Research Article

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Toxoplasma gondii seropositivity and substance use in US adults

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Abstract: The intracellular parasite *Toxoplasma gondii* (Nicolle et Manceaux, 1908) infects humans resulting in acute toxoplasmosis, an infection that in immunocompetent people is typically mild but results in persistent latent toxoplasmosis. In that *T. gondii* appears to affect dopamine synthesis and because addicting drugs affect midbrain dopamine transmission, latent toxoplasmosis could influence substance use. Using both the third and continuous National Health and Nutrition Examination Surveys from the US Centers for Disease Control and Prevention, we used logistic regression to test for associations between *T. gondii* seropositivity and subject self-report of having ever used tobacco, alcohol, marijuana, cocaine, heroin, or methamphetamine. In the third NHANES dataset, which included data for tobacco, alcohol, marijuana and cocaine, *T. gondii* seropositivity was associated with a reduced likelihood of self-reported marijuana (OR = 0.71 [95% CI: 0.58; 0.87]; $p = 0.001$) and cocaine use (OR = 0.72 [95% CI: 0.56; 0.91]; $p = 0.006$). In the continuous National Health and Nutrition Examination Surveys dataset, which included data for all six substances, *T. gondii* seropositivity was associated with a reduced likelihood of self-reported tobacco (OR = 0.87 [95% CI: 0.76; 1.00]; $p = 0.044$), marijuana (OR = 0.60 [95% CI: 0.50; 0.72]; $p < 0.001$), heroin (OR = 0.60 [95% CI: 0.42; 0.85]; $p = 0.005$) and methamphetamine use (OR = 0.54 [95% CI: 0.38; 0.77]; $p = 0.001$). We observed interactions between sex and *T. gondii* seropositivity in the prediction of self-reported use of tobacco and alcohol. Further, *T. gondii* seropositivity appeared to remove the protective effect of education and economic status against self-reported cigarette smoking. These findings suggest that *T. gondii* seropositivity may be inversely associated with some but not all types of substance use in US adults.

Keywords: Toxoplasmosis, tobacco, alcohol, marijuana, cocaine, heroin, methamphetamine, drug use

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The intracellular parasite *Toxoplasma gondii* (Nicolle et Manceaux, 1908) is present in approximately 30 to 50 percent of the world's population (Flegr et al. 2014), including 12 percent of the population in the United States (US) (Jones et al. 2014). The definitive host for *T. gondii* is any member of the family Felidae, from which oocytes can contaminate the environment through excretion. *T. gondii* can infect humans through direct exposure to cat faeces, ingestion of uncooked or under-cooked, infected meat, eating contaminated foods, or congenital transmission (Elmore et al. 2010). *Toxoplasma gondii* disseminates throughout the body, including the brain and muscle where it initiates an encapsulation process protecting it from the host's immune response resulting in a latent infection (Dupont et al. 2012).

Both tachyzoites and bradyzoites of *T. gondii* produce tyrosine hydroxylase, the rate-limiting enzyme in dopa-

mine production. Following infection, *T. gondii* can import the remaining components necessary for dopamine synthesis from the host cell (Martin et al. 2015). In fact, cells infected with *T. gondii* can contain upwards of 350 percent more cytoplasmic dopamine than uninfected cells (Prandovszky et al. 2011). Attempts to determine the extent to which *T. gondii* might influence dopaminergic activity in infected cells and how this activity might affect neural systems have produced mixed results (Martin et al. 2015, Wang et al. 2015, Xiao et al. 2014).

However, because dopamine is associated with numerous aspects of human behaviour including movement, pleasure, attention, mood, memory and addiction (Frank and O'Reilly 2006, Schultz 2007), the modification of dopamine transmission associated with *T. gondii* seropositivity may be one mechanism by which *T. gondii* could affect human behaviour.

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Indeed, multiple studies have reported associations between *T. gondii* seropositivity and schizophrenia (Hinz-Selch et al. 2007, Mortensen et al. 2007, Torrey et al. 2007), a disease associated with abnormal dopamine transmission. Further, one study found an association between *T. gondii* seropositivity and self-directed violence in women (Pedersen et al. 2012) and another found an association with impulsivity and aggression (Cook et al. 2015), behaviours thought to be associated with dopamine transmission or availability (Pine et al. 2010, Trifileff and Martinez 2014a, Okusaga et al. 2016). In contrast to the negative effects associated with *T. gondii* seropositivity, Stock et al. 2014) found improved action control in people seropositive for *T. gondii*, a connection also potentially due to changes in dopaminergic activity in the brain (Ramdani et al. 2015). Taken together, the reported associations between *T. gondii* seropositivity and behaviour suggest that the modifications of dopamine metabolism associated with *T. gondii* seropositivity may be clinically relevant.

