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Latent ;*Toxoplasma gondii*; Infection Moderates the Association Between the C677T MTHFR Polymorphism and Cognitive Function in U.S. Adults

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Doctor of Philosophy

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ABSTRACT

Latent *Toxoplasma Gondii* Infection Moderates the Association Between the C677T MTHFR Polymorphism and Cognitive Function in U.S. Adults

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Sufficient blood concentrations of folate and the products from its metabolism are necessary for several cellular functions. The C677T MTHFR polymorphism, present in over half of the U.S. population, reduces the efficiency of folate metabolism and has been linked to the onset of multiple psychiatric disorders and cognitive decline. The intracellular parasite *Toxoplasma gondii* can infect the human brain and is associated with increased prevalence of psychiatric disorders and cognitive decline. *In vitro* studies have found that *Toxoplasma gondii* may salvage unmetabolized folate from host cells. Since the C677T MTHFR polymorphism and infection by *Toxoplasma gondii* both affect folate metabolism or availability, I used data from the third National Health and Nutrition Examination Survey to test the hypothesis that latent toxoplasmosis and the C677T MTHFR polymorphism interact to predict worse cognitive functioning in U.S. adults. I found a statistically significant interaction effect between *Toxoplasma gondii* infection and the C677T MTHFR polymorphism in predicting performance on a test of reaction time. Subjects who were not infected with *Toxoplasma gondii* experienced declines in reaction time with the presence of one or two alleles for the C677T MTHFR polymorphism. However, this association was reversed for subjects who were seropositive for *Toxoplasma gondii.* No interaction effects were observed when predicting performance on a test of processing speed or a test of short term memory. In conclusion, these findings suggest that the co-occurrence of *Toxoplasma gondii* infection and the C677T MTHFR polymorphism may be associated with improved reaction time.

Keywords: *Toxoplasma gondii*, MTHFR, folate, cognitive function, reaction time

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Latent *Toxoplasma Gondii* Infection Moderates the Association Between the C677T MTHFR Polymorphism and Cognitive Function in U.S. Adults

Healthy cognitive functioning is dependent upon several factors including genetics, nutrition, environmental influences, and others. Often, these endogenous or exogenous factors interact in ways that either impair or improve cognitive function. One such case involves the bioavailability and metabolism of folate. A sufficient blood folate concentration is necessary for healthy neuronal and cognitive function. Genetic mutation of the methylenetetrahydrofolate reductase (MTHFR) enzyme and infection with the apicomplexan parasite *Toxoplasma gondii* (*T. gondii*) have each been shown to independently interfere with folate metabolism or availability. The purpose of this study is to determine the neuropsychological impact of an interaction between the C677T MTHFR polymorphism and *T. gondii* infection.

The Folate Cycle

Folates are key nutrients utilized by the human body in several biological processes including (but not limited to) DNA synthesis and repair, various methylation reactions, and the conversion of homocysteine to methionine. Sufficient dietary folate is also required for healthy neurologic development and function (Lan, Guhaniyogi, Horn, Xia, & Graham, 2007). Despite the multiple uses of folates within the body, humans lack the ability to synthesize them *de novo*. Thus, folates must be obtained in the diet via naturally folate-rich plants, artificially fortified foods (via folic acid), or dietary supplements. While plant-derived folates primarily exist in the already reduced dihydrofolate (DHF) state, folic acid must first be converted to DHF by dihydrofolate reductase before it can be further metabolized. Ultimately, whether from natural folates or from synthetic folic acid, DHF is further converted to the more stable tetrahydrofolate (THF) state by dihydrofolate reductase (Bailey, 1995). In this form, THF enters the one-carbon

(1-C) folate cycle in which THF accepts or donates methyl groups to or from its N^5 and N^{10} active sites. Methyl groups are utilized in numerous biochemical pathways and are exchanged between biomolecules to initiate methylation and other biochemical reactions. A variety of molecules, including THF, act as transporters of these methyl groups. In folate metabolism, hydroxymethyltransferase transfers methyl groups from two serines to THF to form 5,10-methylenetetrahydrofolate (5,10-MTHF). In the 5,10-MTHF state, this form of folate is prepared to donate its newly acquired methyl groups to other biological processes.

Several coenzymes assist in the reception or donation of methyl groups to or from THF. One such enzyme is MTHFR, which acts as a catalyst in the irreversible removal of a single methyl group from the N^{10} position of 5,10-MTHF to generate 5-methyltetrahydrofolate (5-MTHF). In this form, 5-MTHF is unalterably dedicated to the singular role of methyl donation to homocysteine with vitamin B_{12} acting as a co-factor (Bailey, 1995). In other words, once removed, a methyl group cannot be reattached to 5-MTHF to reform 5,10-MTHF.

The transfer of the final methyl group from 5-MTHF to homocysteine is completed in a two-step process. The methyl group is briefly handed to an awaiting cobalamin (vitamin B_{12}) forming methylcobalamin (methyl-B_{12}). Methyl-B_{12} then transfers the methyl group to homocysteine thereby converting it into methionine. The process of transferring the methyl group from 5-MTHF to homocysteine is facilitated by the enzyme methionine synthase. In other words, methionine synthase simultaneously interacts with 5-MTHF, vitamin B_{12}, and homocysteine to ultimately transfer the methyl group from 5-MTHF to homocysteine and thereby convert homocysteine to methionine.

In elevated quantities, homocysteine can become neurologically toxic by interacting with glutamate and glycine binding sites on N-methyl-D-aspartate receptors and promoting the

accumulation of reactive oxygen species in the brain. As these reactive oxygen species are severely damaging to cells, apoptotic and necrotic cell death typically follows their accumulation (Boldyrev, Bryushkova, Mashkina, & Vladychenskaya, 2013). Therefore, the conversion of homocysteine to methionine is essential for healthy neurologic function. Following methyl group donation from 5-MTHF to vitamin B_{12}, the resulting THF product is either recycled or eliminated.

The C677T MTHFR Polymorphism

Ongoing research efforts have been aimed at uncovering the potential physiologic consequences of various 1-C folate cycle failures. Potential sources of these failures appear to vary immensely and may include behavioral, environmental, and/or genetic causes. For instance, limited availability of folate-rich or fortified foods (such as in developing countries) may increase the risk of folate deficiency. Alternatively, genetic mutation of key folate cycle enzymes, such as the MTHFR enzyme, can also significantly alter the efficiency of folate metabolism (Ho, Massey, & King, 2013).

The most common mutation of the MTHFR enzyme is the C677T single nucleotide polymorphism (Botto & Yang, 2000). Genetic code for the human MTHFR enzyme is found in the p36.3-36.2 region of chromosome 1 (Goyette et al., 1994). Mutation of this enzyme may occur when a cytosine in position 677 of the MTHFR gene is replaced with a thymine. This point mutation results in the generation of the amino acid valine instead of the expected alanine. The point mutation reduces the thermostability and, subsequently, enzymatic efficiency of the enzyme in a step-wise manner depending on whether an individual is heterozygous (C/T) or homozygous (T/T) for the mutation with the T/T genotype being the fully mutated form (Liew & Gupta, 2015). One report has predicted that MTHFR efficiency may be reduced by upwards of

40 to 50 percent in T/T homozygous mutants (Rai, 2017). Due to this reduction in MTHFR efficiency, less 5-MTHF is produced. Reduced availability of 5-MTHF directly influences the rate at which methionine synthase can convert homocysteine to methionine.

The epidemiology of the C677T MTHFR polymorphism depends on factors such as ethnicity with the highest prevalence appearing in countries such as Mexico and Italy and lowest among individuals of African descent (Botto & Yang, 2000; Wilcken et al., 2003). Previous reports have suggested that approximately 45% of the United States (U.S.) population may be CC homozygous (non-mutated), 45% C/T heterozygous, and 10% T/T homozygous (Ho et al., 2013; "RS1801133,").

The consequences of an MTHFR polymorphism appear to be relatively widespread due to the multiple uses of folate and the products of folate metabolism throughout the body. In fact, it is estimated that nearly every type of cell in the human body contains a folate transporter of some type (Mattson & Shea, 2003). However, due to the neurotoxic properties of elevated homocysteine and the relatively heavy use of folate in the brain, the neurological and psychological consequences of MTHFR mutation have received added attention over recent years. For example, a human embryo developing in a folate-depleted environment is at a significantly greater risk of developing neural tube defects or spontaneously aborting (Liu et al., 2014). Further, the frequency of autism spectrum disorder and Down syndrome have each been reported to be higher in children with MTHFR mutations (Boris, Goldblatt, Galanko, & James, 2004; Liu et al., 2011; Pu, Shen, & Wu, 2013; Wu et al., 2013). Beyond associations with developmental outcomes, the C677T MTHFR polymorphism has also been associated with increased rates of migraines, depression, Parkinson's disease, and Alzheimer's disease in adults (Azimova et al., 2013; Bousman, Potiriadis, Everall, & Gunn, 2014; Lok et al., 2014; Rai, 2017).

It is possible that the link between the C677T MTHFR polymorphism and neurological or psychological outcomes is related to an elevation of homocysteine. Hyperhomocysteinemia due to an MTHFR polymorphism may result in impaired DNA repair (Kruman et al., 2002), elevated concentrations of inflammatory mediators (Mansoori et al., 2012), and even an enhanced production of molecules such as neurofilaments, tau proteins, and beta-amyloid precursor proteins associated with neurodegenerative diseases (Hasegawa et al., 2012). Ultimately, prior literature suggests that the presence of at least one copy of the C677T MTHFR polymorphism may be related to clinically relevant cognitive decline and increased odds of developing a psychiatric disorder such as depression.

Toxoplasma gondii

The C677T MTHFR polymorphism is not the only means by which folate metabolism could become compromised. Several other factors, including environmental ones, may also negatively affect folate metabolism. One such factor may include the intracellular, apicomplexan parasite *Toxoplasma gondii.* While the definitive host for *T. gondii* is any member of the cat family, epidemiologic studies suggest that approximately 12 to 13 percent of humans in the United States (Jones, Kruszon-Moran, Rivera, Price, & Wilkins, 2014) and between 20 to 60 percent of people living in developing countries around the world (Pappas, Roussos, & Falagas, 2009) may be latently infected with the parasite. No one primary route of transmission has currently been identified. However, congenital transmission, ingestion of improperly cooked foods, contaminated water, or exposure to the feces of infected cats may all be potential candidates (Sakikawa et al., 2012). Upon successful invasion of the host, *T. gondii* typically locates to and resides in muscle and neural tissue, including the brain. To preserve itself against the host immune system, the parasite encapsulates itself in a cyst-like structure formed by a

parasitophorous vacuole membrane (Weiss & Dubey, 2009). Inside the cyst is a densely packed cluster of *T. gondii* bradyzoites. In this form, *T. gondii* is semi-dormant and cellular division is slow. The tissue cysts containing *T. gondii* generally persist for the life of the host and maintain an ongoing, latent infection often termed "latent toxoplasmosis". Despite the longevity of *T. gondii* cysts, recent findings show that a very small percentage may rupture and form new cysts (Hill, Sreekumar, Jones, & Dubey, 2007), thereby recycling aged cyst structures with new ones.

