



ORIGINAL INVESTIGATION

Latent toxoplasmosis reduces gray matter density in schizophrenia but not in controls: Voxel-based-morphometry (VBM) study

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Abstract

Objectives. To address the role of latent T. gondii infection in schizophrenia we studied the influence of latent toxoplasmosis on brain morphology. Methods. An optimized voxel-based morphometry of magnetic resonance imaging was analyzed by analysis of variance with diagnosis and seropositivity as factors in 44 schizophrenic patients (12 T. gondii positive) and 56 controls (13 T. gondii positive). Results. Grey matter (GM) volume was reduced in schizophrenia patients compared with controls in the cortical regions, hippocampus and in the caudate. In the schizophrenia sample we found a significant reduction of GM volume in T. gondii positive comparing with T. gondii-negative patients bilaterally in the caudate, median cingulate, thalamus and occipital cortex and in the left cerebellar hemispheres. T. gondii-positive and -negative controls did not differ in any cluster. Among participants seropositive to T. gondii the reduction of GM in the schizophrenia subjects was located in the same regions when comparing the entire sample (11,660 over-threshold voxels (P ≤ 0.05, FWR corrected). The differences between T. gondii-negative patients and controls consisted only of 289 voxels in temporal regions. Conclusions. Our study is the first to document that latent toxoplasmosis reduces GM in schizophrenia but not in controls.

Keywords: Schizophrenia, Toxoplasma gondii, latent toxoplasmosis, magnetic resonance imaging, voxel-based-morphometry (VBM)

Introduction

The role of coccidian protozoa Toxoplasma gondii represents one of the most enigmatic and unexplained questions in the field of pathophysiology of schizophrenia. T. gondii is a very successful parasite, infecting approximately 30% of the human population (Jones et al. 2001). The congenital toxoplasmosis is linked to severe neuropsychiatric and ophthalmological symptoms (Carruthers and Suzuki 2007). The more frequent postnatal infection is caused by ingesting T. gondii tissue cysts from meat or by oocysts present in water and soil contaminated by infected cat feces. This postnatal infection consists of an acute stage in which the rapidly growing T. gondii tachyzoites infect a wide range of tissues and are transported to the brain. An activated immune system induces tachyzoites to differentiate into slowly

replicating bradyzoites which form cysts in neurons and glias (Dubey 1998). This stage results in a latent infection that persists over an entire lifetime. The latent toxoplasmosis has been thought to be asymptomatic and the clinical consequences of this long-standing, infection have been largely ignored.

However, accumulating evidence from epidemiological and neurobiological approaches suggests that the latent toxoplasmosis is associated with an increased incidence of schizophrenia. The recent meta-analysis including nearly four thousand patients with schizophrenia clearly documented higher prevalence of T. gondii seropositivity in patients than in controls with an odds ratio of 2.73, and 2.54 for first episode patients (Torrey et al. 2007). Interestingly, the effect size of latent toxoplasmosis is in schizophrenia greater than in any of

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the identified candidate genes and other environmental risk factors (Torrey et al. 2007). It is unlikely that these impressive epidemiological findings would be an artifact of antipsychotic medication. Antipsychotics have been shown to inhibit *T. gondii* in vitro and the prevalence of seropositivity is high in drug-naïve patients as well (Leweke et al. 2004). The higher prevalence of latent *T. gondii* infection was found to be specific for schizophrenia but not for bipolar disorder (Wang et al. 2006).

However, these epidemiological data indicating the influence of *T. gondii* on the risk of schizophrenia are only correlative in nature. The high prevalence of toxoplasmosis in non-schizophrenic subjects limits causal interpretation and indicates the importance of the interaction between toxoplasmosis and other pathogenetic factors. The hypotheses for a causal relationship between latent toxoplasmosis and schizophrenia proposed that *T. gondii* increases dopaminergic activity (Flegr et al. 2003; Gaskell et al. 2009) or tryptophan metabolites levels (Schwarcz and Hunter 2007).

We suggest that the subjects with genetic vulnerability would be more sensitive to *T. gondii* induced biochemical changes resulting in more pronounced neurobiological and morphological damage underlying the higher risk of schizophrenia. To address the question of schizophrenia and latent toxoplasmosis interaction we studied the influence of seropositivity for latent toxoplasmosis on brain morphometry in schizophrenia patients investigated by magnetic resonance imaging (MRI). Neuroimaging data were analyzed by optimized voxel-based morphometry (VBM), a fully automated approach for whole brain structural mapping allowing voxel-wise statistical comparison of grey (GM) and white (WM) matter. We expected that the effect of latent *T. gondii* infection on brain morphology will be more pronounced in schizophrenia patients than in controls and that these differences will be located in brain regions which are involved in the pathology of schizophrenia.

