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Effects of Toxoplasma Serointensity on Cognitive Function Amongst Schizophrenic Patients

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B.S. The College of New Jersey, 2010

Thesis Committee Chair: Brad Pearce, PhD

An abstract of a thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

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Abstract

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By Marylynn Fisch

The link between the parasitic infection caused by *Toxoplasma gondii* (abbreviated "toxo") and neurological function has been well established in both humans and animal models. Meta-analysis shows the highly significant risk for toxo sero-positivity (toxo positive) amongst schizophrenic patients to be 2.7 times that of non-schizophrenic patients. In addition, numerous animal models have shown significant cognitive impairment in toxo positive animals. In addition to investigating effects of toxo infection upon specific tests of cognitive function, in schizophrenic subjects, the role of sera-intensity on cognitive function will also be examined in this thesis using regression models

In this study, 169 schizophrenic patients were given a series of well-validated tests of cognitive and symptomatic function, including IQ, finger tap, Wisconsin Card Sort Test, the MATRICS Battery, and the Positive and Negative Syndrome Scale (PANSS). Blood was drawn from each subject and both positive or negative toxo sera status, as well as sera-intensity, was determined.

Subjects who were toxo positive scored lower on the Wisconsin Card Sort Test ($p=0.05$), as well as the MATRICS test processing speed ($p=0.04$) and for verbal learning ($p=0.007$). A positive association was seen between sera-intensity and cognitive performance, with higher titers performing significantly better on the verbal learning test. Similar trends were seen amongst several other cognitive tests, with near-significant results. Among non-schizophrenic controls, toxo positive subjects scored significantly lower on IQ and the Wisconsin Card Sort Test.

This study adds to the current body of literature associating toxo infection with decreased performance in tests of cognitive function. It also shows a positive correlation between sera-intensity and cognitive performance. As sera-intensity generally declines

with duration of infection, it may show that cognitive function declines with length of time infected.

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Contents

Introduction

Toxoplasmosis is a parasitic infection caused by *Toxoplasma gondii* found in humans worldwide, with an estimated 20% of the United States population infected [1]. Felines are the definitive host, with all warm-blood mammals, including humans, serving as intermediate hosts to the protozoa. Although widely asymptomatic, the infection has received more attention in the years following the advent of the HIV epidemic, as infection in an immunocompromised person can lead to toxoplasmic encephalitis [2]. Along with causing serious problems in the immunocompromised, the damaging effects of congenital transmission have lead to warning labels on all packages of cat litter sold in the United States, warning both pregnant woman and the immunocompromised to take extra precautions when handling feline excrement to prevent toxo transmission. In recent past, multiple studies have shown a link of toxoplasma seropositivity among people with schizophrenia [3]. Due to the high prevalence of toxo infection, along with the ability for toxoplasma (toxo) cysts to live in neural tissue in the brain, investigations of neurological, psychological, psychophysiso, and cognitive abnormalities among those infected are of great research interest [1, 4-6].

The *T. gondii* Organism and Infection

Toxoplasma gondii is single-celled, eukaryotic, protozoa which has evolved a complex life cycle that has ensured its success all across the world. The reproductive portion of the life cycle must be conducted in a feline host, but other mammals can be

infected, often with rodents serving as intermediate hosts [1]. However, toxo often infects pigs, cows, and birds such as chicken, and other livestock. Although not generally thought of as such, toxo is also a food-borne infection [7]. After acute infection cysts develop in the host tissue with greatest concentration in muscle and brain. These cysts are infectious to the felines who eat the intermediate host (primarily rodents), or humans who consume undercooked meat infected with cysts (can be pork, poultry, or beef). The organism reproduces sexually in the intestine of the feline host and creates highly infectious oocysts that are spread by defecation to set in motion a new round of infection. Humans become infected by indirectly ingesting fecal matter or consuming infected meat products. In non-feline based transmission, humans can become infected from consuming undercooked muscle tissue infected with cysts (pork, beef, or poultry). Acute infection in humans can be accompanied by systemic symptoms such as fever, fatigue, and lymphadenopathy. In the immunocompetent, it is often mistaken for a cold or flu and rarely leads to medical attention and diagnosis of acute toxoplasmosis [7]. In rare cases, retinal problems and blindness may occur, although these are rare in those with competent immune systems. Chronic infection generally involves cysts persisting in the brain and muscle tissue of an infected mammal. The effects of chronic infection in brain tissue in humans are not fully understood.

Cell Invasion and Tropism of *T. gondii*

The *T. gondii* protozoan has a crescent-shaped cell from which its name is derived (toxon being Greek for “ark”). The conoid end is believed to be central in breeching the

host's cell membrane for entry. The more pointed part of the cell is where the three main secretary organelles are located, whose main functions are attachment and infiltration of host cells. These are the micronemes, rhoptries and dense-granule organelles. As the protozoa has no other discernible means of motility (flagella, cilia, etc), it is thought that the micronemes play a role movement. In addition, the micronemes are known to secrete the M2AP-MIC2 complex that aids in adhesion and attachment to the host cell. During the infiltration process, rhoptry proteins are also secreted, which reside within the parasitophorous vacuole. The dense-granule proteins are in charge of altering this vacuole for maximal survival once it enter the host cell, and also assists in the replication process. These organelles are also thought to be the source of secreted lactate dehydrogenase; although the function of this protein has yet to be understood, it has been found to be vital to the parasite's ability to survive once it enters the host [8, 9].

Once the infectious cysts (sporozoites) enter a host's body, toxo invades primarily the muscle and nervous tissue, although can infect almost any tissue in a mammal's body. Although sexual reproduction only occurs in felines, they can multiply asexually by endodyogeny in intermediate hosts. At this point the sporozoites are called tachyzoites. They multiply quickly forming pseudocysts inside host cells, which burst open and release more tachyzoites. After several days or more of infection, the tachyzoites have transitioned, and are then referred to as bradyzoites. They multiply more slowly and stop bursting out of pseudocysts. They finally form encased cysts which remain, usually benign, in the host's tissues until it is consumed and passed along to another host. Inside the feline host, the cysts go through gametogony formation of zygotes, then oocysts, then sporonts, then sporoblasts, and finally sporozoites to complete the sexual life cycle [10].

Not being prey in modern times, humans are the final host and cannot transmit toxo infection to felines. Those with competent immune systems are often spared serious symptoms through an innate immune response and adaptive immunity.

Chronic infection in humans is largely asymptomatic and generally involves cysts persisting in the brain and muscle tissue. Cysts in the brain are too small to be detected by MRI or cerebrospinal fluid analysis. The brain areas with the greatest concentration of cysts are the nucleus accumbens and amygdala [11] Because the relatively small burden of brain cysts their microscopic size, it is thought unlikely that direct physical brain damage causes behavioral changes [12]. However, the effects of *T. gondii* in the brain have shown to play an integral part in the parasite's transmission by altering the behavior of the host.

Manipulation of the Host by Toxo

One of the most definitive findings supporting behavioral changes in the host due to toxo infection has been shown in rodents. Rodents experimentally infected with *T. gondii* infection have reduced fear of cats, which increases the possibility of toxo transmission [13]. Experiments in laboratory-bred that have never been exposed to cats have shown that fear of cats is a hard-wired response in rodents. Those rodents with a toxo infection are less fearful of cat scent. This behavioral change increases the likelihood of a rodent being consumed by a feline predator, helping to perpetuate the *T. gondii* lifecycle [13]. Although it has been hypothesized that this behavior was primarily due to decreased olfactory sensitivity due to toxo infection, studies show their olfactory learning remains intact and the change is specific to the relationship between rodent and

feline. A series of experiments demonstrate that in addition to losing fear of felines, infected rats actually *attracted* to the smell of cats [14] It was also found that the loss of fear was highly specific to the smell of cat and did not generalize to changes in anxiety-like behavior, non-aversive hippocampal based learning, or even a simple paradigm involving conditioned fear to foot shock as measured by freezing behavior. Experiments showed that feline-scent based learning did slow [13]. The psycho-motor slowing that occurs in infected rodents has lead research to look at the psycho-motor and cognitive effects toxo infection may have upon humans.

Schizophrenia

Schizophrenia is a complex, chronic, and debilitating disorder that affects about 1% of all Americans. Schizophrenia affects men and women equally, with onset of symptoms generally in a person's 20's, between ages 16 and 30 [15]. Although symptoms vary from person to person, there are three basic groups of schizophrenic symptoms: positive symptoms, negative symptoms, and cognitive symptoms. Positive symptoms are behaviors and thought processes such as delusions, hallucinations, thought disruptions, and movement disorders. Negative symptoms are disruptions of normal emotions and activities and are often harder to notice at first; these include a lack of facial expression, inability to carry out routine activities such as personal hygiene, and lack of conversation with others, even when forced to interact [16]. As with negative symptoms, cognitive symptoms are often subtle and hard to recognize and treat in schizophrenia, and include trouble focusing and paying attention, and having a limited "working memory,"

which is the ability to use information soon after learning it. Although medications can treat symptoms, patients often struggle for life with the disorder. Symptoms such as delusions and hallucinations often make patients reluctant to seek treatment, and destructive behaviors such as substance abuse and homelessness are common among those suffering from schizophrenia.

Although much progress has been made in treating schizophrenia, very little has been established in discovering a common cause. While there is definitely a genetic component, with the offspring of schizophrenic patients much more likely to develop the disorder themselves, no single gene has shown significant promises in identifying a causal model [17]. In addition to having an unknown causality, the exact neurological basis of the disease is also unclear. One of the most accepted theories is the dopamine (DA) hypothesis. Although not strong enough to completely explain the disorder, empirical validation from antipsychotic treatment and direct testing from imaging studies have given credence to a direct relationship between symptoms and treatment [18].The first formulation of the DA hypothesis of schizophrenia proposed that hyperactivity of DA transmission was responsible for the disorder [19]. This was based on the early observations that dopamine receptors are activated by psychostimulants, that non-reserpine neuroleptics are dopamine antagonists and that dopamine plays an important role in the extrapyramidal motor system. Much of the evidence for this was from characterizing the presence of dopamine in the brain and the effects of neuroleptics on monoaminergic indices [20].

The classical dopamine hypothesis received further support from the correlation between clinical doses of antipsychotic drugs and their potency to block DA D2 receptors and on the psychotogenic effects of DA-enhancing drugs [21]. Over the years, as negative and cognitive symptoms (lack of affect, poor working memory, etc) have become better acknowledged as a part of the disease, their resistance to D2 receptor antagonism led to a reformulation of the classical DA hypothesis. A wealth of preclinical studies emerged documenting the importance of prefrontal DA transmission at D1 receptors (the main DA receptor in the neocortex) for optimal PFC performance. This leads to the hypothesis that a deficit in DA transmission at D1 receptors in the PFC might be implicated in the cognitive impairments and negative symptoms of schizophrenia, while excess DA transmission may be related only to positive symptoms, such as hallucinations and delusions [20, 22, 23].

