A PROPOSED MECHANISM FOR CEREBRAL TOXOPLASMOSIS AS A CONTRIBUTING FACTOR IN SCHIZOPHRENIA

By

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Abstract

Schizophrenia is a devastating mental disorder that affects around 1% of the world's population, characterized by the presence of positive symptoms including hallucinations and delusions, negative symptoms including depression and anxiety, and cognitive impairment including deficits in speech and memory. The complete etiology of schizophrenia is not yet understood, though it is known that both genetics and environmental factors play a role. One environmental factor, a chronic cerebral infection by the parasite *Toxoplasma gondii*, has one of the highest correlations with schizophrenia of any environmental factor, and may play a role in the pathology of the disease. This is especially true in the case of Type I toxoplasma, which is the most virulent of the three common strains of the parasite. Toxoplasmosis causes an increase in dopamine levels in the striatum and substantia nigra through the production of two enzymes that mimic the rate limiting enzyme in dopamine synthesis, tyrosine hydroxylase. Increased dopamine concentrations in these areas are experimentally correlated with positive schizophrenia symptoms. In addition, toxoplasmosis causes chronic upregulation of the kynurenine pathway via INF-γ release, leading to chronically elevated kynurenic acid levels. This leads to dysfunction of the glutamatergic system via (1) the binding and inhibition of α7-nicotinic receptors, leading to decreased GABAergic inhibitory activity in the hippocampus and decreased glutamate release in the prefrontal cortex, and (2) NMDA and AMPA receptor hypofunction, causing decreased inhibitory signaling by GABAergic neurons leaving glutamatergic neurons in a hyper-excitable state. These mechanisms, compounded by commonly identified mutations in the genes of schizophrenic individuals affecting the dopaminergic system, the kynurenine pathway, α7-nicotinic receptors, and the glutamatergic system, create a viable theory as to how the interplay between genetics and toxoplasmosis could cause schizophrenia.

Introduction:

Schizophrenia is a devastating mental illness that prevents sufferers from living normally, even with expensive and intricate combinations of medications. Schizophrenia sufferers experience a combination of positive symptoms (hallucinations and delusions), negative symptoms (depression and apathy), and cognitive impairments (poor memory and low IQ) (DSM IV 2000). The pathological mechanisms underlying the symptoms of schizophrenia are not fully understood, although many investigators have proposed theories to attempt to explain the etiology of the disease. The prevailing theory for the last 50 years has been the dopamine theory, which proposes that schizophrenia symptoms are caused by either an increase in dopamine or an increase in dopamine receptors in the brain. However, a newer theory, the glutamate theory, suggests that loss of glutamate mediated activation of glutamate receptors is the primary cause of symptoms. This theory states that reduced signaling through glutamate receptors via (1) limited glutamate release through decreased a7-nicotinic acetylcholine receptor (α7-nAchR) functionality, (2) N-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) inhibition, or (3) a reduced number of NMDA or AMPA receptors, is directly and indirectly responsible for all of the symptoms of schizophrenia (Howes 2015). The abnormal function of these systems is thought to be caused by heritable gene mutations in combination with environmental factors (Gejman 2010).

Evidence has been found that chronic cerebral toxoplasmosis may be one of these environmental factors, with correlational links to behavioral changes and mental disorders, in particular, schizophrenia (Jones-Brando 2003, Webster 2006, Torrey 2007, Goodwin 2008, Goodwin 2011, Torrey 2012, Horacek 2012, Smith 2014, Sutterland 2015, Fabiani 2015). *Toxoplasma gondii* (*T. gondii*) is a protozoan parasite that affects an estimated one-third of the world's population, and causes the chronic condition toxoplasmosis. In the majority of cases, infection with *T. gondii* is asymptomatic and remains undetected. Until recently, infection with *T. gondii* was thought to be innocuous unless it occurred in pregnant or immunocompromised individuals. However, current research has overwhelmingly concluded that toxoplasmosis is far more insidious than originally theorized (Montoya & Liesenfeld, 2004). One of the three strains, Type I toxoplasma, was found to be especially virulent and effective at causing altered gene expression (Sibley & Howe, 1995 & Xiao 2011).

