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Toxoplasma gondii impairs memory in infected seniors

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ABSTRACT

Almost 30% of humans present a *Toxoplasma gondii* positive antibody status and its prevalence increases with age. The central nervous system is the main target. However, little is known about the influence of asymptomatic i.e. latent Toxoplasmosis on cognitive functions in humans. To investigate neurocognitive dysfunctions in asymptomatic older adults with *T. gondii* positive antibody status a double-blinded neuropsychological study was conducted. The participants were classified from a population-based sample ($N = 131$) of healthy participants with an age of 65 years and older into two groups with 42 individuals each: Toxoplasmosis positive (T-pos; IgG > 50 IU/ml) and Toxoplasmosis negative (T-neg; IgG = 0 IU/ml). The outcome measures were a computer-based working-memory test (2-back) and several standardized psychometric tests of memory and executive cognitive functions. T-pos seniors showed an impairment of different aspects of memory. The rate of correctly detected target symbols in a 2-back task was decreased by nearly 9% ($P = 0.020$), corresponding to a performance reduction of about 35% in working memory relative to the T-neg group. Moreover, T-pos seniors had a lower performance in a verbal memory test, both regarding immediate recall (10% reduction; $P = 0.022$), delayed recognition (6%; $P = 0.037$) and recall from long-term memory assessed by the word fluency tests (12%; $P = 0.029$). In contrast, executive functions were not affected. The effects remained mostly unchanged after controlling for medication. The impairment of memory functions in T-pos seniors was accompanied by a decreased self-reported quality of life. Because of the high prevalence of asymptomatic Toxoplasmosis and an increasing population of older adults this finding is of high relevance for public health.

1. Introduction

Almost one third of world's population is infected with *Toxoplasma gondii* (Tenter et al., 2000). The oocysts of the parasite are resistant against stomach acid and infect nearly each cell type in the intestinal tract. When intestinal cells rupture released parasites pass the blood–brain barrier and may cause life-long infections of astrocytes and neurons (Carruthers and Suzuki, 2007). Toxoplasma infection of the human brain is well known since 1923 by discovering the parasite in the eye of a congenitally infected infant (Weiss and Dubey, 2009). Since the 1950s serious neurological and psychiatric diseases like schizophrenia or other psychotic disorders, anxiety disorders with obsessions or Parkinson disease, or even suicidal attempts were identified to be associated with *T. gondii* infection (Dalimi and Abdoli, 2012). A possible common mechanism of these disorders is Toxoplasma-related modification of dopaminergic signalling in the central nervous system (McConkey et al., 2013; Prandovszky et al., 2011).

Besides associations with neurological diseases *T. gondii* has been reported to modify behaviour in animals. Whereas uninfected rodents avoid predators, infected ones show risky behaviour (Webster et al., 1994; House et al., 2011). Particularly, elimination of the aversion to odour of cat urine in rodents enhances the probability to be devoured by the cat and to transmit the parasite to its final host (House et al., 2011; Berdoy et al., 2000). This may be interpreted as an extended phenotype where the parasite manifests its influence outside the physical confines of its body (Dawkins, 1982). In humans infections with *T. gondii* have been reported to be associated with subtle behavioural and personality alterations (Flegr, 2013; Webster et al., 2013). Moreover, the Big-Five personality questionnaire (NEO-P-IR) revealed higher scores for extraversion and less conscientiousness in infected than non-infected persons (Lindová et al., 2012).

Because of the high prevalence it is important to know whether "asymptomatic" *T. gondii* infection impairs cognitive functions. Asymptomatic means that no symptoms of the infection are manifest and recognizable either for the person itself or for the environment. However, current investigations in our lab suggest specific deficits in asymptomatic *T. gondii* infected seniors in an auditory distraction paradigm (Beste et al., in press). To our knowledge no further studies were conducted to investigate the association between *T. Gondii* and aspects of fluid cognition like attention,

