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The Effect of *Toxoplasma gondii* on Acoustic Startle Response in an Inner City Population

By

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Master of Public Health

Epidemiology

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2014

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An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology

2016

Abstract

The Effect of *Toxoplasma gondii* on Acoustic Startle Response in an Inner City Population

By Nicholas Marlin Massa

Toxoplasma gondii (TOXO) infection affects approximately 1.1 million people in the United States annually. TOXO has recently been implicated in the etiology of psychiatric diseases, namely schizophrenia (Scz), and has been correlated with a decrease in cognition. The objective of this study is to assess the effect of TOXO on the acoustic startle response (ASR), a physiological measure of neural processing, accounting for sociodemographic and psychiatric factors. Physiological and psychological data along with biological specimens were collected from a primary care clinic at Grady Memorial Hospital serving the inner city of Atlanta, GA. From a cohort of 611 patients, those missing either demographic, biologic, or physiologic data were excluded leaving a final sample of 364 patients. A series of linear regression models were used to assess the impact of a TOXO seropositivity indicator, or TOXO serointensity, the amount of antibody produced by a subject, on ASR latency and amplitude controlling for demographics and psychiatric diagnoses. Both TOXO seropostivity and serointensity did not significantly associate with the latency of the acoustic startle response ($F=0.23$, $p=0.6305$; $F=0.06$, $p=0.8014$). However PTSD, and no other psychiatric covariates, did positively correlate ASR latency in seropositive individuals, but the correlation was attenuated when serointensity was taken into consideration ($F=5.15$, $p=0.0238$; $F=3.51$, $p=0.062$). ASR amplitude was significantly increased in TOXO seropositive subjects, and the effect was stronger using serointensity ($F=7.41$, $p=.0068$; $F=10.05$, $p=0.0017$). This significant increase in amplitude was measured when controlling for all other psychiatric covariates. An increase in amplitude has been attributed to a decrease in habituation to repeated stimuli, through dysregulation in both the hypothalamic-pituitary-adrenal axis, and through the amygdala. The increase in amplitude attributed to TOXO, potentially implicates these regions of the brain, and their respective neurotransmitters in TOXO's effects on the brain. In future studies we would hope to assess cytokines and other biomarkers to potentially discern a biological effect of TOXO that could contribute to the increased startle amplitude.

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Acknowledgements

This document could not have been completed without the help and support from many individuals, who, unbeknownst to them, have facilitated my growth both as a scientist and as a person. Namely, I would like to thank Drs. Pearce and Duncan for their tremendous support and guidance throughout my academic journey at Emory. They both welcomed me into their collaboration at both Emory, and the Atlanta VA, and helped to shape my view of both research and mental illness pathology. The experiences in both labs have enhanced my skillset from both a biological and physiological perspective, and allowed me the opportunity to experience the trials and tribulations of human-based research. I would like to thank members of both labs for their support and assistance throughout this process especially Lei, Bruce, Krissy and Alex.

I would also like to thank Karl Schmidt for his wisdom and leadership both on and off the field. He has enabled me to revisit and come to love lacrosse again, and provide me with a unique opportunity to be a leader among our team. His commitment to the program, and to me as an athlete has helped me retain balance and a physical outlet throughout the trials and tribulations of the MPH degree.

Finally, I would like to thank my friends and family, who regardless of distance, have always been a constant source of strength and support. Your individual contributions have helped me become who I am today, and have pushed me onwards and upwards. Wherever we may end up, I know I can always count on you to be there for me, and I can’t thank you enough for that.

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Introduction

Post-traumatic stress disorder (PTSD) is the result of a modulated "fight-or-flight" response after a trauma. It often leads to a perpetual worry of recurrent trauma, when the likelihood of such trauma is very low. PTSD usually stems from a traumatic ordeal in which the individual, their loved ones, or the people around them were afflicted by some harm. The etiology of PTSD is unknown as many people can experience the same stressor, and only a few will develop PTSD. The type of trauma endured, the age at which said trauma occurred, the personality of the individual, and the genetics of the individual each play a significant role in the development of PTSD. The type of trauma whether physical or emotional, and the age at which it occurs, has the potential to significantly impact the neurobiology and psychology of the individual putting them at greater risk of PTSD. Others may inherently be at risk of PTSD due to differential neuroanatomy or a genetic susceptibility which leads to a dysregulation of the processes necessary to mitigate emotional responses to trauma. The signs of PTSD are classified into three primary categories: re-experiencing, avoidance, and hyperarousal. Re-experiencing symptoms include cognitive flashbacks, bad dreams, and frightening thoughts with concurrent physiological responses. Avoidance symptoms include withdrawal from triggers of the experience, negative affect, anhedonia, amnesia, and depression. Hyperarousal symptoms include irritability, insomnia, and anxiety (NIMH, 2015). PTSD can develop in both adults and children. The lifetime prevalence of PTSD in the USA among adults was estimated at 6.8%, and the annual prevalence was estimated at 3.5% (Kessler et al., 2005, Kessler et al., 2005). PTSD also tends to occur more frequently in women as compared to men as the lifetime and annual prevalence of PTSD in women was significantly higher than that of men in the general community (Lifetime: 9.7% vs. 3.6%, Annual: 5.2% vs. 1.8%) (National Comorbidity Survey, 2005). The Grady Trauma Project (GTP), a cross-sectional study of Atlanta's minority urban population who are of low socioeconomic status and highly traumatized, attempts to provide a means of discerning the relative risk of acquiring PTSD given genotypic polymorphisms, a lifetime history of trauma, and emotional responses in response to the trauma.

The primary objectives of the study are to discern the associations between PTSD and stress-related genetic polymorphisms, physiological symptomatology, and amount of trauma the individual experienced (Ressler et al., 2016). Another subset of the GTP population of particular significance is those with schizophrenia (Scz).

Scz is a debilitating brain disorder characterized by an abnormal interpretation of reality. Patients with Scz manifest positive symptoms, negative symptoms, and cognitive impairment. Positive symptoms are hallucinations, delusions, thought disorders, and movement disorders, whereas negative symptoms are flat affect, social withdrawal, and anhedonia. Cognitive impairment is characterized by problems with attention, concentration, psychomotor speed, learning, memory, and executive functions (Mueser et al., 2004). The point prevalence of Scz is approximately 5/1,000 in the population, and the estimated annual incidence is approximately 2/10,000. Scz diagnoses usually occur between the ages of 15 and 24. Men are significantly more likely to develop Scz, and do so at a younger age as compared to women (Messias et al., 2007). A study conducted by Bresnahan et al. (2007) also found that those of African American descent were 3 times more likely to develop Scz as compared to whites, and this relationship was slightly attenuated when adjusting for socioeconomic status. While the etiology of Scz is unknown, some primary risk factors are genetics, environmental factors, and abnormal pathophysiology (Mueser et al., 2004). One means of assessing the physiological symptomatology associated with both PTSD and SCZ by the GTP was through the use of the acoustic startle response.

The acoustic startle response (ASR), is the culmination of a series of involuntary reflexes brought about by a sudden, intense auditory stimulus. The primary ASR pathway begins with stimulation of the cochlear nuclei, propagates to the caudal pontine reticular formation, and stimulates motor neurons. The ASR is usually measured by electromyogram from contractions of the musculus orbicularis oculi in response to the auditory stimuli (Koch 1999). Reactions to the startle response are modulated by the hippocampus, amygdala, bed nucleus of the stria terminalis (BNST) of the forebrain, and the locus coeruleus of the hindbrain (Flandreau et. al., 2014). Similar regions associated with the ASR, are also

associated with the neurobiological and physiological etiology of PTSD through the corticotropin-releasing factor, the hypothalamic-pituitary-adrenal axis system (HPA axis), and the noradrenergic system (Bremner, 2006). Given the ASR's ability to assess neural processing speed, it has been used to study the effects of toxoplasmosis on cognition (Pearce et al., 2013).

Toxoplasma gondii (TOXO) is a protozoan parasite which is able to induce toxoplasmosis. Felines are a primary host of TOXO, where it will complete its sexual cycle, and be expelled in the form of oocysts in fecal matter for 1-2 weeks after a primary infection. Once expelled, the oocysts undergo sporulation within the first 5 days in the environment in order to become infectious. If ingested by a warm-blooded animal, the parasite will transition into a tissue-infective stage in which it can form cysts in the eyes and central nervous system of the new host. TOXO is predominately acquired from consumption of raw or undercooked meat from infected animals, or consumption of food, soil or water contaminated by cat feces. Congenital transmission can occur if a pregnant woman becomes newly infected with TOXO during the early stages of her pregnancy. TOXO can also be transmitted when a previously uninfected individual acquires a blood transfusion or an organ transplant from an infected donor (Jones, et al. 2014).

TOXO infection is especially common among those who are black or Hispanic, born outside of the United States, and of low socioeconomic status and educational attainment. A significant risk factor is an occupation in which there is significant exposure to soil. Toxoplasmosis was the second leading cause of deaths attributable to a food-borne illness, and is known to infect approximately 1.1 million people each year in the United States. Serologic surveys from the National Health and Nutrition Examination Survey (NHANES) found a significantly decreasing seroprevalence of TOXO from 1988-1994 (14.1%) as compared to 1999-2004 (9.0%). Despite the decreasing seroprevalence, an estimated 400-4000 infants are born with congenital toxoplasmosis, with 30% of women who acquire TOXO transmitting it to the neonate. Congenital infection of the fetus with TOXO can lead to a miscarriage, a stillbirth, developmental delays, blindness, and epilepsy. It is also possible for those who experience a congenital infection to be devoid of symptomatology at birth, but develop a neurologic or developmental illness later

in life. Among those who are immunocompromised and co-infected with TOXO, tend to develop encephalitis. Healthy individuals may present symptoms of malaise, but most infections are subclinical, as the immune system is able to combat the parasite without presenting significant symptomatology (Jones, et al. 2014).

Within the last couple of decades, research has begun to elucidate an association between TOXO and a variety of psychiatric and neurologic disorders. Markovitz et al. (2014) found that there was no association between TOXO and either PTSD or depression, all the while demonstrating a significant association between TOXO and generalized anxiety disorder. These findings are relatively preliminary, as to our knowledge no one else has looked at the association between TOXO and PTSD. Despite the lack of an association, there are correlates between toxoplasmosis and PTSD, as both conditions induce the production of similar inflammatory markers which impact similar regions of the brain, and have similar neurotransmission dysfunction.

