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Association Between Latent Toxoplasma gondii Infection and Alzheimer's Disease

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ABSTRACT

Association Between Latent *Toxoplasma gondii* Infection and Alzheimer's Disease

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Many studies have found an association between *Toxoplasma gondii* seropositivity and behavioral and cognitive changes in animal models and in humans. In addition, early findings have suggested an association between *T. gondii* seropositivity and Alzheimer's disease (AD). This work sought to determine whether there is an association between *T. gondii* seropositivity and AD as well as cognitive functioning (including memory, working memory, processing speed, language functioning, executive functioning) in a large, well-characterized sample of subjects with AD and matched controls without dementia. Using enzyme-linked immunosorbent assay (ELISA) assays, anti-*T. gondii* IgG antibody titers were determined in 114 control subjects and in 105 subjects diagnosed with AD through an Alzheimer's Disease Research Center. The seroprevalence between the two groups were compared using propensity score matching (PSM). Associations between *T. gondii* seropositivity and cognitive functioning were also compared using both PSM and linear regressions. No differences were found between groups in age, ethnicity, or gender. Education and socioeconomic status was slightly higher in the control group. Using PSM, no significant differences were found in having AD due to *T. gondii* seropositivity between the two groups. Using PSM, *T. gondii* seropositivity was associated with worse performance on the WAIS-R Digit Symbol test. Within the AD group, *T. gondii* seropositivity was associated with worse performance on the WAIS Block Design and Trail Making B tests. In this sample, no evidence of an association between *T. gondii* seropositivity and AD was found in a larger study than previous studies. However, evidence of a negative association between processing speed and *T. gondii* seropositivity as well as a negative association between processing speed, executive functioning, and *T. gondii* seropositivity in those with AD was found.

Keywords: *Toxoplasma gondii,* Toxoplasmosis, Alzheimer disease, neurocognitive function, aging, processing speed, executive function

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Alzheimer's disease (AD) is the most common form of dementia, the most common neurodegenerative disorder, and an estimated 5.4 million Americans had AD in 2012 (Gu et al., 2017). The disease causes progressive brain deterioration and is characterized by marked cognitive decline (Alzheimer's Association, 2013). AD is a growing public-health concern and is the sixth leading cause of death in the United States. A variety of factors are associated with the development of AD including neurological changes such as the well-known accumulation of beta-amyloid and tau proteins, genetic mutations (such as in the presenilin and ApoE genes), family history, environmental stress, illiteracy or lack of early education, physical activity, cognitive activity, social activity, nutrition, and cardiovascular risks (Polidori, Nelles, & Pientka, 2010). Additionally, there is growing interest in the role of infectious diseases, including parasites, and their impact on development of AD as well as cognitive function. For example, Katan, Moon, Wright, and Elkind (2013) found an association between a history of exposure to bacterial and viral pathogens and cognitive function. Other studies show an association between the protozoal parasite *Toxoplasma gondii* and cognitive, behavioral, and neurological changes in human and animal models, making the parasite a possible factor in the development of cognitive decline and AD (Dalimi & Abdoli, 2012; Fekadu, Shibre, & Cleare, 2010).

Toxoplasma gondii

Toxoplasma gondii is a common parasite that has particular affinity for the central nervous system (CNS) in intermediate hosts. Felines are the definitive host (the host in which the parasite sexually replicates) for *T. gondii* and intermediate hosts (hosts in which the parasite is metabolically active, lives, and reproduces through asexual cell division) include almost all warm-blooded animals including humans (Fekadu et al., 2010; Gulinello et al., 2010). Although

humans are not the definitive hosts of *T. gondii*, prevalence rates for IgG seropositivity (having IgG antibodies to *T. gondii* after the acute stage of the infection) in humans are surprisingly high. Worldwide prevalence is reported to be between 30 to 50 percent (Carter, 2013; Hermes et al., 2008). In some parts of the world, the seroprevalence (prevalence of those seropositive for the parasite) rates can be as high as 80 percent (Dalimi & Abdoli, 2012). In the United States, the seroprevalence rates tend to be lower than the worldwide prevalence for adults, ranging from approximately nine to 22 percent (Dubey & Jones, 2008; Montoya & Liesenfeld, 2004). Additionally, prevalence rates in age groups older than 40 to 49 years tend to be higher than those of younger age. One study found prevalence in those over 70 years as high as 42 percent (Jones et al., 2001).

T. gondii infection of humans by oocysts and cysts can happen through vertical transmission (from a woman to her child during or after birth), eating undercooked meat infected with *Toxoplasma* cysts, or contaminated food or water that contains oocysts[1], or handling soil contaminated with oocysts (Kannan & Pletnikov, 2012). Thus, transmission of the parasite need not involve the definitive host at all, as ingesting cysts from the parasite can transmit the parasite to another host. After ingestion of the parasite, the acute phase of disease consists of tachyzoites[2] proliferating in any nucleated cell in the body and destroying the host cell in the process. After the acute phase, the second stage consists of bradyzoite[3] tissue cysts in skeletal muscle and the brain, including cysts in astrocytes, microglia, and neurons (Fabiani, Pinto, Bonuccelli, & Bruschi, 2015; Fekadu et al., 2010). These cysts can become dormant and are

[1] Oocysts are cysts containing zygotes.

[2] Tachyzoites are part of the asexual stage of rapid growth of *T. gondii*.

[3] Bradyzoites are the slowly multiplying, encysted form of *T. gondii*.

characteristic of chronic, or latent, infection, which is lifelong and can cause continuous infection of the brain as well as persistent seropositivity for the parasite (Dalimi & Abdoli, 2012; Fekadu et al., 2010; Kannan & Pletnikov, 2012; Prandovszky et al., 2011). All infected humans develop an acute immune reaction to *T. gondii*, and then if the person is not immunocompromised, the infection most often becomes dormant. Development of an immune response (seropositivity) can provide life-long immunity against toxoplasmosis (Jung et al., 2012). However, *T. gondii* infection can be reactivated if host immunity wanes. Feline infection results in the production of oocysts by the parasite, which are then excreted into the environment and can be transmitted to other organisms (Kannan & Pletnikov, 2012). Sexual reproduction of the parasite can only happen in felines, but asexual reproduction can occur in any infected animal, including humans.

Certain risk factors are associated with developing *T. gondii* seropositivity humans, which are often dependent on the region of the world examined. In the United States, increasing age and lower levels of education are associated with *T. gondii* seropositivity. In addition, Hispanic ethnicity and ethnicity reported as "other" have statistically higher seroprevalence than non-Hispanic whites (Gale, Brown, Erickson, Berrett, & Hedges, 2014). Additionally, Mexican Americans and non-Hispanic black persons also tend to have a higher seroprevalence. Seroprevalence also tends to vary based on region in the United States. The northeastern United States has a seroprevalence rate of 29.2 percent, the south at 22.8 percent, the midwest at 20.5 percent, and the west with the lowest rate at 17.5 percent. Lastly, other risk factors for developing *T. gondii* seropositivity in the United States include living in a crowded conditions, being foreign-born, and working in soil-related jobs (Dubey & Jones, 2008). A study from Mexico found *T. gondii* seropositivity is associated with having no formal education, female gender, presence of dogs at home, eating raw dried meat, and eating goat, turkey, and pigeon

meat. Additionally, those who raised animals also had a higher rate of *T. gondii* seropositivity than those who did not (Alvarado-Esquivel et al., 2014). Why these risk factors are related to *T. gondii* seropositivity is unclear but various hypotheses have been proposed, including undercooking of meat before eating, poor sanitation, soil-related occupation, and crowded households (Alvarado-esquivel et al., 2014; Pearce, Kruszon-moran, & Jones, 2014).

In immunocompetent humans, most infections with *T. gondii* result in *T. gondii* seropositivity, a chronic, asymptomatic, latent infection in the brain, rather than an acute infection. The most replicated association with *T. gondii* seropositivity is schizophrenia, with over 38 studies finding an association between *T. gondii* seropositivity and schizophrenia (Webster, Kaushik, Bristow, & Mcconkey, 2013). Other associations include depression, bipolar disorder, obsessive-compulsive disorder, suicide attempts, lower IQ scores, Parkinson's disease, epilepsy, and intellectual disability, but these associations have not be studied thoroughly or consistently replicated (Dalimi & Abdoli, 2012; Fekadu et al., 2010).

Potential Connections Between AD and *T. gondii*

In addition to the other diseases listed above, a few studies show an association between *T. gondii* seropositivity and AD (Dalimi & Abdoli, 2012; Flegr, Lenchova, Hodny, & Vondrova, 2011). Kusbeci, Miman, Yaman, Aktepe, and Yazar (2011) found a significantly increased prevalence of *T. gondii* seropositivity of anti-*T. gondii* IgG antibodies in individuals with Alzheimer's disease as compared to control groups (44.1% and 24.3% respectively, $p = 0.005$) in a sample size of 71. Another study by Rashno, Fallahi, and Bahrami (2017) examined 87 adults with AD and 87 healthy controls for *T. gondii* seropositivity. In this study, they did not find an association between *T. gondii* seropositivity and AD (Rashno et al., 2017). In addition, Mahami-oskouei et al. (2016) examined 75 patients with AD and 75 healthy controls and again found no

association between AD and *T. gondii* seropositivity. Thus, the evidence of association between AD and *T. gondii* seropositivity based on seroprevalence between AD groups and healthy controls is mixed and more evidence is needed. Two lines of evidence support the possibility of *T. gondii* seropositivity as a factor contributing to the development of AD, specifically cognitive changes (and accompanying behavioral changes) associated with *T. gondii* infection and the pathology of *T. gondii* in its latent form of cysts in the human brain.

Cognitive Changes Associated with *T. gondii*

Some studies found overall cognitive changes in *T. gondii* seropositive humans and mice. For example, Kannan and Pletnikov (2012) report *T. gondii* seropositive young adult men had lower verbal intelligence compared to age-matched uninfected controls. Additionally, Ibrahim et al. (2016) found higher anti-*T. gondii* IgG antibody titers are associated with worse accuracy on a line orientation test, measured emotion differentiation test, and emotion recognition test.

Importantly, not all associations between *T. gondii* seropositivity and cognition indicate a negative effect on cognition. Some studies found a positive association between *T. gondii* seropositivity and some cognitive measures. For example, Ibrahim et al. (2016) found those with higher anti-*T. gondii* IgG antibody titers had faster reaction times on the Penn Conditional Exclusion Test.

Cognition can be defined many ways, but it is often defined by placing different cognitive abilities into various cognitive domains. In particular, neuropsychological tests (which are often used to assess cognition in humans) can divide into the following domains: memory (including working memory), processing speed, language functioning, executive functioning, and visuospatial abilities (Nuechterlein et al., 2004; Sheline et al., 2006). We will discuss these

domains expect visuospatial abilities with relevance to their association with *T. gondii* seropositivity, in both human and mouse studies, and for both positive and negative effects on cognition.

Memory and working memory – Mouse studies. The results from mouse models examining memory and *T. gondii* infection are mixed. Some studies show impaired recognition memory for chronically infected (six to nine weeks post infection), while other studies show no impairment (Kannan & Pletnikov, 2012). For example, Gulinello et al. (2010) found normal spatial memory, levels of novel object exploration, recognition memory, and familiar object exploration in chronically infected (seven weeks post infection) mice. However, Mahmoudvand et al. (2016) infected mice with *T. gondii* and found that *T. gondii* IgG seropositive mice had impaired learning and spatial memory functions compared to control mice as tested by the Morris water maze. In addition, the authors also infected mice with AD pathology with Toxoplasmosis. Again, using the Morris water maze, the authors found that the mice with both AD pathology and *T. gondii* IgG seropositivity had significantly more impairment in some learning and memory functions (e.g. distance to reach the platform, escape latency) than only *T. gondii* IgG seropositive mice and only AD pathology mice. In other areas of learning and memory tasks, however, the *T. gondii* IgG seropositive and AD pathology mice performed similarly to mice with only AD pathology. Lastly, the study found that *T. gondii* IgG seropositivity led to the promotion of neuroinflammation as well as the progression of AD in mice (Mahmoudvand et al., 2016).

Using the same study design as in Mahmoudvand et al. (2016), the authors examined the effect of donepezil (an acetylcholinesterase inhibitor, often used in the treatment of AD) on memory functioning in *T. gondii* IgG seropositive mice. *T. gondii* IgG seropositive mice had

delayed learning (as measured by a longer distance traveled and longer time to reach the platform in the Morris Water Maze). However, when the mice were treated with donepezil, the mice had significantly reduced time and distance traveled to the platform compared to the untreated, infected mice. Additionally, *T. gondii* IgG seropositive mice had longer escape latency than uninfected mice. Again, when treated with donepezil, *T. gondii* IgG seropositive mice had significantly shorter escape latency times than the untreated mice. As found previously, *T. gondii* IgG seropositive mice had short-term memory impairment, which was reversed by treatment with donepezil. Importantly, no differences were found in swimming speed between any of the groups, indicating no differences in visual or motor abilities (Mahmoudvand, Sheibani, Keshavarz, et al., 2016).

Another study used a Morris water maze to test infected mice's spatial reference memory by having them find a platform submerged in water (Daniels, Sestito, & Rouse, 2015). The study found that infected mice demonstrated worse memory for the location of the platform after it had been moved (as measured by the mice crossing the area where the platform used to be). Additionally, the infected mice performed more poorly at finding the moved platform after longer delays from the learning trial (e.g. two hours versus 24 hours). Three other studies found similar impairment in spatial memory based on infected mice's worse performance in maze navigation. However, another study found no impairment in spatial memory in infected rats (Worth, Thompson, & Lymbery, 2014).

In another study, infected mice showed impaired learning and memory as measured by a two-way active avoidance equipment as compared to control mice and mice infected with the parasite *Toxocara canis.* Interestingly, infected mice also showed increased anxiety as measured by the elevated plus maze (Corrêa, Chieffi, Lescano, & Vieira, 2014).

While not always part of conscious memory, studies often consider fear-conditioning memory as a type of memory. Ihara et al. (2016) used a fear-conditioning task (pairing an auditory cue with a foot shock) to determine if infected mice varied in their memory of fear-eliciting cues. Infected mice had significantly less freezing behavior when the auditory cue was played (after the mice had been conditioned), suggesting reduced fear conditioning memory (Ihara et al., 2016). Additionally, another study found impaired olfactory memory in infected male mice (Worth et al., 2014). However, these findings of reduced fear conditioning and olfactory memory have not been well-replicated by other studies.

Importantly, different strains of *T. gondii* may have different behavioral and cognitive effects on mice. For example, Kannan et al. (2010) examined the Prugniaud and ME49 strains of *T. gondii* and how they may affect mouse working memory. Mice IgG seropositive with the ME49 strain showed impaired spatial working memory while IgG seropositive mice with the Prugniaud strain did not. While some research has been performed on different strains in mice, to our knowledge, no such research has been performed on *T. gondii* seropositivity in humans and as such, these results need to be replicated in human studies.

In sum, most of the studies examining *T. gondii* infection in mice found impaired spatial, short-term, and delayed memory in infected mice compared to control mice. These results show replication by other studies with fewer studies in the literature finding no association between *T. gondii* seropositivity and memory in mice. Interestingly, one study found mice with AD pathology and *T. gondii* IgG seropositivity displayed significantly worse learning and memory functioning in some tasks compared to mice with only AD pathology, but this study has yet to be replicated. Thus, overall, infected mice seem to consistently show impairment in many areas of memory functioning.

Memory and working memory – Human studies. In humans, a study in school-aged children examined *T. gondii* seropositivity and its effects on cognitive function, specifically memory. A large study (n=1755), Mendy, Vieira, Albatineh, and Gasana (2015) found *T. gondii* seropositive children had worse working memory and short-term memory skills (as measured by the WISC-R Digit Span subtest) compared to seronegative children. The authors also found that *T. gondii* seropositive children who also had serum vitamin E levels below the median had significantly worse working memory and short-term memory scores than children with vitamin E serum levels at or above the median.

At different stages in the lifespan, *T. gondii* seropositive young adult and elderly men had greater impairment in delayed and immediate memory compared to controls (Kannan & Pletnikov, 2012). In addition, *T. gondii* seropositive subjects performed worse on the N-back neuropsychological test compared to seronegative controls. When the researchers adjusted the scores for age and sex, however, the significance between scores was lost (Guenter, Bieliński, Deptuła, & Zalas-więcek, 2012). Also, when Flegr, Guenter, Bieli, Deptuła, and Zalas-więcek (2012) re-analyzed the data separately for males and females, *T. gondii* seropositive females performed more poorly than did seronegative females on the N-back test.

Hamdani et al. (2017) examined the effect of exposure to many viruses and parasites including *T. gondii* on memory functioning. The subjects with previous exposure to HSV-1, HSV-2, CMV, and *T. gondii* had worse scores on a test of working memory (backward digit span and letter number sequencing) than those without exposure to all four infectious agents. However, the researchers did not find an association between exposure to only *T. gondii* and memory functioning.

While Gale, Erickson, Brown, and Hedges (2015) did not find a main effect for *T. gondii* seropositivity on cognition in a large study (n= 1785), they did find that those seropositive for both *T. gondii* and *H. pylori* had lower functioning on a serial-digit learning task (testing working memory), suggesting that having more than one infectious disease may result in greater loss of memory function than having just one infectious disease (Gale, Erickson, Brown, & Hedges, 2015).

