



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 ($P \leq 0.05$, 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.

Key words: 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 glia (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

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.

positivity index > 1), combined with a negative or equivocal IgM ELISA test (positivity index < 0.9).

MRI data acquisition and VBM protocol

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

Structural MRI analysis was performed using an optimized VBM protocol in Statistical Parametric Mapping v.5, SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5>) implemented in MATLAB 7.3 (Math Works, Natick, MA). VBM has proven useful in characterizing subtle changes in brain structure in schizophrenia (Glahn et al. 2008; Fornito et al. 2009). The optimized version of VBM is complemented by correcting for the deformations introduced during the spatial normalization procedure (modulation) and reflects an estimate of absolute grey matter volume. Each 3D volume of T1W images was segmented into GM, WM, and cerebrospinal fluid (CSF), and total volumes were obtained via the 'get_totals' matlab script (<http://www.cs.ucl.ac.uk/staff/G.Ridgway/vbm/>). Spatial normalization parameters were estimated by matching GM tissue with the standard GM template provided by SPM5. The normalization parameters were applied to the original T1W images. Optimally normalized T1W images were then segmented into GM, WM, and CSF segments. In order to restore tissue volumes modified during normalization processing, Jacobian modulation was applied to normalized GM and WM segments. Modulated normalized GM, WM and CSF images were smoothed by a Gaussian kernel of 8 mm full width at half maximum (FWHM). The full factorial model (analysis of variance, ANOVA) with diagnosis and latent toxoplasmosis positivity/negativity as factors was used to address the differences in gray and white matter. For all SPM5 analyses, total brain volume (TBV) was used as a nuisance covariate (Pell et al. 2008). For rigorous control type I error we accepted as significant only conservative family-wise error (FWE) corrected findings with the height threshold $T = 5.00$ ($P \leq 0.05$) with the extent threshold consisting of 50 voxels per cluster. The coordinates for local maxima in MNI (Montreal Neurological Institute) template were converted to stereotactic Talairach x, y, z coordinates (Lancaster et al. 2007). Results were displayed using a study-specific 3D template and

slices derived from the mean image of all normalized 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 volume, and between *T. gondii*-positive and -negative patients in PANSS, duration of antipsychotic treatment, chlorpromazine equivalents and age of onset were performed by independent sample *t*-tests. Two-way ANOVA with a group and *T. gondii* serological status as factors followed by a Bonferroni post hoc test, when appropriate, was used to compare *T. gondii*-positive and -negative patients and controls in absolute and relative brain volumes.

Results

Demographic and clinical data

The schizophrenia group and final sample of controls did not differ in age ($t = 1.78$, $df = 98$, $P = 0.08$), sex ($\chi^2 = 0.79$, $df = 1$, $P = 0.37$), education ($\chi^2 = 5.68$, $df = 2$, $P = 0.06$), 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 chlorpromazine 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 volumes 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

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 ³	1679 (236.9)^a	1688.4 (251.6)	1654.6 (200.4)	1526 (194.8)^a	1539 (189.4)	1484 (213.8)
GM in cm ³	685.8 (88.19)^b	682.9 (98.1)	693.6 (56.5)	699.4 (65.94)^b	704.8 (58.19)	685.2 (89.29)
WM in cm ³	520 (79.63)^b	528.5 (83.7)	497.4 (65.4)	501.5 (65.99)^b	507.3 (57.20)	482.3 (89.37)
CSF in cm ³	473.3 (162.4)^{a,b}	479.8 (172.2)	463.6 (139.4)	325.5 (112.5)^{a,b}	328.2 (122.5)	316.3 (73.53)
Age of onset	24.74 (8.354)	22.77 (6.689)⁺	28.41 (9.952)⁺	–	–	–
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 ≥ 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).

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

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	-

L or R, left or right hemisphere; x, y, z, Talairach coordinates of voxel of maximum significance; BA, Brodmann's area; -, no BA for the cluster.