Though latent toxoplasmosis is not typically associated with severe or observable symptoms, the infection itself is not necessarily silent. Latent toxoplasmosis has been associated with several psychological and neurological outcomes. An abundance of literature centers on associations between *T. gondii* infection and dopamine-related behaviors or disorders. For instance, *T. gondii* infection may be associated with increased odds of developing schizophrenia (Fabiani, Pinto, Bonuccelli, & Bruschi, 2015; Flegr, 2013; Henriquez, Brett, Alexander, Pratt, & Roberts, 2009). Such an association is potentially explained by the parasite's unique ability to generate significant concentrations of dopamine, a neurotransmitter linked to multiple behaviors and neurological functions (Martin et al., 2015; Prandovszky et al., 2011). Dopamine-related behaviors such as risk-taking (Pedersen, Mortensen, Norgaard-Pedersen, & Postolache, 2012), addiction (Sutterland et al., 2015), and aggression and impulsivity (Cook et al., 2015) have each been shown to be potentially modified in infected humans or animals. Beyond the potential links between *T. gondii* dopamine production and changes in behavior, the parasite may affect the human brain in additional ways. For example, the general inflammatory burden of infectious diseases has been associated with cognitive impairments and neurologic health (Elkind et al., 2010; Gale, Erickson, Berrett, Brown, & Hedges, 2015; Katan et al., 2013).

Another potential way in which *T. gondii* may affect both the psychological and neurological health of human hosts involves a possible association between *T. gondii* infection and cellular concentrations of folate and other factors involved in the 1-C folate cycle. Massimine et al. (2005) demonstrated that *T. gondii* may be capable of salvaging folate from infected host cells. If indeed true, it is possible that infected neural cells could suffer from *T. gondii*-related folate depletion and subsequent homocysteine elevation resulting in a decline in cognitive functioning. However, no studies have yet quantified the amount of folate that is salvaged from infected cells, nor has any research been conducted to determine the extent to which *T. gondii* folate salvaging might impair cognitive functioning. To explore the latter question, I previously conducted a study in which I used data from Center for Disease Control and Prevention's (CDC) third National Health and Nutrition Examination Survey (NHANES) to determine whether infection with *T. gondii* might interact with concentrations of multiple folate cycle factors in the prediction of cognitive functioning (Berrett, Gale, Erickson, Brown, & Hedges, 2017).

The third NHANES includes data for multiple infectious diseases, nutritional biochemistries, and cognitive functioning for those who were eligible. Cognitive functioning was assessed via performance on computerized versions of the symbol digit substitution (SDS), serial digit learning (SDL), and reaction time (RT) tests as described by Krieg et al. (2001). By predicting performance on three separate tests of cognitive functioning, I could more specifically identify which components of cognitive functioning might be particularly affected by reductions in concentrations of folate factors due to *T. gondii* infection. The SDS assesses processing speed but also requires intact working memory and attention to perform well. The SDL is generally considered a measure of learning and attention and requires normal ability in short term memory.

The RT is a measure of reaction time involving both sensory and cognitive control systems (Krieg et al., 2001).

Briefly, the results of the study revealed significant interaction effects between *T. gondii* infection status and each of the folate factors examined (folate, vitamin B_{12}, and homocysteine) in the prediction of performance on the SDL. Specifically, subjects who did not have antibodies specific for *T. gondii* exposure performed similarly on the SDL no matter the concentrations of folate, vitamin B-12, or homocysteine. However, for subjects seropositive for *T. gondii* infection, SDL performance significantly worsened as folate and vitamin B-12 concentrations decreased and as homocysteine increased. Since these interaction effects were limited to the SDL, I could conclude that reduced folate concentrations, potentially due to *T. gondii* salvaging, may particularly affect brain regions that are active in learning and attention such as the hippocampus or pre-frontal cortex.

Interacting Effects of MTHFR Mutation and *T. gondii* Infection

Since MTHFR mutation and *T. gondii* infection each affect the 1-C folate cycle, albeit in unique ways, it is possible that folate metabolism could be affected by an interaction between both factors. Especially given the relatively large prevalence of both MTHFR mutation and *T. gondii* infection, a significant percentage of the population may be heterozygous or homozygous for the C677T MTHFR polymorphism while also infected with *T. gondii*. In this case, cellular concentrations of folate would initially be reduced due to *T. gondii* folate salvaging. With a reduced concentration of available folate, enzymes of the 1-C folate cycle such as MTHFR would be starved of this initial ingredient necessary for product generation. In people void of any folate-cycle-related genetic mutations, the consequences of depleted MTHFR product could be enough to affect cognitive function. However, an individual homo- or heterozygous for an

MTHFR mutation would simultaneously possess MTHFR enzymes that are less efficient in the processing or conversion of the now reduced concentration of folate factors. Thus, production of 5-MTHF, the primary product of MTHFR, would be substantially hampered. In this case, infection by *T. gondii* would reduce the availability of THF to be metabolized by an MTHFR enzyme that is already less efficient due to genetic mutation. Figure 1 provides a graphical presentation of this conceptual model. The downward pointing arrows indicate that, depending on the color of the arrow, *T. gondii* infection or the C677T MTHFR polymorphism is responsible for a decrease in concentration or efficiency of that folate cycle component. Upward pointing arrows indicate elevations in concentrations or enzymatic efficiency.

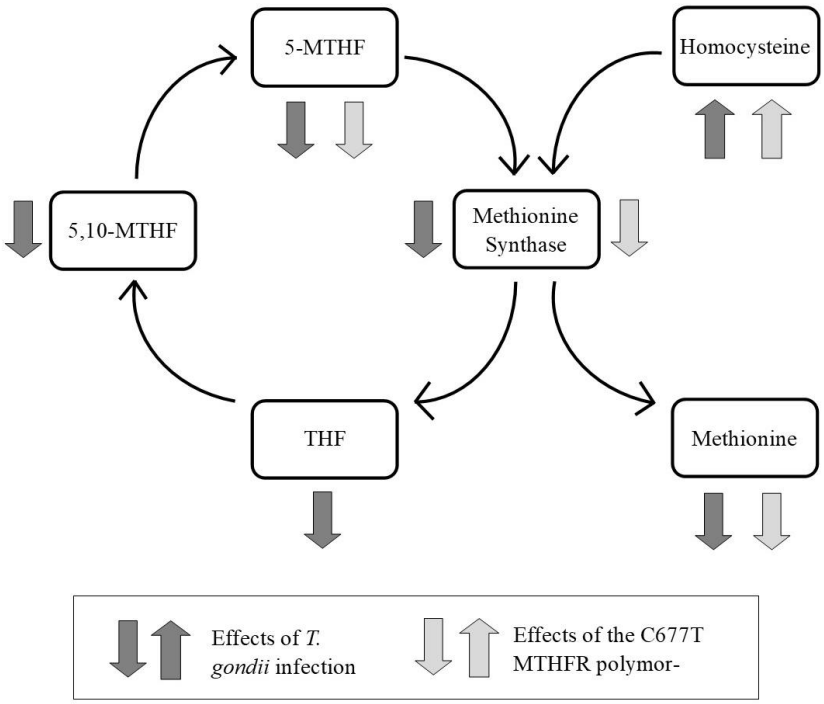

Figure 1. Conceptual model of the effects of *T. gondii* infection and the C677T MTHFR polymorphism on folate metabolism.

Helicobacter pylori (H. pylori), a bacterium that resides in the gastric epithelium (Hunt & Tytgat, 2003; Mobley, Mendz & Hazell, 2001) has also been purported to interfere with folate metabolism (Akcam, Ozdem, Yilmaz, Gultekin, & Artan, 2007; Berrett, Gale, Erickson, Brown, & Hedges, 2016a; Beydoun, Dore, Canas, Beydoun, & Zonderman, 2015). Though *H. pylori* is indeed biologically different from *T. gondii* in many ways, the downstream effects of infection on folate availability by either organism may be generally similar. In 2016, I and an interdepartmental research group designed a study (Berrett, Gale, Erickson, Brown, & Hedges, 2016b) in which we used data from the CDC's 1999-2000 NHANES data set to test for the possibility of an interaction between *H. pylori* seropositivity and total serum 5-MTHF in the prediction of cognitive function. Though we were unable to utilize genetic data for this study, the 1999-2000 cycle of the NHANES survey included data for multiple folate cycle factors including total serum 5-MTHF, vitamin B_{12}, and homocysteine concentrations. Data for serum 5-MTHF concentration was particularly useful as an indirect measure of MTHFR efficiency because, since MTHFR is the sole producer of 5-MTHF, a reduced concentration of 5-MTHF suggests a potential reduction in MTHFR efficiency.

In the 2016 study, cognitive function was assessed through the administration of the Digit Symbol Coding (DSC) task adapted from the WAIS-III (Wechsler, 1997). The availability of cognitive assessment data varies by NHANES survey cycle and because this was the only NHANES survey cycle with 5-MTHF data, we were limited to the DSC as the only measure of cognitive functioning. This cognitive task is generally considered a measure of processing speed but optimal performance in this task also requires healthy ability in working memory and attention (Lezak, Howieson, Bigler, & Tranel, 2012). Performance is measured by the number of correct substitutions performed within a two-minute time limit.

Ultimately, our analyses revealed a statistically significant interaction effect between 5-MTHF concentration and *H. pylori* infection in the prediction of DSC task performance. For uninfected subjects, progressively lower concentrations of 5-MTHF were associated with fewer correct matches on the DSC. This effect was relatively minor but statistically significant. However, for subjects who were seropositive for *H. pylori* infection, the slope of this association significantly increased suggesting that subjects who were infected with *H. pylori* and who simultaneously also had less bioavailability of 5-MTHF (at the time of examination) performed substantially worse on this test of processing speed compared to uninfected subjects (Berrett et al., 2016b).

The Current Study

The results of the studies discussed above provide initial evidence and rationale for a potential interaction effect between *T. gondii* infection and genetic mutation of the MTHFR enzyme. The first study presented evidence that *T. gondii* modifies the association between serum folate concentration and cognitive functioning (Berrett et al., 2017). The study investigating the interacting effects between *H. pylori* infection and serum concentrations of 5-MTHF, vitamin B-12, and homocysteine in the prediction of cognitive functioning supports the theory that folate concentration and metabolism could be affected by organisms such as *H. pylori* or *T. gondii* as well as genetic polymorphisms (Berrett et al., 2016b). However, neither of the above studies directly test for an interaction effect between *T. gondii* infection and the C677T MTHFR polymorphism in the prediction of cognitive functioning. Further, 5-MTHFR or folate concentrations cannot be used to determine MTHFR genotype. Instead, with MTHFR genotype data, it would be possible to determine whether *T. gondii* infection interacts with MTHFR genotype to predict differences in cognitive functioning.

In fact, the same data sets created by the CDC that were utilized to explore an interaction between *T. gondii* and folate concentrations also contain genetic data for MTHFR genotype. However, access to this data is limited to researchers who submit and receive approval for a study proposal. Therefore, I submitted such a proposal to the CDC to gain access to this genetic data and thereby test my hypotheses. I expected that subjects who were hetero- or homozygous for the MTHFR C677T polymorphism would perform incrementally worse on tests of cognitive function compared to those without an MTHFR polymorphism. Further, I expected that *T. gondii* infection status would interact with MTHFR genotype to predict worse performance on tests of cognitive functioning. Specifically, I hypothesized that the degree to which the MTHFR C677T polymorphism might affect cognitive task performance would be conditional upon *T. gondii* infection status.