Propensity towards substance use appears to be related to both genetic and behavioural influences (Noble 2000). Though specific mechanisms differ, substances of abuse such as marijuana, cocaine and heroin are associated with increased dopamine transmission in mesolimbic brain regions (Adinoff 2004). Further, the frequency of substance use may be associated with the degree of substance-induced modification of dopamine-receptor densities and basal dopaminergic activity (Devoto et al. 2016, Trifileff and Martinez 2014b). Moreover, people with the A1 allele for the dopamine-2 receptor might use substances to compensate for decreased dopamine transmission (Noble 2000). While only a few studies have investigated the association between *T. gondii* seropositivity and substance use, a meta-analysis investigating the association between latent toxoplasmosis and psychiatric disorders (Sutherland et al. 2015) included four studies that found a positive association between latent toxoplasmosis and heroin addiction, suggesting a possible link between latent toxoplasmosis and opioid use. More recently, Alvarado-Esquivel et al. (2015) found that behaviours associated with substance abuse do not appear to increase the risk of *T. gondii* infection. Rather, their conclusions suggest that latent toxoplasmosis may be more likely to influence the degree of substance use as opposed to substance use increasing the likelihood of *T. gondii* infection. In this regard, childhood exposure to Epstein-Barr virus and cytomegalovirus in childhood is associated with later substance-use disorder, although *T. gondii* seropositivity was not (Vanyukov et al. 2017).

Because addictive drugs increase dopamine transmission in brain-reward pathways (Nutt et al. 2015), the potential influence of *T. gondii* seropositivity on these dopaminergic circuits might affect the propensity for substance use. Based on this hypothesis, we sought to investigate the association between *T. gondii* seropositivity and self-reported use of multiple substances of abuse in two large datasets representative of the US population. Using data from the third and continuous National Health and Nutritional Examination Surveys collected and made publicly availa-

ble by the US Centers for Disease Control and Prevention, we evaluated the association between *T. gondii* seropositivity and self-reported use of alcohol, tobacco, marijuana, cocaine, heroin and methamphetamine. As prior literature has suggested that infection by *T. gondii* might affect men and women differently (Flegr et al. 2000, Lindová et al. 2010), we also tested for sex-specific effects.

MATERIALS AND METHODS

Study sample

We analysed data from the third and continuous National Health and Nutritional Examination Surveys (NHANES) administered from 1988 to 1994 and from 2009 to 2014, respectively, by the National Center of Health Statistics at the Centers for Disease Control and Prevention (CDC) in the U.S.A. The NHANES surveys represent the US population via statistical weighting and complex multi-stage sampling and were collected in compliance with federally established ethical standards. For each NHANES cycle, the CDC recruited participants from 15 randomly selected counties across the US and informed approximately 600 to 700 randomly selected households from those counties of their eligibility to participate. The CDC offered participants personal health information and cash payment as compensation for participation. The CDC obtained blood samples only from subjects who completed the relevant consent documentation. Finally, the CDC deidentified all collected information to ensure confidentiality.

As not all subjects were surveyed for each of the substances used in this study, each of our statistical analyses included differing sample sizes. In the third NHANES dataset, subjects between the ages of 17 to 90 years were surveyed for tobacco and alcohol use while subjects between 17 to 59 years old were asked about cocaine and marijuana use. This resulted in analyses incorporating between 9,789 to 14,158 subjects depending on the substance assessed. In contrast, subjects in the continuous NHANES (2009–2014) data sets were surveyed for tobacco and alcohol use if they were 18 years or older (up to 90 years). Subjects between the ages of 18 and 59 years were also surveyed for marijuana while subjects aged 18 to 69 years were surveyed for cocaine, heroin and methamphetamine use. Between 8,257 and 13,745 subjects were included in the analyses using continuous NHANES data. The reduced NHANES data sets compiled for this study can be freely accessed online at <https://doi.org/10.6084/m9.figshare.6160526.v1>.

Toxoplasma gondii

Detection of latent *Toxoplasma gondii* infection was done using indirect enzyme immunoassay, in which an optical density of human gamma chain immunoglobulin is compared to a World Health Organisation calibrated standard curve (Gunter et al. 1996). As the criteria for *T. gondii* seropositivity have been regularly updated over the past two decades, the minimum optical density values required for diagnosis of latent toxoplasmosis differed across the NHANES datasets based on the assay manufacturer's guidelines. In the third NHANES dataset, samples with test results below 7 international units (IU/ml) were considered negative for *T. gondii* infection, while results equal to or higher than 7 IU/ml were considered positive for infection. In the continuous NHANES datasets, 33 IU/ml was set as the determina-

Table 1. Descriptive statistics of dependent, independent and control variables: NHANES III

	Full sample		<i>T. gondii</i> (Nicolle et Manceaux, 1908) positive		<i>T. gondii</i> negative	
	Mean	SE	Mean	SE	Mean	SE
Substance use ^{a†}						
Tobacco	0.53	0.01	0.57	0.01	0.52	0.01
Alcohol	0.86	0.01	0.85	0.01	0.87	0.01
Marijuana	0.45	0.01	0.33	0.02	0.48	0.01
Cocaine	0.14	0.01	0.10	0.01	0.15	0.01
<i>T. gondii</i> seropositive ^a	0.20	0.01	-	-	-	-
Age (years)	35.98	0.22	40.07	0.55	35.00	0.22
Female ^a	0.50	0.01	0.49	0.02	0.51	0.01
Poverty-Income Ratio	3.10	0.07	3.00	0.11	3.12	0.07
Race-ethnicity ^a						
Non-Hispanic White	0.76	0.01	0.71	0.02	0.77	0.01
Non-Hispanic Black	0.11	0.01	0.11	0.01	0.11	0.01
Mexican American	0.06	0.01	0.06	0.01	0.06	0.01
Other	0.08	0.01	0.12	0.02	0.07	0.01
Education	12.70	0.08	12.05	0.16	12.86	0.07

^a Proportions used for categorical variables

[†] Sample sizes differed per substance predicted (Tobacco – 14,158; Alcohol – 13,814; Marijuana – 9,791; Cocaine – 9,789)

NHANES – National Health and Nutrition Examination Survey; SE – standard error; *T. gondii* – *Toxoplasma gondii*; Other – non-Mexican-American Hispanics, Asian-Americans and multi-racial subjects

tion point for *T. gondii* seropositivity. In our models, we coded subjects with a positive *T. gondii* titre result as 1 and those with a negative result as 0. We could not determine the time of initial infection with *T. gondii* based on the available information.