Toxoplasmosis has been associated with an increase in expression of (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF- α), interferon-γ (IFN- γ), and interleukin-12 (IL-12) (Fabiani et al., 2015). Similarly, PTSD has been found to be associated with an increased level of IL-6, IL-1β, TNF- α, and IFN- γ (Passos et al., 2015), and an increase in expression of IL-12 (Bam et al., 2016). The HPA axis in response to cytokines produced by TOXO leads to the production of glucocorticoids. These glucocorticoids in turn reduce neuroplasticity and cellular resistance through a negative-feedback loop with the HPA axis, which creates an imbalance between glucocorticoids and their high-density receptor (Fabiani et al., 2015). Research into the stress response in those with PTSD has brought about two potential etiologies. One of the proposed etiologies of PTSD is through the hypoactivity of the HPA axis in response to trauma. The hypoactivity in turn leads to a hyposecretion of cortisol which has been implicated in avoidance behaviors of PTSD. Another etiology is hyperactivation of the sympathetic nervous system leading to hyperarousal and re-experiencing symptomology (Morris et al., 2013). Along

with the differential immune responses induced by both TOXO and PTSD, both conditions also lead to neurotransmission dysregulation in the serotonergic, dopaminergic and noradrenergic systems.

Cytokines produced in response to a TOXO infection have been seen to affect the serotonergic system through activation of the indoleamine-2,3-dioxygenase enzyme (IDO), activation of the mitogen-activated protein kinase (MAPK) pathways, alteration in tetrahydropterin (BH4) enzyme activity, and excitotoxicity and oxidative stress (Parlog et al., 2015). The primary impact of TOXO is through IDO activation in immune cells in response to IFN-γ. TOXO impacts neurotransmission through the degradation of the main precursor to serotonin, L-tryptophan into N-formylkynurenine. Depletion of tryptophan then leads to the production of catabolites such as 3-hydroxykynurenine, and anthranilic, kynurenic, xanthurenic and quinolinic acids, which have the ability to impact neuronal function (Henriquez et al., 2009). The serotonergic system has also been implicated in some of the etiology of PTSD, as selective serotonin (5-HT) reuptake inhibitors (SSRIs) have been useful in the treatment of PTSD, there was a significantly lower number of platelet 5-HT transporters in PTSD patients as compared to controls (Maes et al., 1998), and a positive association found between serotonin transporter (5-HTT) genotype and PTSD (Broekman et al., 2007). Serotonergic dysfunction has also been associated with PTSD, as decreased serotonin levels have been associated with some of the symptomatology of PTSD such as impulsivity, aggression, fear, anxiety and depression (Albucher et al. 2002). This serotonergic dysfunction could be multifold, as serotonin acts as a modulator to ensure homeostasis between dopaminergic, noradrenergic, and GABAergic neurons which are all culpable in the symptomatology of PTSD (Vaswani et al., 2003). Decreased 5-HT has been seen to contribute to the pathology of TOXO and PTSD, leading to both the upregulation of biologic markers, and increased symptoms. Similar associations can be made between TOXO and PTSD in the dopaminergic system.

One of the neuromodulatory effects of TOXO is its ability to affect dopamine (DA) metabolism through the upregulation of genes which encode for tyrosine hydroxylase, the rate-limiting component in DA synthesis. This genetic upregulation, and increased DA production has been seen both *in vitro* as well

as *in vivo*. The excessive production of DA within humans has been shown to impair both neurologic and psychologic factors such as locomotion, cognition, memory, learning, and mood. An indirect function of TOXO on the dopaminergic system is through the production of kynurenic acid (KYNA) through the degradation of L-tryptophan. The production of KYNA leads to the antagonism of N-methyl-D-aspartate receptors (NMDARs) which impacts the neurotransmission of dopamine (Parlog et al., 2015). This dysregulation of DA, also has a significant impact on PTSD. Animal models have found that the agonism of the dopamine D1 receptor has led to an enhanced startle response, consistent with symptomatology of PTSD. In human studies, urinary and plasma DA concentrations were correlated with the severity of PTSD symptoms experienced by the patient. The physiological associations led to an assessment of the association between dopaminergic gene expression and PTSD. While not all studies have shown a positive association between dopaminergic genes and PTSD, a couple of studies have found a positive association between the dopamine D2 receptor (DRD2) and the dopamine transporter gene (DAT) with PTSD (Broekman et al., 2007). The enhanced production of dopamine attributed to toxoplasmosis, and the association between DA and PTSD etiology both contribute to the neurotransmitter dysregulation implicated in both pathologies. Another neurotransmitter which has been correlated with toxoplasmosis and PTSD is norepinephrine (NE).

Alterations in NE have been attributed to neuro-inflammation in mice infected with TOXO. A study by Stibbs (1985) found that NE levels decreased by 28% in acutely infected mice, however NE levels normalized during a chronic infection. However, since then not many have looked into the association between TOXO and NE. Nevertheless, TOXO could still be implicated in the noradrenergic system, as TOXO has a significant impact on amygdalar DA, and DA is a precursor to NE (Prandovsky et al., 2011; Medina et al., 2003). Given this connection, and the utility of the amygdala in the fear response, there are potential downstream consequences of TOXO on NE which could significantly impact PTSD (Prandovsky et al., 2011). NE is one of the predominate neurotransmitters implicated in PTSD etiology. NE is involved in attention discernment through a “signal to noise ratio”, made up of strong excitatory or

inhibitory post-synaptic potentials. The locus coeruleus (LC) mediates the level of arousal through stimulation of NE neurons, especially in the presence of threatening stimuli. In patients with PTSD there is increased responsivity of noradrenergic neurons in response to stressors. This sensitization of the noradrenergic system has been seen to correlate with symptoms of PTSD such as hypervigilance, enhanced startle response, anger, and insomnia. There is a significant overlap in the cytokines production, neurotransmitter dysfunction, and HPA-axis activation in both toxoplasmosis and PTSD. While there are some differences, namely the way in which the HPA-axis is activated, and the utility of the noradrenergic system, there are parallels in the neurobiology which could exacerbate the effects of either TOXO or PTSD. The etiology of PTSD, while starkly different from Scz, has some parallels psychologically, physiologically, and neurobiologically.

PTSD is a significantly more prevalent disease as compared to Scz, however there is a relatively high amount of comorbidity, as 29% of those with Scz tend to also exhibit symptomatology consistent with PTSD. Other comorbidities which tend to be exhibited by both those with PTSD and Scz are depression, anxiety, and substance abuse. The PTSD and Scz comorbidity has also been associated with more severe cognitive impairments, higher rates of suicidal ideation and suicidal behaviors, along with more frequent hospitalizations (Buckley et al., 2009). Along with similar behavioral manifestations in the etiology of both PTSD and Scz, there are also similarities in pro-inflammatory markers. A review conducted by Passos et al. (2015) found that IL-6, IL-1β, and TNF-α were all significantly elevated in PTSD patients, and similarly, a review by Upthegrove et al. (2014) found the same cytokines were elevated in Scz patients. Along with physiological similarities, there are also neurobiological comorbidities, as studies using proton magnetic resonance spectroscopy (MRS) were able to detect diminished levels of N-acetyl aspartate (NAA) in the right hippocampi of both PTSD and Scz patients (Hull, 2002). Given the similarities between PTSD and Scz, it is possible that some of the etiology behind the incidence of each disease could be attributed to similar neurological dysfunction, which would be exacerbated by TOXO.

The hypothesis that there is an etiological association between TOXO and Scz is derived from a variety of different studies, namely epidemiologic, animal-model, behavioral, and cell culture (Halonen,Weiss. 2014). Epidemiologic studies which have found that the concentration of TOXO antibodies was significantly higher in those with Scz as compared to controls (OR=2.73) (Yolken et al., 2009). Studies using rodents found that TOXO significantly impacted dopamine levels causing a shift in behavior, synonymous with Scz patients. Studies assessing behavior in humans, have found that TOXO infection leads to a significant shift in behaviors, namely personality traits and a decreased level of novelty seeking. Cell culture studies have found that anti-psychotic medications are able to inhibit the replication of TOXO (Halonen,Weiss. 2014). Given the parallels between PTSD and Scz, and the strong association between TOXO and Scz, there may be associations between TOXO and PTSD, which have previously been unexplored. We hypothesize that TOXO seropositivity and TOXO serointensity will have a significant impact on acoustic startle amplitude and latency in subjects from the GTP. Initially we will investigate the effect of TOXO seropositivity on acoustic startle latency and amplitude. From there we will investigate the relationship of PTSD, other psychiatric comorbidities such as MDD and Scz, substance abuse, and demographic factors in discerning the association between TOXO and acoustic startle latency and amplitude, using both TOXO seropositivity and serointensity.

Methods

Subject Recruitment: The Grady Trauma Project (GTP) recruited subjects from the primary care and obstetric-gynecological outpatient medical clinics at Grady Memorial Hospital, Atlanta, Georgia. Subjects were approached at random in waiting rooms from Monday to Friday during regular clinic hours, and asked to partake in the study if they met the inclusion criteria. The study called for subjects who were between 18 and 65 years old, were proficient in English, and willing to undergo the initial interview (Nylocks et al., 2015). Subjects were also asked to provide saliva sample and participate in a blood draw (Kaminsky et al., 2015). Prior to the initiation of any study procedures, GTP study volunteers and staff explicitly explained the time commitment and monetary compensation associated with the study, and collected written informed consent from the subject. All subjects were assigned a number for de-identification purposes. All study procedures underwent the approval processes required by both Emory University Institutional Review Board and the Grady Hospital Research Oversight Committee (Nylocks et al., 2015).

Subject Inclusion: The total number of subjects in our dataset initially began with 650 individuals. It was then parsed down to 611 subjects due to both repeated individuals, those missing a reported age, and those missing demographic variables. The data set was narrowed to 394 subjects based upon those missing blood samples utilized to assess TOXO, and further narrowed to a final total of 364 subjects based upon 30 subjects missing a component of the acoustic startle response (Figure 1.). Demographic information such as age, sex, race, employment status, education and income were collected from each subject. Dichotomous variables were created for sex (male or female), race (African American or other), and employment status (yes or no). Education was collected as a 4 level categorical variable (less than 12^{th} grade, high school graduate or GED, some college or technical school, college graduate or graduate school), but analyzed as a dichotomous variable based upon whether or not the subject graduated from high school or obtained their GED. Monthly income was collected as a 5 level categorical variable ($0-$249, $250-$499, $500-$999, $1000-$2000, or greater than $2000), but analyzed as a dichotomous

variable based upon the federal poverty guidelines which were set at an income of approximately $12,000 per year (GDCH, 2016).

Toxoplasmosa Seropositivity: Sera specimens from participants underwent assessment for Toxoplasma IgG antibodies as per manufacturer's instructions (Bio-Rad, Redmond, WA). Seropositivity was determined in an ELISA through a quotient of a weakly positive single calibrator index value and its absorbance at 450nm, multiplied by the absorbance of the sample, in order to find the sample index. An index value greater than 0.9 was indicative of toxoplasma seropositivity. For all seropositive subjects, a discrete titer was determined using a three point curve of the blank, the weakly positive calibrator, and a strongly positive calibrator, as per manufacturer's instructions.