Aims of the Study

The primary aim of the present study was to determine if mutation of the MTHFR gene and infection by the intracellular parasite *T. gondii* would interact to impair cognitive function. The specific aims and corresponding hypotheses of this study included:

1. Confirm the presence of an association between *T. gondii* infection and cognitive functioning as measured by the SDS, SDL, and RT tests. Previous reports (Gale et al., 2015) have indicated that *T. gondii* infection alone may be sufficient to affect cognitive function. However, a confirmation of this association would aid in establishing the rationale for this study.
2. Test for associations between individual folate cycle factors (folate, vitamin B12, homocysteine) and cognitive performance. At the time of this study, prior literature still disagrees concerning the magnitude of effect that folate, vitamin B12, and homocysteine

levels have on cognitive functioning. Again, confirming such associations would further justify the rationale for this study. However, the absence of significant main effects between folate, vitamin B12, or homocysteine with cognitive functioning would not limit the possibility that interactions with *T. gondii* might be associated with impaired cognitive function.

3. Test for an association between the C677T MTHFR mutation and performance on the SDS, SDL, and RT cognitive assessments. Based on prior literature (Cheng et al., 2010; Schiepers et al., 2011), I hypothesized that subjects who were hetero- or homozygous for the MTHFR mutation would perform worse on each of the cognitive assessments compared to non-mutated subjects. Further, I expected this association to follow a dose-response pattern. In other words, subjects who were heterozygous for the MTHFR polymorphism would perform worse than subjects who did not possess the polymorphism while subjects who were T/T homozygous for the MTHFR polymorphism would perform worse than either C/C homozygotes or C/T heterozygotes with the biggest contrast being between T/T homozygotes and C/C homozygotes.
4. Test for an interaction effect between MTHFR genotype and *T. gondii* infection status in the prediction of SDS, SDL, and RT test performance. Based on previous findings, I expected to observe an interacting effect between MTHFR genotype and *T. gondii* infection status and that this interaction would significantly relate to SDS, SDL, and RT test performance. Specifically, I hypothesized that, in uninfected subjects, MTHFR genotype (C/C, C/T, T/T) would be associated with step-wise reductions in performance on each cognitive measure. However, I expected that this effect would be substantially magnified in subjects who were seropositive for *T. gondii.*

5. If a statistically significant interaction effect was observed between *T. gondii* infection and MTHFR genotype, I would perform a follow-up analysis to further illuminate potential mechanisms. Specifically, I intended to test if homocysteine concentration mediates the relationship between the MTHFR genotype and *T. gondii* interaction in the prediction of performance on the SDS, SDL, and RT tests. I hypothesized that such a mediation effect would be revealed and that the MTHFR and *T. gondii* interaction would lead to increased concentrations of homocysteine that would subsequently relate to worse performance on each cognitive assessment.

Materials and Methods

Study Sample

Approximately 34,000 participants were recruited for the third National Health and Nutrition Examination Survey conducted between 1988 to 1994. The NHANES III was divided into two phases. Phase 1 spanned the years of 1988 to 1991 while phase 2 was from 1991 to 1994. While much of the data was collected for the entirety of the survey, some was only collected for a single phase. Further, not all participants were eligible for or chose to participate in all components of the survey. *T. gondii* infection status was assessed in all subjects aged 12 years and above for both phases of the NHANES III. MTHFR genotype was acquired for all subjects aged 12 years and above who were surveyed in phase 2. Total folate and vitamin B12 concentrations were acquired for all eligible participants aged 12 years and above. Homocysteine concentration was assessed in phase 2 subjects aged 12 years and above. A sub-sample of subjects aged 20 to 59 years old were administered the SDS, SDL, and RT cognitive assessments. Thus, the age of the participants in the sample ranged from 20 to 59 years. Subjects with missing data on any of the above-mentioned measures or covariates were not

included in my analyses. Due to the restrictions imposed by the remote access system used to access the necessary data sets, multiple imputation or other methods used to address missing data were not possible. Ultimately, a total of 1,537 subjects were included in all analyses. Finally, all data retrieved from the NHANES data sets is cross-sectional by nature. Therefore, any statistical associations exposed within these NHANES data sets must be considered with this context in mind.

Toxoplasma gondii

CDC lab technicians tested for the presence of *T. gondii* antibodies via indirect enzyme immunoassay. The process requires combining participant serum with *T. gondii*-specific antigens and peroxidase labelled anti-human IgG. The addition of a peroxidase substrate and chromogen substrate serum initiates a color reaction. The resulting color change is proportional to the concentration of *T. gondii* antibody in the participant's serum and can be interpreted and quantified via spectrophotometer. According to the recommendation of NHANES documentation, a value of less than 7.00 IU/mL was considered negative for infection while values at or above 7.00 IU/mL were considered positive for infection (Gunter, Lewis, & Koncikowski, 1996). Following this convention, I coded all positive results as 1 and all negative results as 0.

MTHFR Genotype

MTHFR genotyping was performed by CDC technicians. DNA samples were collected and isolated from 10 mL aliquots of whole blood and normalized to concentrations of approximately 50 ng/μL ("National Health and Nutrition Examination Survey Biospecimen Program: NHANES III (1988–1994) and NHANES 1999–2014," 2015). I coded subjects who were homozygous for the non-mutated C/C MTHFR genotype as 0 and subjects who were

heterozygous (C/T) or homozygous (T/T) for the C677T MTHFR polymorphism as 1 or 2 respectively. Further, to test for more generalized differences between subjects with or without a C677T MTHFR mutation, I created a separate variable in which subjects who were C/C homozygous were coded as 0 and subjects who were either hetero- or homozygous for the mutation were coded as 1.

Folate Cycle Factors

Serum folate (ng/mL) and vitamin B_{12} (pg/mL) concentrations were previously determined by CDC lab technicians using a Quantaphase II folate/vitamin B_{12} radioassay kit provided by Bio-Rad Laboratories (Bio-Rad Laboratories, 1993). Following preparation, labeled and unlabeled folate and vitamin B_{12} compete for binding sites. Unbound folate and vitamin B_{12} is discarded while bound substrate collects in pellet form. Radioactivity from the pellets is measured and compared to a standard curve to determine the concentration of folate and vitamin B_{12} in the participant's blood**.** To address a right data skew, folate and vitamin B_{12} concentrations were logarithmically transformed prior to implementation in any statistical models.

Total homocysteine concentration (μmol/L) was determined by CDC lab technicians using reverse-phase high-performance liquid chromatography and fluorescence detection. The degree of fluorescence is compared to a standard curve to determine the concentration of homocysteine in the sample. As with folate and vitamin B_{12}, homocysteine concentration was logarithmically transformed to account for a right skew.

Cognitive Function

A random half-sample of survey participants between the ages of 20 to 59 years old were administered computerized versions of the Symbol Digit Substitution (SDS), Serial Digit

Learning (SDL), and Reaction Time (RT) tests. Each of these assessments are components of the Neurobehavioral Evaluations System 2 that was specifically designed for use in epidemiological studies (Krieg et al., 2001). The SDS is considered a measure of processing speed but also requires intact memory and attention (Lezak et al., 2012). The SDS is a timed test requiring participants to match symbols with their corresponding numbers as outlined by a key placed at the top of the form. Performance on the test is assessed by computing the mean of the two lowest latencies between matched pairs. Since a lower average latency indicates better or faster performance, a lower score on the SDS indicates better cognitive functioning.

The SDL is a measure of learning and recall requiring participants to memorize and recall a series of eight digits over eight trials. For each trial, a score of 0 is given when all eight digits are successfully recalled. A score of 1 is given when two-thirds of the digits are correctly recalled, and a score of 2 is given when less than two-thirds of the digits are correctly recalled. Therefore, a higher score indicates worse performance.

The RT is a test of reaction time requiring participants to press a designated button every time a box-shaped stimulus appears on a computer screen. Each participant completes 10 practice trials and 40 test trials and the average time recorded (in milliseconds) from the 40 test trials is used as the final score. Again, since the RT is a time-based task, a lower score (or faster response) indicates better performance. Participants with physical limitations such as blindness or those who could not successfully complete an initial practice phase were excluded from cognitive testing. Additionally, data from participants who stopped a test before the allotted time were not reported in the NHANES.

Covariates

I included several covariates in my analyses to control for potential confounding. Categorical covariates were sex and race-ethnicity. The race-ethnicity variable included categories for non-Hispanic whites, non-Hispanic blacks, Mexican Americans, and "other" ethnicities as suggested by NHANES documentation (National Center for Health Statistics, 2011). Continuous covariates were education, poverty-to-income ratio (PIR), and age. Education was recorded as total years of completed education. PIR is defined as the ratio between household income and the federally established poverty level at the time of the survey. Values above 1 are above the poverty threshold whereas values below 1 are in poverty. Age was measured in years.

Statistical Analysis

Use of the limited access data sets provided by the CDC requires performing statistical and data processing operations through a remote-access interface called the ANDRE system. This system utilizes Statistical Analysis Software (SAS) version 9.4 to execute all tasks and requires researchers to submit prepared SAS code via File Transfer Protocol (FTP). Following submission, the ANDRE system processes the submitted SAS code and returns all output via e-mail in a text file. I performed all analyses using SAS's survey-specific commands that allow for the inclusion of sampling weights to adjust parameter estimates to be representative of the U.S. civilian non-institutionalized population. Traditionally, in analyzing NHANES data, the use of provided strata and cluster variables are sufficient for adjusting standard-error estimates to account for the complex-sampling design of the NHANES data sets. However, due to the restricted sample included in this study, it was likely that some strata would be limited to a single cluster. Therefore, as an alternative, I utilized the 52 replicate weights included with the

NHANES III data to implement Fay's method of balanced repeated replication (Rao & Shao, 1999) to account for the survey design.

I used linear regression (*proc surveyreg*) to independently estimate performance on the SDS, SDL, and RT cognitive assessments from *T. gondii* seropositivity and each of the folate cycle factors (folate, vitamin B_{12}, and homocysteine) with included covariates. Next, I used linear regression to test for significant differences in SDS, SDL, and RT performance between each of the C677T MTHFR genotypes. To replicate previous findings (Berrett et al., 2017), I then used linear regression to test for interaction effects between *T. gondii* infection and each of the folate factors (folate, vitamin B_{12}, and homocysteine) as separate models in the prediction of SDS, SDL, and RT performance. Finally, I used linear regression to test for potential interaction effects between *T. gondii* infection status and MTHFR C677T genotype in predicting performance on the SDS, SDL, and RT cognitive assessments. Each analysis included sex, race-ethnicity, education, PIR and age as controlling covariates.

Results

Of the 1,537 subjects included in my analyses, 19% were seropositive for a latent *T. gondii* infection. Nearly 38% of the total study sample possessed a single C677T MTHFR polymorphism while 10% were homozygous for the C677T polymorphism. Most subjects were non-Hispanic White (78%). The sample was balanced between men and women. On average, subjects were 37 years old, they had achieved 13 years of education, and were earning an income about 3 times greater than the federal poverty level. These summary statistics and others are presented in Table 1.

