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Thesis of Antonella Quimbayo

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science Biological Sciences

Nova Southeastern University Halmos College of Arts and Sciences

August 2024

Approved: Thesis Committee

Committee Chair: Christopher Blanar

Committee Member: Jonathan Banks

Committee Member: Omar Eldakar

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NOVA SOUTHEASTERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

Emotional Response in Working Memory of Individuals with Latent Toxoplasmosis

Antonella Quimbayo

Submitted to the Faculty of

Halmos College of Arts and Sciences

in partial fulfillment of the requirements for

the degree of Master of Science with a specialty in:

Biological Sciences

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Abstract

Toxoplasma gondii is a prevalent parasite that infects about 30% of the world population. House cats and other felids serve as definitive hosts and acquire Toxoplasma by consuming infected prey, which are intermediate hosts. This includes rats and avians, but also large mammals such as wolves and deer. Toxoplasma gondii is known to manipulate prey behavior to enhance its transmission. For example, infected rodents demonstrates attraction to and curiosity around cat urine. Wolves infected with *Toxoplasma* were 11 times more likely to disperse from their pack and start a new pack. The manipulation of intermediate host behavior observed in rodents raises the possibility that *Toxoplasma* may similarly affect infected humans, and several studies have begun to explore this theory. There is anecdotal and epidemiological evidence that infected humans may be more likely to engage in risky activities such as participating in thrill-seeking activities, entrepreneurship, and experience increased rates of traffic accidents. This project explored the relationship between Toxoplasma infection, emotional response to aversive stimuli, reported anxiety, and risky behaviors in college-age adults. A total of 316 college-aged adults participated in the study. The Working Memory Delayed Recognition Task with Valanced Distractors and Image Rating Task was used to assess emotional response. The State-Trait Anxiety Inventory was used to assess participants' current state of anxiety and predisposition to have anxiety symptoms. The Risky, Impulsive, & Self-destructive behavior Questionnaire (RISQ) was used to assess the extent to which participants engaged in risky or self-destructive behaviors. Toxoplasma seropositivity was assessed using TOXOPLASMOSE ICT IgG-IgM rapid blood tests. Seropositivity rates were unusually low (2.2%) in the target population. The results were inconclusive, due to the extremely low rate of toxoplasmosis in our sample, and this experiment should be reformed and administered to a known population of individuals with toxoplasmosis.

KEYWORDS: T. gondii, parasite, human behavior, risk assessment, anxiety inventory

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Introduction

Toxoplasmosis is a parasitic infection caused by the protozoan parasite *Toxoplasma gondii*. It is a significant medical and veterinary disease, and it is prevalent across the globe. A common estimate is that a third of the human population worldwide is living with toxoplasmosis (Robert-Gangneux & Darde, 2012). The disease is associated with behavioral changes in infected animals, particularly feline prey, the intermediate hosts of *T. gondii*. From the prey, the parasite must travel to a cat, its primary host, where it can complete sexual reproduction. Studies demonstrate that rats infected with *T. gondii* engage in risky behavior and are attracted to cat urine, making them easier prey for cats and facilitating transmission of the parasite to its primary host (Berdoy et al., 2000). Such behavior manipulation of intermediate hosts is a common tactic used by parasites to ensure or enhance transmission to their definitive hosts (da Silva et al., 2009; Hughes et al., 2019).

Humans are also susceptible to toxoplasmosis, and act as an intermediate host while the parasite attempts to infiltrate a feline. It has long been speculated that we are subject to *T*. *gondii's* behavioral influence as well. Previous studies indicate that humans that have toxoplasmosis have poorer psychomotor performance (Flegr, 2007), are more likely to display risky behavior and impulse control and are at increased risk for psychiatric disorders (Fernandes, 2021) and (Fond, 2013). Toxoplasmosis may also affect motivation and drive: individuals with latent toxoplasmosis are less motivated by rewards to perform better in a task, while non-infected individuals are (Stock et al., 2017). Despite the global distribution and prevalence of this parasite in human populations, the behavioral alterations associated with latent infection remain largely unknown.

Toxoplasmosis

Toxoplasmosis is a disease caused by an intracellular parasite called *Toxoplasma gondii*. It is a protozoan belonging to the phylum Apicomplexa, and it lives inside host cells. *Toxoplasma gondii* is an obligate parasite, meaning it requires at least one host to complete its life cycle. This parasite typically has two hosts: the intermediate or secondary host, and the definitive or primary host. The definitive host- which can be any member of the Felidae - is where it can reproduce sexually; while the intermediate host is any warm-blooded animal, (Dubey, 1996) ideally one that is prey to a feline. The parasite is acquired when an intermediate host consumes fecal matter of an infected definitive host, or raw or undercooked meat of another infected intermediate host. It may also be transmitted vertically in definitive and intermediate hosts.

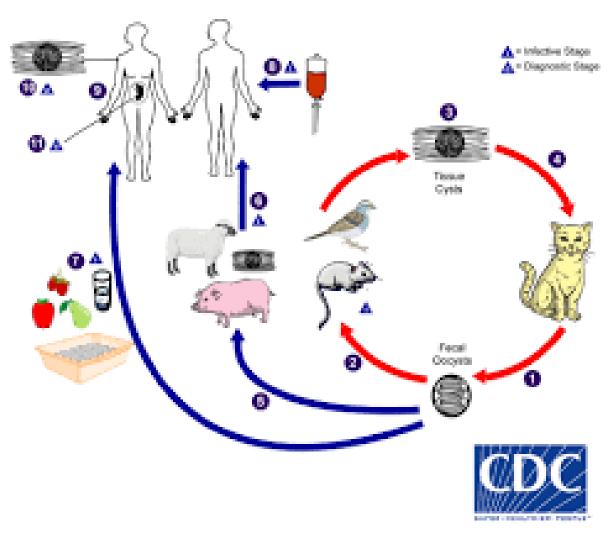


Figure 1: Life Cycle of *Toxoplasma gondii* (Centers for Disease Control and Prevention, 2019) About 30% of the human population across the world is infected with *Toxoplasma gondii* (Robert-Gangneux & Darde, 2012). In North America, Northern Europe, and Southeast Asia, about 10-30% of the population is seropositive and in Central and Southern Europe, about 30-50% of the population is seropositive (Robert-Gangneux & Darde, 2012). In Latin America and tropical African countries, the seropositivity rate can be as high as 80% (Daher et al., 2021). Several factors affect *T. gondii* seroprevalence, such as climate, hygiene customs, and agricultural regulations. In 2016, Lykins and colleagues surveyed an American private insurance company for cases of toxoplasmosis. They identified 9,260 patients between 2003 and 2012 that showed clinical manifestations of toxoplasmosis. The disease was more prevalent in the south, where climate is ideal for *T. gondii*, and 73% of identified seropositive females were of reproductive age (Lykins et al., 2016). 38% of identified patients presented ocular disease and 12% of identified patients presented other critical manifestations (Lykins et al., 2016). Patients with toxoplasmosis were likely tested because they were diagnosed with HIV or other autoimmune diseases (Lykins et al., 2016).

While Lykins's study focused on reporting statistics from patients diagnosed with clinical toxoplasmosis, their approach may have missed patients under that insurance with subclinical toxoplasmosis. There are two stages of the disease: acute and latent. During acute toxoplasmosis, the initial clinical stage of infection, a patient may experience headache, fatigue, fever, and swollen lymph nodes (Hahari, 2018). If present, symptoms of acute toxoplasmosis usually subside within a week. After acute infection, the next stage is the latent stage. In healthy individuals, latent toxoplasmosis does not present any clinical manifestations (Flegr, 2014), but they carry the parasite the rest of their life, unless it is eliminated with medication. Toxoplasmosis only becomes a concern when individuals have a severely compromised immune system. Patients with HIV, patients who are on chemotherapy or take immunosuppressants, or organ transplant patients, do not have a strong enough immune system to keep the parasite from reproducing and causing damage to the body (Jones et al., 2001), regardless of whether the disease is in its acute or latent stage. Also, pregnant women with the infection can pass it on to their fetus, causing miscarriage or birth defects. Otherwise healthy individuals who are not pregnant are not at risk for serious disease if they contract toxoplasmosis (Jones et al., 2001).