Research indicates that chronic toxoplasmosis disrupts dopaminergic and glutamatergic signaling in the brain, which, coupled with genetic mutations commonly found in schizophrenic individuals, could potentially account for all of the symptoms of schizophrenia, negative, positive, and cognitive. After invasion of host cells, toxoplasma causes alterations in the

expression levels of thousands of genes, culminating in dysfunction in the levels of numerous neurotransmitters and disruption of the function of many neuronal receptors (Xiao 2011, Hill 2012, Xiao 2013). The most significant of these changes are (1) the immune response to parasitic invasion initiating the release of INF-ɣ and (2) the production of tyrosine hydroxylase by toxoplasma parasites. Among the 200 known effects of INF-ɣ is the upregulation of the kynurenine pathway through the overproduction of Indoleamine-2,3 Dioxygenase (IDO) and Tryptophan-2,3 Dioxygenase (TDO2), the rate limiting enzymes of the pathway. This decreases the amount of tryptophan available to the active stage of the parasite (tachyzoite), thereby starving it and forcing it into its slow-growing encysted (bradyzoite) form (Lang 2007). The other effect of upregulating the kynurenine pathway is an increase in the concentrations of the metabolites formed during its intermediate steps, of which kynurenic acid (KYNA) remains elevated chronically. KYNA directly inhibits multiple glutamate receptors including NMDA, AMPA, and α7-nicotinic receptors. The inhibition of which has been shown to cause both positive and negative symptoms of schizophrenia (Moroni 2012). Tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis is produced by the encysted parasites, causing a chronic increase in dopamine synthesis and release. Increased levels of dopamine have been consistently linked to psychosis (Connell 1957, Lieberman 1987). Therefore, through the disruption of receptor function via increased kynurenine metabolites and increased tyrosine hydroxylase in the brain, coupled with pre-existing genetic mutations, toxoplasmosis could lead to the manifestation of the various symptoms of schizophrenia.

Schizophrenia:

Schizophrenia is a complex disorder characterized by a number of clinical symptoms that can be broadly classified into three categories: negative symptoms, positive symptoms, and cognitive impairment. Negative symptoms can include, from most to least common: anhedonia (the inability to feel pleasure or interest in relationships or activities), flattening or blunting of emotional responses, decrease in motivation or apathy, and alogia (a lack of speech or meaningful speech caused by disordered thinking). Negative symptoms often manifest in social withdrawal or isolation (DSM IV 2000).

A core feature of schizophrenia that was initially classified as a negative symptom, but is now seen as distinct, is cognitive impairment. Individuals with schizophrenia often have a markedly lowered IQ as compared to the population at large, and cognition tends to worsen over time (Kupfer 2015). Negative and cognitive symptoms are often overlooked in the mainstream

perception of schizophrenia, though both categories of symptoms must be present for clinical diagnosis of the disease (DSM IV 2000)

The positive symptoms of schizophrenia are what most people tend to think of as the main attributes of the disease. These symptoms are broken into four categories. From most to least common these are: (1) delusions, the belief in something that is contradicted by reality, (2) bizarre behaviors, including an impairment of social skills, strange appearance, hoarding, poor hygiene, dysfunctional sexual behaviors, aggression during a psychotic episode, and/or repetitive behaviors, (3) hallucinations, which can include any of the senses, and (4) disordered thought, mainly affecting speech patterns (DSM IV 2000).

Schizophrenia tends to occur in multiple stages (Fig. 1). The prodromal stage is when subtle iterations of common schizophrenia symptoms may occur before the acute stage of the disease. These predictive symptoms tend to present as milder forms of acute stage symptoms including: cognitive decline, psychomotor disturbances, decrease in recognizing self-identity and agency, difficulty in maintaining social norms, reduction in motivation, speech