In a large study similar to the studies by Gale et al. (2014) and Gale, Erickson, Berrett, Brown, and Hedges (2016), Pearce, Kruszon-Moran, and Jones (2014) found those *T. gondii* seropositive had worse performance on the symbol-digit substitution test and the serial-digit learning test, both measures of working memory (as well as processing speed), in models without any adjustments made for covariates. When the researchers added age, race/ethnicity, gender, and foreign birth to the models, those *T. gondii* seropositive were more likely to have symbol-digit substitution test scores in the worst quartile. However, when the researchers added all the covariates to the models (including life-style variables, demographic factors, and medical conditions), the likelihood that *T. gondii* seropositive subjects would have worse scores was the same as those who were seronegative. When the authors examined interactions between *T. gondii* seropositivity and poverty level, however, for those in the lowest income category, *T. gondii* seropositive subjects had worse performance on the symbol-digit substitution test even after controlling for covariates. Additionally, those born outside the United States had worse performance on the symbol-digit substitution test while controlling for covariates.

Older adults. Gajewski, Falkenstein, Hengstler, and Golka (2014) and Gajewski, Falkenstein, Hengstler, and Golka (2016) examined working memory in older adults (mean age: 70 years) and found *T. gondii* seropositive older adults had lower scores on a verbal memory test

(Verbal Learning and Memory Test) for both immediate memory and delayed recognition portions. Additionally, seropositive older adults had lower performance on the Word Fluency Test testing long-term semantic memory. On the N-back task, *T. gondii* seropositive older adults performed more poorly, which is likely indicative of worse working memory. Interestingly, *T. gondii* seropositive subjects with older age had worse performance on the verbal memory task, which was not true for seronegative subjects (Gajewski et al., 2014, 2016).

In another study, Mendy, Vieira, Albatineh, and Gasana (2015) examined memory functioning in a large sample of older adults (N=4485; 60 years and older) using the verbal memory portion of the Mini Mental State Examination (MMSE) and the East Boston Memory Test. They found that *T. gondii* seropositive older adults had associated decreased immediate memory functioning but no associations for delayed memory. Additionally, the researchers found higher serointensity of anti-*T. gondii* antibodies (a higher concentration of antibodies) was negatively associated with immediate memory scores. Lastly, the authors found more significant associations between *T. gondii* seropositivity and serointensity and worse immediate memory scores in white Americans compared to other ethnicities (Mendy et al., 2015).

Overall, the most replicated results show *T. gondii* seropositivity seems to be associated with working memory impairment in certain circumstances such as participants having other infections (including *H. pylori,* HSV, CMV), being born outside the United States, and having lower income. These interactions may be due to several factors. For example, having multiple infections may result in increased inflammation which may reduce cognitive efficiency and/or capacity. Additionally, lower incomes are often associated with lower educational levels, which lower education levels may result in fewer neuronal connections allowing for more negative impact from *T. gondii* on memory (Borroni, Premi, Bozzali, & Padovani, 2012). If true, high

education may protect against the negative effects on memory of *T. gondii* seropositivity by providing more neural connections and more neuronal paths for use in case some paths become damaged by *T. gondii.* Overall, researchers tend to find no or few main effects for *T. gondii* seropositivity on working memory for adults (Gale et al., 2015; Hamdani et al., 2017; Brad D Pearce, Kruszon-Moran, & Jones, 2014). In older adults, more studies found main effects associated with *T. gondii* seropositivity including decreased verbal memory (both immediate and delayed), working memory, and long-term semantic memory (Gajewski et al., 2014, 2016). Increasing age may amplify the influence *T. gondii* seropositivity has on cognition, making main effects more common in studies of older adults. However, the studies for older adults tend to have much smaller sample sizes than the studies performed on middle-age or younger adults. The few studies performed in children examining *T. gondii* seropositivity and memory functioning have not been consistently replicated.

Processing speed. In a large, community-based sample (n=4178), Gale et al. (2014) did not find a correlation between *T. gondii* seropositivity and cognitive functioning (specifically for a test of processing speed with attention and short-term memory components) for adults between the ages of 20-59 years. While no main effects were found, the authors did see an association between *T. gondii* seropositivity and decreased processing speed among non-Hispanic Blacks and other race-ethnicities when compared to non-Hispanic Whites. Additionally, the study found having low levels of education was associated with worse processing speed. They also found those subjects who were *T. gondii* seropositive and had a lower poverty-to-income ratio (meaning closer to the official United States poverty threshold) also had slower processing speed.

In addition to working memory, the previously-mentioned study by Pearce et al. (2014) examined processing speed using the Simple Reaction Time Test. Like their findings with working memory, those *T. gondii* seropositive had slower reaction times than those seronegative. However, when the researchers added all covariates to the model, they did not find any significant differences between those *T. gondii* seropositive or seronegative (Pearce et al., 2014).

In another study by Pearce et al. (2013), researchers measured acoustic startle response latency in both subjects with schizophrenia and controls in order to measure neural processing speed. The study found larger acoustic startle latency (indicating slower processing speed) for *T. gondii* seropositive subjects in both subjects with schizophrenia and controls. These findings may reflect similar findings of slowed psychomotor speed in *T. gondii* seropositive mice (Pearce et al., 2013).

Massa et al. (2016) measured the acoustic startle response in a sample of mostly African Americans that included subjects with and without psychiatric disorders, such as MDD and schizophrenia. The authors indicate that acoustic startle response is an index of early information processing ability. They found a larger acoustic startle response for those *T. gondii* seropositive, even when adjusting for psychiatric illness and demographic factors, indicating those *T. gondii* seropositive had slower early information processing (Massa et al., 2016).

Fewer studies have been performed examining processing speed and *T. gondii* seropositivity than for memory functioning. Overall, the findings seem to be mixed in terms of main effects of *T. gondii* seropositivity. Some studies (particularly those with larger sample sizes) have not found a main effect for *T. gondii* seropositivity on processing speed but have found certain circumstances that seem to interact with *T. gondii* seropositivity to effect processing speed, specifically ethnicity, lower levels of education, and lower income (Gale et al.,

2014; Pearce et al., 2014). As with the memory studies, some of the interaction effects seen in studies may again be due to a larger cognitive reserve for those with higher levels of education and income. Other studies found *T. gondii* seropositivity was associated with decreased processing speed, regardless of other demographic variables (Massa et al., 2016; Pearce et al., 2013). Because the studies that found main effects used different measures (Simple Reaction Time Test versus acoustic startle response), the differences in main effects may be due to differences in measures.

Language functioning. Compared to other domains, researchers have performed the least amount of research on the effect of *T. gondii* seropositivity on language functioning. One study performed in children by Mendy et al. (2015) found *T. gondii* seropositive children had lower reading skills (as measured by the WRAT-R). Additionally, two studies in older adults used the Word Fluency Test to measure long-term semantic memory (Gajewski et al., 2014, 2016). However, some researchers may also consider the Word Fluency Test to measure verbal fluency. The studies found those *T. gondii* seropositive had lower performance on the Word Fluency Test, which may indicate worse verbal fluency for those *T. gondii* seropositive. Overall, however, we can make few conclusions about the potential effect of *T. gondii* seropositivity on language functioning due to the scarcity of the research and lack of replication.

Executive functioning. Compared to language functioning, more studies have been conducted specifically examining executive functioning and *T. gondii* seropositivity, but there are still relatively few. Beste, Getzmann, Gajewski, Golka, and Falkenstein (2014) assessed "goal-directed behavior" in healthy older adults using an auditory distraction task. They found that *T. gondii* seropositive older adults had slower reaction times to the tasks and therefore, less

proficient "goal-directed behavior." Specifically, individuals with higher anti-*T. gondii* IgG antibody titers had slower reaction times (Beste et al., 2014).

In a longitudinal study of older adults, researchers examined multiple cognitive domains over a five-year span (Nimgaonkar et al., 2016). *T. gondii* seropositive older adults had a faster decline in executive functioning as well as faster overall changes in cognition as measured by the MMSE than those seronegative.

Few studies have examined possible associations between *T. gondii* seropositivity and executive functioning and more replication studies are needed. The few studies performed have been done in older adults and have found decreases in executive functioning for those *T. gondii* seropositive (Beste et al., 2014; Nimgaonkar et al., 2016). However, in the study by Nimgaonkar et al. (2016), the authors do not explain clearly what measures of executive functioning were used, making interpretation and replication by other researchers difficult. Executive functioning is made of many different components (planning, inhibition, switching, etc.) and Nimgaonkar et al., (2016) could be measuring any or all of these components while Beste et al. (2014) is likely measuring inhibition. To properly assess how *T. gondii* seropositivity may influence executive functioning, researchers need to examine the different components of executive functioning rather than just inhibition.

Null or opposite findings. While many studies mentioned above have found an association between *T. gondii* seropositivity/infection and effects on cognitive functioning, not all studies have found an association. For example, one study found motor coordination and sensory function problems in chronically infected mice (seven weeks post infection) but did not find any changes in cognitive functioning (as assessed by object recognition and object placement tasks; Gulinello et al., 2010). Additionally, Dickerson et al. (2014) examined the

association between cognitive functioning as measured by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) in non-psychiatric controls seropositive for both acute and latent toxoplasmosis. The researchers found a low total score on the RBANS was associated with acute toxoplasmosis infection but did not find any associations for those chronically *T. gondii* seropositive. However, the RBANS has been shown by Duff, Hobson, Beglinger, & Bryant (2010) to have lower sensitivity in detecting mild impairments so mild impairments associated with *T. gondii* seropositivity may have gone unnoticed in the study.

Many studies find a negative association between *T. gondii* seropositivity and cognitive functioning, or a decrease in cognitive functioning associated with seropositivity. However, a few have found a positive association – *T. gondii* seropositivity enhancing aspects of cognitive functioning. One such study examined young adults' executive functioning abilities of switching and inhibition, and found those *T. gondii* seropositive performed better at switching tasks (Stock, von Heinegg, Köhling, & Beste, 2014). However, this positive finding has yet to be replicated by other studies.

Lastly, many studies have examined *T. gondii* seropositivity in groups with various disorders, such as bipolar disorder, HIV, and schizophrenia in comparison to healthy controls. While Ene et al. (2016) found significant association between *T. gondii* seropositivity and decreased overall cognitive performance in those who were HIV positive, they did not find an association between seropositivity and cognition in healthy controls. In another study, a similar result was seen for subjects with bipolar disorder (Hamdani et al., 2015). In contrast, two studies did not find an association between cognitive functioning (such as memory and executive functioning) and *T. gondii* seropositivity in people with schizophrenia (Shirts et al., 2009; Yolken, Torrey, Lieberman, Yang, & Dickerson, 2011). In studies examining bipolar disorder,

T. gondii may interact with neuroinflammation seen in the disorder, influencing changes in cognition or vice versa (Hamdani et al., 2015). However, cognitive deficits in schizophrenia tend to be larger than those common to other psychological disorders so deficits associated with *T. gondii* seropositivity may be masked.

Behavioral Changes Associated with *T. gondii*

Given the widely accepted notion that cognition is associated with behavior, researchers expect to find behavioral changes due to *T. gondii* infection in addition to cognitive changes, and, indeed, researchers have found behavioral changes in rodent models and humans. In studies examining rats, *T. gondii* infected rats were observed to be more active and have less fear of novelty than uninfected rats, as well as to behave in more potentially self-destructive ways such as demonstrating a lack of fear of and attraction to cat odors, specifically to the odor of cat fur (Fekadu et al., 2010; Prandovszky et al., 2011; Worth et al., 2014). Specifically, Hutchinson, Aitken, and Wells (1980) and Hay, Hutchinson, Aitken, and Graham (1983) found infected mice showed decreased exploration of novel areas. Additionally, Berdoy, Webster, and Macdonald (1995) found infected rats were more likely to approach a human observer than control rats. However, in mice, Gulinello et al. (2010) found no difference in the amount of time spent exploring novel objects between chronically infected and control mice. The most cited and replicated result in this area seems to an attraction to cat fur odor with seven out of fifteen studies replicating the result (Worth et al., 2014).

The area of behavior changes most studied in rodents by researchers seems to be motor deficits associated with *T. gondii* infection. Chronically infected mice had motor coordination deficits as tested by a balance beam assay and gait analysis. Specifically, mice had increased number of slips on the balance beam and increased time to cross the balance beam (Gulinello et

al., 2010). In addition, other studies found that infected mice fell more often from a rotating cylinder (Worth et al., 2014). Lastly, approximately equal number of studies have found a difference in activity level in infected mice (either decreased or increased activity) while other studies have found no difference in activity (Worth et al., 2014). The differences in results of motor abilities may be due to decreased levels of dopamine and other catecholamines in infected mice brains, which has been seen in other studies (Gulinello et al., 2010). Additionally, these differences in results may be due to differences in mouse strains and/or *T. gondii* strains used across studies (Worth et al., 2014).

Along with decreased novelty seeking and motor deficits, some studies have found increased anxiety in mice infected with *T. gondii.* Anxiety in rodents in such studies is measured by decreased time spent on open arms in the elevated plus maze, decreased time spent in social interaction, and decreased time spent in the middle of an open field (Worth et al., 2014). Some studies using the open field test found *T. gondii* infected mice spent less time in the central part of the open field and one study found a sex difference – infected female mice spent less time in the middle of the open field but not infected male mice. However, another study found decreased anxiety in infected mice while another found no difference between infected and control mice (Worth et al., 2014). The differences seen in anxiety in mice may be due to differences of how studies measure and define anxiety.

In a human study examining the possible behavior changes due to *T. gondii* seropositivity, Flegr (2007) found personality differences based on gender using Cattell's 16 Personality Factor questionnaire and the Cloninger's Temperament and Character Inventory. In *T. gondii* seropositive males, the researchers found evidence of higher vigilance, suspicion, and jealousy, but lower superego strength. However, *T. gondii* seropositive females demonstrated

higher superego strength, and more factors of warmth, conscientiousness, and moral adherence. They also found higher levels of apprehension and lower levels of novelty seeking in both *T. gondii* seropositive men and women as compared to controls. In addition, Flegr, Kodym, and Tolarova (2000) found a positive correlation between the duration of *T. gondii* seropositivity and personality changes, but only in women. Personality changes associated with *T. gondii* seropositivity have rarely been studied in the literature, however, and the results are often difficult to translate into observable, functional differences between those seropositive and seronegative.

Some studies have found additional behavior changes in humans including reduced concentration and a longer reaction time for *T. gondii* seropositive individuals as well as increased traffic accidents, especially in those with higher titers of anti-*T. gondii* IgG antibodies (Fekadu et al., 2010). Other studies have shown diminished psychomotor performance in *T. gondii* seropositive individuals (Dalimi & Abdoli, 2012). Another study did not find an association between financial risk-taking and *T. gondii* seropositivity in young adult females, however (Lanchava, Carlson, Blanka, Flegr, & Nave, 2015). Overall, the studies assessing reaction time are more well-replicated than those assessing personality factors or financial risk-taking. Additionally, a slower reaction time (and subsequently more traffic accidents) are theoretically related to results showing slower processing speed for those seropositive (Gale et al., 2014; Massa et al., 2016; Pearce et al., 2013).

From the existing research, the reasons for these behavior modifications in the host of the parasite are still unclear but one hypothesis is that the parasite may change the host behavior in order to enhance its transmission rate (Flegr, 2007). Additionally, Worth et al. (2014) suggest three possibilities for mechanisms on how *T. gondii* might change host behavior, namely: 1)

localization of cysts in the brain leading to possible structural damage; 2) variation of neurotransmitters; and 3) change in immune response of the brain. We will discuss the specifics of these three possibilities in the next section.

Pathology of *T. gondii*

In addition to cognitive and behavioral changes associated with *T. gondii* seropositivity, studies have found various neurological differences in infected brains, changes that might may be associated with the development or progression of AD and have been hypothesized to interact with behavioral and cognitive changes associated with *T. gondii* seropositivity. These differences include immune-response changes, specifically inflammation in the CNS, structural neurological changes due to *T. gondii* cysts, and dopamine-transmission abnormalities. Additionally, we will also discuss specific connections to AD pathology in this section.

Inflammation in the CNS. Infection in the brain by *T. gondii* initiates an immune response driven by increased CD4 and CD8 T cells. The immune response to *T. gondii* generates long-term antigen persistence to the parasite (Brake, 2003). In addition to T cell activation, *T. gondii* infection can cause neuroinflammation due to microglia and over-expression of proinflammatory cytokines. In fact, continuous production of proinflammatory cytokines is needed for the human body's resistance to *T. gondii* (Webster & Mcconkey, 2010). Such neuroinflammation is present in both *T. gondii* seropositivity and AD (Freidel, Martin-Solch, & Schreiter-Gasser, 2002). In a study of chronically infected mice, Hermes et al. (2008) found increased inflammation in the brain causing neuronal damage including increased CD4 and CD8 T cells and activated microglia, particularly in perivascular areas. Additionally, mice genetically more resistant to *T. gondii* infection still showed evidence of increased inflammation in perivascular areas of the brain but less inflammation as compared to other infected mice (Hermes

et al., 2008). Specifically in AD, autoaggressive microglia can produce neurotoxins in response to amyloid exposure (Li et al., 2015). Similarly, in *T. gondii* infection, pro-inflammatory cytokines can release nitric oxide which may lead to neuronal and behavioral dysfunction and/or alter neurotransmitter systems leading to mood and behavior changes (Fekadu et al., 2010).

Of interest is a case of an immunocompromised patient with cerebral toxoplasmosis that was associated with dementia of the Alzheimer type (Freidel et al., 2002). The patient was infected with *T. gondii* one to one and a half years before the presentation of cognitive impairment. Given these findings, inflammation associated with chronic *T. gondii* infection could function as a factor in neurodegenerative diseases in individuals who are genetically susceptible (Hermes et al., 2008).