## Methods

### Participants

A group of 44 patients with schizophrenia diagnosed by two psychiatrists according to DSM-IV criteria with the Structured Clinical Interview-Patient Version (SCID-I/P) was recruited from Prague Psychiatric Centre (Czech Republic). The patients were ill for 6.6 (SD = 4.47) years and were studied in clinical remission.

A standard physical examination, medical history evaluation, biochemistry, and EEG were performed to exclude neurological disorders (except schizophrenia),

severe medical illness, and drug or alcohol abuse. The clinical condition was assessed by the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987) on the day of MRI investigation. Forty patients were on antipsychotic medication, 38 of them were on atypical antipsychotics (olanzapine 5–20 mg, risperidone 1–8 mg, clozapine 150–500 mg, sulpiride 200–600 mg, aripiprazole 15 mg, amisulpride 200 mg), two were on classical antipsychotics (levomepromazine 200 mg and flupenthixol depot 40 mg/3 weeks). Chlorpromazine equivalents were calculated based on the equivalence ratios (Woods 2003; Lehman et al. 2004). Four patients were without medication (three in the *T. gondii*-positive and one in *T. gondii*-negative groups).

Healthy control subjects were recruited via a local advertisement from a similar sociodemographic background respecting the age and gender characteristics of the schizophrenia sample. The main exclusion criteria for control subjects were a personal history of any psychiatric disorder or substance abuse established by the Structured Clinical Interview for DSM-IV, and a psychotic disorder in first- or second-degree relatives. The final sample (56 controls with 13 *T. gondii*-positive and 44 patients with 12 *T. gondii*-positive cases) respected a larger control than patient group allowing optimal detection of volume changes in VBM (Pell et al. 2008). The hand preference of participants was measured by Edinburgh Handedness Inventory (Oldfield 1971) with laterality index > 70 indicating right-handed subjects.

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki, written informed consent was obtained from all subjects and the local ethics committee approved the study.

### Immunological tests for toxoplasmosis

Serological tests were carried out in the National Reference Laboratory for Toxoplasmosis (National Institute of Public Health, Prague, Czech Republic). Specific anti-*Toxoplasma* IgG (SEVAC, Prague) in all subjects and IgM (TestLine, Brno; optimized for early detection of acute toxoplasmosis) in high IgG subjects were determined by ELISA. A complement fixation test (CFT) (SEVAC, Prague), which is more reliable in established (“old”) *T. gondii* infections (Kodym et al. 2007), was performed in all subjects. *T. gondii* antibody titres in the sera were measured at dilutions between 1:8 and 1:1024. Latent toxoplasmosis was diagnosed on the basis of a positive CFT (titre equal or higher than 1:8) and a positive IgG ELISA test (using a control positive cut-off serum according to manufacturer recommendations, i.e.

1 positivity index  $> 1$ ), combined with a negative or  
 2 equivocal IgM ELISA test (positivity index  $< 0.9$ ).  
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#### 4 *MRI data acquisition and VBM protocol*

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 6 Magnetic resonance images were obtained with a  
 7 1.5-T system Magnetom Vision (Siemens, Erlangen,  
 8 Germany). Subjects were scanned using a T1-weighted  
 9 (T1W) 3D-MPRAGE sequence (repetition time TR  
 10 of 2000 ms; echo time TE of 4.4 ms; bandwidth 130  
 11 Hz/pixel; field of view 256 mm; matrix  $256 \times 256$ ;  
 12 slice thickness 1 mm; 160 contiguous sagittal slices;  
 13 voxel size of  $1 \times 1 \times 1$  mm and total acquisition  
 14 time 8 min).