In the discipline of parasitology, there is phenomenon called the manipulation hypothesis; it posits that parasites can deliberately alter host behavior to bipofacilitate transmission between intermediate and definitive hosts (Berdoy et al., 2000). Berdoy et al (2000) demonstrated a well-known example of manipulation by *T. gondii*. They set up mazes in pens with corners of rat scent, cat scent, rabbit scent, and a neutral scent and observed the exploration patterns of toxopositive rats and toxo-negative rats. Toxo-positive rats avidly explored the pens and did not avoid corners doused with cat scent, while toxo-negative rats explored less and avoided cat scent

corners (Berdoy et al., 2000). Both groups of rats behaved similarly in the presence of rat scent, rabbit scent, and neutral scent, indicating that parasitic manipulation is specific to feline predator scent, and not odor in general. In another study, Meyer and colleagues (2022) surveyed seropositive and seronegative gray wolves that lived among cougars in Yellowstone National Park, Wyoming. They found that seropositive wolves were more likely to disperse from their pack and go off on their own, eventually starting a new pack. This action goes against the hierarchical structures of wolf packs, and it may be attributed to *Toxoplasma gondii*, and its attempts to increase transmission of dispersed wolves and neighboring cougars (Meyer et al., 2022). These studies show robust evidence to support the manipulation hypothesis and has inspired observations in the effects of toxoplasmosis on human behavior.

In the 1990s, scientists began investigating the relationships between toxoplasmosis and human behavior. The most common topics of interest in these studies are psychiatric disorders, personality profiles, and psychomotor function. Some psychiatric disorders that have been studied in relation to toxoplasmosis are schizophrenia, major depressive disorder, bipolar disorder, and obsessive-compulsive disorder. One meta-analysis of 38 studies of schizophrenia and T. gondii seroprevalence revealed that patients with schizophrenia were infected with Toxoplasma 2.7 times more than healthy individuals (Arias et al., 2011; McConkey et al., 2013). Another meta-analysis of 24 studies showed that 14 of the studies provided support for a relationship between toxoplasmosis and schizophrenia, while 10 did not (Fernandes et al., 2021). Most studies of major depressive disorder conclude that there is no association between the illness and infection (Chegeni et al., 2019; Fernandes et al., 2021; Pearce et al., 2012). Two studies found that seroprevalence was higher in bipolar patients than in healthy individuals (de Barros et al., 2017; Pearce et al., 2012), and a meta-analysis showed that six out of 11 studies indicated an association between bipolar disorder and T. gondii seroprevalence (Fernandes et al., 2017). A meta-analysis of 12 studies in 2019 found that seropositivity was higher in OCD (Obsessive Compulsive Disorder) patients than neurotypical individuals (Nayeri Chegeni et al., 2019). It is not clear whether the psychiatric disorders lead to the infection, or if the infection exacerbates symptoms of the psychiatric disorders. More studies of T. gondii seroprevalence and psychiatric disorders must be conducted to better explore the relationship between the two.

Personality profiles and observable behavior are also believed to differ in individuals with latent toxoplasmosis and healthy individuals. Flegr, known for his extensive research in toxoplasmosis, conducted personality profiles on women who tested positive for *T. gondii* antibodies during routine pregnancy exam and on women who had suffered acute toxoplasmosis. Pregnant toxo-positive women showed higher intelligence, guilt proneness, tension, and radicalism (Flegr et al., 1999). He also found that certain personality aspects like being easygoing, being enthusiastic, being adventurous, and being suspecting were correlated with the length of their infections, which they measured by evaluating antibody titres (Flegr et al., 2000). Interestingly, toxoplasmosis in men was found to be correlated with a decrease in superego – referring to one's conscious or the internal moral standards one has from observing society and community – strength (Flegr et al., 2000). Additionally, a meta-analysis of nine studies of toxoplasmosis and traffic accidents showed that five of the nine determined a significant association between infection and accidents, where infection increased the risk of traffic accidents in individuals under 45 (Gohardehi, 2018).

Working Memory and Emotional Stimuli

In human psychology and neuroscience, working memory concerns the systems involved in retaining information while completing a complex task (Baddeley, 2010). There are two relevant types of memory for the purposes of this study: long-term memory and working memory. Long-term memory is a person's entire reservoir of past events (Chai et al., 2018; Cowan, 2008). Baddeley's model of working memory involves storage and manipulation (Baddeley, 2012). Over three decades, Baddeley and Hitch have described several systems that are a part of working memory. These are the phonological loop (Broca's and Wernicke's), the visuospatial sketchpad (Occipital Lobe), the central executive control (Prefrontal Cortex), and the episodic buffer (Parietal Lobe) (Chai et al., 2018). Working memory is a system of mechanisms, one area of the brain, like the temporal cortex, may not be functioning properly. This might affect visual working memory, but not spatial working memory (Eriksson et al., 2015). Because toxoplasmosis may cause cysts in the brain, it is worth considering if cysts that imbed themselves on the frontal or parietal lobes can cause inflammation in this significant region which can therefore impair working memory function. Working memory is still a recent topic in psychology and several alternative models exist to explain it. Working memory may be affected by psychiatric disorders, head trauma, age, sleep, diet, genetics, learning ability, fluid intelligence, attentional control, and active maintenance (Banks et al., 2022; Chai et al., 2018; Eriksson et al., 2015). Variations in working memory also exist due to simple individual differences in normal healthy adults, like differences in hair color or flavor preference.

Researchers in the 21st century have studied the effects of various psychiatric disorders on working memory. Patients with generalized anxiety disorder, schizophrenia, major depressive disorder, bipolar affective disorder, and attention deficit disorder all performed poorer on working memory tests than healthy individuals (Chai et al., 2018). It is relevant to note that psychiatric disorders are associated with poorer working memory and possibly toxoplasmosis. Working memory systems rely on the fronto-parietal region of the brain and patients with traumatic brain injury in the frontal or parietal lobes also perform poorer in working memory tests than healthy individuals (Chai et al., 2018). Psychiatric disorders and brain trauma seem to be correlated with poorer performance on working memory tasks.

In working memory and other cognitive processes, emotional stimuli can affect an individual's ability to perform well. The presence of emotional stimuli while performing a working memory task can result in one of three results. An individual may perform poorly when exposed to emotional stimuli, especially negative, because the stimuli draw attention away from the task (Garrison et al., 2018; Williams et al., 1996). Conversely, an individual may perform better when exposed to emotional stimuli because emotion triggers upregulation of cognitive control (Banks et al, 2022; Witkin et al., 2020). The presence of emotional stimuli may not yield evidence of association with performance in a working memory task at all. An example of the first possible result can be identified in the emotional Stroop task. In general, patients were slower to name the color of words associated with their clinical conditions (Williams et al., 1996). In another study, participants completed an operation span task where they were presented a series of simple math problems and an emotional or neutral word to remember. Once they finished all the equations, they had to remember the words. The results of this study demonstrated that emotional words reduced working memory ability compared to neutral words (Garrison et al., 2018).

Other working memory studies exist that evidence increase of cognitive function after exposure to negative emotional stimuli. Witkin and colleagues performed a delayed-recognition working memory task in which participants were asked to remember a collection of faces or shoes that was interrupted by distracting imagery (Witkin et al., 2020) The mnemonic load was either low or high, one or two shoes or faces. The imagery was neutral or negative, like clothespins or gun violence. Participants performed better on subsequent trials after being exposed to high mnemonic load and negative distractors than they did on the current trial, and performance is overall poorer on high load trials (Witkin et al., 2020). The study was later replicated by Banks and yielded consistent results. The newer study found, however, that better subsequent performance depended more on high mnemonic load than negative stimuli (Banks et al., 2022). These results suggest that better performance, or upregulation of cognitive control occurs because of the need for control instead of the threat of conflict (Banks et al., 2022).

