The association of *Toxoplasma gondii* infection with neurocognitive deficits in a population based analysis

Brad D. Pearce[a], **Deanna Kruszon-Moran**[b], and **Jeffrey L. Jones**[c]

[a]Dept of Epidemiology. Emory University. 1518 Clifton Rd. NE. Atlanta, GA 30322

[b]Centers for Disease Control and Prevention, Division of Health and Nutrition Examination Statistics, National Center for Health Statistics (NCHS), Hyattsville, MD 20782

[c]Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, 30322

Abstract

Purpose—To examine the relationship between infection with *Toxoplasma gondii* (toxo) and cognition.

Methods—Multivariate logistic regression was used to test the association of toxo seropositivity with indices of cognitive function among over 4200 adults in the third National Health and Nutrition Examination Survey.

Results—Toxo-seropositive participants were more likely than seronegative participants to score in the worst quartile of the Simple Reaction Time Test (OR, 1.3, 95% CI 1.0,1.6), Symbol Digit Substitution Test (SDST, OR 1.5, 95% CI 1.2,1.9) and the Serial Digit Learning Test(trials to criterion) (SDLTNT, OR 1.4, 95% CI 1.1,1.8) in models adjusted for age, race/ethnicity, gender and foreign birth. After further adjustment for all cofactors, the association between toxo seropositivity and these outcomes was no longer significant. However, seropositivity was associated with worse scores on the SDST (OR 2.9, 95% CI 1.8,4.8) among those in the lowest income category and the SDLTNT (OR 1.5, 95% CI 1.1,2.5) among those foreign born.

Conclusions—Toxo seropositivity may be associated with poor cognitive test scores in certain subgroups, however, causation cannot be established in this cross sectional study.

Keywords

Infection; Cognition; Neuroimmunology; Population-based study

Corresponding author: Brad D. Pearce, Ph.D. Emory University, Dept. of Epidemiology, Rollins School of Public Health, 1518-002-3BB, Atlanta, GA 30322, Office 404 727-914, Fax 404 727-8737, bpearce@emory.edu.
Deanna M. Kruszon-Moran ScM, Division of Health and Nutrition Examination Statistics, National Center for Health Statistics, Centers for Disease Control and Prevention, 3311 Toledo Rd. Room 4308, Hyattsville, MD 20782, (301)458-4328
Jeffrey L. Jones, M.D., M.P.H. Parasitic Diseases Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Bld. 24, 1600 Clifton Road, NE, Mailstop A-06, Atlanta, GA 30333, Phone: 404-718-4747, Fax: 404-718-4816

INTRODUCTION

Toxoplasma gondii (toxo) is a protozoan parasite that can infect most warm-blooded species. The prevalence of toxo infection in humans is approximately 25–30% worldwide, with a somewhat lower prevalence (10–30%) in the United States, Northern Europe, South East Asia and parts of central Africa [1,2]. Humans are an intermediate host for toxo yet the infection rarely comes to clinical attention except when transmitted vertically or complicated by immunosuppression. Nevertheless, toxo establishes a lifelong infection in all or most humans by forming parasite-containing cysts in muscle and brain.

The toxo lifecycle requires transmission from an intermediate host (e.g. rodent) to a definitive host, where sexual reproduction occurs. Because felids (cats) are the only known definitive hosts, consumption of rodents by cats is the main mechanism for transmission of the parasite through this essential life cycle stage [2]. Accordingly, toxo gains a reproductive advantage by facilitating predation of rodents by cats, and it achieves this in part by altering rodent behavior to increase the likelihood that infected hosts succumb to feline predation and consumption [3]. Thus, infected mice are hyperactive yet have slower reaction times and possibly a preference for open spaces compared to uninfected mice [4,3]. Toxo-infected mice and rats have reduced fear of cat odor, and a subset even become attracted to cat urine [5,3]. These behavioral alterations cannot be attributed to non-specific effects on olfaction (e.g. responses to rabbit odor are unaffected) or to a generalized brain dysfunction because many social and memory functions remain intact [5,3]. One prominent mechanism by which toxo is thought to manipulate rodent behavior is by modulating neurotransmitters, either by acting directly (e.g. increasing dopamine via parasite-encoded tyrosine hydroxylase), or indirectly through neuroimmune mechanisms [6,7].

Therefore, toxo appears to have evolved a specific neurobiological mechanism for behavioral manipulation of rodent prey. Emerging data suggests latent toxo infection can also affect human behavior. While it is difficult to envision a reproductive advantage for toxo due to behavioral manipulation of humans in modern times, there is no reason to believe the parasite has lost its ability to modulate neurotransmitters in such "dead end" intermediate hosts. Indeed, toxo seropositivity is associated with risk for some psychiatric illnesses, lower intelligence quotient, and impaired psychomotor performance [8–12]. This slowed psychomotor reaction time has been proposed to explain the higher rate of automobile and work related accidents among toxo seropositive individuals [13–16]. However, these studies of accident rates did not directly measure visual-motor reaction time, and most did not adjust for possible confounders other than gender and age. The study by Alvarado-Esquivel et al., which did consider possible toxo exposure sources and socioeconomic status in regression models, found an association of toxo and work-related accidents in patients with low socioeconomic status, though these analyses were based on only 8 seropositive accident victims in the low SES strata [15].

Most prior studies of toxo that included psychometric assessments of reaction time or cognitive function had small sample sizes (<100 seropositive subjects), or examined sub-populations such as blood donors, young military recruits, or patients with schizophrenia [17,8,18–20]. Thus despite numerous intriguing studies suggesting a link between toxo and

cognitive function, there is a lack of a large scale population-based studies with individual-level psychometric assessments of cognition as well as toxoplasma serology and assessment of covariates. In the current study, we examined the association between *T. gondii* exposure and neurocognitive tests scores among individuals in the Third National Health and Nutrition Examination Survey (NHANES III), controlling for sociodemographic and other possible confounders.

