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Sydney Hubbard Date

The Effect of *Toxoplasma gondii* Infection on Prepulse Inhibition and Other Startle Responses: Implications for Schizophrenia Research

By

Sydney Hubbard

MPH

Department of Epidemiology

_____

Brad Pearce, Ph.D.

Committee Chair

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Sydney Hubbard

B.S., The University of Dayton, 2009

Thesis Committee Chair: Brad Pearce, Ph.D.

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ABSTRACT

The Effect of *Toxoplasma gondii* Infection on Prepulse Inhibition and Other Startle Responses: Implications for Schizophrenia Research

By Sydney Hubbard

Introduction: Affecting approximately one percent of the world's population, schizophrenia is a debilitating, psychiatric disease whose exact cause is unknown. There are many genetic and environmental etiologic theories behind schizophrenia. The present study examines a genetic-environmental interaction theory by gathering prepulse inhibition (PPI) and other startle response data as well as blood plasma samples on a group of schizophrenic cases and healthy controls. Prepulse inhibition is a type of endophenotype (quantitative, trait-related deficit in cognitive functioning) that could possibly be the key to linking an environmental exposure, such as the parasite *Toxoplasma gondii*, to a genetic susceptibility.

Methods: Forty-three schizophrenic patients from the Atlanta VAMC and sixy-one controls from the Atlanta community were sampled for this study. Each subject was put through a sensorimotor gating study to collect startle response data and had their blood drawn through standard methods. Hierarchically well-formulated, multivariate linear regression models were constructed to examine the effect of the main exposures, patient status and *Toxoplasma* exposure, on the startle outcome data. The R^2 statistic was used to measure the proportion of the variation in the dependent variable accounted for the by independent variables. P-values were also calculated to determine the significance of each term in each regression model.

Results: There were a total of nine *Toxoplasma* seropositive subjects in the study, two cases and seven controls. Overall, the regression models all had low R^2 statistics. The models with PPI as the outcome showed significant beta values in the covariates age and African American race. The patient status and *Toxoplasma* variables were closest to significance in the models with PPI at the interstimulus interval of 120 ms as the outcome.

Conclusions: Future studies will need a larger sample size to establish a significant difference between cases and controls for *Toxoplasma* seropositivity. Promising findings occurred with the 120 ms PPI outcome due to the large effect sizes of patient status and *Toxoplasma* variables that both showed trends towards deficits in PPI. These trends are consistent with the literature. Future studies with a larger sample size will need to focus on this interstimulus interval to reveal a significant association.

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TABLE OF CONTENTS

LIST OF TABLES

BACKGROUND

Schizophrenia

Schizophrenia is a debilitating psychiatric disease that affects approximately one percent of the world's population. The syndrome usually does not become overtly manifest until adolescence or usually early adulthood. However, in many cases subtle neurointegrative function impairments are present from birth. The symptoms can be divided into positive symptoms such as auditory hallucinations and delusions, and negative symptoms such as social withdrawal, flattened affect, poor motivation and depressed mood. Incidence of the disease is hard to measure, and the only global study that directly generated incidence data (WHO 10-nation study) found annual incidence to range from 16 to 40 per 100,000 per year. The point prevalence averages approximately 4.5 per population of 1,000 (36). Even though it has been studied for the past century, the exact etiology of schizophrenia is still unknown. Schizophrenia has been shown to run in families; thus, supporting the idea of a genetic etiology. Many other studies show environmental factors that are linked to a higher likelihood of developing schizophrenia. And still, many studies point to gene-environment interactions as the causation of the disease.

Etiologic Theories

Genetic Theories

It is well known that schizophrenia aggregates in families (23). Even though most of the cases occur sporadically, the chances of having the disease increase substantially when a family member has the disease. Twin, adoption, and family studies provide consistent evidence for the strong genetic component to the etiology of schizophrenia. In

twin studies, concordance rates between monozygotic twins are reported to be approximately 30-65% compared with 5-15% in dizygotic twins (24). Thus, if one twin has schizophrenia, the relative risk of the other having the disorder in monozygotic twins may increase to 65 and in dizygotic twins to approximately 5-15 (24). Greater concordance rates in monozygotic twins, who share 100% of their genetic material, compared to dizygotic twins, who share about 40-50% of their genetic material, points to a genetic component of schizophrenia but also indicates a non-genetic component. Adoption studies examine the risk of schizophrenia for offspring of parents with the disorder who were adopted by parents without the disorder, versus the risk of schizophrenia in offspring without the disorder adopted by parents with the illness. These studies consistently showed that the risk of schizophrenia was the greatest in the offspring of biological parents with the disease (37). These findings once again point to the genetic basis of the disorder.

Given the strong evidence for the genetic heritability of schizophrenia, researchers have been trying for years to determine the genetic architecture of this disease. Linkage studies and association studies are the two common means through which scientists have been trying to pinpoint a susceptibility gene(s) for schizophrenia. Linkage studies aim at finding a pattern of co-transmission of the disease in families with several affected members with a DNA marker whose chromosomal location is known (23). In other words, researchers try to identify the regions of the genome linked to the illness. Tandon et. al's meta-analysis identified these chromosomal regions as containing possible susceptibility genes (listed in descending likelihood): 2p12-q22, 5q23-q34, 3p25-p22, 11q22-q24, 6pter-p22, 2q22-q23, 1p13-q23, 22pter-q12, 8p22-p21, 6p22-p21,

20p20-p11, 14pter-q13, 16p13-q12, 18q22-qter, 10pter-p14, 1q23-q31, 15q21-q26, 6q15-q23, and 17q21-q24. The regions listed approximate 4,000 genes, which indicates the extreme imprecision of this approach. Furthermore, no linkage appears to be consistently replicable across large studies. This brings into question the suitability of this strategy for detecting a genetic contribution to schizophrenia.

Association studies can be conducted on unrelated individuals or related individuals (23). They evaluate the relationship between specific gene variants and the risk of developing schizophrenia. These studies pick up where linkage studies leave off. Linkage studies fail to pinpoint an exact susceptibility gene(s), which leaves the possibility that the disorder might be epigenetic (related to gene expression rather than sequence variation) (11). In association studies, variations in specific gene sequences are compared between individuals with and without schizophrenia. Variants found with significantly different frequency among those with schizophrenia are considered to confer susceptibility to the disease. The next steps are to examine if the protein products of the alleged “susceptibility gene” are expressed in the brain and to examine the brain’s function. Researchers then analyze whether the brain function is relevant to the illness. Across numerous studies, there is once again a failure to replicate the gene variants that could possibly cause schizophrenia. Thus, no particular gene variant can be ascertained as a definitive factor that would increase an individual’s risk of schizophrenia.

Environmental Theories

Despite strong evidence for a genetic component to schizophrenia, 85% of individuals with the disorder have no first-degree relative with the disorder (24). Thus, there must be some sort of environmental etiological factors at play with this disease.

