Striatal Volume Increase After Six Weeks of Selective Dopamine $D_{2/3}$ Receptor Blockade in First-Episode, Antipsychotic-Naïve Schizophrenia Patients

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Abstract

Patients with chronic schizophrenia often display enlarged striatal volumes, and antipsychotic drugs (APD) may contribute via dopamine $D_{2/3}$ receptor ($D_{2/3}R$) blockade. However, previous exposure to multiple antipsychotics with rich receptor profiles compromises a causal inference.
In this prospective study of antipsychotic-naïve, first-episode schizophrenia patients, we tested if selective blockade of $D_{2/3}R$ would induce a dose-dependent striatal volume increase. Twenty-one patients underwent magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), and symptom severity ratings before and after six weeks of amisulpride treatment. Twenty-three matched healthy controls underwent baseline and follow-up MRI and baseline SPECT. Data were analyzed using repeated measure- and multiple regression analyses.

Striatal volumes did not differ between patients and controls at baseline or follow-up, but a Group*Time interaction was found. This interaction was explained by a significant striatal volume increase of 2.1% in patients. Volume increase was predicted by dose, but not by $D_{2/3}R$ occupancy or baseline symptom severity. A significant reduction in symptom severity was observed at a mean dose of 233.3 (SD=109.9) mg, corresponding to $D_{2/3}R$ occupancy of 44.65%.

Our data provides clinical evidence for a causal link between $D_{2/3}R$ blockade and striatal volume increase in antipsychotic-naïve schizophrenia patients.

Introduction

Schizophrenia is a mental disorder, primarily characterized by so-called positive symptoms such as delusions and/or hallucinations[1]. Studies using MRI have shown structural brain abnormalities to be linked to the disorder, most likely progressing over the course of the illness[2]. Amongst most consistent abnormalities are ventricular volume enlargement, cortical thinning and basal ganglia enlargement in medicated patients[2–5].

Antipsychotic medication is the only effective treatment against positive symptoms[1], but the long-term effect on brain structure is unknown[6]. Antipsychotics function by antagonizing dopamine D_2 receptors (D_2R) in striatum, thereby blocking the down-stream signaling of dopamine[1,7]. However, most ADPs are characterized by broad receptor profiles, and bind to e.g. serotonin 2A-, histaminergic- and muscarinergic receptor systems[8]. The complex pharmacology has limited the investigations of causal mechanisms between antipsychotic treatment and structural brain changes. Nevertheless, meta-analyses and reviews have reported associations between antipsychotic exposure and volumetric increase in basal ganglia[9–11].

In the current prospective study, we examined a cohort of first-episode, antipsychotic-naïve schizophrenia patients, before and after six weeks of treatment with amisulpride, a relatively selective dopamine $D_{2/3}$ receptor antagonist[12]. Baseline- and follow-up examinations included MRI, SPECT and Positive and Negative Syndrome Scale (PANSS) examinations.

We hypothesized that selective blockade of dopamine $D_{2/3}R$ would lead to a dose-dependent striatal volume increase.

Materials and methods

The study was conducted in accordance with the Helsinki declaration II, and approved by the Danish research ethics committee (H-D-2008-088), as well as the Danish Data Committee (RHP-2016-025, Isuite nr 05181). All participants gave written consent.

Participants

We included participants between the age of 18-45 years. Patients were first-episode, antipsychotic-naïve, and were recruited from Danish hospitals, as a part of the Pan European Collaboration Antipsychotic-naïve Studies cohort (PECANS 1). All patients met the International Classification of Diseases (ICD-10) criteria for schizophrenia (F20), verified by the structured diagnostic interview SCAN (Schedule of Clinical Assessment in Neuropsychiatry, version 2.1). Exclusion criteria included previous exposure to antipsychotic medication, methylphenidate, or use of antidepressants less than 1 month prior to baseline examinations. Healthy controls were recruited through advertisement. Exclusion criteria were identical to criteria for patients, but also comprised any former or current psychiatric illnesses, psychiatric diagnoses within firstdegree relatives and/or any drug-abuse (classified by ICD-10). For all participants, previous or current medical history of serious head trauma, neurological diseases, developmental disorders or drug dependency (by ICD-10 classification), and current pregnancy were exclusion criteria. All participants were screened for drug-use with urine samples (Rapid Response, Jepsen HealthCare) prior to SPECT scan. The PECANS 1 cohort data is included in other published studies[13].