Another example of a cognitive function is reward modulation. Stock and colleagues (2017) investigated the relationship between latent toxoplasmosis and reward modulation and cognitive control. After completing a computer-based cognitive task, researchers found that toxo-positive and toxo-negative participants did not differ in response times but did differ in performance and reward response. Toxo-positive participants demonstrated a diminished response to monetary rewards compared to toxo-negative participants (Stock et al., 2017). Toxopositive participants also responded with more accuracy than their counterparts. The results of this study imply that latent toxoplasmosis may not influence cognitive ability directly. Instead, it is the ability to process emotional elements that influence human cognition, like reward, risk, or perceived threat, that is affected by infection. This study, along with many others, suggests that toxo-positive individuals behave differently than healthy individuals, and they deviate from the norm.

Given this backdrop, we predicted that seropositive individuals will be less affected by the emotional load of the distraction images, than seronegative individuals. Therefore, they will perform better overall with higher accuracy than their counterparts. The questionnaires will answer other research questions: are toxo-positive individuals more risky than toxo-negative individuals? Do toxo-positive individuals report higher or lower levels of anxiety? All participants will perform the same working memory task, blood test, and questionnaires. The entire study will take approximately one hour.

Measures

Toxoplasma seropositivity was assessed using TOXOPLASMOSE ICT IgG-IgM rapid blood tests. A lancet was used to collect one drop of blood from participants, which was placed in the well of the rapid test. Four drops of eluent were added to the blood and the test was left for 20 to 30 minutes to develop results. One line indicated a negative result, and two lines indicated a positive result.



Figure 2: LDBIO T. gondii rapid test negative and positive

Working Memory Delayed Recognition Task with Valenced Distractors (Jha et al., 2017). This task instructed participants to remember either faces or shoes over a delayed period. The trials begin with an encode phase (S1) that contains either two memory items (high mnemonic load) or one memory item paired with a noise mask (low mnemonic load) presented for 3000 ms. Afterwards, a fixation cross is shown for for 500 ms. Then, a task-irrelevant

distraction image is displayed for 2000 ms which is followed by another fixation cross for 500 ms, these screens serve as the active maintenance phase. Following this is the retrieval phase where the test item (S2) is presented for 2500 ms. On half the trials, S2 is a single image that appeared in S1 (match), while on the other half, S2 is a novel image that did not appear in S1 or elsewhere in the experiment (non-match). Participants indicate a match or non-match between the S1 and S2 stimuli by pressing a designated 'Y' or 'N' key. Participants are instructed to respond quickly and accurately, as the task moves on without a response after 2.5 seconds. Half of the trials consisted of faces as stimuli and the other half consisted of shoes – both of which were intermixed randomly throughout the study. There are three blocks with 30 trials, adding up to 90 total trials (Banks et al., 2022).

With regards to the variables that will be discussed later, Negative/Neutral Image refers to the type of distraction image that a participant was shown. High/Low Load refers to mnemonic load and whether the participant was tasked with remembering two or one shoe(s)/face(s). Accuracy refers to how often they answered a question correctly. Response Time refers to how quickly the participant answered a question. This measure was scored by averaging trial types (negative image or neutral paired with high load or low load) and each trial type had two measures – accuracy and response time, creating four trial types for analysis. Due to computer failures, eight samples were left out of analysis.

Image Rating Task was presented as a follow-up to the Working Memory Delayed Recognition Task. Participants were shown each distraction image again in a different order and asked to rate each image on a scale of emotional valence. The scale was 1, highly negative emotional content, to 9, highly positive emotional content. They were then shown the distraction images a third time and asked to rate each image on a scale of arousal. The scale was 1, the lowest level of arousal, to 9, the highest level of arousal.

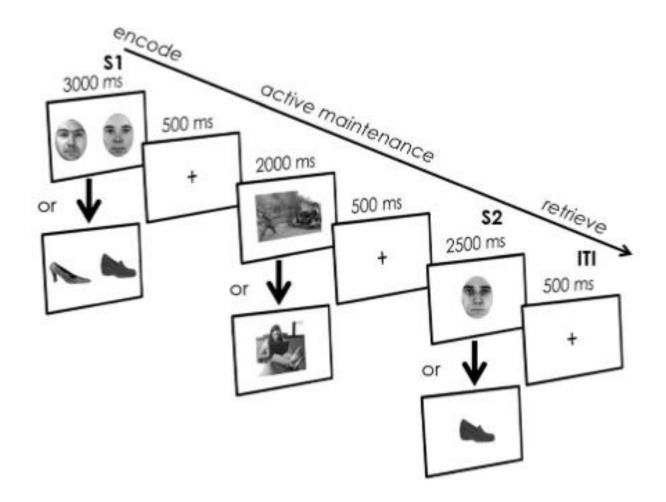


Figure 3: Delayed recognition task

State-Trait Anxiety Inventory (Spielberger et al., 1983) questionnaire consists of two 20item scales used to assess anxiety. Each item has a zero to four scale which the participant uses to indicate how anxious they feel in the moment they are completing the questionnaire, and then in general. One scale is used to measure the current state of anxiety, while the second scale is used to measure the participants' tendency to experience anxiety everyday (Banks et al., 2022). The measure is scored by adding all 20 item responses for state anxiety, then adding all 20 items for trait anxiety. The higher sum a participant scores, the more anxious they tend to be. Due to issues with computer failure, eighteen samples were left out of analysis.

STAI-State

INSTRUCTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and circle an answer to indicate how you feel *right now*, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement, but give the answer which seems to describe your present feelings best.

		Not at all	Somewhat	Moderately so	Very Much so
1.	I feel calm	0	1	2	3
2.	I feel secure	0	1	2 2 2 2 2 2 2 2 2 2	3
3.	I am tense	0	1	2	3 3
4.	I feel strained	0	1	2	3
5.	I feel at ease	0	1	2	3 3
6.	I feel upset	0	1	2	3
7.	I am presently worrying over possible misfortunes	0	1	2	3
8.	I feel satisfied	0	1	2	3
9.	I feel frightened	0	1	2 2 2	3
10.	I feel comfortable	0	1	2	3
11.	I feel self-confident	0	1	2	3
12.	I feel nervous	0	1	2	3
13.	I am jittery	0	1	2	3 3
14.	I feel indecisive	0	1	2	3
15.	I am relaxed	0	1	2	3
16.	I feel content	0	1	2	3
17.	I am worried	0	1	2	3
18.	I feel confused	0	1	2	3
19.	I feel steady	0	1	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	3
20.	I feel pleasant	0	1	2	3

Figure 4: State Anxiety Inventory

STAI-Trait

INSTRUCTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then circle an answer sheet to indicate how you <u>generally</u> feel. There are no right or wrong answers. Do not spend too much time on any one statement, but give the answer which seems to describe how you generally feel.

