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## **Toxoplasma gondii seropositivity and cognitive functioning in older adults: An analysis of cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014**

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3 1 **Toxoplasma gondii seropositivity and cognitive functioning in older adults: An analysis of**  
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5 2 **cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014**  
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3 41 **Toxoplasma gondii seropositivity and cognitive functioning in older adults: An analysis of**  
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5 42 **cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014**  
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9  
10 44 **Abstract**

11  
12 45 **Objectives:** This study sought to examine the relationship between *Toxoplasma gondii*  
13  
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15 46 seropositivity and cognitive functioning in older adults.

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17 47 **Design:** A cross-sectional study.

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19 48 **Setting:** The National Health and Nutrition Examination Survey (NHANES) study took place at  
20  
21  
22 49 participants' homes and mobile exam centers.

23  
24 50 **Participants:** A total of 2,956 older adults aged 60 and above from the NHANES from 2011 to  
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26  
27 51 2014 were included in the study.

28  
29 52 Exposure of interest: Serum *Toxoplasma gondii* antibody was analyzed in the lab. A value > 33  
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31  
32 53 IU/mL was categorized as seropositive for *Toxoplasma gondii* infection; <27 IU/mL was  
33  
34  
35 54 categorized as seronegative for *Toxoplasma gondii* infection.

36 55 **Primary and secondary outcome measures:** Cognitive tests included the Consortium to  
37  
38 56 Establish a Registry for Alzheimer's Disease Word Learning subtest (CERAD-WL) immediate  
39  
40  
41 57 and delayed memory, the Animal Fluency test (AFT), and the Digit Symbol Substitution Test  
42  
43  
44 58 (DSST).

45 59 **Results:** About half of the 2,956 participants (mean age 70.0) were female (51.0%), non-  
46  
47  
48 60 Hispanic White (48.3%), and completed some college or above (48.3%). A total of 703  
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50  
51 61 participants were positive for *Toxoplasma gondii* infection (23.8%). Adjusted linear regression  
52  
53  
54 62 showed that compared with participants with negative *Toxoplasma gondii* infection, those with  
55  
56  
57 63 positive *Toxoplasma gondii* infection had lower CERAD-WL immediate memory (beta [ $\beta$ ] -0.16,

64 95% confidence interval [CI] -0.25, -0.07), CERAD-WL delayed memory ( $\beta$  -0.15, 95% CI -  
65 0.24, -0.06), AFT ( $\beta$  -0.15, 95% CI -0.24,-0.06), DSST ( $\beta$  -0.34, 95% CI -0.43, -0.26), and global  
66 cognition ( $\beta$  -0.24, 95% CI -0.32,-0.16) z scores after controlling for the covariates.

67 **Conclusions:** Toxoplasma gondii seropositivity is associated with worse immediate and delayed  
68 verbal learning, language proficiency, executive functioning, processing speed, sustained  
69 attention, working memory, as well as global cognition in older adults. Public health measures  
70 aiming at preventing toxoplasma gondii infection may help preserve cognitive functioning in  
71 older adults.

72  
73 **Keywords:** Toxoplasma gondii; cognitive functioning; older adults; neurotoxoplasmosis;  
74 neuropsychological; psychomotor performance

75  
76 **Strengths and limitations:**

- 77 • This study is one of the few studies that examined the cognitive effect of Toxoplasma  
78 gondii seropositivity on cognitive outcomes in older adults.
- 79 • With the NHANES data, the study population was nationally representative of U.S. older  
80 adults.
- 81 • A wide range of sociodemographic, lifestyle, mental health, and physical health  
82 covariates was adjusted, reducing the possibility of residual confounding.
- 83 • A cross-sectional study hinders the assessment of longitudinal relationships.

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85  
86 **1. Introduction**

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3 87 Alzheimer's disease and related dementia (ADRD) is a serious public health threat  
4  
5 88 worldwide. In 2016, a total of 43.8 million people had dementia in the world. With a growing  
6  
7 89 number of people with ADRD, families, communities, and healthcare systems around the world  
8  
9  
10 90 are heavily burdened (1). Although dementia is currently not curable, identifying modifiable risk  
11  
12 91 factors associated with ADRD can help reduce the burden of the disease. Cognitive test  
13  
14 92 performance in older adults is an important indicator of their cognitive functioning (2). By  
15  
16 93 examining their correlations to cognitive tests, risk factors for cognitive decline can be identified  
17  
18 94 and intervened.

19  
20  
21 95 *Toxoplasma gondii* is among the most prevalent human zoonoses (3) and affects about  
22  
23 96 30% of the global population (4). *Toxoplasma gondii* infection can happen prior to or after a  
24  
25 97 person is born, with postnatal infection being more common. By consuming oocysts found in cat  
26  
27 98 feces-contaminated soil or water (5, 6) or eating tissue cysts in undercooked meat (7, 8), humans  
28  
29 99 can be infected with *toxoplasma gondii* after birth. While most people with a healthy immune  
30  
31 100 system are asymptomatic following acute infection with *toxoplasma gondii*, some may  
32  
33 101 experience unspecific symptoms lasting from several weeks to months, including fever, malaise,  
34  
35 102 and lymphadenopathy (9).

36  
37  
38 103 A limited number of studies have found that *toxoplasma gondii* infection is associated  
39  
40 104 with neurocognitive changes in humans (10-13). However, their direction of findings and effect  
41  
42 105 sizes are inconsistent. In addition, this area is understudied, given the prevalence of *toxoplasma*  
43  
44 106 *gondii* infection in humans. In a systematic review and meta-analysis of the association of  
45  
46 107 *toxoplasma gondii* seropositivity and cognitive function in healthy people, only thirteen studies  
47  
48 108 were included, most of which had small sample sizes (4). In addition, two included studies (14,  
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50 109 15) utilized the National Health and Nutrition Examination Survey (NHANES) 1988-1994 cycle  
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3 110 data and thus could not reflect the current epidemic of toxoplasma gondii infection. The findings  
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5 111 of this study showed that seropositivity to toxoplasma gondii was modestly but significantly  
6  
7 112 associated with poorer processing speed, working memory, verbal short-term memory, and  
8  
9 113 executive functioning. However, studies using large, the latest, and nationally representative  
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11 114 population-based data are needed to better elucidate the cognitive effects of toxoplasma gondii  
12  
13 115 seropositivity.  
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17 116 In this study, taking advantage of the NHANES, we aimed to examine the relationship  
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19 117 between toxoplasma gondii seropositivity and cognitive functioning in a nationally  
20  
21 118 representative sample of U.S. older adults. The findings of this study will provide implications  
22  
23 119 for understanding the cognitive effects of toxoplasma gondii infection and developing tailored  
24  
25 120 public interventions to protect cognitive functioning in the growing number of older adults in the  
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27 121 US.  
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## 32 33 123 **2. Method**

### 34 35 124 ***2.1 Study design and setting***

36  
37 125 The NHANES is an ongoing, cross-sectional survey of civilian, non-institutionalized  
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39 126 adults and children in the United States conducted by the National Center for Health Statistics of  
40  
41 127 the Centers for Disease Control and Prevention. A nationally representative sample of children  
42  
43 128 and adults across the country are surveyed bi-annually (16). Their sociodemographic, health, and  
44  
45 129 nutritional status are evaluated using in-person interviews and physical exams. The interviews  
46  
47 130 are conducted at participants' homes; health exams are conducted in specially equipped mobile  
48  
49 131 exam centers. Health exams include laboratory testing of urine and blood specimens and  
50  
51 132 medical, dental, and physiological assessments. Participants' serum toxoplasma gondii antibody  
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3 133 levels and cognitive functioning were measured in the NHANES 2011-2014 cycles. In this study,  
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5 134 two survey cycles (2011-2012 and 2013-2014) were merged to increase sample size and power.  
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8 135 Between 2011 and 2014, a total of 19,151 individuals participated in the NHANES. They were  
9  
10 136 recruited from a selection of census blocks or clusters of census block area segments. The  
11  
12 137 detailed sampling method has been published elsewhere (17).

13  
14  
15 138 Of the 19,151 individuals, we excluded individuals aged < 60 (n = 15,679) or had  
16  
17 139 missing data on serum *Toxoplasma gondii* IgG (n=511). Participants with equivocal serum  
18  
19 140 *Toxoplasma gondii* IgG ( $\geq 27$  and <33 IU/mL) were further excluded (n=5). Finally, a total of  
20  
21 141 2,956 participants aged 60 and above were included in the analysis. The characteristics of the  
22  
23 142 excluded participants due to missing data (n=516) were presented in the Appendix. Compared  
24  
25 143 with the included participants, people who were excluded were more likely to be of other  
26  
27 144 ethnicities than Non-Hispanic Whites, overweight/obese, completed lower education and had  
28  
29 145 higher systolic blood pressure, lower Digit Symbol Substitution Test (DSST) score, and lower  
30  
31 146 Animal Fluency test (AFT) score.