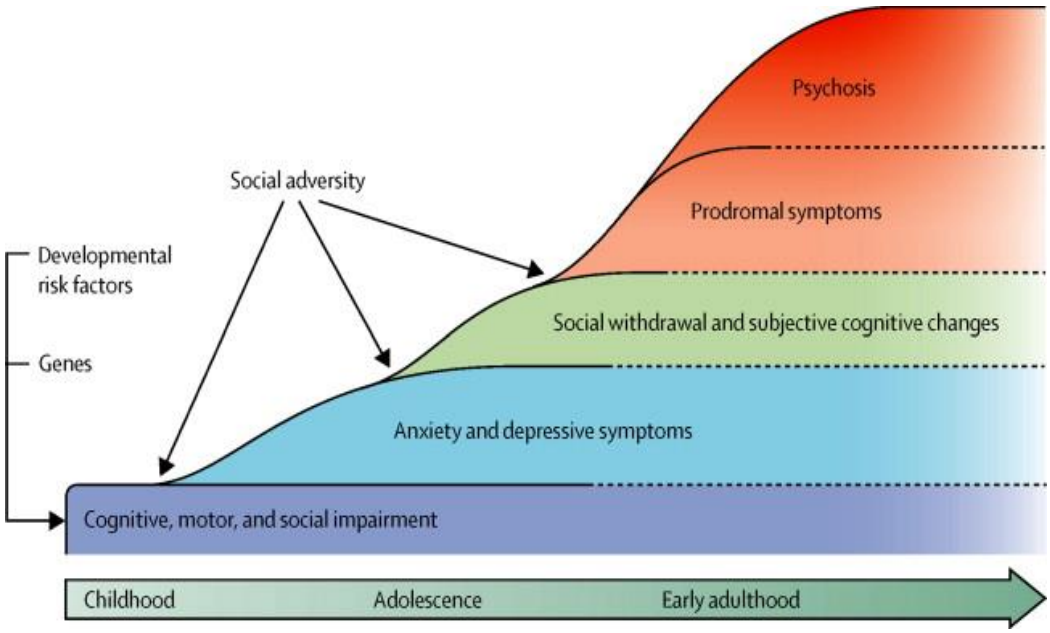

Fig. 1 Disease progression of schizophrenia.
From Howes & Murray 2014

abnormalities, difficulties in processing emotion, abnormalities in perception, decreased ability to concentrate, excessive suspicion, and mild disorganization of thought. Additional physical symptoms may also appear including decreased insulin sensitivity, impaired eye tracking, substance abuse, and changes in brain structure, function, and chemistry. The acute stage of

schizophrenia includes mainly positive symptoms and may include episodes of euphoria, anxiety, or depression. During this stage, the prevalence of suicide and violent outbursts are the highest. Chronic schizophrenia is characterized by negative symptoms, where an individual may neglect their hygiene or responsibilities, and may have long periods of inactivity. These individuals are incapable of caring for themselves and require assistance for common tasks like cleaning and feeding (Wright 2012). The risk of suicide is drastically increased in schizophrenia sufferers as compared to the unaffected population, with 20% of sufferers attempting suicide at some point in their life. In addition, 25% of schizophrenia diagnoses are comorbid with depression, and up to 50% are comorbid with substance abuse, the most common of which is nicotine addiction (Kupfer 2015).

Epidemiology and Demographics of Schizophrenia:

Schizophrenia is a disease that is difficult to diagnose and thus is often underdiagnosed. It is estimated that schizophrenia affects approximately 1% of the world's population, with a lifetime prevalence of 4.0 per 1,000 individuals (Fig. 2). Developed countries have higher rates of diagnosis as compared to less developed countries, likely due to the stigma associated with diagnosis or a lack of education about the disease in these countries (Peluso 2008, Massuda 2011). Men have a slightly higher incidence of schizophrenia, with a ratio of 1.4 to every 1 woman diagnosed (Owen, 2016).

Though schizophrenia is known to have a genetic basis with an estimated heritability of up to 80%, it is important to note the role environmental factors play in the incidence of disease (Gejman 2010). From before conception to adolescence, numerous risk factors have been found

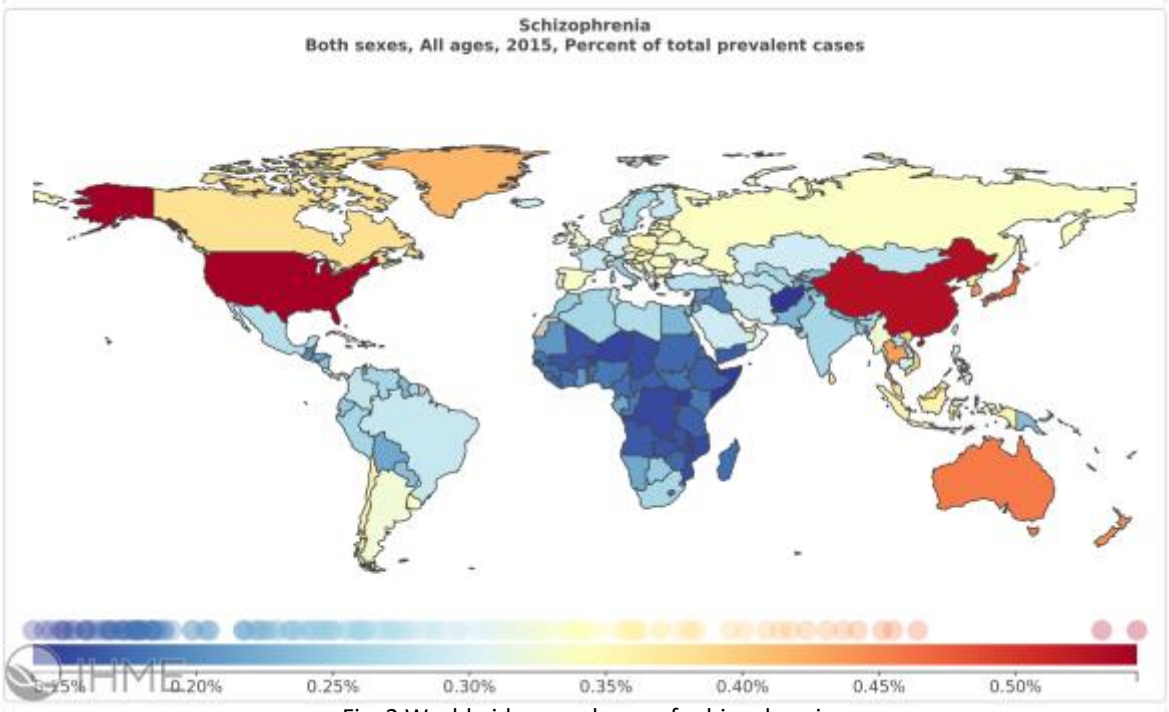

Fig. 2 Worldwide prevalence of schizophrenia
From The Institute for Health Metrics and Evaluation 2016

to have some correlation with schizophrenia incidence, leading to the theory that the disease requires a combination of genetics and environment to arise (Brown 2012).