Table 1

Unweighted Summary Statistics of the Study Sample

	Mean/Proportion	**SE**
Symbol Digit Substitution Score	2.59	.04
Serial Digit Learning Score	4.08	.16
Reaction Time (ms)	236.01	1.38
Toxoplasma Gondii Seropositivity	.19	.02
MTHFR Genotype		
C/C	.52	.02
C/T	.38	.01
T/T	.10	.01
Folate (mg/dL)	7.24	.24
Vitamin B-12 (mg/dL)	491.57	12.50
Homocysteine (mg/dL)	9.52	.26
Race-ethnicity		
Non-Hispanic White	.78	.02
Non-Hispanic Black	.09	.01
Mexican American	.06	.01
Other	.07	.02
Male	.50	.01
Age (years)	37.26	.72
Education (years)	13.03	.13
Poverty-to-income Ratio	3.35	.12

Note. Means were calculated for continuous variables and proportions for categorical and binary variables.

N = 1,537

Ordinary least squares regression analysis with *T. gondii* seropositivity predicting performance on each of the three cognitive measures revealed a statistically significant effect between *T. gondii* seropositivity and performance on the SDL (β = .76 [95% CI: -.17, -1.36], p = .01). On average, subjects who were seropositive for *T. gondii* achieved an SDL score .76 points higher than seronegative subjects indicating worse performance (Table 2). *T. gondii* seropositivity did not predict performance on the SDS or RT.

Table 2

Toxoplasma Gondii Seropositivity Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Toxoplasma Gondii	.08	[-.02, .19]	.76*	[-.17, -1.36]	4.62	[-2.72, 11.97]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.28***	[.19, .36]	1.97***	[1.36, 2.58]	5.23	[-.70, 11.15]
Mexican American	.26***	[.14, .39]	2.42***	[1.73, 3.11]	.171	[-7.02, 7.36]
Other	.24**	[.07, .42]	1.88**	[.55, 3.21]	-4.95	[-14.39, 4.48]
Male	-.17***	[-.25, -.10]	.21	[-.14, .57]	4.75	[-1.45, 10.96]
Age	.03***	[.02, .03]	.08***	[.05, .10]	.37**	[.10, .64]
Education	-.10***	[-.12, -.08]	-.49***	[-.63, -.35]	-2.67***	[-3.70, -1.64]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.20**	[-.32, -.08]	-3.52***	[-4.79, -2.26]
Constant	3.10***	[2.77, 3.43]	8.37***	[6.37, 10.38]	270.06***	[252.82, 287.30]

Note. CI = confidence interval, ref = reference category

$N = 1{,}537$

* $p < .05$ ** $p < .01$ *** $p < .001$

No statistically significant associations were observed for folate, vitamin B_{12}, or homocysteine concentrations in predicting performance on any of the three cognitive assessments while controlling for race-ethnicity, sex, age, education, and PIR (Tables 3-5). Similarly, the C677T MTHFR polymorphism, whether defined by genotype (CC, CT, TT) or the presence or absence of a T-allele (CC, CT or TT), did not predict performance on the SDS, SDL, or RT assessments (Tables 6-7). As stated previously, statistically significant associations between these factors and the cognitive assessments were not required to establish the case for moderation by another factor. Therefore, tests of moderation by *T. gondii* were still warranted.

Table 3

Folate Concentration Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Folate	-.00	[-.06, .06]	-.17	[-.61, .27]	1.98	[-2.99, 6.95]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.28***	[.19, .36]	1.95***	[1.32, 2.58]	5.71	[-.09, 11.51]
Mexican American	.26***	[.14, .39]	2.41***	[1.70, 3.12]	.24	[-6.87, 7.35]
Other	.25**	[.07, .43]	1.92**	[.58, 3.26]	-4.64	[-13.79, 4.52]
Male	-.17***	[-.25, -.10]	.23	[-.16, .62]	4.48	[-1.96, 10.92]
Age	.03***	[.02, .03]	.09***	[.06, .11]	.39**	[.11, .68]
Education	-.11***	[-.12, -.09]	-.50***	[-.63, -.36]	-2.77***	[-3.77, 1.78]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.21**	[-.33, -.09]	-3.61***	[-4.93, -2.30]
Constant	3.02***	[2.71, 3.34]	7.82***	[5.62, 10.02]	263.61***	[245.91, 281.31]

Note. CI = confidence interval, ref = reference category

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 4

Homocysteine Concentration Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Homocysteine	-.04	[-.14, .05]	-.40	[-1.11, .32]	-3.65	[-9.79, 2.48]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.28***	[.19, .37]	1.98***	[1.35, 2.61]	5.27	[-.69, 11.23]
Mexican American	.26***	[.13, .39]	2.38***	[1.69, 3.06]	-.22	[-7.46, 7.02]
Other	.25**	[.07, .43]	1.98**	[.64, 3.33]	-4.18	[-13.38, 5.02]
Male	-.18***	[-.27, -.10]	.11	[-.31, .52]	3.80	[-2.48, 10.07]
Age	.03***	[.02, .03]	.09***	[.06, .11]	.43**	[.14, .73]
Education	-.11***	[-.13, -.09]	-.51***	[-.64, -.37]	-2.75***	[-3.78, -1.73]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.22**	[-.34, -.10]	-3.64***	[-4.97, -2.31]
Constant	3.11***	[2.77, 3.46]	8.52***	[6.01, 11.03]	273.63***	[250.77, 296.45]

Note. CI = confidence interval, ref = reference category

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 5

Vitamin B_{12} Concentration Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Vitamin B_{12}	.05	[-.01, .10]	-.01	[-.49, .48]	-2.43	[-8.46, 3.60]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.27***	[.18, .36]	1.99***	[1.34, 2.63]	5.78	[-.45, 12.00]
Mexican American	.26***	[.13, .38]	2.42***	[1.70, 3.14]	.42	[-6.86, 7.70]
Other	.25**	[.07, .43]	1.93**	[.57, 3.28]	-4.94	[-14.12, 4.31]
Male	-.17***	[-.25, -.10]	.21	[-.14, .56]	4.69	[-1.49, 10.87]
Age	.03***	[.02, .03]	.08***	[.06, .11]	.40**	[.12, .69]
Education	-.10***	[-.12, -.08]	-.50***	[-.64, -.37]	-2.73**	[-3.75, -1.72]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.21***	[-.33, -.10]	-3.53***	[-4.80, -2.27]
Constant	2.74***	[2.24, 3.24]	7.68***	[4.49, 10.86]	280.70***	[244.33, 317.07]

Note. CI = confidence interval, ref = reference category

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 6

C677T MTHFR Genotype Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
MTHFR Genotype						
C/C (ref)						
C/T	.01	[-.06, .08]	.19	[-.24, .61]	-.34	[-6.44, 5.75]
T/T	.03	[-.08, .15]	.53	[-.14, 1.20]	2.91	[-6.80, 12.61]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.29***	[.20, .37]	2.09***	[1.50, 2.67]	5.58	[-.60, 11.75]
Mexican American	.26***	[.13, .39]	2.36***	[1.65, 3.07]	.05	[-7.19, 7.2]
Other	.25**	[.07, .43]	1.95**	[.64, 3.27]	-4.66	[-14.00, 4.69]
Male	-.17***	[-.25, -.10]	.18	[-.16, .53]	4.60	[-1.69, 10.89]
Age	.03***	[.02, .03]	.08***	[.06, .11]	.41**	[.12, .71]
Education	-.11***	[-.13, -.09]	-.50***	[-.64. -.37]	-2.71***	[-3.74, -1.68]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.21**	[-.32, -.09]	-3.56***	[-4.89, -2.23]
Constant	3.01***	[2.71, 3.31]	7.53***	[5.44, 9.63]	265.20***	[248.32, 282.09]

Note. CI = confidence interval, ref = reference category

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 7

C677T MTHFR T-allele Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	B	95% CI	β	95% CI	β	95% CI
C677T MTHFR T-allele						
C/C (ref)						
C/T or T/T	-.02	[-.08, .05]	-.26	[-.66, .14]	-.34	[-6.69, 6.01]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.28***	[.20, .37]	2.07***	[1.49, 2.65]	5.42	[-.77, 11.61]
Mexican American	.26***	[.13, .39]	2.36***	[1.65, 3.08]	.08	[-7.16, 7.31]
Other	.25**	[.07, .43]	1.96**	[.63, 3.28]	-4.64	[-13.94, 4.67]
Male	-.17***	[-.25, -.10]	.19	[-.16, .55]	4.72	[-1.44, 10.88]
Age	.03***	[.02, .03]	.08***	[.06, .11]	.41**	[.11, .70]
Education	-.11***	[-.13, -.09]	-.50***	[-.64, -.37]	-2.72***	[-3.76, -1.69]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.21**	[-.32, -.09]	-3.57***	[-4.91, -2.23]
Constant	3.03***	[2.72, 3.34]	7.83***	[5.64, 10.02]	265.89***	[248.12, 283.65]

Note. CI = confidence interval, ref = reference category

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

Statistically significant interactions were observed between *T. gondii* seropositivity and folate concentration in predicting performance on the SDL (β = -1.28 [-2.13, -.43], p = .001) and RT assessments (β = 10.20 [.09, 20.32], p = .05) directly replicating the results I obtained in a previous study (Berrett et al., 2017) (Table 8). Subjects who were seronegative for *T. gondii* varied little in their performance on the SDL as folate concentrations increased or decreased. In contrast, subjects who were *T. gondii* seropositive performed worse on the SDL as folate concentration decreased. An interaction effect was also observed between *T. gondii* seropositivity and homocysteine concentration in predicting SDL performance (β = 3.06 [1.81, 4.31], p <.001) (Table 9). The interaction between *T. gondii* seropositivity and homocysteine concentration was like that of *T. gondii* and folate except that for *T. gondii* seropositive subjects, SDL performance worsened as homocysteine increased. No interaction effects were observed between *T. gondii* infection and vitamin B_{12} concentration in predicting any of the cognitive measures (Table 10).