METHODS

Study Participants

To examine the relationship between prevalence of immunoglobulin G (IgG) antibodies to *T. gondii* and several neurobehavioral measures we used data from NHANES III, a cross-sectional survey conducted between 1988 and 1994 by the National Center for Health Statistics, Centers for Disease Control and Prevention. The NHANES III was designed to obtain nationally representative statistics on health measures and conditions through household interviews, standardized physical examinations, and collection of biological specimens in mobile examination centers [21]. The NHANES III was based on a stratified, multistage, probability cluster design from which a sample representative of the civilian, noninstitutionalized United States population 2 months of age or older was drawn. Institutional Review Board (IRB) approval and documented consent was obtained from participants. Details of the design of the survey and the sample have been described elsewhere [21].

Measures of Neurocognitive Function

During the physical examination, three computerized tests were used to evaluate neurocognitive function [22]. The simple reaction time test (SRTT) was a basic measure of motor response speed to a visual stimulus; the symbol-digit substitution test (SDST) was a test of coding ability; and the serial-digit learning test (SDLT) was a short-term memory test. Krieg et al. present a detailed description of how these neurocognitive outcome variables where measured and created [23]. These tests were administered to only a random half-sample of examinees aged 20–59 years at the time of the examination. A subset of these had available data for toxo serology testing and analysis weights were further adjusted to account for the differential probability of selection for these individuals.

SRTT is recorded as the latency (in milliseconds) between the display of an object on the computer screen and the pressing of a button by the participant to signal the appearance of this object. SDST measured the speed to correctly match a digit to a corresponding symbol according to a guided scheme presented to the participant in each of four trials, We used the NHANES SDST summary measure (coded as CNPCBEST), which is the error-corrected latencies (in seconds per correct digit) on the two best (lowest latency) of the four trials. SRTT and SDST were measured as continuous variables whose values were not normally distributed, and thus two outcome measures were created for each: 1) a dichotomous variable based on whether the value fell into the highest quartile of the distribution of the sample for that outcome measure (values >246.15 ms for SRTT and values >2.88 ms/correct digit for SDST); and 2) a continuous variable created by transforming the original

value to approximate a normal distribution using the inverse function, reflecting that value (multiplying by -1), and adding a constant to bring the minimum value back above 1.0 so the ordering of values will be the same as the original data.

For SDLT, participants attempted to reproduce a previously-displayed digit series using the keyboard. All the trials presented the same 8-digit sequence, and the number of trials to criterion (SDLTNT), and sum of the error scores (SDLTSC) were used as our outcome variables. Dichotomous variables were created based on the highest quartile, with cut points established on weighted percentages. Scores of 7–9 for SDLTNT and 7–16 for SDLTSC were assigned as the worst quartile.

Selection of Covariates

Sociodemographic factors possibly related to these neurocognitive tests as well as cofactors previously shown to be related to *T. gondii* seropositivity were assessed [2,23]. These data were collected by questionnaire and included, age, gender, race/ethnicity, place of birth, poverty index, education, and metropolitan residence.

Health status and risk behavior variables posited to be associated with the cognitive function outcome measures [24,25,23] were collected by questionnaire and evaluated in our analyses. They included: smoking; alcohol use; video game familiarity, sleep before the exam; diagnosis of diabetes; and hypertension diagnosis and hypertension medication use. Body mass index (kg/m^2) was determined by examination. Details on all questions can be found in the survey documentation [22] and categories used in the analysis are outlined in Table 1. Age was grouped as a categorical variable in the univariate analysis (Table 1) but was analyzed as a continuous variable (in years) in the logistic models.

Lower socioeconomic status is correlated with worse scores on some neurocognitive tests [23] and may be correlated with labor occupations involving prolonged contact with soil (possibly increasing toxo oocyst exposure)[26]. We examined the possible confounding effects of soil-related occupation (measured as longest held occupation in farming, nursery work or agriculture compared to all other occupations) [27] on the association of cognitive function and poverty.

Laboratory Testing

Sera was tested previously for *T. gondii* IgG antibodies with the Patelia Toxo-G immunoglobulin G enzyme immunoassay (Sanofi Diagnostics Pasteur, BioRad, Hercules, California), according to the manufacturer's instructions. Before the study the Patelia Toxo-G kit was evaluated by comparison with the Centers for Disease Control and Prevention Toxoplasma immunofluorescence assay-immunoglobulin G test and Sabin-Feldman dye test (Dr. Jack Remington, Palo Alto, California) and found to have a sensitivity and specificity of 100% [26]. *T. gondii* antibody titers of 7 IU/ml or greater were categorized as positive according to the instructions of the manufacturer.

Response rates

There were 14,883 individuals age 20–59 years sampled in NHANES III, 12,229 (82%) were interviewed, 11,271 (92%) of those interviewed were examined, and 5662 (50%) were part of the cognitive tested subsample and given sample weights for that portion of the exam. Of the 5662 with subsample weights, 5064 (89%) were tested for *Toxoplasma gondii* antibody, 4383 (77%) were tested for *T. gondii* and had complete valid data on SRTT, 4526 (80%) for SDST, and 4438 (78%) for SDLT. Outcome data was set to missing for individuals who used alcohol in the past 3 hours (N=58), and SRTT data was set to missing for those who did not use their preferred hand (N= 195) and therefore excluded from all analyses. Differences in those with complete data for both *T. gondii* serologic testing and all three cognitive function tests varied significantly by levels of several cofactors associated with either toxoplasma seropositivity or the outcome measures and included race/ethnicity, foreign birth, poverty index, education, alcohol use, video game use (p<0.05 from a chi square analysis). All, individuals without complete data were more likely to be both seropositive to *T. gondii* and have poorer performance on the neurobehavioral tests, which would bias our findings towards the null.

Statistical Analysis

Prevalence estimates were weighted to represent the total US non-institutionalized population and to account for oversampling and nonresponse to the household interview and physical examination [28,29]. Because cognitive testing was completed on a random subsample of those 20–59 years of age, sample weights that account for the additional probability of selection were used in our analysis. Statistical analyses were conducted with SUDAAN (version 10.0.1), a family of statistical procedures for analysis of data from complex sample surveys (Research Triangle Institute, Research Triangle Park, North Carolina). Standard error estimates were calculated using the Taylor Series Linearization method. Ninety-five percent confidence limits were estimated using the exact binomial method [30]. All relative standard errors (RSE) of the estimates met our standard of stability (RSE<30%).