During pregnancy, the central nervous system is vulnerable to a variety of factors as the fetus develops. Recently one prenatal factor, maternal exposure to infectious agents, has become a topic of great interest to researchers. Herpes simplex virus, polio, rubella, influenza, genital and reproductive infections, respiratory infections, and particularly *T. gondii* infections have all been found to increase schizophrenia incidence, as well as the incidence of numerous other mental disorders, throughout the child's lifetime (Brown 2012). Additional prenatal risk factors include maternal stress or anxiety, which could arise during acute infection with any of the above diseases (Khashan 2008, Opler 2013).

Postnatally, risk factors include the environment in which an individual was raised, and internal conditions such as inflammation arising from a number of autoimmune disorders or chronic infections (Benros 2014). Environmental factors can also include the use of psychoactive drugs including LSD, cannabis, PCP, or other mind altering substances (Moore 2007). In addition, events during childhood that have significant odds ratios in increased schizophrenia risk are immigration, traumatic brain injury, living in an urban setting, low socioeconomic status, and being born in winter or spring (Cantor-Graae 2005, Orlovska 2014, Dealberto 2010, Byrne 2004, Mortenson 1999, Torrey 2012).

Many of the risk factors correlated with the onset of schizophrenia are identical to those found in individuals with chronic toxoplasmosis. As both diseases are underreported in less developed countries where toxoplasma flourishes due to a lack of access to medical professionals, societal beliefs about mental illness, or the asymptomatic nature of most toxoplasmosis infections, the risk factors of both diseases are speculative at best. To draw conclusions about which risk factors are of real significance, far more data, better diagnostic methods, and more complete histories of affected individuals would need to be gathered. Given the nature of both diseases, this is unlikely and so the similarities in risk factors will, for now, remain an interesting side note in the investigation into the link between the diseases, rather than conclusive proof.

Neuroanatomical Structures Involved in Schizophrenia:

To fully understand the mechanism by which toxoplasma may contribute to schizophrenia symptoms, it is important to understand the functions and interconnectivity between the structures in the brain that have been implicated in schizophrenia, namely: the prefrontal cortex, the basal ganglia, and the limbic system. Within each of these systems,

specific structures are thought to have more significant roles in the pathology of schizophrenia than others. The prefrontal cortex makes up the anterior portion of the brain and is divided into three areas, the dorsolateral, orbital, and mesial, all which control functions that are disrupted in schizophrenia. The dorsolateral area is involved in (1) planning and (2) organizing behavior and memories for encoding and retrieval. In humans, this area is also involved in organizing speech and writing. The orbital area is thought to be involved in the control of automatic behaviors and attributing emotional significance to stimuli. The mesial area, which includes the anterior and posterior cingulate, is involved in drive and motivation. The prefrontal cortex receives and processes stimuli from all sensory areas and transmits information to the limbic system and basal ganglia (Sira, 2014).

In the basal ganglia, the caudate nucleus and putamen (collectively referred to as the caudate putamen), substantia nigra, and nucleus accumbens are the structures that have consistently shown the most significant abnormalities in the brains of schizophrenic individuals. The basal ganglia receives input from the entire cerebral cortex including the prefrontal cortex, and the thalamus via the caudate putamen and the nucleus accumbens, collectively known as the striatum. The input then travels through the globus pallidus and substantia nigra to the limbic system via the thalamus (Gerfen 2010). The basal ganglia controls motor functions and learning of motor functions, as well as transmitting impulses from the cerebral cortex to behavior effector centers of the brain. Dysfunction in these areas could lead to the abnormalities in movements seen in schizophrenic patients, as well as the behavioral symptoms should impulses be incorrectly transmitted from the prefrontal cortex to behavior centers (Gerfen 2010).

The limbic system is responsible for processing emotion, memory, personality, evaluation of stimuli, and other cognitive processes. The main areas of the limbic system implicated in the pathology of schizophrenia are the thalamus, hippocampus, and amygdala (Stephani, 2014). The thalamus is responsible for sensory gating and processing of stimuli, receiving all of the sensory information in the brain except olfactory input. The relaying of this sensory information makes the thalamus the most important area of the brain for how stimuli are perceived by an individual (Katz 2014). The hippocampus is responsible for processing memories including personal recollections, general knowledge, spatial awareness, imagination, and contextual associations (Vago 2014). The amygdala is responsible for recognition and creation of emotional and behavioral cues, receiving input from the hippocampus, prefrontal cortex, and all of the sensory areas (McDonald 2014). Once again, the disruption of normal function of any of these structures would very likely cause many symptoms of schizophrenia

including emotional abnormalities, improper response to stimuli, delusions, hallucinations, and learning difficulties.