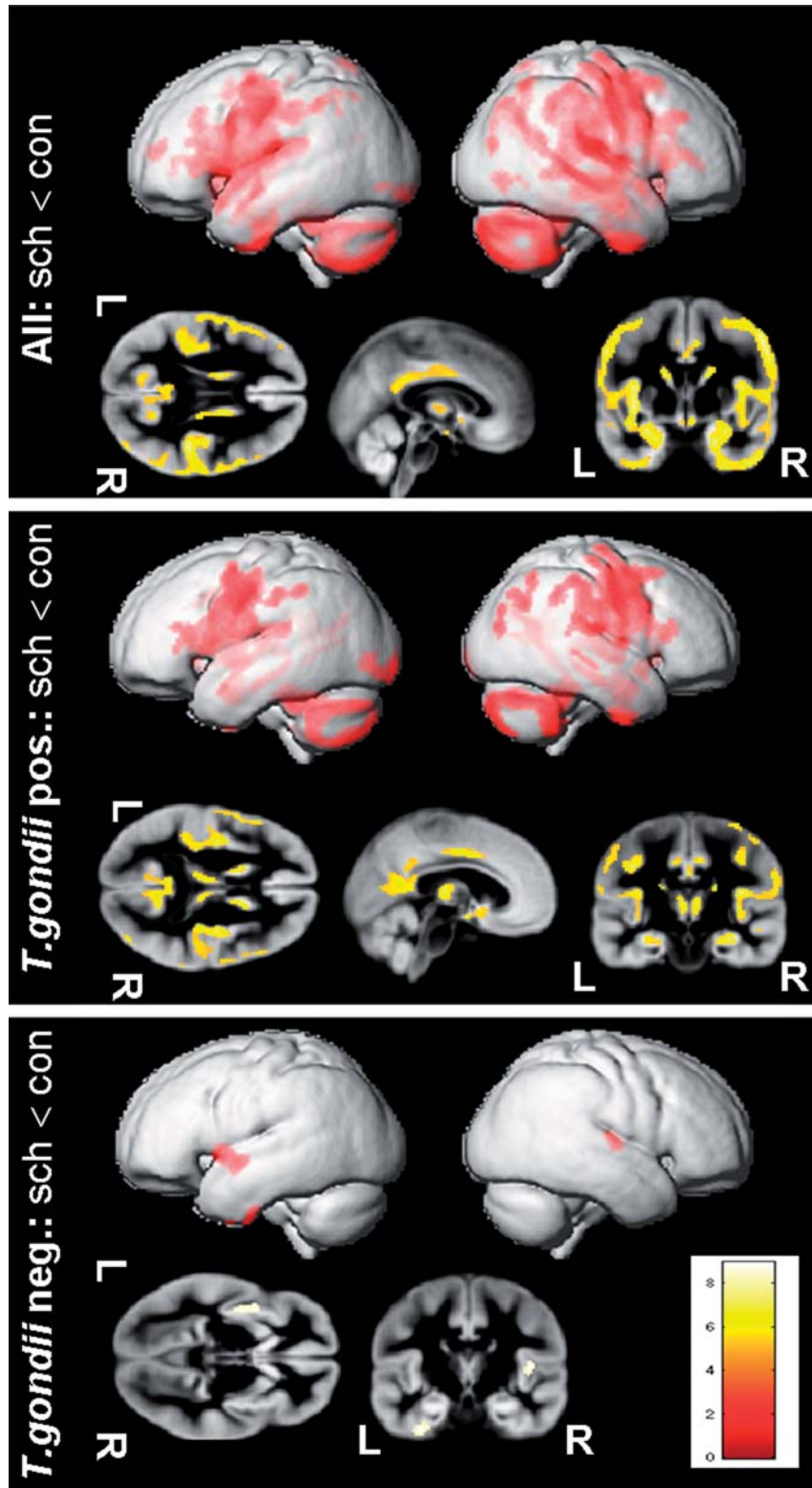


Figure 1. The regional gray matter volume reduction in schizophrenia for the whole sample (upper image), *T. gondii*-positive (middle image) and *T. gondii*-negative (lower image) subjects. Significant results ($P \leq 0.05$, FWE, cluster ≥ 50 voxels) are displayed on study specific 3D template and mean 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.