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## 36 37 148 **2.2 Ethical considerations**

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40 149 The National Center for Health Statistics Research Ethics Review Board approved the  
41  
42 150 NHANES. Participants in the NHANES provided written informed consent before enrolling in  
43  
44 151 the study. The University of Houston-Downtown Committee for the Protection of Human  
45  
46 152 Subjects granted this study an exemption because only publicly accessible and de-identified data  
47  
48 153 were used.

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## 52 53 155 **2.3 Public and Patient involvement**

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2  
3 156 Patients or the public were NOT involved in the design, conduct, reporting, or  
4  
5 157 dissemination plans of our research.  
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7  
8 158

#### 9 10 159 **2.4 Independent variable: Toxoplasma gondii seropositivity**

11  
12 160 Serological tests that detect Toxoplasma. gondii Immunoglobulin G (IgG) and  
13  
14 161 Immunoglobulin M (IgM) antibodies are often used for clinical diagnoses of toxoplasmosis.  
15  
16 162 While the IgM antibody test can validate acute phases, the IgG antibody test can identify acute or  
17  
18 163 chronic phases (18). In the NHANES, an enzyme immunoassay (EIA) measuring IgG against  
19  
20 164 toxoplasma gondii was used to measure the presence or absence of toxoplasma gondii (16).  
21  
22 165 Toxoplasma gondii IgG was measured with two enzyme immunoassay kits (19). Strict quality  
23  
24 166 control was implemented for every plate. A value between 27 and 33 IU/mL was deemed as  
25  
26 167 “equivocal”; a value  $\geq 33$  IU/mL was deemed as “positive”; a value  $< 27$  IU/mL was deemed as  
27  
28 168 “negative” (16). Samples with equivocal results ( $\geq 27$  IU/mL and  $< 33$  IU/mL) were repeated  
29  
30  
31 169 twice and confirmed as negative. For our analysis, we categorized participants into “seropositive  
32  
33 170 for toxoplasma gondii infection” or “seronegative for toxoplasma gondii infection”. This cutoff  
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35  
36 171 is consistent with previous NHANES studies (20, 21).  
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#### 41 42 173 **2.5 Dependent variable: Cognitive functioning**

43  
44 174 Three cognitive tests were used to assess participants’ various domains of cognitive  
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46 175 functioning, including the Consortium to Establish a Registry for Alzheimer’s Disease Word  
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48 176 Learning subtest (CERAD-WL), the AFT, and the DSST. The detailed method has been  
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50 177 published elsewhere (22).  
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3 178 1) The CERAD-WL assessed participants' capacity for both immediate (immediate  
4  
5 179 memory) and delayed (delayed memory) verbal learning (23). It consisted of a delayed recall  
6  
7 180 after three consecutive learning trials. For each learning trial, participants must read aloud ten  
8  
9 181 randomly selected words that are displayed on a computer screen in huge, bolded characters, one  
10  
11 182 at a time. Following the presentation of the words, participants were encouraged to retain and  
12  
13 183 recall as many words as they could. In each of the three trials, the order of the ten words was  
14  
15 184 changed. There was a ten-point maximum for each trial. A participant's immediate memory score  
16  
17 185 was the sum of their three trials' scores, ranging from zero to thirty. After the AFT and the  
18  
19 186 DSST, participants took the delayed recall test, which asked them to recall as many words from  
20  
21 187 the same ten-word list as they could. The delayed memory score, which varied from zero to ten,  
22  
23 188 depended on how many accurate words a subject could recall.  
24  
25  
26  
27

28 189 2) Participants' language proficiency and executive function were assessed by the AFT  
29  
30 190 (24). Each animal a participant named received one point, and they had one minute to name as  
31  
32 191 many animals as they could. Participants were first prompted to identify three pieces of clothing  
33  
34 192 as a warm-up.  
35  
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37

38 193 3) The DSST measured the participants' working memory, sustained attention, and  
39  
40 194 processing speed (25). A paper form with a top-mounted key that included nine numbers and  
41  
42 195 paired symbols was used to conduct the exam. Participants were instructed to copy various  
43  
44 196 symptoms to the matching symbols in the 133 boxes that were placed next to the numbers. They  
45  
46 197 had two minutes to complete this task. The DDST score was based on the total number of correct  
47  
48 198 matches (26). Before participants started the formal test, a sample practice test was provided.  
49  
50 199 The possible score range of the DSST was between zero and 133 (27).  
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## 201 **2.6 Covariates**

202 Covariates of this study were selected according to literature review and included age  
203 (years), sex (male or female), race/ethnicity (Mexican Americans, other Hispanics, non-Hispanic  
204 White, or non-Hispanic Black), education (below high school, high school graduate, or some  
205 college or above), depressive symptoms, smoking status (never, former, or current smokers),  
206 BMI ( $<18.5$  kg/m<sup>2</sup>, 18.5-24.9 kg/m<sup>2</sup>, 25-29.9 kg/m<sup>2</sup>, or  $\geq 30$  kg/m<sup>2</sup>), prevalent coronary heart  
207 disease (CHD) (yes or no), stroke (yes or no), and systolic blood pressure (mmHg). The  
208 information was either self-reported or collected at health exams. The Patient Health  
209 Questionnaire (PHQ-9) was used to measure depressive symptoms (28). It is a nine-item  
210 screening tool on the frequency of depressive symptoms over the past two weeks and has a total  
211 score ranging from 0-27. A higher score indicates more severe depression symptoms. The PHQ-  
212 9 total score was used to indicate depressive symptoms.

## 214 **2.7 Statistical analysis**

215 Means (standard deviation [SD]) were used to describe the characteristics of the study  
216 population for continuous data that followed a normally distributed distribution. Medians  
217 (interquartile range) were used for non-normally distributed continuous data. Data for categorical  
218 variables were summarized using frequency (percentages). Independent T-tests were used to  
219 compare group differences for continuous variables between the two groups. Chi-square tests  
220 were used to compare group differences for categorical variables between the two groups.

221 The CERAD-WL immediate memory, CERAD-WL delayed memory, AFT, and DSST  
222 were standardized with mean zero and variance one to compute cognitive test-specific z-scores.  
223 The cognitive test-specific z scores of the four tests were then averaged to calculate the global

224 cognition z scores. Linear regression models were constructed between toxoplasma gondii  
225 seropositivity (seronegative or seropositive) and each of the four cognitive test-specific and  
226 global cognition z scores. All models were adjusted for the covariates mentioned above. We  
227 considered a 95% confidence interval excluding one as statistically significant. All the analyses  
228 were performed using SPSS 25.0.

229

### 230 3. Results

231 The sociodemographic and health information of the study population, stratified by  
232 toxoplasma gondii seropositivity, was presented in **Table 1**. Of the 2,956 participants, 1, 403  
233 were from the 2011-2012 cycle and 1,553 from the 2013-2014 cycle. A total of 703 participants  
234 were seropositive for toxoplasma gondii infection (23.8%). They had a mean age of 70.0 (SD  
235 7.0). Most of the 2,952 participants (mean age of 70.0 [SD 7.0]) were female (51.0%), non-  
236 Hispanic White (48.3%), completed some college or above (48.3%), were never smokers  
237 (49.8%), and had a BMI  $\geq 30$  (35.6%) and an average of 8.7 (SD 10.7) hours of physical activity  
238 every week. Their mean total cholesterol and systolic blood pressure were 190.2 mg/dL and  
239 133.5 mmHg. Their mean delayed memory, immediate memory, AFT, and DSST score were 5.8  
240 (SD 2.4), 18.5 (SD 5.0), 16.4 (SD 5.6), and 45.8 (SD 17.5), respectively. Compared with  
241 participants with negative toxoplasma gondii seropositivity, participants with positive  
242 toxoplasma gondii seropositivity were older, less educated, and more likely to be male, current  
243 smokers, and had more physical activities. They were also more likely to have different  
244 ethnicities and lower CERAD W-L immediate recall, CERAD W-L delayed recall, AFT, and  
245 DSST scores.