Logistic regression modeling was used to determine the association of toxoplasma seropositivity with each outcome with various levels of adjustment. Steps included adjustment by age alone, adjusting for the major demographic variables (age, race/ethnicity, gender and foreign birth), adjusting for these demographic cofactors and including socio-economic variables (poverty index and education) previously associated with both toxoplasma seropositivity and the outcome measures, and adjusting for these and all other variables possibly associated with the neurobehavioral outcome measures (video game usage, alcohol use, smoking, sleep, body mass index, diagnosis of diabetes, and hypertension - the "full" model). A "final" model was determined using a backwards stepwise procedure where cofactors whose p-value was > 0.05 were deleted from the full model. Because some cofactors were logical candidates for effect modification of toxo on cognition (e.g. diabetes), and many cofactors were associated with both toxo seropositivity and cognitive measures, we tested for interactions with toxoplasma status prior to deletion as well as among the remaining variables in the "final" model. Any significant interactions were further examined in stratified models. In addition, SRTT and SDST were coded as

transformed continuous outcomes as described previously, and examined using linear regression.

RESULTS

Table 1 presents toxo seroprevalence and each of the three neurobehavioral outcomes by demographic factors and covariates that may be associated with either toxo seroprevalence or these behavioral outcomes. Percentages in Table 1 are weighted as described in the methods, and all percentages (except age group rows) were adjusted for age to account for the known effect of age on toxo seroprevalence and neurobehavioral outcomes. Definitions of highest quartile for all 3 outcomes were determined on the weighted but non-age standardized data. Age standardization of the original percentages for each subgroup of the sociodemographic and health variables in Table 1 may have resulted in percentages greater than 25% in all subgroups for a particular variable. The actual unweighted number of respondents is reported only to indicate sample size in each subgroup.

Age adjusted toxo seroprevalence in the sample with data for all 3 outcomes (n=4234) was 20.9%. The prevalence of toxo was greater among those older, foreign born, of lower socioeconomic status, with lower education, obese, and with a diabetes diagnosis. Toxo seroprevalence was lower among participants who reported current alcohol use and familiarity with video games (Table 1). Poor performance on simple reaction time (SRTT) (p<0.05), symbol coding speed (SDST, p<0.001) and learning/working memory (SDLT, p<0.001) tests was greater among those toxo seropositive.

Because almost all socio-demographic and health related variables were also associated with at least one of these neurobehavioral outcomes (Table 1), we used logistic regression to examine the relationship between toxo and poor performance on these cognitive test outcomes adjusting for these possible confounders.

Table 2 shows the odds ratio (and 95% confidence interval) of poorer performance on SRTT and SDST with toxo seropositivity. In age adjusted analysis, toxo seropositive respondents were more likely to have test scores in the worst quartile for SRTT (OR 1.3, 95% CI, 1.1,1.6, p<0.05) and SDST (OR 1.7, 95% CI 1.4,2.2, p<0.001). These associations were attenuated after adjusting for core demographic variables (race/ethnicity, gender, and foreign birth) but remained statistically significant (Table 2, p<0.05). In full models controlling for all demographic factors, lifestyle variables, and medical conditions examined previously, the odds ratios for performance in the worst quartile in toxo seropositive compared to seronegative respondents were similar (OR 1.1) for all outcomes, with the 95% confidence intervals including 1.0 (Table 2). Final reduced models in which each outcome was adjusted only for significant cofactors gave similar results as the full models and toxo seropositivity remained a nonsignificant cofactor (Table 2).

In additional analysis, the SRTT and SDST outcomes were coded as continuous variables and examined in linear regression models using the same procedure as described above. The mean SRTT latency for the toxo seropositive group was 231 milliseconds (95% CI 227, 235) versus 226 milliseconds (95% CI 224,228) in the toxo seronegative group. For SDST the

mean error corrected latency for toxo seropositive respondents was 2.71 seconds/correct digit (95% CI 2.65, 2.78) compared to 2.51 seconds/correct digit (95% CI 2.48,2.56) in the seronegative group. The association of poorer performance on these tests among toxo seropositive respondents was similar to the dichotomized analysis except that statistical significance was retained in the SDST continuous models but not SRTT even after adjustment for age, race/ethnicity, gender, foreign birth, poverty and education (Table 2, P<0.05). For both continuous outcomes, the association was no longer significant in the final model.

Toxo seropositivity was also associated with scores in the worst quartile on learning/working memory as measured by trials to criterion (SDLTNT, OR 1.6, 95% CI 1.2,2.0, p<0.001) or total error scores (SDLTSC, OR 1.8, 95% CI 1.4,2.3, p<0.001) (Table 3). These associations were no longer significant after adjustment for all confounders (Table 3).

Interactions that could modify the association between toxo and the neurobehavioral test scores (Tables 2 and 3) were also examined. No significant interactions were found for SRTT. For SDST, our models suggested an interaction between toxo and poverty level (p-value for interaction term, p=0.003). Among respondents in the lowest income strata (PIR <1.3), toxo was associated with worse performance on the SDST (OR 2.9, 95% CI 1.8, 4.8, P< 0.001) even after controlling for age, race, gender, education, video game use, smoking, alcohol consumption, and sleep. No significant association between toxo and SDST performance was found in other income strata. The possible confounding effect of soil occupations on toxo and SDST was considered. Individuals who had their longest occupation in soil-related jobs had a higher toxo seroprevalence than all other job categories (OR 2.2; 95% CI 1.2, 4.0) in models adjusted for age, race/ethnicity, gender, foreign birth, poverty index, and education. However, this correlation could not explain the association between toxo and worse SDST scores in the lowest socioeconomic category (PIR <1.3) because toxo was still associated with worse SDST scores in the model for the PIR < 1.3 strata that excluded individuals with mainly soil related occupations and adjusted for covariates from the final model (Table 2, OR 3.0; 95% CI 1.8,5.2).