Structural neurological changes. *T. gondii* seropositivity often indicates cysts in the brains of both humans and rodents. However, there is inconclusive evidence on whether the number of cysts in a brain (in most studies, rodent brains) plays a role on behavioral or cognitive changes (Worth et al., 2014). Structurally, there is some evidence that *T. gondii* infections have the greatest impacts on the hippocampus and amygdala, areas of importance in AD as well. Fabiani et al. (2015) reported *T. gondii* cysts are mainly found in the hippocampus, amygdala, basal ganglia, cerebellum, cerebral cortex, brain stem, and olfactory bulb. Interestingly, researchers also found *T. gondii* cysts in areas high in dopamine including the amygdala and nucleus accumbens (Fabiani et al., 2015). One study found that cysts in certain combinations of brain areas were associated with increased risk taking behavior, indicating the parasite may inhabit particular functional areas of the brain rather than certain areas or structures (Worth et al., 2014). However, other studies have not found *T. gondii* cysts to have a preference for particular areas of the mouse brain (Ihara et al., 2016). The weights of chronically infected mice brains are

often decreased compared to control brain weights, however. Other studies have observed mild to moderate ventricular dilation in infected brains of mice (Dalimi & Abdoli, 2012; Worth et al., 2014).

Dopamine transmission abnormalities. Along with structural brain changes, studies have found differences in dopamine levels in the brains of mice infected with *T. gondii*, albeit with mixed results. Some studies have found increases in dopamine levels (Hodkova, Kodym, & Flegr, 2007; Prandovszky et al., 2011), whereas others have found decreased levels of dopamine (Gulinello et al., 2010). In one study, Prandovszky et al. (2011) found increased dopamine in the *T. gondii* cyst-containing neural cells and that infected cells release up to 350 percent more dopamine than uninfected cells. In support of a dopamine imbalance in the brain of infected mice and humans is the association between schizophrenia and *T. gondii* seropositivity found by numerous studies, particularly the imbalance of dopamine between mesolimbic and mesocortical regions of the brain (Hodkova et al., 2007). Specifically, even in chronic infection, *T. gondii* can synthesize L-DOPA and tyrosine, both precursors to dopamine. This production of dopamine precursors by the parasite has resulted in increased dopamine in rodent brains (Webster et al., 2013). While lower levels of dopamine have been observed in AD (Dalimi & Abdoli, 2012), because *T. gondii* has the ability to make its own dopamine, the parasite may contribute to dopamine imbalances seen in infection, making the possible variation in dopamine levels found in *T. gondii* infection of significance for AD pathology.

AD-associated pathology. ApoE allele E4 is a well-known risk factor for developing AD. One study examined ApoE genotype and *T. gondii* seropositivity in persons with and without dementia and found higher prevalence of dementia in those who were *T. gondii* seropositive and who were also non-carriers for the E4 allele. Additionally, when using a

regression to predict dementia, *T. gondii* seropositive subjects had a higher risk to develop dementia, even when researchers included covariates such as age, sex, and ApoE E4 status in the model (Yahya et al., 2017). Thus, *T. gondii* seropositivity was a predictor for dementia in this study while ApoE E4 status was not. This is contradictory to most findings on ApoE and development of dementia but may provide some insight into the role of *T. gondii* seropositivity on development AD.

Another common finding in the pathology of AD is the deposition and lack of clearance of beta-amyloid plaques. In AD, microglia cells go into a state of senescence and do not remove beta-amyloid plaques effectively. These microglia also increase neurodegeneration by releasing proinflammatory cytokines at later stages of AD. In a study investigating the effect of *T. gondii* seropositivity on beta-amyloid deposition in mice that rapidly develop beta-amyloid pathology, the authors found *T. gondii* infection resulted in reduced number and volume of beta-amyloid plaques as compared to control mice (Möhle et al., 2016). This change in beta-amyloid plaques can be viewed as either beneficial or detrimental due to the other immune cells associated with the removal of beta-amyloid, which cascade of immune cells can worsen neuroinflammation at certain points in AD. However, the authors speculate that *T. gondii* infection may act as a strong stimulus for the immune system, which may ultimately overcome some of the pathology of AD (Möhle et al., 2016). Similar results were found in two other studies. Specifically, in a line of AD-model mice (Tg2576, the Swedish APP mutation), one study showed *T. gondii* infection reduced cerebral beta-amyloid deposition and increased levels of anti-inflammatory cytokines. The researchers attributed this to the immunosuppressant effects of *T. gondii* infection (Carter, 2013). Another mouse study found that *T. gondii* seropositivity in an AD model of mice (Tg2576) caused less neuronal death in the hippocampus and fewer beta-amyloid plaques in

infected mice. AD-model mice that were *T. gondii* seronegative had reduced cognitive capacities compared to chronically infected (six months post infection) mice (Jung et al., 2012). Lastly, evidence from another study suggested opposite reasons for beta-amyloid deposition: a host may make beta-amyloid plaques to protect against *T. gondii* because they sequester iron and iron is needed for replication of tachyzoites (Prandota, 2011). Thus, there seems to be contradictory evidence on the relationship between *T. gondii* infection and beta-amyloid disposition and clearance. Some evidence suggests the parasite is associated with less beta-amyloid while others suggest beta-amyloid is a response to infection.

Anosmia is often associated with AD as well as other neurodegenerative disorders and disorders associated with or potentially started by neuroinflammation. Indeed, olfactory dysfunction is often one of the first signs of AD. Several studies have found that *T. gondii* invades and encysts in olfactory bulb cells in mice (Prandota, 2014). Additionally, one study found that in male mice, *T. gondii* infection modulated genes associated with olfactory functioning. However, overall olfactory tests were normal in infected mice (Prandota, 2014). On the other hand, one author goes so far as to suggest that the damage caused to the olfactory bulb by *T. gondii* may be responsible for the anosmia and olfactory dysfunctions seen in neurodegenerative diseases such as AD (Prandota, 2014). It is clear that both *T. gondii* infection and AD are associated with olfactory dysfunction but the relationship between the two is unclear.

Other results show associations between *T. gondii* seropositivity and AD that do not involve the "classic" pathology of AD. Particularly, Prandota et al. (2011) reported changes in cell cycle in both *T. gondii* infection and AD as well as disturbances of cerebral metabolic rate for glucose. Lastly, as mentioned previously, Mahmoudvand, Sheibani, Keshavarz, et al. (2016) examined the effect of donepezil on memory functioning in *T. gondii* IgG seropositive mice.

Importantly, donepezil is a medication often used in the treatment of cognitive problems associated with AD. Mice treated with donepezil had the short-term memory impairment, purportedly caused by *T. gondii* IgG seropositivity, reversed.

Given the evidence of cognitive and behavioral changes, neurological structural differences and pathology as well as some preliminary evidence of higher *T. gondii* seroprevalence in persons with AD, we hypothesize that *T. gondii* seropositivity may be an important risk factor in the development of AD. Additionally, given the previous evidence in both mouse and human studies, we predict that *T. gondii* seropositivity will negatively affect cognition in older adults with and without AD and will more negatively impact those with AD than those without. In the present study, we will assess a larger sample size of participants than in previous studies performed for associations between *T. gondii* seropositivity, AD, and cognitive function in older adults.

Hypotheses

In this study, we used subjects from the Alzheimer's Disease Research Center (ARDC) at Washington University (WU) to examine our hypotheses. Specifically, we had a group of patients with AD and a control group of patients without dementia for comparison. We had data on *T. gondii* seropositivity, gender, age, education, socioeconomic status, ethnic background, neuropsychological data assessing multiple cognitive domains, and other variables that we used as covariates in our hypotheses and models. In particular, we hypothesized that:

1. *T. gondii* seropositivity will differ between AD and control groups. That is, *T. gondii* seropositivity will be more prevalent in patients with AD. We will evaluate this hypothesis using *t*-tests.

2. *T. gondii s*eropositivity will predict AD diagnosis in matched groups. We will evaluate this hypothesis using propensity score matching.
3. Memory will be impaired for *T. gondii* seropositive subjects in both the AD and control groups. We will assess memory functioning by using scores on Wechsler Memory Scale Revised (WMS-R) Logical Memory IA subtest, WMS-R Logical Memory IIA subtest, WMS-R Associate Learning subtest, and Selective Reminding Test (SRT). We will assess this hypothesis using linear regressions.
4. *T. gondii* seropositivity will predict worse memory functioning in matched groups, using the same neuropsychological tests as in the previous hypothesis. We will assess this hypothesis using propensity score matching.
5. Working memory will be impaired for *T. gondii* seropositive subjects in both the AD and control groups. We will assess working memory functioning by using scores on Wechsler Adult Intelligence Scale (WAIS) Digit Span Forward subtest, WAIS Digit Span Backward subtest, WMS Mental Control subtest, and WMS Letter Number Sequencing subtest. We will assess this hypothesis using linear regressions.
6. *T. gondii s*eropositivity will predict worse working memory functioning in matched groups, using the same neuropsychological tests as in the previous hypothesis. We will assess this hypothesis using propensity score matching.
7. Processing speed will be impaired for *T. gondii* seropositive subjects in both the AD and control groups. We will assess processing speed by using scores on the WAIS Digit Symbol subtest and Trail Making Test A and B. We will assess this hypothesis using linear regressions.

8. *T. gondii* seropositivity will predict worse processing speed in matched groups, using the same neuropsychological tests as in the previous hypothesis. We will assess this hypothesis using propensity score matching.
9. Language functioning will be impaired for *T. gondii* seropositive subjects in both the AD and control groups. We will assess language functioning by using scores on the Boston Naming Test and WAIS Information subtest. We will assess this hypothesis using linear regressions.
10. *T. gondii* seropositivity will predict worse language functioning in matched groups, using the same neuropsychological tests as in the previous hypothesis. We will assess this hypothesis using propensity score matching.
11. Executive functioning will be impaired for *T. gondii* seropositive subjects in both the AD and control groups. We will assess executive functioning by using scores on the WAIS Block Design subtest, Category Fluency (Animals and Vegetables subtests), and Word Fluency S & P test. We will assess this hypothesis using linear regressions.
12. *T. gondii* seropositivity will predict worse executive functioning in matched groups, using the same neuropsychological tests as in the previous hypothesis. We will assess this hypothesis using propensity score matching.

Statistical Analyses Theory

In order to test the above hypotheses, we will use two statistical methods. The first is propensity score matching and the second is linear regression.

Propensity Score Matching

One difficulty with observational or non-experimental settings is that subjects are seen in either the treatment condition or the control condition; the experimenters cannot observe the outcome of the same subject in both conditions. Thus, observational experiments often compare different subjects in the treatment and control conditions, which means different covariates are present in each group. This can lead to biases in the data because there can be significant differences between groups in either measured or unmeasured variables (Andrade, 2017). Ideally, in our study, we would have liked to observe participants before they became *T. gondii* seropositive and then after they became *T. gondii* seropositive, both under the same conditions, which would have eliminated any confounding variables that could potentially account for some of the effect of developing AD due to *T. gondii* seropositivity or the effect of *T. gondii* seropositivity on neuropsychological test scores. However, that was not possible with this experiment as data were collected by WU before *T. gondii* seropositivity status was determined. As such, we determined that using propensity score matching (PSM) would help ameliorate the difficulties presented with the confounding variables in each treatment group and help come closer to determining the causal role of *T. gondii* seropositivity on development of AD and/or decreased neuropsychological test scores.

PSM is a statistical method used in non-experimental settings to estimate causality. Specifically, PSM is one technique used to estimate average treatment effects by adjusting for differences in observable covariates. In PSM, propensity scores are estimated and used to match similar participants in each treatment group. The propensity score for a subject is the probability that subject will receive treatment (in this case, be seropositive for *T. gondii*) given all the control variables we want to account for in the statistical model (such as age, gender, education, etc.;

StataCorp, 2013). In our model, we used a logistic regression to make the propensity scores, using presence or absence of *T. gondii* seropositivity as the outcome variable and age, education level, SES status, ethnicity, gender, and the presence or absence of ApoE E4 allele as covariates for the model. Matches for each subject in the treatment condition (*T. gondii* seropositive) are found by determining the closest subject in the control condition (*T. gondii* seronegative) based on their propensity scores. Overall, the matched treatment and control groups are similar on average across the covariates included in the model. The PSM technique estimates the average treatment effects (ATE) which is the average of the difference between the observed and potential outcomes of each participant (having AD or not). Additionally, ATE determines if any differences in outcome between the treatment and matched "control" group are due to the result of the treatment (e.g. being *T. gondii* seropositive). Thus, if a large or significant difference is seen in outcome (having AD) between the treatment group (*T. gondii* seropositive) and matched control group (*T. gondii* seronegative) then we can conclude that treatment (*T. gondii* seropositivity) significantly influences the development of AD (StataCorp, 2013). We use the same technique and theory for the outcome of neuropsychological test scores and how they are affected by the treatment (*T. gondii* seropositivity).

Assumptions. PSM has three assumptions associated with its use. The first is the independent and identically distributed assumption. This assumption specifies that the outcome and treatment status of each subject need to be separate or unrelated to the outcome and treatment status of all the other participants included in the study. In other words, in our study, one participant being *T. gondii* seropositive cannot influence another participant in the study being *T. gondii* seropositive, as well as one participant developing AD cannot influence another participant developing AD and one person having lower neuropsychological test scores cannot

influence another person having lower neuropsychological test scores. This assumption excludes the use of hierarchical or longitudinal data in PSM techniques. In our study, it also makes using data from immediate family members problematic due to the potential influence of genetics in developing AD (Alzheimer's Association, 2012) as well as the potential influence of family members having the same potential factors that would promote becoming seropositive for *T. gondii* (such as cat ownership, eating undercooked meat, place of residence, etc.). Unfortunately, we could not determine if family members of the participants were included in the study. This data was not available in the dataset received from WU. As such, we acknowledge that this is a limitation for the study because it can potentially affect causation if immediate family members are included in the study (StataCorp, 2013).

Another assumption associated with PSM is the conditional independence assumption, which states that once all the observable variables have been controlled for, the outcome (in this case, AD or neuropsychological test scores) is independent of assignment to the treatment group (developing *T. gondii* seropositivity). In other words, there cannot be any unobserved factors that influence *T. gondii* seropositivity, developing AD, and neuropsychological test scores. This assumption is fairly robust but can be violated if the unobserved variables are on the "causal pathway" of *T. gondii* seropositivity leading to the development of AD or influencing neuropsychological test scores. Known factors influencing *T. gondii* seropositivity that are not included in our study include cat ownership and meat-consumption behaviors such as eating undercooked meat (Alvarado-esquivel et al., 2014; Wei, He, Yang, Lindsay, & Peng, 2016). However, to our knowledge, these factors do not directly relate to the development of AD or influence neuropsychological test scores. Country of residence has been associated with *T. gondii* seropositivity as well as loosely associated with the development of AD (Brookmeyer,

Johnson, Ziegler-graham, & Arrighi, 2007; Dubey & Jones, 2008). However, all participants in the study lived in the United States, eliminating the variation in this factor (StataCorp, 2013).

Lastly, the overlap assumption associated with PSM states that each participant needs to have a positive probability of receiving treatment, ensuring that each participant could potentially have all treatment levels. This means in our study, that each participant has to have a positive probability of being *T. gondii* seropositive. Given that all participants lived in the United States, a country known to have an infections with *T. gondii*, it is likely that each participant has a positive chance of contracting the disease (StataCorp, 2013).

Proposed models. The models we used with PSM include testing the hypothesis that *T. gondii* seropositivity influences the development of AD as well as the hypotheses that *T. gondii* seropositivity will negatively affect different domains of cognition including memory, working memory, language functioning, executive functioning, and processing speed. In each model, we used the following covariates: age, Hollingshead SES, education level, gender, ethnicity, and presence or absence of the ApoE E4 allele. We chose these variables as covariates as they all potentially play a role in influencing the development of AD as well as neuropsychological test scores (Abate, Marziano, Rungratanawanich, Memo, & Uberti, 2017; Dorey, Chang, Liu, Yang, & Zhang, 2014; Fiest et al., 2016; S D Gale et al., 2014; Levy et al., 2004). By using PSM in these proposed models, we will be able to better control for potential cofounding variables as well as better establish a causal link between *T. gondii* seropositivity and the development of AD, if such a link is present. Also, for the neuropsychological test scores outcomes, if a large or significant difference is seen in outcome (neuropsychological test scores) between the treatment (*T. gondii* seropositivity) and matched control group (*T. gondii* seronegativity) then we can

conclude that treatment (*T. gondii* seropositivity) significantly influences the neuropsychological test scores.

Linear Regression

Bivariate linear regression is a statistical method used to predict an outcome variable (Y) using a predictor variable (X) with a linear function. Such a method assumes X and Y are related linearly. In the case of our study, we use linear regression to predict scores on various neuropsychological tests (Y) based on the presence of *T. gondii* seropositivity or anti-*T. gondii* IgG antibody concentration (X). We determined neuropsychological test scores to be the outcome variable because it is more probable that *T. gondii* seropositivity would influence neuropsychological test scores than the other way around. The linear function of a regression produces as a result multiple R, which indicates how well the predicted scores for Y match the actual scores for Y. The larger the R is the closer the actual and predicted Y scores are (Warner, 2013). Additionally, many factors may contribute to the correlation between neuropsychological test scores and *T. gondii* seropositivity including gender, age, SES, education, and ApoE status. We included each of these as a covariate in the regression models.