Table 8

Interaction Effects Between T. gondii Seropositivity and Folate Concentration Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Toxoplasma Gondii	.22	[-.02, .47]	2.97***	[1.34, 4.60]	-12.97	[-28.53, 2.59]
Folate Concentration	.01	[-.04, .07]	.11	[-.36, .58]	-.06	[-4.72, 4.61]
T. gondii x Folate Interaction	-.08	[-.22, .06]	-1.28**	[-2.13, -.43]	10.20*	[.09, 20.32]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.28***	[.19, .36]	1.92***	[1.30, 2.53]	5.82*	[.01, 11.62]
Mexican American	.27***	[.14, .39]	2.45***	[1.76, 3.14]	-.00	[-7.14, 7.13]
Other	.24**	[.07, .41]	1.85**	[.53, 3.16]	-4.68	[-13.91, 4.56]
Male	-.17***	[-.25, -.10]	.21	[-.17, .60]	4.63	[-1.79, 11.06]
Age	.03***	[.02, .03]	.08***	[.06, .11]	.33*	[.07, .58]
Education	-.10***	[-.12, -.08]	-.48***	[-.62, -.35]	-2.77***	[-3.76, -1.77]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.20**	[-.32, -.08]	-3.54***	[-4.84, -2.23]
Constant	2.98***	[2.67, 3.29]	7.13***	[5.02, 9.24]	268.44***	[252.04, 284.84]

Note. *T. gondii* = *Toxoplasma gondii*, *CI* = confidence interval, ref = reference category

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 9

Interaction Effects Between T. gondii Seropositivity and Homocysteine Concentration Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Toxoplasma Gondii	-.31	[-1.29, .66]	-5.91***	[-8.67, -3.15]	-14.85	[-47.78, 18.07]
Homocysteine Concentration	-.07	[-.16, .02]	-.85*	[-1.53, -.18]	-4.96	[-12.20, 2.28]
T. gondii x Homocysteine Interaction	.18	[-.27, .63]	3.06***	[1.81, 4.31]	8.92	[-7.76, 25.60]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.28***	[.19, .37]	2.01***	[1.40, 2.62]	5.30	[-.67, 11.28]
Mexican American	.26***	[.13, .39]	2.45***	[1.76, 3.13]	.00	[-7.18, 7.19]
Other	.25**	[.08, .42]	1.97**	[.62, 3.29]	-4.36	[-13.78, 5.06]
Male	-.19***	[-.27, -.10]	.08	[-.34, .51]	3.74	[-2.59, 10.07]
Age	.03***	[.02, .03]	.09***	[.06, .11]	.41**	[.13, .69]
Education	-.10***	[-.12, -.08]	-.49***	[-.63, -.36]	-2.69***	[-3.73, -1.65]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.21***	[-.33, -.10]	-3.60***	[-4.91, -2.29]
Constant	3.15***	[2.84, 3.47]	9.23***	[6.73, 11.74]	275.56***	[252.59, 298.54]

Note. T. gondii = Toxoplasma gondii, CI = confidence interval, ref = reference category

$N = 1{,}537$

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 10

Interaction Effects Between T. gondii Seropositivity and Vitamin B_{12} Concentration Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Toxoplasma Gondii	1.30*	[.01, 2.60]	7.60*	[.00, 15.20]	9.84	[-117.49, 137.17]
Vitamin B_{12} Concentration	.08**	[.03, .14]	.19	[-.34, .71]	-2.43	[-8.42, 3.57]
T. gondii x Vitamin B_{12} Interaction	-.20	[-.41, .01]	-1.12	[-2.36, .12]	-.84	[-21.39, 19.71]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.27***	[.18, .36]	1.98***	[1.35, 2.61]	5.72	[-.49, 11.93]
Mexican American	.26***	[.14, .39]	2.46***	[1.75, 3.16]	.49	[-6.76, 7.74]
Other	.25**	[.07, .42]	1.88**	[.55, 3.20]	-5.24	[-14.70, 4.21]
Male	-.17***	[-.25, -.10]	.21	[-.13, .56]	4.70	[-1.46, 10.86]
Age	.03***	[.02, .03]	.08***	[.05, .10]	.36*	[.09, .64]
Education	-.10***	[-.12, -.08]	-.49***	[-.63, -.35]	-2.68***	[-3.73, -1.63]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.20**	[-.32, -.08]	-3.48***	[-4.71, -2.24]
Constant	2.50***	[2.06, 2.94]	6.37***	[3.03, 9.71]	280.41***	[241.32, 319.51]

Note. T. gondii = Toxoplasma gondii, CI = confidence interval, ref = reference category

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

While no interaction effects were observed between *T. gondii* and C677T MTHFR genotype in predicting performance on the SDS or SDL, a statistically significant interaction effect was observed in predicting RT performance (β = -19.85 [-35.92, -3.78], p = .017) (Table 11; Figure 2). For subjects who were *T. gondii* seronegative, heterozygous and homozygous mutations of the MTHFR gene were associated with worse reaction time with the homozygous mutation being associated with the most substantial increases in reaction time. In contrast, in subjects seropositive for *T. gondii*, heterozygous and homozygous C677T mutations of the MTHFR gene were associated with faster reaction time with the homozygous mutation being associated with the fastest reaction time. In a more generalized format, the same effect was observed if genotype for the C677T MTHFR polymorphism was operationalized as the presence (CT or TT) or absence (CC) of a T-allele (β = 15.14 [1.71, 28.57], p = .03) (Table 12; Figure 3).

Table 11

Interaction Effects Between T. gondii Seropositivity and C677T MTHFR Genotype Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Toxoplasma Gondii	.13	[-.00, .27]	.88	[-.07, 1.82]	11.97*	[1.65, 22.29]
C677T MTHFR Genotype						
C/C (ref)						
C/T	.03	[-.03, .09]	.20	[-.27, .68]	2.26	[-3.81, 8.33]
T/T	.05	[-.05, .15]	.70	[-.10, 1.51]	7.03	[-4.24, 18.30]
T. gondii x MTHFR Genotype Int.						
T. gondii x C/C (ref)						
T. gondii x C/T	-.10	[.32, .12]	-.04	[-1.73, 1.65]	-13.84	[-29.08, 1.39]
T. gondii x T/T	-.10	[-.47, .27]	-.93	[-2.87, 1.01]	-19.85*	[-35.92, -3.78]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.28***	[.20, .36]	2.07***	[1.51, 2.63]	5.18	[-1.09, 11.44]
Mexican American	.26***	[.13, .39]	2.36***	[1.67, 3.05]	.03	[-7.17, 7.23]
Other	.24**	[.07, .42]	1.94**	[.64, 3.24]	-4.53	[-14.00, 4.93]
Male	-.18***	[-.25, -.11]	.19	[-.13, .51]	4.16	[-2.34, 10.66]
Age	.03***	[.02, .03]	.08***	[.05, .10]	.38**	[.11, .66]

Table 11 Continued

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Education	-.10***	[-.12, -.08]	-.50***	[-.64, -.36]	-2.71***	[-3.72, -1.70]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.19**	[-.31, -.08]	-3.51***	[-4.84, -2.18]
Constant	3.00***	[2.70, 3.31]	7.48***	[5.39, 9.57]	264.09***	[247.58, 280.60]

Note. *T. gondii* = *Toxoplasma gondii*, CI = confidence interval, ref = reference category, int. = interaction

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

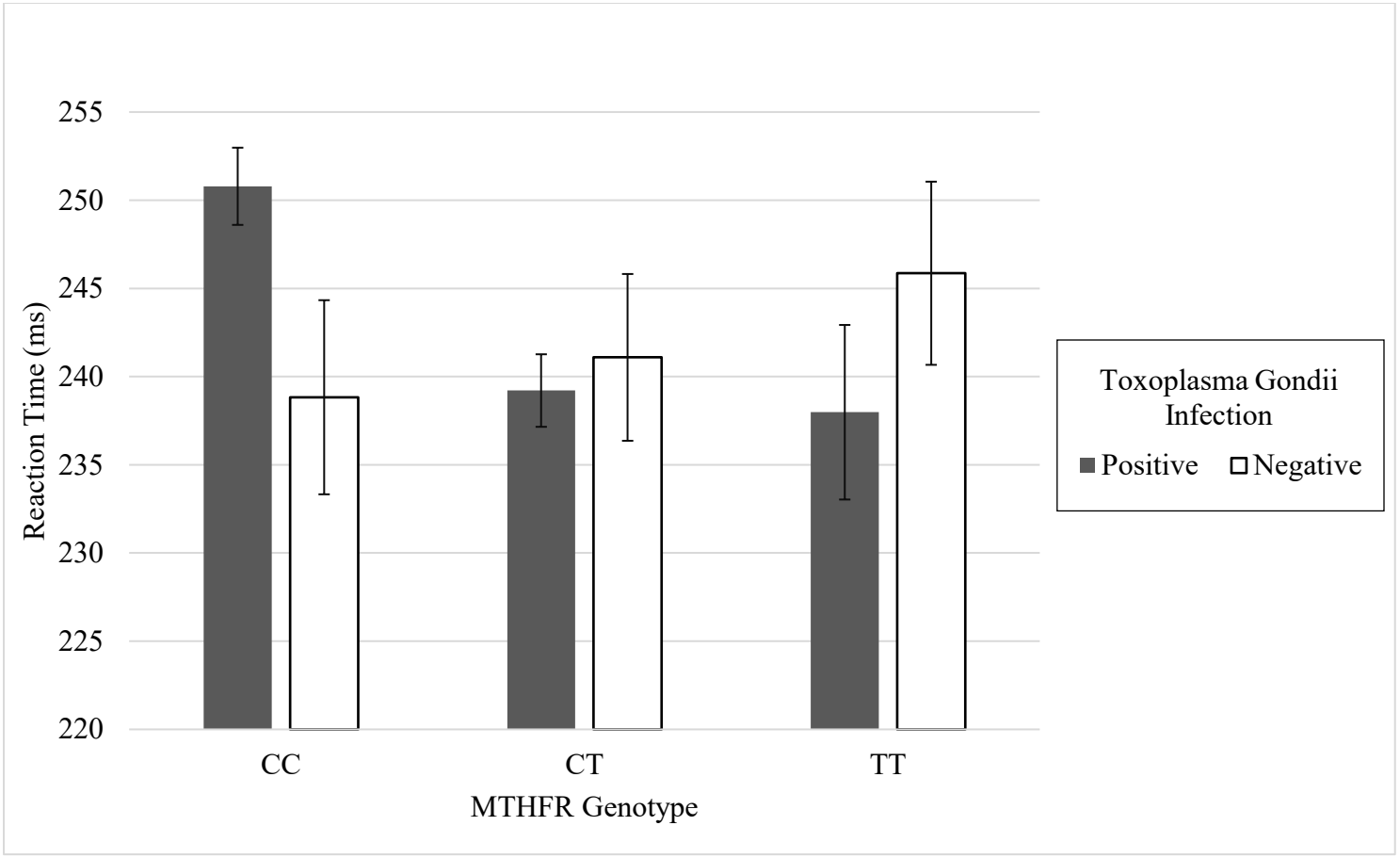

Figure 2. Interaction effect between C677T MTHFR genotype and *T. gondii* seropositivity predicting reaction time.