For both SDLT outcomes, toxo seroprevalence interacted with foreign birth. Among foreign born respondents, toxo infection was associated with poor performance on the SDLT after controlling for confounders in the final model, with the SDLTSC showing the largest effect (OR 2.4; 95% CI 1.4–3.9).

DISCUSSION

Based on univariate analyses, toxo seropositivity was associated with worse scores on cognitive tests measuring visual-motor response time (SRTT), coding ability (SDST), and learning/memory (SDLT). These associations could not be fully attributed to confounding by age, race, gender, or foreign birth, but they did not remain statistically significant after adjustment for all confounders in final models. In our data set, toxo, SES and cognition are all measured in adulthood and it is not possible to model the temporal relationships. One possible mechanism is that lower socioeconomic status (SES) early in life or foreign birth can lead to higher toxo seroprevalence through increased oocyst exposure (poor sanitation,

soil related occupation, and crowded households). Our analysis of serologic data for the youngest NHANES III participants (those age 12–19 years) (n=2749) did find toxo seropositivity was related to low SES (Crude OR 1.9; CI 1.2–3.0, p<0.05) and foreign birth (Crude OR 2.5; CI 1.4–4.6, p<0.05), suggesting these demographic variables were risk factors for infection in childhood. Nevertheless, a limitation of this cross-sectional study is that we cannot determine from our data whether infection with toxo predated cognitive deficits. Difficulties in school and in cognition can be the result of either low SES, toxo or both. Poor school performance could then lead to lower SES later in life. What is unknown is whether toxo has a direct effect on cognition or if the observed relationship between toxo and cognition is due to the fact that low SES in childhood causes both increased rates of toxo infection and cognitive difficulties. To the extent that poverty in adulthood is related to poverty in childhood, there may be no direct relationship between toxo and cognition. To the extent that adult poverty is the result of toxo exposure operating through cognitive limitations, controlling for SES in our models is a misspecification. Thus a prospective design that tested the timing of toxo seroconversion and followed cognitive development longitudinally with comprehensive tests such as IQ would be preferable, though logistically difficult.

Flegr et al. have argued that infection with toxo may be a neurobiological instigator of decreased cognitive abilities; a direct causal effect [9]. Ferreira et al. reported an association between toxo seroprevalence and poor scholastic achievement in children, and proposed that infection with toxo in childhood could impair educational attainment [31]. Mechanistically, toxo could induce persistent inflammation in a subset of individuals, leading to adverse effects on psychomotor performance. However, the proportion of our sample with high C-reactive protein levels (CRP>=2.2 mg/L, an indicator of inflammation) were comparable between T. *gondii* seronegative (24.7%; CI 22.0–27.6) and seropositive (26.3%; CI 22.2–31.0) (p=0.81) individuals. Nevertheless, CRP could exert an independent additive effect on cognitive parameters, as has been suggested for herpes simplex infection [32]. Toxo may also induce behavioral changes by usurping immune responses and exploiting immune-neurotransmitter interactions [7]. Specifically, it is hypothesized that in order to keep the persistent toxo infection in check, the host upregulates the cytokine interferon gamma (IFNγ), which prevents toxo replication via depletion of tryptophan—an amino acid the parasite cannot produce itself. IFNγ achieves local tryptophan depletion by shunting tryptophan degradation along a series of enzyme-controlled steps that generate kynurenic acid (KYNA) [7]. KYNA in turn acts as an inhibitor of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors and alpha7 nicotinic acetylcholine receptors, which are involved in cognitive function [7,33]. *T. gondii* also encodes enzymes that regulate dopamine metabolism, and this neurotransmitter has likewise been implicated in cognition [6].

Alternatively, toxo seropositivity could be a surrogate biomarker for another infection with similar prevalence and risk factors, or could indicate an underlying immunogenetic factor influencing cognition such as major histocompatibility complex molecules [34]. The persistence of toxo in non-neural tissues (e.g heart muscle) could in theory increase risk of cardiovascular antecedents of poor cognition, but considering the young age of our sample

this mechanism could not likely be teased-out from other physiological factors influencing cognition [35]. Many such physiological variables affecting cognition are also related to lower socioeconomic status and could interact with toxo to exacerbate its effects on these neurobehavioral tests. Accordingly, we controlled for many such factors (e.g. smoking, BMI, diabetes, hypertension), including them in our full models and testing for potential interactions with toxo seropositivity before deleting them from the final models.

In stratified analyses, toxo seropositivity remained a strong risk factor for scoring in the worst quartile among respondents in the lowest income group for SDST and among those foreign born for both SDLT measures, even after adjusting for demographic and behavioral variables (e.g education level, alcohol use, smoking).

This association remained significant even after removing respondents with soil-related occupations, arguing against the theory that low cognitive skills increase the likelihood of soil-related occupations and consequently toxo exposure. A prior study in Brazil found a greater risk of intellectual disability in children among toxo seropositives and this effect was greater among those in the lower socioeconomic group. [36]. Interestingly, Krieg et al. found that differences between racial-ethic groups in performance levels on these cognitive tests tended to diminish as family income increased, suggesting the relative importance of modifiable factors such as socioeconomic status on these cognitive test scores [23].

The effect of toxo on cognitive function was not consistent across our 3 outcome measures. This was not unexpected since these outcomes measure different aspects of cognitive function – simple visuomotor speed (SRTT), coding speed (SDST), and learning and recall (SDLT). Moreover, scoring in the worst quartile for any of these neurocognitive indices cannot readily be construed as clinically significant for either intellectual disability or cognitive decline. Other studies using these cognitive measures have found different levels of significance among various predictors for each outcome measure [37,25,38,39,35]. In addition, other studies have shown the combined effects of multiple significant predictors may be greater than either alone and vary between outcome measures [40].

While the determinants of cognitive function are undoubtedly multifactorial, our findings suggest a possible association between toxo seroprevalence and worse performance on the SDST among adults in the lowest income group and SDLT among foreign born adults residing in the US.