246 Table 1. Characteristics of the participants by toxoplasma gondii seropositivity

Variables	Negative (n=2,253)	Positive (n=703)	Total (n=2,956)	P-Value
Age, years	69.7(6.9)	70.9(7.0)	70.0(7.0)	<b>&lt;0.001</b>
Sex, n (%)				<b>&lt;0.001</b>
Male	1,057(46.9%)	390(55.5%)	1,447(49.0%)	
Female	1,196(53.1%)	313(44.5%)	1,509(51.0%)	
Race/ethnicity, n (%)				<b>&lt;0.001</b>
Mexican Americans	231(10.3%)	43(6.1%)	274(9.3%)	
Other Hispanics	161(7.1%)	131(18.6%)	292(9.9%)	
Non-Hispanic Whites	1,111(49.3%)	317(45.1%)	1,428(48.3%)	
Non-Hispanic Blacks	479(21.3%)	165(23.5%)	644(21.8%)	
Other	271(12.0%)	47(6.7%)	318(10.8%)	
Education, n (%)				<b>&lt;0.001</b>
Below high school	594(26.3%)	256(36.4%)	850(28.7%)	
High school graduate	510(22.6%)	165(23.5%)	421(14.2)	
Some college or above	1,147(50.9%)	281(39.9%)	1,428(48.3%)	
Depressive symptoms	3.5(4.8)	3.3(4.5)	3.5(4.8)	0.334
Smoking, n (%)				<b>0.043</b>
Never	1,130(50.2%)	342(48.6%)	1,472(49.8%)	
Former	846(37.5%)	249(35.4%)	1,095(37.0%)	
Current	274(12.2%)	111(15.8%)	385(13.0%)	
Body mass index, n (%)				0.444
<18.5 kg/m <sup>2</sup>	35(1.6%)	9(1.3%)	44(1.5%)	
18.5-24.9 kg/m <sup>2</sup>	592(26.3%)	166(23.6%)	758(25.6%)	
25.0-29.9 kg/m <sup>2</sup>	784(34.8%)	262(37.3%)	1,046(35.4%)	
≥30 kg/m <sup>2</sup>	800(35.5%)	251(35.7%)	1,051(35.6%)	
Physical activity, hours/week	8.2(10.3)	10.6(12.1)	8.7(10.7)	<b>0.025</b>
Total cholesterol, mg/dL	190.7(42.5)	188.4(43.4)	190.2(42.7)	0.209
Systolic blood pressure, mmHg	133.2(19.8)	134.5(42.5)	133.5(27.0)	0.271
CERAD W-L immediate recall	18.7(5.0)	17.9(4.8)	18.5(5.0)	<b>&lt;0.01</b>
CERAD W-L delayed recall	5.9(2.4)	5.5(2.4)	5.8(2.4)	<b>&lt;0.01</b>
Animal Fluency Test	16.6(5.6)	15.8(5.3)	16.4(5.6)	<b>&lt;0.01</b>
Digit Symbol Substitution Test	47.2(17.4)	41.2(16.8)	45.8(17.5)	<b>&lt;0.001</b>

247 Data are presented as means (standard deviation) for continuous variables and n (%) for categorical  
 248 variables. Bolded values mean statistical significance (P<0.05).  
 249

250  
 251 The means and 95% confidence intervals (CI) of cognitive test-specific z-scores by  
 252 toxoplasma gondii infection status were presented in **Table 2**. The mean of CERAD W-L  
 253 immediate call, CERAD W-L delayed recall, AFT and DSST was 0.04 (95% CI -1.94, 2.01),

254 0.03 (95% CI -1.92, 1.99), 0.03 (95% CI -1.95, 2.01) and 0.08 (95% CI -1.88, 2.03),  
 255 respectively, among participants with negative toxoplasma gondii infection. Among participants  
 256 with seropositive toxoplasma gondii infection, the mean of CERAD W-L immediate call,  
 257 CERAD W-L delayed recall, AFT and DSST was -0.12 (95% CI -2.01, 1.77), -0.12 (95% CI -  
 258 2.07, 1.83), -0.11 (95% CI -1.99, 1.76) and -0.26 (95% CI -2.15, 1.63), respectively. For the  
 259 global cognition z-scores, the mean was 0.06 (95% CI -1.92, 2.03) for participants with negative  
 260 toxoplasma gondii infection and -0.18 (95% CI -2.06, 1.70) for those with positive toxoplasma  
 261 gondii infection.

262 Table 2. Cognitive z-scores and 95% confidence intervals by toxoplasma gondii seropositivity

	Negative (n=2,253)	Positive (n=703)
CERAD W-L immediate recall	0.04 (-1.94, 2.01)	-0.12 (-2.01, 1.77)
CERAD W-L delayed recall	0.03 (-1.92, 1.99)	-0.12 (-2.07, 1.83)
Animal fluency test	0.03 (-1.95, 2.01)	-0.11 (-1.99, 1.76)
Digit Symbol Substitution Test	0.08 (-1.88, 2.03)	-0.26 (-2.15, 1.63)
Global cognition	0.06 (-1.92, 2.03)	-0.18 (-2.06, 1.70)

263  
 264 Adjusted linear regression (**Table 3**) showed that compared with participants with  
 265 seronegative toxoplasma gondii infection, those with seropositive toxoplasma gondii infection  
 266 had lower CERAD-WL immediate memory (beta [ $\beta$ ] -0.16, 95% CI -0.25, -0.07), CERAD-WL  
 267 delayed memory ( $\beta$  -0.15, 95% CI -0.24, -0.06), AFT ( $\beta$  -0.15, 95% CI -0.24, -0.06), and DSST  
 268 ( $\beta$  -0.34, 95% CI -0.43, -0.26) z scores controlling for age, race/ethnicity, education, depressive  
 269 symptoms, smoking status, BMI, prevalent CHD, stroke, and systolic blood pressure. For the  
 270 global cognition z score, which is calculated by averaging the four cognitive test-specific z-  
 271 scores, the negative association remained ( $\beta$  -0.24, 95% CI -0.32, -0.16).

272 Table 3. The independent associations of toxoplasma gondii seropositivity (reference: Negative) with  
 273 cognitive specific test and global cognition z-scores  
 274

	Beta	95% CI
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CERAD W-L immediate recall	-0.16	<b>(-0.25, -0.07)</b>
CERAD W-L delayed recall	-0.15	<b>(-0.24, -0.06)</b>
Animal fluency test	-0.15	<b>(-0.24, -0.06)</b>
Digit Symbol Substitution Test	-0.34	<b>(-0.43, -0.25)</b>
Global cognition	-0.24	(-0.32, -0.16)

95% CI means 95% confidence interval

Bolded values mean statistical significance (96% CI excluding zero).

#### 4. Discussion

In this sample of 2,956 nationally representative older adults in the US, positive toxoplasma gondii infection is independently associated with worse immediate and delayed verbal learning, language proficiency, executive functioning, processing speed, sustained attention, working memory, as well as global cognition. This relationship is independent of age, race/ethnicity, education, depressive symptoms, smoking status, BMI, prevalent CHD, stroke, and systolic blood pressure. Although our findings should be validated using longitudinal studies, they suggest that serum toxoplasma gondii seropositivity may be associated with cognitive impairment and that preventing toxoplasma gondii infection should be a target of public health interventions to protect cognitive functioning in older adults. This is very important given the high prevalence of toxoplasma gondii infection and the increasing population aging in the US.

A limited number of studies have examined the associations of toxoplasma gondii seropositivity with cognitive outcomes in humans. Two studies utilized the NHANES 1988-1994 cycle data and thus could not reflect the current epidemic of toxoplasma gondii infection (14, 15). In addition, one of them only included school-aged children (15). To the best of our knowledge, only two relevant studies exclusively targeted older adults (29, 30). In one study of 84 older adults aged 65 years and above in Germany, toxoplasmosis-positive participants showed impaired working memory, attention, and word fluency, but not processing speed measured by



1  
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3 297 DSST, compared with those who were toxoplasmosis negative (29). It is important to note that  
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5 298 the sample size of that study is very small. Then, in another study including older adults in the  
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7  
8 299 US, while no statistically significant association was found between toxoplasma gondii IgG  
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10 300 levels and memory performance or attention, toxoplasma gondii IgG levels were inversely  
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12 301 associated with global cognition measured by Mini-Mental State Examination (MMSE) (30).  
13  
14 302 However, in that study, researchers did not adjust BMI, exercise, or depressive symptoms. In  
15  
16 303 another longitudinal study targeting adults aged >30 years with eleven years' follow-up,  
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18 304 researchers found no associations of toxoplasma gondii infections with verbal fluency and verbal  
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20 305 learning assessed by CERAD (3). However, their study population was middle-aged, which was  
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22 306 different from our participants. Overall, the findings of cross-sectional or longitudinal studies are  
23  
24 307 inconsistent. Most of these studies were based on relatively small sample sizes, had  
25  
26 308 methodological limitations, or targeted a different age group. However, in our study, we took  
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28 309 advantage of a nationally representative and relatively large sample and calculated global  
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30 310 cognition, adding stronger evidence on the negative relationship between toxoplasma gondii  
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32 311 infection and cognitive functioning.