Toxoplasmosis, Schizophrenia and Human Behavior

The epidemiological link between toxo infection and schizophrenia is strong; a recent meta-analysis of 23 studies that were judged methodically sound found that the risk for toxo seropositivity was 2.7 times higher in schizophrenic patients than the general population, and this was highly significant (95% confidence interval, 2.10–3.60; $P < .000001$) [3]. This odds ratio is far larger than any odds ratio identified by studies of vulnerability genes in schizophrenia, including recent meta-analyses and genome wide association studies, which give odds ratios only between 1.6 and 1.8 [3, 4]. While there is one hypothesis that symptoms of schizophrenia may lead to actions that increase likelihood of acquisition of the parasite, investigations of toxo status in first-episode

cases of schizophrenia imply that sero-conversion still occurs more frequently amongst schizophrenic patients, prior to the onset of symptoms [24-26]. A large study of U.S. military personnel diagnosed with schizophrenia to have not only an increased likelihood of toxo infection, but higher serointensity, measured by IgG levels than their non-schizophrenic counterparts [26].

Using the behavioral changes seen in rodents as groundwork, several studies have looked at the effects of the toxo infection upon the human brain. Psychomotor slowing has been reported in toxo seropositive humans without psychiatric illness, in striking accord with a similar finding in rodents [27]. One study performed in 2002 showed an increased rate of motor vehicle accidents in people, seemingly asymptomatic, with chronic toxo infection [28]. This study has hence been repeated three times [29-31]. Other studies have shown that seropositive humans without psychiatric history had worse performance in memory tasks indicative of impaired learning than their toxo negative counterparts [32]. Personality traits have also been found to associate with toxo seropositivity without a history of psychiatric illness [32, 33]. Using lower titres as an indication of longer time since initial infection, the Flegr group reported that the personality changes were more pronounced in subjects with a longer history of chronic infection [32]. These findings suggest that chronic toxo infection can induce behavioral change in human hosts as it can in rodent hosts.

Neurochemical Basis for Behavioral Modification

There are two neurochemical mechanisms suggested in the literature by which *T.*

gondii can modify behavior and contribute towards schizophrenia. Toxo infection is known to cause an over-production of dopamine. This is performed by enzymes located within the *T. gondii* organism itself. Studies show that levels of dopamine (DA) in the brains of experimentally infected mice were 14% increased during the chronic phase of toxo infection [34]. Assuming the dopamine hypothesis is a valid contributing factor to schizophrenia, the effect of toxo infection is a logical link towards the disease.

Subsequent work reveals that the toxoplasma genome contains two genes that code for tyrosine hydroxylase, the enzyme that is the rate limiting step in neuronal synthesis of DA [35]. Functional studies confirm that this enzyme is indeed active in the host [36] and leads to the synthesis of levodopa (L-DOPA), which is then rapidly converted to DA in dopaminergic neurons [37]. The *T. gondii* genome is able to induce both forms of tyrosine hydroxylase, thus providing evidence of a mechanism by which the organism could induce a state of hyperdopaminergia in the host [37].

The second mechanism by which behavior can be modified is due to the ability of *T. gondii* to take over the host's neuroimmune response and modify metabolic pathways for serotonergic and glutamatergic transmitters [37]. In addition to modulating the behavior of rodents, these are the same neurotransmitters that have been widely cited as playing a role in the neural underpinnings of schizophrenia [38].

Toxoplasmosis and Cognitive Function

Recent studies have associated prenatal toxo exposure to poorer performance on the Wisconsin Card Sort Test (WCST), a widely used marker of set-shifting ability in schizophrenia [39]. Studies in humans have also shown that those with latent toxo

infection tend to perform more poorly on tests of psychomotor function, and show delayed reaction times [27]. Studies by the Flegr group have also demonstrated that men with who are seropositive for toxo have lower IQs than their seronegative counterparts [40]. These studies also show that those who are toxo positive perform significantly worse in tests of memory, with non-significant trends towards worse performance on the WCST and tests of cognitive function [36].

Novelty of Research

While a 2008 study by Shirts, et al, did not show any significant correlation between toxo serointensity and cognitive function, only one test of cognitive function was administered [41]. Our study is to our knowledge, the first of its kind to look at the effects of serointensity on a wide range of tests of cognitive and psychophysio- function amongst schizophrenic patients. In addition, this study also looks at the effects of toxo infection and serointensity upon some cognitive variables amongst non-schizophrenic controls as well.

Hypothesis

As toxo infection has been shown to decrease cognitive and psychomotor functions in multiple animal models, we believe that standardized tests of memory, cognitive and motor function will show significant differences between those who are and are not infected with *Toxoplama gondii*. In addition, we believe that serointensity, rather than dichotomization based on positive or negative status, will also be a significant predictor of cognitive, memory, and motor function among schizophrenic patients. In addition, because of the clinical associations between toxo infection and psychosis and

schizophrenic symptoms [2, 6, 24], it is hypothesized that toxo infection will have some effect on symptomatic scores.

Methods

Subjects

Subjects were recruited from a population from a local Veteran's Affairs hospital in accordance with both Emory University and Veteran's Affairs IRB procedures (**Table 1)**. Research is conducted through a grant entitled, "Sensorimotor Gating in Schizophrenia," funded by the VA Merit Review, principle investigator Dr. Erica Duncan. There were 118 age, sex, and race matched-controls recruited, 93 of which had data collected on cognitive function. However, due to the small number of toxo positive controls for whom cognitive data was collected**,** analysis is concentrated upon schizophrenic subjects. Of the 169 schizophrenic subjects, 133 (79%) were male, and 36 (21%) female. There was an equal mix of smokers (49%) and non-smokers (51%). 110 subjects (65%) were self-identified as black, 49 (29%) were white, and 10 (6%) were of "other" race. 138 (82%) of subjects were on an atypical antipsychotic, and 36 (21%), on typical antipsychotics. The subjects ranged from 20 to 66 years of age, with a mean age of 44.5 years and a standard deviation of 10 years **(Table 1)**.

Determination of Toxo Status and Titer

Blood samples were collected from all subjects and an IgG ELISA using whole blood was used to determine titer. Samples were assayed in the laboratory of Patty Wilkinson with Hilda Rivera, and consultation with Jeff Jones, Parasitic Disease Branch, Center for Global Health, Centers for Disease Control and Prevention. The main analysis measured IgG antibodies in human test sera to whole toxo organisms using a

commercially available indirect enzyme immuno assay (Platelia toxo-G immunoglobulin G enzyme immunoassay test, Sanofi Diagnostics Pasteur, BioRad, Hercules, California). This measure is determined based on a dilution series of specific toxo antibody standards assayed along with each assay. OD values were converted to arbitrary units to determine serointensity. A cut off value of 30 arbitrary units was used to consider a subject toxo-positive.

Description of Variables

Cognitive Tests

Intelligence Quotient (IQ)

IQ is a score from one of several tests to measure general intelligence. Some subjects received a RIST IQ exam, while other received the WASI IQ exam. As both are highly validated measures of IQ, we used the score from either of these measures in order to maximize the sample size for IQ.

Wisconsin Card Sort Test

This is an extensively used test in schizophrenia research that is widely accepted as a measure of executive and frontal lobe function [42]. It is a test of "set-shifting," the ability to display flexibility in the face of changing schedules of reinforcement. Initially, a number of stimulus cards are presented to the participant. The shapes on the cards are different in color, quantity, and design. The cards are to be matched first by color, then by design and then by quantity. The participant is given a stack of additional cards and asked to match each one to one of the stimulus cards, thereby forming separate piles of cards

for each. The participant is not told how to match the cards; however, he or she is told whether a particular match is right or wrong. The resulting score is the number of perseverative errors [42]. As such, this is the only cognitive variable in our analysis where a higher score implies lower function.

Finger Tap

This is a motor test of the speed at which the subject is able to repeatedly press a key in a specified length of time. Psychomotor speed in this test is measured as the number of clicks the subject is able to make on the counter in 60 s by pressing their finger. This measure is completed on the dominant hand. Studies show this is not only an indicator of motor function, but of general learning ability in schizophrenic subjects [43].

The MATRICS Consensus Cognitive Battery (MCCB) [34]

Speed of Processing.

This component is comprised of three subtests:

(a) Brief Assessment of Cognition in Schizophrenia (BACS): Symbol-Coding test. This is a timed paper-and-pencil visuomotor test in which respondent uses a key to write digits that correspond to nonsense symbols. Processing speed in this test is measured as the correct number of coded symbols the subject is able to write in 90 seconds.

(b) Category Fluency: Animal Naming. Oral test in which respondent names as many animals as she/he can in 1 minute as a verbal index of processing speed. Processing speed in this test is measured as the total number of animal names the subject is able to say in

60 seconds.

(c) Trail Making Test: Part A. This is a timed paper-and-pencil visuomotor test in which respondent draws a line to connect consecutively numbered circles placed irregularly on a sheet of paper. Processing speed in this test is measured as the number of seconds it takes the subject to complete the connection of all the numbers. The MATRICS software computes a composite score from these three subtests that serves as a measure of processing speed.

Working Memory.

This domain is comprised of two subtests:

(a) Wechsler Memory Scale-Third Edition: Spatial Span. On a board with 10 irregularly spaced cubes, the subject taps the cubes in the same or reverse sequence as the test administrator.

(b) Letter-Number Span. The subject mentally reorders strings of numbers and letters and repeats them. The MATRICS software computes a composite score from these three subtests that serves as a measure of working memory

Attention.

Computer-administered measure of sustained attention in which respondent presses a response button to consecutive matching numbers.

Verbal Learning.

Orally administered test in which a list of 12 words from three taxonomic categories

is presented and the respondent is asked to recall as many as possible after each of three learning trials.

Visual Learning.

A test that involves reproducing six geometric figures from memory.

Reasoning and Problem Solving.

Seven timed paper-and-pencil mazes of increasing difficulty that measure foresight and planning.

Social Cognition.

This is measured by the Mayer-Salovey-Caruso Emotional Intelligence Test: Managing Emotions. This is a paper and pencil test that assesses how people manage their emotions [44].

Symptom Ratings

Positive and Negative Syndrome Scale (PANSS)

PANSS is a widely-used test of symptoms amongst schizophrenic patients, particularly with regards to medication efficacy [45]. There are three scores, one for positive symptoms, which refer to an excess or distortion of normal functions, such as hallucinations and delusions, and negative symptoms, which represent a diminution or loss of normal functions, such as social withdrawal. There is also a total score that is the sum of all items in the PANSS [45].