Acknowledgments

REFERENCES

1. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 2012; 25(2):264–296. [PubMed: 22491772]

2. Dubey JP, Jones JL. Toxoplasma gondii infection in humans and animals in the United States. Int J Parasitol. 2008; 38(11):1257–1278. [PubMed: 18508057]

3. Webster JP. The effect of Toxoplasma gondii on animal behavior: playing cat and mouse. Schizophr Bull. 2007; 33(3):752–756. [PubMed: 17218613]

4. Hrdá Š, Votýpka J, Kodym P, Flegr J. Transient nature of Toxoplasma gondii-induced behavioral changes in mice. 2009

5. Vyas A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky RM. Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(15):6442–6447. [PubMed: 17404235]

6. Prandovszky E, Gaskell E, Martin H, Dubey J, Webster JP, McConkey GA. The Neurotropic Parasite Toxoplasma Gondii Increases Dopamine Metabolism. PloS one. 2011; 6(9):e23866. [PubMed: 21957440]

7. Schwarcz R, Hunter CA. Toxoplasma gondii and schizophrenia: linkage through astrocyte-derived kynurenic acid? Schizophr Bull. 2007; 33(3):652–653. [PubMed: 17434932]

8. Havlicek J, Ga�ová Z, Smith AP, Zvára K, Flegr J. Decrease of psychomotor performance in subjects with latent 'asymptomatic'toxoplasmosis. Parasitology. 2001; 122(05):515–520. [PubMed: 11393824]

9. Flegr J, Preiss M, Klose J, Havlicek J, Vitakova M, Kodym P. 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. 2003; 63(3):253–268. [PubMed: 12853170]

10. Torrey EF, Bartko JJ, Yolken RH. Toxoplasma gondii and Other Risk Factors for Schizophrenia: An Update. Schizophrenia bulletin. 2012; 38(3):642–647. [PubMed: 22446566]

11. Hamdani N, Daban-Huard C, Lajnef M, Richard J-R, Delavest M, Godin O, Guen EL, Vederine F-E, Lépine J-P, Jamain S. Relationship between *Toxoplasma gondii* infection and bipolar disorder in a French sample. Journal of affective disorders. 2012

12. Pearce BD, Kruszon-Moran D, Jones JL. The Relationship Between Toxoplasma Gondii Infection and Mood Disorders in the Third National Health and Nutrition Survey. Biological psychiatry. 2012; 72:290–295. [PubMed: 22325983]

13. Kocazeybek B, Oner YA, Turksoy R, Babur C, Cakan H, Sahip N, Unal A, Ozaslan A, Kılıc S, Saribas S. Higher prevalence of toxoplasmosis in victims of traffic accidents suggest increased risk of traffic accident in Toxoplasma-infected inhabitants of Istanbul and its suburbs. Forensic Sci Int. 2009; 187(1):103–108. [PubMed: 19356869]

14. Flegr J, Havlícek J, Kodym P, Malý M, Smahel Z. Increased risk of traffic accidents in subjects with latent toxoplasmosis: a retrospective case-control study. BMC Infect Dis. 2002; 2(1):11. [PubMed: 12095427]

15. Alvarado-Esquivel C, Torres-Castorena A, Liesenfeld O, Estrada-Martínez S, Urbina-Álvarez JD. High seroprevalence of Toxoplasma gondii infection in a subset of Mexican patients with work accidents and low socioeconomic status. Parasit Vectors. 2012; 5:13. [PubMed: 22236539]

16. Yereli K, Balcio lu IC, Özbilgin A. Is Toxoplasma gondiia potential risk for traffic accidents in Turkey? Forensic Sci Int. 2006; 163(1):34–37. [PubMed: 16332418]

17. Yolken R, Dickerson F, Fuller Torrey E. Toxoplasma and schizophrenia. Parasite Immunol. 2009; 31(11):706–715. [PubMed: 19825110]

18. Novotná M, Havlí ek J, Smith A, Kolbeková P, Skallová A, Klose J, Gašová Z, Písa ka M, Sechovská M, Flegr J. Toxoplasma and reaction time: role of toxoplasmosis in the origin, preservation and geographical distribution of Rh blood group polymorphism. Parasitology. 2008; 135(11):1253–1261. [PubMed: 18752708]

19. Flegr J, Preiss M, Klose J, Havlí ek J, Vitáková M, Kodym P. Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite<i>Toxoplasma gondii</i>Dopamine, a missing link between schizophrenia and toxoplasmosis? Biological psychology. 2003; 63(3):253–268. [PubMed: 12853170]

20. Holub D, Flegr J, Dragomirecká E, Rodriguez M, Preiss M, Novák T, ermák J, Horá ek J, Kodym P, Libiger J. Differences in onset of disease and severity of psychopathology between toxoplasmosis-related and toxoplasmosis-unrelated schizophrenia.

21. NCHS. Vital and health statistics. Vol. series 2. Washington, DC: US GPO; 1992. Sample design: Third National Health and Nutrition Examination Survey, 1988–1994. DHHS publication no. (PHS) 92–1387.

22. NCHS. [Accessed 09/14/11 2011] NHANES III Examination Data File Documentation. 1996. ftp://ftp.cdc.gov/pub/Health_Statistics/NCHS/nhanes/nhanes3/1A/exam-acc.pdf.

23. Krieg EF Jr, Chrislip DW, Letz RE, Otto DA, Crespo CJ, Stephen Brightwell W, Ehrenberg RL. Neurobehavioral test performance in the third National Health and Nutrition Examination Survey. Neurotoxicol Teratol. 2001; 23(6):569–589. [PubMed: 11792526]

24. Tolppanen AM, Williams DM, Lawlor DA. The association of serum ionized calcium and vitamin D with adult cognitive performance. Epidemiology. 2011; 22(1):113. [PubMed: 20881598]

25. Hailpern SM, Melamed ML, Cohen HW, Hostetter TH. Moderate chronic kidney disease and cognitive function in adults 20 to 59 years of age: Third National Health and Nutrition Examination Survey (NHANES III). Journal of the American Society of Nephrology. 2007; 18(7): 2205–2213. [PubMed: 17554148]

26. Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. Toxoplasma gondii infection in the United States: seroprevalence and risk factors. Am J Epidemiol. 2001; 154(4):357–365. [PubMed: 11495859]

27. NCHS. NHANES III Household Adult Data File Documentation. 1996 ftp://ftp.cdc.gov/pub/Health_Statistics/NCHS/nhanes/nhanes3/1A/adult-acc.pdf. 2013.

