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ORIGINAL PAPER

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

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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 4,200 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

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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 [3, 4]. Toxo-infected mice and rats have reduced fear of cat odor, and a subset even become attracted to cat urine [3, 5]. 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 [3, 5]. 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. [15], which did consider possible toxo exposure sources and socioeconomic status (SES) in regression models, found an association of toxo and work-related accidents in patients with low SES, though these analyses were based on only eight seropositive accident victims in the low SES strata.

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 [8, 9, 17–19]. Thus, despite numerous intriguing studies suggesting a link between toxo and cognitive function, there is a lack of 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 [20]. The NHANES III was based on a stratified, multistage, probability cluster design from which a sample representative of the civilian, non-institutionalized 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 [20].

Measures of neurocognitive function

During the physical examination, three computerized tests were used to evaluate neurocognitive function [21]. 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. [22] present a detailed description of how these neurocognitive outcome variables were measured and created. 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 ms) 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

Table 1 Age-adjusted prevalence of *Toxoplasma gondii* seropositivity and poor outcome on three cognitive function measures by demographic, socioeconomic, behavioral, and health-related factors

Variable	Category	Toxoplasma seropositive			SRTT highest quartile[A]			SDST highest quartile[A]			SDLTNT highest quartile[A]		
		N	Weighted 95 % CI (%)	*p* value	*N*	Weighted 95 % CI (%)	*p* value	*N*	Weighted 95 % CI (%)	*p* value	*N*	Weighted 95 % CI (%)	*p* value
Total		4,234	20.9 (19.1–22.8)		4,383	25.4 (22.7–28.2)		4,526	27.3 (24.5–30.3)		4,438	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				3,439	24.4 (21.5–27.4)		3,571	25.4 (22.5–28.5)		3,508	23.4 (20.7–26.3)	
Age	20–29 years (ref)	1,320	14.5 (11.7–17.6)		1,347	23.7 (20.0–27.7)		1,421	12.6 (10.0–15.6)		1,398	18 (14.4–22.1)	
	30–39 years	1,231	14.9 (12.0–18.3)	n	1,256	22.3 (18.4–26.5)	n	1,300	14.9 (11.7–18.5)	n	1,280	20.6 (17.2–24.2)	n
	40–49 years	1,007	21.1 (17.3–25.4)	a	1,056	27.3 (22.1–33.1)	n	1,088	33.3 (28.2–38.6)	c	1,053	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)	1,927	21.6 (18.7–24.7)		1,999	20.6 (17.3–24.1)		2,057	32.5 (29.0–36.1)		2,020	24.7 (21.8–27.7)	
	Female	2,307	20.1 (17.9–22.5)	n	2,384	30.1 (26.4–34.0)	c	2,469	22.3 (18.8–26.2)	c	2,418	25.8 (22.7–29.0)	n
Race/ethnicity	Non-hispanic white (ref)	1,628	19.5 (17.5–21.6)		1,653	22.9 (19.6–26.5)		1,669	21.7 (18.5–25.2)		1,657	20 (17.6–22.5)	
	Non-hispanic black	1,298	22.7 (19.6–26.0)	n	1,355	34.8 (31.9–37.8)	c	1,381	43.8 (40.6–47.1)	c	1,357	39.6 (36.7–42.6)	c
	Mexican–American	1,156	21 (18.2–24.0)	n	1,218	37.4 (33.8–41.1)	c	1,315	56.4 (51.2–61.6)	c	1,265	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)	3,359	19.6 (17.6–21.7)		3,442	24.5 (21.6–27.5)		3,540	24.8 (21.9–27.9)		3,501	23 (20.7–25.5)	
SES–PIR	PIR <1.3	1,201	29.6 (25.4–34.1)	c	1,272	33.6 (28.0–39.5)	c	1,331	47.1 (41.6–52.7)	c	1,285	45.5 (39.2–51.9)	c
	PIR 1.3–3.49	1,739	20.7 (17.7–23.8)	n	1,795	27.7 (23.7–31.9)	c	1,843	30.2 (26.7–33.9)	c	1,812	28.4 (25.3–31.7)	c
	PIR >=3.5 (ref)	1,022	17.3 (14.5–20.5)		1,029	18.2 (14.8–22.1)		1,057	16.6 (13.3–20.3)		1,052	13.9 (11.4–16.7)	
Education	<High school	1,238	30.2 (26.4–34.2)	c	1,359	36.4 (31.6–41.4)	c	1,396	58.6 (54.5–62.6)	c	1,325	50.9 (46.2–55.7)	c
	Completed high school	1,473	21 (18.1–24.2)	n	1,490	28 (24.0–32.3)	c	1,549	28.2 (24.9–31.8)	c	1,540	29.3 (25.5–33.4)	c
	Completed more than high school (ref)	1,502	17.1 (14.5–19.9)		1,512	19 (15.9–22.3)		1,556	13.2 (10.8–15.9)		1,549	11.4 (9.5–13.4)	
Metro residence	Metro area (ref)	2,113	19.5 (16.9–22.5)		2,190	24.4 (21.5–27.5)		2,243	24.3 (21.4–27.5)		2,187	22.7 (20.3–25.2)	
	Non-metro area	2,121	22 (19.0–25.3)	n	2,193	26.2 (22.0–30.8)	n	2,283	29.6 (25.1–34.5)	a	2,251	27.2 (22.8–31.9)	n
Smoking	Current smoker	1,361	20.6 (17.9–23.6)	n	1,416	25.4 (22.2–28.8)	n	1,460	36.4 (31.8–41.1)	c	1,442	28.3(24.8–32.1)	n
	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)	2,036	19.6 (16.5–23.0)		2,105	27.1 (23.6–30.8)		2,175	25.3 (22.0–28.7)		2,123	26.9 (24.3–29.6)	
Alcohol	Current user	2,258	19.3 (17.1–21.7)	a	2,309	20.4 (17.3–23.8)	c	2,391	23.8 (20.7–27.2)	c	2,369	20 (17.3–22.9)	c
	Past user	1,346	21.6 (18.2–25.2)	n	1,398	30.4 (26.9–34.1)	a	1,430	28.2 (24.5–32.1)	c	1,396	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[B]	Some or a lot	2,537	18.5 (16.4–20.8)	c	2,563	21.6 (18.5–25.0)	c	2,654	16.8 (14.4–19.5)	c	2,635	16.8 (14.8–19.0)	c
	No familiarity (ref)	1,641	27.4 (23.7–31.3)		1,760	33.8 (30.3–37.5)		1,814	49.8 (45.5–54.0)		1,746	43.3 (39.3–47.2)	
Sleep night before exam[B]	Usual sleep (ref)	1,277	20.9 (17.5–24.7)		1,322	27.7 (23.9–31.8)		1,358	31.2 (28.0–34.5)		1,333	26.9 (22.9–31.2)	
	Less sleep	2,515	21.2 (18.8–23.8)	n	2,610	25.1 (21.7–28.8)	n	2,705	26.8 (23.3–30.4)	a	2,647	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