Analytic Method

Choice of Covariates

Many demographic variables have been linked to toxo status, such as age, sex, and race, as well as socio-economic status, smoking status, and being born outside of the United States [1]. Data was not available on socio-economic status and was subsequently left out of analysis. Data was also unavailable with regards to country of origin; however as the population is comprised of United States military veterans, it can be assumed most were born in the US. Data on race was collected in self-identified categories of "black," "white," and "other." It was not shown to be a significant predictor in preliminary analysis and data was missing for many subjects, and as such, was subsequently left out of the final model. Smoking status was not shown to be significant in preliminary analysis and was also left out of the final model. Because over 80% of the study population was on an atypical antipsychotic, as well as some subjects being on both typical and atypical antipsychotics, medication type was not included in final data analysis. All final models control for age and sex.

Challenges of Modeling

Serointensity values were zero-inflated and over-dispersed. Intensity was transformed by taking 1 plus the natural log of the intensity (**Figure 1, Figure 2**). To deal with the zero-inflation of the data, both a dichotomized *T. gondii* serology positive or negative variable, as well as the transformed intensity, were included in all models, unless otherwise noted.

Modeling of serological data has proved problematic for many researchers and many methods have been proposed and utilized in published analysis [46, 47] . Zero-inflation poses a problem because zero-values may represent true lack of exposure, or exposure below the limit of detection. Other methods, such as reversing the equation and using a true zero-inflated model, or creating separate models for zero and non-zero data, have been used [46-49]. New methods are emerging for dealing with the issue but none have been accepted definitively [47]. The decision to deal with the problem by creating separate variables for positive or negative toxo status and serointensity was the simplest method that is appropriate for our data. Much statistical guidance was provided by Drs. Nancy Bliwise and Mary Kelley.

Due to the small number of toxo positive controls for whom cognitive data was collected, most analysis focuses on schizophrenic subjects. Enough data remained for control analysis of IQ and Wisconsin Card Sort Test variables

Poisson Regression

Poisson regression was used to model the outcomes of both the Wisconsin Card Sort Test and the Finger Tap test, as it an appropriate fit for count data. The chi-square distributions of the deviances lead to no significant p-values, giving no reason to reject using a Poisson model [50].

Linear Regression

Because of the normal distribution of the IQ data, an untransformed linear regression, controlling for age and sex, was used to model the effects of toxo status and

serointensity (**Figure 3**). MATRICS cognitive battery test scores were also generally distributed in a Gaussian fashion, and linear regression was used for these variables as well (**Figures 4-11)**. PANNS scores total and positive symptom scores were distributed in a Gaussian fashion but the negative symptom score was skewed (**Figures 12-14**). Moreover, some resulting models had poor fit and as such, alternative models were investigated.

Negative Binomial

A negative binomial regression was also used to model PANSS and this method was also used in exploratory analysis of some MATRICS data. Negative binomial models have been used to model similar data in other published studies [39, 46, 48, 51]. Deviance values/degrees of freedom were all close to one, implying a reasonable level of model fit. The chi-square distributions of the deviances lead to no significant p-values, giving no reason to reject using a negative binomial model [50, 52]**.**

Results

Table 1 shows demographic information including age, race, sex, and smoking status for the study population, stratified by schizophrenic cases and controls. There were no significant differences between smoking status and race between toxo positive and toxo negative subjects across the study population (**Table 2**), and as preliminary analysis showed neither variable to be significant predictors for either the schizophrenic or control population, they were left out of final models. Amongst the total population, schizophrenic status was not a significant predictor of seropositivity (p=.38); it should be noted that the number of toxo-positive controls was small.

Point estimates of the entire sample (both schizophrenic patients and controls) were lower for all variables amongst the toxo positive, excepting the Finger Tap and Wisconsin Card Sort Test (**Table 3**). The mean Finger Tap Score was 46.6 amongst the toxo negative, and 49.7 amongst seropostive. Mean scores on the WCST were 12.01 among toxo negative, and 12.8 among the toxo positive. However, in the WCST, higher score is an indicator of lower performance. Amongst schizophrenic patients, point estimates were lower for the toxo positive group for all variables, excepting the MATRICS visual learning (37.9 and 39.2, respectively) and Finger Tap Test (44.5 to 49.7) (**Table 4**). Amongst controls, toxo positive subjects had lower point estimates for all cognitive variables except Finger Tap (49.6 to 49.9), Wisconsin Card Sort Test (8.4 to 11), and Attention (**Table 5**). Due to the small sample size (n=3) of toxo positive controls with available MATRICS data, further analysis of cognitive function in controls was only performed using the IQ and WCST variables.

Cognitive Function Amongst Schizophrenic patients

MATRICS Variables

Toxo Status

Using a linear regression model, toxo positive subjects performed significantly worse on tests of speed of processing (p=.045) and verbal learning (p=.007) (**Table 6**). By inspection of beta values, all variables, with the exception of social cognition, were negatively associated with toxo positive status (n.s.). Similar results were seen in an exploratory analysis using a negative binomial regression (also adjusted for age and gender) for verbal learning (B=-.61, p=.006)

Serointensity

Using the linear regression model, performance on the test of verbal learning was significantly increased with serointensity (p=.005) (**Table 7**). There was a near-significant positive association with speed of processing (p=.080). By inspection of betas, all variables except reasoning/problem solving and social cognition showed a positive association between performance and serointensity (n.s.).

Symptomatic Scores

No significant association was seen between toxo status or serointensity and any symptomatic scores, using either linear or negative binomial regression (**Tables 8-11**).

Finger Tap Test

Using a Poisson regression, scores on the Finger Tap Test were significantly higher amongst the toxo positive when only age, sex, and toxo status were included in the model (p=.049). When including intensity in the model, a non-significant negative association with toxo status is seen, with near-significant results showing an increase in score with serointensity (p=.081) (**Table 12**).

Cognitive Function Amongst Both Schizophrenic patients and Controls

IQ

Controls

Using a linear regression model, toxo status was associated with a significantly lower IQ amongst controls, when age, sex, and toxo status were included in the model (p=.001). When serointensity was included in the model, a trending, but not significant reduction in IQ was also seen with toxo status (p=.176), with no effect of serointensity (p=.803). However, including sera intensity in the model reduced model fit when compared to the model containing only dichotomized toxo status (Adjusted R^2 .121 to 109) (**Table 13**).

Schizophrenic patients

Among schizophrenic patients, a non-significant reduction in IQ was seen with positive toxo status (p=.131), when both toxo status and serointensity were included in the model. A non-significant, positive association with serointensity was also seen (p=.191). Toxo status was not a significant predictor of IQ when serointensity was not included in the model. However, model fit improved with the addition of serointensity (**Table 13**).

Wisconsin Card Sort Test (WCST)

Controls

Using a Poisson regression, a significant increase in score (indicator of worse performance) was seen amongst toxo positive controls when compared to their toxo negative counterparts (p=.04). A non-significant negative association (improvement in performance) was seen with serointensity (p=.173). A non-significant positive association (decreased performance) was seen when serointensity was excluded from the model (p=.146) (**Table 14**).

Schizophrenic patients

Among schizophrenic patients, a significant decrease in score (increased performance) was associated with toxo seropositivity (p=.05). However, when serointensity was added to the model, a near significant (p=.06) increase in score (worse performance) was seen with toxo status. Increase in serointensity was associated with a significant (p=.017) decrease in score (increased performance) (**Table 14**).

Interpreting the Rate Ratio

As the variable associated with the Wisconsin Card Sort Test is a count of errors, the rate ratio (RR) can be interpreted as a function of the number of errors made. Using the mean value as baseline for toxo negative controls, the average number of errors made is 12.01. Being toxo positive has a significant positive RR of 1.38, meaning on average someone who is toxo positive will make 1.38 times the mistakes (16.57) as someone toxo negative of the same age, sex, and serointensity. Amongst schizophrenic subjects, the RR associated with toxo status is 1.67; if a toxo negative subject were to make 8.4 errors, a toxo positive subject of the same age, sex, and serointensity would make 14.02 based upon this regression (**Table 15**). With regards to serointensity, the RR of .72 shows the magnitude of change for each unit of serointensity (analysis used log transformed intensity). If a toxo positive schizophrenic subject with a serointensity of 30 (log-transformed value 3.4) were to make 8.4 mistakes, based upon this regression a toxo positive subject of the same age and gender with a serointensity of 100 (log-transformed value 4.6) would make 7.2 errors (**Table 16**).

Discussion

This study is consistent with other published literature that shows a decrease in cognitive function associated with toxo, among both the schizophrenic and non-schizophrenic controls. Although the sample of toxo positive controls was small, the effects of toxo infection were still highly significant for both IQ and the Wisconsin Card Sort Test. This is consistent with current published literature and is similar to findings in animal models.[27, 39, 40, 53, 54] Among schizophrenic patients, the effects of toxo can be seen in the Wisconsin Card Sort and verbal learning. While the small sample size did not give great power to the analysis, trends which show toxo infection is associated with poorer outcome on speed of processing and overall MATRICS composite score are seen This is consistent with the findings of previous studies [24, 27, 55], as well as parallel to the effects of infection seen in animal models. Interestingly, the significant decrease in IQ associated with toxo infection in controls is not seen in schizophrenic patients, in spite of there being a larger infected population for analysis. However, the p-value for the regression is .131; with a larger sample size this might show significant reduction amongst schizophrenic patients as well [56]. Also interesting, is that model fit of IQ improves amongst schizophrenic patients when serointensity is added to the model, but actually decreases model fit for controls. This implies that while toxo status seems to uniformly lead to decreases in cognitive function amongst both schizophrenic patients and controls, the effects of serointensity may affect schizophrenic and normal populations differently. The p-value associated with serointensity when modeling IQ in a normal population is .803, which clearly indicates no significant effect. Amongst schizophrenic

patients however, the p-value is .191, which could imply a significant effect may be seen in a larger study population.

While some studies have discussed the association between toxo status and cognitive variables, few have gone as far to determine effects of toxoplasma sera intensity. While a previous study did not show a significant association between serointensity and cognitive function [41], the study only looked at one test of cognitive function, the Trail Making Test, which is only one part of one of the MATRICS variables (speed of processing). In addition, there were differences in the demographics of the study population, which may also contribute to the different results. Although a significant change was only seen for the WCST, verbal learning and processing speed, results suggest that overall MATRICS composite scores are also negatively associated with toxo status, while simultaneously being positively correlated with serointensity. The results of the finger tap test were near significant (p=.08), implying that serointensity also plays a role in motor function.