Psychiatric Assessment: Subjects who were willing to complete the first round of interviews, were asked to return at another date to undergo further assessment for trauma history and symptomatology. Subjects were assessed for PTSD using the Clinician Administered PTSD Scale (CAPS). CAPS was administered by an interviewer, in order to obtain a diagnostic measure of PTSD and assess lifetime and current PTSD. There are 17 diagnostic criteria measured by the CAPS, and each criterion is scored from 0 (absent) to 5 (extremely severe) to account for frequency and intensity of symptoms. Subjects were scored as having PTSD if they met the DSM-IV PTSD criteria from the structured interview. The Structured Clinical Interview for DSM-IV (SCID-DSMIV) was used to assess the presence of comorbid psychiatric disorders, such as major depressive disorder (MDD) in the study population. Other measures used by GTP to assess psychiatric states of subjects were the modified PTSD Symptom Scale (PSS), a 17-item self-report scale which assess PTSD symptomatology over the previous two weeks. PTSD symptom severity was scored from 0 (not at all) to 3 (>5 times a week) to obtain a continuous score of PTSD symptom severity. The Beck Depression Inventory (BDI) was used to discern the depressed mood of the subject. The BDI consists of 21 items which account for symptomatology consistent with depression and are scored in a continuous fashion (Gillespie et al., 2009).

Acoustic Startle Response (ASR): Participants were seated in an audiology booth and requested to look straight ahead while maintaining their eyes open during the session. Acoustic stimuli were sent binaurally through headphones. The startle session began with 60 seconds of 70 dB white noise that persisted throughout the trial. The startle probe was a 108 dB, 40 ms burst of broadband noise with 0 rise time. The noise was delivered binaurally through headphones over the white noise (Braff et al., 1992).

The psychophysiological data was collected using Biopac MP150 for Windows (Biopac Systems, Inc., Aero Camino, CA). Electromyographic (EMG) data was sampled at 1,000 Hz and amplified using the Biopac system. The acquired data was filtered, rectified, and smoothed in MindWare software (MindWare Technologies, Inc., Gahanna, OH). EMG activity was recorded from two 5 mm Ag/AgCl electrodes placed over the *orbicularis oculi* muscle, approximately 1 cm under the pupil and 1 cm below the lateral canthus. The impedances for all participants were less than 6 kilo-ohms. The EMG signal was filtered with low- and high-frequency cutoffs at 28 and 500 Hz, respectively. Startle amplitude was assessed as the peak amplitude of the EMG contraction, and the latency was assessed as the time of the peak amplitude following the acoustic stimulus (Glover et al. 2012).

Statistical Analyses

Variables such as startle amplitude and latency were logarithmically adjusted in order to account for a non-normal distribution. A T-test was conducted to assess the association between TOXO and both acoustic startle amplitude and latency. A series of linear regression models were created to assess the correlation between TOXO and either acoustic startle amplitude or latency, controlling for demographic, psychiatric diagnoses such as PTSD, major depressive disorder (MDD), and Scz status, and substance abuse. Another series of regression models were used to assess the effect of an interaction between TOXO and psychiatric diagnoses on either acoustic startle amplitude or latency. All regression models were assessed via backwards elimination and in a stepwise manner. All statistics and data cleaning were completed using SAS 9.4 (Cary, North Carolina). Figures were created using Microsoft Office (2013, Redmond, Washington).

Results

Subject Characteristics

Demographic characteristics of the participants in the study are described in Table 1, and the study has a TOXO seropositivity of 13.46%. The average age of TOXO positive individuals was 44.02 years (SD=11.89), whereas the average of TOXO negative individuals was 41.32 years (SD=11.46) (T=1.48, p=0.14, Figure 2.). A majority of the participants in the study were women (69.51%) of African American descent (94.23%). Most had a monthly income of less than $1000 per month (68.13%), were unemployed (78.85%), and had either less than a 12^{th} grade education, graduated from high school, or completed GED requirements (86.54%) (Table 1.). Psychiatric and substance abuse characteristics of the study subjects can be seen in Table 2. Approximately half of the participants reported some sort of substance abuse (50.82%), and approximately 29.4% reported using cocaine. The most common psychiatric condition established in the study population was PTSD (57.42%), followed by MDD (42.03%), other non-descript Axis 1 psychoses (16.76%), and Scz (8.24%). Nevertheless, a very slight proportion of the study population did not report either a psychiatric diagnoses, or substance abuse (11.81%) (Table 2.). There was also a significant overlap in both psychiatric conditions and substance abuse (Figures 3.; Figure 4.).

Acoustic Startle Latency and Amplitude

An initial analysis of the distribution of startle latency and amplitude was conducted to find the relative distribution of each outcome variable. The mean latency was 75.32 milliseconds (SD=31.67) to eye-blink, with a corresponding average amplitude of 100.85 μV (SD=140.165) among the whole cohort. The distribution of both latency and amplitude were right skewed, and normalized via Log_{10} transformation prior to analysis (Figure 5.).

Toxoplasma Serointensity

Toxoplasma serointensity was discerned from a direct calculation of absorbance against a three-point curve provided by the manufacturer. The diagnostic criteria for Toxoplama positivity provided by the manufacturer was set at a value of greater than 33 IU/mL, whereas a value greater than 27 IU/mL, but less than 33 IU/mL was indicative of equivocality. A concentration less than 27 IU/mL was an indicator of negative seropositivity. For the purpose of this study both those who were positive and equivocal were grouped together given values significantly higher than the threshold for a negative interpretation. The range of values of serointensity are from 422.66 to 0.493, with the mean serointensity for those considered positive was 113.85 IU/mL (SD= 88.54) as compared to those considered negative, 3.75 IU/mL (SD=4.24) (Figure 6).

T-test Analysis

To test the association of TOXO with both acoustic startle latency and amplitude, a T-test was conducted comparing the latency and amplitude of those who were seropositive to those who were seronegative. Toxoplasma showed no association with acoustic startle latency (T=0.49, p=0.6252), in that those who were seronegative took approximately the same amount of time as seropositive individuals to react to the auditory stimuli (1.85± .1567 vs. 1.84± .1195). Toxoplasma, however, before adjusting for any covariates, did associate with acoustic startle amplitude (T=2.87, p=0.014). The mean acoustic startle amplitude was significantly higher for those with TOXO seropositivity, as compared to those without (1.9257± .5202 vs. 1.7274± .439) (Figure 6.).

Linear Regression and Covariate Analysis

Linear regression analysis looked at the effect of TOXO seropositivity on acoustic startle latency by adjusting for the demographic variables age, race, sex, education, income, and employment status. TOXO seropositivity had no effect on the acoustic startle latency when in a model with demographic factors (F=0.23, p=0.6305) (Table 3.). The next series of analyses stepped in psychiatric and substance abuse variables PTSD, MDD, Scz, substance abuse, Cocaine Use, Psychosis, a diagnosis of at least one

Axis 1 mental disorder, and Mentally-healthy, a subject who was devoid of both a psychological diagnosis and substance abuse. Throughout the course of this individual covariate analysis PTSD was found to have an association with an increase in latency, however TOXO did not (Table 4.). The rest of the psychiatric and substance covariates all displayed $p>0.05$. The same analyses were run using serointensity in lieu of seropositivity. Similar conclusions were made, as TOXO did not have an impact on startle latency, and PTSD alone was significantly associated with an increase in latency (Tables 5. and 7.).

Linear regression analysis also assessed the effect of TOXO seropositivity on acoustic startle amplitude while adjusting for demographic variables. TOXO seropositivity independently contributed to startle amplitude ($F=7.41$, $p=.0068$, Table 7.). When we stepped in psychiatric covariates the significance of TOXO held below a p-value of 0.05 (Tables 8.-14.). Similar analyses were conducted to assess the effect of TOXO serointensity on startle amplitude while adjusting for demographic variables and stepping in psychiatric covariates. When adjusting for demographic variables both race and serointensity were significant contributors to a higher amplitude ($F=4.45$, $p=0.0357$; $F=10.05$, $p=0.0017$; Table 15.). In all subsequent analyses stepping in psychiatric covariates TOXO serointensity remained a significantly associated with an increase in amplitude. Race also remained significantly associated with an increase in startle amplitude except for when Scz and Mentally-healthy were individually stepped into the model (Tables 16.-22.).

Fully Parameterized Models

Fully parameterized models, with a dichotomous TOXO seropositivity variable, were used to assess the impact of each variable on either latency or amplitude when both demographic and psychiatric covariates are within the model. In a fully parameterized model assessing latency, both PTSD and substance abuse significantly contributed to the model. PTSD was associated with an increase in latency whereas substance abuse was associated with a decrease in latency ($F=4.18$, $p= 0.0421$; $F=4.43$,

p=0.0364; Table 23.). A fully parameterized model assessing amplitude found a significant association with both TOXO and Scz. Both TOXO and Scz were associated with an increase in startle amplitude (F= 4.9, p= 0.0279; F= 4.24, p= 0.0406, Table 24.). Fully parameterized models taking serointensity into account became non-significant as compared to seropositivity PTSD on startle latency (F= 2.75, p= 0.099), and enhanced the decrease in latency attributed to substance abuse (F=6.84, p= 0.0096; Table 25.). Fully parameterized models utilizing serointensity to assess the startle amplitude found that race could be attributed to a significant increase in amplitude (F=4.11, p=0.0439). The fully parameterized model using serointensity significantly increased the effect of TOXO (F=9.44, p=0.0024), and the effect of Scz on startle amplitude (F=5.62, p=0.0188; Table 26.).

Discussion

TOXO has been established as a pathogenic agent which can have a significant impact on cognitive function. Throughout the course of this study we assessed the effect of both seropositivity and serointensity on latency and amplitude from the ASR. We initially hypothesized that seropositivity, and furthermore serointensity, would have a significant impact on the ASR. Given previous work conducted by Pearce et al. (2013), in which there was a significant increase in ASR latency among those with TOXO as compared to those who were without, we hypothesized that TOXO seropositivity would have a significant impact on the ASR latency among members of this cohort. While we did not see an effect in latency attributed to TOXO, we did see an increase in latency attributed to PTSD, however it was slightly attenuated, and still significant when implemented into a fully parameterized model. When serointensity was taken into consideration, the increase in latency attributed to PTSD was significantly attenuated in both a discrete and fully parameterized model. Within the fully parameterized model including seropositivity we saw a decrease in latency associated with substance abuse. This value became even more significant when serointensity was taken into consideration. The significance of substance abuse might have been due to the use of stimulants which are known to decrease startle latency (Corcoran et al., 2011). The association between PTSD and longer startle latency in this population contrasts with a study conducted by Vrana et al. (2013), which found a decreased latency. Following this finding, linear regression models were created using demographic variables and covariates which highlighted the impact of TOXO on amplitude.