The differences detected between patients and controls in brain tissue volumes (CSF enlargement and relative GM, WM reduction) as well as in the local maxima of GM reduction correspond with previous studies in schizophrenia. The recent meta-analyses implicate grey matter reductions in the thalamus, caudate, insula, anterior cingulate, lateral prefrontal cortex, and medial and lateral temporal regions associated with schizophrenia (Glahn et al. 2008; Fornito et al. 2009). In accordance with our results, the regions with GM increases in schizophrenia are more discrete and across the studies only small clusters within basal ganglia were detected (Glahn et al. 2008). The finding of GM reduction in our *T. gondii*-positive sample corresponds with the distribution of *T. gondii* cysts in microglia, astrocytes, but primarily in neurons (Ferguson and Hutchison 1987; Halonen et al. 1996) including Purkinje cells in the cerebellum (Bertoli et al. 1995). The regions of GM reduction in seropositive patients are located in the same regions as in the whole sample suggesting that latent toxoplasmosis could be in fact responsible for some if not all differences in the GM morphometry between schizophrenic patients and controls.

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

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

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

production of indoleamine-2, 3-dioxygenase (IDO) (Nagineni et al. 1996; Silva et al. 2002). IDO is a rate-limiting enzyme in catabolism of tryptophan which is an amino acid essential for *T. gondii* replication (Nagineni et al. 1996; Ceravolo et al. 1999). Tryptophan is degraded by IDO to kynurenine which is subsequently metabolized to kynurenic acid (KYNA), an antagonist of the glutamate NMDA (*N*-methyl-D-aspartate) receptor (Kessler et al. 1989) which plays an important role in schizophrenia (Bubenikova-Valesova et al. 2008). The elevation of kynurenine and KYNA in the brains of schizophrenia patients (Schwarcz et al. 2001; Miller et al. 2006) has been interpreted as due to up-regulation of tryptophan 2,3-dioxygenase (TDO), the second enzyme converting tryptophan to kynurenine (Miller et al. 2004). The same study did not find any change in the expression of IDO in schizophrenia. Together, the induction IDO by IFN- γ in latent toxoplasmosis would represent the critical (additional to TDO) environmental risk factor for schizophrenia. This assumption is supported by the observation of 7-fold increase of KYNA in chronically *T. gondii*-infected mice (Schwarcz and Hunter 2007).

Kynurenine is alternatively hydroxylated to quinolinate, a potent NMDA neurotoxic agent (el-Defrawy et al. 1986). Kynurenine hydroxylase is also stimulated by IFN- γ (Berati-Giani et al. 1996) and in latent toxoplasmosis would then accelerate the reduction of gray matter. These changes would be more pronounced in vulnerable subjects with increased quinolinate precursor kynurenine as is the case in schizophrenia (Schwarcz et al. 2001).

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

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

Several methodological factors should be considered 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- γ , 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.

Acknowledgments

This work was supported by grants 1M0517 and VZ 0021620816 from the MEYS CR, GACR P303/11/1398, and MZ0PCP2005 from MH CR. We thank H. Fridrichova for technical assistance and subject coordination, I. Gorman for language correction and Prof. Sir Peter Lachmann and Prof. Martin Alda for helpful suggestions and comments.