In light of the widely-accepted reactivation theory of toxo and schizophrenia, [55] the positive association between sera intensity and performance was unexpected. While the reactivation theory states that in those who are schizophrenic, toxo gets "reactivated" periodically, causing a spike in immune response, and subsequently, a higher titer and decreased cognitive function [55, 57]. Our results suggest that although being toxo positive has a negative effect on outcomes, if a subject is in fact toxo positive, the higher the sera intensity, the better performance on cognitive tests. However, this does not necessarily contradict reactivation theory, as there is no way of knowing whether or not subjects were tested during a peak in reactivation. In addition, our study is of IgG titers,

which are indicative of chronic infection, while acute infection is best determined by IgM titer [55]. It has also been shown that the longer the duration since initial infection, the lower the titer [32]. Assuming the lower the titer the longer duration of infection, it is possible that those with a longer duration of infection, also have undergone more reactivation cycles. This would increase the effects of toxo upon cognitive function, which does not contradict any currently accepted theories on the effects of toxo and function in schizophrenic patients.

Literature and clinical data has shown that especially among the immunocompromised, acute toxo infection can lead to hallucinatory symptoms and psychosis [58, 59]. As such, it was hypothesized for an effect to be seen between toxo and schizophrenic symptoms, particularly positive symptoms relating to hallucination. Interestingly, toxo status does not appear to have any association between types or severity of symptoms using the PANSS variables. While this differs from the findings in a 2005 study [24], there were several key differences. Perhaps most significant is that the Bachmann/Yolken study looked at IgG, IgM, and IgA antibodies, and found that 24% of the variance of the model was accounted for by IgG, while 52% was accounted for by IgM. Our serointensity data was based only on IgG. In addition, all subjects in their study were Caucasian, with our study population being only 29%. As IgM antibodies are also a marker of more recent infection than IgG, this study shows more short-term effects of active infection, while the investigation into IgG titers looks at more long-term results.

Implications for Future Studies

The effects of serointensity upon cognitive function show an important link between immune response and neurological processes. Using serointensity as a marker, future studies could investigate whether effects on cognitive function are caused by the parasite itself, or a result of the immune response to infection. Effects of immune response on cognitive function could help establish links between other infectious moderators of neurological disorder.

While it has been postulated that lower IgG titers for toxoplasma are associated with a longer duration of infection, and smaller titers shorter time since infection, there is no definitive way to determine duration of infection. IgM titers can determine if infection occurred within the last year, but aside from that, length since infection is indeterminable. Among the immunocompetent, active infection is rarely established. Although some spikes can be seen with recent infection, once a person has been infected with toxoplasma, IgG titers can remain for life. As toxo titers are likely to decrease over time, investigation into whether or not cognitive function declines as well could help establish the long-term effects of infection.

From a clinical standpoint, establishing time of infection is imperative for treatment and prevention of congenital toxo transmission. In the last few years, avidity tests have been shown to help to specify the range of time since initial infection. Although current methods cannot establish an exact time of infection, it can exclude that the patient was infected in the prior 3 to 5 months (depends on the diagnostic kit employed). The utility of the avidity test is based on the observation that IgG antibodies

from patients with a recently acquired toxo infection bind antigens weakly (low avidity), whereas IgG antibodies from chronically infected patients have stronger binding capacity (high avidity) [60]. As such, avidity of toxoplasma-specific IgG antibodies for toxoplasma antigens gradually rises over time following acute infection and continues to rise as the acute infection evolves into a chronic infection. This has extraordinary implications for congenital screening, and it has been used in that capacity [61]. Future studies could build upon this method of testing to expand upon the relationship between duration of infection, sera intensity, and cognitive function.

One hypothesis of toxo pathophysiology in schizophrenia is that autoantibodies cross-reacting with toxo and brain tissue cause a local inflammatory response and the induction of soluble mediators (e.g. cytokines), which modulate neurotransmitters. Since the absorbance readings in the immune-assay used in the current study are a reflection of both the antibody number (molarity) and antibody affinity, it would be valuable to examine the relationship between these factors and anti-brain antibodies. For example, it is possible that autoimmune responses are linked to lower affinity antibodies, which could explain why those with lower serointensity have worse cognitive function.

Strengths and Weaknesses

This investigation adds evidence showing an association between toxo status and decrements in cognitive function amongst both schizophrenic patients and normal controls. In addition, it is to our knowledge, the first to go beyond dichotomization of toxo status and investigate effects of serointensity on multiple cognitive domains. Major

strengths of this study are the well-defined study population, comprised of a relatively large number of subjects. In addition, control match was well-performed and based on a uniform population. Measures of cognitive function are performed using highly validated testing methods. Laboratory methods for determination of toxoplasma titer were also reliable and validated. Subjects are representative of a wide range of age, which was also controlled for during analysis.

A limitation of this study is that small number of toxo positive subjects limits statistical power. As most people do not know their toxo titer status, recruitment must begin with a population more than ten times larger than the desired toxo positive population, and all those samples must then be tested for sera status. This is a problem for almost all population-based studies of toxo (although several meta-analyses have yielded significant power in showing the association between toxo and schizophrenia). In addition, significance values were set at a p-value of .05; some statistical experts argue that in a small sample study such as this, a higher cut off for significance may be appropriate [56], thus increasing our significant results. It is thought that analysis with a larger toxo positive population we could assess and include more covariates, and this could lead to more statistically significant findings [56]. Another weakness is that all toxo positive controls and the majority of toxo-positive schizophrenic patients, were male and some studies suggest a differing of effects of toxo based upon gender [40, 62, 63]. In addition, all subjects were selected from a population of veterans that may not be representative of the population at large. Studies suggest that different strains of *T. gondii* may lead to different effects on cognition [32, 64]. In addition to *T. gondii* strain being unknown, veteran deployment status and residential history of these veterans is also

unknown, and some subjects may have become infected with strains endemic to various parts of the world. This goes along with the issues that time of infection is currently undeterminable by available techniques, and as presence of *T. gondii* cysts in the brain can only be determined at autopsy. Other demographic data that has been linked to toxo infection is socioeconomic status, of which information was unavailable [65].

An issue that arose during analysis is that the best statistical method for dealing with serological data is not yet adequately described in the literature and multiple methods had to be considered. Models with best-fit only had adjusted-R^2 value of .17, suggesting a large portion of determinants for these cognitive variables are unexplored. Moreover, p-values were not corrected for multiple comparisons. However, while analysis resulted in different values of effect and significance, trends were consistent for most cognitive variables. This adds strength to the argument for effects of both general toxo status and serointensity.

Conclusions

This study adds to the current body of literature associating a positive toxoplasma sera status with poorer cognitive function compared to those who are toxo negative. These include IQ, set-changing, as measured by the Wisconsin Card Sort Test, and verbal learning. In addition, it provides evidence that the effects are further modulated by serointensity, with a higher serointensity leading to better performance in several areas, including set-changing and verbal learning. This could be due to a shorter duration of infection associated with higher titers. Current literature in addition to this analysis,

suggest analysis of a larger study population may yield significant results in more cognitive areas, particularly speed of processing.

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Figures and Tables

Figures

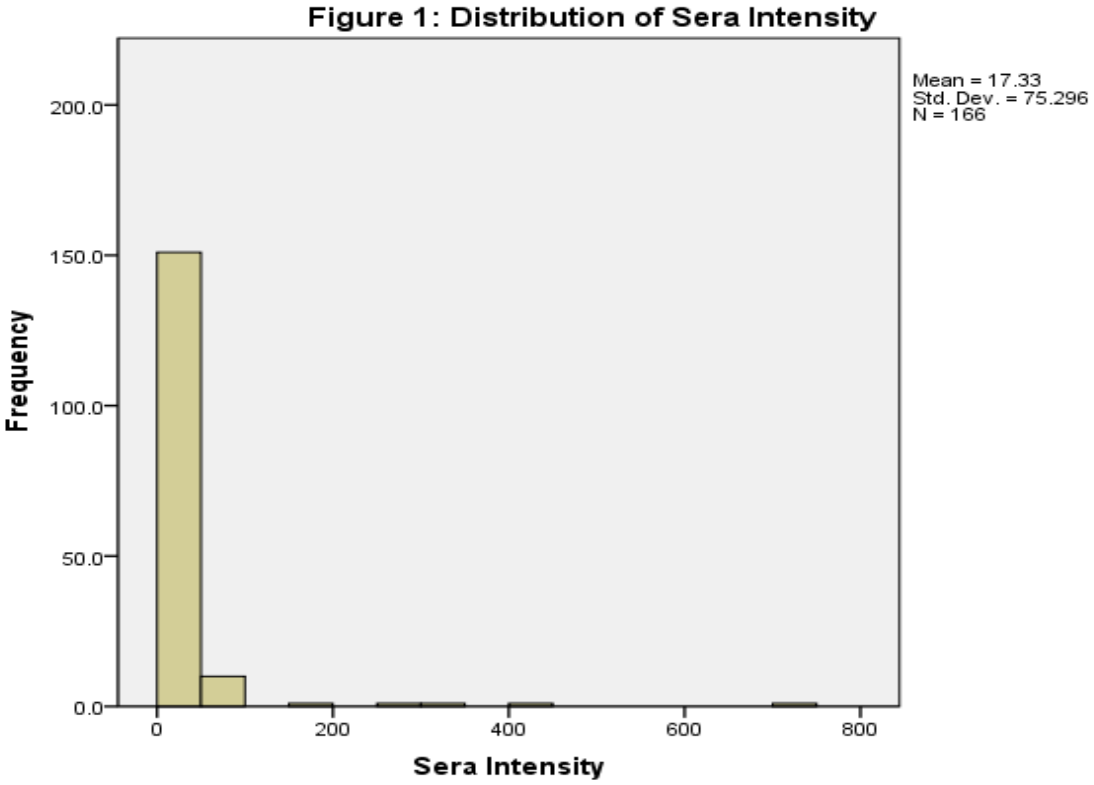


Figure 1: Distribution of Sera Intensity
Mean = 17.33
Std. Dev. = 75.296
N = 166
Frequency
200.0
150.0
100.0
50.0
0.0
0
200
400
600
800
Sera Intensity