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memory or executive functions in older age. Recently, Guenter et al. (2012) investigated cognitive performance of infected young adults using a set of neuropsychological tests. They observed trends towards reduced cognitive functions but the differences did not amount to statistical significance. A possible explanation of the negative result might be the relatively young age of the individuals in the study of Guenter et al. (2012). Previous work has shown that the impact of some endogenous factors like genetic polymorphisms on cognitive functions is larger in older than in younger individuals (Lindenberger et al., 2008). The authors suggested that losses of brain resources associated with normal aging amplify the effect of common genetic polymorphisms on human cognition, thereby contributing to the observed increase in heterogeneity of cognitive performance. This may also apply to effects of congenital or postnatal infections like *T. gondii*. Indeed, Flegr (2013) suggested that most of differences in personality traits associated with Toxoplasmosis increase with the length of time since the onset of infection. Therefore, we performed a double-blinded study on a possible influence of asymptomatic *T. gondii* infection in seniors older than 65 years with relatively high IgG antibody levels using well established quantitative tests of working memory and executive functions. We report for the first time that *T. gondii* infection significantly deteriorates functions of the episodic and working memory, which in turn was associated with reduced self-perceived psychosocial health. Considering the high prevalence and the relatively strong association with cognitive functions, the impact of *T. gondii* infection on public health may be higher than hitherto expected.

2. Materials and methods

2.1. Participants and measures of antibody levels

Participants were recruited through a number of newspaper advertisements and flyers distributed in the city of Dortmund (Germany) advertising for a training study (Gajewski and Falkenstein, 2012). All participants were German native speakers with Caucasian descent. Participants were included in the study after meeting some criteria inquired by a telephone interview: They should be 65 or older, physically and mentally fit, living independently and self-paced (no nursing home), and having sufficient or corrected visual and auditory acuity. Exclusion criterions were: history of cardiovascular, psychiatric, neurological, motor or oncologic diseases and psychopharmacologic or hormonal therapy, current or history of alcohol or drug addiction. Exceptions were made for typical age-related diseases like hypertension, hypo- and hyperthyroidism and enhanced cholesterol level. In total, 467 telephone interviews were completed, 152 persons met the criteria and were included in the study. A subsample of 131 persons aged from 65 to 88 ($M = 70.5$, SD = 4.5) were willing to donate blood for analyzing effects of diverse markers on cognitive functions. The main characteristics of the sample were described in more detail elsewhere (Gajewski et al., 2011, 2012). Identification of Toxoplasma-negative and -positive subjects without any clinical symptoms of acute Toxoplasmosis was performed according to the following procedure: The venous blood of all individuals was sampled and tested for *T. gondii* specific IgG antibodies. The analyses were performed in a certified local clinical laboratory using an enzyme immunoassay (EIA) Enzygnost® Toxoplasmosis/IgG (Siemens Healthcare Diagnostics, Eschborn, Germany). The EIA was processed on a BEP III system (Siemens Healthcare Diagnostics, Eschborn, Germany). The sensitivity threshold of Enzygnost® Toxoplasmosis/IgG is at least 6 IU/ml. The cut-off for the clinical application is 10 IU/ml. The upper measurement limit is about 2000 IU/ml with the initial sample dilution of 1 + 230. The sensitivity after retesting in blood donors ($n = 260$) with initial equivocal results was 100%. The specificity after retesting in blood donors ($n = 259$) with initial equivocal results was 100%, according to the manufacturer (Siemens Healthcare Diagnostics, 2010).

IgG levels of 50 IU/ml or higher qualified the participants to the seropositive (T-pos) group (43 participants). This cut-off was set to get two subsamples: (i) with a clearly distinguishable IgG antibody levels (ii) with approximately the same number of participants (iii) to enhance effects of *T. gondii* which did not reach significance in the study of Guenter et al. (2012) using the cut-off of 35 IU/ml. Only non-infected participants (0 IU/ml) were included into the seronegative (T-neg) group (42 participants). Subjects with intermediate concentrations (1–49 IU/ml) were excluded from the study (46 participants). One participant of the T-pos group was excluded from the analysis as he did not complete all tests. Consequently, the sample consisted of 42 Toxoplasma-negative and 42-positive individuals. The characteristics of both groups were included in Table 1.

The mean IgG antibody level in the T-pos subjects was 143.2 (SD 114.7, range 52–510) IU/ml. Twenty-nine participants of the T-neg group (69%) and 27 of the T-pos group (64.3%), were female ($X^2 = 0.21$, $P = 0.64$). The Chi-square test indicates that gender was equally distributed in T-neg and T-pos groups. Four participants of the T-neg and three participants of the T-pos group were left handed or ambidextrous. All participants had normal or corrected-to-normal vision. Possible dementia and mild cognitive impairment were assessed by using Mini Mental State Examination (MMSE; Folstein et al., 1975). No participant revealed a MMSE score lower than 25, suggesting normal cognitive functioning (Table 1).