Statement of Interest

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

References

- Berati-Giani D, Ricciardi-Castagnoli P, Kohler C, Cesura AM. 1996. Regulation of the kynurenine metabolic pathway by interferon-gamma in murine cloned macrophages and microglial cells. *J Neurochem* 66:996–1004.
- Bertoli F, Espino M, Arosemena JR, Fishback JL, Frenkel JK. 1995. A spectrum in the pathology of toxoplasmosis in patients with acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 119:214–224.
- Brown AS, Schaefer CA, Quesenberry CP Jr, Liu L, Babulas VP, Susser ES. 2005. Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. *Am J Psychiatry* 162:767–773.
- Bubenikova-Valesova V, Horacek J, Vrajova M, Hoschl C. 2008. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neurosci Biobehav Rev* 32:1014–1023.
- Carruthers VB, Suzuki Y. 2007. Effects of *Toxoplasma gondii* infection on the brain. *Schizophr Bull* 33:745–751.
- Ceravolo IP, Chaves AC, Bonjardim CA, Sibley D, Romanha AJ, Gazzinelli RT. 1999. Replication of *Toxoplasma gondii*, but not *Trypanosoma cruzi*, is regulated in human fibroblasts activated with gamma interferon: requirement of a functional JAK/STAT pathway. *Infect Immun* 67:2233–2240.
- Chan RC, Di X, McAlonan GM, Gong QY. 2011. Brain anatomical abnormalities in high-risk individuals, first-episode, and chronic schizophrenia: an activation likelihood estimation meta-analysis of illness progression. *Schizophr Bull* 37:177–188.
- Dubey JP. 1998. Advances in the life cycle of *Toxoplasma gondii*. *Int J Parasitol* 28:1019–1024.
- el-Defrawy SR, Boegman RJ, Jhamandas K, Beninger RJ. 1986. The neurotoxic actions of quinolinic acid in the central nervous system. *Can J Physiol Pharmacol* 64:369–375.
- Ferguson DJ, Hutchison WM. 1987. The host-parasite relationship of *Toxoplasma gondii* in the brains of chronically infected mice. *Virchows Arch A Pathol Anat Histopathol* 411:39–43.
- Fernandes JF, Taketomi EA, Mineo JR, Miranda DO, Alves R, Resende RO, et al. 2010. Antibody and cytokine responses to house dust mite allergens and *Toxoplasma gondii* antigens in atopic and non-atopic Brazilian subjects. *Clin Immunol* 136:148–156.
- Flegel J, Preiss M, Klose J, Havlicek J, Vitakova M, Kodym P. 2003. Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasma gondii* dopamine, a missing link between schizophrenia and toxoplasmosis? *Biol Psychol* 63:253–268.
- Fornito A, Yucel M, Patti J, Wood SJ, Pantelis C. 2009. Mapping grey matter reductions in schizophrenia: an anatomical likelihood estimation analysis of voxel-based morphometry studies. *Schizophr Res* 108:104–113.
- Gaskell EA, Smith JE, Pinney JW, Westhead DR, McConkey GA. 2009. A unique dual activity amino acid hydroxylase in *Toxoplasma gondii*. *PLoS One* 4:e4801.
- Glahn DC, Laird AR, Ellison-Wright I, Thelen SM, Robinson JL, Lancaster JL, et al. 2008. Meta-analysis of gray matter anomalies in schizophrenia: application of anatomic likelihood estimation and network analysis. *Biol Psychiatry* 64:774–781.
- Halonen SK, Lyman WD, Chiu FC. 1996. Growth and development of *Toxoplasma gondii* in human neurons and astrocytes. *J Neuropathol Exp Neurol* 55:1150–1156.
- Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. 2001. *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. *Am J Epidemiol* 154:357–365.
- Kay SR, Fiszbein A, Opler LA. 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* 13:261–276.