Figure 2: Distribution of Transformed Sera Intensity

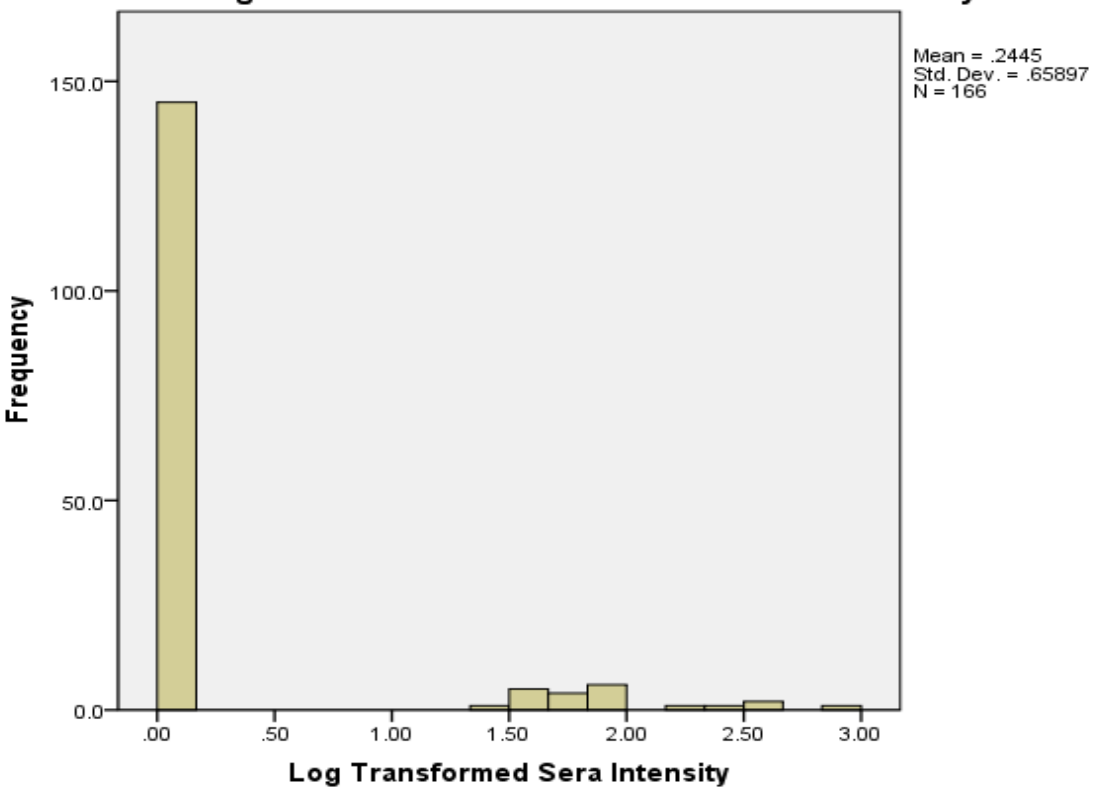


Figure 3: Distribution of IQ

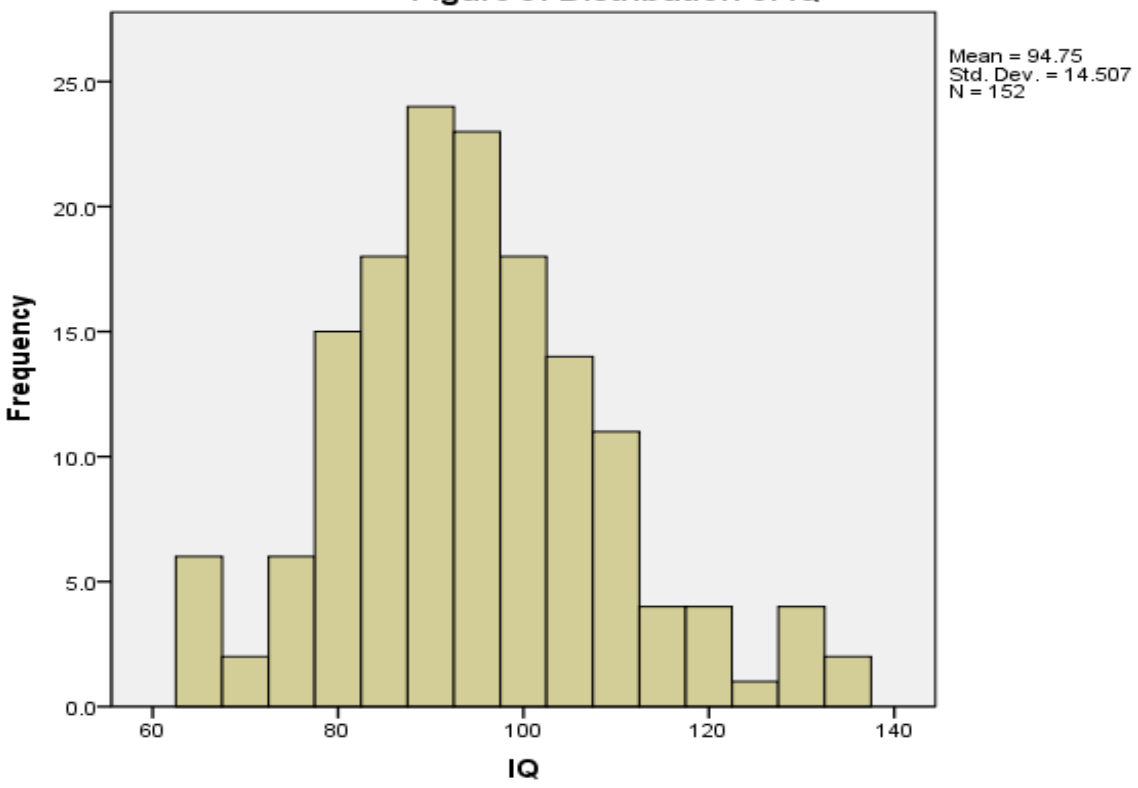


Figure 4: Distribution of MATRICS Speed of Processing Score

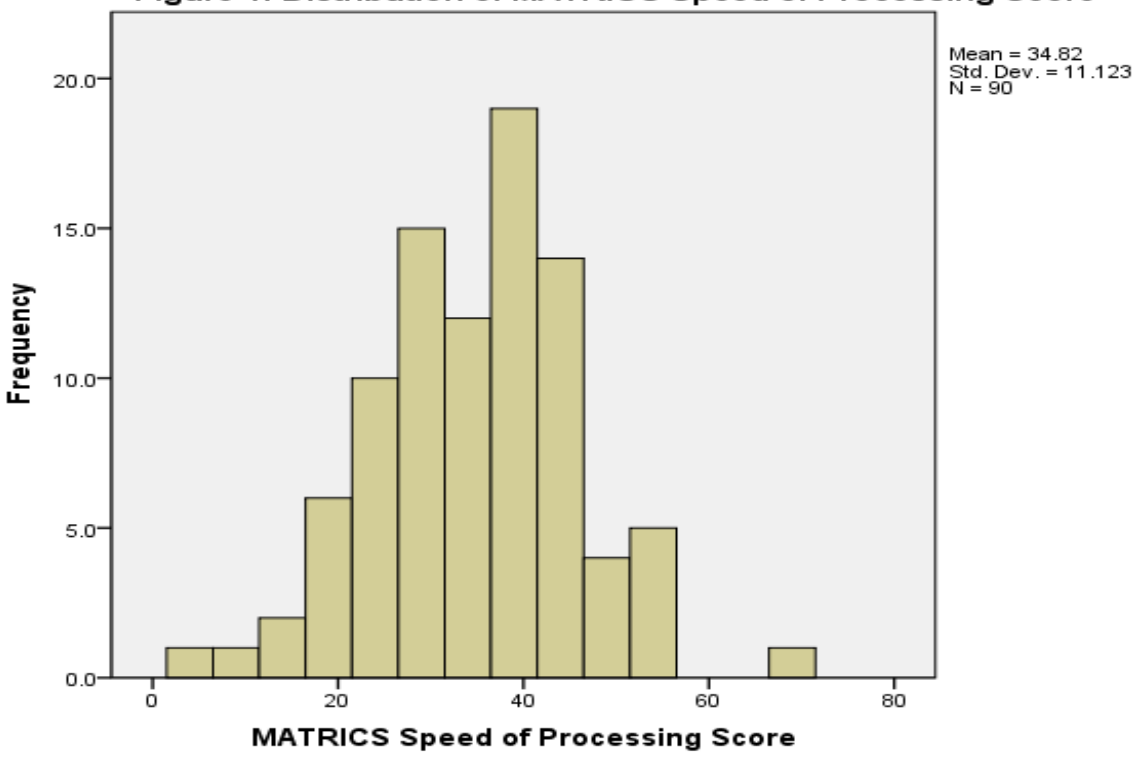


Figure 5: Distribution of MATRICS Attention Scores

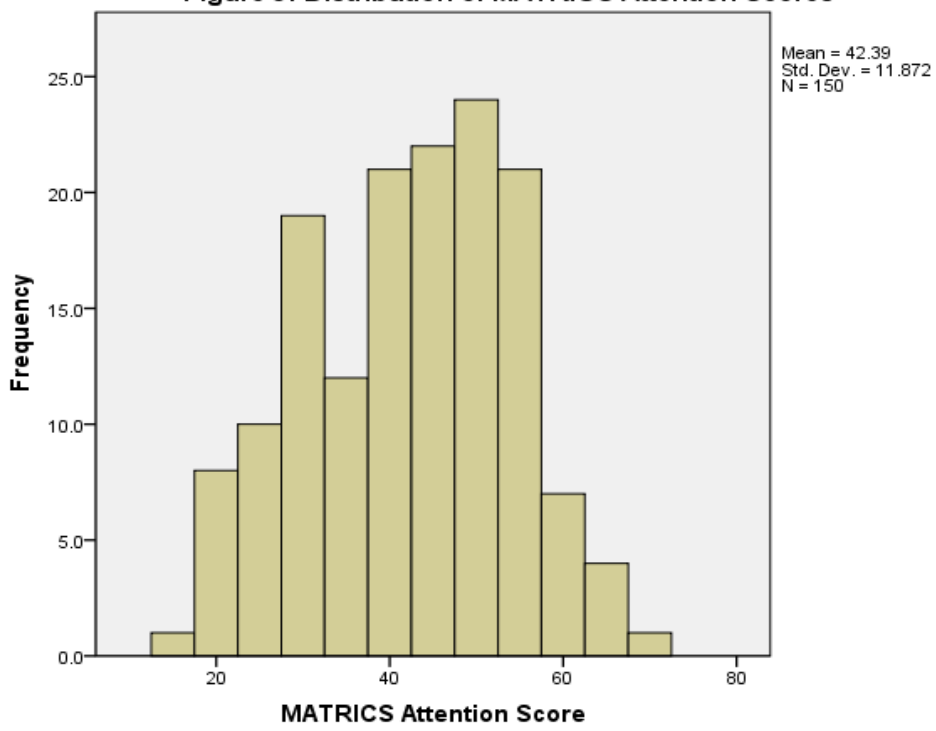


Figure 6: Distribution of MATRICS Working Memory Scores

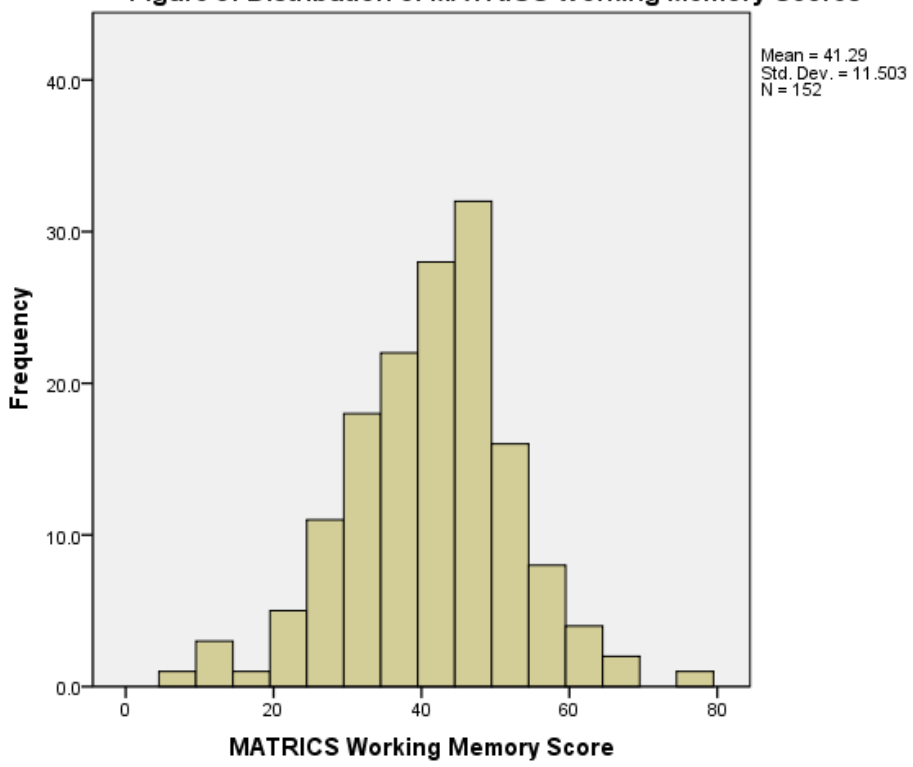


Figure 7: Distribution of MATRICS Verbal Learning Score

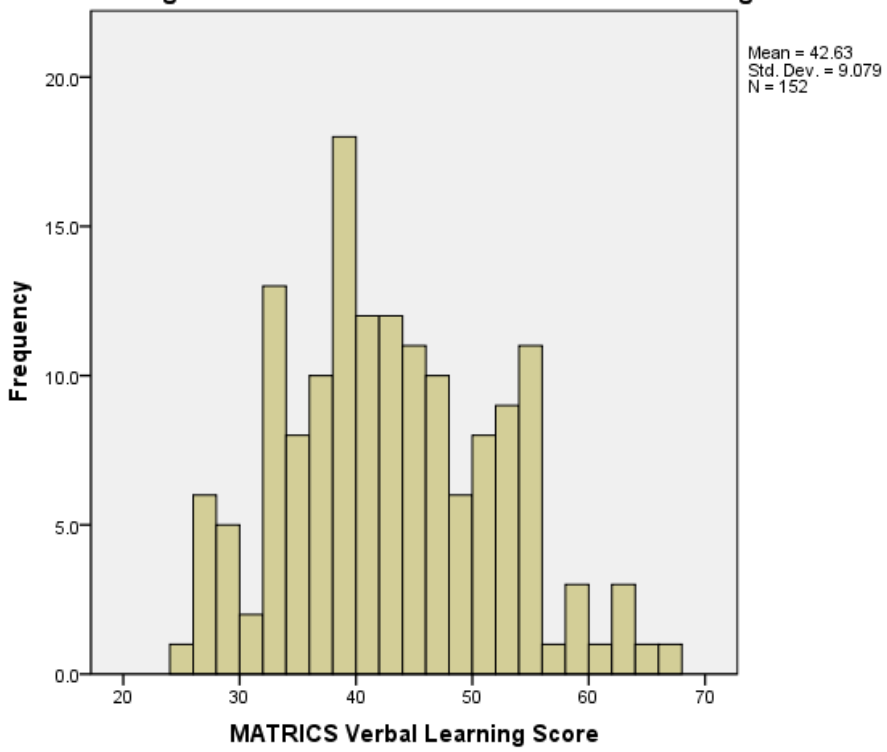


Figure 8: Distribution of MATRICS Visual Learning Score