Substance use

Self-reported history of tobacco, alcohol, marijuana and cocaine use was available in the third NHANES dataset, while tobacco, alcohol, marijuana, cocaine, heroin and methamphetamine use data were available in the continuous NHANES datasets. An exhaustive record of prior substance use was unavailable in either dataset. For marijuana, cocaine, heroin and methamphetamine, available data were limited to the acknowledgement of at least a single use during the subject's lifetime recorded as "Yes" or "No." For example, lifetime use of marijuana was assessed with the question, "Have you ever used marijuana?" For alcohol use, at least 12 drinks during a subject's lifetime was required to categorise the subject as having used alcohol. Finally, subjects were considered as having used tobacco if they had smoked at least 100 cigarettes in their lifetime. We recoded all substance-use questions so that a value of 1 indicated prior use while a value of 0 indicated no prior use of that substance. Although data for recent substance use (previous one or twelve month(s)) were available in the NHANES datasets, we did not use them because the variable structures within and between the datasets were inconsistent and therefore did not permit consistent or reliable comparisons across substances.

Covariates

We included several variables to control for potential confounding. Continuous variables included educational attainment, age in years and poverty-income ratio (PIR). Categorical variables included race-ethnicity and sex. In the third NHANES dataset, education level was determined by the number of years of schooling achieved with a range of 0 to 17 years. In the continuous NHANES datasets, no continuous measure of educational attainment was available; instead, education was categorised into groups based on general educational accomplishment (e.g. less than 9th grade, 9 through 11th grade and so forth). To improve

interpretability, we recoded the categorical education variable into three general categories: less than high school, high school and more than high school. Poverty-income ratio was defined as the ratio between total family income and the US poverty threshold (at the time of the administration of the NHANES surveys). A value above one indicated an income above poverty level. According to CDC guidelines (2011), for both datasets, the race-ethnicity variable was categorised into groups for non-Hispanic White, non-Hispanic Black, Mexican-American and "Other" race-ethnicities with the "Other" group including non-Mexican-American Hispanics, Asian-Americans and multi-racial subjects. Finally, sex was coded 1 for male and 0 for female.

Statistical analysis

We used Stata 14.2 (StataCorp 2015) for all statistical analyses and included the complex sampling characteristics of the NHANES datasets (i.e. sampling weights, strata and clustering in the survey design) in all statistical analyses using the `svy` command prefix to adjust parameter estimates and standard errors to be representative of the US civilian non-institutionalised population to avoid bias in statistical inferences.

We calculated descriptive statistics separately for each NHANES sample and calculated sample proportions and standard errors for each categorical variable and means and standard errors for continuous variables. Due to the binary nature of the dependent variables, we used logistic regression to predict alcohol, tobacco, marijuana, cocaine, heroin and methamphetamine use from *T. gondii* seropositivity in controlled models. We considered a *p*-value of equal to or less than 0.05 to be statistically significant.

We used logistic regression to test for potential interaction effects between *T. gondii* seropositivity and education, age, PIR, race-ethnicity and sex in the prediction of each substance included in this study, considering again a *p*-value of equal to or less than 0.05 to be statistically significant.

RESULTS

Tables 1 and 2 report means and proportions for the dependent, independent and control variables in each

Table 2. Descriptive statistics of dependent, independent and control variables: Continuous NHANES

	Full sample		<i>T. gondii</i> positive		<i>T. gondii</i> negative	
	Mean	SE	Mean	SE	Mean	SE
Substance Use ^{a†}						
Tobacco	0.44	0.01	0.46	0.01	0.44	0.01
Alcohol	0.89	0.01	0.87	0.01	0.90	0.01
Marijuana	0.60	0.01	0.47	0.03	0.62	0.01
Cocaine	0.18	0.01	0.17	0.01	0.18	0.01
Heroin	0.02	0.00	0.02	0.00	0.03	0.00
Methamphetamine	0.07	0.01	0.05	0.01	0.08	0.01
<i>T. gondii</i> Seropositive ^a	0.11	0.01	-	-	-	-
Age (years)	39.59	0.28	47.36	0.59	43.03	0.35
Female ^a	0.50	0.01	0.45	0.02	0.51	0.01
Poverty-Income Ratio	2.95	0.06	2.63	0.10	3.07	0.05
Race-ethnicity ^a						
Non-Hispanic White	0.67	0.02	0.57	0.03	0.70	0.02
Non-Hispanic Black	0.11	0.01	0.12	0.01	0.10	0.01
Mexican American	0.09	0.01	0.12	0.02	0.08	0.01
Other	0.13	0.01	0.19	0.02	0.11	0.01
Education ^a						
Less than High School	0.14	0.01	0.24	0.02	0.12	0.01
High School Diploma	0.21	0.01	0.25	0.01	0.20	0.01
More than High School	0.65	0.01	0.51	0.02	0.67	0.01