When assessing ASR amplitude, we saw that TOXO in regression models contributed to a significant increase in amplitude, in every model accounting for both seropositivity and serointensity. To our knowledge no one has reported a significant change in amplitude attributed to TOXO. Other studies

have been relatively inconclusive when it comes to the trend in startle amplitude when it comes to subjects with PTSD, as Butler et al. (1990) saw an increased ASR among PTSD as compared to controls, whereas Jovanovic et al. (2009) saw no effect in ASR amplitude between subjects with and without PTSD. Similar to Jovanovic et al. (2009), we did not see a significant effect of PTSD on startle amplitude, even when TOXO was excluded from the model. ASR amplitude, a measure of habituation in response to repeated stimuli, has been found to be enhanced by the amygdala and potentially modulated by corticotropin releasing hormone, a component of the HPA axis (Poli, Angrilli, 2015). Given the interconnectivity of both the HPA axis and amygdalar control of the fear response systems, and the way in which TOXO affects both, it provides potential evidence for the potential modulation of the fear response consistent with the effect of TOXO in rodents (Weidenfeld et al., 2002; Fabiani et al., 2015; Mitra et al., 2013).

Strengths and Weaknesses

The present study will add to a growing body of literature investigating the effect of TOXO on the acoustic startle response. The findings, to our knowledge, are novel as a differential in amplitude has not been reported when assessing the acoustic startle response. This study benefits from quantitative markers of both neurophysiology, seropositivity, and serointensity. This limits the amount of confounding which could be attributed to our qualitative covariates. Nevertheless, the use of standardized psychologic metrics to discern diagnoses, can be seen as a strength to the study, as it mitigates the amount of biases which could occur. Our population also exhibited a sufficient number of seropositives which facilitated the analysis and adds validity to the finding. While our study population is very distinct, and has been well studied, those strengths also bring about weaknesses in the study.

Weaknesses which limit the impact of the study is the lack of generalizability, as the study consisted of primarily African-Americans living in the inner-city of Atlanta, GA. Another significant limitation in the study is the inability to discern temporality of the TOXO infection, and the possible

implications an acute TOXO infection may have in comparison to a latent TOXO infection. Temporality also becomes an issue with fluctuating psychiatric diseases such as MDD and PTSD, which develop different symptomatology dependent upon the current state of the patient. This limits the ability of the study to truly discern the potential impact on these psychiatric conditions, and whether they could contribute to their incidence. Along with the fluctuation of psychiatric diseases, the significant amount of comorbidity also has the potential to confound the effect of one disease on the ASR. Certain covariates such as All Psychoses, and All Substance abuse, also limit the effect of the study as it conceals potential diagnoses and substances which could significantly impact the ASR leading to an erroneous result. Another limitation of the study is its' cross-sectional design, as it suffers from an inability to elicit the true effect of TOXO on ASR, as subjects were only studied at one time point.

Future Directions

This study could be taken in a multitude of directions in order to more extensively investigate the effect of TOXO on ASR among patients with differential psychiatric diagnoses. One piece of information which could be useful, would be a panel of metabolites measured from each subject upon entrance into the study to effectively discern whether any substances are affecting the neural processing of the subject. Another potential direction could be to more effectively investigate PTSD and other psychiatric symptomatology in subjects. This would enable research to more effectively correlate particular psychiatric symptoms with the ASR, and account for TOXO as a potential modifier of either behavior or the ASR in this population. Furthermore biomolecular analysis of cytokines and other biomarkers in both psychiatric and TOXO research could help mechanistically discern the effect of TOXO on cognition among those with and without psychiatric diagnoses. The presence of TOXO also needs to be explored in similar populations using other cognitive assessment measures, as this would help to further explore the effect of TOXO on cognition. And finally, our results need to be assessed in a more demographically diverse population, and on a larger scale to effectively generalize the study, and begin to parse the etiologic link between TOXO and psychiatric diseases.

References

Achim AM, Maziade M, Raymond E, Olivier D, Mérette C, Roy MA. How prevalent are anxiety disorders in schizophrenia? A meta-analysis and critical review on a significant association. Schizophr Bull. 2011;37(4):811-21.

Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. Brain Res Brain Res Rev. 2000;31(2-3):302-12.

Albucher RC, Liberzon I. Psychopharmacological treatment in PTSD: a critical review. J Psychiatr Res. 2002;36(6):355-67.

Almli LM, Duncan R, Feng H, et al. Correcting systematic inflation in genetic association tests that consider interaction effects: application to a genome-wide association study of posttraumatic stress disorder. JAMA Psychiatry. 2014;71(12):1392-9.

Altamura AC, Boin F, Maes M. HPA axis and cytokines dysregulation in schizophrenia: potential implications for the antipsychotic treatment. Eur Neuropsychopharmacol. 1999;10(1):1-4.

Bailey JN, Goenjian AK, Noble EP, Walling DP, Ritchie T, Goenjian HA. PTSD and dopaminergic genes, DRD2 and DAT, in multigenerational families exposed to the Spitak earthquake. Psychiatry Res. 2010;178(3):507-10.

Bam M, Yang X, Zhou J, et al. Evidence for Epigenetic Regulation of Pro-Inflammatory Cytokines, Interleukin-12 and Interferon Gamma, in Peripheral Blood Mononuclear Cells from PTSD Patients. J Neuroimmune Pharmacol. 2016;11(1):168-81.

Berenreiterová M, Flegr J, Kuběna AA, Němec P. The distribution of Toxoplasma gondii cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis. PLoS ONE. 2011;6(12):e28925.

Bradley AJ, Dinan TG. A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality. J Psychopharmacol (Oxford). 2010;24(4 Suppl):91-118.

Braff DL, Grillon C, Geyer MA. Gating and habituation of the startle reflex in schizophrenic patients. Arch Gen Psychiatry. 1992;49(3):206-15.

Breier A. Serotonin, schizophrenia and antipsychotic drug action. Schizophr Res. 1995;14(3):187-202.

Bremner JD. Traumatic stress: effects on the brain. Dialogues Clin Neurosci. 2006;8(4):445-61.

Bresnahan M, Begg MD, Brown A, et al. Race and risk of schizophrenia in a US birth cohort: another example of health disparity?. Int J Epidemiol. 2007;36(4):751-8.

Broekman BF, Olff M, Boer F. The genetic background to PTSD. Neurosci Biobehav Rev. 2007;31(3):348-62.

Buckley PF, Miller BJ, Lehrer DS, Castle DJ. Psychiatric comorbidities and schizophrenia. Schizophr Bull. 2009;35(2):383-402.

Butler RW, Braff DL, Rausch JL, Jenkins MA, Sprock J, Geyer MA. Physiological evidence of exaggerated startle response in a subgroup of Vietnam veterans with combat-related PTSD. Am J Psychiatry. 1990;147(10):1308-12.

Corcoran S, Norrholm SD, Cuthbert B, Sternberg M, Hollis J, Duncan E. Acoustic startle reduction in cocaine dependence persists for 1 year of abstinence. Psychopharmacology (Berl). 2011;215(1):93-103.

Davis LL, Suris A, Lambert MT, Heimberg C, Petty F. Post-traumatic stress disorder and serotonin: new directions for research and treatment. J Psychiatry Neurosci. 1997;22(5):318-26.

De kloet CS, Vermetten E, Geuze E, Kavelaars A, Heijnen CJ, Westenberg HG. Assessment of HPA-axis function in posttraumatic stress disorder: pharmacological and non-pharmacological challenge tests, a review. J Psychiatr Res. 2006;40(6):550-67.

Drake CH, Smith JE. Letter: Salivary antibody response to oral vaccine. Lancet. 1975; 2(7935):614-5.

Fabiani S, Pinto B, Bonuccelli U, Bruschi F. Neurobiological studies on the relationship between toxoplasmosis and neuropsychiatric diseases. J Neurol Sci. 2015; 351(1-2):3-8.

Federal Poverty Guidelines. Georgia Department of Community Health. Available at: https://dch.georgia.gov/federal-poverty-guidelines-0. Accessed April 19, 2016.

Flandreau E, Risbrough V, Lu A, et al. Cell type-specific modifications of corticotropin-releasing factor (CRF) and its type 1 receptor (CRF1) on startle behavior and sensorimotor gating. Psychoneuroendocrinology. 2015;53:16-28.

Gaskell EA, Smith JE, Pinney JW, Westhead DR, Mcconkey GA. A unique dual activity amino acid hydroxylase in Toxoplasma gondii. PLoS ONE. 2009;4(3):e4801.

Gillespie CF, Bradley B, Mercer K, et al. Trauma exposure and stress-related disorders in inner city primary care patients. Gen Hosp Psychiatry. 2009;31(6):505-14.

Glover EM, Phifer JE, Crain DF, et al. Tools for translational neuroscience: PTSD is associated with heightened fear responses using acoustic startle but not skin conductance measures. Depress Anxiety. 2011;28(12):1058-66.

Halonen SK, Weiss LM. Toxoplasmosis. Handb Clin Neurol. 2013;114:125-45.

Hamner MB, Gold PB. Plasma dopamine beta-hydroxylase activity in psychotic and non-psychotic post-traumatic stress disorder. Psychiatry Res. 1998;77(3):175-81.

Henriquez SA, Brett R, Alexander J, Pratt J, Roberts CW. Neuropsychiatric disease and Toxoplasma gondii infection. Neuroimmunomodulation. 2009;16(2):122-33.

Hull AM. Neuroimaging findings in post-traumatic stress disorder. Systematic review. Br J Psychiatry. 2002;181:102-10.

Jones JL, Parise ME, Fiore AE. Neglected parasitic infections in the United States: toxoplasmosis. Am J Trop Med Hyg. 2014;90(5):794-9.

Jovanovic T, Norrholm SD, Sakoman AJ, Esterajher S, Kozarić-kovacić D. Altered resting psychophysiology and startle response in Croatian combat veterans with PTSD. Int J Psychophysiol. 2009;71(3):264-8.

Kaminsky Z, Wilcox HC, Eaton WW, et al. Epigenetic and genetic variation at SKA2 predict suicidal behavior and post-traumatic stress disorder. Transl Psychiatry. 2015;5:e627.

Kamkwalala A, Norrholm SD, Poole JM, et al. Dark-enhanced startle responses and heart rate variability in a traumatized civilian sample: putative sex-specific correlates of posttraumatic stress disorder. Psychosom Med. 2012;74(2):153-9.

Karl A, Werner A. The use of proton magnetic resonance spectroscopy in PTSD research--meta-analyses of findings and methodological review. Neurosci Biobehav Rev. 2010;34(1):7-22.

Kessler, R.C., Berglund, P., Delmer, O., Jin, R., Merikangas, K.R., & Walters, E.E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry, 62(6): 593-602.

Kessler, R.C., Chiu, W.T., Demler, O., Merikangas, K.R., & Walters, E.E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry, 62(6): 617-627.

Koch M, Schnitzler HU. The acoustic startle response in rats--circuits mediating evocation, inhibition and potentiation. Behav Brain Res. 1997;89(1-2):35-49.

Koch M. The neurobiology of startle. Prog Neurobiol. 1999;59(2):107-28.