15 Structural MRI analysis was performed using an  
 16 optimized VBM protocol in Statistical Parametric  
 17 Mapping v.5, SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5>) implemented in MATLAB 7.3  
 18 (Math Works, Natick, MA). VBM has proven useful  
 19 in characterizing subtle changes in brain structure  
 20 in schizophrenia (Glahn et al. 2008; Fornito et al.  
 21 2009). The optimized version of VBM is comple-  
 22 mented by correcting for the deformations intro-  
 23 duced during the spatial normalization procedure  
 24 (modulation) and reflects an estimate of absolute  
 25 grey matter volume. Each 3D volume of T1W images  
 26 was segmented into GM, WM, and cerebrospinal  
 27 fluid (CSF), and total volumes were obtained via the  
 28 'get\_totals' matlab script (<http://www.cs.ucl.ac.uk/staff/G.Ridgway/vbm/>). Spatial normalization para-  
 29 meters were estimated by matching GM tissue with  
 30 the standard GM template provided by SPM5. The  
 31 normalization parameters were applied to the orig-  
 32 inal T1W images. Optimally normalized T1W  
 33 images were then segmented into GM, WM, and  
 34 CSF segments. In order to restore tissue volumes  
 35 modified during normalization processing, Jacobian  
 36 modulation was applied to normalized GM and  
 37 WM segments. Modulated normalized GM, WM  
 38 and CSF images were smoothed by a Gaussian ker-  
 39 nel of 8 mm full width at half maximum (FWHM).  
 40 The full factorial model (analysis of variance,  
 41 ANOVA) with diagnosis and latent toxoplasmosis  
 42 positivity/negativity as factors was used to address  
 43 the differences in gray and white matter. For all  
 44 SPM5 analyses, total brain volume (TBV) was used  
 45 as a nuisance covariate (Pell et al. 2008). For rigor-  
 46 ous control type I error we accepted as significant  
 47 only conservative family-wise error (FWE) cor-  
 48 rected findings with the height threshold  $T = 5.00$   
 49 ( $P \leq 0.05$ ) with the extent threshold consisting of  
 50 50 voxels per cluster. The coordinates for local max-  
 51 ima in MNI (Montreal Neurological Institute) tem-  
 52 plate were converted to stereotactic Talairach  $x, y, z$   
 53 coordinates (Lancaster et al. 2007). Results were  
 54 displayed using a study-specific 3D template and  
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slices derived from the mean image of all normal-  
 ized T1W images.

#### Statistical analyses

The categorical measures (handedness, gender and  
 education level in three grades) were compared  
 between patients and controls by the Chi-square  
 test. Further, gender and handedness rates across  
 four groups (*T. gondii*-positive and -negative patients  
 and controls) were tested by a log-linear analysis for  
 a three-way contingency table under the hypothesis  
 of complete independence.

The comparisons between patients and controls  
 in age, total brain volume, GM, WM and CSF vol-  
 ume, and between *T. gondii*-positive and -negative  
 patients in PANSS, duration of antipsychotic treat-  
 ment, chlorpromazine equivalents and age of onset  
 were performed by independent sample *t*-tests.  
 Two-way ANOVA with a group and *T. gondii* sero-  
 logical status as factors followed by a Bonferroni  
 post hoc test, when appropriate, was used to com-  
 pare *T. gondii*-positive and -negative patients and con-  
 trols in absolute and relative brain volumes.

## Results

### *Demographic and clinical data*

The schizophrenia group and final sample of con-  
 trols did not differ in age ( $t = 1.78$ ,  $df = 98$ ,  
 $P = 0.08$ ), sex ( $\chi^2 = 0.79$ ,  $df = 1$ ,  $P = 0.37$ ), edu-  
 cation ( $\chi^2 = 5.68$ ,  $df = 2$ ,  $P = 0.06$ ), handedness  
 ( $\chi^2 = 0.57$ ,  $df = 1$ ,  $P = 0.45$ ) or in proportion of  
*T. gondii*-positive and -negative cases ( $\chi^2 = 0.22$ ,  
 $df = 1$ ,  $P = 0.64$ , Table I).

*T. gondii*-positive and -negative subjects did not  
 differ in either groups in age ( $F = 3.14$ ,  $df = 1.96$ ,  
 $P = 0.08$ ), gender ( $\chi^2 = 3.08$ ,  $df = 4$ ,  $P = 0.54$ ),  
 handedness ( $\chi^2 = 2.08$ ,  $df = 4$ ,  $P = 0.72$ ), or chlo-  
 rpromazine equivalents ( $t = 0.21$ ,  $P = 0.80$ ).

In the schizophrenia group, the *T. gondii*-positive  
 patients had a significantly higher age of onset of  
 schizophrenia than *T. gondii*-negative patients  
 ( $t = 2.51$ ,  $df = 43$ ,  $P = 0.04$ , Table I).

### *MRI results*

The two-way ANOVA of segmented brain tissue vol-  
 umes showed that there was no significant *T. gondii*  
 serological status effect on the TBV ( $P = 0.37$ ) and  
 CSF ( $P = 0.69$ ) and no significant group-serological  
 status interaction ( $P = 0.83$ ;  $P = 0.98$ , respectively).  
 However, we found a significant group effect in TBV  
 ( $F = 10.26$ ,  $df = 1,96$ ,  $P = 0.002$ ) and in CSF

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Table I. Sample description for demographic and clinical data. Data for group and handedness are presented in number of subjects. The other data are displayed in means (standard deviation). Significant results ( $P \leq 0.05$ ) are in bold font and marked by “+” for  $t$ -test. The group effect in two-way ANOVA is marked by “a” for absolute and by “b” for relative volume differences.