Theories of Schizophrenia:

Despite the name schizophrenia being coined over 100 years ago in 1887, the pathology of the disease is still not fully understood. In fact, there may be multiple mechanisms responsible for schizophrenia as the disease is currently diagnosed using a list of symptoms that may vary greatly between patients. This is not to say, however, that researchers have not proposed many hypotheses backed by solid scientific evidence to explain positive, cognitive, and negative schizophrenia symptoms. The two prevailing theories in the field for the last century are the Dopamine theory and the Glutamate theory. For many years, researchers tried to prove these theories independent of one another, though in recent times research has provided evidence that these theories may not be mutually exclusive and the interplay between the two affected pathways, as well as the inclusion of multiple additional factors, may hold the key to gaining a complete understanding of the mechanisms of this disease (Howes 2015).

Toxoplasmosis affects the three major neurotransmitter systems that have been implicated in the pathology of schizophrenia via a sustained increase in KYNA and dopamine levels. The downstream effects of a cerebral toxoplasmosis infection are (1) a marked increase in dopamine levels via the action of parasite disseminated tyrosine hydroxylase, (2) hypofunction of the glutamatergic system via KYNA acting as a non-competitive inhibitor of both NMDA and AMPA receptors, and (3) the inhibition of α7-nicotinic acetylcholine receptors by KYNA to a much higher degree than normal (Gaskell 2009, Prandovsky 2011, Moroni 2012). The combined dysfunction of these three systems leads to (1) abnormal function of the glutamatergic system, which is implicated in negative symptoms and cognitive impairment, and (2) a consistent hyperdopaminergic state, which is highly correlated with psychosis, culminating in the manifestation of schizophrenia symptoms (Moghaddam 2012, Howes 2011).

Dopamine theory:

The original theory of schizophrenia was the Dopamine theory. First proposed over 60 years ago, this theory states that the mechanism behind schizophrenia symptoms lie in the dopaminergic system. This theory was initially supported by the fact that administering amphetamines, and thus causing an increase in extracellular dopamine, would create schizophrenia-like symptoms in the subject (Connell 1957, Lieberman 1987). Further support was found when it was discovered that drugs targeting the D2 dopamine receptors could

decrease symptoms, and drugs like levodopa that act as dopamine agonists can induce schizophrenia-like psychotic symptoms in non-schizophrenic individuals (Creese 1976, Ricciardi 2016).

When dopamine levels were tested in various areas of the brains of deceased schizophrenic individuals, increased dopamine was found in areas including the caudate nucleus, the amygdala, and the nucleus accumbens, though these results are not consistently reproducible (Ellenbrock 2005). A significant increase in the rate-limiting enzyme in dopamine synthesis, tyrosine hydroxylase, has also been found in the substantia nigra and caudate putamen of schizophrenic individuals (Howes 2013, Toru 1988). Increases in D2 receptors have been found, but this may be due to long term antipsychotic use, which specifically blocks D2 receptors thus causing increased receptor synthesis to compensate (Kaalund 2014). This theory has considerable evidence behind it as drug-naive schizophrenic individuals did not show any increase in D2 receptors, while those who had been taking antipsychotics had significantly higher D2 levels (Howes 2015).

Despite this evidence for the involvement of dopamine in schizophrenia, one-third of patients do not respond to D2 blocking drugs, nor do treatment-resistant patients exhibit the same increased dopamine synthesis capacity as treatment-responsive individuals (Mortimer 2010, Demjaha 2012). Lastly, the dopamine theory cannot account for the cognitive decline or negative symptoms central to a schizophrenia diagnosis (Laruelle 1999).

Though dopamine may not be solely responsible for all of the symptoms of schizophrenia, there is no doubt that changes in dopamine levels in the brain cause significant effects on behavior. This is of some importance as toxoplasma parasites have two genes encoding tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of the L-DOPA precursor to dopamine, which share a 53% homology with the human enzyme and contain a signal sequence allowing them to be trafficked to organelles or secreted into host cells. The two enzymes referred to as TgAaaH1 and TgAaaH2 are expressed at different levels based upon the life stage of the parasite. TgAaaH1 is expressed in both the active tachyzoite stage and the encysted bradyzoite stage, while TgAaaH2 is only expressed in the bradyzoite stage, indicating an increased level of L-DOPA synthesis during the chronic stage of infection (Gaskell 2009 & Prandovszky 2011). The action of the two parasite-derived enzymes was confirmed in 2009 as causing an upregulation in CNS dopamine levels in the brains of infected mice. The amount of dopamine released from infected cells increased in a dose-dependent manner with the number of cysts. In some cells, dopamine release was increased by up to 350% (Prandovszky 2011). However, the antibody in this study could have also been staining L-DOPA, the precursor to