Many studies have pointed to both biological and psychosocial factors that could occur in utero, in childhood/adolescence, and/or in early adulthood that could lead to schizophrenia. First, many studies have examined the extent to which maternal factors play a role in the development of schizophrenia. Prenatal infections have been shown to increase the risk of subsequent schizophrenia in offspring (28). A study by Brown et al. (c. 2001), showed a link between prenatal infection and executive dysfunction (as seen in schizophrenia) in adulthood. Before this study, no other studies were able to show a link between prenatal infection and the neurocognitive deficits seen in schizophrenia. Specific prenatal viral infections that other studies have found to be risk factors of schizophrenia include rubella and influenza. Other high risk infections include polio, measles, varicella-zoster, herpes simplex virus type 2, and other unspecified infections (5, 10, 26). Researchers theorize that these types of infections disturb development of the fetal brain which could lead to abnormalities in adulthood (5). Even bacterial infections could play a role in subsequent schizophrenia in offspring. The bacterial endotoxins, lipopolysaccharide (LPS), are likely to cause secondary inflammatory responses (i.e. cytokine induction) in the maternal plasma. Consequences of this maternal infection can contribute to altered fetal neurodevelopment (1). Aside from infections, other studies have found correlations between maternal nutritional factors and risk of adult schizophrenia (32). Deficiencies of several nutrients may give rise to maternal hyperhomocysteinemia (this condition can cause birth defects in pregnant women) (29). Obstetric complications have also been linked to an increased risk of schizophrenia in offspring (7, 13). Some abnormalities of pregnancy and delivery are associated with development of this disease perhaps due to hypoxia, which leads to fetal brain damage.

And lastly, some studies have found that paternal factors, such as advanced paternal age, can affect schizophrenic outcomes in offspring (8).

Other environmental risk factors that occur during pregnancy or in childhood are psychosocial. For instance, many studies have found a higher incidence of schizophrenia in people who grew up in urban environments. Along the same lines, there also appears to be a higher incidence of schizophrenia in migrant populations; that is, schizophrenia is more common among groups of peoples in new homelands (especially among the second generation born in the new land) as compared to native-born individuals (24). Other childhood factors that have been found to increase the risk of schizophrenia include: childhood trauma, head injury, parental separation or death, and adverse child-rearing (37). In adolescence, cannabis use is associated with an increased risk of schizophrenia. Substance abuse can lead to dysfunctions of dopaminergic, serotonergic, and glutamatergic neurotransmission, and these kinds of dysfunctions are associated with schizophrenia. Also, a range of premorbid impairments during adolescence and childhood are risk indicators of schizophrenia. Individuals who later develop schizophrenia have been found to perform lower on IQ tests and other standardized measures of intelligence in childhood. The lower the IQ, the higher is the risk for the later development of the disorder (24).

Gene-Environment Interaction Theories

Even with a large number of possible environmental risk factors for schizophrenia, none of the factors appear sufficient or necessary to have a single cause-effect relationship with the disorder (37). Thus, with the lack of complete genetic or environmental etiology of this disease, many researchers have turned to the possibility of

a gene-environment interplay in schizophrenia (31, 38). The predictive power of each environmental factor is low; that is, the majority of the people exposed to these environmental factors will not get the disease (2). Thus, with the proven heritability of this disorder, it is very likely that the environmental factors operate on genetic risk. A good example of this concept is the Finnish Adoptive Family Study (9). This study followed children of schizophrenic patients who were adopted away by mentally-healthy parents. The risk of developing spectrum disorders was higher in the group of children who were adopted into dysfunctional family rearing environments. This example illustrates that genes alone do not predispose individuals to the disorder. In this situation, there had to be an environmental catalyst as well. This same study also examined schizophrenia on the level of sub-clinical thought disorders. The researchers compared the adoptees (who had schizophrenic biological parents) to adoptees who had no particular known genetic risk. The adoptees with biologically schizophrenic parents did not have a higher risk for the disorder than their counterparts. However, the children of biologically schizophrenic parents were much more likely to display the subclinical thought disorder if they were adopted into families with high communication deviances. Once again, this study adds more weight to the gene-environment interaction theory of schizophrenia.

Toxoplasma gondii

To touch back on the possible environmental risk factors of schizophrenia, there is one particular infection that can occur in utero or in childhood/adolescence (or even adulthood) that reoccurs in the literature. Toxoplasmosis is caused by the ubiquitous intracellular parasite *Toxoplasma gondii*. First described in 1908, this parasitic infection

has been linked to a congenital syndrome that includes deafness, retinal damage, seizures, mental retardation, and intracranial calcifications (39). Postnatal transmission can produce lymphadenopathy and nonspecific symptoms of infection; however, most cases are thought to be asymptomatic (39). The primary hosts of this parasite are felines. It is in these animals that the sexual part of the *Toxoplasma* life cycle takes place. However, the parasite can infect and live in almost any warm-blooded animal, such as humans. The transmission of *Toxoplasma* from cats to humans occurs through the ingestion or inhalation of oocysts shed by infected cats into litter boxes, gardens, sandboxes, or other children's play areas. The organism is also transmitted by the consumption of undercooked meat from an animal that has been infected by a cat. Once it enters the body, *Toxoplasma* usually travels to and remains in the brain of its human host and persists there for the duration of that individual's life. Carriers of this parasite can be asymptomatic their entire life. In fact, in the United States, 1.5 million infections are estimated to occur annually, with 60% of them being asymptomatic (43).

Once it has gained access to a human host, *Toxoplasma*'s mode of action includes invasion ofvarious brain cells where it forms cysts (14). Since *Toxoplasma* displays an affinity for nervous tissue and is associated with congenital brain dysfunction, there has been much interest in the scientific community in investigating the link between this organism and psychiatric disorders (39). Serological methods for detection of *T. gondii* include immunoassays for serum immunoglobulin IgM antibody and elevation of IgG antibody. Elevated *Toxoplasma* IgG may reflect a recent or reactivated infection, but this immune marker can persist in the body for years or the entire lifespan. (12). IgM is a specific indicator of a recent infection (6). Across studies, seroprevalence of *T. gondii*

infection has been more frequently reported to be higher in schizophrenic patients than in control patients. A study done by Wang et al. (c 2006), compared the clinical manifestations of *Toxoplasma* seropositive schizophrenic patients with seronegative schizophrenic patients. This study found that the scores for the seropositive group were significantly higher on the positive symptom and excitement scales than the seronegative group. Likewise, the scores of the seropositive group were significantly lower on the negative component of the scale than the seronegative group; furthermore showing evidence for the link between this infection and specific symptoms of schizophrenia.

Early life infection (in utero and early postnatal exposure) is a concern with *Toxoplasma*. Studies have found an association between mothers with *Toxoplasma* infection and schizophrenic offspring later in life (6, 12). Mortensen et al. found that the evidence suggests a linkage between maternal prevalence of past infection with *T. gondii* and an increased risk of the development of schizophrenia in her offspring, but not a direct effect of an acute maternal infection (no association with IgM prevalence). One hypothesis for the effect of maternal latent infection (IgG) on the schizophrenia outcome is the direct effect of circulating maternal IgG has on fetal brain development. Another possible explanation is that the presence of maternal IgG indicates a lifestyle that is conducive to the contraction of the *Toxoplasma* parasite. For example, chronically infected mothers might have cooking practices that lead to food-borne infection of the parasite (under-cooked meat) (26). Thus, exposure of the proband would happen in childhood. Furthermore, a recent study found that the genotype of the parasite itself plays a role in schizophrenic outcome. Xiao et. al. found that the offspring of mothers with a serological pattern consistent with *Toxoplasma* type I infection were at significantly

increased risk for the development of psychoses compared to unaffected control mothers. In contrast, they did not find an association between maternal antibodies to other genotypes and risk of psychoses in the offspring.