Medication

The atypical APD, amisulpride, was chosen for treatment because of its relative selectivity towards dopamine $D_{2/3}$ receptors[12]. Treatment commenced after baseline examinations. Dosage was slowly increased and adjusted to the individual patient, according to clinical judgement and patients' reports of adverse effects. Pharmaceutical treatment against adverse effects was not allowed. Follow-up examinations were conducted after six weeks, and treatment dose in mg was recorded. Dosage was kept stable in the week prior to follow-up, to ensure a steady concentration at examinations. Controls were not treated.

Symptom severity

Symptom severity was assessed with PANSS within the same week as MRI and SPECT scan examinations. PANSS total score as well as sub-scores (positive, negative, and general) was evaluated at baseline and follow-up. To ensure consistency in PANSS ratings between clinicians, evaluations of ratings were regularly held. Responders were defined as having 30% or higher reduction in positive symptoms as previously described[14]. Controls did not undergo PANSS examinations. Duration of untreated illness was assessed from the patient history of worsening in functions due to symptoms.

Magnetic resonance imaging

High-resolution three-dimensional T1-weighted sagittal scans of the whole head were acquired for each subject on a Philips Achieva 3T whole-body MRI scanner (Philips Healthcare, Best, The Netherlands) at baseline and after six weeks. The details of the scan parameters are described elsewhere[14]. MR images were acquired within the same week as SPECT and PANSS. Image processing was conducted with tools from the FSL, FMRIB software library v5.0.10, and subcortical segmentation and volume extraction (mm^3) was performed[15]. In this study we focused on striatum as our region of interest (ROI), estimated as a sum of volumes from subregions nucleus caudatus, putamen and accumbens.

Single photon emission computed tomography

SPECT acquisition has previously been described[14]. In short, SPECT images were acquired using a Siemens Symbia T2 series SPECT-CT scanner, with the [^{123}I]-Iodobenzamide ([^{123}I]-IBZM) as the radioactive ligand, because of its selectivity towards the striatal dopamine $D_{2/3}R$[16,17]. CT-scout and tomography was performed for positioning and attenuation correction. Patients underwent scan at baseline and follow-up, whereas controls only underwent baseline scan to minimize radiation exposure. At follow-up, individual amisulpride

dose was administered 3 hours prior to scan, serum-amisulpride (s-amisulpride) was measured continuously, and mean levels during scan was calculated.

Image coregistration

To extract SPECT counts (counts/sec) from ROI's, we first co-registered CT- and MR anatomical images, using a statistical parametric mapping method (SPM8) to calculate the transformation matrix. This step was visually inspected with an image overlay method, and manually adjusted if needed[18]. Next, the CT-MR transformation matrix was used to co-register the SPECT images to the MRIs, and FSL subcortical regiondefinitions were resliced to fit the individual SPECT images. Subsequently, SPECT counts were extracted from the FSL-MRI defined regions.
Lastly, extracted SPECT counts were scatter- and decay corrected. Specific binding potentials were calculated by subtracting non-specific binding from the reference region from total binding in the ROI's, divided by the metabolite corrected plasma counts. Cerebellum was used as reference region for non-specific binding[14]. Dopamine receptor occupancy was calculated using following equation:

$$\boldsymbol{Occypancy}\ (\%) = (\mathbf{1} - \mathbf{100}\% \left(\frac{\boldsymbol{Specific\ binding\ potential\ (follow-up)}}{\boldsymbol{Specific\ binding\ potential\ \ baseline)}} \right) *$$

Statistical analyses

Statistical analyses were conducted using IBM SPSS version 25. Normal distributions were assessed by Shapiro-Wilk. Equality of variance was assessed by Box's- or Levene's test. For all analyses, a two-sided pvalue less than 0.05 was accepted as significant. Baseline- and follow-up descriptive between groups were compared with unpaired students t-test for normally distributed data, and Mann-Whitney for non-normally distributed data. Changes within groups were analyzed with paired students t-test or Wilcoxon for nonnormally distributed data. Pearsons Chi^2 was used for nominal data. Pearson's correlation coefficient was used for correlating parametric data, otherwise Spearman's rho was used.