Table 12

Interaction Effects Between T. gondii Seropositivity and C677T MTHFR T-allele Predicting Performance on Three Measures of Cognitive Functioning in U.S. Adults

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Toxoplasma Gondii	.03	[-.13, .19]	.64	[-.30, 1.57]	-3.11	[-12.59, 6.36]
C677T MTHFR T-allele						
C/C (ref)						
C/T or T/T	-.03	[-.09, .02]	-.30	[-.77, .17]	-3.21	[-9.62, 3.20]
T. gondii x MTHFR Genotype Int.						
T. gondii x C/C (ref)						
T. gondii x C/T or T/T	.10	[-.11, .31]	.25	[-1.22, 1.71]	15.14*	[1.71, 28.57]
Race-ethnicity						
Non-Hispanic White (ref)						
Non-Hispanic Black	.28***	[.20, .36]	2.05***	[1.48, 2.61]	4.97	[-1.29, 11.22]
Mexican American	.26***	[.13, .39]	2.37***	[1.67, 3.07]	.08	[-7.14, 7.30]
Other	.24**	[.07, .42]	1.91**	[.60, 3.23]	-4.72	[-13.99, 4.56]
Male	-.18***	[-.25, -.10]	.19	[-.15, .53]	4.21	[-2.05, 10.47]
Age	.03***	[.02, .03]	.08***	[.05, .10]	.37**	[.10, .65]

Table 12 Continued

	Symbol Digit Substitution		Serial Digit Learning		Reaction Time	
	β	95% CI	β	95% CI	β	95% CI
Education	-.10***	[-.12, -.08]	-.50***	[-.64, -.36]	-2.70***	[-3.71, -1.69]
Poverty-to-income Ratio	-.03***	[-.05, -.02]	-.20**	[-.32, -.08]	-3.55***	[-4.86, -2.24]
Constant	3.04***	[2.72, 3.35]	7.82***	[5.58, 10.07]	267.73***	[250.32, 285.13]

Note. T. gondii = *Toxoplasma gondii*, CI = confidence interval, ref = reference category, int. = interaction

N = 1,537

* $p < .05$ ** $p < .01$ *** $p < .001$

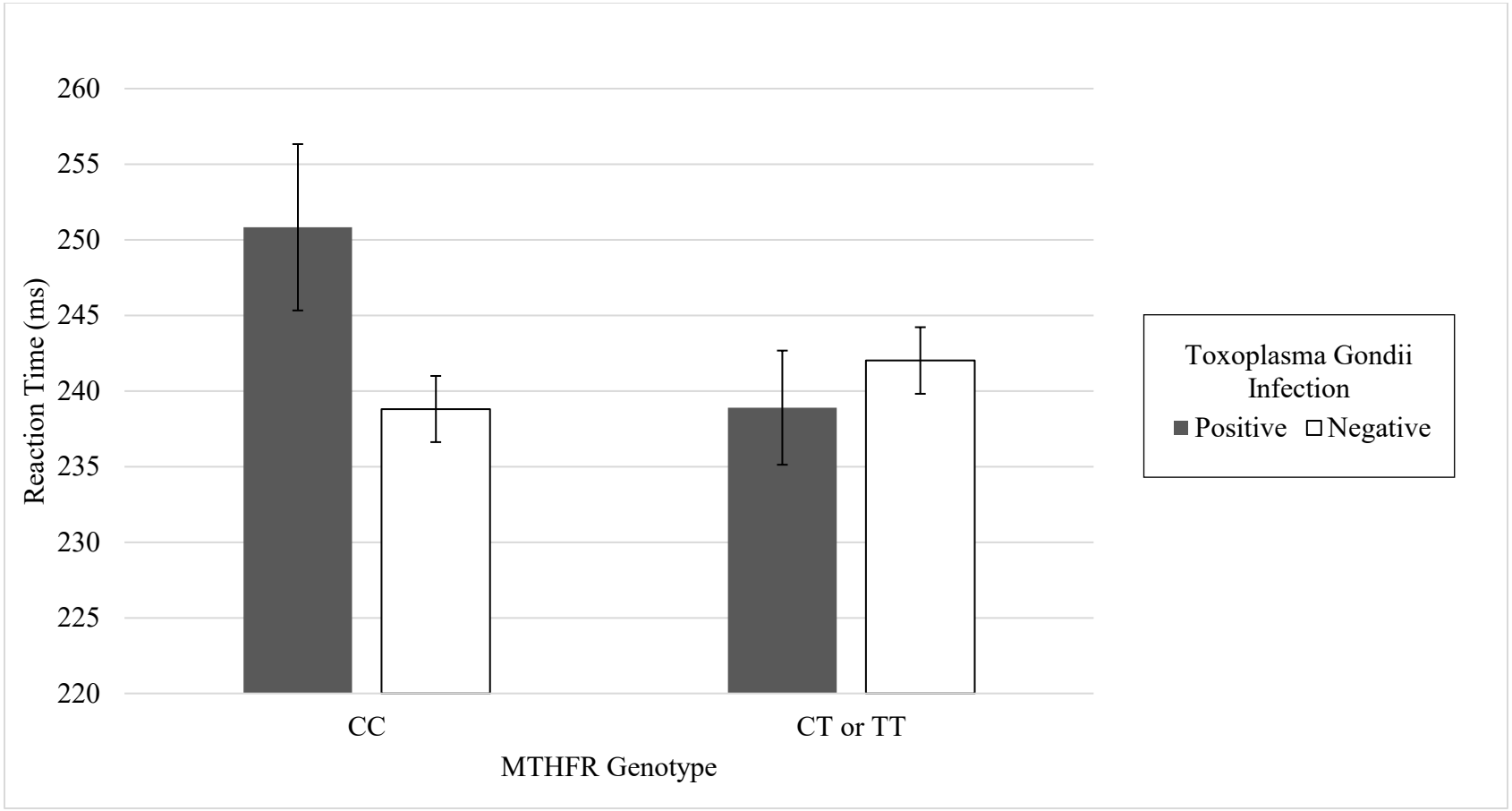

Figure 3. Interaction effect between C677T MTHFR T-allele and *T. gondii* seropositivity predicting reaction time.

Discussion

In this study of 1,537 young to middle-aged U.S. adults, I used data from the third NHANES to test the hypothesis that seropositivity for *T. gondii* moderates the association between the C677T MTHFR polymorphism and impairments in cognitive functioning. No significant interaction effects were observed between *T. gondii* infection and the C677T MTHFR polymorphism in predicting performance on the SDS or SDL. However, a statistically significant interaction effect was observed between *T. gondii* seropositivity and the C677T MTHFR polymorphism in predicting reaction time. Specifically, while subjects who were seronegative for *T. gondii* infection had slower reaction times with increasing degrees of MTHFR mutation (i.e. one or two T-alleles), subjects who were seropositive for *T. gondii* infection achieved better reaction times with increasing degrees of MTHFR mutation. While this finding was not in line with my original hypotheses, it may reveal a unique association between *T. gondii* infection and the C677T MTHFR polymorphism in the context of mental health and cognitive performance.

Over 19 percent of the total sample was seropositive for a latent *T. gondii* infection. This frequency is in line with other reports (Jones et al., 2001). Importantly, this estimate reflects the proportion of U.S. individuals who were seropositive for *T. gondii* infection between the years of 1988 and 1994. Data from more recent epidemiological surveys report a lower *T. gondii* seroprevalence with estimates ranging between 10 to 12 percent (Jones et al., 2014). The reduction in *T. gondii* seroprevalence over the past three decades may be due to improved awareness of the parasite among pet owners and pet care professionals such as veterinarians. Improved awareness could lead to changes in pet care in the home, veterinary screening practices, and new options for eliminating the parasite from carrier organisms (Dabritz &

Conrad, 2010). Despite the encouraging changes in *T. gondii* seroprevalence, the CDC still considers *T. gondii* to be one of the top five neglected parasitic infections in the U.S (Parise, Hotez, & Slutsker, 2014). Further, while more recent estimates of *T. gondii* seroprevalence appear to be lower in the U.S. in comparison to past decades (Jones et al., 2014), seroprevalence in developing nations seems to have changed little and remains much higher than the seroprevalence observed in the U.S. (Tilahun et al., 2016; Wilking, Thamm, Stark, Aebischer, & Seeber, 2016). Therefore, *T. gondii* infection remains both a nation and world-wide concern.

Approximately 48 percent of the sample was either heterozygous (38 percent) or homozygous (10 percent) for the C677T MTHFR polymorphism, a frequency that matches previous national estimates (Liew & Gupta, 2015; Peng, Labelle, Rainey, & Tsongalis, 2001).

In testing for associations between latent toxoplasmosis and performance on the three tests of cognitive functioning, individuals seropositive for *T. gondii* performed significantly worse on the SDL, a test of short-term memory, than their uninfected counterparts. This finding matches that of a previous report using the same data (Gale et al., 2015) and reinforces other literature suggesting that infectious pathogens such as *T. gondii* might be associated with increased risk for cognitive decline in adults (Flegr, 2015; Mendy, Vieira, Albatineh, & Gasana, 2015; Pearce, Kruszon-Moran, & Jones, 2014). However, research is still lacking to describe the potential mechanisms by which *T. gondii* might affect cognitive functioning. Further, limited research has explored the various ways in which *T. gondii* infection might modify the association between other factors, such as folate metabolism, and cognitive functioning.

In a previous report (Berrett et al., 2017), I used data from the NHANES III to test for interaction effects between infection with *T. gondii* and blood concentrations of folate, homocysteine, and vitamin B_{12} in the prediction of scores on the SDS, SDL, and RT cognitive

assessments. In that report, *T. gondii* seropositivity significantly moderated the association between folate and homocysteine concentrations in predicting performance on the SDL and RT cognitive assessments. The same analyses were performed in this study and, expectedly, the same results were obtained. As discussed previously (Berrett et al., 2017), the interaction effects that predict performance on the SDL may suggest that the relationship between folate concentration and cognitive functioning might differ based on the presence or absence of latent toxoplasmosis. Theoretically, this could be due to direct salvaging of intracellular folate by *T. gondii*. Salvaging of intracellular folate by *T. gondii* may lead to elevated levels of homocysteine because less product from folate metabolism (i.e. 5-MTHF) would be available to convert homocysteine into methionine. In this regard, one could suppose that, in the presence of another factor that might also impair folate metabolism, cognitive functioning might be further impaired. Such is the underlying theory of the current report in that the C677T MTHFR polymorphism is indeed another factor, genetic by nature, that could also negatively impact folate metabolism. Therefore, I hypothesized that the impairments in cognitive functioning observed in the previous report (Berrett et al., 2017), as well as the current report, particularly for the SDS or SDL, would be more substantial in subjects who were affected by both *T. gondii* infection and the C677T MTHFR genetic polymorphism.

Since no interaction effect was observed between *T. gondii* infection and folate concentration in predicting performance on the SDS, associations between *T. gondii* and processing speed or other components of cognitive functioning employed in the SDS task must operate through a mechanism other than folate salvaging. A common theory is that infectious pathogens such as *T. gondii* might trigger an inflammatory response that is damaging to both the pathogen and the host. In the case of *T. gondii,* which is resistant to inflammatory defenses

(Laliberte & Carruthers, 2008), damage to the host could continue indefinitely for the life of the parasite. Since latent toxoplasmosis often persists for the life of the host, collateral inflammatory damage could eventually result in clinically relevant changes in host health, particularly if initial infection occurred earlier in life. Unfortunately, the NHANES datasets did not include data for typical measures of the cerebral immune response including pro-inflammatory cytokines (e.g. interleukin-6 and tumor necrosis factor) or anti-inflammatory cytokines (e.g. interleukin-4 and interleukin-10) (Woodcock & Morganti-Kossmann, 2013). Therefore, given the absence of such data, I was unable to determine whether inflammation plays any mediating or moderating role in the prediction of an association between *T. gondii* infection and performance on the SDS.