Until recently, studies of the association between *Toxoplasma* and schizophrenia have always had one major limitation. The main limitation in these studies is the issue of causation. Most studies report the seroprevalence of patients who have already developed the disease. If blood samples were not taken before the illness began, it is difficult to ascertain if the parasite was actually present before the onset of the disease and is therefore causal. It is entirely plausible that the psychological aspects of schizophrenia led to a change in behavior in the patient that led to increased exposure to the parasite (e.g. eating raw meat), which led to a subsequent diagnosis of seroprevalence. In this instance, the *Toxoplasma* infection would not be causal. However, a recent study was released in 2008 that was a major breakthrough in the theory of the causality of *Toxoplasma*. Niebuhr et al. performed a study on a population of U.S. military personnel who had been discharged with a diagnosis of schizophrenia compared to healthy military personnel controls. What is different about this study compared to past studies is the fact that all military personnel regularly have their blood drawn (upon entry, once every two years, and upon dismissal). Thus, for this population, serum specimens were available from before and after diagnosis. Researchers in this study were therefore able to analyze individuals who were healthy prior to schizophrenia diagnosis. They were able to document that elevated levels of *Toxoplasma* antibody existed prior to symptom onset, making it unlikely that exposure was a result of schizophrenic behavior or that schizophrenia was purely a result of a genetic disposition to the disease. While this study

is a major breakthrough, it should be used as a stepping stone for more studies. Future studies should be undertaken that are more generalizable to female and nonwhite populations

Even though *Toxoplasma gondii* is believed to infect one-third of the world's population (14), not all of those people infected are afflicted with schizophrenia. Thus, even though this parasitic infection has been shown to be an environmental risk factor for this disease, other factors need to occur for schizophrenia to arise. It is this idea that bolsters the gene-environment interaction hypothesis mentioned earlier. Yet it is very difficult to pinpoint what kind of interaction with the human genome *Toxoplasma* might have. This difficulty might lie in the broad spectrum of schizophrenia. Genetic vulnerability and *Toxoplasma* infection may interact to produce distinct subsets of symptoms or neuropsychological impairments. Accordingly, schizophrenic patients who have been exposed to *Toxoplasma* may differ in their clinical profile from those who have not been exposed. Due to the broad schizophrenic phenotypes, it is very difficult to pinpoint the exact genetic architecture of the disease. However, if there were an intermediary step between genotype and phenotype, it might be easier to elucidate apathophysiological pathways leading to schizophrenia symptoms. .

Endophenotypes

It is for the above reasons that much effort has been devoted to studying endophenotypes. These endophenotypes are quantitative, trait-related deficits that are usually assessed by laboratory-based methods rather than clinical observation (3). They are also heritable but the magnitude of expression of endophenotypes is modulated by environmental factors. It is believed that endophenotypes will be easier to analyze than

that of the broad phenotypes of psychiatric disease (15). The endophenotype strategy can thus prove to be very useful in the understanding of the genetic architecture of schizophrenia, and the interaction of genetic variation with environmental factors, including infections. Another reason particular interest is being paid to these intermediary steps between genotype and phenotype is because genes predisposing an individual to a psychiatric disorder can be transmitted without expression (the clinical phenotype) (16). Thus, testing for certain endophenotypes would be a means to catch disorders before clinical manifestation of the symptoms. There are several criteria for viable endophenotypes (following Gottesman and Gould's rational): 1) the deficits in the endophenotype are associated with schizophrenia, 2) these deficits are heritable, 3) the endophenotype deficits are stable and trait-related, 4) the endophenotype and disorder show cosegregation, 5) the proband's specific endophenotype deficit is found at higher rates in the proband's relatives than in the general population (3, 20).

Three neurophysiologic endophenotypes are prepulse inhibition (PPI) of the startle response, P50 event-related potential suppression, and the antisaccade task for eye movements. Impaired performance in these endophenotypes has been demonstrated both in schizophrenic subjects and their clinically unaffected relatives, which provides evidence for the heritability of the endophenotype (17). Characterizing the genetic architecture of these endophenotypic measures is likely to help determine which of these measures contributes to the heritable risk of schizophrenia. Even if these endophenotypes turn out to be determined by multiple rather than single genes, their complex genetic architecture probably will still be simpler than the qualitative diagnostic category of schizophrenia (3). It is possible that *Toxoplasma* exposure is an effect modifier in the

relationship between endophenotype and schizophrenia diagnosis. Conversely, *Toxoplasma* infection may mimic some of the endophenotype due to its ability to infect the key brain areas related to these behaviors. The literature is lacking data on *Toxoplasma* infection and endophenotypes related to schizophrenia.

Prepulse Inhibition and Acoustic Startle Response

The endophenotype, prepulse inhibition (PPI) of the startle response, is of particular interest to many researchers due to its apparent heritability.. Patients with schizophrenia have trouble automatically screening or "gating" irrelevant thoughts and sensory information from their conscious awareness (18). These deficits in gating can lead to a sensory overload that usually results in the psychotic symptoms often seen in schizophrenia. An acoustic startle is a good example of irrelevant sensory information that could be gated. The acoustic startle response is a reflex contraction of the skeletal muscles in response to a sudden acoustic stimulus (18). When a non-startling stimulus is presented just before a startling acoustic stimulus, the result is an inhibition of the startle response amplitude (prepulse inhibition). This decrease in amplitude is used as a measure of sensorimotor gating. This gating inhibits the organism's response to additional incoming stimuli and is usually made manifest by a reduction in behavioral responses to these disruptive stimuli. Many past studies have found that schizophrenic subjects exhibit reduced PPI (25), and this common endophenotype could lead researchers to identifying genetic disease variants and possible environmental effect modifiers.

Another reason prepulse inhibition and acoustic startle responses are of particular interest to researchers stems from the fact that the modulation of these responses involves brain pathways and neurotransmitters known to be important in neuropsychiatric

disorders and to be affected by stress and emotion. It has been documented that schizophrenic subjects exhibit reduced PPI and also have a higher prevalence of *Toxoplasma gondii* infection. Therefore, it is plausible to hypothesize that this environmental agent that can be found in the brain cells of humans and other mammals might be playing a role in the abnormal brain function expressed in schizophrenia. This abnormal brain function is expressed in the form of deficits in PPI and other startle responses. The present study looks to explore the possible association between these endophenotypes and the environmental agent *Toxoplasma gondii*.

The protective effect of PPI has been well-documented in humans. For example, presentation of a weak acoustic stimulus 100ms before a startle-eliciting stimulus significantly reduces errors in an aiming task (22). In humans, PPI is usually documented in sensorimotor gating studies that measure change in the eyeblink reflex (one of the components of the startle reflex) (22). These studies usually evoke the "attention-to-prepulse" paradigm. In this paradigm, the prepulse stimuli are a series of high and low pitch tones of short and long durations. Patients are instructed to focus on one tone and tune the other out. Filion et al. demonstrated that participants who attended to the prepulse exhibited larger PPI at the prepulse lead time (time before startle stimulus) of 120 ms compared to participants who ignored the prepulse (22). The same effect was not observed for lead times of 60 ms and 240 ms, displaying the basic principle of "not too high, not too low" for lead times. Thus, in patients with schizophrenia, the inability to focus (attentional deficits) will result in deficient attentional modulation of PPI, which will be reflected in these sensorimotor gating studies (22).

Researchers have come across several variables that should be taken into consideration when looking for associations between PPI and schizophrenia. Swerdlow et al (c. 2006) found that PPI levels were associated with sex (higher in men than in women), medication status, and smoking (higher in smokers than in non-smokers). Medication status might be the most important factor to consider. Numerous studies have shown that schizophrenic patients on atypical antipsychotic medications experience an increase or normalization in PPI deficits. Thus, medication status must be taken into account when assessing this relationship.