Our hypothesis was tested in two steps:

First, striatal volume changes over time were tested with a repeated measure analysis. Significant group*time-interactions were further investigated with *post-hoc* t-tests. Repeated measures analysis was initially performed for striatum, and afterwards separately for striatal subregions, defined as nucleus caudatus, putamen and accumbens.

Second, we applied a multiple regression analysis to investigate variables' individual, predictive effect on striatal volume increase, whilst controlling for one another. The "Enter" function was used. Variables were: amisulpride dose, striatal receptor occupancy, and baseline PANSS positive score. PANSS baseline positive scores were included in the model to control for the disease severity. Multiple regression model assumptions were met. If variables were non-normally distributed, they were transformed to normal distributions using log10- or square root functions.

Results Patients compared to healthy controls

We included 21 patients and 23 controls with full datasets in our analyses (Supplementary Material, Figure S1). Patients had higher use of tobacco and fewer years of education compared to controls. Patients and controls did not differ in other demographic factors (Table 1). No difference in mean striatal volumes between patients and controls was found at baseline (p=0.72) or follow-up (p=0.28). No difference in mean specific binding potentials to dopamine $D_{2/3}R$ was found between patients (2.49±0.82) and controls (2.68±0.71) (p=0.40).

Table 1 **Group; mean ± SD [mean]**

Between-groups	**Patients (n=21)**	**Controls (n=23)**	**ρ value**
Demographics:			
Age, years[b]	23.5 ± 4.8	24.09 ± 5.01	0.92
Sex[c], male:female	10:11	12:11	0.76
Handedness[c], right:ambidextrous:left	16:3:2	20:2:0	*0.31
Handedness score[b], -100:100	59.2 ± 60.7	54.6 ± 68.7	0.78
Parental socioeconomic status[c], high:moderate:low	4:11:6	5:14:4	*0.68
Educational level[c], higher education/self emplyed, medium education, uneducated, student	0:3:4:9	0:2:0:15	*0.06
Years of education[a]	11.9 ± 2.0	14.3 ± 2.5	**0.001**
Weight kg[a]	78.5 ±20.6	68.5 ± 11.0	0.058
Height cm[a]	172.8 ± 9.5	175.1 ± 10.3	0.54
Substance use[c], alcohol, tobacco, cannabis, benzo, opioids, stimulants	16:13:4:0:1:3	20:3:1:0:0:0	***<0.001[e]**
Volumes:			
Striatum baseline[b] (mm^3)	18311.0 ± 2326.8	18042.7 ± 2510.4	0.82
Striatum follow-up[a] (mm^3)	18674.2±2303.6	17915.8±2329.4	0.28
Specific binding potentials (counts/sec)			
Striatum[b]	2.49±0.82	2.68±0.71	0.25
Within-patients	**Baseline**	**Follow-up**	
PANSS clinical scores[d]			
Positive:	19.8 ± 4.0	13.4 ± 3.4	**<0.001**
Negative:	18.7 ±7.2	20.3 ± 5.8	0.081
General:	40.1 ±8.5	30.2 ±7.5	**<0.001**
Total:	78.5 ±16.4	64.0 ±13.8	**<0.001**
Medication:			
Dose amisulpride (mg/day)	-	233.3 ±109.9	
S-amisulpride (ng/ml)	-	399.7 ±283.8	
Duration of untreated illness (weeks)	80.8 ± 96.2	-	
Receptor occupancy			
Striatum	-	44.65% ±18.7%	

SD= standard deviation.
[a]=t-test, [b]=Mann-Whitney U, [c]=Pearsons Chi, [d]= Wilcoxon, [e]= p<0.05 only for tobacco use.
*=Groups have expected counts less than 5. Significant p-values are in bold.