Seventeen participants of the T-neg group (40.5%) and 23 (54.8%) of the T-pos group reported taking medication against hypertension ($X^2 = 1.78$, $P = 0.19$). Eight participants of the T-neg group (19.0%) and 11 (26.2%) of the T-pos group used thyroid hormones ($X^2 = 0.61$, $P = 0.43$). Each three persons of the T-neg

Table 1
Sample characteristics and the results of the questionnaires in relation to *Toxoplasma gondii* immune status.

Characteristic	T-neg	T-pos	*F*(df)	*P* value
Number	$n = 42$	$n = 42$		
Age	69.8 (3.5)	70.8 (4.5)	$F(1,83) = 1.3$	ns.
Years of occupation	34.7 (13.7)	32.3 (14.0)	$F(1,83) < 1$	ns.
Highest educational level[a]	2.3 (1.4)	2.4 (1.3)	$F(1,83) < 1$	ns.
Level of physical activity[b]	2.00 (0.9)	1.93 (0.9)	$F(1,83) < 1$	ns.
MMSE	28.8 (1.5)	28.5 (1.7)	$F(1,83) < 1$	ns.
BDI	4.9 (3.7)	6.1 (4.1)	$F(1,83) = 1.9$	ns.
NEO-FFI[*]				
Neuroticism	1.5 (0.5)	1.5 (0.4)	$F(1,77) < 1$	ns.
Extraversion	2.0 (0.5)	2.0 (0.4)	$F(1,76) < 1$	ns.
Openess to experience	2.2 (0.4)	2.0 (0.5)	$F(1,76) = 1.5$	ns.
Agreeableness	2.4 (0.5)	2.2 (0.6)	$F(1,76) = 1.8$	ns.
Conscientiousness	2.6 (0.5)	2.5 (0.5)	$F(1,76) < 1$	ns.
WHOQOL-BREF				
Physical health	16.1 (2.0)	15.1 (2.1)	$F(1,83) = 5.0$	0.027
Psychological health	14.7 (1.2)	13.7 (1.7)	$F(1,83) = 8.0$	0.013
Social relations	15.0 (2.0)	13.8 (2.4)	$F(1,83) = 6.4$	0.013
Environment	16.0 (1.4)	15.7 (1.7)	$F(1,83) < 1$	ns.

Significance level was set at $P < 0.05$. The values represent mean and standard deviation (SD) in parentheses.
Key: BDI, Beck Depression Inventory; MMSE, Mini Mental State Examination; NEO-FFI, "Big Five" personality factors questionnaire; WHO-Quality of Life Questionnaire.
[a] Highest educational level: (0) No school. (1) Primary school (4th grade). (2) Secondary general school (10th grade). (3) Intermediate secondary school (10th grade). (4) Grammar school (gymnasium, 12th grade).
[b] Level of physical activity: (1) Too low. (2) Meet the minimum requirements. (3) Sufficient. (4) High level.
[*] Reduced number of participants, see (Gajewski et al., 2012) for further information.

and T-pos group (7.1%) used cholesterol lowering drugs. In the whole sample, 90.5% were no-smokers, 6% smoked previously, and 3.5% were active smokers. All participants were informed about the scope of the study and gave written informed consent before any study protocol was commenced. However, the result of the immunoassay on *T. gondii* specific IgG antibodies was unknown to the participants of the study and to the operators who performed the cognitive testing, as the identification of *T. gondii* in the participants was conducted after the cognitive testing was finished. The study was approved by the institutional review board in accordance with the declaration of Helsinki. The informed consent from all participants was obtained and their rights were protected throughout the study.

2.2. Cognitive testing

The participants underwent extensive assessment to document their demographic, socioeconomic, neuropsychological and psychiatric status. All tests were performed according to published standard procedures (Gajewski et al., 2011, 2012). Briefly, they comprised a number of questionnaires, assessing the general cognitive status (MMSE), depressive disorder (BDI) and the Big Five personality traits (NEO-FFI). The quality of life regarding physical and psychological health, social relationships and environment was assessed with the WHOQOL-BREF (World Health Organization, 1996). The neuropsychological tests for non-memory functions measured attentional endurance (d2) and speed of processing (Digit-Symbol-Test). Interference was assessed by the Stroop color-word test and mental rotation by the mirrored figures. The Trail-Making-Test (TMT A and B) was administered to measure the attention shifting. The everyday failures were analyzed using the Cognitive Failures Questionnaire (CFQ). Finally, crystalline intelligence was examined by the multiple choice word test (MWT-B).