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38 312 The possible mechanisms that account for the association between toxoplasmosis gondii  
39  
40 313 infection and worse cognitive functioning are complicated. T gondii infection has been shown to  
41  
42 314 increase dopamine release in vitro and animal trials (31-34). Excess dopamine turnover has been  
43  
44 315 associated with worse cognitive decline (35-37). Dysregulated dopamine may influence neuronal  
45  
46 316 plasticity in the hippocampus in humans, a brain region important for memory and spatial  
47  
48 317 orientation (38, 39). In addition, as a defense mechanism against toxoplasma gondii infection,  
49  
50 318 the host may rapidly catabolize tryptophan and produce more kynurenine and quinolinic acid  
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52 319 (40). It is reported that higher levels of dopamine, kynurenine, and quinolinic acid were  
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3 320 associated with increased neurotoxic effects and impulsive behavior incidence (41). Furthermore,  
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5 321 toxoplasma gondii infection was associated with the dysbiosis of gut microbiota in mice, which  
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7 322 may increase gut-blood-barrier permeability and induce mental disturbances and behavioral  
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9 323 changes (42-44). Future studies are expected to explore the underlying mechanism of the  
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11 324 cognitive effects of toxoplasmosis gondii infection in humans.

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13  
14 325 The major strength of this study is the relatively large, nationally representative sample of  
15  
16 326 older adults in the US. Stringent quality control and assurance measures were implemented  
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18 327 throughout the NHANES study, including the rigorous assessment of toxoplasma gondii IgG and  
19  
20 328 the adoption of validated cognitive tests to assess multiple cognitive functioning domains,  
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22 329 therefore guaranteeing the quality of data used in this study. Moreover, a comprehensive list of  
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24 330 sociodemographic, lifestyle, mental, and physical health covariates were adjusted, minimizing  
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26 331 residual confounding. Thus, the findings of our study are generalizable to U.S. older adults.  
27  
28 332 Importantly, the cognitive effects of toxoplasma gondii infection in humans are understudied in  
29  
30 333 the literature. Thus, our study fills in a research gap. Last but not least, the findings of lower  
31  
32 334 DSST score associated with toxoplasma gondii infection is important as previous studies have  
33  
34 335 shown that lower DSST scores were independently associated with a higher risk of dementia (45,  
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36 336 46).

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38  
39 337 The major limitation of this study is the cross-sectional design which prevented us from  
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41 338 examining whether participants had long-term exposure to toxoplasma gondii or a recent  
42  
43 339 exposure where the IgG immune response to toxoplasma gondii had just started (4). Reverse  
44  
45 340 causation is also possible. Additionally, research has revealed that specific genes affect  
46  
47 341 susceptibility and immune response to toxoplasma gondii infection (47). However, our study did  
48  
49 342 not assess any genetic factors. Additionally, the participants were administered the AFT during  
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3 343 the CERAD-WL delay, which may interfere with their memory formation. Residual confounding  
4  
5 344 is also likely, although we tried to adjust a comprehensive list of covariates. Finally, with three  
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7 345 cognitive tests, we may not assess all domains of participants' cognitive functioning. In addition,  
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9 346 the excluded people (n=516) were different from the included participants (n= 2,956) in several  
10  
11 347 aspects; thus, selection bias is possible (48).

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14 348 Future students are expected to 1) use more advanced methods for identifying specific  
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16 349 strains and stages of toxoplasma gondii infection (49), 2) explore the pathophysiological  
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18 350 mechanisms of cognitive effects of toxoplasma gondii infection, 3) include non-western  
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20 351 populations, and 4) utilize longitudinal designs to assess the temporal relationship between  
21  
22 352 toxoplasma gondii infection and cognitive functioning. These studies may enable the  
23  
24 353 identification of new biomarkers for cognitive impairment and enlighten the development of  
25  
26 354 toxoplasma gondii medications and vaccinations to protect people from toxoplasma gondii  
27  
28 355 infection and its adverse effects.

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31 356 In conclusion, toxoplasma gondii seropositivity is prevalent in U.S. older adults and is  
32  
33 357 independently associated with worse immediate and delayed verbal learning, language  
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35 358 proficiency, executive functioning, processing speed, sustained attention, working memory, as  
36  
37 359 well as global cognition in this population. Future studies are expected to examine the  
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39 360 longitudinal relationship and pathophysiological mechanism between toxoplasma gondii  
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41 361 infection and cognitive functioning. Public health measures are needed to prevent toxoplasma  
42  
43 362 gondii infection, which may help preserve cognitive functioning in older adults.

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## 46 364 **5. Conflict of Interest**

47 365 The authors have no conflict of interest to declare.  
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5 367 **6. Data Availability Statement**

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7 368 The data that support the findings of this study are openly available on the NHANES  
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10 369 website and can be accessed at <https://wwwn.cdc.gov/nchs/nhanes/Default.aspx>

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12 37013  
14 371 **7. Acknowledgment**

15  
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17  
18 37319  
20 374 **9. Authors' contributions**

21  
22 375 ZQ and CY performed data analysis; SG and XL drafted the original manuscript. All the  
23  
24 376 authors significantly provided feedback on the manuscript.

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26 37727  
28 378 **10. Ethics approval and consent to participate**

29  
30 379 This study was exempted from the University of Houston-Downtown Committee for the  
31  
32 380 Protection of Human Subjects because only public-available and de-identified data were used.

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50 389 **References**51  
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Appendix. The characteristics of included and excluded participants due to missing data

Variable	Excluded (n=516)	Included (n=2956)	<i>P</i> Value
Age, years	69.6(7.0)	70.0(7.0)	0.173
Sex, n (%)			0.436
Male	243(47.1%)	1447(49.0%)	
Female	273(52.9%)	1509(51.0%)	
Race/ethnicity, n (%)			<b>&lt;0.001</b>
Mexican Americans	47(9.1%)	274(9.3%)	
Other Hispanics	55(10.7%)	292(9.9%)	
Non-Hispanic Whites	152(29.5%)	1428(48.3%)	
Non-Hispanic Blacks	196(38.0%)	644(21.8%)	
Other	66(12.8%)	318(10.8%)	
Education, n (%)			<b>&lt;0.001</b>
Below high school	170(33.0%)	850(28.7%)	
High school graduate	123(23.8%)	421(14.2)	
Some college or above	219(42.4%)	1428(48.3%)	
Depressive symptoms	3.1(4.2)	3.5(4.8)	0.136
Smoking, n (%)			0.578
Never	265(51.4%)	1472(49.8%)	
Former	189(36.6%)	1095(37.0%)	
Current	59(11.4%)	385(13.0%)	
Body mass index, n (%)			<b>0.001</b>
<18.5 kg/m <sup>2</sup>	16(3.1%)	44(1.5%)	
18.5-24.9 kg/m <sup>2</sup>	134(26.0%)	758(25.6%)	
25.0-29.9 kg/m <sup>2</sup>	142(27.5%)	1046(35.4%)	
≥30 kg/m <sup>2</sup>	202(39.1%)	1051(35.6%)	
Physical activity, hours/week	8.1(10.0)	8.7(10.7)	0.558
Total cholesterol, mg/dL	192.5(45.9)	190.2(42.7)	0.385
Systolic blood pressure, mmHg	136.3(21.7)	133.5(27.0)	<b>0.032</b>
Digit Symbol Substitution Test	43.1(17.1)	45.8(17.5)	<b>0.004</b>
CERAD W-L delayed recall	5.6(2.5)	5.8(2.4)	0.093
CERAD W-L immediate recall	18.2(5.3)	18.5(5.0)	0.300
Animal Fluency Test	15.8(5.5)	16.4(5.6)	<b>0.030</b>



# BMJ Open

## Toxoplasma gondii seropositivity and cognitive functioning in older adults: An analysis of cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014

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1 ***Toxoplasma gondii* seropositivity and cognitive functioning in older adults: An analysis of**  
2 **cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014**

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1  
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3 **76 Abstract**

4  
5 **77 Objectives:** This study sought to examine the relationship between *Toxoplasma gondii*  
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8 seropositivity and cognitive function in older adults.

9  
10 **79 Design:** An observational cross-sectional study.

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12 **80 Setting:** The National Health and Nutrition Examination Survey (NHANES) study took place at  
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14  
15 participants' homes and mobile exam centers.

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17 **82 Participants:** A total of 2,956 older adults aged 60 and above from the NHANES from 2011 to  
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**83** 2014 were included in the study. Exposure of interest: Participants had serum *Toxoplasma gondii*  
**84** antibody analyzed in the lab. A value > 33 IU/mL was categorized as seropositive for  
**85** *Toxoplasma gondii* infection; <27 IU/mL was categorized as seronegative for *Toxoplasma gondii*  
**86** infection.

**87 Primary and secondary outcome measures:** Cognitive tests included the Consortium to  
88 Establish a Registry for Alzheimer's Disease Word Learning subtest (CERAD-WL) for  
89 immediate and delayed memory, the Animal Fluency Test (AFT), and the Digit Symbol  
90 Substitution Test (DSST).