Symptom severity and receptor occupancy in patients after treatment

After six weeks of treatment, patients significantly decreased in PANSS total-, positive- and general symptoms, but not in negative symptoms (Table 1). Sixty-two percent of patients were defined as responders. Patients were treated with a mean dose of 233.3 (SD=109.9) mg amisulpride. Oral dose and samisulpride correlated positively (r^2=0.76, p<0.001). Mean receptor occupancy was 44.65% (SD=18.7%) and correlated positively with oral dose (r^2=0.60, p=0.004) and s-amisulpride (r^2=0.68, p=0.001). Receptor occupancy is illustrated in Figure 1. Amisulpride dose did not correlate to symptom severity (PANSS total) at baseline (r^2=0.292, p=0.199). Likewise, duration of untreated illness did not correlate with oral dose (r^2=0.35, p=0.123).

Figure 1

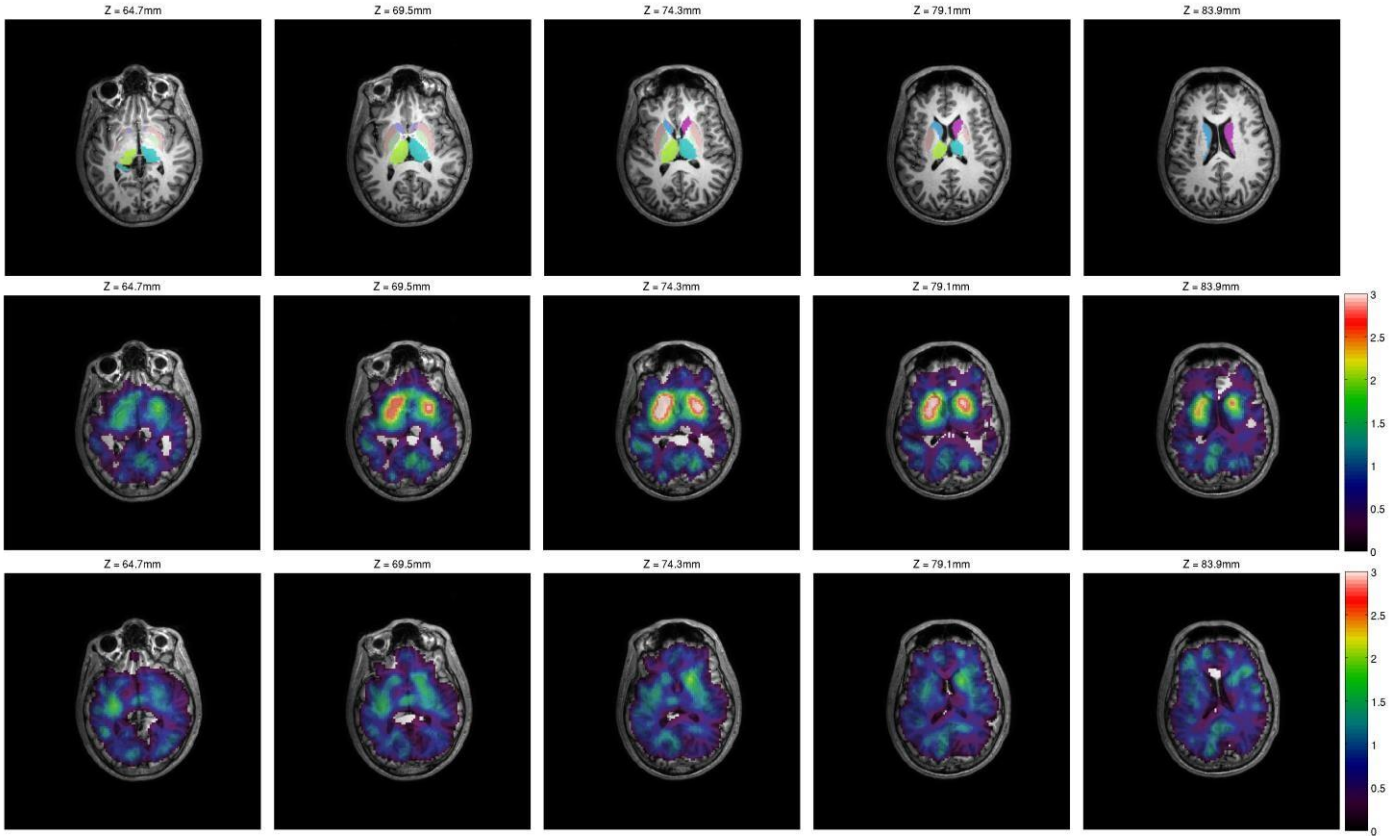

Figure 1: SPECT images of one patient, treated with 350 mg amisulpride, and with a mean receptor occupancy of 48.5%. The first row depicts and MRI with all regions from the subcortical atlas, of which we focused on nucleus caudatus, putamen and accumbens. Row two and three depicts the underlying MRI, with the co-registered SPECT images overlaying, depicting the specific binding potential before treatment (row two), and after six weeks of treatment (row three). Color-scales on the far right shows binding potentials from highest to lowest.

Striatal volume increase predicted by dose