Contrary to my proposed hypotheses, no significant interaction effects were observed between *T. gondii* infection and hetero- or homozygosity for the C677T MTHFR polymorphism in predicting performance on the SDL or SDS cognitive assessments. These null results could potentially be explained by several factors. First, the sample used for these analyses were between the ages of 20 and 59 years. It is possible that interaction effects between *T. gondii* infection and the C677T MTHFR polymorphism might exist in an older population in which cognitive decline is more likely. Relatedly, cognitive decline from either *T. gondii* or the C677T MTHFR polymorphism is not likely an instantaneous process and instead may increase over multiple years or decades. Therefore, by analyzing individuals between the ages of 20 to 59 years, observing significant interaction effects may be less likely because the sample consists of generally healthy (both physically and cognitively) individuals who are less likely to be experiencing cognitive decline usually associated with older age. Alternatively, the cognitive tests used in the NHANES III might not have been sensitive to the effects of the C677T MTHFR polymorphism on cognitive functioning. For example, the polymorphism has been associated

with alterations in attention and executive control, both of which being components of general cognitive functioning but not explicitly assessed by any of the three cognitive assessments available in the NHANES III (Krull et al., 2008; Pathansali et al., 2006). Therefore, detection of statistically significant interaction effects between *T. gondii* infection and mutation of the C677T MTHFR polymorphism may have been possible had more cognitive testing data been available, particularly for components of cognitive functioning more likely to be affected by the C677T MTHFR polymorphism.

Furthermore, because an interaction effect was observed between *T. gondii* and folate but not between *T. gondii* and the C677T MTHFR mutation in predicting performance on the SDL, it is possible that the interplay between *T. gondii* and folate might occur before folate is modified or metabolized by the MTHFR enzyme. Indeed, in the report that provides the first evidence of folate salvaging by *T. gondii*, Massimine et al. (2005) describes a plasma membrane transporter system in *T. gondii* that is highly specific for the THF form of folate. Therefore, *T. gondii* may not salvage the metabolized forms of folate such as 5-MTHF. If so, *T. gondii* salvaging could lead to a depletion of available THF, but not necessarily a depletion of 5-MTHF. Since most available THF is almost immediately converted to 5-MTHF (Bailey, 1995; Obeid, Kirsch, Kasoha, Eckert, & Herrmann, 2011), reduced availability of THF may not significantly drain the available pool of 5-MTHF. Instead, the limited stores of unmetabolized THF may be partially depleted to maintain the rate of THF delivery to the MTHFR enzyme.

Interestingly, the conversion of THF to 5,10-MTHF (the folate form converted by MTHFR to 5-MTHF) leads to the generation of glycine, a molecule necessary for the generation of purines. Purines are, in turn, required for the synthesis of nucleic acids and proteins and are heavily utilized in multiple biochemical reactions requiring energy input. In neurons, purines

such as adenosine are key to functions such as neural plasticity and recovery (Dias, Rombo, Ribeiro, Henley, & Sebastiao, 2013; Kurpius, Nolley, & Dailey, 2007). Therefore, if *T. gondii* were to infect neural cells in highly plastic brain regions such as the hippocampus or prefrontal cortex, specific THF salvaging could lead to the reduced production of purines (or other molecules) necessary for neural plasticity. Further, evidence suggests that *T. gondii* may also directly salvage purines (adenine, guanine, xanthine, and hypoxanthine) from host cells (Chaudhary et al., 2004; Coppens, 2014; Hyde, 2007). Therefore, *T. gondii* may also deplete available stores of purines directly through host cell salvaging and indirectly through THF salvaging. An ongoing, latent infection could perpetuate this scenario for the duration of the hosts life thus potentially increasing the risk for memory loss or other general cognitive deficits later in life by reducing the materials necessary for synaptic plasticity and other energy requiring functions in neural cells.

Also, the experimentation performed by Massimine et al. (2005) was conducted *in vitro* and does not provide any description or characterization of folate salvaging by *T. gondii* within an infected host, particularly in respect to the different stages of infection. For example, it is possible that *T. gondii* might salvage more folate during the initial acute phase of infection to improve survivability and less after it has transitioned into an encapsulated latent infection. The opposite could also be true. Due to the cross-sectional nature of the NHANES data, I was unable to assess blood folate concentrations at different time points across the infection process and no other studies have yet explored this theory. Further, *in vitro* experimentation does not allow for the consideration of potential host adaptation. Perhaps, after the parasite has entered a latent phase, host cells may compensate for the constant drain of THF (or possibly other folate forms) by storing more THF or sourcing it from other parts of the body. Further research (preferably *in*

vivo and in humans) is required to determine whether host cells might adapt to *T. gondii* folate salvaging after long-term infection.

Finally, despite the significant interaction effect observed between *T. gondii* and folate in predicting performance on the SDL, it is also possible that the observed effect is capturing a less direct relationship between *T. gondii* and cellular folate concentration, suggesting that *T. gondii* may not actually influence cellular levels of folate to a clinically significant degree. Instead, the observed interaction between *T. gondii* and folate could be a case of overlapping but very separate effects. For example, reduced folate concentrations may be associated with worse performance on the SDL due to elevated levels of homocysteine, which can eventually be neurotoxic. On the other hand, *T. gondii* infection could also trigger an immune response that results in persistent neuroinflammation. In this scenario, *T. gondii* and folate both influence performance on the SDL, but the point of interaction is not in folate levels, but in inflammation. However, as stated previously, the NHANES datasets do not contain sufficient data for markers of inflammation to test this possibility.

While no interaction effects were observed between latent toxoplasmosis and the C677T MTHFR polymorphism in predicting performance on the SDS or SDL cognitive assessments, an interaction effect was observed in predicting performance on the RTT. The statistically significant effect predicted improved performance on the RTT for subjects seropositive for *T. gondii* infection. Specifically, for subjects who were *T. gondii* seronegative, reaction time was adversely affected in an incremental manner by the presence of one or two T-alleles for the C677T MTHFR polymorphism. *T. gondii* seronegative subjects achieved slower reaction times if they had one or two T-alleles as opposed to seronegative subjects with no C677T MTHFR polymorphism (no T-alleles). In contrast, subjects who were seropositive for *T. gondii* infection

experienced the opposite effect. For those subjects, the presence of one or two T-alleles for the C677T MTHFR polymorphism was associated with incremental improvements in reaction time. Therefore, *T. gondii* seropositive subjects achieved faster reaction times if they had at least one T-allele for the C677T MTHFR polymorphism when compared to seropositive subjects with no T-alleles. Only the difference between homozygous C/C and homozygous T/T subjects was statistically significant.

While this study is the first to report a specific interaction effect between latent toxoplasmosis and the C677T MTHFR polymorphism in predicting reaction time, other reports may indirectly support the validity of the finding. For example, in two previous reports using the same NHANES III data, I observed similar interaction effects between an infectious pathogen and blood folate concentration in predicting faster reaction time. Most recently, I found that latent toxoplasmosis moderated the relationship between blood folate concentration and reaction time (Berrett et al., 2017). In that study, subjects who were seropositive for *T. gondii* infection appeared to achieve faster reaction times as blood folate concentrations decreased. Similarly, in a study exploring the cognitive consequences of infection with *H. pylori*, while not statistically significant, I observed a similar relationship between the bacterium and changes in reaction time performance (Berrett et al., 2016b). Specifically, subjects who were seropositive for the cagA variant of *H. pylori* appeared to achieve faster reaction times as blood folate levels decreased. The agreement between these studies suggests that an underlying connection may exist between infectious pathogens (such as *T. gondii* and *H. pylori*) and reaction time performance. Indeed, while *T. gondii* and *H. pylori* differ in many ways, including their gross physiology, location of infection within the human body, and the way they interact with the host, both may influence the availability of THF, the first component of the folate cycle. *T. gondii* may salvage THF from

infected cells (Massimine et al., 2005) while *H. pylori* may reduce THF absorption via gastric inflammation (Berrett et al., 2016b; Kountouras, Gavalas, Boziki, & Zavos, 2007). Therefore, an important link between infectious pathogens such as *T. gondii* and *H. pylori* and reaction may be the availability of THF or a similar factor related to the metabolism of THF.

Despite the potential theoretical support from studies I previously conducted, each utilize the same data from the NHANES III dataset. Additional findings from studies that do not utilize the NHANES III data may further characterize the association between the *T. gondii*/MTHFR interaction and improvements in reaction time. For example, using a modified stop-change paradigm, Stock, Heintschel von Heinegg, Kohling, and Beste (2014) observed that subjects who were seropositive for *T. gondii* infection exhibited better action control than seronegative subjects. However, like the current study, no direct association was observed between *T. gondii* infection and reaction time suggesting that other moderating or mediating factors should be considered. Further, a report by Sugden et al. (2016) also found no significant differences in reaction time between *T. gondii* seropositive and seronegative subjects. The study sample recruited by Sugden et al. (2016) included individuals in New Zealand who were born between April 1972 and March 1973. While that report did not find any significant differences in reaction time between the two groups, folate concentrations or C677T MTHFR genotype were not considered. It is possible that in the case of low folate concentration or a homozygous C677T MTHFR polymorphism, the effects of *T. gondii* infection could be more pronounced or more potent. In addition to the findings of Sugden et al. (2016), a different report observed a disadvantage in processing speed for schizophrenic and control subjects infected with *T. gondii* (Pearce et al., 2013). However, blood folate concentrations and C677T MTHFR genotype were again not considered.

While some disagreement exists regarding the presence and directionality of an association between latent toxoplasmosis and reaction time, potential mechanisms of such an association can still be posited. One possible theory involves the parasite's unique ability to utilize proteins and other biological compounds available in the host cell to generate large concentrations of dopamine. While the purpose of generating the neurotransmitter is still unclear, the consequences seem to be more apparent thanks to ongoing research on the topic. Possibly the most intuitive and direct evidence that the dopamine generated by *T. gondii* affects human hosts is the numerous reports of associations between *T. gondii* seropositivity and diagnosis with schizophrenia (Hinze-Selch et al., 2007; Yolken, Dickerson, & Fuller Torrey, 2009). Other behavioral or cognitive differences that have been observed between *T. gondii* seropositive and seronegative individuals (de Barros et al., 2017; Suvisaari, Torniainen-Holm, Lindgren, Harkanen, & Yolken, 2017) may be due to the location of the infection in the brain. However, most reports suggest that, once in the brain, *T. gondii* is relatively impartial when choosing a permanent infection site (McConkey, Martin, Bristow, & Webster, 2013). Therefore, depending on the distribution of *T. gondii* cysts throughout the brain, infected individuals will likely experience different consequences of infection.

Optimal reaction time requires contributions from, among others, brain regions involved in executive control, attention, and fine and gross motor control (Chudasama et al., 2003; Prinzmetal, McCool, & Park, 2005). Such brain regions are modulated by multiple neurotransmitters, including dopamine, and function differently when concentrations of the neurotransmitter are altered, whether naturally or artificially. For example, evidence suggest that depletion of dopamine is associated with changes in executive control, attention, and movement (Luo & Levin, 2017; Ramdani et al., 2015; van Dyck et al., 2008) which may lead to

impairments in reaction time (Grant, Kuepper, Wielpuetz, & Hennig, 2014). Therefore, factors that remedy or compensate for dopamine depletion may improve reaction time. For example, in a study by Pezze, Dalley, and Robbins (2007), investigators administered D1 and D2 dopamine receptor agonists to mice and observed significant improvements in attentional control and perseverative responding in a reaction time task when both dopamine receptors were agonized at optimal levels. In contrast, D1 and D2 receptor blockade led to overall impairments in task performance. In another study, investigators observed a significantly higher prevalence of *T. gondii* infection in individuals diagnosed with Parkinson's disease compared to controls suggesting that *T. gondii* may modify the outcomes of the disease (Miman, Kusbeci, Aktepe, & Cetinkaya, 2010). If *T. gondii* invades neural systems involved in attentional control, motor control, or other systems related to reaction time performance, it is possible that the dopamine generated by the parasite may act as a dopamine receptor agonist and thereby modify behavior. However, such a hypothesis is difficult to test due to the seemingly random localization of *T. gondii* cysts in the brain. Also, it is difficult to determine whether *T. gondii* would affect one or many neural systems that might modify reaction time performance.