Maes M, Lin AH, Verkerk R, et al. Serotonergic and noradrenergic markers of post-traumatic stress disorder with and without major depression. Neuropsychopharmacology. 1999;20(2):188-97.

Mahmoud ME, Ihara F, Fereig RM, Nishimura M, Nishikawa Y. Induction of depression-related behaviors by reactivation of chronic Toxoplasma gondii infection in mice. Behav Brain Res. 2016;298(Pt B):125-33.

Mayo Clinic. Schizophrenia. 2014. Retrieved from http://www.mayoclinic.org/diseases-conditions/schizophrenia/basics/definition/con-20021077

Medina MA, Urdiales JL, Rodríguez-caso C, Ramírez FJ, Sánchez-jiménez F. Biogenic amines and polyamines: similar biochemistry for different physiological missions and biomedical applications. Crit Rev Biochem Mol Biol. 2003;38(1):23-59.

Mendy A, Vieira ER, Albatineh AN, Gasana J. Toxoplasma gondii seropositivity and cognitive functions in school-aged children. Parasitology. 2015;142(9):1221-7.

Messias EL, Chen CY, Eaton WW. Epidemiology of schizophrenia: review of findings and myths. Psychiatr Clin North Am. 2007;30(3):323-38.

Mitra R, Sapolsky RM, Vyas A. Toxoplasma gondii infection induces dendritic retraction in basolateral amygdala accompanied by reduced corticosterone secretion. Dis Model Mech. 2013;6(2):516-20.

Morris MC, Rao U. Psychobiology of PTSD in the acute aftermath of trauma: Integrating research on coping, HPA function and sympathetic nervous system activity. Asian J Psychiatr. 2013;6(1):3-21.

Mueser KT, Mcgurk SR. Schizophrenia. Lancet. 2004;363(9426):2063-72.

Müller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. Front Neurosci. 2015;9:372.

National Comorbidity Survey. (2005). NCS-R appendix tables: Table 1. Lifetime prevalence of DSM-IV/WMH-CIDI disorders by sex and cohort. Table 2. Twelve-month prevalence of DSM-IV/WMH-CIDI disorders by sex and cohort. Accessed at:http://www.hcp.med.harvard.edu/ncs/publications.php

Nylocks KM, Michopoulos V, Rothbaum AO, et al. An angiotensin-converting enzyme (ACE) polymorphism may mitigate the effects of angiotensin-pathway medications on posttraumatic stress symptoms. Am J Med Genet B Neuropsychiatr Genet. 2015;168B(4):307-15.

Parlog A, Schlüter D, Dunay IR. Toxoplasma gondii-induced neuronal alterations. Parasite Immunol. 2015;37(3):159-70.

Passos IC, Vasconcelos-moreno MP, Costa LG, et al. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. Lancet Psychiatry. 2015;2(11):1002-12.

Passos IC, Vasconcelos-moreno MP, Costa LG, et al. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. Lancet Psychiatry. 2015;2(11):1002-12.

Pilz PK, Schnitzler HU. Habituation and sensitization of the acoustic startle response in rats: amplitude, threshold, and latency measures. Neurobiol Learn Mem. 1996;66(1):67-79.

Post-Traumatic Stress Disorder. NIMH RSS. Available at: http://www.nimh.nih.gov/health/topics/post-traumatic-stress-disorder-ptsd/index.shtml. Accessed 2015.

Prandovszky E, Gaskell E, Martin H, Dubey JP, Webster JP, Mcconkey GA. The neurotropic parasite Toxoplasma gondii increases dopamine metabolism. PLoS ONE. 2011;6(9):e23866.

PTSD: National Center for PTSD. Epidemiology of PTSD . Available at: http://www.ptsd.va.gov/professional/ptsd-overview/epidemiological-facts-ptsd.asp. Accessed 2015.

Ressler, Kerry J., Bekh Bradley, and Tanja Jovanovic. "Grady Trauma Project." Grady Trauma Project. N.p., n.d. Web. 17 Mar. 2016. <http://gradytraumaproject.com/project/project-overview/>.

Schizophrenia (NIMH RSS). 2015
http://www.nimh.nih.gov/health/publications/schizophrenia/index.shtml

Stibbs HH. Changes in brain concentrations of catecholamines and indoleamines in Toxoplasma gondii infected mice. Ann Trop Med Parasitol. 1985;79(2):153-7.

Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. Int J Parasitol. 2000;30(12-13):1217-58.

Torrey EF, Yolken RH. Toxoplasma gondii and schizophrenia. Emerging Infect Dis. 2003;9(11):1375-80.

Torrey EF, Yolken RH. Toxoplasma gondii and schizophrenia. Emerging Infect Dis. 2003;9(11):1375-80.

Upthegrove R, Manzanares-teson N, Barnes NM. Cytokine function in medication-naive first episode psychosis: a systematic review and meta-analysis. Schizophr Res. 2014;155(1-3):101-8.

Vaswani M, Linda FK, Ramesh S. Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27(1):85-102.

Vrana SR, Calhoun PS, Mcclernon FJ, Dennis MF, Lee ST, Beckham JC. Effects of smoking on the acoustic startle response and prepulse inhibition in smokers with and without posttraumatic stress disorder. Psychopharmacology (Berl). 2013;230(3):477-85.

Watanabe Y, Someya T, Nawa H. Cytokine hypothesis of schizophrenia pathogenesis: evidence from human studies and animal models. Psychiatry Clin Neurosci. 2010;64(3):217-30.

Weidenfeld J, Newman ME, Itzik A, Gur E, Feldman S. The amygdala regulates the pituitary-adrenocortical response and release of hypothalamic serotonin following electrical stimulation of the dorsal raphe nucleus in the rat. Neuroendocrinology. 2002;76(2):63-9.

Xiao J, Li Y, Jones-brando L, Yolken RH. Abnormalities of neurotransmitter and neuropeptide systems in human neuroepithelioma cells infected by three Toxoplasma strains. J Neural Transm (Vienna). 2013;120(12):1631-9.

Tables and Figures

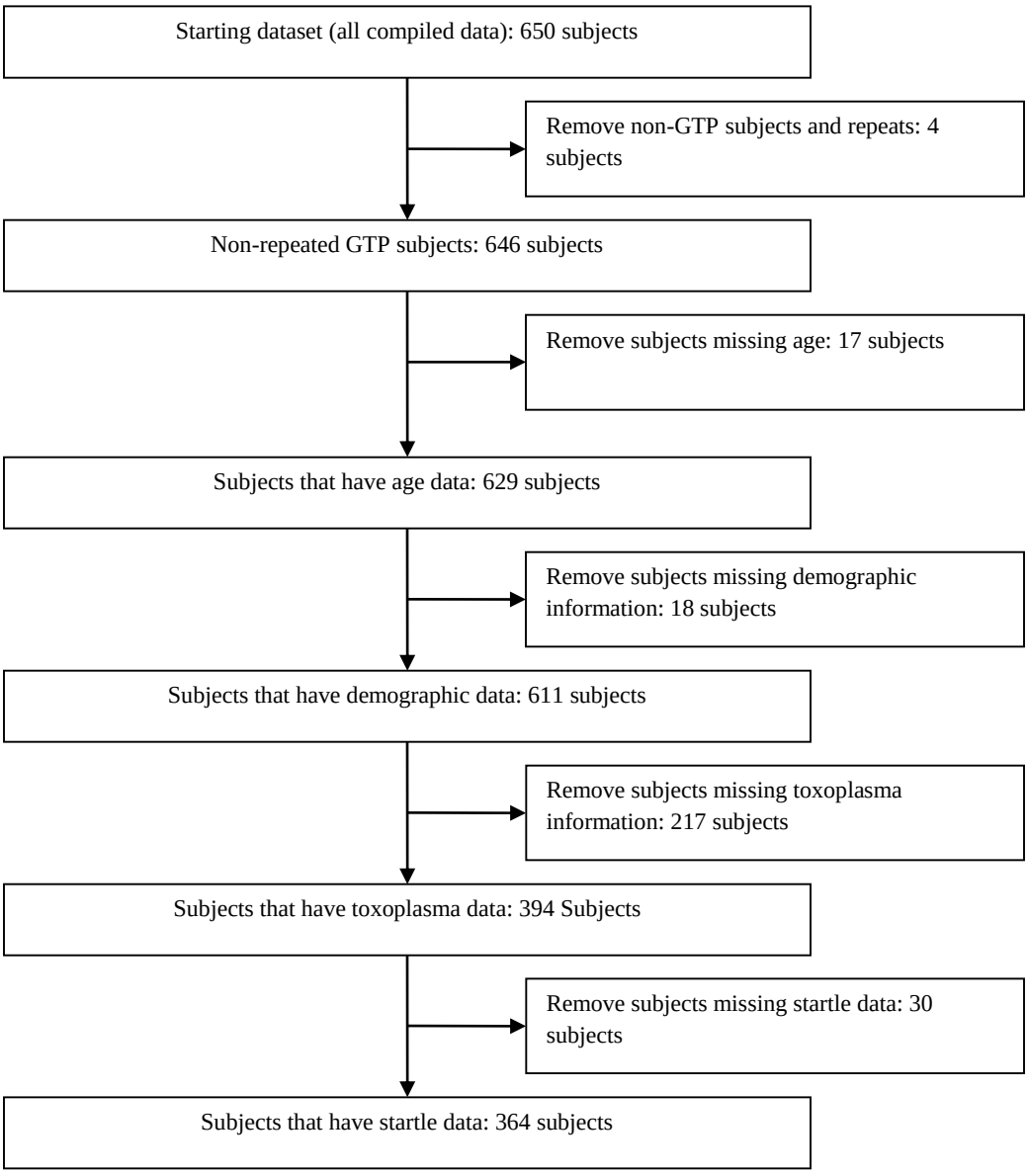

Figure 1. A flow chart depicting the process in which subjects were selected into our final cohort.

Table 1. Demographic Characteristics of a Cohort of 364 Patients from the Grady Trauma Project.

	Toxoplasma Positive[1.]		Toxoplasma Negative			
	No.	%	No.	%	X^2	p
Seropositivity	49	13.46	315	86.54		
Gender						
Male	11	9.91	100	90.09	1.73	0.19
Female	38	15.02	215	84.98		
Race						
African American	44	12.83	299	87.17	2.05	0.18
Other	5	23.81	16	76.19		
Income (monthly)						
< $1000	39	15.73	209	84.27	3.43	0.064
$1000+	10	8.62	106	91.38		
Education						
<12th Grade, or GED/ High School Graduate	43	13.65	272	86.35	0.07	0.79
Some or Completed: College, Technical, or Graduate School	6	12.24	43	87.76		
Employment						
Yes	6	7.79	71	92.21	2.69	0.1
No	43	14.98	244	85.02		
	Mean	SD	Mean	SD	T	p
Age	44.0204	11.8857	41.3238	11.459	1.48	0.14

[1.] Includes subjects whose concentration of Toxoplasma was considered equivocal.