The test battery for measuring memory functions consisted of Verbal Learning and Memory Test (VLMT), a German version of the Auditory-Verbal Learning Test (AVLT), examining immediate verbal memory and delayed recognition of words. Recall from long-term semantic memory was measured by the Word-Fluency-Test (WFT) and short-term memory by the Digit-Span-Test. Visuo-spatial memory was assessed by the Rey-Osterrieth Complex Figure (ROCF). Working memory was assessed using a 2-back task with a 0-back task as non-memory control condition (Wild-Wall et al., 2011; Schapkin et al., 2012). The stimulus material of both tasks consisted of 25 capital letters from the alphabet which were presented in white on a black computer screen. The size of the stimuli was 1.2° width and 1.7° high at a viewing distance of 60 cm. The 0-back condition reflects a control condition with low working memory load. In this task a two-choice decision was required by pressing a response button for the target letter X and no responding when other letters occurred. In the 2-back task inducing high memory load the participants were asked to press a response button if the current letter matches the letter which was presented two trials before (target), requiring a continuous updating of letters. Each block included 20% target and 80% non-target letters. The 2-back task had 156 trials and the 0-back task 102 trials.

2.3. Data analysis

The differences in socio-demographic, psychiatric, personality as well as the neuropsychological parameters between the T-neg and T-pos groups were analyzed using a series of one-way ANOVAs. In the computer-based 0- and 2-back task the first trial of each block, trials with responses faster than 100 ms, or slower than 2500 ms, as well as error trials (i.e. false alarms to non-targets), were excluded from the RT analysis. Mean RTs, false alarms and missing targets were subjected to an ANOVA design including a within-subject factor Task (0-back vs. 2-back) and the between-subject factor Toxoplasmosis (T-neg vs. T-pos) group. Significance level was set at $P < 0.05$. The numbers that follow the ± sign reflect a standard error (SEM). To assess possible confounds between medication and *T. gondii* on cognitive functions, we controlled the intake of hypertension, thyroid and cholesterol medication. In particular, antihypertensive treatment is known to ameliorate cognitive decline (e.g. Sörös et al., 2013). Hence, a possible influence on cognition in aging can be assumed and should be controlled for.

3. Results

3.1. Questionnaires

The T-neg and T-pos subgroups did not differ significantly regarding a number of socio-demographic parameters like highest educational level, duration of occupation, or level of physical activity (Table 1). Also, the Beck Depression Inventar (BDI) did not reveal a statistical difference. Moreover, the infection did not influence the general cognitive status as measured by the MMSE or personality traits assessed by the Big-Five (NEO-FFI). However, Toxoplasmosis negatively affects the quality of life (WHOQOL-BREF), particularly the self-reported physical and psychological health and social relations.

We controlled the significant effects obtained in the WHOQOL-BREF for a potential influence of medication. By including Hypertension Medication, the effect of *T. gondii* on the dimension *physical health* was attenuated but remained significant ($F(1,81) = 3.9$, $P = 0.049$). In contrast, the effect of Toxoplasmosis on *physical health* was no longer significant after controlling for Thyroid ($F(1,81) = 3.6$, $P = 0.061$) and Cholesterol Medication ($F(1,81) = 3.6$, $P = 0.062$).

For the dimension *psychological health* Hypertension Medication did not affect the effect of *T. gondii* ($F(1,81) = 5.2$, $P = 0.025$). The same was obtained for Thyroid ($F(1,81) = 5.3$, $P = 0.024$) and Cholesterol Medication ($F(1,81) = 5.6$, $P = 0.020$). For the dimension *social relations* Hypertension Medication did not influence the effect of *T. gondii* ($F(1,81) = 5.3$, $P = 0.024$). The same was found for Thyroid ($F(1,81) = 6.8$, $P = 0.011$) and Cholesterol Medication ($F(1,81) = 6.7$, $P = 0.011$). In sum, medication diminished the influence of *T. gondii* on physical health but did not affect the dimensions: psychological health and social relations.