Though the dopamine generated by *T. gondii* might lead to improved reaction time, this association may be tempered by the more global effects of infection. Following successful invasion, the parasite ultimately invades and inhabits neurons, thereby triggering both local and systemic inflammatory processes. If necessary, some infected cells will attempt to trigger apoptosis, or programmed cell death, to protect against spreading infection. However, the parasite is equipped with the ability to negate that cellular command (Laliberte & Carruthers, 2008). Built to survive indefinitely within a hosts' neural cells, the immune system is unable to eliminate the parasite and the immune system resorts to maintained systemic inflammation to

limit the effects of the foreign invader. While important for immune function, protracted inflammation has been linked to general cognitive decline. For example, the build-up of amyloid plaques triggers an ongoing inflammatory response and is likely responsible for much of the cognitive decline observed in Alzheimer's disease (Heneka et al., 2015; Tai et al., 2015). Therefore, the neuroinflammation associated with *T. gondii* infection may lead to impairments in various cognitive functions such as attention or executive control which would in turn impair reaction time. Ultimately, the association between *T. gondii* infection and reaction time seems to be relatively complex given that the parasite may affect reaction time both positively via dopamine generation and also negatively via neuroinflammation.

Though no association was observed in this study between the C677T MTHFR polymorphism and performance on the RTT, some indirect links may still exist. For example, one direct consequence of MTHFR mutation is increased blood levels of homocysteine. Elevated homocysteine, or hyperhomocysteinemia, is neurotoxic to the brain and may affect neurotransmitter levels (Bhatia & Singh, 2015; Selhub, Bagley, Miller, & Rosenberg, 2000). In fact, some theories point to hyperhomocysteinemia as an important risk factor for psychiatric diseases such as depression and schizophrenia, both of which are significantly associated with concentrations of certain neurotransmitters including dopamine (Bhatia & Singh, 2015; Selhub et al., 2000). The C677T MTHFR polymorphism reduces the efficiency of the MTHFR enzyme in converting 5,10-MTHF to 5-MTHF, a substrate required for the conversion of homocysteine to methionine. The absence of sufficient 5-MTHF to convert homocysteine leads to a potential decrease in available methionine. The converted methionine product is necessary for several methyl-donating reactions including those involved in the generation of neurotransmitter precursor molecules used to make dopamine, norepinephrine, serotonin and other

neurotransmitters (Selhub, 2000; Almeida 2005; Alpert, 2000). Thus, the C677T MTHFR polymorphism may ultimately lead to reduced production of several neurotransmitters and thereby increase risk for psychiatric or neurologic disease. Indeed, hetero- and homozygosity for the C677T MTHFR polymorphism has been linked to, among others, depression, Parkinson's disease, and schizophrenia (Liew & Gupta, 2015; Nishi et al., 2014; Wu, Ding, Sun, Yang, & Sun, 2013). The C677T MTHFR polymorphism has also been associated with impaired attention and changes in executive control (Krull et al., 2008; Pathansali et al., 2006). Presumably, these associations could be mediated or moderated by reductions in neurotransmitter generation due to the less efficient conversion of homocysteine to methionine. However, additional research is required to confirm such a mechanism.

It is possible that both *T. gondii* infection and the C677T MTHFR polymorphism may be related to reaction time via modulation of neurotransmitter levels. While *T. gondii* might improve reaction time by generating surplus dopamine, the C677T MTHFR polymorphism may impair reaction time by limiting the efficiency of folate metabolism which in turn reduces the production of neurotransmitter precursor molecules. This convergence may explain the interaction effect observed in this study. Specifically, in *T. gondii* seronegative individuals, being hetero- or homozygous for the C677T MTHFR polymorphism was associated with worse reaction time. This pattern is in line with the theory that the C677T MTHFR polymorphism might impair reaction time due to depleted dopamine or other neurotransmitters. However, in individuals seropositive for latent toxoplasmosis, reaction time was better for individuals who were hetero- and homozygous for the C677T MTHFR polymorphism. Therefore, it appears that the surplus dopamine produced by *T. gondii* may counteract the effects of the C677T MTHFR polymorphism. In other words, latent toxoplasmosis appears to be a compensatory factor, at a

significantly significant level, against the effects of C677T MTHFR polymorphism on reaction time.

Importantly, the results of this study do not suggest that the co-presence of *T. gondii* infection and C677T MTHFR mutation promote a more favorable situation over someone who is *T. gondii* seronegative and/or does not possess the C677T MTHFR polymorphism. Instead, *T. gondii* may be at least partially responsible for the recovery of reaction time performance in the event that an individual possesses one or two copies of the C677T MTHFR polymorphism. However, given the cross-sectional nature of the NHANES data, it is impossible to determine the exact mechanistic process by which *T. gondii* infection might influence the relationship between the C677T MTHFR polymorphism and reaction time. The possibility that and infectious pathogen such as *T. gondii* might provide any benefit to the infected host is rarely considered. Generally, infection by an organism such as *T. gondii* is assumed to be accompanied with unfavorable outcomes. Indeed, multiple such consequences or outcomes of *T. gondii* infection have already been identified (Fabiani et al., 2015; Fekadu, Shibre, & Cleare, 2010). However, the results of this study add to the limited literature suggesting potential benefits, so to speak, of *T. gondii* infection (Stock, 2014). In fact, the possibility that *T. gondii* infection might reverse the deleterious effects of another factor on cognitive functioning, such as the C677T MTHFR polymorphism, may provide some evidence that latent toxoplasmosis might also moderate the outcomes of other factors that also affect cognitive functioning. For example, Parkinson's disease is characterized by the degeneration of dopaminergic circuits in the substantia nigra. Of the few studies that have explored possible associations between *T. gondii* infection and Parkinson's disease, none have yet determined whether the dopamine produced by *T. gondii* might affect the outcomes of the disease (Celik et al., 2010; Miman et al., 2010). While the

results of the current study certainly do not translate to Parkinson's disease, they do offer motivation for additional research to explore the potential avenues by which the dopamine generated by *T. gondii* might affect the human brain, if at all.

Another consideration in interpreting the interaction effects between *T. gondii* infection and the C677T MTHFR polymorphism is the possibility that *T. gondii* may be responsible for causing other transcriptional abnormalities that might alter how the C677T MTHFR polymorphism might after cognitive functioning. Investigations into how *T. gondii* might affect a human host post-infection has revealed several transcriptional changes in infected and neighboring cells due to the release of micro-RNA by *T. gondii* (Sacar, Bagci, & Allmer, 2014; Zeiner, Norman, Thomson, Hammond, & Boothroyd, 2010). The genetic influences by *T. gondii* are likely intended to increase the survivability and longevity of the parasite (Thirugnanam, Rout, & Gnanasekar, 2013; Xiao et al., 2014). However, it is possible that the genetic influences of *T. gondii* might have secondary effects. At this point, no studies have yet explored potential intersections between the genetic or physiological influences of *T. gondii* and the C677T MTHFR polymorphism.

Several factors require consideration when interpreting the results of this study. The cross-sectional nature of the NHANES data limits the ability to determine important factors such as the age or time in which an individual was infected with *T. gondii*. It is possible that the results of the current study could vary based on the duration or frequency in which an individual has been exposed to or infected with *T. gondii.* It was also not possible, given the available data in the NHANES III, to determine the location of *T. gondii* cysts within each seropositive subject. Generally, such information is only available post-mortem. Further, the NHANES data set did not include data for all variables or measures necessary to determine the specific mechanisms

involved in the observed findings. For example, subjects were not tested for dopamine or other neurotransmitter levels as such tests were not within the objectives or scope of the NHANES. Also, in the NHANES III, only subjects between the ages of 20 and 59 were assessed for cognitive functioning. It is possible that the observed effects could differ in other populations, such as the elderly, where cognitive decline is more common and a greater risk. While the NHANES III did contain three different assessments of cognitive functioning, performance on those assessments should only be considered as a snapshot of each subject's cognitive health or ability. Additional assessments and/or neuroimaging are typically required to make more conclusive determinations about an individual's cognitive health. Finally, while I attempted to control for several potential confounding factors, it is unlikely that I accounted for all factors that might affect the outcomes of this study. For example, other factors besides the C677T MTHFR polymorphism likely affect folate metabolism and/or alter blood concentrations of folate cycle molecules such as homocysteine.

Despite the potential limitations listed above, this study is also supported by several strengths. The NHANES data sets provide an invaluable opportunity to conduct research using a nationally representative sample. After limiting my analyses to subjects with data for the necessary study variables, each analysis was based on over 1,500 subjects who represent multiple race-ethnicities, socioeconomic groups, and other demographic factors. Therefore, the results of this study can be generalized to the U.S. young to middle-aged adult population. The large sample size also improves statistical power and reduces the likelihood that the observed effects are due to statistical error. Further, by including sociodemographic factors such as PIR, education, race-ethnicity, and others as controlling covariates in each statistical model, I could be more confident that the observed effects were due to the variables of interest and not due to

extraneous factors. Finally, because I used data for three different measures of cognitive functioning as estimates of cognitive ability, I could improve the specificity of my findings by identifying effects that may have been specific to certain components of cognitive functioning.

In conclusion, I found that infection by the apicomplexan parasite *T. gondii* may moderate the association between the C677T MTHFR polymorphism and cognitive functioning. Contrary to my original hypotheses, *T. gondii* infection did not moderate the relationship between the C677T MTHFR polymorphism and performance on the SDS or SDL. Instead, I observed a previously undescribed relationship between *T. gondii* infection and the C677T MTHFR polymorphism in which infection by *T. gondii* appeared to reverse or compensate for the deleterious effects of the C677T MTHFR polymorphism on reaction time. However, given the cross-sectional nature of the data, describing the exact mechanism by with *T. gondii* and the C677T MTHFR polymorphism may interact within the infected host cell was not possible. Thus, the implications of these findings are complex and further research is necessary to better explore the context and mechanisms involved in the observed associations. The importance of these findings is emphasized by the relatively high prevalence of both *T. gondii* infection and mutation of the MTHFR enzyme in U.S. adults (Jones et al., 2014; "RS1801133,"). In fact, given the rates observed in the sample included in this study, it is possible that between five and ten percent of the U.S. population may be simultaneously affected by *T. gondii* and the C677T MTHFR polymorphism. Therefore, additional research is recommended to determine the extent of the interaction effects between *T. gondii* infection and mutation of the C677T MTHFR polymorphism on cognitive function.

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