Table 2. Psychiatric Diagnoses and Assessment Scores from a Cohort from the Grady Trauma Project

		Toxoplasma Positive[1.]		Toxoplasma Negative			
		No.	%	No.	%	X^2	p
Substance Abuse[2.]							
	Yes	26	14.05	159	85.95	0.11	0.74
	No	23	12.85	156	87.15		
Cocaine Use							
	Yes	18	16.82	89	83.18	0.16	0.69
	No	22	14.97	125	85.03		
	Missing	9	8.18	101	91.82		
Psychosis							
	Yes	6	9.84	55	90.16	0.86	0.35
	No	43	14.29	258	85.71		
	Missing	0	0	2	100		
Schizophrenia							
	Yes	4	13.33	26	86.67	0.005	1
	No	45	13.8	281	86.2		
	Missing	0	0	8	100		
PTSD[3]							
	Yes	25	11.96	184	88.04	1.10	0.29
	No	24	15.79	128	84.21		
	Missing	0	0	3	100		
MDD[3]							
	Yes	13	8.5	140	91.5	5.47	0.02
	No	28	17.39	133	82.61		
	Missing	8	16	42	84		
Mentally-healthy Subjects[4]							
	Yes	10	23.26	33	76.74	1.9253	0.16
	No	27	14.59	158	85.41		
	Missing	12	8.82	124	91.18		

[1.] Includes subjects whose concentration of Toxoplasma was considered equivocal.

[2.] The use of any substance considered a drug including but not limited to alcohol and marijuana.

[3.] Includes any subject that has demonstrated the psychiatric disorder at one time or another.

[4.] Includes any subject without a psychiatric disorder and does not abuse substances, if they were missing one of the indicators they were considered missing.

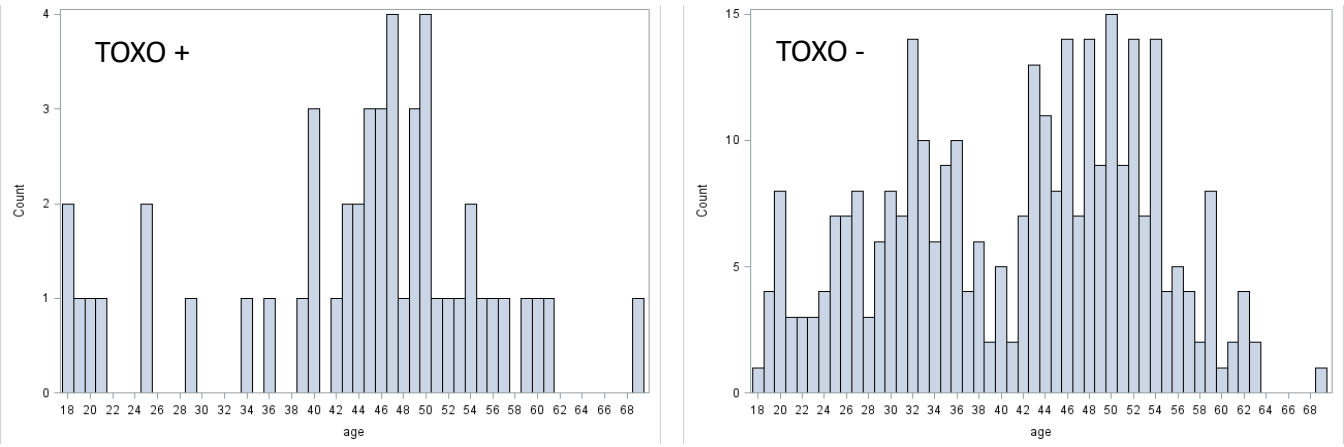

Figure 2. Histograms of the age distribution of both Toxoplasma positive and negative individuals from the GTP.

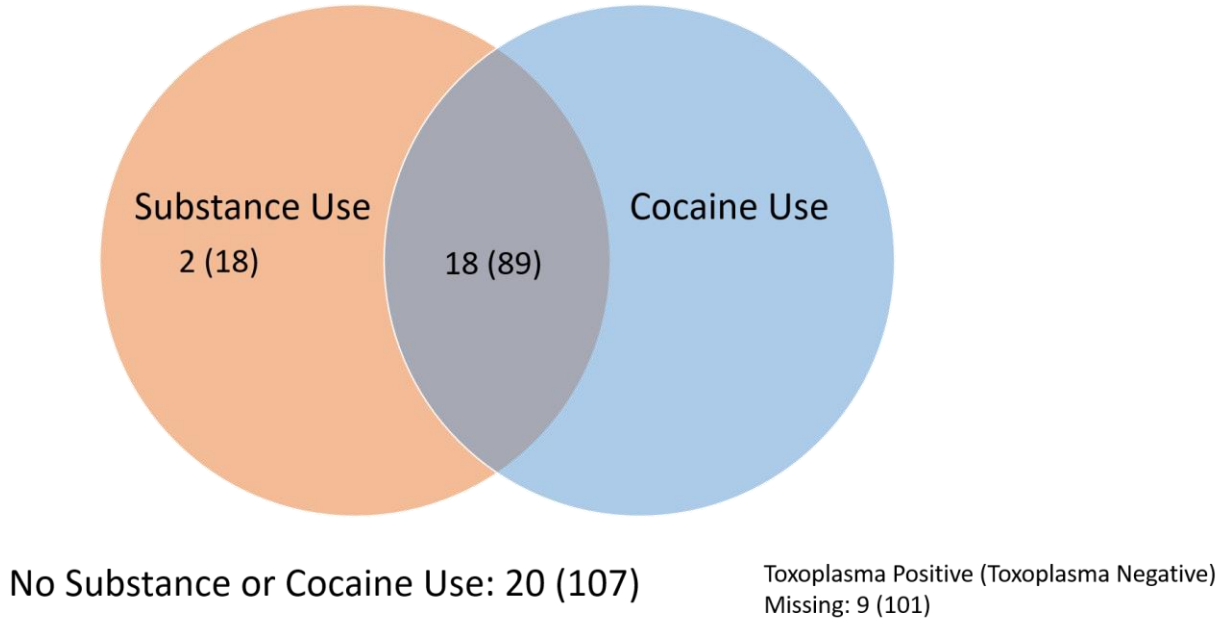

Figure 3. A Venn diagram depicting the comorbidity found between the variables Substance Abuse and Cocaine Use.

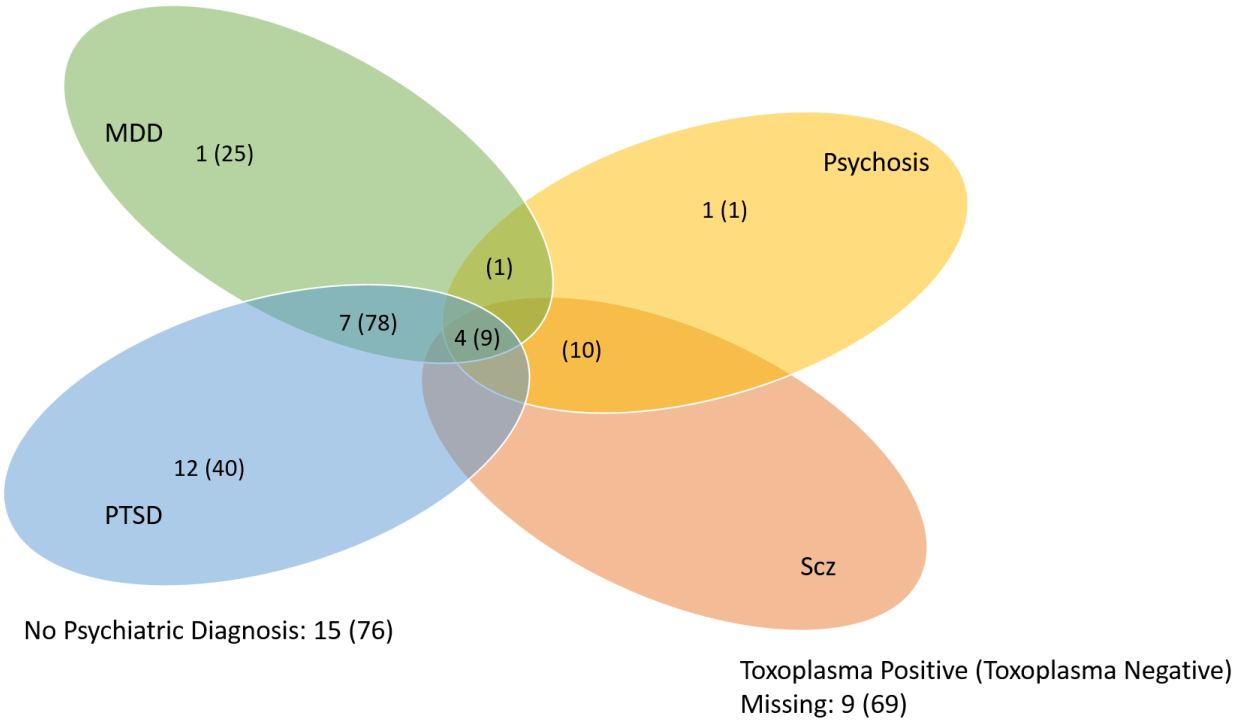

Figure 4. A Venn diagram depicting the psychiatric comorbidities found in our sample population.