^a Proportions used for categorical variables

[†] Sample sizes differed per substance predicted (Tobacco – 13,745; Alcohol – 12,519; Marijuana – 8,257; Cocaine – 10,233; Heroin – 10,233; Methamphetamine – 10,236)

NHANES – National Health and Nutrition Examination Survey; SE – standard error; *T. gondii* – *Toxoplasma gondii* (Nicolle et Manceaux, 1908); Other – non-Mexican-American Hispanics, Asian-Americans and multi-racial subjects

NHANES dataset. Of the substances surveyed, subjects most frequently reported alcohol, tobacco and marijuana use, while less than 20 percent of the sample reported having used cocaine, heroin and methamphetamine at least once. Approximately 20 percent of the study sample were *Toxoplasma gondii* seropositive in the third National Health and Nutritional Examination Surveys (NHANES) dataset, whereas 11 percent were seropositive in the continuous NHANES datasets. In both NHANES datasets, most of the sample was White (third NHANES = 76 percent; continuous NHANES = 67 percent) and was evenly split between men and women.

In the third NHANES dataset, *T. gondii* seropositivity was inversely associated with cocaine and marijuana use, but there were no associations with alcohol or tobacco use (Table 3 and Fig. 1). The odds ratios (OR) for cocaine and marijuana use were 0.72 (95% CI: 0.56; 0.91; $p = 0.006$) and 0.71 (95% CI: 0.58; 0.87; $p = 0.001$), respectively, suggesting that subjects with *T. gondii* seropositivity had 28 percent and 29 percent lower odds of reporting having used cocaine and marijuana compared to the seronegative group. In the continuous NHANES datasets, *T. gondii* seropositivity was significantly associated with a decrease in self-reported use of tobacco (OR = 0.87 [95% CI: 0.76; 1.00]; $p = 0.044$), marijuana (OR = 0.60 [95% CI: 0.50; 0.72]; $p < 0.001$), heroin (OR = 0.60 [95% CI: 0.42; 0.85]; $p = 0.005$) and methamphetamine (OR = 0.54 [95% CI: 0.38; 0.77]; $p = 0.001$). That is, *T. gondii* seropositive subjects had 13 percent lower odds of reporting tobacco use, 40 percent lower odds of reporting marijuana use, 40 percent lower odds of reporting heroin use and 46 percent lower odds of reporting methamphetamine use than were subjects seronegative for *T. gondii*. In contrast, there were

no statistically significant associations with alcohol or cocaine use (Table 4 and Fig. 1).

In the third NHANES dataset, we observed a significant sex $\times$ *T. gondii* interaction related to tobacco use (p for interaction = 0.046) in which there was a significant association of *T. gondii* seropositivity and self-reported tobacco use among males (OR = 1.25 [95% CI: 1.01; 1.54]; $p = 0.039$), but not among females (OR = [0.95; 95% CI: 0.79; 1.16]; $p = 0.614$) (Supplementary Table 1). In the continuous NHANES dataset, we observed a significant sex $\times$ *T. gondii* interaction related to alcohol use (p for interaction = 0.048). However, despite being in opposite directions, neither sex-specific association of *T. gondii* seropositivity with self-reported alcohol use was significant (OR among males = 1.26 [95% CI: 0.85; 1.87]; $p = 0.235$; and OR among females = 0.87 [95% CI: 0.71; 1.05]; $p = 0.142$) (Supplemental Table 2). We did not observe any significant sex $\times$ *T. gondii* interactions related to marijuana, cocaine, heroin, or methamphetamine use (Supplementary Tables 1 and 2).

There were also significant interactions between *T. gondii* seropositivity and education (p for interaction < 0.001) and between *T. gondii* seropositivity and PIR (p for interaction < 0.001) in the prediction of self-report cigarette smoking in the third NHANES dataset (Figs. 2). Subjects seronegative for *T. gondii* had an inverse association between educational attainment and cigarette smoking: those subjects with higher education were less likely to self-report smoking (OR = 0.91 [95% CI: 0.89; 0.94]; $p < 0.001$). In contrast, in subjects seropositive for *T. gondii*, there was no association between cigarette smoking and educational attainment (OR = 0.99 [95% CI: 0.96; 1.03]; $p = 0.659$). Similarly, subjects seronegative for *T. gondii* were less

Fig. 1. Graphical depiction of odds ratios from logistic regression analyses of *T. gondii* (Nicolle et Manceaux, 1908) seropositivity predicting use of substances of abuse. Note: All models include sex, age, race-ethnicity, education and poverty-income ratio as controls.

likely to use tobacco as PIR values increased (OR = 0.93 [95% CI: 0.90; 0.97]; $p = <0.001$). However, in seropositive subjects, there were no associations with PIR (OR = 1.06 [95% CI: 1.00; 1.012]; $p = 0.068$) (Figs. 2).

In the continuous NHANES datasets, there was also a significant interaction between educational attainment and *T. gondii* seropositivity (p for interaction = 0.005) in the prediction of cigarette smoking (more than high school, OR = 1.58 [95% CI: 1.16; 2.16]; $p = 0.005$). Specifically, for seronegative subjects, the probability of having previously smoked was significantly reduced in individuals with increasing degrees of education. In contrast, in subjects seropositive for *T. gondii* infection, the association between tobacco use and education was less pronounced (Figs. 3).