To summarize, schizophrenia is a complex disorder with broad phenotypes that make it difficult to pinpoint its etiology. Over the past century, schizophrenia has been consistently studied with many different risk factors identified but no conclusive causes determined. Some studies point to genetic risk factors, others point to environmental factors, while more recent studies point to a genetic-environmental interplay. With recent interest in endophenotypes, the bridge between genotypes and phenotypes, more hope is arising for pinpointing genetic susceptibility genes and the environmental factors that interact with these genes. It is here that the present study picks up. Many studies acknowledge the relationship between the susceptibility or response to infection and genetic variation, yet, none have yet tried to find the association between this environmental factor and the closest quantifiable genetic measure (endophenotypes). The present study will assess a population of schizophrenic subjects and healthy controls for *Toxoplasma gondii* seropositivity. Then it will examine the relationship of this environmental risk with the endophenotype information gathered on each of the subjects (PPI and other acoustic startle responses from sensorimotor gating study). Adding a

heritable endophenotype to the analysis could help elucidate why some patients with *Toxoplasma* infection have the disorder and others do not. In the end, this work could shed light on a genetic susceptibility exposed by an environmental risk factor.

METHODS

Study Design

A case-control study was performed using data obtained from Dr. Erica Duncan, an attending at the Atlanta VA Medical Center; and Dr. Brad Pearce, a Research Associate Professor at Emory Rollins School of Public Health. This study was reviewed and approved by both Emory University and the Center for Disease Control and Prevention's IRBs.

Study Population

Dr. Brad Pearce collected approximately 80 blood plasma samples from schizophrenic patients and psychiatrically healthy control subjects in 2005. The schizophrenic subjects were sampled from The Atlanta VA Medical Center where Dr. Erica Duncan is an attending. Normal control subjects were recruited from the staff of the Atlanta VAMC, the Emory University community, and the surrounding Atlanta community. These individuals then participated in a sensorimotor gating study and gave informed consent to be part of the study and have their blood drawn. Individuals had to be in between the ages of 18 and 80 to participate.

Exclusion criteria for this study included: 1) substance abuse or dependence within the last six months (nicotine dependence will be allowed), 2) clinically significant medical illness, 3) history of neurological disease including head trauma, CNS infection, loss of consciousness, or seizure disorder at any age, 4) mental retardation, 5) known hearing impairment, 6) visual acuity (corrected if applicable) worse than 20/30 by eye chart screening. For female subjects, current pregnancy or current

hormonal contraception medication were an exclusion, since estrogen levels may influence acoustic startle.

To increase sample power, 24 more blood plasma samples of schizophrenic subjects and controls from 2009 were obtained under similar conditions from Dr. Pearce's lab.

For the purposes of this study, cases were defined as those patients from the Atlanta VAMC that were clinically diagnosed as schizophrenic or schizoaffective. Clinic controls were defined as psychologically health individuals with no history of mental illness.

Data Collection

All subjects underwent testing in a sensorimotor gating study and had their blood drawn. Age, race, and sex were recorded for each patient as well as their medical and psychological history, current symptoms, IQ, and medications. Table 1 shows a description of the sample. Information was also gathered on the psychiatric and medical history of each participant's family members. Additionally, Positive and Negative Syndrome Scale (PANSS) scores were determined for schizophrenic cases.

The research data collected from this population was as follows:

i. Clinical information obtained by medical history and psychiatric interview, symptom ratings, and cognitive testing.

ii. Clinical information obtained by chart review of the subjects' clinical records to help in clarifying diagnostic and course information.

iii. Urine for toxicology. The urine of all subjects was tested for the presence of street drugs of abuse. This data was collected solely for research purposes.

iv. Acoustic startle data was obtained from recordings of surface electrodes under the right eye and behind the right ear. Pulse alone trials consisted of a single startle stimulus followed by 250 ms of Electromyography (EMG) recording. Prepulse + pulse trials consisted of a prepulse stimulus, an interstimulus interval (ISI) of either 30 ms, 60 ms, or 120 ms, a startle stimulus, and 250 ms of EMG recording. This data was collected solely for research purposes.

v. Results of auditory screening. This is a standard audiology screening test done at baseline in order to assure that subjects have normal hearing. This was performed as part of the research protocol.

vi. Blood to be stored for future immunoassays and genetic testing. This was done as part of the research protocol.

Trained hospital personnel drew blood from each participant once using standard methods. Rarely, researchers had to contact study participants to obtain a second sample to verify results. These participants were allowed to decline further participation. These blood samples were then transported to the laboratory of Dr. Brad Pearce at Emory University to be stored and later analyzed.

All plasma samples were stored in sub 80◦C freezers in Dr. Pearce's lab at Emory. The samples were then shipped on dry ice to the CDC's Division of Parasitic Diseases Reference Diagnostic Laboratories for analysis. The measurements of immunoglobulin G (IgG) antibody levels to *Toxoplasma gondii* were performed using enzyme-linked immunosorbent assay (ELISA), a method of antibody detection. The ELISA consists of binding serum to solid-phase antigen and subsequent reactions with enzyme-labeled antihuman IgG and enzyme substrate (27). The amount of color generated in this reaction

was measured in optical density units against the reagent blank. Test results were calculated from a three-point curve comprised of: Calibrator 1 (high-point), Calibrator 2 (mid-point), and the reagent blank (zero/origin), using a point-to-point curve fit. The upper range of the curve was expanded by adding additional points. The results were obtained with the Bio-Rad Toxoplasma IgG EIA test. Samples with a result greater than or equal to 33 IU/mL (International Unites/mL) were considered positive for *Toxoplasma* infection. Samples with a result less than 33 IU/mL were considered negative for *Toxoplasma* infection.

Description of Variables

Startle responses are typically characterized by peak magnitude, latency (following stimulus) to onset, and latency to peak. These different characterizations of the startle response will be used as the outcome variables, while the rest of the variables will be examined as possible independent predictors.

Age: Classified as a continuous variable ranging from 18 to 80 years.

Gender: Classified as male or female (dichotomous variable coded as "1" for male and "0" for female).

Race: Classified as Caucasian (reference group), African American, or other race.

Smoker: Categorized as a dichotomous variable according to whether the subject was a current smoker or not (dichotomous variable coded as "1" for smoker and "0" for non-smoker).

Medication status: Categorized as no medications (reference group), atypical antipsychotic medications, typical antipsychotic medications, or both atypical and typical antipsychotic medications.

Standard Curve Result/Kit Result: Categorized as a dichotomous yes/no variable based on whether the subject tested positive for *Toxoplasma gondii* infection.

Patient status: Classified as a case if Schizophrenic or Schizoaffective, and classified as control if psychologically healthy.

Onset latency for pulse alone: Measured in milliseconds, the onset latency for pulse alone is the time that elapses from the startle stimulus (no prepulse) to the onset of the startle response.

Peak latency for pulse alone: Measured in milliseconds, the peak latency for pulse alone occurs from the startle stimulus (no prepulse) to the time it takes the startle response to reach its largest magnitude.

Pulse alone startle: The amplitude of the baseline startle response when there is only a startle stimulus and no prepulse priming. Startle amplitude was recorded in terms of digital units where each digital unit equals 1.221 microvolts.

PPI30: The inhibition of startle amplitude by a prepulse is expressed in the present study as a percent decrement. Therefore, the PPI30 variable refers to the amplitude inhibition in the 30 ms prepulse trials calculated as a percent decrement over the pulse-alone trials. See formula below:

PPI30 = 100 x [(pulse alone startle amplitude) - (30ms prepulse + pulse amplitude)]/(pulse alone amplitude)

PPI60: The PPI60 variable refers to the amplitude inhibition in the 60 ms prepulse trials calculated as a percent decrement over the pulse-alone trials. See formula below:

PPI60 = 100 x [(pulse alone startle amplitude) - (60ms prepulse + pulse amplitude)]/(pulse alone amplitude)

PPI120: The PPI120 variable refers to the amplitude inhibition in the 120 ms prepulse trials calculated as a percent decrement over the pulse-alone trials. See formula below:

PPI120 = 100 x [(pulse alone startle amplitude) - (120ms prepulse + pulse amplitude)]/(pulse alone amplitude)

*Note: It is possible to get negative values for the PPI outcome variables. When the startle amplitude of the prepulse + pulse amplitude is larger that of the pulse alone startle amplitude, the outcome will be negative. This phenomenon is regularly seen in trials with small interstimulus intervals.