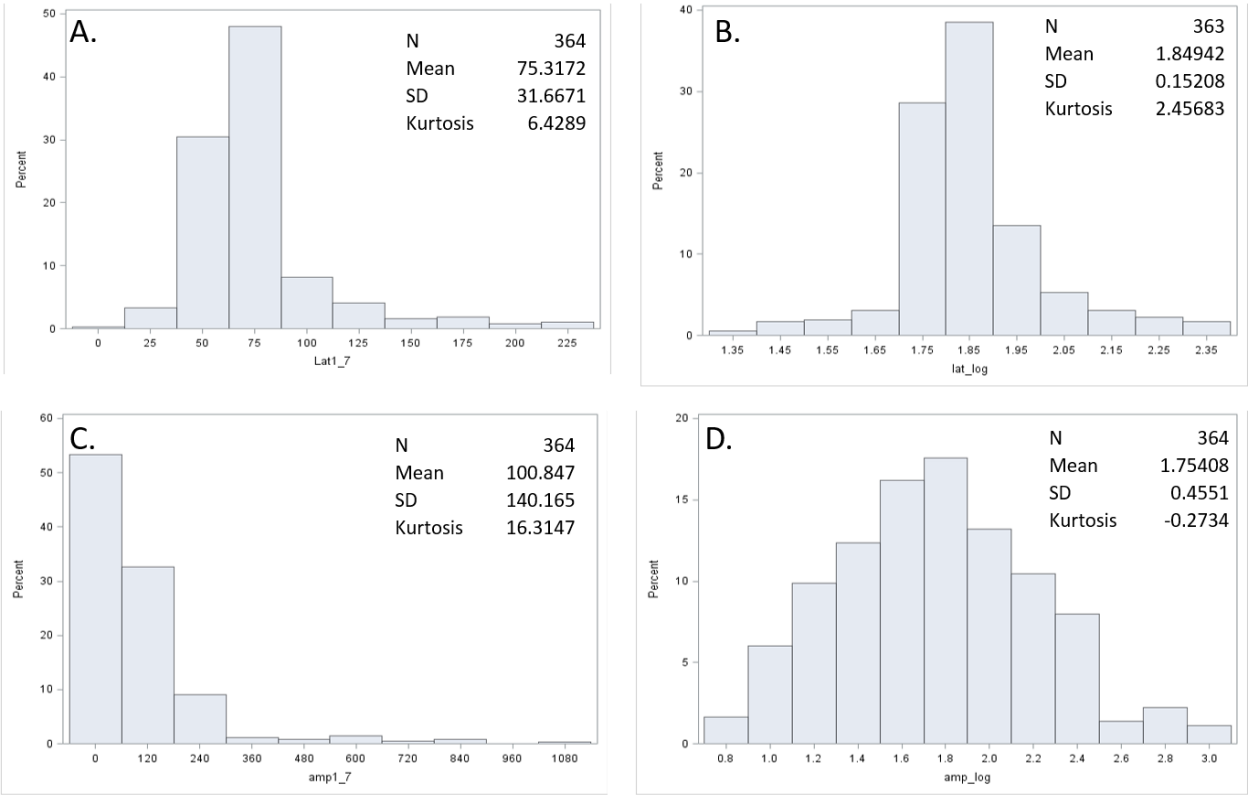

Figure 5. Distribution and basic statistics of startle latency (3A) and startle amplitude (3C), along with their respective logarithmic transformations (3B and 3D).

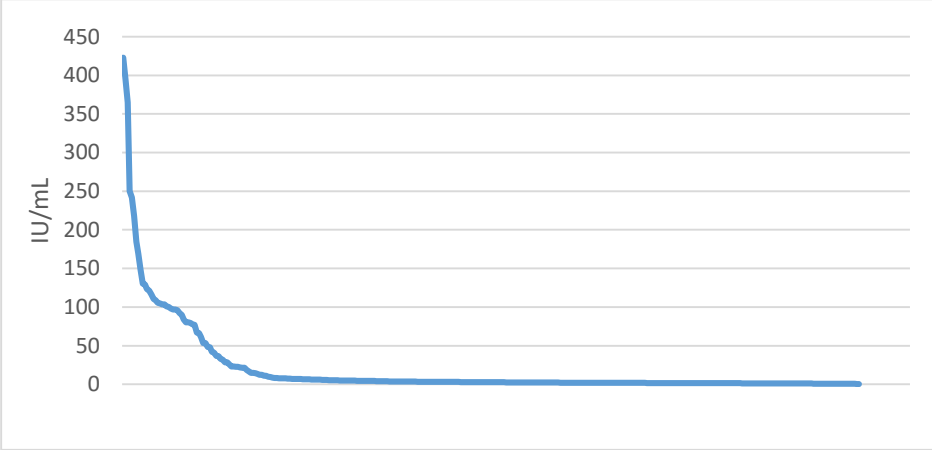

Figure 6. A depiction of the serointensity of TOXO IgG antibodies from the Grady Trauma Project.

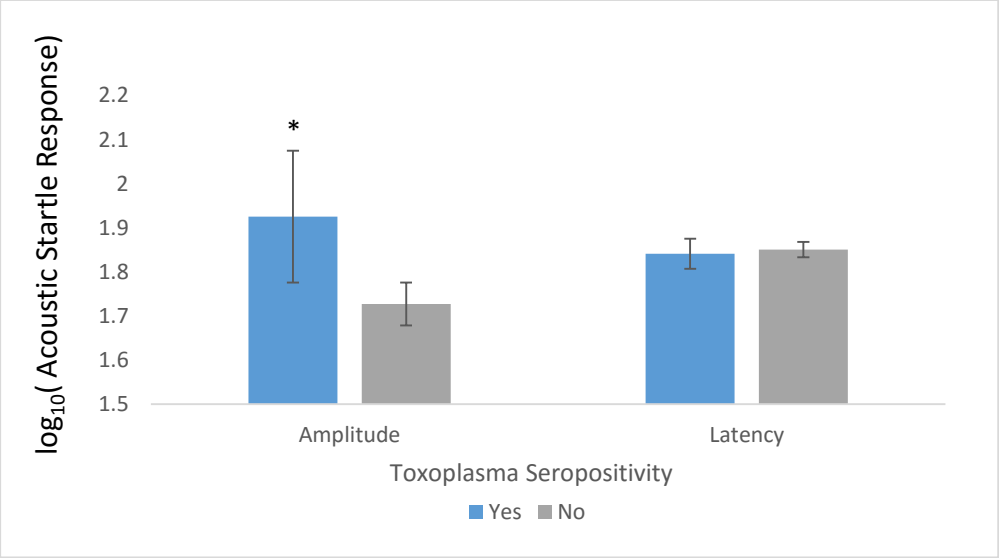

Figure 7. Two T-tests assessing the effect of Toxoplasma seropositivity on both Amplitude (T=2.53, p=0.014) and Latency (T=0.49, p=0.6252). * denotes significance at a level of p< 0.05.

Table 3. The effect of TOXO on Log_{10}(Latency) adjusting for Demographic Variables.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Latency)	Age	363	0.00073	0.00073	1.01	0.3165
	Race (1=African American, 0=Other)		-0.0314	0.0348	0.81	0.3678
	Sex (1=Female, 0=Male)		0.02222	0.01797	1.53	0.2173
	Education (1 >High School, 0 ≤High School)		-0.0151	0.02477	0.37	0.5438
	Income (1> FPL, 0≤ FPL)		0.02392	0.0186	1.65	0.1993
	Employment (1=Yes, 0= No)		-0.0235	0.02085	1.27	0.2597
	Toxoplasma (1= Positive, 0= Negative)		-0.0115	0.0238	0.23	0.6305

Table 4. The effect of TOXO on Log_{10}(Latency) adjusting for Demographic Variables stratifying by PTSD.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Latency)	Age	360	0.00073	0.00072	1.03	0.3116
	Race		-0.0396	0.03449	1.32	0.2517
	Sex		0.01998	0.01789	1.25	0.2647
	Education		-0.0128	0.02448	0.27	0.601
	Income		0.02306	0.01847	1.56	0.2128
	Employment		-0.0195	0.02065	0.9	0.3447
	Toxoplasma		-0.008	0.02359	0.11	0.7355
	PTSD (1= PTSD, 0= No PTSD)		0.03714	0.01636	5.15	0.0238

Table 5. The effect of TOXO IgG serointensity on Log_{10} (Latency) adjusting for Demographic Variables.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Latency)	Age	333	0.000713	0.000755	0.89	0.3455
	Race (1=African American, 0=Other)		-0.04179	0.03564	1.38	0.2418
	Sex (1=Female, 0=Male)		0.02067	0.01883	1.2	0.2732
	Education (1 >High School, 0 ≤High School)		-0.01305	0.02539	0.26	0.6075
	Income (1> FPL, 0≤ FPL)		0.0247	0.01943	1.62	0.2046
	Employment (1=Yes, 0= No)		-0.0161	0.02175	0.55	0.4597
	Serointensity (IU/mL)		-4.1E-05	0.000163	0.06	0.8014

Table 6. The effect of TOXO IgG serointensity on Log_{10} (Latency) adjusting for Demographic Variables stratifying by PTSD.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Latency)	Age	329	0.000743	0.00075	0.98	0.3227
	Race		-0.04816	0.03535	1.86	0.174
	Sex		0.01932	0.01876	1.06	0.3039
	Education		-0.01095	0.02514	0.19	0.6636
	Income		0.02232	0.01932	1.34	0.2487
	Employment		-0.0129	0.02156	0.36	0.55
	Serointensity		-3.4E-05	0.000162	0.05	0.8319
	PTSD (1= PTSD, 0= No PTSD)		0.03175	0.01695	3.51	0.062

Table 7. The effect of TOXO on Log_{10} (Amplitude) adjusting for Demographic Variables.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	364	0.00078	0.00215	0.13	0.7164
	Race (1=African American, 0=Other)		0.17583	0.10292	2.92	0.0884
	Sex (1=Female, 0=Male)		-0.0053	0.05309	0.01	0.9209
	Education (1 >High School, 0 ≤High School)		-0.065	0.07319	0.79	0.3753
	Income (1> FPL, 0≤ FPL)		0.07823	0.05477	2.04	0.154
	Employment (1=Yes, 0= No)		-0.0544	0.06158	0.78	0.3772
	Toxoplasma (1= Positive, 0= Negative)		0.19159	0.07038	7.41	0.0068

Table 8. The effect of TOXO on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by PTSD.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	361	8.7E-05	0.00215	0	0.9677
	Race		0.17713	0.10257	2.98	0.0851
	Sex		-0.0044	0.0531	0.01	0.9338
	Education		-0.0561	0.0727	0.6	0.4409
	Income		0.08452	0.05469	2.39	0.1231
	Employment		-0.0492	0.0613	0.65	0.4224
	Toxoplasma		0.20301	0.07012	8.38	0.004
	PTSD (1= PTSD, 0= No PTSD)		0.02104	0.04855	0.19	0.6651

Table 9. The effect of TOXO on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by MDD.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	314	0.00133	0.00239	0.31	0.5791
	Race		0.22026	0.11127	3.92	0.0487
	Sex		0.0376	0.05777	0.42	0.5156
	Education		-0.0403	0.07863	0.26	0.6087
	Income		0.07898	0.05979	1.74	0.1875
	Employment		-0.0595	0.06796	0.77	0.3819
	Toxoplasma		0.21599	0.07715	7.84	0.0054
	MDD (1=MDD, 0= No MDD)		-0.0531	0.05177	1.05	0.3057

Table 10. The effect of TOXO on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by Schizophrenia.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	356	0.00054	0.0022	0.06	0.8047
	Race		0.1669	0.10882	2.35	0.126
	Sex		-0.0196	0.0544	0.13	0.7193
	Education		-0.0511	0.07445	0.47	0.4933
	Income		0.07376	0.05598	1.74	0.1885
	Employment		-0.0502	0.06217	0.65	0.4204
	Toxoplasma		0.19266	0.07075	7.42	0.0068
	Schizophrenia (1= Yes, 0= No)		0.12123	0.08677	1.95	0.1632

Table 11. The effect of TOXO on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by Substance Abuse.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	364	0.00091	0.00219	0.17	0.6767
	Race		0.17803	0.10325	2.97	0.0855
	Sex		-0.0093	0.05444	0.03	0.864
	Education		-0.0684	0.07394	0.86	0.3556
	Income		0.07734	0.05489	1.99	0.1597
	Employment		-0.0549	0.06167	0.79	0.3737
	Toxoplasma		0.19173	0.07047	7.4	0.0068
	Substance Abuse (1= Yes, 0= No)		-0.0174	0.05035	0.12	0.7296