28. Mohadjer, L.; Waksberg, J. National Center for Health Statistics. Hyattsville, MD: 1996. National Health and Nutrition Examination Survey III: weighting and estimation methodology.

29. Ezzati, T.; Khare, M. 1992 proceedings of the Section on Survey Research Methods. Alexandria, VA: American Statistical Association; 1993. Nonresponse adjustment in a national health survey; p. 339-344.

30. Korn EL, Graubard BI. Confidence intervals for proportions with small expected number of positive counts estimated from survey data. Survey Methodology. 1998; 24:193–201.

31. Ferreira ÉC, Marchioro AA, Guedes TA, Mota DC, Guilherme AL, de Araújo SM. Association between seropositivity for Toxoplasma gondii, scholastic development of children and risk factors for T. gondii infection. Trans R Soc Trop Med Hyg. 2013; 107(6):390–396. [PubMed: 23598948]

32. Dickerson F, Stallings C, Origoni A, Vaughan C, Khushalani S, Yolken R. Additive effects of elevated C-reactive protein and exposure to Herpes Simplex Virus type 1 on cognitive impairment in individuals with schizophrenia. Schizophrenia Research. 2011

33. Hilmas C, Pereira EFR, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits α7 nicotinic receptor activity and increases non-α7 nicotinic receptor expression: physiopathological implications. The Journal of Neuroscience. 2001; 21(19): 7463–7473. [PubMed: 11567036]

34. Shatz CJ. MHC class I: an unexpected role in neuronal plasticity. Neuron. 2009; 64(1):40–45. [PubMed: 19840547]

35. Kobrosly RW, Seplaki CL, Jones CM, van Wijngaarden E. Physiologic Dysfunction Scores and Cognitive Function Test Performance in US Adults. Psychosom Med. 2012; 74(1):81–88. [PubMed: 22155941]

36. Caiaffa WT, Chiari CA, Figueiredo AR, Orefice F, Antunes CM. Toxoplasmosis and mental retardation: report of a case-control study. Mem Inst Oswaldo Cruz. 1993; 88(2):253–261. [PubMed: 8107587]

37. Krieg EF Jr, Butler MA. Blood lead, serum homocysteine, and neurobehavioral test performance in the third National Health and Nutrition Examination Survey. Neurotoxicology. 2009; 30(2):281–289. [PubMed: 19459225]

38. Zhang J, Muldoon MF, McKeown RE. Serum cholesterol concentrations are associated with visuomotor speed in men: findings from the third National Health and Nutrition Examination Survey, 1988–1994. The American journal of clinical nutrition. 2004; 80(2):291–298. [PubMed: 15277148]

39. Beydoun MA, Beydoun HA, Shroff MR, Kitner-Triolo MH, Zonderman AB. Helicobacter pylori Seropositivity and Cognitive Performance Among US Adults: Evidence From a Large National Survey. Psychosom Med. 2013; 75(5):486–496. [PubMed: 23697465]

40. Pavlik VN, Hyman DJ, Doody R. Cardiovascular risk factors and cognitive function in adults 30–59 years of age (NHANES III). Neuroepidemiology. 2004; 24(1–2):42–50. [PubMed: 15459509]

Table 1

Age adjusted prevalence of *Toxoplasma gondii* seropositivity and poor outcome on three cognitive function measures by demographic, socio-economic, behavioral, and health related factors.

Variable	Category	Toxoplasma seropositive*				SRTT Highest Quartile*				SDST Highest Quartile*				SDLTNT Highest Quartile*			
		N	Weighted Percent	95% CI	p-value	N	Weighted Percent	95% CI	p-value	N	Weighted percent	95% CI	p-value	N	Weighted Percent	95% CI	p-value
Total		4234	20.9	(19.1–22.8)		4383	25.4	(22.7–28.2)		4526	27.3	(24.5–30.3)		4438	25.2	(22.8–27.8)	
Toxoplasma status	Positive		Not Applicable			944	29.8	(26.1–33.8)	a	955	36.6	(32.2–41.2)	c	930	32.8	(28.8–37.0)	c
	Negative					3439	24.4	(21.5–27.4)		3571	25.4	(22.5–28.5)		3508	23.4	(20.7–26.3)	
Age	20–29 years (ref)	1320	14.5	(11.7–17.6)		1347	23.7	(20.0–27.7)		1421	12.6	(10.0–15.6)		1398	18	(14.4–22.1)	
	30–39 years	1231	14.9	(12.0–18.3)	n	1256	22.3	(18.4–26.5)	n	1300	14.9	(11.7–18.5)	n	1280	20.6	(17.2–24.2)	n
	40–49 years	1007	21.1	(17.3–25.4)	a	1056	27.3	(22.1–33.1)	n	1088	33.3	(28.2–38.6)	c	1053	28.8	(24.5–33.5)	c
	50–59 years	676	36.2	(31.3–41.3)	c	724	28.8	(24.3–33.7)	n	717	53.6	(47.5–59.6)	c	707	35.2	(30.3–40.3)	c
Gender	Male (ref)	1927	21.6	(18.7–24.7)		1999	20.6	(17.3–24.1)		2057	32.5	(29.0–36.1)		2020	24.7	(21.8–27.7)	
	Female	2307	20.1	(17.9–22.5)	n	2384	30.1	(26.4–34.0)	c	2469	22.3	(18.8–26.2)	c	2418	25.8	(22.7–29.0)	n
Race/ethnicity	Non-Hispanic white (ref)	1628	19.5	(17.5–21.6)		1653	22.9	(19.6–26.5)		1669	21.7	(18.5–25.2)		1657	20	(17.6–22.5)	
	Non-Hispanic black	1298	22.7	(19.6–26.0)	n	1355	34.8	(31.9–37.8)	c	1381	43.8	(40.6–47.1)	c	1357	39.6	(36.7–42.6)	c
	Mexican American	1156	21	(18.2–24.0)	n	1218	37.4	(33.8–41.1)	c	1315	56.6	(51.2–61.6)	c	1265	56.6	(50.2–62.8)	c
	All others	152	31.7	(23.2–41.1)	b	157	31.2	(23.1–40.2)	n	161	46.4	(38.9–54.0)	c	159	44	(33.6–54.7)	c
Birth place	Foreign born	861	30.5	(24.1–37.5)	b	926	31.8	(26.6–37.2)	a	971	45	(38.1–52.0)	c	923	40.7	(33.8–48.0)	c
	US Born (ref)	3359	19.6	(17.6–21.7)		3442	24.5	(21.6–27.5)		3540	24.8	(21.9–27.9)		3501	23	(20.7–25.5)	
SES-PIR	PIR < 1.3	1201	29.6	(25.4–34.1)	c	1272	33.6	(28.0–39.5)	c	1331	47.1	(41.6–52.7)	c	1285	45.5	(39.2–51.9)	c
	PIR 1.3–3.49	1739	20.7	(17.7–23.8)	n	1795	27.7	(23.7–31.9)	c	1843	30.2	(26.7–33.9)	c	1812	28.4	(25.3–31.7)	c
	PIR >=3.5 (ref)	1022	17.3	(14.5–20.5)		1029	18.2	(14.8–22.1)		1057	16.6	(13.3–20.3)		1052	13.9	(11.4–16.7)	
Education	<high school	1238	30.2	(26.4–34.2)	c	1359	36.4	(31.6–41.4)	c	1396	58.6	(54.5–62.6)	c	1325	50.9	(46.2–55.7)	c
	Completed high school	1473	21	(18.1–24.2)	n	1490	28	(24.0–32.3)	c	1549	28.2	(24.9–31.8)	c	1540	29.3	(25.5–33.4)	c
	Completed more than high school (ref)	1502	17.1	(14.5–19.9)		1512	19	(15.9–22.3)		1556	13.2	(10.8–15.9)		1549	11.4	(9.5–13.4)	
Metro residence	Metro area (ref)	2113	19.5	(16.9–22.5)		2190	24.4	(21.5–27.5)		2243	24.3	(21.4–27.5)		2187	22.7	(20.3–25.2)	
	Non-metro area	2121	22	(19.0–25.3)	n	2193	26.2	(22.0–30.8)	n	2283	29.6	(25.1–34.5)	a	2251	27.2	(22.8–31.9)	n
Smoking	Current smoker	1361	20.6	(17.9–23.6)	n	1416	25.4	(22.2–28.8)	n	1460	36.4	(31.8–41.1)	c	1442	28.3	(24.8–32.1)	n