		Almost Never	Sometimes	Often	Almost Always
1.	I feel pleasant	0	1	2	3
2.	I feel nervous and restless	0	1	2 2 2 2	3
3.	I feel satisfied with myself	0	1	2	3 3 3
4.	I wish I could be as happy as others seem to be	0	1	2	3
5.	I feel like a failure	0	1	2	3
6.	I feel rested	0	1	2 2 2 2	3 3 3 3
7.	I am "calm, cool, and collected"	0	1	2	3
8.	I feel that difficulties are piling up so that I cannot overcome them	0	1	2	3
9.	I worry too much over something that really doesn't matter	0	1	2	3
10.	I am happy	0	1	2	3
11.	I have disturbing thoughts	0	1	2	3
12.	I lack self-confidence	0	1	2 2 2 2 2 2 2 2 2	3 3 3 3 3 3 3
13.	I feel secure	0	1	2	3
14.	I make decisions easily	0	1	2	3
15.	I feel inadequate	0	1	2	3
16.	I am content	0	1	2	3
17.	Some unimportant thought runs through my mind and bothers me	0	1	2	3
18.	I take disappointments so keenly that I can't put them out of my mind	0	1	2	3
19.	I am a steady person	0	1	2	3
20.	I get in a state of tension or turmoil as I think about my recent concerns and interests	0	1	2	3

Figure 5: Trait Anxiety Inventory

RISQ (Sadeh, Baskin-Sommers, 2016) a 38-item questionnaire-based measure to assess risky behavior. It is a bifactor model with a general factor and eight-domain specific factors. The RISQ has excellent internal consistency (Cronbach's alpha = .92) and acceptable to excellent reliability most factors (.73 to .92). The Reckless Behaviors factor showed borderline internal reliability (.63). The measure is scored by grouping the 38 items into eight subgroups – for example, all drug-related behaviors are grouped into one item called "drug behaviors." Subitems A, B, C, and D are added together, while subitems E and F are averaged. For the purposes of this thesis, the variables observed from the RISQ were RISQ A, which asked how many times ever a participant had engaged in a certain behavior, RISQ E which asked if the participant engages in this behavior to feel less upset distressed or overwhelmed, and RISQ F which asked if the participant engaged in this behavior to get a thrill or to feel pleasure.

For each behavior, fill-in how many times you did it in your lifetime (A) & the total number of times you did it the past month (B). Enter one number for each time period, even if it is your best guess. Please do not put a range, but enter a single number (e.g., behaviors engaged in everyday for multiple years can be written in as 1000+, behaviors engaged in daily for a single year can be written in as 365, any other frequency should be estimated using your best guess). If you have ever done the behavior, write how old you were the first time (C) and check the box if the behavior ever caused you any problems, regardless of the specific problem (D). For the last two columns (E & F), use the scale in the box to rate how much you agree with each statement from 0 = Strongly Disagree to 4 = Strongly Agree. Please provide ratings for both statements (E & F), and treat them as separate questions. The first two rows are examples of how to complete each item.

					0 trongly isagree	1 Somewhat Disagree	2 Equally Disagree/Ag	3 Somewhat Iree Agree	4 Strongly
_		A	В		С	1	D	E	F
		How many times total have you done this in your life?	How main times ha you done in the pa month	ve this Ist	How old were you the <u>first</u> <u>time</u> ?	proble going to legal tro problem	cause you any ms, such as the hospital	I do this behavior to <u>stop feeling</u> upset, distressed, <u>or overwhelmed</u>	l do this behavior to <u>feel</u> <u>excitement, to</u> <u>get a thrill, or to</u> <u>feel pleasure</u>
	Behavior	# TOTAL	# past MO	NTH	Age	Y	'=YES	Rate 0-4	Rate 0-4
Ex.	Driven a car while intoxicated	10	2		18		Y	4	3
Ex.	Jumped out of a plane	0							
1	Shoplifted things	2							
2	Drove 30mph or faster over the speed limit								
3	Bet on sports, horses, or other animals								
4	Used cocaine or crack								
5	Bought drugs	-							
6	Impulsively bought stuff you did not need & won't use								
7	Had unprotected sex with someone you just met or didn't know well				2				

Figure 6: Risky Impulsive Self-destructive Questionnaire (page 1)

Methods

The recruitment and testing procedures were approved by and carried out according to the protocol submitted to the Nova Southeastern University Institutional Review Board. Participants consisted of 316 college-age adults (70 males, 242 females, 3 non-specified gender ;mean age \pm SD: 20.2 \pm 2.2, range 18–35). were recruited and tested. The only requirement for participants was that they were 18 years or older. One to three participants were tested in one session, and each session took around one hour. Participants arrived and were greeted by the principal investigator or a trained research assistant, then they completed the consent process. The first task participants were given was the Working Memory Delayed Recognition Task with Valenced Distractors; this task was done either on a laptop or a desktop computer on the E-Prime program. During the task, participants were shown three screens per trial The first screen showed either one or two shoes or faces that the participant was instructed to remember. The second screen showed a distraction image. The third screen showed, depending on what they saw on the first screen, a shoe or a face. The participant was to respond 'Y' for yes or 'N' for no, on labelled keyboard keys. This task featured 90 trials over three blocks, and it took about 11 minutes for participants to complete. Participants then completed the image rating task, where they rated the distraction images they were shown in the working memory task. They were shown every image again in a random order and asked to rate each image on a scale of emotional valence and arousal. This image-rating task took about 10 minutes.

After the first participant finished the working memory and image-rating tasks, they were moved to an empty desk and prepped for the *Toxoplasma gondii* antibody rapid test. If there were other participants waiting to get their blood test done, they were started on the questionnaires task. The participants getting their blood drawn was instructed to squeeze the finger that they wanted to prick; this was usually the ring or middle finger on either hand, then wipe that finger with an alcohol prep pad. While wearing gloves, the principal investigator or research assistant would load a sterile lancet into a lancet device, then hold the lancet to the participant's finger and puncture it once. They then guided the participant's finger to the well of the TOXOPLASMOSE ICT IgG-IgM rapid test and milked enough blood from the puncture wound to produce at least one drop. They then squeezed four drops of eluent into the well and

allowed it to mix with the blood. While the participant cleaned their finger and picked out a bandage, the principal investigator or research assistant disposed of the needle in a sharps box, and put the test to the side to develop for at least 20 minutes. The tests were marked with participant ID numbers, and results were given to the participants at the end of the session. The principal investigator or research assistant changed gloves between each participant, and followed the same steps.

After the blood test, participants moved on to the questionnaires. These were completed either on a physical paper packet or on a desktop computer. The first questionnaire was a demographics questionnaire. This was to gain insight on the participants' background, so these questions included gender, race/ethnicity, languages spoken, and highest level of achieved education. Some questions were to gauge how the participant obtained *T. gondii*, if they tested positive, and asked questions about their gardening habits, whether they take care of cats and their food hygiene practices.

The next questionnaire was the State-Trait Anxiety Inventory, a two 20-item scale to assess how anxious the participant is currently feeling, and how anxious they feel in general. The final questionnaire was the Risky, Impulsive and Self-destructive Questionnaire. This 38-item questionnaire assessed risky behavior that participants participated in, including how frequently they participated, how old they were when they first participated, had this behavior caused them issues, and why they participated in these behaviors.

Once the first participant finished the questionnaires, the principal investigator or a research assistant would show a 10 minute decompression video. This video featured silly videos of cats and dogs, and was played to minimize discomfort participants may have felt from the working memory and image-rating tasks, the blood draw, and the potentially distressing questionnaires. It was also shown to pass the time until the full 20 minutes of the rapid test were completed.

Participants were told their *T. gondii* antibody status at the end of all the tasks. If there was a seropositive individual, the whole group was split up and told their status individually only by the principal investigator. Seropositive individuals sat with the principal investigator and all their questions were answered with confidence and compassion. Seropositive individuals were

advised to request a *T. gondii* test during their next blood draw, to confirm infection and proceed with treatment with their doctor.

Participants were encouraged to ask questions throughout the entire session. The principal investigator and research assistants either participated in the study or performed all the tasks and used materials during training, so they were prepared to run the session.