Assumptions. In a bivariate linear regression, the assumption for the outcome variable is that it is quantitative, and the predictor variable is assumed to be either quantitative or dichotomous. Another assumption in bivariate linear regression is that the relationship between the outcome and predictor variables is linear, as mentioned previously. Linear regressions are not immune to the effect of extreme outliers, especially when the sample size is small (below a N of 100, for example). Additionally, large sample sizes are needed in order for the researchers to find differences between correlations. Lastly, scores on both the outcome and predictor variables need to have a range applicable to generalization (Warner, 2013).

Proposed models. The models we used with linear regressions include testing the hypotheses that *T. gondii* seropositivity will negatively affect different domains of cognitions including memory, working memory, language functioning, executive functioning, and processing speed. In each model, we used the following covariates: gender, age, SES (Hollingshead Index), level of education, and presence or absence of ApoE E4 allele. Again, we chose these variables as covariates because they all potentially play a role in influencing the development of AD (Abate et al., 2017; Dorey et al., 2014; Fiest et al., 2016; S D Gale et al., 2014; Levy et al., 2004).

Method

Subjects

We received serum samples from 219 subjects from the ADRC at WU in St. Louis Missouri, Department of Pathology and Immunology. WU deidentified the samples prior to shipment. We used descriptive statistics to characterize the AD and control groups according to age, gender, race/ethnicity, socioeconomic status, educational attainment, ApoE E4 status, and average anti-*T. gondii* IgG antibody titer concentration. Of the samples, 105 of the subjects were diagnosed with AD and 114 subjects were age-matched controls without dementia. The diagnosis of AD was determined by WU using the following: an informant-based semi-structured interview assessing six cognitive domains to give the Clinical Dementia Rating (CDR) score, which interview included 18 relevant scales/questionnaires. The participants in the AD group had a CDR score of 2 (moderate) or 3 (severe) and all control subjects had a CDR score of 0 (no dementia) with a few exceptions. Namely, 26 participants in the AD group had a CDR score of 0.5 (questionable) and 25 participants in the same group had a CDR score of 1 (mild) (Morris, 1993). In the control group, two participants had a CDR score of 0.5, one participant

had a CDR score of 1, two participants had a CDR score of 2 and three participants had a CDR score of 3. Because of the potential confounding of controls who have low cognitive functioning without a diagnosis of AD, we removed from the analysis those controls with a CDR score above 0. Thus, the cognitive status of all subjects was consistent with their diagnosis of either AD or control.

Neuropsychological data. We received neuropsychological data from most participants, which included the following measures: Boston Naming Test, Category Fluency (Animal and Vegetable), Word Fluency, Trail Making Test A and B, and Wechsler Memory Scale-Revised (WMS-R; Digit Span Forward and Backward, Logical Memory Story A: Immediate and Delayed; Associate Learning; Mental Control), Free and Cued Selective Reminding Test, Wechsler Adult Intelligence Scale (WAIS; Block Design, Information), Wechsler Adult Intelligence Scale-Revised (WAIS-R; Digit Symbol), and Wechsler Adult Intelligence Scale-III (WAIS-III; Letter-Number Sequencing). We grouped the neuropsychological test data based on which cognitive domain each test assessed. We used WMS-R Logical Memory IA, WMS-R Logical Memory IIA, WMS-R Associate Learning, and Free and Cued Selective Reminding Test as measures of memory. We used WMS-R Digit Span Forward, WMS-R Digit Span Backward, WMS-R Mental Control, and WAIS-III Letter-Number Sequencing as measures of working memory. We used WAIS-R Digit Symbol and Trail Making Tests A and B as measures of processing speed. We used the Boston Naming Test and WAIS Information as measures of language functioning. Lastly, we used WAIS Block Design, Category Fluency (Animal and Vegetable), and Word Fluency as measures of executive functioning (Nuechterlein et al., 2004; Sheline et al., 2006).

According to the information provided by WU, WU gave the neuropsychological tests used in these analyses in the following manner for each test:

Boston Naming Test: The examiners began at item one and presented all 30 odd-numbered items (pictures of objects) in order, to the subject. They allowed 20 seconds for each response. If the subject gave a response that indicated a misperception of the picture, the examiner would give the assigned stimulus cue. Again, the subject was allowed 20 seconds to respond. If the subject gave an incorrect response following the stimulus cue then the examiner gave the assigned phonemic cue. The total score of the test is the number of items named correctly, including those named correctly following stimulus cues (Fisher et al., 1999; Goodglass & Kaplan, 1983b; Kaplan, Goodglass, & Weintraub, 1983; Mack, Freed, Williams, & Henderson, 1992).

Category Fluency – Animals and Vegetables: For Animal Category Fluency, the examiner asked the subject to name as many different animals as they can for 60 seconds. Similarly, for Vegetable Category Fluency, the examiner asked the subject to name as many different vegetables as they can for a minute. The total score for both Animal and Vegetable Category Fluency was the total number of correct responses in a minute for each (Goodglass & Kaplan, 1983a).

Word Fluency: This test consisted of two parts. First, the examiner asked the subject to name as many words as they can that begin with the letter P. Second, the examiner asked the subject to name as many words as they can that begin with the letter S (Thurstone & Thurstone, 1949). We used the combined score of both P and S subtests in the analyses.

Trailmaking A and B: For Trailmaking A, the examiner asked the subject to connect 25 numbered circles in sequential order. The score is the time in seconds it took the subject to connect the circles. The test was stopped at 150 seconds if the subject had not finished (Armitage, 1945). For Trailmaking B, the examiner asked the subject to connect numbered circles (numbered from 1-13) and lettered circles (from A-L) in alternating sequential order within the 180 second limit. The total score is the time in seconds it took the subject to connect the circles (Armitage, 1945).

WMS-R Digit Span Forward: The examiner gave this test according to the WMS-R manual. Briefly, the examiner gave a series of numbers (of increasing length) which the subject had to repeat in the same order. The score for the test was the longest sequence of numbers the subject repeated correctly (Wechsler, 1987).

WMS-R Digit Span Backward: The examiner gave this test according to the WMS-R manual. The examiner gave a sequence of numbers (of increasing length) which the subject had to repeat in reverse order. The score for the test was the longest sequence of numbers the subject repeated in reverse order correctly (Wechsler, 1987).

WMS-R Logical Memory Story A, Immediate and Delayed: The examiner gave this test according to the WMS-R manual except that the examiner only administered Story A. The examiner read Story A to the subject and asked the subject to tell back as much of the story as he/she could remember immediately after hearing the story, the Immediate part of the test. The examiner then asked the subject to recall as much of the story as he/she could remember at least 30 minutes after originally hearing it, the Delayed part of the test. The score for each part of the test was the number of parts of the story correctly recalled, according to the WMS-R manual (Wechsler, 1987).

WMS-R Associate Learning: The examiner presented the subject with eight word pairs, four "easy" pairs that are easily associated and four "hard" pairs that are not readily associated. The examiner read the word pair list three times, with a memory trial after each reading. Subjects who had not learned the pairs by the third trial had up to three more trials to learn the pairs. The word pairs were randomized in each of the learning trials. About thirty minutes later, the examiner gave a single recall trial. The total score is based on the correctly recalled word pairs on the first three trials (Wechsler & Stone, 1973).

Free and Cued Selective Reminding Test: The examiner presented the subject with sixteen items to be learned, four at a time on a card. The examiner asked the subject to give the name of a pictured item (e.g. grapes) when presented with a category cue (e.g., fruit). To confirm the encoding of the items, the examiner asked the subject to recall the items on each card (four at a time) immediately after reviewing the items on the card. This also provided retrieval practice before the test phase. The subject performed three recall trials with each trial preceded by 20 seconds of interference by counting backwards from 97 by threes. The examiner allowed the subject up to ninety seconds to recall items for each recall trial. Additionally, the examiner gave the category cue for each item not initially recalled. If the subject did not recall the item within ten seconds after the category cue, the examiner told the participant what it was. The score used in these analyses is the total number of items recalled in the three recall trials, for both freely recalled items and items cued with a category cue (Grober, Buschke, Crystal, Bang, & Dresner, 1988).

WAIS Block Design: The examiner asked the subject to replicate models or pictures of two-color designs with blocks. The examiner scored and administered the test according to the

WAIS manual. The score for the test was the total score of points earned for each attempt at replication (Wechsler, 1955).

WAIS Information: The subject answered a series of questions about factual information. The examiner scored and administered the test according to the WAIS manual. The score for the test was the total score of questions answered correctly (Wechsler, 1955).

WAIS-R Digit Symbol: The examiner gave the subject a piece of paper with a code of a symbol for each number (1-9) at the top of the page. The examiner instructed the subject to use the code to fill in empty boxes below symbols. The examiner asked the subject to fill in as many correct numbers as possible, in order, without skipping any, in 90 seconds. One point was given for each correct number entered within the time limit (Wechsler, 1981).

WMS-III Letter-Number Sequencing: The examiner read a combination of numbers of letters and asked the subject to repeat them, saying the numbers first in ascending order and then the letters in alphabetical order. The examiner administered and scored the test according to the WMS-III manual (Wechsler, 1997). The score for the test was the number of points given for each correct sequence of letters and numbers.

WMS Mental Control: This test consisted of a combined score from three subtests of the WMS Mental Control test. First, the examiner asked the subject to count backwards from 20 in 30 seconds and which the examiner scored according to the WMS manual. Second, the examiner asked subject to repeat the alphabet in 30 seconds. Lastly, the examiner asked the subject to count from one to 40 by threes in 45 seconds. (Wechsler & Stone, 1973). The examiner only scored items completed within time limits. The examiner scored the items on a three-point scale: no errors equaled two points; one error equaled one point; and no credit if there

were two or more errors. The score we used in the analyses was the summary score of the three subtests.

We compared the average scores on the neuropsychological measures using *t*-tests in a few different comparisons, namely, comparing neuropsychological scores between those seropositive and seronegative for latent Toxoplasmosis (in both the AD and control groups), between males and females (in both the AD and control groups), and between the AD and control groups.

The BYU IRB office determined IRB approval for this study was not needed due to the lack of direct contact with participants and our use of previously-collected, deidentified data.

Procedure

ELISA assays. We assessed serum samples with quantitative immunoenzymatic determination of *T. gondii* IgG-class antibodies using ELISA analysis with a commercially available kit (GenWay, BioTech). We performed all assays according to the protocol provided by the manufacturer. We diluted all serum samples 1:100 with IgG Sample Diluent by dispensing 10 uL of the sample and 1 mL IgG Sample Diluent and then thoroughly mixing with a vortex. We dispensed 100 uL of each Standard (A, B, C, and D) into the respective wells, leaving the first well (A1) empty as a blank. We covered the wells with foil supplied in the kit and incubated the plate for one hour at 37 degrees Celsius. After completion of the incubation, we aspirated the contents of the wells and washed each well three times with 300 uL of washing solution. After washing, we dispensed 100 uL of the Toxoplasma anti-IgG Conjugate solution into all wells except for the blank well. We then covered the plates covered with foil and incubated them for thirty minutes at room temperature (20 to 25 degrees Celsius). We washed

the wells again as previously stated. We then dispensed 100 uL of the TMB Substrate Solution into each well after which, we covered the wells with foil and incubated them at room temperature (20 to 25 degrees Celsius) in the dark. After incubation, we dispensed 100 uL of the Stop Solution into all wells in the same order and at the same rate as the TMB Substrate Solution. We measured the absorbance of each well at 450mm using an ELISA Microwell Plate Reader directly after we added the Stop Solution. We used the empty well in position A1 as the blank for absorbency measurements. We assayed all samples in duplicate and we calculated the mean absorbency value from the two sample absorbency values. We created a calibration curve by plotting the absorbance values of the Standards included in the assay (Standards A, B, C, D) against their corresponding concentrations (0, 50, 100 and 200 mL, respectively) using a linear regression function in Excel (Microsoft). We determined the anti-*T. gondii* IgG antibody concentration of each sample by using the calibration curve. In accordance with the protocol provided by the manufacturer, we considered samples with an IgG anti-*T. gondii* titer greater than 35 IU/mL as positive. We considered samples below 30 IU/mL non-reactive. We reassayed samples in the equivocal range (30-35 IU/ mL), a total of two samples overall. Additionally, we diluted fivefold and tenfold and reassayed samples with absorbencies outside the standard curve computed for each assay. For the samples we reassayed, we adjusted the absorbencies according to dilution factor. In addition to the positive standard provided in the assay, we included a sample shown to be positive with another assay (Abcam) when assaying each plate as an additional positive control. Lastly, we reassayed three to four samples from each plate a second time as a quality control to ensure reproducibility. In order to reduce the influence of pipetting error on the results, we calibrated all pipettes before we began the assays

and we used the same pipettes for all runs. Additionally, the person conducting the assays was blind to gender and ADRC diagnosis.

The presence of IgG anti-*T. gondii* antibodies in the serum in the absence of IgM antibodies is indicative of a past infection with *T. gondii*, whereas the presence of IgM antibodies is considered indicative of an acute or reactivated *T. gondii* infection. In an effort to distinguish between acute and a latent infection with *T. gondii*, we assayed all samples seropositive or borderline seropositive for IgG antibodies to determine the titer of IgM anti-*T. gondii* antibodies using a commercially available IgM-specific ELISA kit (GenWay, BioTech) following the protocol provided by the manufacturer. We diluted all serum samples 1:40 with Sample Diluent by combining 5 uL of serum sample with 200 uL of Sample Diluent. We diluted the Negative Control, Positive Control, and Calibrator (provided in the kit) 1:40 in the same manner. We then thoroughly mixed the diluted serum samples, Negative Control, Positive Control, and Calibrator with a vortex. We dispensed 100 uL of the diluted Negative Control, Positive Control, Calibrator, and serum samples into their respective wells. In the first well (A1), we dispensed 100 uL of Sample Diluent as a blank. We covered the wells with foil supplied in the kit and incubated the plate for thirty minutes at 37 degrees Celsius. After completion of the incubation, we aspirated the contents of the wells and washed each well five times with 300 uL of washing solution. After washing, we dispensed 100 uL of the Enzyme Conjugate solution (provided by the kit) into all wells. We then gently mixed the wells for ten seconds. Afterwards, we covered the plates with foil and incubated for thirty minutes at 37 degrees Celsius. We again washed the wells as previously stated. We then dispensed 100 uL of the TMB Reagent solution into each well and again mixed gently for 10 seconds. We covered the wells with foil and incubated the plate at 37 degrees Celsius for 15 minutes. After incubation, we dispensed 100 uL of the Stop

Solution into all wells in the same order and at the same rate as the TMB Substrate Solution. We then gently mixed the wells for 30 seconds. We measured the absorbance of each well at 450mm by an ELISA Microwell Plate Reader directly after we added the Stop Solution. We used the well in position A1 filled with Sample Diluent as the blank for absorbency measurements. We assayed all samples in duplicate and calculated the mean absorbency value from the two sample absorbency values. To determine if samples were positive for anti-*T. gondii* IgM antibodies, we calculated the mean cut-off calibrator value, positive control, and negative control. We determined the Toxoplasma IgM Index by dividing the mean values of each sample by the calibrator mean value. According to the protocol, we considered a Toxoplasma IgM Index value greater than one as positive for IgM antibodies to *T. gondii*, an Index between 0.91 and 0.99 as equivocal, and an Index value below 0.90 as negative. In order to determine the assay was performed correctly, we measured the optical density (OD) for the first well (reagent blank) and was below 0.250, the recommended cut-off by the manufacturer for the reagent blank. Additionally, we measured the OD value for the Calibrator and it was below 0.250, again the recommended cut-off value provided by the manufacturer. We performed another quality control check by determining the Toxoplasma IgM Index for the Positive and Negative Controls. The Toxoplasma IgM Index for both the Positive and Negative Controls were within the ranges provided by the kit which is indicative of correct implication of the assay. Only two serum samples were positive for anti-*T. gondii* IgM antibodies, which we removed from the analyses. We performed these analyses in the laboratories of Dr. Brent Nielsen and Dr. Eric Wilson at BYU.

Statistical Analysis

Data cleaning. In order to determine the accuracy of the data being used in the study, we carefully examined the data using STATA software version 13.1 (StataCorp). First, we recoded the anti-*T. gondii* IgG antibody titer concentration variable to become the presence or absence of *T. gondii* seropositivity variable by designating all values of concentration below 29.999 to be "0" in the new variable and all values above 30 to be "1" in the new variable. Because the anti-*T. gondii* IgG antibody titer concentration variable was not normally distributed despite fencing the variable for outliers, we transformed the variable by taking the natural log of the variable. This made the distribution of the antibody-titer variable considerably more normal. Also, we recoded the "dx" variables (cognitive disorder diagnosis by WU) into a dichotomous variable for AD. If the "dx" variable was "DAT" ("dementia of the Alzheimer's type"), "DAT Language dysf with," "DAT w/ProAph w/dement at onset," "DAT w/depression, contribut", "DAT w/depression, not contribut", "DAT w/oth (list B) not contrib", or "DAT/other prior to DAT" we coded the new AD variable as "1". Otherwise, we coded the AD variable as "0". We excluded any participants with diagnoses from WU of "DLBD, primary" ("dementia, Lewy-body dementia"), "Incipient demt PTP", "Unc: ques. Impairment", "uncertain dementia", and "DAT cannot be primary." As mentioned previously, we dropped from the analysis any samples that were not diagnosed with AD but had a CDR rating of 0.5 or greater. We did this to prevent those with some form of cognitive decline (that perhaps had not been diagnosed) from being included in the controls. We excluded a total of eight subjects. We also excluded subjects that only had data for *T. gondii* seropositivity but no other data (clinical, neuropsychological, etc.). We excluded a total of eight subjects because they only had data from the *T. gondii* seropositivity analysis. We decided to keep all races/ethnicities in the analyses (rather than excluding

minorities and only using the majority represented race/ethnicity) because of the ability to match based on race/ethnicity in propensity score matching analyses. Originally, we coded level of education as the number of years of education the participant had achieved. To simplify analyses, we recoded the level of education variable as those with eleven or less years of education as "1"; participants with 12 years of education (a high school education) as "2"; and those participants with 13 or more years of education as "3". In this reclassification, the higher the category classification, the more education the participant had attained. Lastly, we did not fence or modify neuropsychological test variables because the test variables were already bounded by the test themselves. Each test has a limit of how many points are possible as well as the lowest score possible.