Data Analysis

All statistical analyses in this study were performed using The SAS System, version 9.2.

Univariate (Descriptive) analysis: Univariate analysis of the variables was conducted to check for extreme values, outliers, unusual values, and normality of continuous variables (Table 2).

Preliminary univariate analyses showed that several of the continuous outcome variables were grossly skewed. The pulse alone startle response variable was positively

skewed. In order to normalize this outcome for analysis, a value of 10 was added to scale to the variable and then the variable was log transformed. The outcome variables PPI30, PPI60, and PPI120 were all negatively skewed. In order to normalize these outcomes, each variable was first reflected. Reflections of the negatively skewed variables required creating a new variable where the original value of the variable is subtracted from a constant that is equal to one plus the largest value of the original variable. Then each new variable was treated similarly to pulse-alone startle response in that the value 10 was added to scale to the variable and then the variable was log transformed.

Bivariate Analysis: The distribution of each categorical exposure variable was compared between *Toxoplasma* positive and *Toxoplasma* negative subjects utilizing the Mantel-Haenszel chi-square statistic and p-values. From each chi-square statistic and p-value, it was determined whether there was a significant difference in frequency between the seropositive and the seronegative. The mean values of each continuous outcome variable were compared between cases and controls by performing either a pooled or unpooled two-sample independent t-test. Pooled t-tests are used in situations where the two population variances are equal. The unpooled t-test is used when variances are unequal. For each comparison, a folded F-test for equality of variances was performed to determine whether the population variances were equal or not, and then to choose the appropriate two-sample t-test. The null hypothesis for the folded F-test is that the variances for the two populations are equal. Thus, if the p-value for the F-test is less than or equal to the significance level ($\alpha = 0.05$) then the null hypothesis is rejected. Rejecting the null hypothesis means that the unpooled t-test should be used to test for differences

between the two population means. Continuous variables must be normally distributed for these folded F-tests and t-tests.

Main Analysis: In the primary analysis, hierarchically well-formulated, multivariate linear regression models were constructed to examine the effect of the main exposures on the continuous, startle outcome data. The R^2 statistic was used to measure the proportion of the variation in the dependent variable accounted for the by independent variables. The adjusted R^2 was also reported as a more accurate measure of the goodness of fit/association for each model. P-values were also calculated to determine the significance of each term in each regression model. For consistency in data presentation, results derived from reflected variables are presented such that the direction (sign) of the effect is consistent with the original data (e.g. a negative regression coefficient would represent an inverse relationship between predicator and outcome variables in the original data).

RESULTS

Bivariate Analysis

Overall, nine of the subjects tested positive for *Toxoplasma gondii* infection (two schizophrenia cases and seven controls). The distribution of socio-demographic and psychiatric characteristics of the *Toxoplasma* positive individuals and *Toxoplasma* negative individuals can be seen in Table 1. In unadjusted analyses, there was no significant difference between *Toxoplasma* positive and *Toxoplasma* negative subjects across the various categorical variables.

Crude Analysis

Table 3 shows the results of the crude analysis of each of the main exposures of interest on each of the startle response outcome variables alone. Both *Toxoplasma gondii* exposure and schizophrenia proved to be insignificant as sole predictors of each of the six startle outcomes. The R^2 were also very low for each model, indicating poor fitting models. The model with the lowest p-value for the beta coefficient of the main exposure was *Toxoplasma* exposure regressed on the log transformation of the 120 ms prepulse startle response (p-value= 0.2229). This model also had the highest R^2 statistic.

Main Analysis

The primary analysis focused on six different startle response outcome variables: pulse alone startle, onset latency for pulse alone, peak latency for pulse alone, prepulse inhibition with the interstimulus interval of 30 ms, prepulse inhibition with the interstimulus interval of 60 ms, and prepulse inhibition with the interstimulus interval of 120 ms. For all analyses, significance was assessed at $\alpha = 0.05$. For each outcome, three hierarchally well-formulated models were built. The first model examined the effect of

the most relevant confounders (age, gender, race, and smoker status) alone on the dependent startle variable. The second model stepped in the patient status (case or control) to observe its affect on the model. This is designated as "Genetic Variable" to distinguish its contribution from *Toxoplasma* (the environmental variable), though there is no precise family history or genome analysis placed in these equations. The third model then stepped in the environmental exposure of interest (*Toxoplasma* seropositivity) to observe its impact on the model. Due to collinearity issues, interaction between patient status and *Toxoplasma* seropositivity was not able to be examined in the in the final step of the multivariate linear regression models. Also, the medication status variable was left out of the analyses due to some ambiguities in coding of the original data set for some cases, and thus too few individuals who were definitively medication-free. This variable will be reassessed and analyzed in future studies.

Pulse alone startle

In step one of the multivariate regression analysis (Table 4), the covariates age (p-value= 0.0009) and black race (p-value <0.0001) were all found to be significant when regressed on the log transformation of pulse alone startle along with the other confounders. With the addition of the patient status variable in step two, age (p-value= 0.0009) and black race (p-value <0.0001) were still found to be significant. Furthermore, when the environmental variable *Toxoplasma* was added in the final step, age (p-value= 0.0009) and black race (p-value <0.0001) remained significant. The adjusted R^2 value was highest for step one (0.2408) but stayed relatively constant in steps two and three (0.2330 and 0.2360, respectively). These adjusted R^2 values were also the highest of the six startle response outcomes examined.

Onset latency for pulse alone

In step one of the multivariate regression analysis (Table 5), the covariates age (p-value= 0.0380) and black race (p-value= 0.0089) were found to be significant when regressed on onset latency for pulse alone along with the other confounders. In steps two and three, these variables remained significant. The adjusted R^2 was highest for the step one regression model (0.0743).

Peak latency for pulse alone

For peak latency for pulse alone (Table 6), none of the covariates were significant in any of the steps. Overall, the adjusted R^2 values were very low for each model.

30 ms Prepulse Startle Response

In the multivariate regression analysis of the log transformed PPI30 outcome variable (Table 7), the covariate age was found to be significant and remained significant in each step (p-values 0.0291, 0.0347, 0.0366, respectively). The adjusted R^2 values were also very low in each of the models.

60 ms Prepulse Startle Response

In multivariate regression analysis of the log transformed PPI60 outcome variable (Table 8), none of the covariates were found to be significant even when the main exposures of interest were added at each step. Some of the variables were marginally significant. In steps two and three the gender variable had p-values of 0.0592 and 0.0570 respectively. Also in steps two and three, the patient status variable had p-values of 0.0853 and 0.0795, respectively. The adjusted R^2 values were also very low in each of the models.

120 ms Prepulse Startle Response

In the multivariate regression analysis of the log transformed PPI120 outcome variable (Table 9), the age covariate remained significant in all three models (p-values 0.0602, 0.0438, and 0.0374, respectively). Also, the covariate black race was significant in all three steps (0.0044, 0.0105, 0.0071, respectively). The adjusted R^2 values were also very low in each of the models.