3.2. Neuropsychological data

Table 2 shows the results of the neuropsychological tests. Participants of the T-pos group showed an increased number of confused symbols in the d2 attentional endurance test, lower number of generated words as indicated by the Word-Fluency-Test and a smaller number of memorized and later recognized words in the verbal learning and memory test (VLMT). No effect was found in the visuo-spatial memory test (ROCF). The non-memory based tests examining visual attention and speed of processing (Digit-Symbol-Test), executive functions (Stroop, TMT), mental rotation, as well as the Cognitive Failures Questionnaire did not reveal significant differences. Also, the groups did not differ regarding crystalline intelligence (MWT-B).

The significant results of the neuropsychological tests were additionally controlled for effects of medication. By including the Hypertension Medication in the analysis, the Toxoplasmosis effect on the variable *number of confused symbols in the d2 – test* remained unchanged ($F(1,82) = 6.1$, $P = 0.015$). The same was observed for Thyroid ($F(1,82) = 6.5$, $P = 0.013$) and Cholesterol Medication ($F(1,82) = 6.6$, $P = 0.012$). For the VLMT *total score trials 1–5* including

Table 2
Cognitive functions in relation to *Toxoplasma gondii* immune status.

Functions and tests	T-neg	T-pos	*F*(df)	*P* value
Speed of processing, attention				
d2				
Total number of symbols *n* (SD)	414 (83.6)	382 (90.9)	$F(1,81) = 3.0$	ns.
Number omitted symbols *n* (SD)	24.3 (21.6)	18.1 (13.1)	$F(1,81) = 2.6$	ns.
Number confused symbols *n* (SD)	2.8 (3.3)	6.3 (8.1)	$F(1,81) = 6.7$	0.011
Digit-symbol-test				
Total number of symbols *n* (SD)	44.8 (13.4)	45.0 (8.6)	$F(1,82) < 1$	ns.
Number correct *n* (SD)	44.7 (13.4)	45.0 (8.6)	$F(1,82) < 1$	ns.
Interference, inhibition, switching				
Stroop				
Word reading sec. (SD)	14.4 (3.0)	14.5 (2.0)	$F(1,82) < 1$	ns.
Color naming sec. (SD)	22.1 (5.1)	22.0 (3.9)	$F(1,82) < 1$	ns.
Interference list sec. (SD)	44.0 (14.6)	44.3 (9.9)	$F(1,82) < 1$	ns.
TMT[a]				
TMT-A sec. (SD)	38.8 (13.0)	37.1 (11.1)	$F(1,72) < 1$	ns.
TMT-B sec. (SD)	98.8 (45.9)	99.4 (30.2)	$F(1,72) < 1$	ns.
Spatial cognition				
Mental rotation				
Total number *n* (SD)	6.2 (3.1)	5.8 (3.0)	$F(1,82) < 1$	ns.
Number correct *n* (SD)	5.8 (3.2)	4.9 (2.8)	$F(1,82) = 1.4$	ns.
Cognitive failures				
CFQ[a]				
Total score *n* (SD)	27.8 (12.7)	29.4 (11.6)	$F(1,73) < 1$	ns.
Verbal intelligence				
MWT-B				
Correct% (SD)	81.6 (22.1)	86.5 (15.4)	$F(1,82) = 2.4$	ns.
IQ (SD)	116 (12)	118 (11)	$F(1,82) = 1.1$	ns.
Episodic and working memory				
VLMT				
Total score trials 1–5 *n* (SD)	39.9 (6.9)	36.0 (7.9)	$F(1,82) = 5.7$	0.022
Interference list *n* (SD)	4.3 (1.3)	4.5 (1.5)	$F(1,82) < 1$	ns.
Trial 6 *n* (SD)	8.2 (2.3)	7.4 (2.9)	$F(1,82) = 2.2$	ns.
Trial 7 *n* (SD)	8.0 (2.3)	7.5 (2.9)	$F(1,82) = 1.4$	ns.
Delayed recognition *n* (SD)	13.2 (1.4)	12.4 (2.2)	$F(1,82) = 4.0$	0.037
Digit-span				
Forward *n* (SD)	3.7 (1.0)	3.6 (1.0)	$F(1,82) < 1$	ns.
Backward *n* (SD)	2.8 (0.8)	2.8 (0.8)	$F(1,82) < 1$	ns.
Word-fluency *n* (SD)	44.3 (12.9)	39.1 (7.9)	$F(1,82) = 4.9$	0.029
Rey-figur (ROCF)				
Reproduction *n* (SD)	33.8 (3.0)	33.1 (2.9)	$F(1,82) < 1$	ns.
Delayed recall *n* (SD)	15.6 (4.7)	15.2 (5.4)	$F(1,82) < 1$	ns.
0-back				
Reaction times msec. (SD)	461 (42)	467 (56)	$F(1,81) < 1$	ns.
Missed targets % (SD)	0.3 (1.1)	0.1 (0.7)	$F(1,81) < 1$	ns.
2-back				
Reaction times (sec.)	637 (108)	631 (116)	$F(1,81) = 1.0$	ns.
Missed targets % (SD)	14.4 (13.2)	22.9 (22.1)	$F(1,81) = 5.6$	0.020