	Schizophrenia			Controls		
	Total	Negative	Positive	Total	Negative	Positive
Number (M/F)	44 (22/22)	32 (18/14)	12 (4/8)	56 (23/33)	43 (17/26)	13 (6/7)
Age	30.82 (9.758)	29.5 (9.521)	34.33 (9.912)	27.89 (7.3)	27.14 (6.861)	30.38 (8.451)
Total brain in cm <sup>3</sup>	<b>1679 (236.9)<sup>a</sup></b>	1688.4 (251.6)	1654.6 (200.4)	<b>1526 (194.8)<sup>a</sup></b>	1539 (189.4)	1484 (213.8)
GM in cm <sup>3</sup>	<b>685.8 (88.19)<sup>b</sup></b>	682.9 (98.1)	693.6 (56.5)	<b>699.4 (65.94)<sup>b</sup></b>	704.8 (58.19)	685.2 (89.29)
WM in cm <sup>3</sup>	<b>520 (79.63)<sup>b</sup></b>	528.5 (83.7)	497.4 (65.4)	<b>501.5 (65.99)<sup>b</sup></b>	507.3 (57.20)	482.3 (89.37)
CSF in cm <sup>3</sup>	<b>473.3 (162.4)<sup>a,b</sup></b>	479.8 (172.2)	463.6 (139.4)	<b>325.5 (112.5)<sup>a,b</sup></b>	328.2 (122.5)	316.3 (73.53)
Age of onset	24.74 (8.354)	<b>22.77 (6.689)<sup>+</sup></b>	<b>28.41 (9.952)<sup>+</sup></b>	–	–	–
Hand. R/nonR	30//14	21//11	9//3	42//14	31//12	11//2
AP exp. (months)	71.12 (51.27)	71.61 (56.86)	69.79 (34.08)	–	–	–
CHLPMZ in mg	356.1 (237.8)	343.7 (181.6)	360.7 (258.2)	–	–	–
PANSS P	13.22 (7.263)	13.26 (5.504)	14.25 (9.498)	–	–	–
PANSS N	15.78 (8.319)	16.71 (8.705)	13.92 (7.489)	–	–	–
PANSS TOT	61.06 (28.54)	64.7 (24.90)	59.17 (31.32)	–	–	–

Abbreviations: PANSS, Positive and Negative Syndrome Scale (TOT = total score, P = Positive, and N = Negative subscale); AP exp., antipsychotics exposition; CHLPMZ, chlorpromazine equivalents; Hand. R, right handed subjects indicated as Edinburgh handedness inventory  $\geq 70$ ; nonR, non-right handed subjects (Edinburgh handedness inventory  $< 70$ ).

volume ( $F = 21.42$ ,  $df = 1,96$ ,  $P < 0.001$ ). Schizophrenia patients had a larger absolute CSF volume ( $t = 5.37$ ,  $df = 98$ ,  $P < 0.001$ ) corresponding with larger TBV ( $t = 3.54$ ,  $df = 98$ ,  $P < 0.001$ ) (Table I). Further, no significant group and serologic status effect on absolute GM and WM volumes ( $P > 0.1$ ) nor significant group–serologic status interaction ( $P > 0.1$ ) were observed.

For the relative tissue volumes (divided by TBV), only group assignment was a significant factor for GM ( $F = 25.60$ ,  $df = 1,96$ ,  $P < 0.001$ ), WM ( $F = 6.93$ ,  $df = 1,96$ ,  $P = 0.01$ ), and CSF ( $F = 22.60$ ,  $df = 1,96$ ,  $P < 0.001$ ) volume. Schizophrenia patients had a lower relative volume of GM ( $t = 6.16$ ,  $df = 98$ ,  $P < 0.001$ ) and WM ( $t = 2.88$ ,  $df = 98$ ,  $P = 0.005$ ), and a larger relative CSF volume ( $t = 5.65$ ,  $df = 98$ ,  $P < 0.001$ ). We did not detect any significant effect of serologic status or group–serologic status interaction on relative volumes.

Voxel-based morphometry analyses showed a regional GM volume reduction in schizophrenia patients when compared with controls, bilaterally in the lateral frontal, temporal, parietal and occipital cortical regions, hippocampus, in the middle and posterior cingulate region and in the caudate (Table II, Figure 1). An increase of GM volume in patients was observed only in one cluster in globus pallidus.