DISCUSSION

According to a nationally representative study performed in 2003, the prevalence of *Toxoplasma gondii* infection in the United States is approximately 15.8% (44). Thus, in theory, the present study of 104 subjects should have yielded close to 16 *Toxoplasma* seropositive subjects for analysis when, in fact, only 9 subjects tested positive for the parasite (8.7% prevalence in the study population). Furthermore, meta-analyses of the link between schizophrenia and *Toxoplasma* infection suggest that schizophrenic cases have a much higher likelihood of infection over healthy controls. However, in the present study, 7 of the seropositive subjects were controls while only 2 seropositive subjects were actually schizophrenic subjects.

Due to the unexpected low number of *Toxoplasma* seropositive subjects in the study, a case-control analysis of the sample could not be performed. Instead, each startle response outcome was analyzed separately to see if there was an association between schizophrenia and/or *Toxoplasma* infection and the outcome. Of particular interest was the relationship of either schizophrenia or *Toxoplasma* infection to one of the PPI endophenotype variables.

To assess the strength of the linear relationship between each outcome and the independent variables, both the R^2 and the adjusted R^2 statistics were examined for each model. As the variables of interest (schizophrenia and *Toxoplasma* infection) were stepped into each model for each outcome, the adjusted R^2 was assessed as a quantitative measure to see the improvement in the fit of the model. For example, the present study found that the best fitting model was $\mathbf{Y = \beta_0 + \beta_1 Z_1 + \beta_2 Z_2 + \beta_3 Z_3 + \beta_4 Z_4 + \beta_5 Z_5 + E}$ where Y equals the log transformed pulse alone startle response, Z_1 equals age, Z_2 equals

gender, Z_3 equals smoking status, Z_4 equals black race, and Z_5 equals "other" race (Table 4). The covariates age and black race were both found to be highly significant in this model with pulse alone startle amplitude decreasing with age and being lower for African Americans. However, the effect size was relatively small for both. The adjusted R^2 for this model was 0.2408. There was still a good fit for this outcome variable even after the patient status and *Toxoplasma* variables were added (adjusted R^2 of 0.2330 and 0.2360, respectively). Thus, a portion of the variability in the log transformed dependent variable of pulse alone startle was explained by the predictors in the model. The models with prepulse inhibition (the endophenotype of interest) as the outcome variable all had very low adjusted R^2, leading to the conclusions that the addition of the independent variables (including our main predictors schizophrenia and *Toxoplasma*) offered little improvement in predictive power and perhaps not all of the relevant variables for this analysis were taken into account.

For the PPI outcomes, in addition to the main predictors of interest, certain confounders were consistently significant in the analysis of these outcomes. PPI is an indication of better sensorimotor gating. In this study, PPI increased and indicated better sensorimotor gating as age increased at both 30 ms and 120 ms interstimulus intervals. Gender had a marginally significant impact at the ISI 60 ms, but that may be an artifact because the ISIs of 30 ms and 120 ms had no impact. Smoking tended to yield better PPI, but most of these effects were not significant. Black race indicated significantly better PPI at the ISI of 120 ms but had no effect at other ISIs.

For the onset latency pulse alone startle response (measured in milliseconds), both age and black race were found to be significant predictors of this outcome. Onset latency

appears to increase with age, but the effect size of black race is much larger than age in these models. Peak latency pulse alone startle response (measured in milliseconds) showed similar trends to onset latency but was generally not significant.

For patient status, there was no effect at the ISI of 30 ms. Patient status trended towards worse PPI (deficits in sensorimotor gating) for the ISIs of 60 ms and 120 ms (p-values of 0.0853 and 0.1995, respectively). These trends are consistent with previous literature that shows deficits in gating and PPI in schizophrenic subjects. The other main exposure of interest, *Toxoplasma gondii*, was insignificant across all of the regression models. However, there were promising findings that occurred with the 120 ms PPI outcome variable (Table 9). Although the three models for this outcome showed poor goodness of fit, the *Toxoplasma* variable was the closest to significance when regressed on the log transformation of PPI120. And in particular, in this model *Toxoplasma* and patient status showed the largest effect sizes, both showing trends towards deficits in PPI for schizophrenic subjects and *Toxoplasma* seropositive subjects. These findings suggest that a future study with a larger sample size could reveal an association between these exposures of interest and PPI. Thus, future studies need to focus more on the relationship of both of these independent variables with the startle response associated with the prepulse interstimulus interval of 120 ms. This idea follows the principle set out in previous PPI studies of “not too high, not too low” for interstimulus intervals that also found 120 ms to be an appropriate ISI to observe startle response associations with independent predictors (22).

One of the primary objectives of this exploratory study was to determine if there was an association between our main predictor, *Toxoplasma* infection, and any of the

startle outcome variables (specifically the endophenotypes PPI30, PPI60, and PPI120). No study to date has looked at this association yet. In addition, the study initially aimed to determine if there was interaction between *Toxoplasma* infection and schizophrenia in the prediction of startle response. However, the data yielded very little goodness of fit within each of the models, suggesting little association between the independent variables and the outcomes. The most promising results in this study came in the analysis of the log transformed prepulse inhibition interstimulus interval of 120 ms where both patient status and *Toxoplasma* variables trended towards a deficit in PPI for schizophrenic subjects and *Toxoplasma* exposed subjects. Thus, in future studies particular attention should be given to this outcome variable, because while the main exposures were not significant the effect size of these exposures were the largest for PPI120 . Furthermore, future studies will need a much larger sample size to effectively display a higher *Toxoplasma* seroprevalence in schizophrenic cases over healthy controls. Once this disproportionate prevalence has been established, researchers can look more in depth into how this parasite impacts prepulse inhibition and how the interplay between *Toxoplasma* infection and schizophrenia (the gene-environment interaction) impacts PPI. It is important to remember that deficits in endophenotypes such as PPI have been proven in past studies to be heritable between schizophrenic subjects and their clinically unaffected relatives (17). It is this heritability and quantifiability that make endophenotypes the bridge between genotypes and phenotypes. As researchers begin to understand more about these endophenotypes, more hope is rising for the discovery of the genetic architecture of schizophrenia and environmental co-factors. If the gene-environment interaction etiologic theory of schizophrenia has some truth, it would be very important to gain a better

understanding of the interaction *Toxoplasma gondii* has with these susceptibility genes. Further exploration of the association between this parasite and deficits in endophenotypes such as prepulse inhibition could be a crucial first step in discovering gene-environment etiology in schizophrenia.

STRENGTHS AND LIMITATIONS

Strengths

The largest strength of this study was the robustness of the data gathered by both Dr. Erica Duncan and Dr. Brad Pearce over the course of many years. Very few studies have the ability to link such a range of psychological variables (from IQ and PANSS Scores to startle response times) with biological data (each subject had his/her blood drawn and urine specimens taken for testing). It was this unique data set that allowed for such a novel study to occur.

Limitations

Sample

Sample size was one of the main limitations of the present study. The sample size of 104 did not yield a significant difference in the number of seropositive subjects between schizophrenic cases and controls. Future studies will need a much larger sample size to first establish a significant difference in *Toxoplasma* infection between cases and controls before further analysis.

Generalizeability

Schizophrenic participants in the present study were all patients at the Atlanta Veteran Affairs Medical Center (VAMC). Since all of these subjects are veterans of foreign wars, it is hard to determine whether their schizophrenic symptoms can be attributed to the same etiologies as the rest of the general schizophrenic population. Perhaps their symptoms are more attributable other psychological traumas. Furthermore, the healthy controls were taken from the Atlanta community. Perhaps this population is also not representative of the general population. There could have be a lower regional

risk of *Toxoplasma gondii* exposure that ultimately led to a lower overall seroprevalence in the present study.