Finally, in a *post-hoc* analysis, we determined whether there were differences in response rates to the substance-use questions between subjects seropositive and seronegative for *T. gondii* (Table 5). Across both datasets, there was a difference in response rate to substance-use questions for four substances (marijuana, cocaine, heroin and methamphetamine). Because the cocaine, heroin and methamphetamine use questions were all part of a single questionnaire in the continuous NHANES, we expected that each question would have a similar response rate (i.e. a subject who committed to completing the drug use questionnaire was very likely to answer all three drug use questions). It is unknown whether those response rates would have differed had the questions been given separately or in a different format. Generally, subjects who were *T. gondii* positive appeared to be less likely to complete questionnaires related to drug or alcohol use.

DISCUSSION

The main findings of this study suggest inverse relationships between self-reported use of some addictive substances and *Toxoplasma gondii* seropositivity. Specifically, we found associations between *T. gondii* seroposi-

tivity and lower self-reported use of marijuana and cocaine in the third National Health and Nutritional Examination Surveys (NHANES) dataset and lower self-reported use of tobacco, marijuana, heroin and methamphetamine in the continuous NHANES datasets. That is, *T. gondii* seropositivity appeared to be protective against self-reported tobacco, cocaine, marijuana, heroin and methamphetamine use. While the association between *T. gondii* seropositivity and cocaine use was not statistically significant in the continuous NHANES datasets, the direction of the relationship was consistent with the decreased likelihood of self-reported cocaine use in the third NHANES dataset. Similarly, tobacco use was significantly associated with *T. gondii* seropositivity in the continuous NHANES datasets but not the third NHANES dataset. Unlike the findings for cocaine, the direction of the association between *T. gondii* seropositivity and tobacco use did not agree between the third NHANES and continuous NHANES datasets. However, there was only weak evidence for the association between *T. gondii* seropositivity and tobacco use ($p = 0.044$) in the continuous NHANES dataset and we are unable to make firm conclusions about this association.

In the third NHANES dataset, sex moderated the association between *T. gondii* and self-reported tobacco use. In fact, sex-specific models showed that men seropositive for *T. gondii* were significantly more likely to have self-reported tobacco than seronegative men. While we did not find this same result for men in the continuous NHANES dataset, we did observe that seropositive women were significantly less likely to have self-reported tobacco than seronegative women. Further, in the continuous NHANES dataset, seropositive men appeared to be more likely to have reported alcohol use in their lifetime than did seronegative men, but this finding was not present in the third NHANES dataset. Due to the lack of replicability of the sex-specific associations across the NHANES datasets, we recommend additional research to further investigate

Fig. 2. Tobacco use and the interaction of *Toxoplasma gondii* (Nicolle et Manceaux, 1908) seropositivity with PIR and education in NHANES III: Predicted probabilities from logistic regression. Note: The sample size is 14,158. Tobacco use is defined as smoking 100 or more cigarettes. PIR = Poverty-to-income ratio; HS = High school. All models include sex, age, race-ethnicity, education and poverty-income ratio as controls.

Fig. 3. Tobacco use and the interaction of *Toxoplasma gondii* (Nicolle et Manceaux, 1908) seropositivity with education in NHANES 2009-2014: Predicted probabilities from logistic regression. Note: The sample size is 13,745. Tobacco use is defined as smoking 100 or more cigarettes. HS = High school. All models include sex, age, race-ethnicity, education and poverty-income ratio as controls.

Table 3. Logistic regression analysis of *Toxoplasma gondii* (Nicolle et Manceaux, 1908) seropositivity predicting substance use in US adults, third NHANES (1988–1994): Odds ratios from weighted logistic regression.

	Tobacco			Alcohol			Marijuana			Cocaine		
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>
<i>T. gondii</i> seropositive	1.08	[0.93; 1.26]	0.297	1.07	[0.90; 1.28]	0.420	0.71	[0.58; 0.87]	0.001	0.72	[0.56; 0.91]	0.006
Age (years)	1.01	[1.00; 1.01]	<0.001	0.99	[0.99; 1.00]	0.008	0.95	[0.94; 0.96]	<0.001	0.97	[0.96; 0.98]	<0.001
Male	2.11	[1.91; 2.32]	<0.001	3.29	[2.79; 3.88]	<0.001	1.81	[1.60; 2.05]	<0.001	1.96	[1.56; 2.45]	<0.001
Education (years)	0.94	[0.91; 0.96]	<0.001	1.09	[1.06; 1.12]	<0.001	1.05	[1.02; 1.08]	0.001	0.98	[0.95; 1.02]	0.362
Race-ethnicity												
Non-Hispanic White (ref)	-	-	-	-	-	-	-	-	-	-	-	-
Non-Hispanic Black	0.63	[0.54; 0.74]	<0.001	0.63	[0.49; 0.81]	<0.001	0.95	[0.81; 1.12]	0.541	0.96	[0.74; 1.26]	0.781
Mexican-American	0.41	[0.35; 0.48]	<0.001	0.78	[0.61; 1.01]	0.057	0.50	[0.42; 0.60]	<0.001	0.66	[0.51; 0.86]	0.002
Other	0.43	[0.33; 0.56]	<0.001	0.31	[0.21; 0.46]	<0.001	0.31	[0.23; 0.42]	<0.001	0.75	[0.48; 1.16]	0.190
Poverty-Income Ratio	0.96	[0.93; 0.99]	0.026	1.17	[1.10; 1.25]	<0.001	1.03	[0.98; 1.07]	0.239	1.03	[0.97; 1.10]	0.350
Constant	1.76	[1.30; 2.37]	<0.001	1.60	[0.99; 2.62]	0.057	2.41	[1.52; 3.84]	<0.001	0.43	[0.25; 0.72]	0.002
Sample size	14,158			13,814			9,791			9,789		