Significance level was set at $P < 0.05$. The values represent mean and standard deviation (SD) in parentheses.
(*n*) Indicates the number of items, (sec.) the time to perform the task, (%) percent.
Key: d2: attentional endurance test; TMT: Trail Making Test, CFQ: Cognitive Failures Questionnaire.
MWT-B: test of premorbid intelligence; VLMT: Verbal Learning and Memory Test.
ROCF: Rey-Osterrieth Complex Figure test (see text for further information).
[a] Reduced number of participants, see (Gajewski et al., 2012) for further information.

the Hypertension Medication slightly reduced the effect of Toxoplasmosis ($F(1,82) = 3.9$, $P = 0.050$). In contrast, Thyroid Medication did not affect the difference between T-neg and T-pos ($F(1,82) = 5.1$, $P = 0.027$). A similar effect was found for Cholesterol Medication ($F(1,82) = 3.4$, $P = 0.023$).

For the variable VLMT delayed recognition the effect of Toxoplasmosis was also slightly attenuated ($F(1,82) = 3.8$, $P = 0.053$) after including Hypertension Medication. Thyroid Medication did not affect the effect of Toxoplasmosis ($F(1,82) = 4.4$, $P = 0.039$). The same pattern was found for Cholesterol Medication ($F(1,82) = 4.4$, $P = 0.038$).

For the *word fluency test* the effect of *T. gondii* disappeared after correction for Hypertension Medication ($F(1,81) = 2.1$, $P = 0.149$). Thyroid Medication attenuated the effect of Toxoplasmosis ($F(1,81) = 3.8$, $P = 0.052$). Cholesterol Medication also weakened the effect of *T. gondii* on word fluency ($F(1,81) = 3.0$, $P = 0.086$).

In sum, with the exception of the Word-Fluency-Test, some of the results reported in Table 2 were slightly attenuated but did not generally disappeared after controlling for medication.

3.3. Computer-based working memory test

Regarding reaction times (RTs), the ANOVA revealed a main effect of Task ($F(1,81) = 269.3$, $P < 0.001$), suggesting considerably longer RTs in the 2-back than 0-back task (463 ± 5.4 vs. 634 ± 12.3 ms, Table 2). No significant main effect of Toxoplasmosis nor interaction Toxoplasmosis × Task were found in the RT data (both F's < 1). As expected, the rate of omitted targets was higher in

the 2-back than in the 0-back task (18.5 ± 1.9 vs. 0.2 ± 0.1; $F(1,81) = 90.9$, $P < 0.001$). Most importantly, there was an interaction of Task with *T. gondii* ($F(1,81) = 5.6$, $P = 0.020$), suggesting a strongly increased ratio of omitted targets in the T-pos compared to the T-neg group (22.9 ± 2.7% vs. 14.0 ± 2.7%; $P = 0.020$, Table 2) in the 2-back task, while there were no group differences in the 0-back task (0.1 ± 0.1% and 0.3 ± 0.1%). Furthermore, there was also a main effect of the between-subject factor *T. gondii* $F(1,81) = 4.8$, $P = 0.032$) due to the general difference in the rate of omitted targets between the T-pos (11.5%) and T-neg (7.2%).

Finally, for the variable *missed targets in a 2-back task* the effect of *T. gondii* remained unchanged after controlling for Hypertension Medication ($F(1,80) = 4.4$, $P = 0.039$). The same was true for Thyroid ($F(1,80) = 5.0$, $P = 0.028$) and Cholesterol Medication ($F(1,80) = 5.6$, $P = 0.020$).