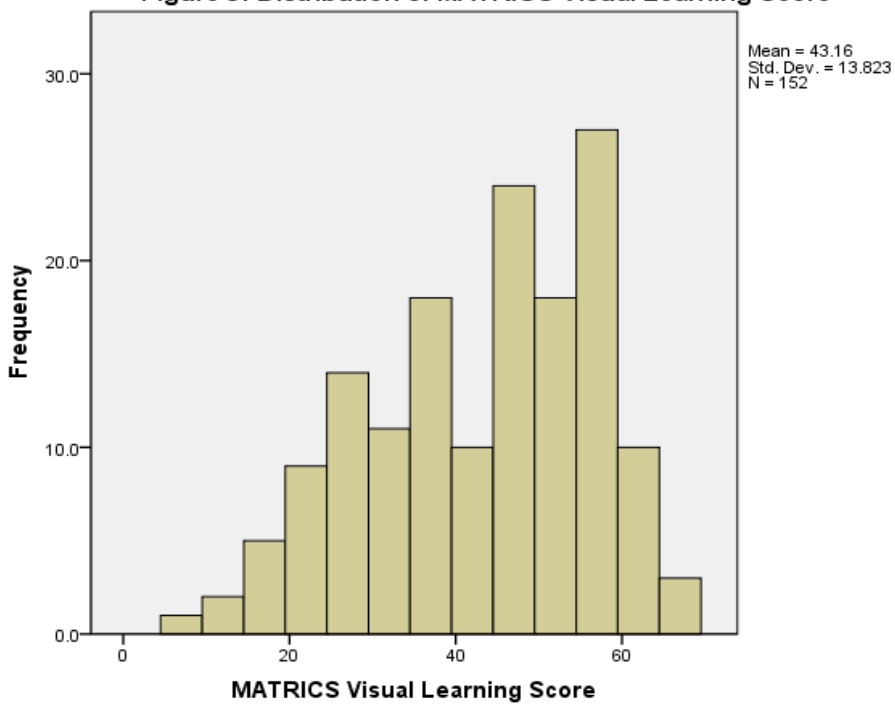


Figure 9: Distribution of MATRICS Reasoning and Problem Solving Score

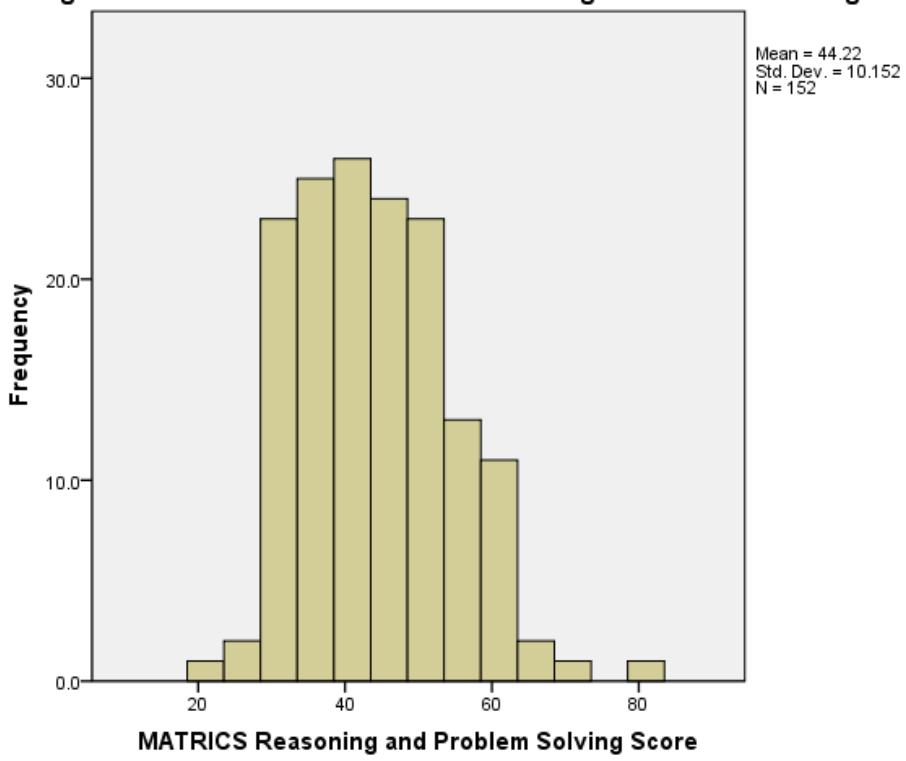


Figure 10: Distribution of MATRICS Social Cognition Score

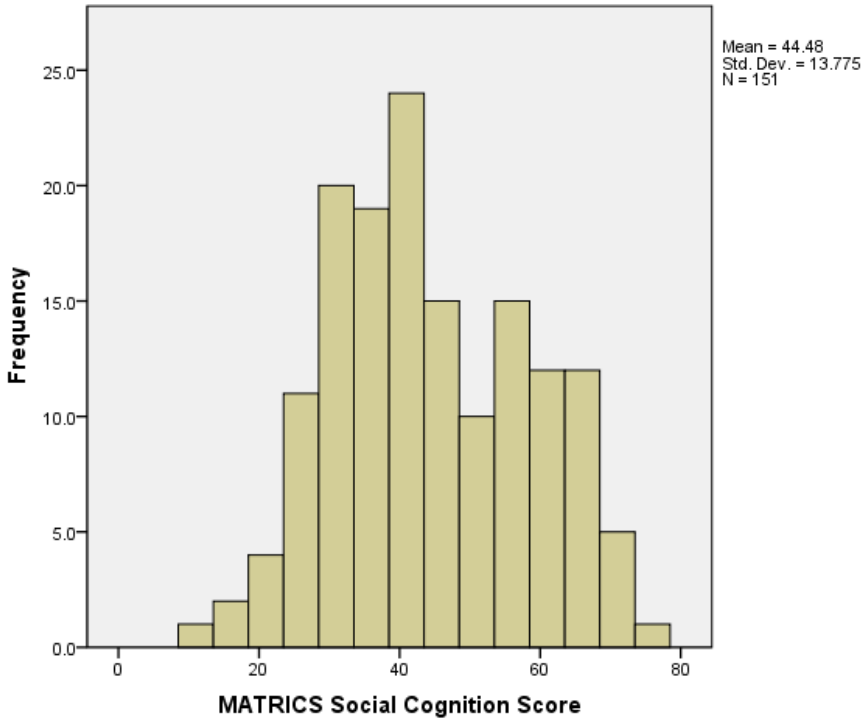


Figure 11: Distribution of MATRICS Composite Score

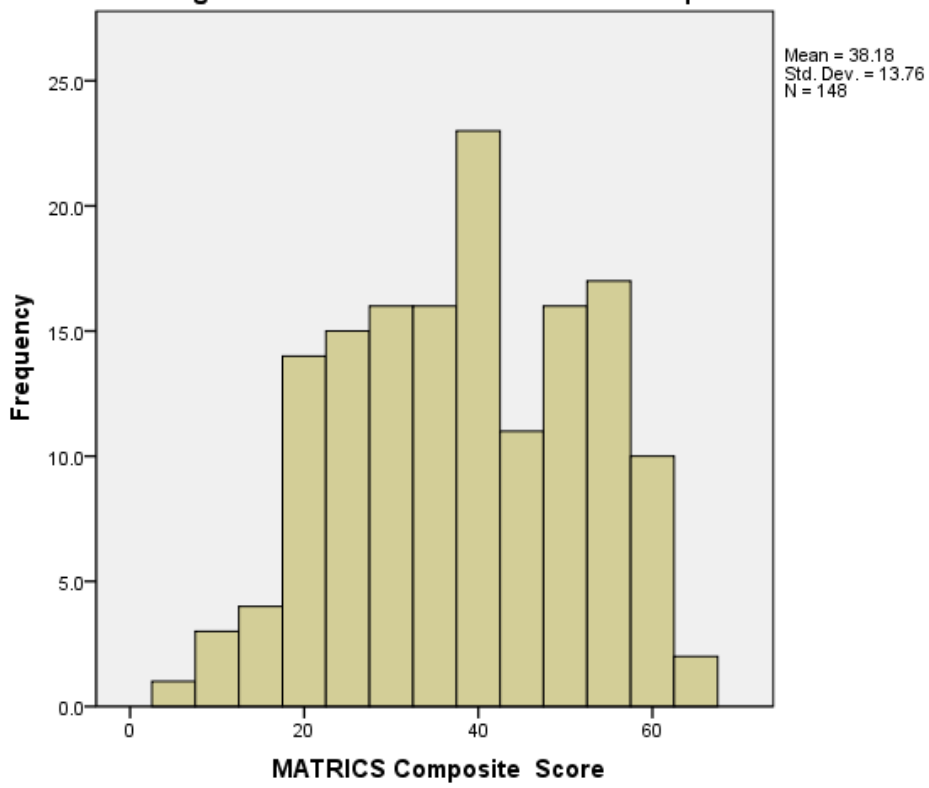


Figure 12: Distribution of PANSS Grand Total Score

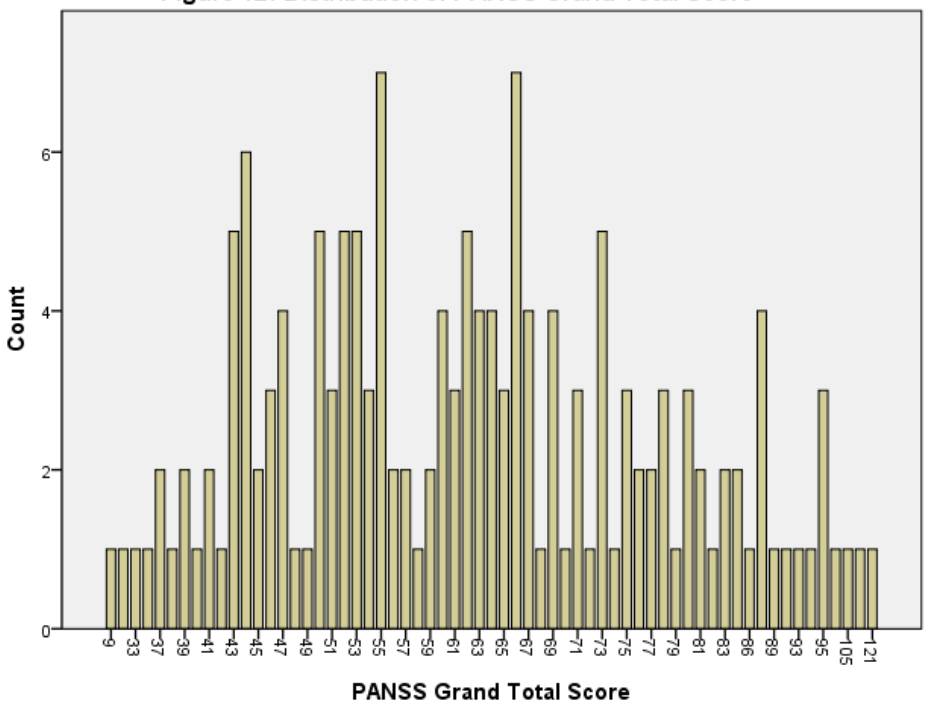


Figure 13 :Distribution of PANSS Positive Score

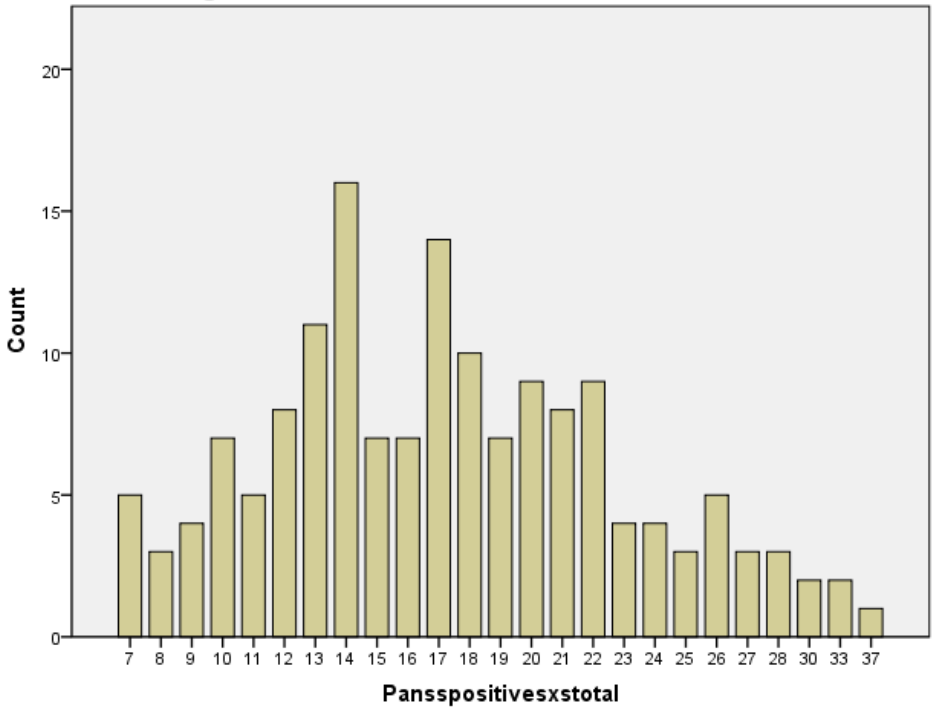
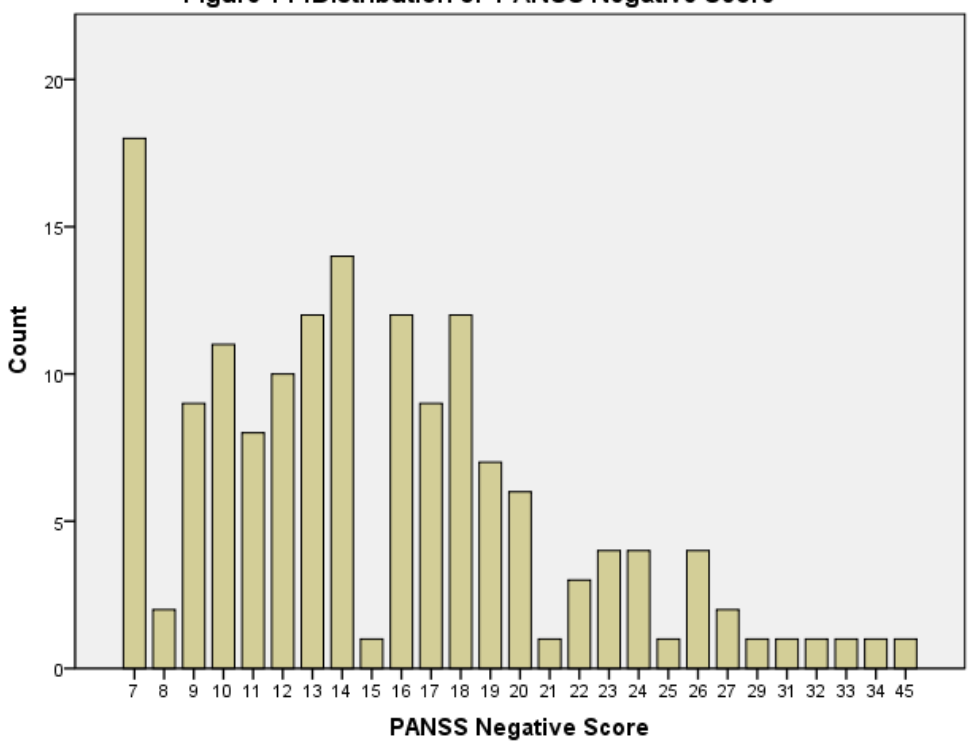


Figure 14 :Distribution of PANSS Negative Score



Tables

Table 1: Demographics of Study Population

Schizophrenic patients, N=169		*Controls, N=116*	
Sex		*Sex*	
Male	133 (79%)	Male	55 (47%)
Female	36 (21%)	Female	61 (53%)
Age in Years		*Age in Years*	
Mean	44.5	Mean	38.09
Minimum	20	Minimum	18
Maximum	66	Maximum	77
Race		*Race*	
White	49 (29%)	White	51 (44%)
Black	110 (65%)	Black	54 (47%)
Other	10 (6%)	Other	11 (9%)
Smoking Status		*Smoking Status*	
Smokers	82 (49%)	Smokers	104 (90%)
Non-Smokers	84 (51%)	Non-Smokers	12 (10%)
Medication Status			
Typical Antipsychotic	36 (21%)		
Atypical Antipsychotic	138 (82%)		

Table 2: Chi-Square Test of Significance For Smoking Status and Race by Toxo Status