Due to missing data in the dataset provided by WU, we considered using multiple imputations to help reduce bias that can occur due to missing data and listwise deletion. Most of our covariates (age, ApoE E4 allele, SES, education level, gender, ethnicity) and predictor variables had very little to no missing data with the highest percent of missing data being 2.7. However, many of our dependent variables (neuropsychological test scores) had missing data that ranged from 10.5 percent to 73.1 percent. Due to the large amount of missing data on some dependent variables, Young and Johnson (2017) suggest the use of the multiple imputation, then deletion (MID) method. In this method, the researcher imputes all variables (including the dependent variables) and then deletes those cases that have imputed data on the dependent variables before performing analyses. Because the majority of missing data are in dependent variables for our dataset, our using the MID method would negate the benefits of multiple imputations because the large majority of the imputed data would be deleted (all except seven

data points). Because of these limitations and due to the majority of the missing data being in the dependent variables, we decided not to use multiple imputations in our analyses.

Propensity score matching. To perform propensity score matching analyses, we used the "psmatch" command in STATA. This command estimates treatment effects from observational data by imputing the missing potential outcome for each participant (either the treatment outcome or no treatment outcome, which is the outcome for *T. gondii* seropositivity or *T. gondii* seronegativity in this study) using the average of similar participants that have the other treatment level. This similarity of participants is determined by making propensity scores, which scores are the probabilities of a participant being assigned to a particular treatment group with a specific set of covariates (StataCorp, 2013). In the case of our study, the "treatment" is a person being *T. gondii* seropositive and the non-treatment group are those *T. gondii* seronegative. The outcome used to determine the propensity scores is diagnosis of AD. The covariates we used in the model to make the propensity scores included age, SES Hollingshead Index, education level, gender, ethnicity, and presence or absence of ApoE E4 allele. We performed a logistic regression (due to the binary nature of the outcome AD) with *T. gondii* seropositivity and the above-mentioned covariates to determine the propensity scores, using the following model:

```
logistic toxo age seshollingsheadindex educ ethn gender apoecoded

predict ps

replace ps = 1/ps if toxo==1

replace ps = 1/(1-ps) if toxo==0
```

To test the first hypothesis, that *T. gondii* seropositivity will predict diagnosis of AD, we used the following model:

teffects psmatch (ad) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

In addition to determining the effect of *T. gondii* seropositivity on diagnosis of AD, we also were interested in determining the effect of *T. gondii* seropositivity on cognitive domains using propensity score matching. As such, we made the following models for each neuropsychological domain/test as the outcome:

Memory tests:

WMS-R Logical Memory IA (Immediate):

teffects psmatch (logimemwmslogicalmemoryiai) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

WMS-R Logical Memory IIA (Delayed):

teffects psmatch (memunitswmslogicalmemoryiia) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

WMS Associate Learning:

teffects psmatch (asscmemwmsassociatelearning) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

SRT Total Score:

teffects psmatch (srttotal) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Working memory:

WMS-R Digit Span Forward:

teffects psmatch (digtordigitspanforward) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

WMS-R Digit Span Backward:

teffects psmatch (digbackdigitspanbackward) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

WMS Mental Control:

teffects psmatch (mentalctrl) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

WAIS-III Letter Number Sequencing:

teffects psmatch (lnsequence) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Processing Speed:

Trailmaking A:

teffects psmatch (tmatrailmakinga) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Trailmaking B:

teffects psmatch (tmbtrailmakingb) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

WAIS-R Digit Symbol:

teffects psmatch (digsymwaisdigitsymbol) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Language Functioning:

Boston Naming Test:

teffects psmatch (bntbostonnamingtesttotalcor) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

WAIS Information:

teffects psmatch (psy019waisinformation) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Executive Functioning:

WAIS Block Design:

teffects psmatch (psy021waisblockdesign) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Category Fluency – Animals:

teffects psmatch (animalscategoryfluency) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Category Fluency – Vegetables:

teffects psmatch (vegcategoryfluency) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Word Fluency S & P:

teffects psmatch (wordfluency) (toxo age seshollingsheadindex educ ethn gender apoecoded, logit)

Lastly, we also employed propensity score matching to determine the effect of *T. gondii* seropositivity on cognitive domains within each group, the AD group and the control group. We separated the analyses because of the great differences in neuropsychological test scores between the two groups. The models for the two groups were the same as those above, just with notation to only analyze one group at a time.

Linear regressions. In addition to performing propensity score matching analyses, we also performed linear regressions to predict scores for each neuropsychological test using two models. The first model included the dichotomous measure of *T. gondii* seropositivity or

seronegativity, and the second included the log-transformed, continuous measure of anti-*T. gondii* IgG antibody titers. Both models included gender, socioeconomic status, education level (categorized), age, race/ethnicity, and ApoE status (presence or absence of E4 allele) as control variables as well as an interaction term to help determine the interaction of group (AD and control) with the main predictor, either *T. gondii* seropositivity/seronegativity or anti-*T. gondii* IgG antibody titer concentration. We included ApoE status as a control variable due to its known association with both Alzheimer's disease and cognitive decline (Abate et al., 2017; Dorey et al., 2014; Fiest et al., 2016). Additionally, Levy et al. (2004) Levy et al. found those carrying an E4 allele of ApoE had lower scores on WMS-R Logical Memory IIA. Given these findings, we wanted to control the potential effect ApoE status may have on memory function and thus included it in our analyses. In each model, the differences in sample size are due to missing data on the dependent variables.

In order to perform the linear regression analyses we used the "reg" command in STATA (StataCorp). Below are the models used for each neuropsychological test domain with models for both *T. gondii* seropositivity status (coded "toxo") and anti-*T. gondii* IgG antibody titer concentration (coded "concln") as predictors with an interaction for group ("ad" coded as "1" for the AD group and "0" for the control group). We coded the control variables as follows: gender as "gender" with male being the reference variable; age as "age" as a continuous variable; SES (from the Hollingshead Index) as "ses" and as a categorical variable; level of education as "edu" and as a categorical variable; presence or absence of ApoE E4 allele as "apoecoded" and as a dichotomous variable with no E4 allele as the reference variable; and ethnicity as "ethn" as a dichotomous variable (because only two ethnicities were represented in the dataset, African American and White) with White as the reference variable. The neuropsychological variables

were coded as follows: WMS-R Logical Memory IA as “logimemwmslogicalmemoryiai”; WMS-R Logical Memory IIA as “memunitswmslogicalmemoryiia”; WMS Associate Learning as “asscmemwmsassociatelearning”; Free and Cued Selective Reminding Test as “srttotal”; WMS-R Digit Span Forward as “digfordigitspanforward”; WMS-R Digit Span Backward as “digbackdigitspanbackward”; WMS Mental Control as “mentalctrl”; WAIS-III Letter Number Sequencing as “lnsequence”; WAIS-R Digit Symbol as “digsymwaisdigitsymbol”; Trailmaking Test A as “tmatrailmakinga”; Trailmaking Test B as “tmbtrailmakingb”; WAIS Information as “psy019waisinformation”; Boston Naming Test as “bntboston”; WAIS Block Design as “psy021waisblockdesign”; Category Fluency – Animals as “animalscategoryfluency”; Category Fluency – Vegetables as “vegcategoryfluency”; and Word Fluency as “wordfluency”.

Memory:

reg logimemwmslogicalmemoryiai ad##c.concln i.gender c.age i.ses i.edu i.apoecoded i.ethn

reg logimemwmslogicalmemoryiai ad##toxo i.gender c.age i.ses i.edu i.apoecoded i.ethn

reg memunitswmslogicalmemoryiia ad##c.concln i.gender c.age i.ses i.edu i.apoecoded i.ethn

reg memunitswmslogicalmemoryiia ad##toxo i.gender c.age i.ses i.edu i.apoecoded i.ethn

reg asscmemwmsassociatelearning ad##c.concln i.gender c.age i.ses i.edu i.apoecoded i.ethn

reg asscmemwmsassociatelearning ad##toxo i.gender c.age i.ses i.edu i.apoecoded i.ethn

reg srttotal ad##c.concln i.gender c.age i.ses i.edu i.apoecoded i.ethn

reg srttotal ad##toxo i.gender c.age i.ses i.edu i.apoecoded i.ethn

Working Memory:

reg digfordigitspanforward ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg digfordigitspanforward ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg digbackdigitspanbackward ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg digbackdigitspanbackward ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg mentalctrl ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg mentalctrl ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg lnsequence ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg lnsequence ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

Processing Speed:

reg digsymwaisdigitsymbol ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg digsymwaisdigitsymbol ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg tmatrailmakinga ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg tmatrailmakinga ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg tmbtrailmakingb ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg tmbtrailmakingb ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg digsymwaisdigitsymbol ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg digsymwaisdigitsymbol ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

Language functioning:

reg psy019waisinformation ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg psy019waisinformation ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg bntboston ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded

reg bntboston ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded

Executive Functioning:

```
reg psy021waisblockdesign ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded
reg psy021waisblockdesign ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded
reg animalscategoryfluency ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded
reg animalscategoryfluency ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded
reg vegcategoryfluency ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded
reg vegcategoryfluency ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded
reg wordfluency ad##c.concln i.gender c.age i.ses i.edu i.ethn i.apoecoded
reg wordfluency ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded
```

Because of the potential for alpha inflation due to the high amount of linear regressions we performed, we performed the Benjamini-Hochberg procedure. We used the Benjamini-Hochberg procedure because it gives much greater power than the Bonferroni technique. We implemented the procedure using the information given in Thissen, Steinberg, and Kuang (2002).

In addition, we also performed power analyses for the linear regressions in the study. We used the "powerreg" command in STATA and estimated the power of each regression rather than estimating the sample size needed to attain a certain power level. Below is an example of the code we used to do a power analysis for WMS-R Logical Memory as an outcome and *T. gondii* seropositivity status as the predictor.

```
reg logimemwmslogicalmemoryiai ad##toxo i.gender c.age i.ses i.edu i.ethn i.apoecoded
local r2f = e(r2)
reg logimemwmslogicalmemoryiai i.ad i.gender c.age i.ses i.edu i.ethn i.apoecoded
local r2r = e(r2)
powerreg, r2f(`r2f') r2r(`r2r') nvar(11) ntest(1) n(117)
```

Results

Sample Characteristics

We used descriptive statistics to characterize the AD and control groups according to age, gender, race/ethnicity, socioeconomic status, educational attainment, ApoE E4 status, and average anti-*T. gondii* IgG antibody titer concentration. Fifty-four percent of the participants diagnosed with AD were female and 52 percent of control participants were female. The average age for those in the AD group was 80.4 years (standard deviation of 7.2 years) and 79.1 years (standard deviation of 7.0 years) in the control group. The percent of participants with greater than a high school education in the AD group was 53 and 72 percent for participants in the control group. We used the Hollingshead two-factor index of social position to estimate socioeconomic status, with a "1" on the scale representing higher socioeconomic status. The range of possible values on the scale are from 1 to 5 (Hollingshead & Redlich, 1958). The average Hollingshead SES index value for the AD group was 3.4 (standard deviation of 1.2) and an average index of 3.8 (standard deviation of 1.0) for the control group. Sixty-one percent of the AD group had at least one E4 allele for the ApoE gene while only 28 percent of the control group had one E4 allele. The percent *T. gondii* seropositive in the AD group was 59 compared to 68 percent *T. gondii* seropositive in the control group. The average anti-*T. gondii* antibody concentration for the control group 140.9 IU/mL with a range of -7.2 to 1351.8 IU/mL. The average anti-*T. gondii* antibody concentration for the AD group was 108.1 IU/mL with a range of 0.3 to 940.8 IU/mL. Additionally, we examined the frequency of a history of hypertension, diabetes, heart disease, thyroid disease, alcohol abuse, tobacco use and hypercholesterolemia in each group, and found no significant difference between them. Only a history of stroke was significantly different between groups, being more prevalent in the AD group than in the control

group (p=0.03; 4.7% versus 0.9%, respectively). We present demographic information, anti-*T. gondii* antibody concentrations and *t*-test results for differences between the two groups on each demographic variable in Table 1.

T-Test Results

Between the two groups there are significant differences between percent Caucasian (p =0.006), level of education (p =0.002), SES (p = 0.01), and presence of ApoE E4 allele (p =

Table 1

Characteristics of AD and Control Groups.

Characteristics	AD	Controls	*t*	*p* value	[95% CI]
Sample size	105	114	.	.	.
Age (years), mean, SD, [range]	80.40, 7.18, [61.57-97.80]	79.06, 7.02, [62.06-98.28]	-1.39	0.17	[78.76-80.65]
Women (%)	54.29	51.75	-0.37	0.71	[1.46-1.60]
Ethnic Background (% Caucasian)	100.00	92.98	2.80	0.01**	[0.01-0.06]
Education (% more than High School)	53.33	71.93	3.07	0.002**	[2.43-2.62]
SES (Hollingshead Index), mean, SD	3.42, 1.20	3.81, 0.99	2.61	0.01**	[3.47-3.77]
T. gondii seropositive (%)	59.05	67.54	-1.30	0.19	[-0.21-0.04]
Anti-*T. gondii* IgG antibody concentration IU/mL, mean, SD, [range]	108.13, 199.97, [0.29-940.81]	140.94, 260.74, [-7.15-1351.84]	-1.05	0.30	[-94.42-28.80]
ApoE status (% with E4 allele)	60.95	28.07	-5.11	0.001***	[0.37-0.51]

Note. * p value = or < 0.05 ** p value < 0.01; *** p value < 0.001.

0.001) with the control group having more African Americans, higher average level of education, lower average level of SES, and lower incidence of ApoE E4 allele than the AD group (Table 1).

The two groups did not significantly differ on the other demographic measures. When we compared performance on neuropsychological measures between the control and AD groups, all neuropsychological tests scores were significantly different between the two groups ($p = 0.001$) with participants in the control group performing better than those in the AD group, which is what we expected (Table 2). When we compared scores on neuropsychological measures between males and females only two tests showed significant differences, namely, the Boston Naming Test ($p = 0.03$) and WAIS Information ($p = 0.01$). On both measures, men performed significantly better than females (Table 3). Lastly, when we compared neuropsychological test scores of those *T. gondii* seropositive and seronegative (in both the control and AD groups), four tests showed significant differences, specifically Selective Reminding Test ($p = 0.05$), Digit Symbol ($p = 0.04$), Block Design ($p =0.03$), and Category Fluency Animals ($p =0.01$). Those *T. gondii* seronegative performed significantly better all on the measures mentioned (Table 4).

Propensity Score Matching Results

After we matched the treatment (*T. gondii* seropositivity) and matched control group (*T. gondii* seronegativity) on the covariates, there was not a significant difference in outcome (having AD) between the groups. Thus, *T. gondii* seropositivity does not seem to significantly influence the development of AD in this sample (Table 5).

In addition to testing the influence of *T. gondii* seropositivity on development of AD, we also tested the effect of *T. gondii* seropositivity on neuropsychological test scores using propensity score matching. For most neuropsychological test scores, we found no significant difference in outcome between the treatment (*T. gondii* seropositivity) and matched control

Table 2

Neuropsychological Test Scores for AD and Control Groups.

Neuropsychological Test	AD	Control	*t*	*p* value	[95% CI]
WMS-R Logical Memory IA, mean, *SD*	5.64, 4.36	14.01, 4.26	9.03	0.001***	[11.01-13.04]
WMS-R Logical Memory IIA, mean, *SD*	3.68, 4.36	12.97, 4.66	9.35	0.001***	[9.66-11.87]
WMS-R Associate Learning, mean, *SD*	7.41, 3.73	14.30, 3.63	12.99	0.001***	[10.53-11.96]
Free and Cued Selective Reminding Test, mean, *SD*	13.83, 8.41	29.15, 6.15	10.77	0.001***	[23.98-27.27]
WMS-R Digit Span Forward, mean, *SD*	5.77, 1.19	6.62, 1.08	5.21	0.001***	[6.07-6.41]
WMS-R Digit Span Backward, mean, *SD*	3.63, 1.35	4.77, 1.25	6.12	0.001***	[4.07-4.47]
WMS-R Mental Control, mean, *SD*	5.13, 2.93	7.47, 1.68	7.03	0.001***	[6.06-6.79]
WAIS-III Letter-Number Sequencing, mean, *SD*	5.67, 4.69	9.06, 2.22	3.47	0.001***	[7.77-9.31]
WAIS-R Digit Symbol, mean, *SD*	23.98, 15.81	46.10, 9.88	11.73	0.001***	[33.90-38.76]
Trails A, mean, *SD*	81.81, 51.38	36.96, 18.10	-8.43	0.001***	[50.50-62.67]
Trails B, mean, *SD*	156.57, 36.36	95.91, 36.70	-10.97	0.001***	[114.25-128.13]
Boston Naming Test, mean, *SD*	40.28, 13.40	54.92, 6.20	10.06	0.001***	[11.77-17.51]
WAIS Information, mean, *SD*	12.80, 6.50	21.68, 4.56	11.19	0.001***	[16.77-18.76]
WAIS Block Design, mean, *SD*	16.93, 11.91	30.08, 8.40	8.85	0.001***	[22.65-26.11]
Category Fluency – Animal, mean, *SD*	8.96, 4.92	20.12, 5.94	13.90	0.001***	[14.21-16.43]
Category Fluency – Vegetables, mean, *SD*	7.34, 3.72	14.11, 3.87	8.26	0.001***	[11.57-13.33]
Word Fluency, mean, *SD*	18.04, 10.12	30.16, 9.88	8.36	0.001***	[23.23-26.53]

Note. * *p* value = or < 0.05; ** *p* value < 0.01; *** *p* value < 0.001.