**91 Results:** About half of the 2,956 participants (mean age 70.0) were female (51.0%), non-  
92 Hispanic White (48.3%), and completed some college or above (48.3%). A total of 703  
93 participants were positive for *Toxoplasma gondii* infection (23.8%). Adjusted linear regression  
94 showed that compared with participants with negative *Toxoplasma gondii* infection, those with  
95 positive *Toxoplasma gondii* infection had lower CERAD-WL immediate memory (beta [ $\beta$ ] -0.16,  
96 95% confidence interval [CI] -0.25, -0.07), CERAD-WL delayed memory ( $\beta$  -0.15, 95% CI -  
97 0.24, -0.06), AFT ( $\beta$  -0.15, 95% CI -0.24, -0.06), DSST ( $\beta$  -0.34, 95% CI -0.43, -0.26), and global  
98 cognition ( $\beta$  -0.24, 95% CI -0.32, -0.16) z-scores after controlling for the covariates.

99 **Conclusions:** *Toxoplasma gondii* seropositivity is associated with worse immediate and delayed  
100 verbal learning, language proficiency, executive functioning, processing speed, sustained  
101 attention, working memory, as well as global cognition in older adults. Public health measures  
102 aiming at preventing *Toxoplasma gondii* infection may help preserve cognitive functioning in  
103 older adults.

104  
105 **Keywords:** *Toxoplasma gondii*; cognitive functioning; older adults; neurotoxoplasmosis;  
106 neuropsychological; psychomotor performance

107  
108 **Strengths and limitations:**

- 109 • This study is one of the few studies that examined the cognitive effect of *Toxoplasma*  
110 *gondii* seropositivity on cognitive outcomes in older adults.
- 111 • With the NHANES data, the study population was nationally representative of older U.S.  
112 adults.
- 113 • A wide range of sociodemographic, lifestyle, mental health, and physical health  
114 covariates was adjusted, reducing the possibility of residual confounding.
- 115 • A cross-sectional study hinders the assessment of longitudinal relationships.
- 116 • Unable to adjust for variables that were not evaluated in NHANES; thus, residual  
117 confounding could not be ruled out.

118  
119 **1. Introduction**

120 Alzheimer's disease and related dementia (ADRD) is a serious public health threat  
121 worldwide. In 2016, a total of 43.8 million people had dementia in the world. With a growing  
122 number of people with ADRD, families, communities, and healthcare systems around the world

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3 123 are heavily burdened (1). Although dementia is currently not curable, identifying modifiable risk  
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5 124 factors associated with ADRD can help reduce the burden of the disease. Cognitive test  
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8 125 performance in older adults is an important indicator of their cognitive functioning (2). By  
9  
10 126 examining their correlations to cognitive tests, risk factors for cognitive decline can provide  
11  
12 127 opportunities for interventions.

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15 128 *Toxoplasma gondii* is among the most prevalent human zoonosis (3) and affects about  
16  
17 129 30% of the global population (4). Vertical *Toxoplasma gondii* infections can happen prior to  
18  
19 130 birth resulting in congenital toxoplasmosis. Transmission after a person is born, postnatal  
20  
21 131 infection, is the more common form of infection (5). By consuming oocysts found in cat feces-  
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23  
24 132 contaminated soil or water a person can develop toxoplasmosis (6, 7). Oocyst contaminated soil  
25  
26 133 can also facilitate transmission via unwashed, nonheat-treated consumable products, like fruits  
27  
28 134 and vegetables, to allow for transmission of this zoonotic disease (8). By eating tissue with cysts  
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30 135 in undercooked meat (9, 10), humans can be infected with *Toxoplasma gondii* after birth.  
31  
32 136 Another route of human exposure to *Toxoplasma gondii* can occur if a person experiences a  
33  
34 137 blood transfusion or organ transplant. While most people with a healthy immune system are  
35  
36 138 asymptomatic following acute infection with *Toxoplasma gondii*, some may experience  
37  
38 139 nonspecific symptoms lasting from several weeks to months, including fever, malaise, muscle  
39  
40 140 ache, lymphadenopathy, along with the miscarriage or stillbirth of a fetus (11, 12).

41  
42  
43  
44 141 A limited number of studies have found that *Toxoplasma gondii* infection is associated  
45  
46 142 with neurocognitive changes in humans (13-16). However, their direction of findings and effect  
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48 143 sizes are inconsistent. In addition, this area is understudied, given the prevalence of *Toxoplasma*  
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50 144 *gondii* infection in humans. In a systematic review and meta-analysis of the association of  
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53 145 *Toxoplasma gondii* seropositivity and cognitive function in healthy people, only thirteen studies  
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3 146 were included, most of which had small sample sizes (4). In addition, two included studies (17,  
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5 147 18) utilized the National Health and Nutrition Examination Survey (NHANES) 1988-1994 cycle  
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7 148 data and thus could not reflect the current epidemic of *Toxoplasma gondii* infection. The findings  
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10 149 of this study showed that seropositivity to *Toxoplasma gondii* was modestly but significantly  
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12 150 associated with poorer processing speed, working memory, verbal short-term memory, and  
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14 151 executive functioning. However, studies using large, the latest, and nationally representative  
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16 152 population-based data are needed to better elucidate the cognitive effects of *Toxoplasma gondii*  
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18 153 seropositivity.

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21 154 In this study, taking advantage of the NHANES, we aimed to examine the relationship  
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23 155 between *Toxoplasma gondii* seropositivity and cognitive functioning in a nationally  
24  
25 156 representative sample of U.S. older adults. The findings of this study will provide implications  
26  
27 157 for understanding the cognitive effects of *Toxoplasma gondii* infection and developing tailored  
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29 158 public interventions to protect cognitive functioning in the growing number of older adults in the  
30  
31 159 US.

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## 33 161 **2. Method**

### 34 162 ***2.1 Study design and setting***

35 163 The NHANES is an ongoing, cross-sectional survey of civilian, non-institutionalized  
36  
37 164 adults and children in the United States conducted by the National Center for Health Statistics of  
38  
39 165 the Centers for Disease Control and Prevention. A nationally representative sample of children  
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41 166 and adults across the country are surveyed bi-annually (19). Their sociodemographic, health, and  
42  
43 167 nutritional status are evaluated using in-person interviews and physical exams. The interviews  
44  
45 168 are conducted at participants' homes; health exams are conducted in specially equipped mobile  
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3 169 exam centers. Health exams include laboratory testing of urine and blood specimens and  
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5 170 medical, dental, and physiological assessments. Participants' serum *Toxoplasma gondii* antibody  
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7 171 levels and cognitive functioning were measured in the NHANES 2011-2014 cycles. Medical  
8  
9 172 conditions, including dementia or neurocognitive disorders, were not exclusion criteria of the  
10  
11 173 NHANES. In this study, two survey cycles (2011-2012 and 2013-2014) were merged to increase  
12  
13 174 sample size and power. Between 2011 and 2014, a total of 19,151 individuals participated in the  
14  
15 175 NHANES. They were recruited from a selection of census blocks or clusters of census block area  
16  
17 176 segments. The detailed sampling method has been published elsewhere (20).  
18  
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20

21 177 Of the 19,151 individuals, we excluded individuals aged < 60 (n = 15,679) or had  
22  
23 178 missing data on serum *Toxoplasma gondii* IgG (n=511). Participants with equivocal serum  
24  
25 179 *Toxoplasma gondii* IgG ( $\geq 27$  and <33 IU/mL) were further excluded (n=5) as the results were  
26  
27 180 inconclusive. Finally, a total of 2,956 participants aged 60 and above were included in the  
28  
29 181 analysis. The characteristics of the excluded participants due to missing data (n=516) were  
30  
31 182 presented in the Appendix. Compared with the included participants, people who were excluded  
32  
33 183 were more likely to be of other ethnicities than Non-Hispanic Whites, overweight/obese,  
34  
35 184 completed lower education and had higher systolic blood pressure, lower Digit Symbol  
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37 185 Substitution Test (DSST) score, and lower Animal Fluency test (AFT) score.  
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## 44 187 **2.2 Ethical considerations**

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46 188 The National Center for Health Statistics Research Ethics Review Board approved the  
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48 189 NHANES. Participants in the NHANES provided written informed consent before enrolling in  
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50 190 the study. The University of Houston-Downtown Committee for the Protection of Human  
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3 191 Subjects granted this study an exemption because only publicly accessible and de-identified data  
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5 192 were used.  
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### 10 194 **2.3 Public and Patient involvement**

11  
12 195 Patients or the public were NOT involved in the design, conducting, reporting, or  
13  
14 196 dissemination plans of our research.  
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17 197