OR – odds ratio, CI – confidence interval, ref – reference category, NHANES – National Health and Nutrition Examination Survey;
Other – non-Mexican-American Hispanics, Asian-Americans and multi-racial subjects

whether potential associations between *T. gondii* and tobacco or alcohol use are indeed sex-specific.

While the interactions between sex and *T. gondii* infection appear to be tentative, prior literature has suggested that *T. gondii* seropositivity may influence the behaviour or personalities of men and women differently (Flegr et al. 2000, 2003). Thus, the inclination or drive to obtain and consume substance of abuse may differ between *T. gondii* infected men and women. In line with these observations, Flegr (2015), summarising preliminary findings from multiple reports, described an association between latent toxoplasmosis and alcohol use. However, the reported findings were not sex-specific and tended to be inconsistent across the reports included in the study, possibly due to variations in geographic region and the degree to which *T. gondii* seropositivity may have affected substance use.

We also found the previously reported inverse association between education and likelihood of cigarette smoking (Higgins et al. 2016) in subjects seronegative for *T. gondii*. However, this inverse association with education was absent in subjects seropositive for *T. gondii* in both datasets. Similarly, in the third NHANES dataset, we also found an inverse association between cigarette smoking and Poverty-Income Ratio (PIR) in seronegative subjects but not in seropositive subjects.

Data from the third NHANES dataset and from the more recent continuous NHANES datasets indicate a possible decrease in the prevalence of *T. gondii* seropositivity in the US. In the third NHANES dataset, 20 percent of the sample were seropositive for *T. gondii*, whereas 11 percent were seropositive in the continuous NHANES datasets. While this apparent decline in *T. gondii* seropositivity in the US is consistent with other reports (Hill et al. 2007, Jones et al. 2014) and suggests that the prevalence of *T. gondii* seropositivity might be decreasing in the US, methodological differences between the two datasets make direct comparisons of *T. gondii* seroprevalence difficult. Changes in the cutoff points to determine *T. gondii* seropositivity might account for some of the difference in *T. gondii* seropositivity between the two datasets.

The Centers for Disease Control and Prevention used immunoassays to determine *T. gondii* seropositivity in both

NHANES datasets, but the third NHANES dataset used a cutoff value of 7 IU/ml to determine seropositivity, whereas the continuous NHANES datasets used a cutoff value of 33 IU/ml. The manufacturers of the ELISA kits used to determine *T. gondii* seropositivity also differed between the two datasets. Nonetheless, the higher cutoff value used in the continuous NHANES dataset could result in possible misclassification in regard to seropositivity with the potential to alter not only the estimated prevalence of *T. gondii* seropositivity in each dataset but also the associations we found between *T. gondii* seropositivity and self-report of substance use.

The mechanisms by which latent toxoplasmosis might be associated with a decreased propensity to use tobacco, marijuana, heroin, methamphetamine and possibly cocaine in the overall sample and the mechanisms for the possible sex differences in self-reported alcohol and tobacco use are unknown. However, our overall results are consistent with a large longitudinal study in which recruitment started at approximately age 11 years and prospective follow-up ended at approximately age 30 years that found associations between childhood seropositivity for some herpes viruses and an increased risk of a substance-use disorder in adulthood but not for *T. gondii* positivity (Vanyukov et al. 2017). Further, the results were not simply due to the lower intellectual function associated with neurotropic infection in childhood because *T. gondii* seropositivity was also associated with lower intellectual function but not substance-use disorder in adulthood.

In terms of a potential mechanism, it is possible that *T. gondii* seropositivity might influence key dopaminergic circuits in the brain leading to a decrease in desire or motivation to seek out rewarding substances that carry a social or legal risk. While *T. gondii* cysts are almost exclusively found in neurons, the parasite appears to be non-selective with respect to the general brain regions it infects (McConkey et al. 2013, Cabral et al. 2016), although the *nucleus accumbens*, a brain region implicated in substance use, might be an area of increased *T. gondii* cyst concentration compared to other brain regions (Fabiani et al., 2015). Therefore, it is possible that *T. gondii* could infect areas of the brain that affect mesolimbic or related regions (Carru-

Table 4. Logistic regression analysis of *Toxoplasma gondii* (Nicolle et Manceaux, 1908) seropositivity predicting substance use in US adults, continuous NHANES 2009–2014: Odds ratios from weighted logistic regression.