Table 3

Neuropsychological Test Scores for Males and Females in AD and Control Groups.

Neuropsychological Test	Males	Females	*t*	*p* value	[95% CI]
WMS-R Logical Memory IA, mean, *SD*	11.8, 5.32	12.22, 5.80	-0.41	0.68	[11.01-1.62]
WMS-R Logical Memory IIA, mean, *SD*	10.51, 5.72	10.98, 6.37	-0.42	0.67	[9.66-11.87]
WMS-R Associate Learning, mean, *SD*	10.65, 4.99	11.80, 5.01	-1.60	0.11	[-2.57-0.27]
Free and Cued Selective Reminding Test, mean, *SD*	25.07, 9.02	26.14, 9.62	-0.64	0.52	[23.98-27.27]
WMS-R Digit Span Forward, mean, *SD*	6.19, 1.20	6.29, 1.20	-0.55	0.58	[-0.44-0.24]
WMS-R Digit Span Backward, mean, *SD*	4.15, 1.26	4.38, 1.54	-1.13	0.26	[-0.63-0.17]
WMS-R Mental Control, mean, *SD*	6.72, 2.50	6.16, 2.65	1.54	0.13	[6.06-6.79]
WAIS-III Letter-Number Sequencing, mean, *SD*	9.26, 2.72	7.75, 3.04	2.01	0.05*	[0.01-3.01]
WAIS-R Digit Symbol, mean, *SD*	36.21, 16.17	36.44, 17.62	-0.09	0.92	[33.90-38.76]
Trails A, mean, *SD*	56.76, 44.13	56.41, 41.63	0.05	0.96	[50.50-62.67]
Trails B, mean, *SD*	117.90, 46.48	124.20, 47.91	-0.89	0.37	[114.25-128.13]
Boston Naming Test, mean, *SD*	50.40, 11.99	46.47, 12.58	2.22	0.03*	[46.59-50.11]
WAIS Information, mean, *SD*	19.09, 6.61	16.53, 7.25	2.56	0.01**	[16.77-18.76]
WAIS Block Design, mean, *SD*	26.08, 11.86	22.74, 11.92	1.92	0.06	[22.65-26.11]
Category Fluency – Animal, mean, *SD*	16.28, 8.25	14.39, 7.28	1.70	0.09	[14.21-16.43]
Category Fluency – Vegetables, mean, *SD*	12.00, 4.92	12.83, 4.72	-0.93	0.35	[11.57-13.33]
Word Fluency, mean, *SD*	25.52, 11.98	24.29, 11.35	0.73	0.47	[23.23-26.53]

Note. * *p* value = or < 0.05; ** *p* value < 0.01; *** *p* value < 0.001.

Table 4

Neuropsychological Test Scores for Those T. Gondii Seropositive and Seronegative in AD and Control Groups.

Neuropsychological Test	Seropositive	Seronegative	*t*	*p* value	[95% CI]
WMS-R Logical Memory IA, mean, *SD*	11.73, 5.61	12.18, 5.57	0.42	0.68	[10.92-13.45]
WMS-R Logical Memory IIA, mean, *SD*	10.61, 6.10	10.84, 6.07	0.20	0.84	[9.66-11.87]
WMS-R Associate Learning, mean, *SD*	10.61, 4.89	11.63, 5.08	1.38	0.17	[10.53-11.96]
Free and Cued Selective Reminding Test, mean, *SD*	23.43, 9.75	26.80, 8.92	1.96	0.05*	[23.98-27.27]
WMS-R Digit Span Forward, mean, *SD*	6.32, 1.20	6.19, 1.21	-0.76	0.45	[6.07-6.41]
WMS-R Digit Span Backward, mean, *SD*	4.08, 1.38	4.38, 1.42	1.44	0.15	[4.07-4.47]
WMS-R Mental Control, mean, *SD*	6.16, 2.92	6.59, 2.36	1.12	0.26	[6.06-6.79]
WAIS-III Letter-Number Sequencing, mean, *SD*	9.00, 3.17	8.33, 2.78	-0.82	0.42	[7.77-9.31]
WAIS-R Digit Symbol, mean, *SD*	32.91, 16.44	38.27, 16.90	2.11	0.04*	[33.90-10.37]
Trails A, mean, *SD*	62.89, 46.40	52.88, 40.21	-1.57	0.12	[50.56-62.67]
Trails B, mean, *SD*	129.25, 45.04	116.74, 47.97	-1.71	0.09	[114.25-128.13]
Boston Naming Test, mean, *SD*	46.16, 13.64	49.67, 11.50	1.92	0.06	[46.59-50.11]
WAIS Information, mean, *SD*	17.08, 7.31	18.18, 6.88	1.06	0.29	[16.77-18.76]
WAIS Block Design, mean, *SD*	21.96, 12.06	25.83, 11.74	2.16	0.03*	[22.65-26.11]
Category Fluency – Animals, mean, *SD*	13.55, 7.66	16.40, 7.73	2.49	0.01**	[14.21-16.43]
Category Fluency – Vegetables, mean, *SD*	11.76, 4.10	12.82, 5.13	1.14	0.26	[11.57-13.33]
Word Fluency, mean, *SD*	22.97, 11.81	26.04, 11.43	1.79	0.07	[23.23-26.53]

Note. * *p* value = or < 0.05; ** *p* value < 0.01; *** *p* value < 0.001.

Table 5

Propensity Score Matching Results for AD Outcomes: Presence of T. Gondii Seropositivity Predicting Outcome of Group Membership.

Outcome	Coefficient	Std. Error	*z*	*p* value	[95% CI]
AD	-0.05	0.08	-0.64	0.52	[-0.19-0.10]

Note. The following covariates were included as part of the matching criteria in the analysis: age, SES status, education level, ethnicity, gender, and presence or absence of ApoE E4.

group (*T. gondii* seronegativity). However, for the processing speed test of WAIS-R Digit Symbol, we found a significant difference ($p = 0.052$) between the treatment and matched control group, indicating *T. gondii* seropositivity may play a role in the performance of this neuropsychological test (Table 6).

As mentioned previously, in addition to performing propensity score matching with neuropsychological tests for the whole group, we also employed the procedure for the AD and control groups separated. For most neuropsychological test scores, we again found no significant difference in outcome between the treatment (*T. gondii* seropositivity) and matched groups (*T. gondii* seronegativity) for those in the AD group. However, for the processing speed test of Trailmaking B and executive functioning test of WAIS Block Design, we found a significant difference ($p = 0.011$ and $p=0.026$, respectively) between the treatment and matched groups, indicating *T. gondii* seropositivity may play a role in the performance of these neuropsychological tests in the AD group (Table 7). When assessing the control group only, no significant differences were seen between treatment and matched groups (Table 8).

Table 6

Propensity Score Matching Results for Neuropsychological Test Outcomes: Presence of T. Gondii Seropositivity Predicting Outcome of Each Test.

Neuropsychological test outcome	Coefficient	Std. Error	*z*	*p* value	[95% CI]
Logical Memory IA	-0.76	1.33	-0.57	0.57	[-3.38-1.86]
Logical Memory IIA	-0.21	1.45	-0.14	0.89	[-3.05-2.64]
Associate Memory	-0.53	0.83	-0.64	0.52	[-2.15-1.10]
SRT Total	0.08	0.61	0.13	0.90	[-1.12-1.28]
Digit Span – Forward	0.19	0.21	0.88	0.38	[-0.23-0.60]
Digit Span – Backward	-0.22	0.21	-1.06	0.29	[-0.63-0.19]
Mental Control	-0.28	0.44	-0.64	0.53	[-1.13-0.58]
Letter Number Sequencing	0.90	0.51	1.75	0.08	[-0.11-1.90]
Trails A	3.81	8.70	0.44	0.66	[-13.24-20.85]
Trails B	4.12	9.21	0.45	0.66	[-13.94-22.18]
Digit Symbol	-5.26	2.70	-19.5	0.05	[-10.55-0.04]
Boston Naming Test	-1.08	2.04	-0.53	0.60	[-5.07-2.91]
WAIS Information	0.01	1.12	0.00	0.99	[-2.19-2.20]
WAIS Block Design	-2.16	1.76	-1.22	0.22	[-5.61-1.29]
Category Fluency – Animals	-0.33	1.19	-0.28	0.78	[-2.68-2.00]
Category Fluency – Vegetables	-0.24	0.85	-0.28	0.78	[-1.91-1.43]
Word Fluency S & P	-1.93	1.94	-1.00	0.32	[-5.73-1.87]

Note. The following covariates were included as part of the matching criteria in the analysis: age, SES status, education level, ethnicity, gender, and presence or absence of ApoE E4.

Table 7

Propensity Score Matching Results for Neuropsychological Test Outcomes for AD Group Only: Presence of T. Gondii Seropositivity Predicting Outcome of Each Test.

Neuropsychological test outcome	Coefficient	Std. Error	*z*	*p* value	[95% CI]
Logical Memory IA	-0.89	0.99	-0.90	0.37	[-2.84, 1.06]
Logical Memory IIA	-0.11	1.13	-0.10	0.92	[-2.32, 2.10]
Associate Memory	-0.69	0.72	-0.96	0.34	[-2.11, 0.72]
SRT Total	0.59	0.97	0.60	0.55	[-1.32, 2.49]
Digit Span – Forward	0.23	0.31	0.74	0.46	[-0.38, 0.85]
Digit Span – Backward	-0.35	0.37	-0.94	0.35	[-1.08, 0.38]
Mental Control	-0.72	0.68	-1.06	0.29	[-2.06, 0.61]
Trails A	7.45	11.49	0.65	0.52	[-15.07, 29.98]
Trails B	20.47	8.05	2.54	0.01	[4.69, 36.24]
Digit Symbol	1.49	3.22	0.46	0.64	[-4.81, 7.80]
Boston Naming Test	-0.84	3.11	-0.27	0.79	[-6.94, 5.26]
WAIS Information	0.05	1.48	0.03	0.98	[-2.86, 2.95]
WAIS Block Design	-4.69	2.11	-2.23	0.03	[-8.82, -0.56]
Category Fluency – Animals	-1.53	0.99	-1.55	0.12	[-3.47, 0.41]
Category Fluency – Vegetables	1.59	1.84	0.86	0.39	[-2.02, 5.19]
Word Fluency S & P	-0.80	1.98	-0.40	0.69	[-4.67, 3.08]

Note. The following covariates were included as part of the matching criteria in the analysis: age, SES status, education level, ethnicity, gender, and presence or absence of ApoE E4.

Table 8

Propensity Score Matching Results for Neuropsychological Test Outcomes for Control Group Only: Presence of T. Gondii Seropositivity Predicting Outcome of Each Test.

Neuropsychological test outcome	Coefficient	Std. Error	*z*	*p* value	[95% CI]
Logical Memory IA	-0.02	0.97	-0.02	0.98	[-1.92, 1.87]
Logical Memory IIA	0.46	1.09	0.42	0.67	[-1.68, 2.60]
Associate Memory	0.41	0.74	0.55	0.58	[-1.05, 1.86]
SRT Total	0.02	0.12	0.17	0.87	[-0.22, 0.26]
Digit Span – Forward	0.40	0.22	1.82	0.07	[-0.03, 0.83]
Digit Span – Backward	-0.22	0.24	-0.95	0.34	[-0.68, 0.24]
Mental Control	-0.31	0.38	-0.82	0.41	[-1.06, 0.44]
Letter Number Sequencing	0.88	0.48	1.83	0.07	[-0.06, 1.82]
Trails A	0.79	2.20	0.36	0.72	[-3.51, 5.10]
Trails B	2.31	9.89	0.23	0.82	[-17.09, 21.70]
Digit Symbol	-2.03	2.07	-0.98	0.33	[-6.08, 2.03]
Boston Naming Test	-0.37	1.21	-0.31	0.76	[-2.73, 2.00]
WAIS Information	-0.11	0.86	-0.13	0.90	[-1.79, 1.57]
WAIS Block Design	-3.50	1.89	-1.84	0.07	[-7.21, 0.22]
Category Fluency – Animals	-0.04	1.65	-0.02	0.98	[-3.28, 3.20]
Category Fluency – Vegetables	-0.30	0.97	-0.30	0.76	[-2.20, 1.61]
Word Fluency S & P	-0.32	1.77	-0.18	0.85	[-3.78, 3.14]

Note. The following covariates were included as part of the matching criteria in the analysis: age, SES status, education level, ethnicity, gender, and presence or absence of ApoE E4.

Additionally, we performed post-estimation commands for all significant results found using propensity score matching. The post-estimation command of "overlap" allowed us to examine the overlap of the propensity scores made for each group (seropositive and seronegative groups). For each significant result, the overlap analyses showed the propensity scores from each group overlapped each other and neither group had many scores around zero or one probability. Given these results, we can determine the overlap assumption of propensity score matching has not been violated in our analyses (StataCorp, 2013).

Linear Regression Results

Memory neuropsychological measures. In the linear regression analyses, anti-*T. gondii* antibody titer was not significantly associated with any of the memory measures in the control or AD groups in the model including control variables. Additionally, we found *T. gondii* seropositivity was not significantly associated with any of the memory measures in the control or AD groups (Table 9).

Working memory neuropsychological measures. In the linear regression analyses, we found anti-*T. gondii* antibody titer was positively associated with performance on the WMS-R Digit Span Forward test ($p = 0.052$) and WMS Letter-Number Sequencing test ($p = 0.050$; Table 10). Additionally, we found *T. gondii* seropositivity was positively associated with WMS Letter-Number Sequencing test ($p = 0.046$; Table 10). We found no other associations between working memory measures and seropositivity.

Table 9

Main Effects Models of Anti-T. Gondii Antibody Titer or Presence of T. Gondii Seropositivity and Each Memory Test Score: Unstandardized Coefficients (Standard Errors) From Linear Regression.

	Logical Memory IA		Logical Memory IIA		Associate Memory		Selective Reminding Test	
	b	SE	b	SE	b	SE	b	SE
Antibody titer	0.308	0.301	0.387	0.322	0.147	0.234	-0.099	0.323
Seropositive & AD (Group interaction)	-0.880	0.592	-1.190	0.633	-0.255	0.334	0.567	0.583
Female	0.588	0.862	0.939	0.923	1.463**	0.551	-1.228	0.886
Age	-0.006	0.060	0.012	0.064	-0.024	0.040	-0.039	0.061
SES (Hollingshead Index)								
2	5.028	4.557	4.011	4.877	-2.247	2.156	-0.338	4.874
3	4.968	4.477	2.828	4.791	-1.140	2.177	0.254	4.843
4	4.637	4.462	2.524	4.775	-2.354	2.198	0.454	4.818
5	5.171	4.574	2.206	4.895	-1.540	2.257	0.503	4.913
Education								
High School	1.605	1.916	0.813	2.051	-0.815	1.007	-3.998*	1.995
More than High School	2.664	2.047	3.484	2.191	0.109	1.096	-5.674**	2.120
African American	-0.750	1.621	-1.950	1.735	-3.478**	1.339	0.174	1.752
ApoE (presence of E4)	-0.843	0.935	-0.558	1.001	-0.814	0.593	-3.004**	0.966
Constant	6.158	7.191	5.020	7.696	17.270	4.176	57.330	7.298
R^2	0.476		0.491		0.535		0.464	
n	113		113		187		122	
Power	0.09		0.19		0.74		0.75	

Table 9 Continued

	Logical Memory IA		Logical Memory IIA		Associate Memory		Selective Reminding Test	
	b	SE	b	SE	b	SE	b	SE
Presence of *T. gondii* seropositivity	-0.155	0.984	0.131	1.056	-0.119	0.770	0.034	1.055
Seropositive & AD (Group interaction)	-0.782	1.948	-0.971	2.090	-0.083	1.107	2.480	2.043
Female	0.486	0.839	0.791	0.900	1.286*	0.540	-1.215	0.869
Age	0.002	0.060	0.017	0.064	-0.014	0.039	-0.046	0.061
SES (Hollingshead Index)								
2	5.210	4.548	4.313	4.879	-2.152	2.163	-1.099	4.885
3	5.194	4.472	3.261	4.798	-0.866	2.187	-0.683	4.859
4	4.814	4.452	2.916	4.776	-1.824	2.199	-0.123	4.837
5	5.521	4.566	2.796	4.898	-1.077	2.265	-0.190	4.940
Education								
High School	1.564	1.836	0.861	1.970	-0.792	0.998	-4.039*	1.907
More than High School	2.713	1.930	3.451	2.070	-0.119	1.054	-5.483**	1.988
African American	-0.578	1.621	-1.723	7.839	-3.350**	1.346	0.247	1.757
ApoE (presence of E4)	-1.273	0.894	-0.930	0.959	-0.888	0.583	-3.430**	0.942
Constant	6.520	7.275	5.693	7.806	16.914	4.136	58.247	7.400
R^2	0.473		0.487		0.518		0.443	
n	117		117		193		125	
Power	0.09		0.08		0.06		0.32	

Note. * p value = or < 0.05; ** p value < 0.01; *** p value < 0.001.