### 19 198 **2.4 Independent variable: *Toxoplasma gondii* seropositivity**

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21 199 Serological tests that detect *Toxoplasma gondii* Immunoglobulin G (IgG) and  
22  
23 200 Immunoglobulin M (IgM) antibodies are often used for clinical diagnoses of toxoplasmosis.  
24  
25 201 While the IgM antibody test can validate acute phases, the IgG antibody test can identify acute or  
26  
27 202 chronic phases (21). In the NHANES, an enzyme immunoassay (EIA) measuring IgG against  
28  
29 203 *Toxoplasma gondii* was used to measure the presence or absence of *Toxoplasma gondii* (19).  
30  
31 204 *Toxoplasma gondii* IgG was measured with two enzyme immunoassay kits (22). Strict quality  
32  
33 205 control was implemented for every plate. A value between 27 and 33 IU/mL was deemed as  
34  
35 206 “equivocal”; a value  $\geq 33$  IU/mL was deemed as “positive”; a value  $< 27$  IU/mL was deemed as  
36  
37 207 “negative” (19). Samples with equivocal results ( $\geq 27$  IU/mL and  $< 33$  IU/mL) were repeated  
38  
39 208 twice and confirmed as negative. For our analysis, we categorized participants into “seropositive  
40  
41 209 for *Toxoplasma gondii* infection” or “seronegative for *Toxoplasma gondii* infection”. This cutoff  
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43 210 is consistent with descriptions on the NHANES website and previous NHANES publications  
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45 211 (23, 24).  
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### 54 213 **2.5 Dependent variable: Cognitive functioning**

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3 214 Three cognitive tests were used to assess participants' various domains of cognitive  
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5 215 functioning, including the Consortium to Establish a Registry for Alzheimer's Disease Word  
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7 216 Learning subtest (CERAD-WL), the AFT, and the DSST. The detailed method of assessing  
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9  
10 217 cognitive function has been published elsewhere (25).

11  
12 218 1) The CERAD-WL assessed participants' capacity for both immediate (immediate  
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14 219 memory) and delayed (delayed memory) verbal learning (26). It consisted of a delayed recall  
15  
16 220 after three consecutive learning trials. For each learning trial, participants must read aloud ten  
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18 221 randomly selected words that are displayed on a computer screen in huge, bolded characters, one  
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20 222 at a time. Following the presentation of the words, participants were encouraged to retain and  
21  
22 223 recall as many words as they could. In each of the three trials, the order of the ten words was  
23  
24 224 changed. There was a ten-point maximum for each trial. A participant's immediate memory score  
25  
26 225 was the sum of their three trials' scores, ranging from zero to thirty. After the AFT and the  
27  
28 226 DSST, participants took the delayed recall test, which asked them to recall as many words from  
29  
30 227 the same ten-word list as they could. The delayed memory score, which varied from zero to ten,  
31  
32 228 depended on how many accurate words a subject could recall.

33  
34 229 2) Participants' language proficiency and executive function were assessed by the AFT  
35  
36 230 (27). Each animal a participant named received one point, and they had one minute to name as  
37  
38 231 many animals as they could. Participants were first prompted to identify three pieces of clothing  
39  
40 232 as a warm-up.

41  
42 233 3) The DSST measured the participants' working memory, sustained attention, and  
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44 234 processing speed (28). A paper form with a top-mounted key that included nine numbers and  
45  
46 235 paired symbols was used to conduct the exam. Participants were instructed to copy various  
47  
48 236 symbols to the matching symbols in the 133 boxes that were placed next to the numbers. They

237 had two minutes to complete this task. The DDST score was based on the total number of correct  
238 matches (29). Before participants started the formal test, a sample practice test was provided.  
239 The possible score range of the DSST was between zero and 133 (30).

240

## 241 **2.6 Covariates**

242 Covariates of this study were selected according to literature review and included age  
243 (years), sex (male or female), race/ethnicity (Mexican Americans, other Hispanics, non-Hispanic  
244 White, or non-Hispanic Black), education (below high school, high school graduate, or some  
245 college or above), depressive symptoms, smoking status (never, former, or current smokers),  
246 BMI ( $<18.5$  kg/m<sup>2</sup>, 18.5-24.9 kg/m<sup>2</sup>, 25-29.9 kg/m<sup>2</sup>, or  $\geq 30$  kg/m<sup>2</sup>), prevalent coronary heart  
247 disease (CHD) (yes or no), stroke (yes or no), and systolic blood pressure (mmHg). The  
248 information was either self-reported or collected at health exams. The Patient Health  
249 Questionnaire (PHQ-9) was used to measure depressive symptoms (31). It is a nine-item  
250 screening tool on the frequency of depressive symptoms over the past two weeks and has a total  
251 score ranging from 0-27. A higher score indicates more severe depression symptoms. The PHQ-  
252 9 total score was used to indicate depressive symptoms.

253

## 254 **2.7 Statistical analysis**

255 Means (standard deviation [SD]) were used to describe the characteristics of the study  
256 population for continuous data that followed a normally dispersed distribution. Medians  
257 (interquartile range) were used for non-normally distributed continuous data. Data for categorical  
258 variables were summarized using frequency (percentages). Independent T-tests were used to

259 compare group differences for continuous variables between the two groups. Chi-square tests  
260 were used to compare group differences for categorical variables between the two groups.

261 The CERAD-WL immediate memory, CERAD-WL delayed memory, AFT, and DSST  
262 were standardized with mean zero and variance one to compute cognitive test-specific z-scores.  
263 The cognitive test-specific z-scores of the four tests were then averaged to calculate the global  
264 cognition z-scores. Linear regression models were constructed between *Toxoplasma gondii*  
265 seropositivity (seronegative or seropositive) and each of the four cognitive test-specific and  
266 global cognition z-scores. All models were adjusted for the covariates mentioned above. We  
267 considered a 95% confidence interval (CI) excluding one as statistically significant. All the  
268 analyses were performed using SPSS 25.0.

269

### 270 3. Results

271 The sociodemographic and health information of the study population, stratified by  
272 *Toxoplasma gondii* seropositivity, was presented in **Table 1**. Of the 2,956 participants, 1, 403  
273 were from the 2011-2012 cycle and 1,553 from the 2013-2014 cycle. A total of 703 participants  
274 were seropositive for *Toxoplasma gondii* infection (23.8%). The participants had a mean age of  
275 70.0 (SD 7.0). Most of the 2,952 participants (mean age of 70.0 [SD 7.0]) were female (51.0%),  
276 non-Hispanic White (48.3%), completed some college or above (48.3%), were never smokers  
277 (49.8%), and had a BMI  $\geq 30$  (35.6%) and an average of 8.7 (SD 10.7) hours of physical activity  
278 every week. Their mean total cholesterol and systolic blood pressure were 190.2 mg/dL and  
279 133.5 mmHg. Around 63% of the participants had stroke. Their mean delayed memory,  
280 immediate memory, AFT, and DSST scores were 5.8 (SD 2.4), 18.5 (SD 5.0), 16.4 (SD 5.6), and  
281 45.8 (SD 17.5), respectively. Compared with participants with negative *Toxoplasma gondii*

282 seropositivity, participants with positive *Toxoplasma gondii* seropositivity were older, less  
 283 educated, and more likely to be male, current smokers, and had more physical activities. They  
 284 were also more likely to have different ethnicities and lower CERAD W-L immediate recall,  
 285 CERAD W-L delayed recall, AFT, and DSST scores.

286 Table 1. Characteristics of the participants by *Toxoplasma gondii* seropositivity

Variables	Negative (n=2,253)	Positive (n=703)	Total (n=2,956)	P-Value
Age, years	69.7(6.9)	70.9(7.0)	70.0(7.0)	<b>&lt;0.001</b>
Sex, n (%)				<b>&lt;0.001</b>
Male	1,057(46.9%)	390(55.5%)	1,447(49.0%)	
Female	1,196(53.1%)	313(44.5%)	1,509(51.0%)	
Race/ethnicity, n (%)				<b>&lt;0.001</b>
Mexican Americans	231(10.3%)	43(6.1%)	274(9.3%)	
Other Hispanics	161(7.1%)	131(18.6%)	292(9.9%)	
Non-Hispanic Whites	1,111(49.3%)	317(45.1%)	1,428(48.3%)	
Non-Hispanic Blacks	479(21.3%)	165(23.5%)	644(21.8%)	
Other	271(12.0%)	47(6.7%)	318(10.8%)	
Education, n (%)				<b>&lt;0.001</b>
Below high school	594(26.3%)	256(36.4%)	850(28.7%)	
High school graduate	510(22.6%)	165(23.5%)	421(14.2)	
Some college or above	1,147(50.9%)	281(39.9%)	1,428(48.3%)	
Depressive symptoms	3.5(4.8)	3.3(4.5)	3.5(4.8)	0.334
Smoking, n (%)				<b>0.043</b>
Never	1,130(50.2%)	342(48.6%)	1,472(49.8%)	
Former	846(37.5%)	249(35.4%)	1,095(37.0%)	
Current	274(12.2%)	111(15.8%)	385(13.0%)	
Body mass index, n (%)				0.444
<18.5 kg/m <sup>2</sup>	35(1.6%)	9(1.3%)	44(1.5%)	
18.5-24.9 kg/m <sup>2</sup>	592(26.3%)	166(23.6%)	758(25.6%)	
25.0-29.9 kg/m <sup>2</sup>	784(34.8%)	262(37.3%)	1,046(35.4%)	
≥30 kg/m <sup>2</sup>	800(35.5%)	251(35.7%)	1,051(35.6%)	
Physical activity, hours/week	8.2(10.3)	10.6(12.1)	8.7(10.7)	<b>0.025</b>
Total cholesterol, mg/dL	190.7(42.5)	188.4(43.4)	190.2(42.7)	0.209
Systolic blood pressure, mmHg	133.2(19.8)	134.5(42.5)	133.5(27.0)	0.271
CERAD W-L immediate recall	18.7(5.0)	17.9(4.8)	18.5(5.0)	<b>&lt;0.01</b>
CERAD W-L delayed recall	5.9(2.4)	5.5(2.4)	5.8(2.4)	<b>&lt;0.01</b>
Animal Fluency Test	16.6(5.6)	15.8(5.3)	16.4(5.6)	<b>&lt;0.01</b>
Digit Symbol Substitution Test	47.2(17.4)	41.2(16.8)	45.8(17.5)	<b>&lt;0.001</b>