PUBLIC HEALTH IMPLICATIONS

Being able to find the link between an environmental exposure such as *Toxoplasma gondii* and a genetic susceptibility gene(s) for schizophrenia through endophenotype research would make a large impact in the world of public health. If the population at large were aware that an environmental agent could have a role in the pathogenesis of such a disorder, there would be more efforts towards primary prevention of *Toxoplasma gondii* infection (e.g. more thorough cooking of meat). Also, more research might be allocated to the discovery of new, more effective medications for the treatment of *Toxoplasma* infection. If our data supports the concept that *Toxoplasma* participates in the etiopathogenesis of even a small subset of schizophrenia cases, this could have major implications for treatment. For example, the recent discoveries of an etiologic connection between Helicobacter pylori and peptic ulcer disease, and between human papillomavirus and cervical cancer, have both resulted in major advances in treatment and prevention. Furthermore, the discovery of a schizophrenic susceptibility gene(s) affecting the connection with this infection could potentially lead to the ability to test for this disease biologically. Since the symptoms (phenotypes) of schizophrenia are so varied it would be advantageous to define subsets of patients with certain types of schizophrenia based on genetics and etiological mechanisms. Early diagnosis would lead to more effective treatment of this psychiatric disorder. Economically, a test that could detect schizophrenia early and target subtypes with respect to prognosis would greatly reduce the financial burden treating a disease with such a broad clinical spectrum. Thus, with greater awareness and primary prevention of one of the main environmental risk factors for schizophrenia and a better understanding/better diagnostic testing for the

genetic susceptibility gene(s), a greater number of cases of the disorder could be prevented or at least treated more effectively, which would greatly reduce both the health and economic burden of schizophrenia.

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TABLES

Table 1. Characteristics of a Cohort of 104 Patients at the Atlanta VA Medical Center by Patient Status Based on an on-going Sensorimotor Gating Study from 2005-2010

	***Toxoplasma* Positive**		***Toxoplasma Negative***		**Total Sample**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Patient Status						
Schizophrenic	2	22.22	41	43.16	43	41.35
Control	7	77.78	54	56.84	61	58.65
Gender						
Male	6	66.7	59	62.1	65	37.5
Female	3	33.3	36	37.9	39	62.5
Age, years						
20-24	0	0.0	11	11.6	11	10.6
25-29	2	22.2	9	9.5	11	10.6
30-34	1	11.1	9	9.5	10	9.6
35-39	1	11.1	10	10.5	11	10.6
40-44	1	11.1	8	8.4	10	9.6
45-49	2	22.2	17	17.9	19	18.3
50-54	0	0.0	12	12.6	12	11.5
55-59	1	11.1	10	10.5	11	10.6
60 and >	0	0.0	9	9.5	9	8.7
Race/Ethnicity						
Caucasian	2	22.2	42	44.2	44	42.3
African American	5	55.6	42	44.2	47	45.2
Other	2	22.2	11	11.6	13	12.5
Smoker						
Yes	1	11.1	22	23.2	23	22.1
No	8	88.9	71	74.7	79	76.0
Missing	.	.	2	2.1	2	1.9
Medication Status						
No Medications	7	77.8	55	57.9	62	59.6
Typical Antipsychotics	.	.	2	2.1	2	1.9
Atypical Antipsychotics	1	11.1	29	30.5	30	28.8
Atypical and Typical Antipsychotics	.	.	4	4.2	4	3.8
Missing	1	11.1	5	5.3	6	5.8

Table 2. Univariate Analyses of Startle Response Outcome Variables by Toxoplasma Seropositivity

	***Toxoplasma* Positive**			***Toxoplasma* Negative**			**Total Sample**		
Outcome Variable	Mean (SD)	Range	Interquartile Range	Mean (SD)	Range	Interquartile Range	Mean (SD)	Range	Interquartile Range
Pulse Alone Startle Response[1]	124.96 (136.04)	0.00 - 440.67	23.67 - 164.33	88.99 (103.37)	0.00 - 597.67	18.00 - 107.33	92.20 (106.35)	0 - 597.67	20.33 - 129.00
30ms Prepulse Startle Response[2]	41.56 (29.38)	8.32 - 100.00	22.19 - 58.12	26.62 (61.90)	-183.93 - 100.00	7.66 - 68.77	27.95 (59.78)	-183.93 - 100.00	12.39 - 67.84
60ms Prepulse Startle Response[2]	49.65 (45.50)	-20.24 - 100.00	16.46 - 95.85	50.00 (56.57)	-188.39 - 100.00	32.93 - 100.00	49.97 (55.46)	-188.39 - 100.00	31.54 - 100.00
120ms Prepulse Startle Response[2]	48.14 (39.95)	12.71 - 100.00	18.10 - 93.88	64.92 (46.77)	-114.85 - 100.00	51.00 - 100.00	63.43 (46.26)	-114.85 - 100.00	43.33 - 100.00
Onset Latency Pulse Alone Startle Response[3]	60.46 (20.56)	36.33 - 91.00	43.17 - 75.42	60.58 (16.50)	30.33 - 99.00	46.00 - 72.00	60.57 (16.76)	30.33 - 99.00	46.00 - 72.00
Peak Latency Pulse Alone Startle Response[3]	89.04 (12.03)	70.00 - 100.50	78.25 - 99.33	89.68 (16.78)	54.00 - 150.00	78.33 - 99.00	89.63 (16.36)	54.00 - 150.00	78.33 - 99.00

[1]amplitude measured in digital units

[2]percent decrement in amplitude

[3]milliseconds

Table 3. Unadjusted Regression Analyses of the Main Exposure Variables on the Startle Response Outcome Variables

	Toxoplasma gondii infection (alone)		Patient status (alone)	
Outcome Variable	β (Pr > |t|)	R^2	β (Pr > |t|)	R^2
Pulse Alone Startle Response[1]	0.25171 (0.4901)	0.0048	0.21052 (0.8399)	0.0004
30ms Prepulse Startle Response[2]	-0.29094 (0.8796)	0.0003	0.1613 (0.3361)	0.0105
60ms Prepulse Startle Response[2]	-0.04079 (0.8963)	0.0002	-0.10923 (0.5444)	0.0042
120ms Prepulse Startle Response[2]	-0.37585 (0.2229)	0.0127	-0.05327 (0.7652)	0.0027
Onset Latency Pulse Alone Startle Response[3]	-0.125 (0.9841)	0	-0.75506 (0.8342)	0.0005
Peak Latency Pulse Alone Startle Response[3]	-0.64126 (0.9164)	0.0001	-0.96188 (0.7847)	0.0009

[1]amplitude measured in digital units and log transformed (+10)

[2]percent decrement in amplitude reflected and log transformed (+10)

[3]measured in milliseconds

Table 4. Multiple Regression Analyses of Pulse Alone Startle Response**

Variable		Step 1 β (Pr > |t|)	Step 2 β (Pr > |t|)	Step 3 β (Pr > |t|)
Control Variables				
Age		-0.02518 (0.0009)	-0.02531 (0.0009)	-0.02548 (0.0009)
Gender		-0.06565 (0.7387)	-0.07714 (0.7063)	-0.09785 (0.6334)
Smoking status		-0.01308 (0.955)	-0.03614 (0.8875)	-0.01368 (0.9573)
Race*				
	Black	-0.98369 (<0.0001)	-0.97591 (<0.0001)	-1.00124 (<0.0001)
	Other	-0.28345 (0.3461)	-0.26794 (0.3882)	-0.30286 (0.331)
Genetic Variable				
Patient status			0.04951 (0.8271)	0.06963 (0.759)
Environmental Variable				
Toxoplasma gondii infection				0.37756 (0.2465)
	R^2	0.2791	0.2795	0.2900
	Adjusted R^2	0.2408	0.2330	0.2360

