235.83 [-359.41, -112.26]	-2.63 (3.51)	0.00 (1.60)	-2.63 (3.63)
Caudate	8456.97 ± 1194.26	8193.08 ± 1204.06	0.189	8052.13 ± 1174.16	8190.45 ± 1200.68	0.597	> <b>0.001</b>	-402.21 [-501.83, -302.59]	-4.83 (2.49)	-0.03 (2.38)	-4.80 (3.63)
Putamen	12781.62 ± 1790.28	12459.92 ± 1761.53	0.388	11993.99 ± 1610.45	12457.48 ± 1798.72	0.073	> <b>0.001</b>	-785.20 [-947.18, -623.22]	-6.07 (2.50)	-0.03 (2.18)	-6.04 (3.78)
Pallidus	4200.93 ± 669.20	3964.46 ± 706.62	0.088	4116.39 ± 612.12	3951.75 ± 704.01	0.243	0.282	-71.83 [-166.04, 22.39]	-1.74 (4.94)	-0.27 (2.98)	-1.47 (5.34)
Amygdala	3210.78 ± 339.72	3295.14 ± 426.97	0.428	3141.08 ± 364.80	3265.88 ± 410.05	0.213	0.364	-40.45 [-114.05, 33.15]	-2.16 (4.88)	-0.77 (4.03)	-1.39 (5.28)
Nucleus Accumbens	1188.58 ± 199.04	1198.76 ± 213.56	0.518	1145.21 ± 198.17	1194.95 ± 220.84	0.169	0.060	-39.57 [-74.71, -4.43]	-3.50 (6.24)	-0.32 (5.02)	-3.18 (7.60)

Note: the table shows the difference between treatment-resistant schizophrenia patient and healthy control groups on subcortical structures at baseline, follow-up and over time. *Post-hoc* analyses corrected for multiple comparisons (Bonferroni,  $\alpha = 0.006$ ). Legend: GLM= generalized linear model. C.I = confidence interval; % Vol. Diff. = percentage volume difference; calculated as follows:  $100 \times [(volume\ at\ follow-up - volume\ at\ baseline) / volume\ at\ baseline]$ , Negative value indicates a % volume decrease over time. TRS= treatment resistant-schizophrenia patients; HC= healthy controls; **Bold** = significant values.







## Supplementary Data.

Supplementary Table 1. Uncorrected means (SD) in mm<sup>3</sup> for each subcortical structure at baseline and results of statistical comparisons

	SCHIZOPHRENIA (n=39)	HEALTHY CONTROL (n=40)	GLM Baseline	Effect size <i>Cohen's d</i>
STRUCTURES	BASELINE	BASELINE	F (8,66) = 1.83, p= 0.088	
	Means ± SD	Means ± SD	<i>p</i>	
Lateral Ventricle	16645.83 ± 9038.13	14397.65 ± 8612.52	0.023	0.5
Thalamus	17375.83 ± 2130.64	17553.04 ± 2238.17	0.112	0.3
Hippocampus	8852.54 ± 829.88	9134.66 ± 760.41	0.022	0.5
Caudate	8409.65 ± 1151.67	8044.83 ± 1177.15	0.188	0.3
Putamen	12630.16 ± 1746.00	12044.24 ± 1801.58	0.350	0.2
Pallidus	4138.89 ± 659.62	3817.94 ± 708.69	0.119	0.1
Amygdala	3202.95 ± 360.02	3252.12 ± 393.17	0.399	0.1
Nucleus Accumbens	1189.09 ± 186.98	1182.88 ± 219.69	0.456	0.1

Note: the table shows the difference between treatment-resistant schizophrenia patient and healthy control groups (Whole initial recruited sample) on subcortical structures at baseline. Cohen's d was calculated from the F-value of Analyses of Covariance and therefore age, gender and ICV were included.

Supplementary Table 2. Correlations between neuroanatomical change and clinical variables change in patients

STRUCTURES	PANSS Negative		PANSS Positive		PANSS General		PANSS Total		SAPS		SANS		GAF	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Lateral Ventricle	-0.08	0.692	0.22	0.243	0.08	0.692	0.08	0.686	-0.03	0.879	-0.03	0.891	0.03	0.895
Thalamus	0.32	0.086	0.34	0.063	<b>0.39</b>	<b>0.034</b>	<b>0.42</b>	<b>0.021</b>	0.19	0.320	<b>0.36</b>	<b>0.049</b>	-0.32	0.088
Caudate	0.19	0.328	0.21	0.275	-0.00	0.996	0.13	0.491	0.04	0.823	0.23	0.218	-0.14	0.467
Putamen	0.31	0.097	0.34	0.067	0.34	0.066	<b>0.39</b>	<b>0.033</b>	<b>*0.39</b>	<b>0.035</b>	<b>0.40</b>	<b>0.027</b>	-0.36	0.052
Hippocampus	-0.16	0.411	-0.02	0.902	0.03	0.874	-0.05	0.795	-0.08	0.676	0.03	0.877	0.00	0.988

Note: PANSS: Positive and Negative Syndrome Scale; SANS: The Scale for the Assessment of Negative Symptoms; SAPS: The Scale for the Assessment of Positive Symptoms. In the correlations (Follow-up - Baseline)/Baseline×100) was used to express the volumetric change in subcortical structures and (Follow-up - Baseline) was used to express change in the clinical variables. Correlations controlled for age, sex and ICV. \* After removing 1 outlier this correlation lost significance (r=0.31; p= 0.102).

Supplementary Figure 1.