dopamine, which requires the enzyme DOPA decarboxylase to create dopamine. This enzyme is only found in dopaminergic and serotonergic neurons. This could indicate that toxoplasma infected cells synthesize and release massive quantities of L-DOPA, which travel to dopaminergic and serotonergic neurons, which convert it to dopamine. The greatest concentrations of dopaminergic neurons are in the substantia nigra, which was confirmed in postmortem brain imaging to contain higher than normal amounts of tyrosine hydroxylase in schizophrenic individuals. The higher the concentration of the enzyme, the greater the uptake of the precursor to dopamine became, which was positively correlated with severity of positive symptoms (Howes 2013). Through this mechanism, specific cells could be targeted despite the nonspecific nature of toxoplasma infection (McConkey 2013).

The existence of behavioral effects caused by the toxoplasma-induced increase in dopamine synthesis was inadvertently confirmed in a 2006 study that tested the behavioral changes in toxoplasma-infected mice after treatment with common antipsychotics. When treated with the antipsychotic Haloperidol, which is known to act through the inhibition of D2 receptor activity, or Valproic acid, a mood stabilizer that is not thought to act on the dopaminergic system, the mice treated with Haloperidol saw significant improvement in symptoms to a greater degree than those treated with valproic acid. Thus, the mice with decreased dopaminergic activity showed fewer behavioral abnormalities than those who had no dopaminergic modulation (Webster 2006).

Despite the knowledge that increases in dopamine are tied to positive symptoms, particularly psychosis, in schizophrenia sufferers, the discovery that many of the most effective antipsychotics are not specific to D2 receptors and target additional neuronal receptors led researchers to modify the Dopamine theory to include glutamate in the 1990s (Howes 2015).

Glutamate theory:

Glutamate theory emerged as a way to incorporate numerous proposed mechanisms behind schizophrenia into a single unified theory. This theory explains several factors that the dopamine theory alone cannot, including why one-third of patients don't respond to typical antipsychotics, why the brains of schizophrenic individuals exhibit structural changes over time, and how negative symptoms may arise. This theory also allows for the development of a straightforward, evidence-based mechanism by which toxoplasmosis may contribute to schizophrenia symptoms through the inhibitory actions of KYNA on NMDA, AMPA, and α7-nAchR receptors. While this theory encompasses a number of neurotransmitters and pathways,

glutamatergic hypofunction is the proposed central dysfunction from which all other abnormal signaling pathways arise.

The glutamatergic system makes up between 60% and 80% of the total metabolic activity of the brain (Rothman 2003). Glutamatergic neurons are in the highest concentrations in the hippocampus and the entire cortex by way of the limbic system and the basal ganglia. Glutamate is an excitatory neurotransmitter that exerts an influence over nearly every other neurotransmitter system in the brain through NMDA receptor activation (Bleich & Kornhuber 2005). NMDA receptors are ion channels that are held in an inactive state through the binding of a magnesium ion in the pore of the receptor until they are activated via the binding of both glutamate and glycine, after which they allow positively charged ions, including calcium, to pass through the neuronal membrane. The influx of calcium causes the activation of numerous signaling pathways in the neurons, including (1) long-term potentiation, which increases receptor density and contributes to learning and memory, and (2) excitatory neurotransmission (Goff 2001).

The hypofunction of the glutamatergic system was originally hypothesized as having involvement in the pathology of schizophrenia when it was observed that individuals who took the NMDA receptor antagonists phencyclidine (PCP) or ketamine experienced schizophrenia-like symptoms, including hallucinations, delusions, thought disturbances, dissociation, impaired recall and, most significantly, negative symptoms (Javitt 1991, Krystal 1994, Morgan 2009). In 1995, neurodegenerative changes mimicking those seen in schizophrenic individuals were observed in rats that were repeatedly dosed with NMDA channel blockers (Olney 1995). Postmortem studies of the amount and localization of NMDA receptors in the brains of schizophrenic individuals have had inconsistent results, with some studies finding increases in the thalamus, prefrontal cortex, basal ganglia, and hippocampus, while others found no significant difference from healthy individuals (Howes 2015). Given the lack of conclusive evidence as to NMDA receptor increases in schizophrenic brains, research instead focused on dysregulation of NMDA receptor subunits, and their possible links to schizophrenia symptoms. Knockdowns of the NR1 subunit, the NR1 glycine binding site, or the NR2 subunits that make up NMDA receptors all caused symptoms including hyperactivity, behavioral abnormalities, and decreased learning ability, mimicking schizophrenia in mice that could be treated with antipsychotics (Mohn 1999, Ballard 2002, Miyamoto 2001).