*reference group= white race

** the outcome variable "pulse alone startle" is an amplitude measured in digital units and log transformed (+10) for the purpose of this analy

Table 5. Multiple Regression Analyses of Onset Latency Pulse Alone Startle Response**

Variable		Step 1 β (Pr > |t|)	Step 2 β (Pr > |t|)	Step 3 β (Pr > |t|)
Control Variables				
Age		0.28706 (0.038)	0.29578 (0.0346)	0.2974 (0.0347)
Gender		-0.18808 (0.9589)	0.42302 (0.9119)	0.53056 (0.8906)
Smoking status		3.0217 (0.4823)	4.25401 (0.3815)	4.21461 (0.3886)
Race*				
	Black	10.24402 (0.0089)	9.80086 (0.0144)	9.91835 (0.0142)
	Other	6.66413 (0.2107)	5.89459 (0.2854)	6.04558 (0.2775)
Genetic Variable				
Patient status			-2.45121 (0.579)	-2.67929 (0.551)
Environmental Variable				
Toxoplasma gondii infection				-2.07465 (0.7352)
	R^2	0.1269	0.1302	0.1314
	Adjusted R^2	0.0743	0.0665	0.0563

*reference group= white race

**the outcome variable "onset latency pulse alone" is measured in milliseconds

Table 6. Multiple Regression Analyses of Peak Latency Pulse Alone Startle Response**

Variable		Step 1 β (Pr > |t|)	Step 2 β (Pr > |t|)	Step 3 β (Pr > |t|)
Control Variables				
Age		0.12366 (0.3892)	0.12364 (0.3953)	0.12498 (0.3932)
Gender		-0.09789 (0.9796)	-0.09931 (0.9803)	-0.01036 (0.998)
Smoking status		-0.67742 (0.8805)	-0.68029 (0.8938)	-0.71287 (0.8894)
Race*				
	Black	2.62288 (0.515)	2.62391 (0.5261)	2.72109 (0.515)
	Other	5.55266 (0.3194)	5.55445 (0.3379)	5.67933 (0.3316)
Genetic Variable				
Patient status			0.00571 (0.999)	-0.18294 (0.9691)
Environmental Variable				
Toxoplasma gondii infection				-1.71599 (0.7902)
	R^2	0.0171	0.0171	0.0179
	Adjusted R^2	-0.0422	-0.0549	-0.0670

*reference group= white race

**the outcome variable "peak latency pulse alone" is measured in milliseconds

Table 7. Multiple Regression Analyses of 30ms Prepulse Startle Response**

Variable		Step 1 β (Pr > |t|)	Step 2 β (Pr > |t|)	Step 3 β (Pr > |t|)
Control Variables				
Age		0.01476 (0.0291)	0.01444 (0.0347)	0.01438 (0.0366)
Gender		-0.01319 (0.9409)	-0.03532 (0.8501)	-0.03954 (0.8339)
Smoking status		0.12276 (0.5585)	0.07813 (0.7417)	0.07967 (0.7383)
Race*				
	Black	0.08718 (0.6417)	0.10323 (0.5915)	0.09862 (0.6115)
	Other	0.06102 (0.8135)	0.0889 (0.7408)	0.08297 (0.7596)
Genetic Variable				
Patient status			0.08879 (0.6807)	0.09773 (0.6561)
Environmental Variable				
Toxoplasma gondii infection				0.08139 (0.786)
	R^2	0.0689	0.0708	0.0717
	Adjusted R^2	0.0128	0.0028	-0.0085

*reference group= white race

**the outcome variable "30 ms Prepulse Startle Response" is presented as percent decrement in amplitude. For the purpose of this analysis, it was reflected and log transformed (+10)

Table 8. Multiple Regression Analyses of 60ms Prepulse Startle Response**

Variable	Step 1 β (Pr > |t|)	Step 2 β (Pr > |t|)	Step 3 β (Pr > |t|)
Control Variables			
Age	-0.00846 (0.2359)	-0.00707 (0.3189)	-0.00697 (0.3286)
Gender	0.27569 (0.149)	0.37355 (0.0592)	0.3803 (0.057)
Smoking status	0.19276 (0.3896)	0.39012 (0.119)	0.38765 (0.1233)
Race*			
Black	0.19471 (0.3308)	0.12373 (0.5395)	0.1311 (0.5195)
Other	-0.15789 (0.5675)	-0.28113 (0.3196)	-0.27167 (0.3401)
Genetic Variable			
Patient status		-0.39257 (0.0853)	-0.40687 (0.0795)
Environmental Variable			
Toxoplasma gondii infection			-0.13005 (0.6789)
R^2	0.0801	0.1129	0.1148
Adjusted R^2	0.0246	0.0480	0.0383

*reference group= white race

**the outcome variable "60 ms Prepulse Startle Response" is presented as percent decrement in amplitude. For the purpose of this analysis, it was reflected and log transformed (+10)

Table 9. Multiple Regression Analyses of 120ms Prepulse Startle Response**

Variable		Step 1 β (Pr > |t|)	Step 2 β (Pr > |t|)	Step 3 β (Pr > |t|)
Control Variables				
Age		0.03049 (0.0602)	0.03285 (0.0438)	0.03369 (0.0374)
Gender		0.14812 (0.7297)	0.31347 (0.4825)	0.36955 (0.4058)
Smoking status		0.28806 (0.5685)	0.62149 (0.2733)	0.60095 (0.2855)
Race*				
	Black	1.316 (0.0044)	1.19609 (0.0105)	1.25736 (0.0071)
	Other	0.3933 (0.5281)	0.18508 (0.7728)	0.26381 (0.6792)
Genetic Variable				
Patient status			-0.66325 (0.1995)	-0.78218 (0.1323)
Environmental Variable				
Toxoplasma gondii infection				-1.08186 (0.2855)
	R^2	0.1388	0.1560	0.1800
	Adjusted R^2	0.0870	0.0943	0.1092

*reference group= white race

**the outcome variable "120 ms Prepulse Startle Response" is presented as percent decrement in amplitude. For the purpose of this analysis, it was reflected and log transformed (+10)

EMORY UNIVERSITY | Institutional Review Board

TO:
Erica
Duncan,
MD
Principal
Investigator

CC:

Gross	Robin	Psychiatry - Main
Egan	Glenn	Psychiatry - Main
Pearce	Bradley	Epidemiology

DATE: November 5, 2010

RE: **Notification of Amendment Approval**
AM6_IRB00021861
Amendment 6 for IRB Study #IRB00021861
Sensorimotor gating in schizophrenia

This is your notification that your above referenced amendment was reviewed and APPROVED by the IRB on 11/4/2010.

Changes to Protocol Document(s): Merit Genetics Immune Addendum revised 10/21/2010
This is a VA study and the amendment must also be approved by the VA.

All correspondence and inquiries concerning this research study must include the IRB ID, the name of the Principal Investigator and the Study Title.

Sincerely,

Jim Henderson, MA, CIP
Sr. Research Protocol Analyst
VA-Emory IRB Liaison
This letter has been digitally signed

ver
sity
1599 Clifton Road, 5th Floor - Atlanta,
Georgia 30322
Tel: 404.712.0720 - Fax: 404.727.1358 - Email: irb@emory.edu - Web:
http://www.irb.emory.edu/
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11/5/2010