Table 10

Main Effects Models of Anti-T. Gondii Antibody Titer or Presence of T. Gondii Seropositivity and Each Working Memory Test Score: Unstandardized Coefficients (Standard Errors) from Linear Regression.

	Digit Span Forward		Digit Span Backward		WMS Mental Control		WMS Letter-Number Sequencing	
	b	SE	b	SE	b	SE	b	SE
Antibody titer	0.145*	0.074	-0.004	0.083	0.122	0.144	0.485*	0.213
Seropositivity & AD (Group interaction)	-0.088	0.106	-0.020	0.119	-0.221	0.205	-0.253	0.644
Female	0.211	0.173	0.391*	0.194	-0.132	0.336	-0.994	0.640
Age	-0.001	0.013	0.004	0.014	-0.033	0.025	-0.182**	0.049
SES (Hollingshead Index)								
2	-0.819	0.681	0.296	0.766	0.698	1.325	.	.
3	-0.190	0.689	0.471	0.775	2.224	1.338	4.051**	1.221
4	-0.117	0.694	0.879	0.781	1.906	1.351	3.891**	1.183
5	-0.059	0.713	0.927	0.802	2.423	1.387	4.799**	1.307
Education								
High School	0.077	0.331	0.459	0.372	-0.242	0.619	-5.650**	1.829
More than High School	0.008	0.357	0.500	0.402	0.291	0.672	-6.293**	1.960
African American	-0.103	0.423	-0.422	0.476	-1.592	0.823	-1.023	0.970
ApoE (presence of E4)	0.152	0.188	0.242	0.212	0.295	0.363	-2.075**	0.754
Constant	6.189	1.340	3.045	1.507	7.625	2.553	24.816	4.348
R^2	0.184		0.244		0.346		0.600	
n	188		188		189		56	
Power	0.51		0.43		0.58		0.68	

Table 10 Continued

	Digit Span Forward		Digit Span Backward		WMS Mental Control		WMS Letter-Number Sequencing	
	b	SE	b	SE	b	SE	b	SE
Presence of *T. gondii* seropositivity	0.352	0.240	-0.128	0.272	0.343	0.466	1.393*	0.680
Seropositivity & AD (Group interaction)	-0.109	0.346	0.028	0.392	-0.867	0.670	-1.573	1.937
Female	0.174	0.168	0.353	0.190	-0.213	0.327	-1.041	0.640
Age	0.000	0.013	0.008	0.014	-0.024	0.024	-0.193**	0.049
SES (Hollingshead Index)								
2	-0.766	0.677	0.285	0.767	0.781	1.318	.	.
3	-0.148	0.686	0.565	0.777	2.356	1.333	3.711**	1.262
4	-0.063	0.688	1.007	0.779	2.149	1.339	3.248**	1.193
5	-0.043	0.709	1.067	0.802	2.560	1.379	4.112**	1.350
Education								
High School	0.085	0.325	0.298	0.368	-0.336	0.608	-5.822**	1.870
More than High School	-0.002	0.341	0.298	0.386	0.114	0.641	-5.961**	2.029
African American	-0.046	0.421	-0.396	0.477	-1.517	0.820	-0.917	0.978
ApoE (presence of E4)	0.130	0.183	0.190	0.208	0.320	0.353	-2.273**	0.727
Constant	6.467	1.317	2.801	1.491	7.222	2.507	27.337	4.382
R^2	0.181		0.233		0.336		0.594	
n	194		194		195		58	
Power	0.44		0.09		0.27		0.63	

Note. * p value = or < 0.05; ** p value < 0.01; *** p value < 0.001.

Processing speed neuropsychological measures. In the linear regression analyses, we found anti-*T. gondii* antibody titer was not significantly associated with any of the processing speed measures in the models including control variables. Additionally, we found *T. gondii* seropositivity was not significantly associated with any of the processing speed measures (Table 11).

Language functioning neuropsychological measures. In the linear regression analyses, we found anti-*T. gondii* antibody titer was not significantly associated with any of the language functioning measures in the models including control variables. Additionally, we found *T. gondii* seropositivity was not significantly associated with any of the language functioning measures (Table 12).

Executive functioning neuropsychological measures. In the linear regression analyses, we found anti-*T. gondii* antibody titer was not significantly associated with any of the executive functioning measures the models including control variables. Additionally, we found *T. gondii* seropositivity was not significantly associated with any of the executive functioning measures (Table 13).

We found other expected associations between many neuropsychological measures and covariates such as AD, gender, level of education, presence of ApoE E4 allele, ethnicity, and SES for both *T. gondii* seropositivity and anti-*T. gondii* antibody titer models. We expected these associations and they were not the main purpose of the analyses.

Benjamini-Hochberg results. Due to the potential for alpha inflation from the many linear regressions we performed, we performed the Benjamini-Hochberg procedure to counteract potential alpha inflation. Based on the Benjamini-Hochberg critical values produced, the two

Table 11

Main Effects Models of Anti-T. Gondii Antibody Titer or Presence of T. Gondii Seropositivity and Each Processing Speed Test Score: Unstandardized Coefficients (Standard Errors) from Linear Regression.

	WAIS Digit Symbol		Trails A		Trails B	
	b	SE	b	SE	b	SE
Antibody titer	-0.564	0.780	-0.415	2.309	-0.598	2.195
Seropositivity & AD (Group interaction)	0.716	1.121	3.016	3.322	3.268	3.201
Female	1.945	1.957	-1.205	5.423	2.130	5.303
Age	-0.338*	0.138	0.644	0.410	1.539**	0.412
SES (Hollingshead Index)						
2	4.627	7.157	2.608	21.234	-28.234	20.202
3	13.511	7.244	-23.064	21.531	-41.688*	20.497
4	10.848	7.294	-16.421	21.641	-31.014	20.603
5	14.010	7.508	-20.231	22.226	-36.480	21.254
Education						
High School	0.525	3.392	13.966	10.464	7.346	10.578
More than High School	1.654	3.724	15.163	11.258	0.981	11.988
African American	-11.403**	4.445	35.365**	13.182	41.424**	12.486
ApoE (presence of E4)	-0.634	2.004	-3.131	5.958	4.954	5.765
Constant	61.891	14.201	-10.958	42.274	3.204	41.789
R^2	0.540		0.367		0.538	
n	181		185		173	
Power	0.61		0.51		0.82	

Table 11 Continued

	WAIS Digit Symbol		Trails A		Trails B	
	b	SE	b	SE	b	SE
Presence of *T. gondii* seropositivity	-2.622	2.602	-0.404	7.707	-2.027	7.321
Seropositivity & AD (Group interaction)	0.960	3.760	6.968	11.146	16.422	11.061
Female	1.286	1.821	-2.975	5.373	4.020	5.225
Age	-0.321*	0.135	0.806*	0.407	1.513**	0.407
SES (Hollingshead Index)						
2	4.854	7.182	2.967	21.507	-28.046	20.391
3	13.591	7.279	-20.769	21.831	-42.370*	20.711
4	11.939	7.299	-13.281	21.859	-34.788	20.714
5	14.562	7.534	-18.745	22.510	-37.482	21.424
Education						
High School	1.379	3.359	7.591	10.455	8.102	10.490
More than High School	1.707	3.570	7.583	10.913	3.278	11.473
African American	-11.449**	4.469	35.622**	13.376	40.718**	12.639
ApoE (presence of E4)	-0.271	1.967	-3.085	5.898	5.336	5.682
Constant	58.862	14.038	-19.448	42.321	2.803	41.638
R^2	0.531		0.356		0.525	
n	187		191		179	
Power	0.23		0.14		0.45	

Note. * p value = or < 0.05; ** p value < 0.01; *** p value < 0.001.

Table 12

Main Effects Models of Anti-T. Gondii Antibody Titer or Presence of T. Gondii Seropositivity and Each Language Functioning Test Score: Unstandardized Coefficients (Standard Errors) from Linear Regression.

	WAIS Information		Boston Naming Test	
	b	SE	b	SE
Antibody titer	0.301	0.329	0.047	0.617
Seropositivity & AD (Group interaction)	-0.247	0.470	-0.422	0.879
Female	-1.655*	0.773	-2.737	1.451
Age	-0.034	0.056	-0.193	0.107
SES (Hollingshead Index)				
2	-0.185	3.034	-1.950	5.679
3	1.867	3.064	3.403	5.740
4	2.401	3.094	3.662	5.787
5	4.284	3.175	5.039	5.946
Education				
High School	1.392	1.417	-0.782	2.653
More than High School	2.274	1.542	-1.125	2.889
African American	-4.223*	1.884	-12.532**	3.529
ApoE (presence of E4)	-0.189	0.834	0.303	1.578
Constant	20.112	5.877	69.956	11.108
R^2	0.534		0.469	
n	188		187	
Power	0.45		0.30	

Table 12 Continued

	WAIS Information		Boston Naming Test	
	b	SE	b	SE
Presence of *T. gondii* seropositivity	-0.254	1.072	-0.635	2.035
Group interaction	0.218	1.545	-1.721	2.909
Female	-1.748*	0.754	-3.043*	1.416
Age	-0.004	0.055	-0.164	0.105
SES (Hollingshead Index)				
2	0.211	3.028	-2.041	5.669
3	2.223	3.062	3.688	5.738
4	2.760	3.079	4.022	5.762
5	4.717	3.169	5.270	5.939
Education				
High School	1.425	1.397	-1.941	2.615
More than High School	2.195	1.475	-2.077	2.762
African American	-4.107*	1.884	-12.398**	3.530
ApoE (presence of E4)	-0.153	0.815	-0.170	1.541
Constant	18.597	5.789	69.053	10.922
R^2	0.525		0.468	
n	194		193	
Power	0.06		0.23	

Note. * p value = or < 0.05; ** p value < 0.01; *** p value < 0.001.

Table 13

Main Effects Models of Anti-T. Gondii Antibody Titer or Presence of T. Gondii Seropositivity and Each Executive Functioning Test Score: Unstandardized Coefficients (Standard Errors) from Linear Regression.

	WAIS Block Design		Category Fluency – Animals		Category Fluency – Vegetables		Word Fluency S & P	
	b	SE	b	SE	b	SE	b	SE
Antibody titer	0.137	0.598	-0091	0.341	-0.219	0.273	-0.507	0.622
Seropositivity & AD (Group interaction)	-1.018	0.865	-0.415	0.491	0.446	0.531	0.197	0.896
Female	-2.249	1.425	-1.652*	0.800	0.636	0.786	0.301	1.468
Age	-0.097	0.108	-0.126*	0.059	-0.082	0.053	-0.028	0.107
SES (Hollingshead Index)								
2	1.363	5.497	-0.157	3.136	7.094	4.106	-1.658	5.730
3	5.992	5.558	0.212	3.177	7.959*	4.064	0.047	5.790
4	3.483	5.594	1.811	3.203	7.713	4.040	-0.101	5.863
5	7.073	5.749	1.715	3.286	7.917	4.142	1.723	6.007
Education								
High School	-4.597	2.643	0.036	1.526	-1.235	1.599	2.420	2.683
More than High School	-1.143	2.853	0.077	1.649	-1.425	1.740	5.021	2.920
African American	-12.180**	3.407	-2.814	1.947	-2.158	1.470	-10.539**	3.559
ApoE (presence of E4)	0.119	1.552	-0.633	0.869	-0.569	0.832	0.483	1.584
Constant	36.615	10.999	30.182	6.101	14.905	6.269	30.030	11.150
R^2	0.478		0.570		0.402		0.369	
n	180		186		113		186	
Power	0.97		0.22		0.74		0.22	

Table 13 Continued

	WAIS Block Design		Category Fluency – Animals		Category Fluency – Vegetables		Word Fluency S & P	
	b	SE	b	SE	b	SE	b	SE
Presence of *T. gondii* seropositivity	-1.108	2.007	-0.702	1.099	-1.240	0.878	-1.366	2.013
Seropositivity & AD (Group interaction)	-3.529	2.940	-0.513	1.602	2.560	1.708	-0.397	2.924
Female	-2.684	1.415	-1.736*	0.774	0.844	0.747	0.487	1.422
Age	-0.074	0.107	-0.134*	0.057	-0.092	0.052	-0.030	0.104
SES (Hollingshead Index)								
2	1.835	5.583	-0.366	3.100	-7.241	4.013	-1.999	5.686
3	6.300	5.654	0.175	3.145	7.950*	3.973	-0.257	5.752
4	4.591	5.668	1.639	3.157	7.410	3.942	-0.354	5.798
5	7.644	5.840	1.567	3.248	7.750	4.045	1.640	5.959
Education								
High School	-4.032	2.651	0.038	1.491	-0.717	1.508	2.267	2.631
More than High School	-0.825	2.777	0.161	1.561	-0.671	1.614	5.267	2.775
African American	-12.071**	3.468	-2.849	1.928	-2.383	1.438	-10.677**	3.537
ApoE (presence of E4)	0.070	1.543	-0.741	0.840	-0.525	0.780	0.123	1.539
Constant	34.852	11.022	30.908	5.974	14.689	6.211	29.003	10.916
R^2	0.452		0.577		0.452		0.368	
n	186		192		117		192	
Power	0.64		0.24		0.43		0.20	

Note. * p value = or < 0.05; ** p value < 0.01; *** p value < 0.001.

regressions that had significant results (for the WMS-R Digit Span Forward and WMS Letter-Number Sequencing tests) are no longer significant and were likely spurious results due to the large number of regressions performed (Table 14).

Power analysis results. Taking 0.80 as the cutoff for detecting an effect when there is an effect to be detected (Cohen, 1988), only two regressions were at or above 0.80 (association between anti-*T. gondii* antibody titer and Trailmaking B; association between anti-*T. gondii* antibody titer and WAIS Block Design). We determined all other regressions had power estimates much lower than 0.80 except for three: association between anti-*T. gondii* antibody titer WMS Associate Memory (power estimate of 0.74), Free and Cued Selective Reminding Test (power estimate of 0.75), and Category Fluency Vegetables (power estimate 0.74; Tables 9-13).

Discussion

In contrast to the study by Kusbeci et al. (2011), we did not find a significantly higher prevalence of *T. gondii* seropositivity in those with AD compared to control subjects without dementia. Moreover, we did not find significant differences in the average natural log-transformed anti-*T. gondii* IgG antibody titers between the two groups. Additionally, *T. gondii* seropositivity did not predict diagnosis of AD. However, other studies examined the association between *T. gondii* seropositivity and AD and also did not find an association (Mahami-oskouei et al., 2016; Rashno et al., 2016). Our findings are similar to the latter studies, although our study had a larger sample size than any of the previous studies (219 compared to 71, 174, and 150, respectively). Interestingly, some mouse studies have supported the idea that *T. gondii* seropositivity actually protects from AD infection, an idea similar to our results that *T. gondii* seropositivity did not predict AD diagnosis. Specifically, one study found that *T. gondii*

Table 14

Results of Benjamini-Hochberg Procedure for Each Linear Regression.

Comparison	Benjamini-Hochberg Critical Value	*p* value less than critical value
Logical Memory IA – Antibody titer	0.006	No
Logical Memory IA – Presence of seropositivity	0.021	No
Logical Memory IIA – Antibody titer	0.004	No
Logical Memory IIA – Presence of seropositivity	0.022	No
Associate Memory – Antibody titer	0.013	No
Associate Memory – Presence of seropositivity	0.021	No
Selective Reminding Test – Antibody titer	0.015	No
Selective Reminding Test – Presence of seropositivity	0.025	No
Digit Span Forward – Antibody titer	0.002	No
Digit Span Forward – Presence of seropositivity	0.003	No
Digit Span Backward – Antibody titer	0.024	No
Digit Span Backward – Presence of seropositivity	0.013	No
Mental Control – Antibody titer	0.007	No
Mental Control - Presence of seropositivity	0.010	No
Letter-Number Sequencing – Antibody titer	0.001	No
Letter-Number Sequencing - Presence of seropositivity	0.001	No
Digit Symbol – Antibody titer	0.010	No
Digit Symbol - Presence of seropositivity	0.006	No
Trails A – Antibody titer	0.020	No
Trails A - Presence of seropositivity	0.023	No
Trails B – Antibody titer	0.018	No

Table 14 Continued

Comparison	Benjamini-Hochberg Critical Value	*p* value less than critical value
Trails B - Presence of seropositivity	0.016	No
Information – Antibody titer	0.007	No
Information - Presence of seropositivity	0.018	No
Boston Naming – Antibody titer	0.024	No
Boston Naming - Presence of seropositivity	0.015	No
Block Design – Antibody titer	0.019	No
Block Design - Presence of seropositivity	0.014	No
Category Fluency (Animals) – Antibody titer	0.017	No
Category Fluency (Animals) - Presence of seropositivity	0.012	No
Category Fluency (Vegetables) – Antibody titer	0.009	No
Category Fluency (Vegetables) - Presence of seropositivity	0.004	No
Word Fluency – Antibody titer	0.008	No
Word Fluency - Presence of seropositivity	0.011	No

seropositive mice had reduced number and volume of beta-amyloid plaques compared to seronegative mice (Möhle et al., 2016). Additionally, two other studies examining mice models of AD found less beta-amyloid deposition and less hippocampal neuronal death (Carter, 2013; Jung et al., 2012). In fact, if these mice studies are similar to what happens in humans, *T. gondii* seropositivity would predict inclusion in the control group (those without AD). However, this evidence of how *T. gondii* seropositivity affects AD pathology is in mice making definite conclusions difficult. In sum, our analyses did not support our first hypothesis that *T. gondii* seropositivity would significantly predict AD.