287 Data are presented as means (standard deviation) for continuous variables and n (%) for categorical  
 288 variables. Bolded values mean statistical significance (P<0.05).

289

290 The means and 95% confidence intervals (CI)s of cognitive test-specific z-scores by  
 291 *Toxoplasma gondii* infection status were presented in **Table 2**. The mean of CERAD W-L  
 292 immediate call, CERAD W-L delayed recall, AFT and DSST was 0.04 (95% CI -1.94, 2.01),  
 293 0.03 (95% CI -1.92, 1.99), 0.03 (95% CI -1.95, 2.01) and 0.08 (95% CI -1.88, 2.03),  
 294 respectively, among participants with negative *Toxoplasma gondii* infection. Among participants  
 295 with seropositive *Toxoplasma gondii* infection, the mean of CERAD W-L immediate call,  
 296 CERAD W-L delayed recall, AFT and DSST was -0.12 (95% CI -2.01, 1.77), -0.12 (95% CI -  
 297 2.07, 1.83), -0.11 (95% CI -1.99, 1.76) and -0.26 (95% CI -2.15, 1.63), respectively. For the  
 298 global cognition z-scores, the mean was 0.06 (95% CI -1.92, 2.03) for participants with negative  
 299 *Toxoplasma gondii* infection and -0.18 (95% CI -2.06, 1.70) for those with positive *Toxoplasma*  
 300 *gondii* infection.

301 Table 2. Cognitive z-scores and 95% confidence intervals by *Toxoplasma gondii* seropositivity

	Negative (n=2,253)	Positive (n=703)
CERAD W-L immediate recall	0.04 (-1.94, 2.01)	-0.12 (-2.01, 1.77)
CERAD W-L delayed recall	0.03 (-1.92, 1.99)	-0.12 (-2.07, 1.83)
Animal fluency test	0.03 (-1.95, 2.01)	-0.11 (-1.99, 1.76)
Digit Symbol Substitution Test	0.08 (-1.88, 2.03)	-0.26 (-2.15, 1.63)
Global cognition	0.06 (-1.92, 2.03)	-0.18 (-2.06, 1.70)

302  
 303 Adjusted linear regression (**Table 3**) showed that compared with participants with  
 304 seronegative *Toxoplasma gondii* infection, those with seropositive *Toxoplasma gondii* infection  
 305 had lower CERAD-WL immediate memory (beta [ $\beta$ ] -0.16, 95% CI -0.25, -0.07), CERAD-WL  
 306 delayed memory ( $\beta$  -0.15, 95% CI -0.24, -0.06), AFT ( $\beta$  -0.15, 95% CI -0.24, -0.06), and DSST  
 307 ( $\beta$  -0.34, 95% CI -0.43, -0.26) z-scores controlling for age, race/ethnicity, education, depressive  
 308 symptoms, smoking status, BMI, prevalent CHD, stroke, and systolic blood pressure. For the

309 global cognition z-score, which is calculated by averaging the four cognitive test-specific z-  
 310 scores, the negative association remained ( $\beta$  -0.24, 95% CI -0.32, -0.16).

311 Table 3. The independent associations of *Toxoplasma gondii* seropositivity (reference: Negative) with  
 312 cognitive specific test and global cognition z-scores  
 313

	Beta	95% CI
CERAD W-L immediate recall	-0.16	<b>(-0.25, -0.07)</b>
CERAD W-L delayed recall	-0.15	<b>(-0.24, -0.06)</b>
Animal fluency test	-0.15	<b>(-0.24, -0.06)</b>
Digit Symbol Substitution Test	-0.34	<b>(-0.43, -0.25)</b>
Global cognition	-0.24	(-0.32, -0.16)

314 95% CI means 95% confidence interval

315 Bolded values mean statistical significance (96% CI excluding zero).

#### 317 4. Discussion

318 In this sample of 2,956 nationally representative older adults in the US, positive  
 319 *Toxoplasma gondii* infection is independently associated with worse immediate and delayed  
 320 verbal learning, language proficiency, executive functioning, processing speed, sustained  
 321 attention, working memory, as well as global cognition. This relationship is independent of age,  
 322 race/ethnicity, education, depressive symptoms, smoking status, BMI, prevalent CHD, stroke,  
 323 and systolic blood pressure. Although our findings should be validated using longitudinal  
 324 studies, they suggest that serum *Toxoplasma gondii* seropositivity may be associated with  
 325 cognitive impairment and that preventing *Toxoplasma gondii* infection should be a target of  
 326 public health interventions to protect cognitive functioning in older adults. This is very important  
 327 given the high prevalence of *Toxoplasma gondii* infection and the increasing population aging in  
 328 the US.

329 A limited number of studies have examined the associations of *Toxoplasma gondii*  
 330 seropositivity with cognitive outcomes in humans. Two studies utilized the NHANES 1988-1994  
 331 cycle data and thus could not reflect the current epidemic of *Toxoplasma gondii* infection (17,



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3 332 18). In addition, one of them only included school-aged children (18). To the best of our  
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5 333 knowledge, only two relevant studies exclusively targeted older adults (32, 33). In one study of  
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7 334 84 older adults aged 65 years and above in Germany, toxoplasmosis-positive participants showed  
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9 335 impaired working memory, attention, and word fluency, but not processing speed measured by  
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11 336 DSST, compared with those who were toxoplasmosis negative (32). It is important to note that  
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13 337 the sample size of that study is very small. Then, in another study including older adults in the  
14  
15 338 US, while no statistically significant association was found between *Toxoplasma gondii* IgG  
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17 339 levels and memory performance or attention, *Toxoplasma gondii* IgG levels were inversely  
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19 340 associated with global cognition measured by Mini-Mental State Examination (MMSE) (33).  
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21 341 However, in that study, researchers did not adjust BMI, exercise, or depressive symptoms. In  
22  
23 342 another longitudinal study targeting adults aged >30 years with eleven years' follow-up,  
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25 343 researchers found no associations of *Toxoplasma gondii* infections with verbal fluency and  
26  
27 344 verbal learning assessed by CERAD (3). However, their study population was middle-aged,  
28  
29 345 which was different from our participants. Overall, the findings of cross-sectional or longitudinal  
30  
31 346 studies are inconsistent. Most of these studies were based on relatively small sample sizes, had  
32  
33 347 methodological limitations, or targeted a different age group. However, in our study, we took  
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35 348 advantage of a nationally representative and relatively large sample and calculated global  
36  
37 349 cognition, adding stronger evidence on the negative relationship between *Toxoplasma gondii*  
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39 350 infection and cognitive functioning.