The comparison of *T. gondii*-negative subjects between schizophrenia and the control group revealed a reduction in GM volume in patients in the left uncus and middle temporal gyrus, and bilaterally in the insula. However, the difference between patients and controls was more pronounced for *T. gondii*-positive participants. *T. gondii*-positive patients had a significantly reduced GM volume bilaterally in the

precentral gyri, caudate and thalamus, temporal and mediotemporal regions including the parahippocampal gyri and hippocampus, middle and posterior cingulate, occipital cortex and cerebellum (Table II, Figure 1).

*T. gondii*-positive and -negative controls did not differ in any cluster. In the schizophrenia sample we found a significant reduction of GM volume in *T. gondii*-positive comparing with *T. gondii*-negative patients bilaterally in the caudate, median cingulate, thalamus and occipital cortex and in the left cerebellar hemispheres. In *T. gondii*-positive patients we did not find any regions of increase in GM. The interaction between group and *T. gondii* positivity has been detected bilaterally in the thalamus, caudate and posterior cingulate (Table II). VBM did not reveal any influence of group or serological status on WM.

## Discussion

The main finding of our study is the effect of latent toxoplasmosis on regional gray matter volume (detected by VBM) in schizophrenia but not in the controls. This effect is expressed in two ways. First, we found the differences between latent toxoplasmosis-positive and -negative subjects in patients but not in the control group. Second, the *T. gondii*-seropositive patients exerted reduced GM in comparison with *T. gondii*-seropositive controls in regions consisting of 11,660 over-threshold voxels. At the same time, in *T. gondii*-negative samples, the differences between patients and controls were only in the temporal regions and were significantly less pronounced (289 voxels).

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1 Table II. Voxel-based morphometry results. Two-way ANOVA significant results for clusters consisting of  $\geq 50$  voxels and exceeding 57  
2 height threshold  $T = 5.00$  ( $P \leq 0.05$  after FWE correction across the whole brain) are displayed. 58  
3

Brain region	L or R	Size in voxels	Talairach coordinates			BA
			x	y	z	
All: schizophrenia < controls						
Precentral and parahippocampal gyrus, hippocampus	R	8765	35	-18	39	4 and 6
Insula and claustrum	L	8014	-34	-7	-1	13
Cerebellum	R	4151	18	-79	-39	-
Caudate Body	R	242	15	-9	23	-
Caudate Body	L	151	-16	-9	23	-
Superior Parietal Lobule	L	64	-11	-63	56	7
Inferior occipital gyrus, Inferior and middle temporal gyrus	R	71	45	-71	-5	19 and 37
Inferior and middle occipital Gyrus, fusiform gyrus	L	107	-33	-86	-15	18
Lingual Gyrus	L	53	-12	-44	-4	19
Precuneus and Posterior Cingulate	L	71	-11	-67	18	31
Median cingulate and paracingulate gyri	L	62	-7	-15	42	24
Middle Frontal Gyrus	L	71	-36	42	22	10
Superior Parietal Lobule	R	73	9	-61	56	7
All: schizophrenia > controls						
Medial and lateral globus pallidus	L	55	-12	-8	-4	-
<i>T. gondii</i> positive: schizophrenia < controls						
Precentral Gyrus	R	2650	35	-18	39	4 and 6
Thalamus (pulvinar), parahippocampal gyrus and hippocampus	R	1228	8	-27	9	-
Caudate body and putamen	R	439	15	-9	23	-
Caudate body and head	L	189	-16	-7	21	-
Precentral Gyrus	L	1172	-40	-20	34	4 and 6
Cerebellum	L	1190	-25	-35	-25	-
Insula, parahippocampal gyrus and hippocampus	L	1020	-33	3	11	13 and -
Bilateral posterior and median cingulate, R cerebellum	L and R	3294	-3	-46	18	30
Middle Temporal Gyrus and inferior parietal lobule	R	140	45	-65	30	39 and 7
Fusiform, Inferior Occipital and lingual gyri	L	212	-18	-92	-16	18 and 17
Superior and middle temporal gyri	R	55	45	-24	-3	22
Middle Occipital and Middle Temporal Gyri	R	71	37	-78	14	19
<i>T. gondii</i> negative: schizophrenia < controls						
Uncus and middle temporal gyrus	L	84	-30	-11	-32	20 and 38
Insula, claustrum	L	150	-36	-5	-2	-
Insula	R	55	38	-14	9	-
Schizophrenia: <i>T. gondii</i> positive < negative						
Caudate head and body	L	171	-12	15	4	-
Caudate Body	R	156	10	15	6	-
Median cingulate	R and L	443	6	2	41	24
Thalamus (pulvinar) and parahippocampal gyrus	L	91	-10	-30	7	- and 27
Thalamus (pulvinar)	R	63	8	-27	9	-
Cerebellum	L	119	-34	-71	-32	-
Cerebellum	L	84	-14	-76	-43	-
Parahippocampal and lingual gyrus, posterior cingulate	R	125	10	-49	2	30 and 18
Fusiform Gyrus	L	63	-20	-92	-14	18
Interaction: diagnosis vs TG						
Thalamus (pulvinar and medial dorsal nucleus)	R and L	279	8	-27	7	-
Caudate body	L	56	-16	-7	21	-
Caudate body	R	62	14	-2	21	-
Posterior cingulate	R and L	57	-1	-46	18	-