Many more studies have examined the association between *T. gondii* seropositivity and cognitive functioning than those that examined AD and *T. gondii* seropositivity. Our study only found an association between one processing speed cognitive measure (WAIS-R Digit Symbol) and *T. gondii* seropositivity for both AD and control groups combined, specifically that *T. gondii* seropositivity may play a role in decreased performance on this neuropsychological test for those seropositive. Additionally, when we analyzed the AD and control groups separately, we found an association between the processing speed test of Trailmaking B and the executive functioning test of Block Design and *T. gondii* seropositivity, specifically that *T. gondii* seropositivity may play a role in decreased performance on these neuropsychological tests for those both with AD and seropositive. With these analyses, we did control for confounding variables of age, SES, education level, ethnicity, gender, and ApoE E4 allele status with a statistically stronger method (propensity score matching) than those used in previous studies. However, for our analyses for the AD group only, we stress that we did not include an interaction term for group membership (AD or control group) in the analyses and as such, we cannot compare the AD group results to the control group results. Additionally, we did not perform a direct statistical test to compare the effect sizes of the control group and the AD group on each neuropsychological test. Thus, we cannot conclude that executive functioning is negatively affected due to *T. gondii* seropositivity in those with AD but not in those without AD. We can only conclude that in this group of persons with AD, *T. gondii* seropositivity seems to have a negative effect on some processing speed and executive functioning tests (Nieuwenhuis, Forstmann, & Wagenmakers, 2011).

Overall, our finding is largely in line with previous findings of cognitive function and *T. gondii* seropositivity which have mainly reported a negative association. Specifically, in regard to processing speed, other studies have found a negative association between *T. gondii*

seropositivity and slower processing speed as a main effect as well as for those of different ethnicities and with lower income levels (Gale, Brown, Erickson, Berrett, & Hedges, 2014; Massa et al., 2016; Pearce et al., 2013). Thus, our results in regard to processing speed support our hypothesis that those *T. gondii* seropositive would have worse performance on measures of processing speed. Our study provides some support for previous work performed on processing speed and *T. gondii* seropositivity.

For our hypotheses that *T. gondii* seropositivity would be associated with worse cognitive functioning for those both seropositive and having AD, we did find a negative association between *T. gondii* seropositivity and processing speed and executive functioning in the AD group. These results supported our hypotheses. However, as mentioned previously, we cannot determine if on these tests, the *T. gondii* seropositive participants in the AD group performed more poorly than the *T. gondii* seropositive participants in the control group because we did not directly compare the two groups. To our knowledge, no other studies have directly assessed cognitive functioning in those with AD who are also *T. gondii* seropositive. Thus, ours is the first study to examine the possibility that *T. gondii* seropositivity may negatively impact cognition in AD. While other studies examining *T. gondii* seropositivity and cognition have not specifically assessed persons with AD, other studies have examined executive functioning in *T. gondii* seropositive older adults. Those studies have found overall worse performance on executive functioning tasks for those seropositive including worse "goal-directed behavior" and faster declines in executive functioning over time (Beste et al., 2014; Nimgaonkar et al., 2016). Overall, our results are in line with these previous studies examining non-demented older adults.

It is unclear how *T. gondii* seropositivity may negatively affect processing speed and executive functioning abilities. Other studies have found declines in processing speed in older

adults associated with structural declines in the prefrontal cortex (Eckert, Keren, Roberts, Calhoun, & Harris, 2010). As mentioned previously, *T. gondii* cysts have been found by other studies throughout the brain, making it a possibility that cysts in the prefrontal cortex could be causing structural changes and thus reducing processing speed. Additionally, researchers have shown small vessel disease to be associated with reduced processing speed (Eckert et al., 2010). A secondary effect of small vessel disease is neuroinflammation and recruitment of microglia and macrophages, which is similar to the inflammatory response seen for *T. gondii* seropositivity (Hermes et al., 2008; Rosenberg, 2017). Overall, *T. gondii* seropositivity may be causing structural changes as well as increased neuroinflammation which could lead to slower processing speed for those seropositive. Another possible reason *T. gondii* seropositivity may decrease cognitive function is the parasite's putative effect on dopamine. As mentioned previously, studies have found both increases and decreases in dopamine in mice brains infected with *T. gondii.* However, *T. gondii* can synthesize L-DOPA and tyrosine and these dopamine precursors could upset the balance of dopamine in the human brain. For example, Beste et al. (2014) explained that increased dopamine in the prefrontal cortex can result in increased processing capacity but make cognitive processing more prone to interference which may make executive functioning worse. Thus, increased dopamine from *T. gondii* seropositivity may interfere with executive functioning in some way. With the prefrontal degeneration seen in AD, increased dopamine in this area may accentuate the already problematic executive functioning associated with the prefrontal cortex. Additionally, any structural changes caused by *T. gondii* cysts in the prefrontal cortex could also potentially affect executive functioning, as the prefrontal cortex has been associated with executive functioning in many studies (Funahashi & Andreau, 2013). Thus, changes in dopamine and/or structure in the prefrontal cortex due to *T. gondii* seropositivity

could account for decreases in processing speed and executive functioning seen in our study. However, because we did not directly assess dopamine, inflammation, or structural changes due to *T. gondii* seropositivity, we cannot be certain of any of these proposed mechanisms.

In regard to our other hypotheses, we did not find any other associations between other cognitive domains (memory, working memory, language functioning, or executive functioning) and *T. gondii* seropositivity using either propensity score matching or linear regressions for the AD and control groups combined. Thus, our analyses did not support our hypotheses of *T. gondii* seropositivity predicting worse cognitive functioning for the combined groups in these other cognitive domains. This is different than other published studies which do find an association between *T. gondii* seropositivity and cognitive functioning, most of which find a negative association between the two. A few studies have not found an association between cognitive functioning and *T. gondii* seropositivity (Dickerson et al., 2014; Gulinello et al., 2010).

There could be several factors that would account for the differences in our study from previous studies. The age, education, socioeconomic status, and ethnicity of participants in different studies are often significantly different or not reported by the authors. Our study has the strength of using propensity score matching which matched the participants on these variables, reducing the influence of these variables on the outcome of analyses. To our knowledge, no other study has used propensity score matching to control for these potentially confounding variables when examining the association between *T. gondii* seropositivity and AD or the association between *T. gondii* seropositivity and cognition in humans. However, it is possible that an association between AD and *T. gondii* seropositivity or cognition and *T. gondii* seropositivity in our study may have been attenuated in some manner. For example, if the participants in our study were significantly older than those in other studies, it is possible that *T.*

gondii seropositive subjects may have died at a higher rate than those seronegative. Additionally, as lower education and lower SES has been associated with worse cognitive performance in *T. gondii* seropositive participants in other studies, the high education level and higher SES in both the AD and control groups in our study could have protected against detrimental effects of *T. gondii* seropositivity, masking associations between AD, cognition, and *T. gondii* seropositivity (Gale et al., 2014).

Another possible reason for our study finding different results than other studies may be the difference in human infection between different strains of *T. gondii,* as different strains of the parasite are associated with differences in virulence (Xiao & Yolken, 2016). We did not identify specific strains of *T. gondii* in our sample and therefore, could neither control for strain difference in our sample nor compare the strain composition between our sample and those of other studies. In addition, host factors likely influence the virulence of *T. gondii* (Xiao & Yolken, 2016). We did not evaluate genetic or other host factors in our sample that could affect the virulence of *T. gondii* seropositivity on human disease and potentially cognition.

The result of our anti-*T. gondii* IgM antibody analysis (with only one subject positive for IgM antibodies in both of the AD and control groups) indicate that the participants in our sample who were positive for anti-*T. gondii* antibodies were seropositive for latent but not acute Toxoplasmosis. The low prevalence of anti-*T. gondii* IgM antibodies also suggests that in older adults, AD does not reactivate *T. gondii* seropositivity, making it unlikely that any association between *T. gondii* seropositivity and AD is due to a reactivated infection.

Strengths and Limitations

Our study has several strengths, particularly its use of PSM as a statistical method. PSM helps determine causality more so than other statistical methods because it can answer three of the seven requirements for inferring a causal relationship between two factors (Kazdin, 2003). First, in this study, we have clear definitions for our outcome and predictor variables. We defined the outcome, AD, by using standard clinical procedures by WU including: an informant based semi-structured interview assessing six cognitive domains to give the Clinical Dementia Rating (CDR) which interview included 18 relevant scales/questionnaires. We defined the predictor variable by the presence or absence of anti-*T. gondii* IgG antibodies, an objective measure. The use of PSM also reduces the chance of selection bias between the control and AD groups because of the inclusion and matching on several covariates. We included many known covariates that can affect development of both *T. gondii* seropositivity including gender, education, SES, age, ethnicity, and ApoE E4 allele status. Because of the matching procedure of PSM, those covariates can be ruled out as potential explanations of the relationship (or lack of relationship) between *T. gondii* seropositivity and AD. However, there may be other unknown covariates of both *T. gondii* seropositivity and AD that were not accounted for in our study. Additionally, because of the use of PSM, we can have more ability to generalize our results than previous studies using other statistical methods. However, because we included so few people of ethnicities other than White in the study along with the higher levels of education and SES in our sample, generalizations still may be limited. Additionally, we cannot determine the temporal precedence of *T. gondii* seropositivity before development of AD because we do not have data of when each participant became seropositive. Lastly, our results do not show a relationship statistically between *T. gondii* seropositivity and development of AD, which relationship is

required for a causal relationship. Other strengths of the study include the larger sample size (compared to previous studies), the analysis of many cognitive areas (rather than just one, like in other studies), and the relative homogeneity of the control and AD group.

We should consider several limitations in the interpretation of our findings. The cross-sectional design we used does not allow us to evaluate survivor effects. *T. gondii* seropositive subjects with AD may have differentially died before being assessed for *T. gondii* seropositivity, making the seroprevalence more equal in the control and AD groups. Also, the cross-sectional design of our study does not allow us to assess how anti-*T. gondii* IgG antibodies changed over time and the effect that may have on AD status and/or cognitive functioning. For example, Konishi (1989) showed that longitudinally those with higher anti-*T. gondii* IgG antibody levels tended to have decreases in antibody levels over time. However, many of the subjects in that study demonstrated no significant change in antibody titer levels over time and others showed increases in antibody titers over time making it difficult to say all *T. gondii* seropositive persons will have a decrease in antibody titers over time. That being said, a decrease in anti-*T. gondii* IgG antibody levels over time could have led to false negatives in our study, resulting in a misclassification of *T. gondii* seropositivity status. This could have led to the high rate of no associations we found in our study because older adults infected before being enrolled in the study could have a low anti-*T. gondii* titer due to an anamnestic antibody response (lowering of antibody titers). However, since our study data are cross-sectional, we cannot directly assess the length of infection and we also cannot assume that seropositive participants with lower titers are those with the longest length of *T. gondii* seropositivity. In addition, we cannot determine whether any of the subjects had been exposed to *T. gondii* more than once or whether a previous

infection had been reactivated at some time. In animals, a reactivation of *T. gondii* seropositivity is associated with increases in IgG and IgA antibodies but not IgM antibodies (Singh et al., 2011).

Importantly, we did not include an interaction term for group (AD or control) when we performed our analyses using propensity score matching. Due to this, we cannot compare our results for the AD group to the control group, which is a limitation of our study. Additionally, we did not assess or include all of the potential risk factors for both AD and development of *T. gondii* seropositivity in the models used in our study such as living in foreign countries (outside the US), having dogs at home, eating raw dried meat, and raising animals (Alvarado-esquivel et al., 2014). Further, the demographic characteristics of our sample limit the generalizability of these findings to other groups. For example, very few of our sample were not White (seven percent in the AD sample and zero percent in the control sample). Given the widespread distribution and relatively high seroprevalence of *T. gondii,* and the need to determine potentially modifiable risk-factors for AD, any association between *T. gondii* seropositivity and neurodegeneration needs thorough study, particularly given the neuroinflammation and other neurological changes associated with *T. gondii* seropositivity.

Conclusion

In conclusion, despite one previous report showing a possible association between *T. gondii* seropositivity and AD, we found no evidence of an association between *T. gondii* seropositivity and AD in a cross-sectional study. Additionally, we found limited evidence of a negative association between processing speed and *T. gondii* seropositivity. We also found evidence of negative association between processing speed, executive functioning, and *T. gondii* seropositivity in those with AD. Limitations associated with this study, however, indicate that

the research field needs additional work investigating the possible associations between *T. gondii* seropositivity, AD, and cognitive functioning.

Given the continuing emerging evidence that *T. gondii* seropositivity may play a role in declining cognition for older adults, employable ideas for prevention of *T. gondii* infection are needed, especially for countries with high levels of seroprevalence. As cats are most prevalent on farms and in rural areas, one possibility for prevention is to vaccinate cats on farms by having cats ingest a strain of *T. gondii* oocysts, immunizing the cats without making the cats produce oocysts. This technique was tested in Illinois in the United States and proved to greatly reduce the seroprevalence in pigs and mice on the farms tested (Dubey & Jones, 2008). While no vaccine to *T. gondii* currently exists for humans, vaccinating cats may help prevent the transmission of *T. gondii.* In addition, research into a possible vaccine for humans against *T. gondii* may help reduce seroprevalence rates, particularly for those countries with high rates. Until such a vaccine can be created, other preventative techniques could be promoted by health departments, healthcare facilities, and even schools and universities. One of the most common forms of transmission of *T. gondii* to humans is through contaminated meat and accordingly, education about properly cooking meat, especially in rural communities could be useful. In addition, prevalence of *T. gondii* in wild game and venison is very high in the United States (Dubey & Jones, 2008). Education for hunters on how to prepare game for human consumption as well as the proper disposal of game viscera (by burying) may help reduce transmission of *T. gondii* due to hunting. This education could be provided by local community centers, places where hunting guns are sold, or even by shooting ranges. For more populated areas, education for families about the potential contamination of sandboxes with cat feces may help reduce infection rates for children. Lastly, as of 2008, there are over 150 million cats in the United

States (Dubey & Jones, 2008). Increased emphasis on campaigns to spay and neuter cats (including feral cats) such as efforts by the Best Friends Animal Society, may help contain an expanding cat population and as a consequence, reduce transmission of *T. gondii.*

In addition to prevention efforts, more research is needed by the field to expand and solidify the connections between *T. gondii* seropositivity and cognition. Important holes in the current research include a lack of studying potential differences on cognition due to different *T. gondii* strains, interactions between *T. gondii* and different host factors, and use of longitudinal techniques. There are three main genotypes of *T. gondii* strains with a fourth atypical strain (Webster et al., 2013). Very few studies have examined the differences in cognitive effects for mice due to strain and to our knowledge, no study in humans has examined the possible effect of *T. gondii* strain on cognition. Different *T. gondii* strains may affect humans differently. For example, some strains have higher levels of expression of tyrosine hydroxylase genes which may interact differently when encysted in the human brain (Webster et al., 2013). More differences have been seen genetically between strains which may likely result in differences in how *T. gondii* affects cognition in humans. Along with different *T. gondii* strains, different strains of mice have been used in many experiments. Worth et al. (2014) point out the importance of differences in susceptibility to *T. gondii* between mice strains. Different models of mice, such as mouse models of AD or other neurological disorders could also be useful for researchers in elucidating how *T. gondii* seropositivity affects cognition when combined with different disorders.

Just as different strains of mice may play a role in *T. gondii* seropositivity, individual differences in humans may also affect how seropositivity affects cognition. Human differences in genetics and immune responses may be of particular interest for researchers. For example, of

the 432 known genes associated with AD, 27.3% of them are involved in the *T. gondii* interactome. This is significantly higher than would be expected by chance according to chi-square analysis. The overlapping genes in *T. gondii* and AD include genes for amyloid precursor protein (APP) processing, cholesterol and lipoprotein function, complement and immune-related genes, and oxidative stress, apoptosis and ubiquitin genes, which are all important genes for both AD and *T. gondii* seropositivity (Carter, 2013). Given the genetic overlap between one neurological disorder and *T. gondii,* other human neurological disorders may also have similar genes to *T. gondii*, which may influence the effect of the parasite on humans. Also, differences in common genes may protect humans from detrimental effects of *T. gondii* seropositivity. As mentioned previously, some studies did not find an effect of *T. gondii* seropositivity on cognition or found that seropositivity was associated with better cognition (Gulinello et al., 2010; Stock et al., 2014). These differences in results may be due to differences in genetic factors that interact between humans and the parasite, but these factors have yet to be studied by researchers. Another important individual factor is the differences in the immune response to *T. gondii* seropositivity. For example, a few studies have examined the potential interactions between *T. gondii* seropositivity, cytokines, and cognition in mice but none, to our knowledge, have examined this relationship in humans (Hamdani et al., 2015; Mahmoudvand, Sheibani, Shojaee, et al., 2016). A different immune response by a host, such as more or less neuroinflammation, may result in different cognitive outcomes.

Finally, an important way to bring clarity and resolution to the research on *T. gondii* seropositivity and human cognition would be for research to use longitudinal studies. Currently, there is debate in the literature on how antibodies to *T. gondii* change over time and how that relates to cognition (Flegr et al., 2003). Additionally, researchers know little about how the time

of infection may differentially affect cognition. For example, does infection in childhood lead to more detrimental effects on cognition than in adulthood because of the brain's continuing development? Or could infection in adulthood lead to more susceptibility to or early onset of dementia because of increased neuroinflammation? These questions would best be answered through researchers using longitudinal techniques.

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