The repeated measure analysis revealed no volume difference between groups at either time-point, but instead a significant Group*Time-interaction (p=0.01). The *post hoc* analysis revealed that the interaction was driven by a significant volume increase in striatum of 2.1% (95% CI=0.52%-3.68%) in patients. Subregional increases were observed in left and right nucleus caudatus (2.6%) and right putamen (2.4%) (Table 2). The multiple regression model significantly predicted striatal volume increase (r^2=0.411, p=0.026) (Figure 2), with oral dose as the only unique predictor (beta=0.553, p=0.028) (Supplementary Material, Table S1).

Table 2	Patients			Controls		
Area (mm^3)	**Baseline**	**Follow-up**	**p-value**	**Baseline**	**Follow-up**	**p-value**
Striatum	18311	18674.2	**0.003**	18042.7	17915.8	0.107[a]
Caudatus	7682.1	7877.7	**<0.001**	7449.2	7374.4	0.187
Left	3743.8	3877.8	**0.003**	3675.2	3609.9	0,067
Right	3938.3	3999.9	**0.004**	3774.1	3764.6	0.770
Putamen	9663.1	9819.5	**<0.001**	9632.8	9570.9	0.224[a]
Left	4869.5	4911.3	0.347	4812.7	4774.2	0.670[a]
Right	4793.6	4908.1	**0.007**	4820.1	4796.7	0.212[a]
Accumbens	965.8	977.0	0.732	960.7	970.5	0.484[a]
Left	535.1	545.6	0.627	547.1	546.7	0.879[a]
Right	430.7	431.4	0.614[a]	413.5	423.8	0.346[a]

Equal variances assumed. Areas marked with [a] was tested with Wilcoxon. All other areas were tested with students t-test. Significant p-values are in bold.