3.4. Correlational analyses

In order to assess additive vs. synergistic effects of the factor Age and *T. gondii* on the cognitive functions we conducted a correlational analysis between Age and cognitive tests. Age was significantly correlated with some variables of the VLMT in the Toxoplasmosis positive group (T-pos) only: initial learning trial (*trial 1*; $r = -0.32$, $P = 0.04$), total of initial learning trials (*total score trials 1–5*; $r = -0.35$, $P = 0.024$), immediate learning of an interference list (*interference list*; $r = -0.47$, $P = 0.002$), freedom from distraction (*trial 6*; $r = -0.38$, $P = 0.012$), long-term recall (*trial 7*; $r = -0.51$, $P < 0.0001$), loss of learned contents (*trial 5–trial 7*; $r = 0.32$, $P = 0.038$), interference score (*trial 6 + trial 7*; $r = 0.32$, $P = 0.038$), false positives in the recognition task (*list S*; $r = -0.35$, $P = 0.023$). Moreover, RTs of the 0-back task were positively correlated with Age in the T-pos group ($r = 0.35$, $P = 0.024$). There were no correlations between the IgG antibody level and cognitive parameters or age in the sample including participants with IgG levels larger than 0 IU/ml. In other words we did not find any dose–effect relationship.

In sum, the older were the infected seniors the lower their memory scores and the higher their memory interference scores. Moreover, response speed was slower the older the infected participants. These relationships were evident in infected (T-pos) seniors only.

4. Discussion

The influence of asymptomatic *T. gondii* infection on cognitive functions is currently unclear. To answer this question we designed a double-blinded study which fulfilled the following criteria: (i) We focused on older age (65 years and older) as the effects may become more apparent when the losses of brain resources associated with normal aging enhance the effect of infection on cognition. Such interactions with age have for example already been observed for polymorphisms of genes involved in cognitive processes (Lindenberger et al., 2008). (ii) Classification criteria for seropositivity were IgG levels of more than 50 IU/ml. To avoid ambiguity individuals with 1–50 IU/ml were excluded. These are more stringent conditions compared to a previous study where 35 IU/ml were chosen as a cut-off to differentiate between seropositive and -negative individuals (Guenter et al., 2012). (iii) We used standard procedures of quantitative cognitive tests which have already been successfully applied in our lab under stringent quality criteria in previous studies (Gajewski et al., 2011, 2012; Wild-Wall et al., 2011).

The results clearly show deteriorated memory functions but not executive functions in infected elderly individuals. Some of the memory effects were slightly attenuated after controlling for medication intake. Medication diminished the *T. gondii* effects in the word-fluency test and in the physical fitness dimension of the WHOQOL questionnaire. *T. gondii* positive seniors showed lower performance in the verbal memory test, both regarding immediate recall and delayed recognition. Both parameters are correlated with intelligence (Bleecker et al., 1988). The mean size of the difference in the immediate recall between seropositive and seronegative individuals was four points. This corresponds to approximately the performance difference observed between 60- and 70-year-old healthy males (Helmstaedter et al., 2001). Substantial differences were also obtained in recall from long-term semantic memory as assessed by the word fluency test. The results of Guenter et al. (2012) in the word fluency showed exactly the same numerical difference between seropositive vs. seronegative individuals as in the present study. Due to a higher statistical power this effect reached significance in our study. The mean difference of four words between the groups corresponds to 10 percentiles in the age group 60–79 (Tombaugh et al., 1999). These results are complemented by the finding in the computer based 2-back working memory task: whereas no group differences were found in the simple version of the test with low memory load (0-back), the rate of correctly detected target symbols was strongly reduced in the version with high memory load (2-back), indicating a reduced working memory capacity, or difficulties in maintenance of stimulus representation over time. The size of the effect is remarkable. Whereas the difference between seropositive vs. seronegative young participants in Guenter et al. (2012) was 4%, the rate in our study amounted to 8.5%. The difference in the rate of undetected target symbols between seropositive and seronegative individuals in the present study is even larger than the rate between middle aged and young (2.2% in Wild-Wall et al., 2011 and 4.3% in Schapkin et al., 2012), or old vs. young participants (5.3% in Missonnier et al., 2004), and comparable to the decrease in the rate between controls and individuals with progressive mild cognitive impairment 8% (Missonnier et al., 2007). Overall the results clearly show deteriorated memory functions including working memory in seniors who are *T. gondii* positive. The significant negative relationship between age of the infected participants and a number of variables of the verbal learning and memory test (VLMT) suggests that the older the T-pos participants the lower the memory performance. Moreover, the positive correlation of age and response time in the simple task indicates psychomotor slowing in infected persons reported previously (Flegr, 2013). In sum, the fact that the age range in this group was relatively small (65–88) and these correlations were significant for infected seniors only indicates that *T. gondii* infection interacts with age in a synergistic manner.