Further evidence for the glutamate theory has been found in the variation in glutamate activity in schizophrenic individuals. Early studies disagreed on whether schizophrenic individuals had consistently elevated or decreased glutamate levels in the CNS, with some

studies publishing very significant reductions in glutamate that were inversely correlated with positive symptoms, while others found increased glutamate in various brain areas (Marsman 2013, Song 2014, Merritt 2016). This could be due to the lack of advanced imaging technology, as the current method used in all of the analyzed studies cannot differentiate between intra and extracellular glutamate (Howes 2015). Later postmortem studies, coupled with advances in technology, were able to settle this debate to an extent when it was found that glutamate levels were only reduced in the hippocampus and frontal cortex, AMPA receptors were decreased in the hippocampus, and there were lower levels of NMDA, AMPA, and glutamate in the thalamus. These studies also found that NMDA receptors on GABAergic interneurons were several times more sensitive to NMDA antagonists than those on pyramidal neurons (Goff 2001).

GABAergic Interneuron Hypothesis:

After establishing the presence of a dysfunction within the glutamatergic system, the next logical step was to determine how this dysfunction causes the manifestation of schizophrenia symptoms. In 1972, it was proposed that schizophrenia symptoms may arise from defects in inhibitory GABAergic interneurons (Blum 2002). Glutamatergic neurons and GABAergic interneurons maintain balance in the brains of healthy individuals, with glutamatergic neurons being excitatory and GABAergic interneurons being inhibitory. In the case of schizophrenia, it was theorized that the hypofunction of NMDA receptors causes GABAergic neurons to interpret the decrease in NMDA receptor function as too little excitatory activity, and thus lowering inhibitory activity in an attempt to restore balance. This lack of inhibitory activity on glutamatergic neurons leaves excitatory activity in the brain unregulated, leading to dysregulation of neuron firing rendering them unable to react to stimuli normally and ultimately causing inappropriate behavioral responses (Gordon 2010). The consequences of an impaired excitation to inhibition balance was confirmed as having cognitive effects in a 2011 mouse study which found that an elevation in excitation, with no compensatory elevation in inhibition, led to impaired social behavior and decreased cognition. Normal behavior was restored when inhibitory signals were raised to balance the excitatory activity (Yizhar 2011).

The neurotransmitter responsible for inhibiting the activity of glutamatergic neurons is γ-aminobutyric acid (GABA). Studies found evidence of decreased GABA synthesis in the brains of schizophrenic individuals through measuring the levels of the rate-limiting enzyme in GABA synthesis, glutamic acid decarboxylase (GAD), via directly measuring GAD protein levels or by measuring GAD mRNA. Though results were varied, three studies found significant decreases in GAD mRNA in the brains of schizophrenic individuals, while two studies found lower GAD

protein levels. Other studies tested GABA concentration directly, some of which found decreases in GABA in the hippocampus and thalamus, while others found no difference. However, GABA was consistently found to be significantly lowered in the amygdala of schizophrenic brains (Blum 2002).

GABAergic neurons can be classified into numerous categories based upon function, molecular markers, or shape. One type of GABAergic neuron contains the calcium-binding protein parvalbumin (PV) and was found to be the main type of neuron that is affected in schizophrenic individuals. This type of GABAergic neuron, abbreviated as PV+ GABA neurons, makes up only 24% of the 11% of total neurons that are GABAergic, meaning it represents 2.6% of the total neuron population in the brain. PV+ GABA neurons associate exclusively with the axons of pyramidal neurons, and have long reaching dendrites that are covered in a dense population of synapses and extensively branched axons that can deliver inhibitory signals to vast numbers of neurons at once. Despite the small number, PV+GABA neurons play a large role in neural function. Experimental models in mice in which the PV+GABA neurons were made dysfunctional due to the deletion of a growth factor specific to the cell type, caused behaviors in the mice that mimicked schizophrenia (Hu 2014). Further studies found that reduction in PV+GABA neurons led to deficits in attention, disorganized behavior, and aberrant emotional responses in both rodent and human models (Maric 2015).