Figure 2

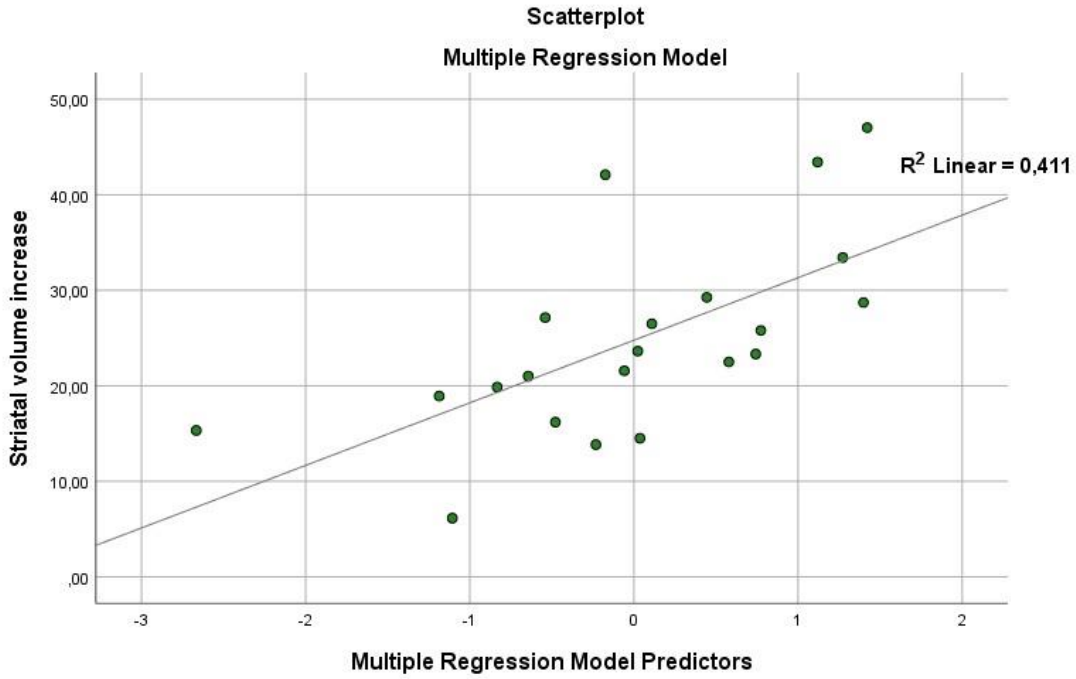

Figure 2: Scatter plot of Multiple Regression Model. Variables included in the model were dose, striatal receptor occupancy and PANSS positive score. Dependant variable 'striatal volume increase' depicted on y-axis, independent variables on x-axis. The model significantly (r^2=0.411, p=0.026) predicted striatal voluminal change. Only dose was a unique predictor of volume increase (r^2=0.553, p=0.028) when controlling for the other variables. All model coefficients can be seen in supplementary material, Table s1.

Discussion

Primary findings

Twenty-one antipsychotic-naïve first-episode schizophrenia patients were treated with amisulpride for six weeks with a mean dose of 233.3 mg. Patients significantly decreased in positive and negative symptoms at a mean receptor occupancy of 44.65%. In line with our hypothesis, we found a significant volume increase in striatum in patients (2.1%). Our predictive model showed that dose, but not positive symptom severity at baseline, or $D_{2/3}R$ occupancy explained the volume increase.

Strengths and limitations

We conducted a clinically challenging prospective, multimodal study on a cohort of antipsychotic-naïve first-episode schizophrenia patients and well matched, healthy controls. Men and women were equally represented. Confounding effects of previous exposure to APDs could be ruled out. Amisulpride was chosen for treatment because of its selectivity towards dopamine $D_{2/3}R$, thereby excluding potential involvement of other neuroreceptors.

Because of the extensive examination program, the study included a limited sample of patients. and a selection bias cannot be ruled out. Still, with mean baseline PANSS total score of 78.5, patients can be considered moderately to severely ill[19]. Follow-up period was limited to six weeks to ensure adherence to the trial, and results may have been different with a longer follow-up. Controls and patients differed on years of education and smoking habits. The difference in years of education was interpreted as a possible symptom of the disease. It is unresolved if nicotine affects the dopamine system, but studies have found no differences in dopamine synthesis capacity between smokers and non-smokers, and no influence of nicotine on brain volumes[20,21]. The limited number of patients restricted the degrees of freedom in the multivariate linear regression model, and therefore it was not possible to include- and control for further variables in our analyses.