In contrast to the impaired memory functions in T-pos individuals, almost all of the non-memory tasks were unaffected. As the only exception from this pattern we found a substantially enhanced rate of confused symbols in the d2 attentional endurance test. Since the remaining tests of attention showed no effects, the d2 effect may be due to a reduced memory capacity in the infected group when the cognitive representation of the target symbol is impaired. Furthermore, a significantly lower self-estimated quality of life was obtained for *T. gondii* positive individuals. Interestingly, lower quality of life was estimated particularly in regard to psychological health and social relationship but not in regard to environmental factors like financial resources and perceived safety and security.

Earlier reports have suggested that *T. gondii* may affect brain functions by increasing concentrations of dopamine in infected hosts and humans (McConkey et al., 2013; Prandovszky et al., 2011). As outlined above this may explain the association between *T. gondii* and schizophrenic disease (Dalimi and Abdoli, 2012; Yolken, 2010), behavioral changes like novelty seeking (Flegr,

2013; Webster et al., 2013), as well as slower psychomotor responses and thereby increased risk of traffic accidents in infected persons (Flegr, 2013). Previous studies have demonstrated that dopamine plays a critical role in the modulation of neuronal activities in the hippocampus which is essential for learning and memory (Bäckman et al., 2010; Jay, 2003). The evidence that chronic Toxoplasma infection has its greatest impacts on the hippocampus (Berenreiterová et al., 2011) may explain the impaired memory functions.

It is tempting to speculate that the reduced scores of the self-reported quality of life (Table 1) may be a consequence of the impaired memory functions. However, as the subjectively reported quality of life results from a large number of factors such as genetic disposition, medication, education, or social support this conclusion must be treated with caution. There are some obvious limitations of the present study. First, the sample was relatively small. Second, the effects of hypertension and/or antihypertensive medication as well as thyroid disorders and/or thyroid medication attenuated some of the observed effects. This suggests that typical age-related physical disorders and their medication are important factors which affect cognitive functions in elderly but are often underestimated in the cognitive aging research. Third, this is a cross-sectional study. To understand development of the *T. gondii* effect on cognitive functions a longitudinal study would be necessary. Thus, further studies are required to analyze whether similar associations between Toxoplasma infection and deteriorated memory functions can be confirmed in independent and larger cohorts. Future neuroimaging studies should investigate directly the relationship between the imbalance of dopamine levels in infected persons on the one hand and cognitive performance and the quality of life on the other.

Considering the high prevalence and the observed influence on cognitive functions the relevance of asymptomatic *T. gondii* infection for public health may currently be underestimated (Kirby, 2012). Presently, large efforts are undertaken to develop vaccines against *T. gondii* (Liu et al., 2012; Jongert et al., 2009) to reduce congenital Toxoplasmosis. If our results will be confirmed in independent cohorts the recommendation of the WHO (World Health Organisation, 1992) will be fueled.

5. Conclusions

T. gondii is a common parasitic intracellular protozoon that affects organisms and behaviour of its carriers. Nearly one third of the world population is infected and the infection prevalence increases with age. Whereas some behavioural effects were described in rodents little is known about the influence of Toxoplasmosis on cognitive functions in humans. The present study found a decline of several aspects of memory even after controlling for medication but no decline of executive functions in asymptomatic *T. gondii* infected older individuals. The observed association is relevant for public health considering the high prevalence of Toxoplasmosis and an increasing number of older individuals. The relatively large effects of *T. gondii* on memory functions observed here correspond to the mean decrease in performance usually observed between years 60 and 70, which may have considerable socioeconomic consequences.

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Author contributions

P.G. is responsible for the study design, data collection, data analysis, data interpretation and writing. J.H. contributed to data analysis and writing. M.F. contributed to study design, data interpretation and writing. K.G contributed to study design, literature search, data analysis and interpretation.

Conflict of interest

All authors declare that there are no conflict of interest.

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