	OR	Tobacco 95% CI	<i>p</i>	OR	Alcohol 95% CI	<i>p</i>	OR	Marijuana 95% CI	<i>p</i>
<i>T. gondii</i> seropositive	0.87	[0.76; 1.00]	0.044	0.99	[0.80; 1.22]	0.895	0.60	[0.50; 0.72]	<0.001
Age	1.01	[1.01; 1.02]	<0.001	0.99	[0.98; 0.99]	<0.001	1.00	[0.99; 1.00]	0.658
Male	1.66	[1.51; 1.84]	<0.001	2.40	[2.06; 2.81]	<0.001	1.58	[1.42; 1.76]	<0.001
Education									
Less than High School (ref)	-	-	-	-	-	-	-	-	-
High School	0.80	[0.68; 0.95]	0.011	1.06	[0.86; 1.31]	0.572	1.13	[0.92; 1.39]	0.226
More than High School	0.51	[0.44; 0.59]	<0.001	1.35	[0.99; 1.84]	0.056	1.10	[0.90; 1.35]	0.354
Race-ethnicity									
Non-Hispanic White (ref)	-	-	-	-	-	-	-	-	-
Non-Hispanic Black	0.63	[0.54; 0.73]	<0.001	0.58	[0.43; 0.79]	0.001	0.72	[0.61; 0.87]	0.001
Mexican American	0.39	[0.33; 0.46]	<0.001	0.60	[0.46; 0.79]	<0.001	0.32	[0.26; 0.40]	<0.001
Other	0.57	[0.48; 0.67]	<0.001	0.43	[0.34; 0.56]	<0.001	0.38	[0.31; 0.47]	<0.001
Poverty-Income Ratio	0.86	[0.84; 0.89]	<0.001	1.19	[1.13; 1.26]	<0.001	0.97	[0.92; 1.02]	0.290
Constant	1.10	[0.84; 1.44]	0.498	7.96	[4.81; 13.16]	<0.001	1.78	[1.35; 2.34]	<.001
Sample size		13,745			12,519				

	OR	Cocaine 95% CI	<i>p</i>	OR	Heroin 95% CI	<i>p</i>	OR	Methamphetamine 95% CI	<i>p</i>
<i>T. gondii</i> seropositive	0.90	[0.76; 1.06]	0.206	0.60	[0.42; 0.85]	0.005	0.54	[0.38; 0.77]	0.001
Age (years)	1.00	[1.00; 1.01]	0.589	1.01	[0.99; 1.02]	0.340	1.01	[1.00; 1.01]	0.063
Male	1.92	[1.68; 2.19]	<0.001	2.36	[1.61; 3.46]	<0.001	1.99	[1.66; 2.38]	0.000
Education									
Less than High School (ref)	-	-	-	-	-	-	-	-	-
High School	1.11	[0.88; 1.41]	0.370	1.09	[0.73; 1.62]	0.670	1.28	[0.92; 1.77]	0.134
More than High School	0.93	[0.76; 1.13]	0.454	0.75	[0.48; 1.17]	0.201	0.96	[0.72; 1.26]	0.754
Race-ethnicity									
Non-Hispanic White (ref)	-	-	-	-	-	-	-	-	-
Non-Hispanic Black	0.67	[0.55; 0.82]	<0.001	1.01	[0.62; 1.65]	0.952	0.24	[0.15; 0.38]	<0.001
Mexican American	0.77	[0.63; 0.95]	0.014	0.38	[0.21; 0.69]	0.002	0.84	[0.57; 1.24]	0.383
Other	0.56	[0.46; 0.69]	<0.001	0.62	[0.37; 1.04]	0.070	0.74	[0.54; 1.01]	0.055
Poverty-Income Ratio	0.94	[0.89; 0.99]	0.014	0.75	[0.68; 0.83]	<0.001	0.83	[0.77; 0.90]	<0.001
Constant	0.20	[0.14; 0.31]	<0.001	0.03	[0.01; 0.08]	<0.001	0.09	[0.05; 0.14]	<0.001
Sample size		10,233			10,233			10,236	

OR – Odds ratio, CI – Confidence interval, ref – reference category, NHANES – National Health and Nutrition Examination Survey;
 Other – non-Mexican-American Hispanics, Asian-Americans and multi-racial subjects

thers and Suzuki 2007, Hermes et al. 2008). Accordingly, the putative effect of *T. gondii* on dopamine synthesis (Prandovszky et al., 2011) could counteract the deficient dopaminergic transmission associated with the A1 allele of the dopamine-2 receptor gene (Noble 2000) to decrease substance use in subjects with this genetic variant or even in subjects with normal dopamine transmission. *Toxoplasma gondii* seropositivity also has been associated with decreased novelty seeking in both men and women possibly via increased dopamine synthesis (Flegr et al. 2003, Skallová et al. 2005), which could be another mechanism accounting for the association between *T. gondii* seropositivity and decreased self-report of some substances.

Another potentially important finding in this study was the interaction between *T. gondii* seropositivity and educational attainment and PIR in predicting tobacco use. Although some research suggests that higher education might be associated with a lower prevalence of smoking (Higgins et al. 2016), the association between smoking and education is complex and the research findings are mixed (Maralani 2013). We found that subjects seronegative for *T. gondii* had an inverse relationship between educational attainment and cigarette smoking, but we did not find this association in the group seropositive for *T. gondii*, suggest-

ing that *T. gondii* seropositivity might alter this association. Similarly, we found an inverse association between PIR and smoking in seronegative subjects but not in seropositive subjects in the third NHANES dataset. Together, these factors suggest that *T. gondii* seropositivity might modify the associations between cigarette smoking and educational attainment and PIR (Higgins et al. 2016). Of course, because these data are cross sectional and because we do not know age at time of infection with *T. gondii* or age when subjects started smoking, we cannot address causation.