54 Lor R, left or right hemisphere; x, y, z, Talairach coordinates of voxel of maximum significance; BA, Brodmann's area; -, no BA for the 110  
55 cluster. 111  
56 112

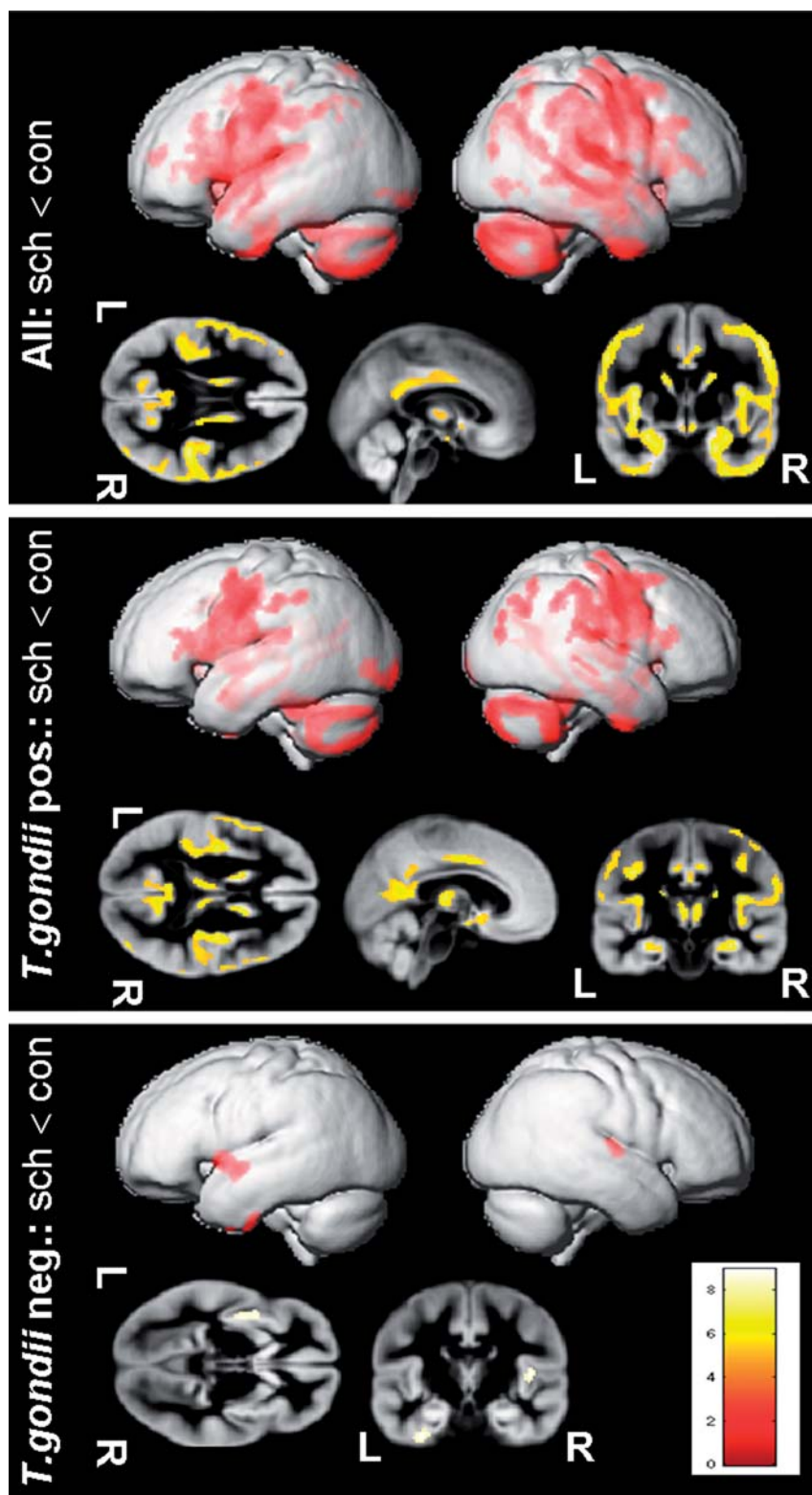


Figure 1. The regional gray matter volume reduction in schizophrenia for the whole sample (upper image), *T. gondii*-positive (middle image) and *T. gondii*-negative (lower image) subjects. Significant results ( $P \leq 0.05$ , FWE, cluster  $\geq 50$  voxels) are displayed on study specific 3D template and mean image slices. L or R, left or right hemisphere; sch or con, schizophrenia or control subjects, the bar in the lower left corner represents *T* value for slices.

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1 The differences detected between patients and  
 2 controls in brain tissue volumes (CSF enlargement  
 3 and relative GM, WM reduction) as well as in the  
 4 local maxima of GM reduction correspond with pre-  
 5 vious studies in schizophrenia. The recent meta-  
 6 analyses implicate grey matter reductions in the  
 7 thalamus, caudate, insula, anterior cingulate, lateral  
 8 prefrontal cortex, and medial and lateral temporal  
 9 regions associated with schizophrenia (Glahn et al.  
 10 2008; Fornito et al. 2009). In accordance with our  
 11 results, the regions with GM increases in schizophre-  
 12 nia are more discrete and across the studies only  
 13 small clusters within basal ganglia were detected  
 14 (Glahn et al. 2008). The finding of GM reduction in  
 15 our *T. gondii*-positive sample corresponds with the  
 16 distribution of *T. gondii* cysts in microglia, astrocytes,  
 17 but primarily in neurons (Ferguson and Hutchison  
 18 1987; Halonen et al. 1996) including Purkynje cells  
 19 in the cerebellum (Bertoli et al. 1995). The regions  
 20 of GM reduction in seropositive patients are located  
 21 in the same regions as in the whole sample suggesting  
 22 that latent toxoplasmosis could be in fact responsible  