Table 12. The effect of TOXO on Log_{10}(Amplitude) adjusting for Demographic Variables stratifying by Cocaine Use.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	254	0.00031	0.00266	0.01	0.9065
	Race		0.19303	0.11744	2.7	0.1015
	Sex		0.04971	0.06328	0.62	0.4328
	Education		0.00972	0.09639	0.01	0.9198
	Income		0.11745	0.06611	3.16	0.0769
	Employment		-0.1015	0.07444	1.86	0.1742
	Toxoplasma		0.19351	0.07905	5.99	0.0151
	Cocaine Use (1= Yes, 0= No)		0.0514	0.06255	0.68	0.412

Table 13. The effect of TOXO on Log_{10}(Amplitude) adjusting for Demographic Variables stratifying by Psychosis.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	362	0.00052	0.00218	0.06	0.8105
	Race		0.18224	0.10275	3.15	0.077
	Sex		-0.0106	0.05322	0.04	0.842
	Education		-0.0551	0.07318	0.57	0.4521
	Income		0.07008	0.05487	1.63	0.2024
	Employment		-0.0455	0.06168	0.54	0.4615
	Toxoplasma		0.1977	0.07031	7.91	0.0052
	Psychosis (1= Yes, 0= No)		0.09993	0.06374	2.46	0.1178

Table 14. The effect of TOXO on Log_{10}(Amplitude) adjusting for Demographic Variables stratifying by Mentally-healthy Subjects.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	228	0.00124	0.00286	0.19	0.6655
	Race		0.21117	0.14053	2.26	0.1344
	Sex		0.03868	0.06863	0.32	0.5736
	Education		0.02443	0.1039	0.06	0.8143
	Income		0.13002	0.07392	3.09	0.08
	Employment		-0.1016	0.08267	1.51	0.2203
	Toxoplasma		0.21024	0.08506	6.11	0.0142
	Mentally-healthy (1= No Psychiatric or Substance Use, 0= Psychiatric Condition or Substance Abuse)		-0.0029	0.08092	0	0.9715

Table 15. The effect of TOXO IgG serointensity on Log_{10} (Amplitude) adjusting for Demographic Variables.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	334	0.000736	0.0022	0.11	0.738
	Race (1=African American, 0=Other)		0.21921	0.10396	4.45	0.0357
	Sex (1=Female, 0=Male)		0.00797	0.05488	0.02	0.8846
	Education (1 >High School, 0 ≤High School)		-0.04609	0.07399	0.39	0.5337
	Income (1> FPL, 0≤ FPL)		0.08271	0.0564	2.15	0.1434
	Employment (1=Yes, 0= No)		-0.02794	0.06336	0.19	0.6595
	Serointensity (IU/mL)		0.00151	0.000477	10.05	0.0017

Table 16. The effect of TOXO IgG serointensity on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by PTSD.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	330	4.79E-05	0.00219	0	0.9826
	Race		0.22361	0.10342	4.67	0.0313
	Sex		0.01142	0.05482	0.04	0.8351
	Education		-0.03691	0.07346	0.25	0.6156
	Income		0.08745	0.05623	2.42	0.1209
	Employment		-0.02409	0.06299	0.15	0.7024
	Serointensity		0.00154	0.000473	10.57	0.0013
	PTSD (1= PTSD, 0= No PTSD)		0.01426	0.04948	0.08	0.7734

Table 17. The effect of TOXO IgG serointensity on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by MDD.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	285	0.00153	0.00242	0.4	0.5266
	Race		0.27621	0.11206	6.08	0.0143
	Sex		0.06829	0.05954	1.32	0.2523
	Education		-0.01465	0.07924	0.03	0.8535
	Income		0.08439	0.06143	1.89	0.1706
	Employment		-0.03155	0.06962	0.21	0.6508
	Serointensity		0.00179	0.000509	12.43	0.0005
	MDD (1=MDD, 0= No MDD)		-0.03742	0.05268	0.5	0.4781

Table 18. The effect of TOXO IgG serointensity on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by Schizophrenia.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	326	0.000782	0.00223	0.12	0.7259
	Race		0.21585	0.11008	3.84	0.0508
	Sex		0.00111	0.05607	0	0.9842
	Education		-0.03237	0.07524	0.19	0.6673
	Income		0.08082	0.05773	1.96	0.1625
	Employment		-0.02159	0.06397	0.11	0.7359
	Serointensity		0.00154	0.000479	10.37	0.0014
	Schizophrenia (1= Yes, 0= No)		0.14137	0.08775	2.6	0.1081

Table 19. The effect of TOXO IgG serointensity on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by Substance Abuse.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	333	0.00088	0.00223	0.16	0.6929
	Race		0.22252	0.1044	4.54	0.0338
	Sex		0.00331	0.05608	0	0.953
	Education		-0.04944	0.07452	0.44	0.5075
	Income		0.0812	0.05659	2.06	0.1523
	Employment		-0.02878	0.06347	0.21	0.6506
	Serointensity		0.00151	0.000477	10.01	0.0017
	Substance Abuse (1= Yes, 0= No)		-0.0213	0.05126	0.17	0.678

Table 20. The effect of TOXO IgG serointensity on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by Cocaine Use.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	230	0.000759	0.00263	0.08	0.7735
	Race		0.23128	0.11479	4.06	0.0451
	Sex		0.08057	0.06453	1.56	0.2131
	Education		0.03807	0.09637	0.16	0.6932
	Income		0.12306	0.06785	3.29	0.071
	Employment		-0.05753	0.07591	0.57	0.4493
	Serointensity		0.0016	0.000506	10.04	0.0017
	Cocaine Use (1= Yes, 0= No)		0.06598	0.06383	1.07	0.3024

Table 21. The effect of TOXO IgG serointensity on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by Psychosis.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	332	0.000717	0.00221	0.11	0.7454
	Race		0.22903	0.10371	4.88	0.0279
	Sex		0.01089	0.05473	0.04	0.8424
	Education		-0.03463	0.07395	0.22	0.6399
	Income		0.07422	0.05645	1.73	0.1895
	Employment		-0.01617	0.0634	0.07	0.7989
	Serointensity		0.00158	0.000477	11.06	0.001
	Psychosis (1= Yes, 0= No)		0.1154	0.06419	3.23	0.0731

Table 22. The effect of TOXO IgG serointensity on Log_{10} (Amplitude) adjusting for Demographic Variables stratifying by Mentally-healthy Subjects.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	204	0.00183	0.00285	0.41	0.5223
	Race		0.26012	0.13736	3.59	0.0597
	Sex		0.06942	0.07028	0.98	0.3244
	Education		0.05677	0.1043	0.3	0.5869
	Income		0.12961	0.07589	2.92	0.0892
	Employment		-0.05733	0.08423	0.46	0.4969
	Serointensity		0.00167	0.000528	10	0.0018
	Mentally-healthy (1= No Psychiatric or Substance Abuse, 0= Psychiatric Condition or Substance Abuse)		-0.01179	0.07997	0.02	0.883

Table 23. The effect of TOXO on Log_{10} (Latency) in a fully parameterized model.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Latency)	Age	228	0.00152	0.000913	2.77	0.0976
	Race		-0.03557	0.04425	0.65	0.4224
	Sex		-0.00779	0.02263	0.12	0.731
	Education		-0.01645	0.03251	0.26	0.6133
	Income		0.00938	0.02315	0.16	0.6858
	Employment		-0.00454	0.02594	0.03	0.8611
	Toxoplasma		0.000773	0.02672	0	0.977
	PTSD		0.04514	0.02207	4.18	0.0421
	Depression		-0.01841	0.02128	0.75	0.3879
	Schizophrenia		0.01945	0.04398	0.2	0.6587
	Substance Abuse		-0.08089	0.03843	4.43	0.0364
	Cocaine Use		0.03252	0.03861	0.71	0.4006
	Psychosis		0.00612	0.03045	0.04	0.84

Table 24. The effect of TOXO on Log_{10} (Amplitude) in a fully parameterized model.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	228	0.00072	0.00291	0.06	0.8039
	Race		0.21039	0.14131	2.22	0.138
	Sex		0.06076	0.07226	0.71	0.4014
	Education		0.03904	0.10383	0.14	0.7073
	Income		0.13746	0.07392	3.46	0.0643
	Employment		-0.09279	0.08284	1.25	0.2639
	Toxoplasma		0.18888	0.08534	4.9	0.0279
	PTSD		0.03409	0.07048	0.23	0.6291
	MDD		-0.06357	0.06794	0.88	0.3505
	Schizophrenia		0.28926	0.14043	4.24	0.0406
	Substance Abuse		-0.04346	0.12271	0.13	0.7236
	Cocaine Use		0.09614	0.1233	0.61	0.4364
	Psychosis		0.00525	0.09724	0	0.957

Table 25. The effect of TOXO serointensity on Log_{10} (Latency) in a fully parameterized model.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Latency)	Age	213	0.00134	0.00093	2.08	0.1509
	Race		-0.0304	0.04424	0.47	0.4933
	Sex		-0.0118	0.02367	0.25	0.6175
	Education		-0.0025	0.03321	0.01	0.9391
	Income		5.8E-05	0.02434	0	0.9981
	Employment		0.00803	0.02698	0.09	0.7664
	Serointensity		-8E-06	0.00017	0	0.9612
	PTSD		0.03828	0.0231	2.75	0.099
	Depression		-0.0174	0.02221	0.61	0.4352
	Schizophrenia		0.02108	0.04391	0.23	0.6317
	Substance Abuse		-0.106	0.04055	6.84	0.0096
	Cocaine Use		0.05551	0.04122	1.81	0.1796
	Psychosis		0.01183	0.03081	0.15	0.7

Table 26. The effect of TOXO serointensity on Log_{10} (Amplitude) in a fully parameterized model.

Dependent	Independent	N	β	SE	F	P value
Log_{10}(Amplitude)	Age	213	0.00095	0.00288	0.11	0.7412
	Race		0.27896	0.13757	4.11	0.0439
	Sex		0.09065	0.07359	1.52	0.2194
	Education		0.07254	0.10326	0.49	0.4832
	Income		0.13686	0.07568	3.27	0.072
	Employment		-0.0396	0.08389	0.22	0.6377
	Serointensity		0.00163	0.00053	9.44	0.0024
	PTSD		0.00588	0.07182	0.01	0.9348
	Depression		-0.0248	0.06907	0.13	0.7197
	Schizophrenia		0.32355	0.13653	5.62	0.0188
	Substance Abuse		-0.1377	0.12608	1.19	0.2762
	Cocaine Use		0.18949	0.12817	2.19	0.1409
	Psychosis		0.02659	0.09581	0.08	0.7817