- Kessler M, Terramani T, Lynch G, Baudry M. 1989. A glycine site associated with N-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. *J Neurochem* 52:1319–1328.
- Kodym P, Machala L, Rohacova H, Sirocka B, Maly M. 2007. Evaluation of a commercial IgE ELISA in comparison with IgA and IgM ELISAs, IgG avidity assay and complement fixation for the diagnosis of acute toxoplasmosis. *Clin Microbiol Infect* 13:40–47.
- Lancaster JL, Tordesillas-Gutierrez D, Martinez M, Salinas F, Evans A, Zilles K, et al. 2007. Bias between MNI and Talairach coordinates analyzed using the ICBM-152 brain template. *Hum Brain Mapp* 28:1194–1205.
- Lehman AF, Lieberman JA, Dixon LB, et al. 2004. Practice guideline for the treatment of patients with schizophrenia. 2nd ed. *Am J Psychiatry* 161(2 Suppl):1–56.
- Leweke FM, Gerth CW, Koethe D, Klosterkotter J, Ruslanova I, Krivogorsky B, et al. 2004. Antibodies to infectious agents in individuals with recent onset schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 254:4–8.
- Miller CL, Llenos IC, Dulay JR, Barillo MM, Yolken RH, Weis S. 2004. Expression of the kynurenine pathway enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. *Neurobiol Dis* 15:618–629.
- Miller CL, Llenos IC, Dulay JR, Weis S. 2006. Upregulation of the initiating step of the kynurenine pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. *Brain Res* 1073–1074:25–37.
- Mortensen PB, Norgaard-Pedersen B, Waltoft BL, Sorensen TL, Hougaard D, Yolken RH. 2007. Early infections of *Toxoplasma gondii* and the later development of schizophrenia. *Schizophr Bull* 33:741–744.
- Naginani CN, Pardhasaradhi K, Martins MC, Detrick B, Hooks JJ. 1996. Mechanisms of interferon-induced inhibition of *Toxoplasma gondii* replication in human retinal pigment epithelial cells. *Infect Immun* 64:4188–4196.
- Oldfield RC. 1971. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9:97–113.
- Pell GS, Briellmann RS, Chan CH, Pardoe H, Abbott DF, Jackson GD. 2008. Selection of the control group for VBM analysis: influence of covariates, matching and sample size. *Neuroimage* 41:1324–1335.
- Schwarz R, Hunter CA. 2007. *Toxoplasma gondii* and schizophrenia: linkage through astrocyte-derived kynurenic acid? *Schizophr Bull* 33:652–653.
- Schwarz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. 2001. Increased cortical kynurenate content in schizophrenia. *Biol Psychiatry* 50:521–530.
- Silva NM, Rodrigues CV, Santoro MM, Reis LF, Varez-Leite JJ, Gazzinelli RT. 2002. Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with *Toxoplasma gondii*: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. *Infect Immun* 70:859–868.
- Smythies J. 1999. The neurotoxicity of glutamate, dopamine, iron and reactive oxygen species: functional interrelationships in health and disease: a review-discussion. *Neurotox Res* 1: 27–39.
- Suzuki Y, Claflin J, Wang X, Lengi A, Kikuchi T. 2005. Microglia and macrophages as innate producers of interferon-gamma in the brain following infection with *Toxoplasma gondii*. *Int J Parasitol* 35:83–90.
- Tiwari AK, Zai CC, Muller DJ, Kennedy JL. 2010. Genetics in schizophrenia: where are we and what next? *Dialogues Clin Neurosci* 12:289–303.
- Torrey EF, Bartko JJ, Lun ZR, Yolken RH. 2007. Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. *Schizophr Bull* 33:729–736.
- Wang HL, Wang GH, Li QY, Shu C, Jiang MS, Guo Y. 2006. Prevalence of *Toxoplasma* infection in first-episode schizophrenia and comparison between *Toxoplasma*-seropositive and *Toxoplasma*-seronegative schizophrenia. *Acta Psychiatr Scand* 114: 40–48.
- Wilke I, Arolt V, Rothermundt M, Weitzsch C, Hornberg M, Kirchner H. 1996. Investigations of cytokine production in whole blood cultures of paranoid and residual schizophrenic patients. *Eur Arch Psychiatry Clin Neurosci* 246: 279–284.
- Woods SW. 2003. Chlorpromazine equivalent doses for the newer atypical antipsychotics. *J Clin Psychiatry* 64:663–667.