 23 for some if not all differences in the GM morphom-  
 24 etry between schizophrenic patients and controls.

25 The cross-sectional design of our study does not  
 26 allow a decision on whether these GM changes were  
 27 induced by the acute and latent stage of the infec-  
 28 tion. However, a substantial role of acute infection  
 29 in schizophrenia is less likely due to several reasons.  
 30 Firstly, IgM as a marker of acute *T. gondii* infection  
 31 is not elevated in first episode patients (Torrey et al.  
 32 2007). Secondly, the GM changes detected in our  
 33 *T. gondii*-positive patients are specific for schizophre-  
 34 nia in first episodes and even in high risk studies  
 35 (Glahn et al. 2008; Chan et al. 2009; Fornito et al.  
 36 2009). Moreover, a higher risk of schizophrenia was  
 37 also documented in offspring following maternal  
 38 *T. gondii* infection (Brown et al. 2005; Mortensen et  
 39 al. 2007). Hence, a more plausible explanation is  
 40 related to the effect of latent toxoplasmosis.

41 Compatible with our findings is possibility that in  
 42 vulnerable subjects the latent infection induces gray  
 43 matter changes and represents an additional environ-  
 44 mental risk factor for schizophrenia. The reduction  
 45 of gray matter in schizophrenia might be mediated  
 46 by two distinct mechanisms. The first is an immune  
 47 reaction of the brain to *T. gondii*, and the second is  
 48 the biochemical activity of the parasite itself.

49 Interferon- $\gamma$  (IFN- $\gamma$ ) secreted in response to *T.*  
 50 *gondii* infection plays an essential role in maintaining  
 51 toxoplasmosis in a latent form. Owing to the fact that  
 52 IFN- $\gamma$  is reduced schizophrenia (Wilke et al. 1996)  
 53 but elevated in latent toxoplasmosis (Suzuki et al.  
 54 2005; Fernandes et al. 2010), the induction of this  
 55 cytokine would represent an additive neurotoxic  
 56 factor. In concrete, IFN- $\gamma$  induces in astrocytes a