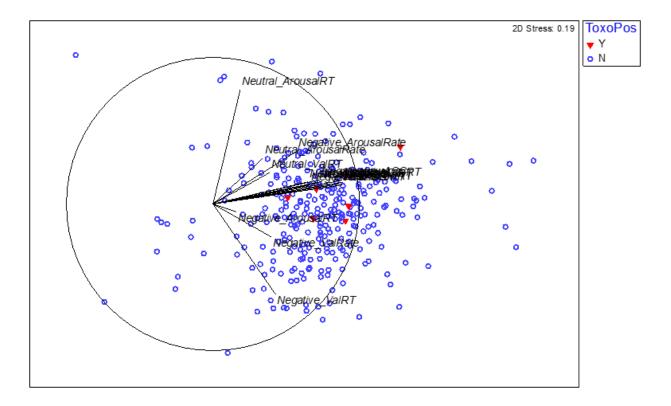
Results

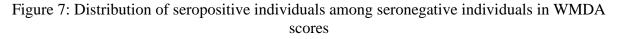
Out of the 315 participants that were screened for toxoplasmosis, only 7 tested positive. This 2.2% positivity rate was much lower than we expected, considering the national average is 11% (Centers for Disease Control and Prevention, 2019). Due to this, it is difficult to draw meaningful conclusions, and if this study is replicated with a higher positive population, it is possible that that study would yield different results.

In total, we compared and analyzed four variables: *T. gondii* rapid test results, the WMDA results, the RISQ results, and the STAI results. A multivariate approach was used to compare the rates of toxoplasmosis seropositivity and each cognitive/behavioral measure. Each cognitive/behavioral measure was also compared against each other to determine if there was correlation between any of them.

T. gondii infection vs WMDA

A Multidimensional (MDS) plot was used to map the relationship between *T. gondii* infection and working memory and image rating scores on the WMDA. Each point is a study participant and points that are close together gave similar responses. The red triangles represent seropositive individuals, and the blue circles represent seronegative individuals. The red triangles do not cluster, indicating that seropositive individuals do not share patterns of risky behavior. The vectors shown are Pearson Correlation vectors.





An Analysis of Similarity (ANOSIM) is used to compare groups of samples. The p-value indicates the proportion of permutations that resulted in an R-value greater or equal to the R-value calculated with the grouping factor. An R-value close to 1.0 suggests dissimilarity between groups, while an R-value closer to 0 suggests an even distribution of similarity. The ANOSIM was used to verify the results of the MDS plots. This ANOSIM resulted in a p-value of 0.95, concluding that the similarity was not significant. The sample statistic (R) is -0.162, and 999 permutations were run. Toxoplasmosis does not significantly affect working memory accuracy, response time, or valence rates, arousal rates, or rating response times.

T. gondii infection vs RISQ

An MDS plot was used to map the relationship between *T. gondii* infection and RISQ A, how many times a participant engaged in a certain risky behavior. Instead of testing all 38 items, the items were grouped together by type of behavior. The data for RISQ A was transformed into more cohesive data, as responses varied from zero to the thousands. The red triangles do not cluster, indicating that seropositive individuals do not share patterns of risky behavior. The

vectors shown are Pearson Correlation vectors and the distribution of points suggests most common risky behaviors are budgeting, eating, self-harm and driving.

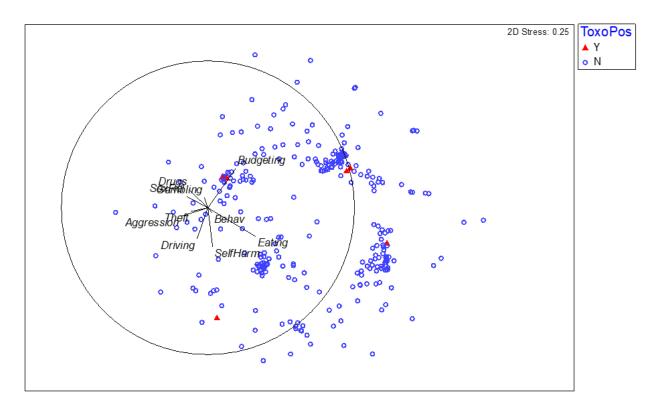


Figure 8: Distribution of seropositive individuals among seronegative individuals in RISQ A scores

An ANOSIM resulted in a p-value of 0.62, concluding that the similarity was not significant. The sample statistic (R) is -0.028, and 999 permutations were run. Toxoplasmosis does not significantly affect occurrence of risky behavior.

A second MDS plot was used to map the relationship between *T. gondii* infection and RISQ F, if the participant engages in the behavior for thrill-seeking. Instead of testing all 38 items, the items were grouped together by type of behavior. This data was not transformed, as RISQ F responses only varied from zero to four. The red triangles do not cluster, indicating that seropositive individuals do not share patterns of thrill-seeking. The vectors shown are Pearson Correlation vectors.

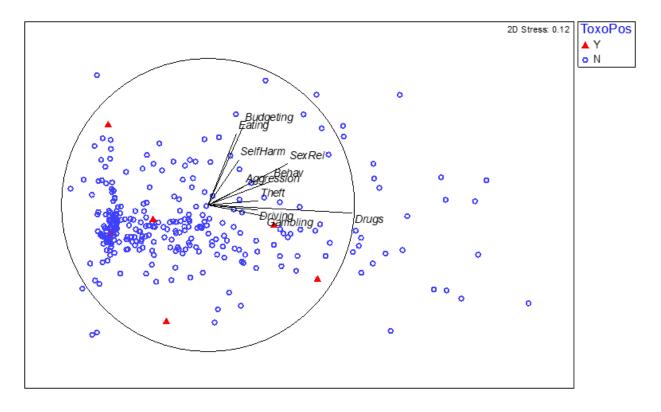


Figure 9: Distribution of seropositive individuals among seronegative individuals in RISQ F scores

An ANOSIM resulted in a p-value of 0.232, concluding that the similarity was not significant. The sample statistic (R) is 0.09, and 999 permutations were run. Toxoplasmosis does not significantly affect thrill-seeking associated with these 36 behaviors.

T. gondii infection vs Trait Anxiety

The Trait and State segments of the measure were calculated separately. An MDS was used to map the relationship between *T. gondii* infection and reported Trait Anxiety. The red triangles do not cluster, indicating that seropositive individuals do not share patterns of trait anxiety. The vectors shown are Pearson Correlation vectors.

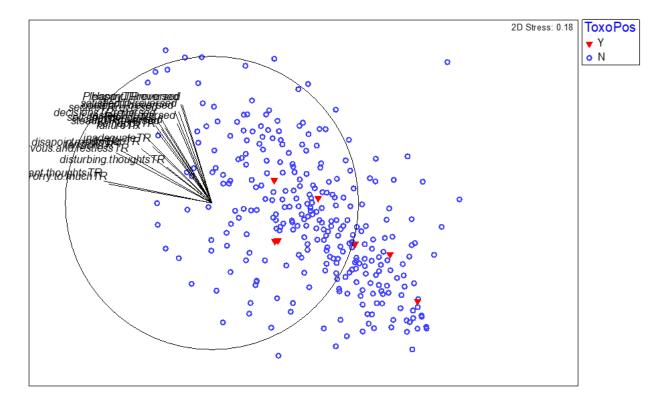


Figure 10: Distribution of seropositive individuals among seronegative individuals and their overall Trait Anxiety scores

An ANOSIM resulted in a p-value of 0.904, concluding that the similarity was not significant. The sample statistic (R) is -0.136, and 999 permutations were run. Toxoplasmosis does not significantly reported trait anxiety.

T. gondii vs State Anxiety

An MDS was used to map the relationship between *T. gondii* infection and reported State Anxiety. The red triangles do not cluster, indicating that seropositive individuals do not share patterns of state anxiety. The vectors shown are Pearson Correlation vectors.