Because substance use is associated with dopamine transmission (Payer et al. 2014) and because dopamine release following substance use might be primarily responsible for continued substance use, potential modifications of dopaminergic activity or reactivity by *T. gondii* seropositivity may decrease the likelihood of initial substance use but increase substance use following first-time use. Unfortunately, data were not available in the NHANES datasets to adequately assess active versus prior substance use or to address substance-use disorders. Data for prior-year substance use were available in the NHANES datasets, but models including these data resulted in too few subjects to ensure statistically robust results. Future research could explore the differential effects of *T. gondii* seropositivity

Table 5. Percent of subjects who responded to each substance use question by *Toxoplasma gondii* (Nicolle et Manceaux, 1908) infection status.

	NHANES III <i>T. gondii</i>				Continuous NHANES <i>T. gondii</i>			
	Positive	Negative	<i>t</i>	<i>p</i>	Positive	Negative	<i>t</i>	<i>p</i>
Tobacco	100.00	100.00	-	-	99.98	99.94	0.98	0.334
Alcohol	98.12	98.51	-1.22	0.227	92.58	91.01	2.07	0.044
Marijuana	97.66	98.39	-1.52	0.134	90.74	88.58	1.83	0.073
Cocaine	97.65	98.39	-1.55	0.128	91.16	88.94	2.57	0.013
Heroin	-	-	-	-	91.17	88.92	2.59	0.013
Methamphetamine	-	-	-	-	91.18	88.94	2.59	0.013

Note: Positive – percent of *T. gondii* seropositive and Negative – percent of *T. gondii* seronegative. NHANES – National Health and Nutrition Examination Survey

on substance use in nonusers, occasional users and heavy users.

Several considerations temper the interpretation of these findings. Because the data we used were cross sectional, we could not evaluate cause and effect relationships, precluding a determination of whether *T. gondii* seropositivity was present prior to the self-reported cocaine and marijuana use. Although the age of initial infection with *T. gondii*, as well as the specific strain of latent toxoplasmosis (Xiao and Yolken 2015), could affect the propensity for later substance use, we could not determine either the age of initial infection or the strain of toxoplasmosis from information available in the NHANES datasets.

Additionally, it is possible that the findings could differ had the NHANES dataset included other operationalisations of substance use, such as recent use, frequency of use, or diagnosis of a substance-use disorder. Unfortunately, in this study, variables that assessed the frequency or recent use of each substance were inconsistent in structure and definition across and within each NHANES dataset and therefore precluded valid comparisons. While we controlled for several potentially confounding variables, other variables for which we did not control could have confounded the associations we found. The NHANES datasets lacked data regarding measures of dopamine concentrations. Because of this, further research directly evaluating for dopamine mediation between *T. gondii* seropositivity and substance use is needed. Further, although not the focus of this study, we observed race differences in substance use when controlling for *T. gondii* indicating that race-ethnic minorities use these substances less often than non-Hispanic whites. This could be because of truly different levels of use, self-report use, or an artifact of the data collection method given findings showing that *T. gondii* seropositivity might influence personality factors such as novelty seeking (Flegr et al. 2003, Skallová et al. 2005) and other traits such as suspiciousness (Flegr 2013).

Indeed, we found that subjects seropositive for *T. gondii* were less likely than seronegative subjects to complete the drug and alcohol questionnaires in the third and continuous NHANES datasets. While the differences in response rates were small, they suggest subtle differences in behaviour

between *T. gondii* seropositive and seronegative subjects and could indicate the presence of error in our estimates of the effect of *T. gondii* seropositivity on substance use. However, we were limited in our ability to determine the direct cause of the response rate differences and the extent or degree to which these differences affected the other analyses. Finally, it is possible that other unmeasured factors such as *T. gondii* strain type (Xiao and Yolken 2015), cultural and regional differences in substance use among different populations (Hartka and Fillmore 1989), or other factors might influence potential associations between *T. gondii* infection and substance use.

Despite these limitations, this study has a number of strengths. First, the study benefits from large sample sizes that represent the general non-institutionalised US population. The use of both the third and continuous NHANES datasets acts as a check against the methods we used and demonstrates the replicability of our findings. Indeed, we found the association between *T. gondii* seropositivity and self-reported marijuana use in both datasets. Further, while not statistically significant in the continuous NHANES dataset, the direction of the association between *T. gondii* seroprevalence and cocaine use also appeared to be consistent across the two datasets. Finally, the use of multiple substances provides a more thorough investigation of the association between *T. gondii* seropositivity and substance use.

In conclusion, in this sample representative of the US population, subjects seropositive for *T. gondii* were less likely to self-report that they had ever used tobacco, cocaine, marijuana, heroin, or methamphetamine in their lifetime than were subjects seronegative for *T. gondii*. In contrast, there were no differences for alcohol use between the seropositive and seronegative groups. Sex, PIR and education moderated the association between *T. gondii* seropositivity and tobacco use and sex moderated the association between *T. gondii* seropositivity and alcohol use. Further research is needed to better characterise these associations, including evaluating the age of initial exposure to *T. gondii* and identifying potential biological underpinnings of these putative associations.

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