production of indoleamine-2, 3-dioxygenase (IDO) 57  
 (Nagineni et al. 1996; Silva et al. 2002). IDO is a 58  
 rate-limiting enzyme in catabolism of tryptophan 59  
 which is an amino acid essential for *T. gondii* replica- 60  
 tion (Nagineni et al. 1996; Ceravolo et al. 1999). 61  
 Tryptophan is degraded by IDO to kynurenine 62  
 which is subsequently metabolized to kynurenic acid 63  
 (KYNA), an antagonist of the glutamate NMDA 64  
 (*N*-methyl-D-aspartate) receptor (Kessler et al. 1989) 65  
 which plays an important role in schizophrenia 66  
 (Bubenikova-Valesova et al. 2008). The elevation of 67  
 kynurenine and KYNA in the brains of schizophre- 68  
 nia patients (Schwarcz et al. 2001; Miller et al. 2006) 69  
 has been interpreted as due to up-regulation of trypt- 70  
 ophan 2,3-dioxygenase (TDO), the second enzyme 71  
 converting tryptophan to kynurenine (Miller et al. 72  
 2004). The same study did not find any change in 73  
 the expression of IDO in schizophrenia. Together, 74  
 the induction IDO by IFN- $\gamma$  in latent toxoplasmosis 75  
 would represent the critical (additional to TDO) 76  
 environmental risk factor for schizophrenia. This 77  
 assumption is supported by the observation of 7-fold 78  
 increase of KYNA in chronically *T. gondii*-infected 79  
 mice (Schwarcz and Hunter 2007). 80

81 Kynurenine is alternatively hydroxylated to quino-  
 82 linate, a potent NMDA neurotoxic agent (el-Defrawy  
 83 et al. 1986). Kynurenine hydroxylase is also stimu-  
 84 lated by IFN- $\gamma$  (Berati-Giani et al. 1996) and in  
 85 latent toxoplasmosis would then accelerate the  
 86 reduction of gray matter. These changes would be  
 87 more pronounced in vulnerable subjects with  
 88 increased quinolinate precursor kynurenine as is the  
 89 case in schizophrenia (Schwarcz et al. 2001).

90 Alternatively, it is possible that the physiology of the  
 91 parasite itself would also mediate the GM reduction.  
 92 Two genes analogical to the human gene for tyrosine  
 93 hydroxylase were recently identified in *T. gondii* bra-  
 94 dyzoites (Gaskell et al. 2009). By the expression of  
 95 these genes *T. gondii* enhances dopaminergic activity  
 96 which would be the critical step for both schizophre-  
 97 nia onset and neurotoxic changes with morphological  
 98 sequelae (Smythies 1999). The differences in GM as  
 99 well as the later onset of psychotic symptoms in  
 100 *T. gondii*-positive patients in our sample indicate that  
 101 *T. gondii*-associated schizophrenia could represent a  
 102 separate phenotype.

103 Interestingly, the recent genome-wide association  
 104 (GWA) studies identified an association with gene  
 105 variants in the major histocompatibility complex  
 106 (MHC, 6p22.1) and interleukin 3 receptor alpha  
 107 (IL3RA, Xp22.3, reviewed in Tiwari et al. 2010). It  
 108 would be important to test an impact on morphom-  
 109 etry of an interaction between these genes and  
 110 *T. gondii* seropositivity.

111 Several methodological factors should be consid-  
 112 ered in our findings. The optimized protocol for VBM

restores the tissue volumes by the step of modulation and lessens the risk of false positive results (Fornito et al. 2009). However, the results would be affected by other methodological factors as smoothing and covariates. In VBM studies, images are typically smoothed to 8, 10, and exceptionally to 12 mm. With respect to the subtle expected structural changes related to toxoplasmosis we applied smoothing to 8 mm. Due to the collinearity between age, gender and TBV, we only used TBV as a confound (Pell et al. 2008). However, the alternative analyses with smoothing to 10 mm and age and/or gender as additional covariates did not substantially affect our results.

There are several limitations to the present study, including the small sample size of seropositive cases and the use of a cross-sectional study design. Both studies of high-risk population and first episode patients, and prospective analyses evaluating the influence of latent toxoplasmosis would determine if *T. gondii*-associated reduction of GM precedes onset of schizophrenia or accelerates the loss of GM tissue. Analyses of dopaminergic activity, IFN- $\gamma$ , MHC and IL3RA gene variants would elucidate the mechanisms mediating the influence of *T. gondii* on GM in vulnerable subjects.

The use of antipsychotics in our clinical sample might represent a limitation. But *T. gondii*-positive and -negative patients differed neither in duration of antipsychotic treatment nor in chlorpromazine equivalents, and hence the substantial influence of medication on our results is less likely.

In conclusion, our study is the first to document that latent toxoplasmosis is connected with the reduction of GM in schizophrenia. *T. gondii* can affect gray matter by several mechanisms including kynurenine metabolites and dopamine overactivity. Another implication of our findings is the fact that *T. gondii* seropositivity would be taken into account in morphometry studies in schizophrenia.

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## Statement of Interest

All authors confirmed their agreement to submission and declared that they have no competing financial interests.

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