Variable	Category	Toxoplasma seropositive				SRTT Highest Quartile*				SDST Highest Quartile*				SDLTNT Highest Quartile*			
		N	Weighted Percent	95% CI	p-value	N	Weighted Percent	95% CI	p-value	N	Weighted percent	95% CI	p-value	N	Weighted Percent	95% CI	p-value
	Past smoker	833	21.8	(18.0–25.9)	n	858	23.6	(17.9–30.0)	n	886	19.7	(16.6–23.0)	a	868	17.8	(14.2–21.9)	c
	Never smoker (ref)	2036	19.6	(16.5–23.0)		2105	27.1	(23.6–30.8)		2175	25.3	(22.0–28.7)		2123	26.9	(24.3–29.6)	
Alcohol	Current user	2258	19.3	(17.1–21.7)	a	2309	20.4	(17.3–23.8)	c	2391	23.8	(20.7–27.2)	c	2369	20	(17.3–22.9)	c
	Past user	1346	21.6	(18.2–25.2)	n	1398	30.4	(26.9–34.1)	a	1430	28.2	(24.5–32.1)	c	1396	28.7	(25.5–31.9)	b
	Never used (ref)	596	25.6	(20.2–31.7)		639	37.9	(31.6–44.5)		653	43.5	(37.9–49.2)		625	42.3	(34.4–50.4)	
Familiarity with video games **	Some or a lot	2537	18.5	(16.4–20.8)	c	2563	21.6	(18.5–25.0)	c	2654	16.8	(14.4–19.5)	c	2635	16.8	(14.8–19.0)	c
	No familiarity	1641	27.4	(23.7–31.3)		1760	33.8	(30.3–37.5)		1814	49.8	(45.5–54.0)		1746	43.3	(39.3–47.2)	
Sleep night before exam **	Usual sleep (ref)	1277	20.9	(17.5–24.7)		1322	27.7	(23.9–31.8)		1358	31.2	(28.0–34.5)		1333	26.9	(22.9–31.2)	
	Less sleep	2515	21.2	(18.8–23.8)	n	2610	25.1	(21.7–28.8)	n	2705	26.8	(23.3–30.4)	a	2647	25.2	(22.4–28.1)	n
	More sleep	386	19.1	(14.2–24.7)	n	391	17	(11.2–24.3)	b	405	23	(16.7–30.4)	a	401	21.6	(16.4–27.6)	n
BMI ***	BMI Normal (ref)	1688	18.4	(16.1–20.8)		1749	23.5	(20.3–26.9)		1800	24.5	(21.9–27.4)		1771	22.7	(19.8–25.8)	
	BMI Overweight	1326	22.3	(18.7–26.2)	n	1376	25.4	(22.3–28.8)	n	1414	28.3	(24.0–32.9)	n	1383	24.9	(21.1–29.0)	n
	BMI Obese	1122	24.9	(21.3–28.8)	b	1158	30.6	(25.0–36.8)	b	1201	31.6	(27.4–36.0)	b	1176	30.4	(26.0–35.1)	b
Diabetes	Diabetes diagnosis	150	30.2	(20.9–40.8)	a	158	33.4	(21.8–46.7)	a	167	37.3	(26.5–49.1)	n	159	38.6	(24.6–54.1)	n
	No Diabetes diagnosis (ref)	4080	20.4	(18.5–22.4)		4221	24.9	(22.2–27.8)		4355	26.9	(24.0–30.0)		4275	24.5	(22.0–27.2)	
Hypertension	Hypertensive and on medication	319	23.5	(11.7–39.3)	n	340	26.4	(20.4–33.2)	n	342	35.2	(25.5–45.7)	n	332	39.5	(27.7–52.2)	b
	Hypertensive not on medication	467	23.8	(18.7–29.4)	n	483	27	(20.1–34.9)	n	502	29.6	(23.9–35.7)	n	492	22	(16.7–27.9)	n
	Not diagnosed hypertensive (ref)	3405	20.1	(18.2–22.2)		3510	25	(22.1–28.0)		3633	26.3	(23.5–29.3)		3568	24.1	(21.5–26.9)	

n= not significant

[a] p<0.05 vs ref group

[b] p<0.01 vs ref group

[c] p<0.001 vs ref group

* Highest quartile defined as > 246.15 ms for SRTT, >2.88 ms/correct digit for SDST, scores of 7–9 for SDLTNT and scores of 7–16 for SDLTSC