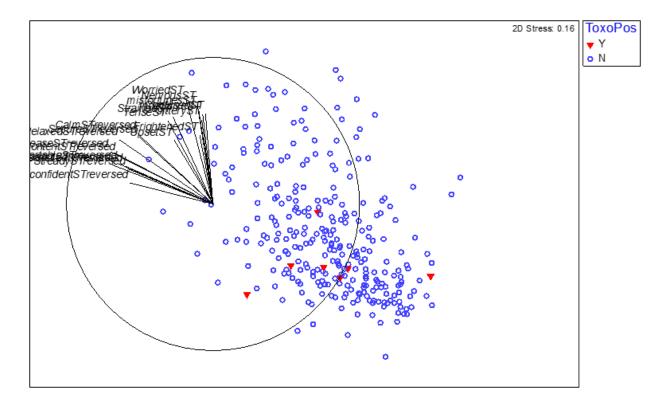


Figure 11: Distribution of seropositive individuals among seronegative individuals and their overall State Anxiety scores

An ANOSIM resulted in a p-value of 0.788, concluding that the similarity was not significant. The sample statistic (R) is -0.089, and 999 permutations were run. Toxoplasmosis does not significantly reported state anxiety.

STAI vs RISQ vs Working Memory Accuracy and Response Time

Table 1. Correlation matrix of STAI vs RISQA vs WMDA accuracy and response times.Correlations are bolded for clarity.

	statesums	traitsums	drugA	aggrA	gambA	riskysexA	alcA	selfharmA	eatA	recklessA
statesums										
traitsums	0.68									
drugA	-0.02	-0.07								
aggrA	-0.07	-0.04	0.01							
gambA	-0.01	0.01	-0.01	0.10						
riskysexS	0.07	0.00	0.12	0.00	-0.02	2				
alcA	-0.01	-0.01	0.11	0.12	0.00	0.11				
selfharmA	0.11	0.23	0.10	0.03	-0.01	0.01	-0.02			
eatA	0.17	0.15	0.43	-0.03	-0.03	0.08	0.18	0.19)	
recklessA	0.03	0.09	0.04	0.09	0.12	0.03	0.22	0.10	0.26	i
drugE	0.07	0.23	0.02	-0.13	-0.14	0.01	0.08	0.01	0.00	-0.05
aggrE	0.09	0.10	-0.09	0.18	-0.07	-0.01	0.11	0.04	-0.05	o 0.13
gambE	0.12	0.21	-0.03	0.05	0.34	-0.09	-0.10	0.16	6 -0.03	-0.06
riskysexE	0.30	0.49	0.04	0.09	0.03	0.15	-0.08	0.17	0.23	3 0.07
alcE	0.14	0.27	0.00	-0.09	0.08	3 0.08	0.05	-0.02	-0.01	0.05
selharmE	0.14	0.16	-0.07	-0.16	-0.15	0.14	0.02	0.10	0.10	0.07
eatE	0.22	0.21	-0.21	-0.16	0.02	0.09	0.09	-0.05	5 0.10	-0.02
recklessE	0.22	0.22	0.03	-0.08	0.05	o.08	0.02	0.08	3 0.07	-0.03
NegativeHiACC	-0.11	-0.09	0.01	0.02	0.09	0.08	-0.08	-0.11	-0.05	-0.07
NegativeLowACC	-0.09	-0.08	-0.01	-0.06	0.07	0.01	-0.02	-0.23	3 0.03	-0.06
NeutralHiACC	-0.11	-0.12	0.05	-0.03	0.06	0.06	0.06	-0.16	6 0.06	6 0.02
NeutralLowACC	-0.04	-0.09	0.07	-0.03	0.03	3 0.05	0.05	-0.37	0.06	6.09
NegativeHiRT	-0.04	-0.03	0.05	-0.07	0.02	2 0.07	-0.10	-0.04	0.03	-0.11
NegativeLowRT	-0.03	0.00	-0.01	-0.09	-0.02	-0.01	-0.12	-0.07	0.01	0.08
NeutralHiRT	-0.03	0.00	0.02	-0.09	0.01	0.03	-0.10	-0.03	8 0.05	-0.07
NeutralLowRT	-0.04	-0.01	-0.03	-0.09	-0.01	0.02	-0.13	-0.09	0.02	-0.07

Table 2. Correlation matrix of STAI vs RISQE vs WMDA accuracy and response times. Correlations are bolded for clarity.

	drugE	aggrE	gambE	riskysexE	alcE	selfharmE	eatE	recklessE
statesums			0					
traitsums								
drugA								
aggrA								
gambA								
riskysexS								
alcA								
selfharmA								
eatA								
recklessA								
drugE								
aggrE	0.2	:5						
gambE	0.0	-0.0	8					
riskysexE	0.2	.4 0.0	8 0.50					
alcE	0.5	5 0.2	9 0.19	0.6	2			
selharmE	0.1	.6 0.0	9 -0.03	-0.09	э о .	36		
eatE	0.3	9 0.2	7 0.07	0.0	6 0 .	45 0.3	9	
recklessE	0.2	2 0.1	3 0.28	0.3	ЭО.	29 0.1	4 0.3 :	L
NegativeHiACC	0.0	0.0	5 0.07	0.0	7 -0.	02 0.0	8 0.03	1 -0.03
NegativeLowACC	-0.0	2 0.1	1 0.04	-0.1	50.	01 0.0	9 0.12	2 0.04
NeutralHiACC	-0.1	.7 0.1	3 -0.04	-0.20	0.	04 -0.0	3 0.13	3 0.01
NeutralLowACC	0.0	3 0.1	5 0.00	0.09	э 0.	11 0.0	6 0.13	3 0.05
NegativeHiRT	0.1	.0 -0.1	3 0.15	-0.0	3 0.	06 0.0	3 -0.07	7 0.05
NegativeLowRT	0.0	9 -0.0	9 0.06	-0.0	1 0.	03 0.0	1 -0.06	6 -0.02
NeutralHiRT	0.1	.4 -0.0	8 0.16	0.1	1 0.	11 0.0	5 -0.0	5 0.00
NeutralLowRT	0.1	.6 -0.0	8 0.15	0.0	5 -0.	01 0.0	6 -0.06	6 -0.03

Table 3. Correlation matrix of WMDA accuracy and response times. Correlations are bolded for clarity.

	and a second						Contraction of the loss	
	NegativeHiACC	NegativeLowACC	NeutralHiACC	NeutralLowACC	NegativeHiRT	NegativeLowRT	NeutralHiRT	NeutralLowRT
statesums								
traitsums								
drugA								
aggrA								
gambA								
riskysexS								
alcA								
selfharmA								
eatA								
recklessA								
drugE								
aggrE								
gambE								
riskysexE								
alcE								
selharmE								
eatE								
recklessE								
NegativeHiACC								
NegativeLowACC	0.38	8						
NeutralHiACC	0.46	6 0.4	4					
NeutralLowACC	0.3	7 0.5	2 0.5:	1				
NegativeHiRT	0.03	3 0.0	7 0.0	7 0.1	1			
NegativeLowRT	-0.1	5 -0.03	3 -0.08	B -0.0	2 0.8	5		
NeutralHiRT	-0.04	4 0.03	3 0.00	0.0	5 0.8	6 0.8	8	
NeutralLowRT	-0.10	-0.04	4 -0.07	7 0.0	3 0.8	1 0.9	1 0.89	9

We examined the correlations between the responses for state anxiety, trait anxiety, the amount of times an individual participated in a risky behavior (RISQ A), whether or not they participated in the behavior to feel less upset (RISQ F), overwhelmed or distressed, and the following WMDA variables: Negative Image High Load Accuracy, Negative Image Low Load Accuracy, Neutral Image High Load Accuracy, Neutral Image Low Load Accuracy, Negative Image High Load Response Time, Negative Image Low Load Response time, Neutral Image High Load Response Time, Neutral Image Low Load Response Time.