Variable	DF	Chi-square value	p-value
Smoking Status	1	0.77	0.38
Race	2	0.35	0.84

Table 3: Cognitive Function Amongst Total Population, Stratified by Status as Toxo Positive or Negative

	Toxo Negative		Toxo Positive	
Variable	Mean (SD)	Range	Mean (SD)	Range
Matrics Variables				
Speed of Processing	N=139, 42.1 (13.9)	(4-74)	N=12, 35.3 (5.6)	(23-42)
Attention	N=138, 42.6 (11.9)	(15-70)	N=12, 39.8 (11.1)	(21-55)
Working Memory	N=140, 41.5 (11.7)	(7-78)	N= 12, 38.9 (8.9)	(21-54)
Verbal Learning	N=140, 42.8 (8.7)	(25-67)	N=12, 40.8 (12.8)	(27-64)
Visual Learning	N=140, 43.4 (13.9)	(7-68)	N=12, 40.4 (13.8)	(22-61)
Reasoning/Problem Solving	N=140, 44.5 (10.4)	(21-79)	N=12, 40.5 (6.3)	(31-53)
Social Cognition	N= 139, 45.0 (13.9)	(11-74)	N=12, 38.7 (11.3)	(23-67)
Matrics Composite Score	N=136, 38.7 (14.0)	(5-64)	N=12, 32.1 (9.6)	(19-49)
IQ	N=198, 99.8 (15.3)	(65-135)	N=26, 94.2 (12.7)	(67-130)
Finger Tap Score	N= 89, 46.4 (11.1)	(19.6-66.8)	N=14, 49.7 (6.3)	(40-64)
Wisconsin Card Sort Test	N=223, 12.01(8.7)	(3-46)	N=27, 12.8 (7.2)	(4-33)

Table 4: Cognitive Function Amongst Schizophrenic patients, Stratified by Status as Toxo Positive or Negative

	Toxo Negative		Toxo Positive	
Variable	Mean (SD)	Range	Mean (SD)	Range
MATRICS Variables				
Speed of Processing	N=81, 34.9 (11.6)	(4-68)	N=9 34.1 (5.8)	(23-42)
Attention	N=80, 37.1 (11.0)	(15-66)	N=9, 35.2 (8.7)	(21-44)
Working Memory	N=81, 36.5 (10.7)	(7-60)	N= 9, 37.6 (9.9)	(21-54)
Verbal Learning	N=81, 39.4 (7.4)	(25-58)	N=9, 39.0 (12.5)	(27-64)
Visual Learning	N=81, 37.9 (13.4)	(7-67)	N=9, 39.2 (14.9)	(22-61)
Reasoning/Problem Solving	N=81, 42.26 (9.9)	(21-70)	N=9, 39.2 (5.7)	(31-47)
Social Cognition	N= 80, 38.7 (11.2)	(11-69)	N=9, 34.1 (6.6)	(23-42)
Matrics Composite Score	N=79, 30.7 (11.1)	(5-56)	N=9, 28.4 (7.7)	(19-45)
Finger Tap Score	N= 34, 49.6 (10.0)	(19.6-65.6)	N=5, 49.9 (4.6)	(40.4-63.9)
Wisconsin Card Sort Test	N=93, 8.4 (4.6)	(4-46)	N=10, 11.0 (5.9)	(4-33)
IQ	N=65, 110.1 (11.2)	(65-135)	N=7, 94.3 (8.2)	(67-130)

Table 5: Cognitive Function Amongst Controls, Stratified by Status as Toxo Positive or Negative

	Toxo Negative		Toxo Positive	
Variable	Mean (SD)	Range	Mean (SD)	Range
MATRICS Variables				
Speed of Processing	N=58, 52.1 (10.1)	(30-74)	N=3, 38.7 (4.26)	(34-42)
Attention	N=59, 50.2 (8.6)	(20-70)	N=3, 53.7 (2.3)	(51-55)
Working Memory	N=59, 48.3 (9.4)	(31-78)	N= 3, 43.0 (2.0)	(41-45)
Verbal Learning	N=59, 47.5 (8.2)	(33-67)	N=3, 46.0 (14.9)	(35-63)
Visual Learning	N=59, 50.9 (10.6)	(19-68)	N=3, 44.0 (11.3)	(37-57)
Reasoning/Problem Solving	N=59, 47.7 (10.3)	(27-79)	N=3, 43.3 (8.5)	(37-53)
Social Cognition	N= 59, 53.6 (12.6)	(22-74)	N=3, 52.3 (12.7)	(44-67)
Matrics Composite Score	N=57, 49.8 (9.1)	(29-64)	N=3, 43.0 (5.3)	(39-49)
Finger Tap Score	N= 34, 49.6 (10.0)	(30.6-66.8)	N=5, 49.9 (4.6)	(44.3-55.2)
Wisconsin Card Sort Test	N=93, 8.4 (4.6)	(3-29)	N=10, 11.0 (5.9)	(5-20)
IQ	N=65, 110.1 (11.2)	(84-133)	N=7, 94.3 (8.2)	(80-105)

Table 6: Toxo Status (Positive or Negative)as a Predictor of Cognitive Variables Amongst Schizophrenic patients Using Linear Regression, Controlling for Age, Gender, and Serointensity

Variable	β	p-value	Adjusted R^2
Combined IQ	-.344	.131	.13
MATRICS Variables			
Speed of Processing	-.458	.045	.17
Attention	-.268	.458	.09
Working Memory	-.258	.481	.06
Verbal Learning	-.987	.007	.08
Visual Learning	-.247	.518	-.03
Reasoning/Problem Solving	-.038	.920	.02
Social Cognition	.140	.710	.02
MATRICS Composite Score	-.456	.209	.10

Table 7: Serointensity as a Predictor of Cognitive Variables Amongst Schizophrenic patients Using Linear Regression, Controlling for Age, Gender, and Positive or Negative Toxo Titer Status

Variable	B	p-value	Adjusted R^2
Combined IQ	0.298	0.191	.13
MATRICS Variables			
Speed of Processing	0.401	0.080	.17
Attention	0.298	0.412	.09
Working Memory	0.361	0.325	.06
Verbal Learning	1.049	0.005	.08
Visual Learning	0.311	0.417	-.03
Reasoning/Problem Solving	-0.020	0.956	.02
Social Cognition	-0.248	0.511	.02
MATRICS Composite Score	0.481	0.186	.10

Table 8: Toxo Status as a Predictor of Symptomatic Variables Amongst Schizophrenic patients Using Linear Regression, Controlling for Age, Gender, and Serointensity

Variable	β	p-value	Adjusted R^2
Symptomatic Scores			
PANSS Grand Total	-.082	.795	-.02
PANSS Negative Total	-.119	.702	.00
PANSS Positive Total	-.094	.765	-.02

Table 9:Toxo Status (Positive or Negative)as a Predictor of Symptomatic Variables Amongst Schizophrenic patients Using Negative Binomial Regression, Controlling for Age, Gender, and Serointensity

Variable	β	p-value	Chi-Square p-value
Symptomatic Scores			
PANNS Grand Total	0.044	0.732	0.29
PANNS Negative Total	-0.142	0.719	0.42
PANNS Positive Total	-0.090	0.759	0.35

*Chi-square p-value of the deviance and degrees of freedom. A significant p-value would give reason to reject the use of a negative binomial model.

Table 10: Serointensity as a Predictor of Symptomatic Variables Amongst Schizophrenic patients Using Linear Regression, Controlling for Age, Gender, and Positive or Negative Toxo Titer Status

Variable	β	p-value	Adjusted R^2
Symptomatic Scores			
PANSS Grand Total	0.122	0.699	-.02
PANSS Negative Total	0.254	0.416	.00
PANSS Positive Total	0.034	0.914	-.02

Table 11: Serointensity as a Predictor of Symptomatic Variables Amongst Schizophrenic patients Using Negative Binomial Regression, Controlling for Age, Gender, and Positive or Negative Toxo Titer Status

Variable	β	p-value	Chi-Square p- value
Symptomatic Scores			
PANNS Grand Total	-0.059	0.823	0.29
PANNS Negative Total	0.033	0.867	0.42
PANNS Positive Total	0.105	0.465	0.35

*Chi-square p-value of the deviance and degrees of freedom. A significant p-value would give reason to reject the use of a negative binomial model.

Table 12: Effects of Toxoplasmosis and Serointensity on Finger Tap Test Using a Poisson Regression Model Amongst Schizophrenic Patients

	Toxo			*Intensity*		
Variables in Model	B	RR	p-value	B	RR	p-value
Toxo, Age, Sex	0.082	1.09	0.049			
Toxo, Serointensity, Age, Sex	-0.2881	.75	0.239	0.224	1.25	0.081

Table 13: Effects of Toxoplasmosis and Serointensity on IQ Using a Linear Regression Model, Stratified by Schizophrenic Status

	Toxo		*Intensity*		
Variables in Model	B	p-value	β	p-value	Adjusted R^2
Controls					
Age, Sex					-0.022
Age, Sex, Toxo	-0.402	0.001			0.121
Age, Sex, Toxo, Serointensity	-0.486	0.176	0.09	0.803	0.109
Schizophrenic patients:					
Age, Sex					0.184
Toxo, Age, Sex	0.052	0.49			0.181
Toxo, Serointensity, Age, Sex	-.344	0.131	0.298	0.191	0.188

Table 14: Effects of Toxoplasmosis and Serointensity on Wisconsin Card Sort Test Using a Poisson Regression Model, Stratified by Schizophrenic Status

	Toxo			*Intensity*		
Variables in Model	B	RR	p-value	β	RR	p-value
Controls						
Toxo, Age, Sex	0.153	1.17	0.146			
Toxo, Serointensity, Age, Sex	0.324	1.38	0.04	-0.0022	1.00	0.173
Schizophrenic patients:						
Toxo, Age, Sex	-0.136	0.87	0.051			
Toxo, Sera Intensity, Age, Sex	0.515	1.67	0.062	-0.33	.72	0.017

Table 15: Interpreting the RR as a Measure of Magnitude of Effect of Toxo Status on WCST

Group	Toxo Status	RR	Number of Errors
Control	Negative	1	12.01*
Control	Positive	1.38	16.57
Schizophrenic	Negative	1	8.4*
Schizophrenic	Positive	1.67	14.02

*Number of errors based on mean sample for toxo negative sample

Table 16: Interpreting RR as a Measure of Magnitude of Effect of Serointensity on WCST

Group	Toxo Titer	Log-Transformed Unit of Serointensity	RR for unit of serointensity	Number of Errors
Schizophrenic	30^{+}	3.4	1	8.4
Schizophrenic	100	4.6	0.72	7.2

+': 30 is the lowest possible serointensity for a subject to be considered toxo positive