** Assessed by self report as part of the cognitive function test in the exam questionnaire using the categories specified

*** BMI defined as follows: normal (BMI <25), overweight (BMI>=25 and BMI<30), and obese (BMI>=30)

Table 2

Odds Ratio's (95% CI's) and p-values for Toxoplasma seropositivity from logistic models using highest quartile outcome measure, and p-values from linear regression models using continuous transformed outcome measure for SRTT and SDST

Outcome		OR for Toxoplasma Seropositivity	95% CI		p-value Logistic Model	p-value Linear Model
SRTT	Adjusted for age[a]	1.3	1.1	1.6	0.015	0.009
	Adjusted for age and demographic factors[b]	1.3	1.0	1.6	0.022	0.012
	Adjusted for age, demographic factors and poverty[c]	1.2	1.0	1.5	0.129	0.055
	Adjusted for age, demographic factors and education[d]	1.2	0.9	1.5	0.173	0.096
	Adjusted for all of the above[e]	1.1	0.9	1.4	0.292	0.135
	Full[f]	1.1	0.9	1.4	0.523	0.236
	Final[g]	1.1	0.9	1.4	0.238	0.145
SDST	Adjusted for age[a]	1.7	1.4	2.2	<0.001	<0.001
	Adjusted for age and demographic factors[b]	1.5	1.2	1.9	<0.001	<0.001
	Adjusted for age, demographic factors and poverty[c]	1.3	1.0	1.7	0.034	0.001
	Adjusted for age, demographic factors and education[d]	1.2	0.9	1.6	0.167	0.007
	Adjusted for all of the above[e]	1.1	0.8	1.5	0.436	0.041
	Full[f]	1.1	0.8	1.4	0.607	0.155
	Final[g]	1.1	0.9	1.5	0.395	0.067
	PIR < 1.3[h]	2.9	1.8	4.8	<0.001	0.004
	PIR 1.3–3.49	1.1	0.7	1.6	0.791	0.065
	PIR >= 3.5	0.6	0.3	1.2	0.120	0.406

OR=Odds Ratio

CI= confidence interval

[a] adjusted for age in years as a continuous variable.

[b] adjusted for age, race/ethnicity, gender and foreign birth

[c] adjusted for age, race/ethnicity, gender, foreign birth and poverty

[d] adjusted for age, race/ethnicity, gender, foreign birth and education

[e] adjusted for age, race/ethnicity, gender, foreign birth, poverty and education

[f] full model - adjusted for age, race/ethnicity, gender, foreign birth, poverty, education, metro residence, sleep, video game familiarity, smoking, alcohol use, body mass index, hypertension, and diagnosis of diabetes.

[g] final model - adjusted for significant cofactors: SRTT highest quartile - adjusted for race/ethnicity, gender, education, video game use, sleep, and alcohol use. SDST highest quartile - adjusted for age, race/ethnicity, gender, poverty, education, video game familiarity, sleep, alcohol use and any smoking.

[h] stratified models - adjusted for cofactors in the final model and stratified on significant interaction term - poverty index

Table 3

Odds Ratio's (95% CI's) and P-values for Toxoplasma Seropositivity from Logistic Models For SDLTNT and SDLTSC Highest Quartile Outcome Measures

Outcome		OR for Toxo	SDLTNT 95% CI		p-value Highest Quartile	OR for Toxo	SDLTSC 95% CI		p-value Highest Quartile
SDLT	Adjusted for age[a]	1.6	1.2	2.0	0.000	1.8	1.4	2.3	<0.001
	Adjusted for age and demographic factors[b]	1.4	1.1	1.8	0.007	1.7	1.3	2.2	<0.001
	Adjusted for age, demographic factors and poverty[c]	1.2	1.0	1.6	0.094	1.5	1.1	1.9	0.010
	Adjusted for age, demographic factors and education[d]	1.2	0.9	1.5	0.196	1.4	1.1	1.9	0.023
	Adjusted for all of the above[e]	1.1	0.9	1.5	0.430	1.3	1.0	1.8	0.068
	Full[f]	1.1	0.9	1.5	0.370	1.4	1.0	1.9	0.061
	Final[g]	1.1	0.8	1.5	0.439	1.3	1.0	1.9	0.081
	Foreign born[h]	1.6	1.1	2.5	0.027	2.4	1.4	3.9	0.002
	US born	1.0	0.8	1.3	0.986	1.2	0.8	1.7	0.332

OR=Odds Ratio, CI=confidence interval

[a] adjusted for age in years as a continuous variable

[b] adjusted for age, race/ethnicity, gender and foreign birth

[c] adjusted for age, race/ethnicity, gender, foreign birth and poverty

[d] adjusted for age, race/ethnicity, gender, foreign birth and education

[e] adjusted for age, race/ethnicity, gender, foreign birth, poverty and education

[f] full model - adjusted for age, race/ethnicity, gender, foreign birth, poverty, education, metro residence, sleep, video game usage, smoking, alcohol use, body mass index, hypertension and diagnosis of diabetes.

[g] final model - adjusted for significant cofactors: Both SDLTNT and SDLTSC were adjusted for age, race/ethnicity, foreign birth, poverty, education, video game use, alcohol use, and on hypertension medicine.

[h] stratified models - adjusted for cofactors in the final model and stratified on significant interaction term - foreign birth.