Of all the p-values, aggrE vs drugE was the closest to 0.05 (p=0.0498, r=0.252) and eatE vs aggrE was just above 0.05 (p=0.05028, r=0.270). Therefore, all correlation coefficients at or above 0.27 were found to be correlated. According to the correlation matrix, state and trait anxiety had a positive correlation (r=0.68). State anxiety and riskysexE had a positive correlation (r=0.30). Trait anxiety had a positive correlation with riskysexE (r=0.49) and alcE (r=0.27).SelfharmA and NeutralLowACC had a negative correlation (r=-0.37). DrugE had a positive correlation with alcE (r=0.55) and eatE (r=0.39). AggrE had a positive correlation with

alcE (r=0.29) and eatE (r=0.27). GambE had a positive correlation with riskysexE (r=0.50) and recklessE (r=0.28). RiskysexE had a positive correlation with alcE (r=0.62), and recklessE (r=0.39). AlcE had a positive correlation with selfharmE (r=0.36), eatE (r=0.4), and recklessE (r=0.29). SelfharmE had a positive correlation with eatE (r= 0.39). EatE had a positive correlation with recklessE (r=0.3). NegativeHiACC had a positive correlation with NegativeLowACC(r=0.38), NeutralHiACC(r=0.46), NeutralLowACC(r=0.37). NegativeLowACC had a positive correlation with NeutralHiACC (r=0.44), and NeutralLowACC (r=0.51). NeutralHiACC had a positive correlation with NeutralHiACC (r=0.51). NeutralHiACC had a positive correlation with NeutralLowACC (r=0.51). NeutralHiACC had a positive correlation with NeutralLowACC (r=0.85), NeutralHiRT (r=0.86), NeutralLowRT (r=0.81). NegativeLowRT had a positive correlation with NeutralHiRT (r=0.88), and NeutralLowRT (r=0.91). NeutralHiRT had a positive correlation with NeutralHiRT (r=0.88), and NeutralLowRT (r=0.91). NeutralHiRT had a positive correlation with NeutralHiRT (r=0.88), and NeutralLowRT (r=0.91). NeutralHiRT had a positive correlation with NeutralLowRT (r=0.89).

STAI vs RISQ vs Valence and Arousal Image Rating

Table 4. Correlation matrix of STAI vs RISQA, vs Image Rating Task valence and arousal
ratings. Correlations are bolded for clarity.

-	statesums ti	raitsums	drugA	aggrA	gambA	riskysexA	alcA	selfharmA eat	A	recklessA
statesums										
traitsums	0.68									
drugA	-0.02	-0.07								
aggrA	-0.07	-0.04	0.01							
gambA	-0.01	0.01	-0.01	0.10						
riskysexS	0.07	0.00	0.12	0.00	-0.02					
alcA	-0.01	-0.01	0.11	0.12	0.00	0.11				
selfharmA	0.11	0.23	0.10	0.03	-0.01	0.01	-0.02			
eatA	0.17	0.15	0.43	-0.03	-0.03	0.08	0.18	0.19		
recklessA	0.03	0.09	0.04	0.09	0.12	0.03	0.22	0.10	0.26	
drugE	0.07	0.23	0.02	-0.13	-0.14	0.01	0.08	0.01	0.00	-0.05
aggrE	0.09	0.10	-0.09	0.18	-0.07	-0.01	0.11	0.04	-0.05	0.13
gambE	0.12	0.21	-0.03	0.05	0.34	-0.09	-0.10	0.16	-0.03	-0.06
riskysexE	0.30	0.49	0.04	0.09	0.03	0.15	-0.08	0.17	0.23	0.07
alcE	0.14	0.27	0.00	-0.09	0.08	0.08	0.05	-0.02	-0.01	0.05
selharmE	0.14	0.16	-0.07	-0.16	-0.15	0.14	0.02	0.10	0.10	0.07
eatE	0.22	0.21	-0.21	-0.16	0.02	0.09	0.09	-0.05	0.10	-0.02
recklessE	0.22	0.22	0.03	-0.08	0.05	0.08	0.02	0.08	0.07	-0.03
Negative_ValRate	-0.13	-0.05	-0.09	0.08	-0.06	-0.05	-0.06	0.04	-0.04	-0.01
Neutral_ValRate	0.08	0.05	0.00	-0.04	0.01	-0.05	-0.06	0.03	0.09	-0.02
Negative_ArousalRate	0.18	0.07	0.01	-0.05	0.03	0.01	0.03	-0.05	0.06	0.02
Neutral_ArousalRate	0.11	0.07	0.06	-0.03	0.09	0.04	-0.02	-0.07	0.16	0.00

······································	drugE	aggrE	gambE	riskysexE	alcE	selfharmE	eatE	recklessE
statesums								
traitsums								
drugA								
aggrA								
gambA								
riskysexS								
alcA								
selfharmA								
eatA								
recklessA								
drugE								
aggrE	0.2	25						
gambE	0.0	06 -0	.08					
riskysexE	0.2	24 0	.08 0	.50				
alcE	0.5	5 0	. 29 0	.19 0.6	2			
selharmE	0.1	.6 0	.09 -0	.03 -0.0	9 0 .	.36		
eatE	0.3	19 0	.27 0	.07 0.0	6 0 .	.45 0.39	ņ.	
recklessE	0.2	22 0	.13 0	.28 0.3	9 0 .	29 0.14	0.31	
Negative_ValRate	-0.1	.2 -0	.06 0	.07 0.0	95 -0.	.11 -0.08	-0.08	-0.12
Neutral_ValRate	-0.1	.1 0	.07 -0	.18 0.0	01 0.	.12 0.11	0.08	0.11
Negative_ArousalRate	-0.0	02 0	.05 -0	.15 -0.0	01 0.	.09 0.10	0.11	0.10
Neutral_ArousalRate	-0.0	-0	.07 0	.02 0.1	.5 0.	.11 -0.01	0.16	0.07

 Table 5. Correlation matrix of STAI vs RISQE, vs Image Rating Task valence and arousal ratings. Correlations are bolded for clarity.

	Negative_ValRate	Neutral_ValRate	e Negative_ArousalRate	Neutral_ArousalRate
statesums				
traitsums				
drugA				
aggrA				
gambA				
riskysexS				
alcA				
selfharmA				
eatA				
recklessA				
drugE				
aggrE				
gambE				
riskysexE				
alcE				
selharmE				
eatE				
recklessE				
Negative_ValRate				
Neutral_ValRate	-0.03			
Negative_ArousalRate	-0.53).20	
Neutral_ArousalRate	-0.12		0.17 0.2	0

 Table 6. Correlation matrix of Image Rating Task valence and arousal ratings. Correlations are bolded for clarity.

We examined the correlations between the responses for state anxiety, trait anxiety, the amount of times an individual participated in a risky behavior (RISQ A), whether or not they participated in the behavior to feel less upset (RISQ F), overwhelmed or distressed, and the following WMDA variables: Negative Image Valence Rate, Neutral Image Valence Rate, Negative Image Arousal Rate, Neutral Image Arousal Rate.

In addition to the previous correlations listed about state anxiety, trait anxiety, RISQ A and RISQ E, we found a negative correlation between Negative_ValenceRate and Negative_ArousalRate (r=-0.53).

Discussion

Due to our seropositivity rate being so low, the results of any analysis involving *Toxoplasma* infection are inconclusive. For the sake of this thesis, I will report the results based on the analyses we completed, even though they are not reliable enough to publish. At the beginning of this experiment, I posed several research questions. The first being: will toxopositive individuals perform a working memory task with emotionally-reactive images with higher accuracy compared to toxo-negative individuals? The results show that there is no significant difference between seropositive and seronegative individuals when it comes to working memory performance. The next questions are: are toxo-positive individuals more likely to engage in risky behavior? The results show that there is no significant difference in the frequency of engagement of risky behavior, the type of risky behavior, or whether they participated in the behavior to feel less upset, overwhelmed or distressed, or to feel thrill between seropositive individuals. Will toxo-positive individuals report higher or lower levels of anxiety than the standard? The results show that there is no significant difference between seropositive individuals when questioned about state or trait anxiety.