Results compared to previous findings

Studies have found structural brain differences between patients and healthy controls apparent at the preclinical stages or time of diagnosis, suggesting that some structural changes are a part of the disease[2]. We did not find any differences in striatal volumes at either time-points. Specific $D_{2/3}R$ binding potentials did not differ between patients and controls prior to treatment, a replication of previous findings[22,23].
Mean amisulpride dose was lower than normal. Our patients were treated with less than half the dose used in the OPTiMiSE study[24]. PANSS baseline scores and symptom reduction were almost identical in the studies, but OPTiMiSE patients decreased slightly more, partly because our patients had an increase in negative symptoms. The low treatment dose could not be explained by a high degree of responders, since approximately 1/3 of our patients were non-responders. However, some of the difference could be explained by the OPTiMiSE patients not being completely antipsychotic-naïve. Studies have found a possible compensatory upregulation of D_2R in response to treatment[25,26], which could increase the needed treatment dose in the OPTiMiSE patients. Treatment dose is inherently linked to occupancy, and it is generally accepted that 65-80% occupancy is necessary for clinical response[27]. However, we found a significant decrease in symptoms at mean 44.65% occupancy. Most recent studies investigating this use estimated occupancies in chronic patients[10,28], but a prospective PET study found an optimal therapeutic window between 50-60% receptor occupancy on clinically stable patients with late-life schizophrenia[29]. This could indicate that lower doses and occupancies can have good clinical effect in newly diagnosed patients, as well as decrease risk of adverse effects such as extrapyramidal symptoms.
We had expected the volume increase to be predicted by striatal occupancy, but this was not the case, a result shared by Di Sero et. al[10]. It may be due to issues regarding the calculation of receptor occupancy. As previously mentioned, a compensatory upregulation of D_2R would affect follow-up receptor availability and thereby occupancy. Changes in endogen dopamine levels could also influence receptor availability and occupancy. Lastly, our multiple regression analysis assumes linearity, which might not be the case between volume increase and occupancy.

Volume increase

It is yet unknown what this volume increase consists of. Investigations into mice striatum following APD treatment found increased microglial cell density and changed morphology[30], and a recent PET study found increased microglial activity in patients[31]. A fMRI study om healthy males found augmented blood flow to striatum, after one dose of APD[32], and the increased blood is possibly an “apparent” volume increase. Taken together, microglial activation and increased blood flow could indicate a proinflammatory reaction in striatum.

Conclusion

We found a dose-dependent striatal volume increase in antipsychotic-naïve schizophrenia patients, in response to six weeks dopamine $D_{2/3}$ receptor blockade. What the volume increase consists of is yet unknown.

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Conflicts of interest

BHE has received lecture fees and/or is part of Advisory Boards of Bristol-Myers Squibb, Eli Lilly and Company, Janssen-Cilag, Otsuka Pharma Scandinavia AB, Takeda Pharmaceutical Company and Lundbeck Pharma A/S. All other authors confirm no conflict of interest in this study.

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Supplementary Material Figure S1

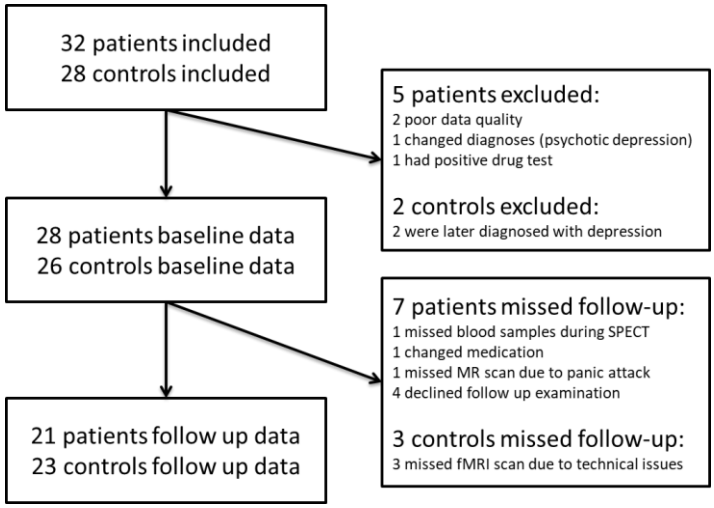

Figure s 1: Consort Flow Diagram[14].

Table S1

Multiple Model	Standardized Coefficients Beta	t	Sig.
(Constant)		-.855	.404
Dosis	.553	2.394	.028

Receptor occupancy	-.017	-.070	.945
PANSS positive	.244	1.251	.228
Model		0.026 regression	

Table s 1: Multiple regression model coefficients. The model significantly predicted volume increase (p=0.026), with dose as the only unique, predictive variable (p=0.028).