Table 1 continued

Variable	Category	Toxoplasma seropositive			SRTT highest quartile[A]			SDST highest quartile[A]			SDLTNT highest quartile[A]		
		N	Weighted 95 % CI (%)	*p* value	*N*	Weighted 95 % CI (%)	*p* value	*N*	Weighted 95 % CI (%)	*p* value	*N*	Weighted 95 % CI (%)	*p* value
BMI[C]	BMI normal (ref)	1,688	18.4 (16.1–20.8)		1,749	23.5 (20.3–26.9)		1,800	24.5(21.9–27.4)		1,771	22.7 (19.8–25.8)	
	BMI overweight	1,326	22.3 (18.7–26.2)	n	1,376	25.4 (22.3–28.8)	n	1,414	28.3 (24.0–32.9)	n	1,383	24.9 (21.1–29.0)	n
	BMI obese	1,122	24.9 (21.3–28.8)	b	1,158	30.6 (25.0–36.8)	a	1,201	31.6 (27.4–36.0)	b	1,176	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)	n	167	37.3 (26.5–49.1)	n	159	38.6 (24.6–54.1)	n
	No diabetes diagnosis (ref)	4,080	20.4 (18.5–22.4)		4,221	24.9 (22.2–27.8)		4,355	26.9 (24.0–30.0)		4,275	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)	3,405	20.1 (18.2–22.2)		3,510	25 (22.1–28.0)		3,633	26.3 (23.5–29.3)		3,568	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

[A] 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

[B] Assessed by self-report as part of the cognitive function test in the exam questionnaire using the categories specified

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

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, 22]. 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 [22–24] 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 [21] 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 SES is correlated with worse scores on some neurocognitive tests [22] and may be correlated with labor occupations involving prolonged contact with soil (possibly increasing toxo oocyst exposure) [25]. 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) [26] 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 % [25]. *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 5,662 (50 %) were part of the cognitive tested subsample and given sample weights for that portion of the exam. Of the 5,662 with subsample weights, 5,064 (89 %) were tested for *Toxoplasma gondii* antibody, 4,383 (77 %) were tested for *T. gondii* and had complete valid data on SRTT, 4,526 (80 %) for SDST, and 4,438 (78 %) for SDLT. Outcome data was set to missing for individuals who used alcohol in the past 3 h ($N = 58$), 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). However, 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 [27, 28]. 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 [29]. 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 socioeconomic 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 three 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 >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 three outcomes ($N = 4,234$) was 20.9 %. The prevalence of toxo was greater among those older, foreign born, of lower SES, with lower education, obese, and with a diabetes diagnosis. Toxo seroprevalence was lower

Table 2 Odds ratios (95 % CIs) 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	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

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 sociodemographic 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 % CI) 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 % CI 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 non-significant 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 ms (95 % CI 227, 235) versus 226 ms (95 % CI 224, 228) in the toxo seronegative group. For SDST the mean error-corrected latency for toxo-seropositive respondents was 2.71 s/correct digit (95 % CI 2.65, 2.78) compared to 2.51 s/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, 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 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) ($N = 2{,}749$) 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. [9] have argued that infection with toxo may be a neurobiological instigator of decreased cognitive abilities; a direct causal effect. Ferreira et al. [30] reported an association between toxo seroprevalence and poor scholastic achievement in children, and proposed that infection with toxo in childhood could impair educational attainment. Mechanistically, toxo could induce persistent inflammation in a subset of individuals, leading to adverse effects on psychomotor performance. However, the

Table 3 Odds ratios (95 % CIs) 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

proportion of our sample with high C-reactive protein levels (CRP $\geq$ 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 [31]. 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, 32]. *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 [33]. 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 [34]. Many such physiological variables affecting cognition are also related to lower SES 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 [35]. Interestingly, Krieg et al. [22] found that differences between racial-ethnic groups in performance levels on these cognitive tests tended to diminish as family income increased, suggesting the relative importance of modifiable factors such as SES on these cognitive test scores.

The effect of toxo on cognitive function was not consistent across our three 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 [24, 34, 36–38]. In addition, other studies have shown the combined effects of multiple significant predictors may be greater than either alone and vary between outcome measures [39].

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.

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