In addition to exploring the relationship between *T. gondii* infection and working memory, image rating, state/trait anxiety, and risky behavioral tendencies, we explored the relationship between the psychological measures. We did not find significant correlations between tests, but we did find them among the tests. The results for state anxiety and trait anxiety are significantly correlated, and so are the results for RISQ A and RISQ E, and RISQ A and RISQ F. Some variables between working memory accuracy and response times were correlated, as was the case between ratings. The figure below shows the correlations between measures; RISQ A and RISQ F are correlated to each other and no other test, state anxiety and trait anxiety are correlated to each other and no other test, and WMDA scores are not correlated to any test.

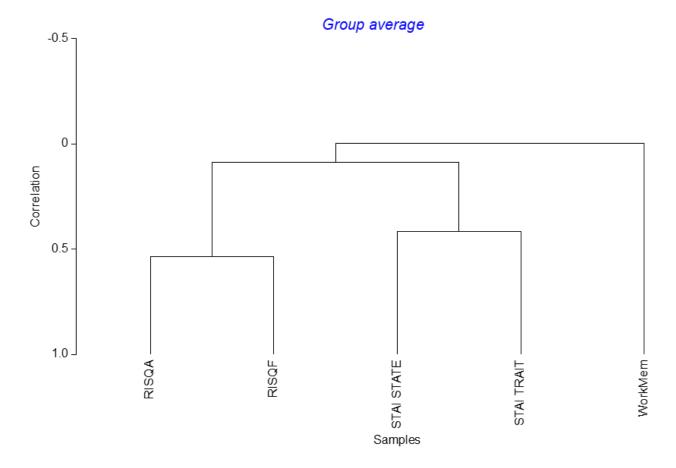


Figure 12: The RELATE measure looks for correlation in multivariate patterns across tests

Although the CDC estimates that 11% of the American population of 6+ years is infected with *Toxoplasma* (Centers for Disease Control and Prevention, 2019), with some studies reporting numbers as high as 22.5% (Jones et al., 2001), our study only found a seropositivity rate of 2.2% among 315 college-age adults. This statistic is smaller than the American standard, and much smaller than the accepted worldwide statistic, which is 30% (Hill et al., 2005). Interestingly, we saw participants of many nationalities, including those with higher seropositivity rates such as Colombia: 50% (Cañón-Franco et al., 2014) and Brazil: 50-80% (Dubey et al., 2012), and our seropositive numbers were still significantly lower than expected. Participants from these countries ranged from born there but immigrated in early life to having lived there most of their lives and only immigrated recently for high school or college. In fact, it is believed that toxoplasmosis is an underreported infection, since most people do not present

clinical symptoms unless they have immune system dysfunction and toxoplasmosis is not an infection that is routinely tested for.

Unfortunately, toxoplasmosis is not a nationally notifiable disease, and there is no national-level surveillance data available (McCall, 2022). While pregnant individuals are routinely tested for toxoplasmosis to prevent congenital toxoplasmosis, there is no national maternal screening program. As of April 2021, only eight out of 50 states consider toxoplasmosis a reportable disease; these include Arkansas, Delaware, Hawaii, Kentucky, Minnesota, Nebraska, Pennsylvania, and Wisconsin. Interestingly, no state collects nonhuman data as a routine part of surveillance (McCall, 2022). Previous studies like the ones conducted by Lykins and colleagues take existing data, in this case from insurance companies. These data points would have been tested for toxoplasmosis, likely because the patients had some immunodeficiency-causing illness or disease. Because these datapoints are biased, it is not comparable to the average American population. All of these factors unfortunately make it impossible to accurately compare our numbers to national or state-wide numbers.

One of the reasons we had such a small toxo-positive sample could be that since toxoplasmosis is a lifelong infection, it is more common in older adults (Wyman et al., 2017). The older someone is, the more opportunities they have to acquire the parasite and most of our samples were in their late-teens to early twenties, the oldest being 35. Another reason could be that T. gondii infection is largely influenced by socioeconomic status (Al-Malki, 2021; Bahia-Oliveira et al., 2003, Hotez, 2008). For example, congenital toxoplasmosis disproportionately affects poor individuals in the American South, disadvantaged inner cities, the US-Mexico borders, and Arctic Alaska (Hotez, 2008). In Brazil, Bahia-Oliveira and colleagues surveyed 1,436 individuals of three socioeconomic statuses: lower, middle, and upper. They found seropositivity to be 84% among the lower socioeconomic sample, 62% among the middle socioeconomic sample, and 23% among the upper socioeconomic sample (Bahia-Oliveira et al., 2003). While we did not survey socioeconomic background of our own participants, most of them were students currently enrolled in a program at Nova Southeastern University, so I found family income data reported by NSU. In 2017, the median family income was \$81,200. 33% of students came from the top 20%, which means a family income of \$110,000(+). Only 9.1% of students came from the bottom 20%, which means a family income of \$20,000 or less

("Economic Diversity and Student Outcomes at Nova Southeastern," 2017). It can be inferred that the average participant surveyed was not of a low socioeconomic status, leading to less positives.

Study Limitations

Several study limitations could have led to skewed or partial results. The most significant limitation is the severely underwhelming number of seropositive participants. We built this study around the common statistic, 11% of the American population is infected with *Toxoplasma gondii* (Centers for Disease Control and Prevention, 2019). Considering other factors like the infection being underrepresented, having many participants from different countries with high seroprevalence, such as Brazil, Colombia, France, and living in a hot, wet climate that *T. gondii* prefers, we expected around 30 out of 315 positives, but we only found seven positives. This left us unable to draw meaningful conclusions in our data analysis, when comparing infection to emotional response in working memory and image rating, the tendency to engage in risky behaviors, and the tendency to experience symptoms of anxiety.

While 315 is a large sample size, and we saw vast diversity in race, ethnicity, and cultural background, the sample pool was homogenous in terms of age, education, place of living, and assumed socioeconomic status. Age ranges of participants were 18 to 32 years old, the levels of achieved education ranged from associate's to bachelor's, with the majority of participants actively enrolled in a bachelor's or master's program at NSU at the time of data collection. While there were almost certainly different results on the study's measures due to race, ethnic, and cultural backgrounds, the unifying factors made for an overall homogenous participant pool.

The 315 samples were collected throughout the year, with the first participant coming in July of 2023 and the last participant coming in May of 2024. Measures such as the WMDA and STAI can be affected depending on the academic season (as in start of the semester, midterms, finals), especially when testing college students. Stressful times can cause distraction and sensitivity, which can affect working memory and image rating tasks, and anxiety which would affect STAI scores. Participants were tested throughout those nine months, and notable times of stress could be the beginning of the semester, midterms, finals, the end of the semester, winter holidays, winter break, and spring break.

From August 2023 to December 2023, the study was run in one of two rooms, either in the psychology lab in Parker 245C during the evenings, or the biology department conference room in Parker 390 during the afternoons. The computers in the psychology lab had the cognitive measures and questionnaires on desktops, but in the afternoon, the cognitive measures had to be performed on laptops and the questionnaires were given on paper. The differences in testing tools affected the WMDA and the STAI, and made it so that some data had to be left out.

The limitations that the RISQ faced were due to the homogenous participant population. While there was a great variety of risky behaviors reported, there were some that were barely reported and some that were surprisingly highly reported. These instances may be attributed to factors like socioeconomic status, place of living, and age. For example, no participants reported heroin use, RISQ 20. The DEA reports that the northeast and midwest report the highest availability levels of heroin (DEA, 2016), which could be a reason that there were no instances in this group. Conversely, this college student demographic is too homogenous in their habits and tendencies; a national survey published in 2005 found that 12% of college students reported nonmedical prescription opioid use, although this study reported anecdotal usage of prescription medications such as hydrocodone, and not heroin. (McCabe et al., 2005). Furthermore, only one person reported having sex for drugs or money, RISQ 10, and only one person reported robbing someone, RISQ 25. This can be related to the above average socioeconomic background of many NSU students.

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