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41 351 In this study, both groups demonstrated cognitive scores within the normal range, and while  
42  
43 352 there was a statistically significant effect observed, its clinical relevance remains modest and  
44  
45 353 somewhat ambiguous. The possible mechanisms that account for the association between  
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47 354 *Toxoplasma gondii* infection and worse cognitive functioning are complicated. *Toxoplasma*  
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49 355 *gondii* infection has been shown to increase dopamine release in vitro and animal trials (34-37).  
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3 356 Excess dopamine turnover has been associated with worse cognitive decline (38-40).  
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5 357 Dysregulated dopamine may influence neuronal plasticity in the hippocampus in humans, a brain  
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7 358 region important for memory and spatial orientation (41, 42). Furthermore, evidence suggests  
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9  
10 359 that dysregulation of neurotransmitters, particularly norepinephrine, is involved in the  
11  
12 360 neuroimmune responses to brain infection (43). In the brains of animals infected with  
13  
14 361 *Toxoplasma gondii* and in vitro studies involving infected human and rat neural cells, the  
15  
16 362 noradrenergic system was shown to be suppressed with decreased norepinephrine levels. This  
17  
18 363 reduction in norepinephrine levels was attributed to the downregulation of the dopamine  $\beta$ -  
19  
20 364 hydroxylase gene expression, which encodes the enzyme responsible for synthesizing  
21  
22 365 norepinephrine from dopamine (44). This altered synthesis of norepinephrine may partly explain  
23  
24 366 the infection-related behavioral effects and the associations with mental illness. In addition, as a  
25  
26 367 defense mechanism against *Toxoplasma gondii* infection, the host may rapidly catabolize  
27  
28 368 tryptophan and produce more kynurenine and quinolinic acid (45). It is reported that higher  
29  
30 369 levels of dopamine, kynurenine, and quinolinic acid were associated with increased neurotoxic  
31  
32 370 effects and impulsive behavior incidence (46). Furthermore, *Toxoplasma gondii* infection was  
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34 371 associated with the dysbiosis of gut microbiota in mice, which may increase gut-blood-barrier  
35  
36 372 permeability and induce mental disturbances and behavioral changes (47-49). Future studies are  
37  
38 373 expected to explore the underlying mechanism of the cognitive effects of *Toxoplasma gondii*  
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40 374 infection in humans.

41  
42 375 The major strength of this study is the relatively large, nationally representative sample of  
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44 376 older adults in the US. Stringent quality control and assurance measures were implemented  
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46 377 throughout the NHANES study, including the rigorous assessment of *Toxoplasma gondii* IgG  
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48 378 and the adoption of validated cognitive tests to assess multiple cognitive functioning domains,  
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3 379 therefore guaranteeing the quality of data used in this study. Moreover, a comprehensive list of  
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5 380 sociodemographic, lifestyle, mental, and physical health covariates were adjusted, minimizing  
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7 381 residual confounding. Thus, the findings of our study are generalizable to U.S. older adults.  
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10 382 Importantly, the cognitive effects of *Toxoplasma gondii* infection in humans are understudied in  
11  
12 383 the literature. Thus, our study fills in a research gap. Last but not least, the findings of lower  
13  
14 384 DSST score associated with *Toxoplasma gondii* infection is important as previous studies have  
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16 385 shown that lower DSST scores were independently associated with a higher risk of dementia (50,  
17  
18 386 51).

21 387 The major limitation of this study is the cross-sectional design which prevented us from  
22  
23 388 examining whether participants had long-term exposure to *Toxoplasma gondii* or a recent  
24  
25 389 exposure where the IgG immune response to *Toxoplasma gondii* had just started (4). Reverse  
26  
27 390 causation is also possible. Additionally, research has revealed that specific genes affect  
28  
29 391 susceptibility and immune response to *Toxoplasma gondii* infection (52). However, our study did  
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31 392 not assess any genetic factors. Additionally, the participants were administered the AFT during  
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33 393 the CERAD-WL delay, which may interfere with their memory formation. Residual confounding  
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35 394 is also likely, although we tried to adjust a comprehensive list of covariates. Finally, with three  
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37 395 cognitive tests, we may not assess all domains of participants' cognitive functioning. In addition,  
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39 396 the excluded people (n=516) were different from the included participants (n= 2,956) in several  
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41 397 aspects; thus, selection bias is possible (53).

46 398 Future students are expected to 1) use more advanced methods for identifying specific  
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48 399 strains and stages of *Toxoplasma gondii* infection (54), 2) explore the pathophysiological  
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50 400 mechanisms of cognitive effects of *Toxoplasma gondii* infection, 3) include non-western  
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52 401 populations, and 4) utilize longitudinal designs to assess the temporal relationship between  
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3 402 *Toxoplasma gondii* infection and cognitive functioning. These studies may enable the  
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5 403 identification of new biomarkers for cognitive impairment and enlighten the development of  
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7 404 *Toxoplasma gondii* medications and vaccinations to protect people from *Toxoplasma gondii*  
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10 405 infection and its adverse effects.

11  
12 406 In conclusion, *Toxoplasma gondii* seropositivity is prevalent in U.S. older adults and is  
13  
14 407 independently associated with worse immediate and delayed verbal learning, language  
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16 408 proficiency, executive functioning, processing speed, sustained attention, working memory, as  
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18 409 well as global cognition in this population. However, the clinical relevance remains modest and  
19  
20 410 somewhat ambiguous. Future studies are expected to examine the longitudinal relationship and  
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22 411 pathophysiological mechanism between *Toxoplasma gondii* infection and cognitive functioning.  
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24 412 Public health measures are needed to prevent *Toxoplasma gondii* infection, which may help  
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26 413 preserve cognitive functioning in older adults.  
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### 32 33 415 **5. Conflict of Interest**

34  
35 416 The authors have no conflict of interest to declare.  
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### 39 40 418 **6. Data Availability Statement**

41  
42 419 The data that support the findings of this study are openly available on the NHANES  
43  
44 420 website and can be accessed at <https://wwwn.cdc.gov/nchs/nhanes/Default.aspx>  
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50  
51 423 We would like to thank NHANES participants for providing data for this study.  
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## 9. Authors' contributions

WM, CS, QZ designed the project; ZQ and CY performed data analysis; SG, XL, ML, HC, and ZZ drafted the original manuscript. CS worked to revise the manuscript. All the authors significantly provided feedback on the manuscript.

## 10. Ethics approval and consent to participate

This study was exempted from the University of Houston-Downtown Committee for the Protection of Human Subjects because only public-available and de-identified data were used.

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For peer review only

## Appendix. The characteristics of included and excluded participants due to missing data

Variable	Excluded (n=516)	Included (n=2956)	<i>P</i> Value
Age, years	69.6(7.0)	70.0(7.0)	0.173
Sex, n (%)			0.436
Male	243(47.1%)	1447(49.0%)	
Female	273(52.9%)	1509(51.0%)	
Race/ethnicity, n (%)			<b>&lt;0.001</b>
Mexican Americans	47(9.1%)	274(9.3%)	
Other Hispanics	55(10.7%)	292(9.9%)	
Non-Hispanic Whites	152(29.5%)	1428(48.3%)	
Non-Hispanic Blacks	196(38.0%)	644(21.8%)	
Other	66(12.8%)	318(10.8%)	
Education, n (%)			<b>&lt;0.001</b>
Below high school	170(33.0%)	850(28.7%)	
High school graduate	123(23.8%)	421(14.2)	
Some college or above	219(42.4%)	1428(48.3%)	
Depressive symptoms	3.1(4.2)	3.5(4.8)	0.136
Smoking, n (%)			0.578
Never	265(51.4%)	1472(49.8%)	
Former	189(36.6%)	1095(37.0%)	
Current	59(11.4%)	385(13.0%)	
Body mass index, n (%)			<b>0.001</b>
<18.5 kg/m <sup>2</sup>	16(3.1%)	44(1.5%)	
18.5-24.9 kg/m <sup>2</sup>	134(26.0%)	758(25.6%)	
25.0-29.9 kg/m <sup>2</sup>	142(27.5%)	1046(35.4%)	
≥30 kg/m <sup>2</sup>	202(39.1%)	1051(35.6%)	
Physical activity, hours/week	8.1(10.0)	8.7(10.7)	0.558
Total cholesterol, mg/dL	192.5(45.9)	190.2(42.7)	0.385
Systolic blood pressure, mmHg	136.3(21.7)	133.5(27.0)	<b>0.032</b>
Digit Symbol Substitution Test	43.1(17.1)	45.8(17.5)	<b>0.004</b>
CERAD W-L delayed recall	5.6(2.5)	5.8(2.4)	0.093
CERAD W-L immediate recall	18.2(5.3)	18.5(5.0)	0.300
Animal Fluency Test	15.8(5.5)	16.4(5.6)	<b>0.030</b>

## STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2	abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	abstract
<b>Introduction</b>				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4	introduction
Objectives	3	State specific objectives, including any prespecified hypotheses	6	introduction
<b>Methods</b>				
Study design	4	Present key elements of study design early in the paper	6	method
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7	method
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants		
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6	measure
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6	method
Bias	9	Describe any efforts to address potential sources of bias	15	limitation
Study size	10	Explain how the study size was arrived at	7	method

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10	measure
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	10	Statistical analysis
		(b) Describe any methods used to examine subgroups and interactions	10	Statistical analysis
		(c) Explain how missing data were addressed	10	Statistical analysis
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy		
		(e) Describe any sensitivity analyses		
<b>Results</b>				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11	results
		(b) Give reasons for non-participation at each stage	11	results
		(c) Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11	results
		(b) Indicate number of participants with missing data for each variable of interest	12	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)		
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time		results
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure		
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures		
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13	results
		(b) Report category boundaries when continuous variables were categorized	13	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	13	

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	14 discussion
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17 limitation
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).