α7-nicotinic Receptor Theory:

A7-nicotinic acetylcholine receptors (α7-nAchRs) belong to the group of acetylcholine receptors called fast ionotropic cationic nicotinic receptor channels, and are found in the largest concentration in the hippocampus, hypothalamus, and cortex. α7-nAchRs are non-specific ion channels that, when open, allow an influx of Na+, leading to membrane depolarization, and Ca2+, causing the release of neurotransmitters including GABA, glutamate, and dopamine. The normal ligands for this family of receptors are nicotine and acetylcholine, though α7-nAchRs can also be bound by KYNA, which non-competitively inhibits some receptor activity under physiological conditions at very low concentrations (Albuquerque 2009). Unlike other nicotinic receptors, α7-nAchRs are highly permeable to calcium, open for a very short period of time, and undergo desensitization, or decreased response to the same level of stimulus, quickly (Alkondon 2004). α7-nAchRs are thought to play a role in long term potentiation, a form of strengthening of the synapse via increased receptor concentration that improves learning and memory (Yakel 2013). Mutations in the gene CHRNA7 which decreases the number of functional α7-nAchRs have been found to be significantly higher in individuals with a P50 sensory gating deficit, which

stops the gating of simple auditory stimuli leading to sensory overload, which is common in schizophrenic individuals. When nicotine, an agonist of α7-nAchRs, is given in high concentrations to individuals with schizophrenia, their sensory gating abilities improve to the point that α7-nAchR agonists are currently being researched as treatments for schizophrenia (Freedman, 2014). When coupled with additional inhibition through the actions of increased KYNA, the presence of the CHRNA7 mutation could provide additional data as to why some individuals with toxoplasmosis do not experience schizophrenia symptoms (Stephens 2009).

Under physiological conditions, KYNA binds to α7-nAchRs preferentially over NMDA and AMPA receptors, and inhibits some of their activity, which causes multiple effects depending upon the brain region. In the hippocampus, the inhibition of α7-nAchRs causes a decrease in GABAergic activity. To determine the extent to which KYNA levels influence GABAergic activity in the brain, mice with a homozygous mutation in the gene encoding kynurenine aminotransferase II (KATII), a key enzyme in the production of KYNA, were created and subjected to variety of behavioral and molecular measurements. When KYNA levels were reduced by 55% in the hippocampi of 21 day old mKATII-/- mutants, the activity of α7-nAchRs increased by 65%, consistent with the premise that KYNA inhibits some activity of α7-nAchRs *in vivo*. To confirm that the change in activity was due solely to the decrease in KYNA, 100nM KYNA was added to mKATII-/- mouse hippocampal slices, and GABAergic activity levels significantly decreased (Alkondon 2004). Should KYNA levels increase in the brain, as in the case of toxoplasmosis infection, hippocampal α7-nAchRs experience a level of inhibition greater than that which occurs under normal physiological conditions. This decreased activity of α7-nAchRs and the subsequent effects on GABAergic neurons has been linked to deficits in learning and memory, which are could explain the cognitive decline or other behavioral symptoms seen in schizophrenia (Gotti 2004).

Throughout the brain, α7-nAchRs are frequently found on glutamatergic neurons in the presynaptic terminals, where they control glutamate release, and as such the number and functionality of α7-nAchRs is critical to the proper regulation of glutamatergic activity. In the presence of an antagonist, such as KYNA, the inhibition of α7-nAchRs will decrease the amount of glutamate released. In a 2010 study, 100nM KYNA was perfused into the prefrontal cortices of rats, and extracellular glutamate levels significantly decreased by 26% as a result. To confirm that the decrease was caused by KYNA, the next trial decreased the extracellular KYNA levels in the prefrontal cortex by 35% and glutamate levels raised 244%. To prove that this result was caused by α7-nAchR inhibition, galantamine, an agonist of the receptor that binds at the allosteric site, was added systemically and prevented the inhibitory effects of KYNA, proving

that KYNA binds to the allosteric site of α7-nAchRs, preventing glutamate release(Wu 2010). The prevention of glutamate release via α7-nAchR inhibition in conjunction with NMDA hypofunction caused by KYNA likely decreases the ability of NMDA receptors to function properly.

α7-nAchRs have been implicated in the pathology of schizophrenia through a number of mechanisms, as they control the release of numerous neurotransmitters in various areas of the brain (Stephens 2009). While the literature may still disagree as to the exact mechanism by which α7-nAchRs cause specific schizophrenia symptoms, it is clear from the evidence that the inhibition of these receptors via the CHRNA7 mutation and further inhibition by KYNA in toxoplasma-positive schizophrenic individuals contributes to the pathology of the disease, particularly with respect to learning, memory, and sensory gating.

Interconnecting the theories:

The first evidence that all of the above theories may actually be one interconnected mechanism came from a 1990 study that blocked the activity of NMDA receptors of mice with selective antagonists and found that dopamine release increased in a dose-dependent manner with the level of antagonist (Imperato 1990). A 2001 study that created mice with a mutation in one of the key genes in the NR1 subunits of NMDA receptors corroborated earlier findings. The mutation caused significant reductions in NMDA receptors, simulating the NMDA receptor hypofunction present in the brains of schizophrenic individuals. Dopamine release in the striatum of the mice with NMDA hypofunction was significantly increased, while GABA release was significantly decreased as compared to controls. The effect of the changes in dopamine

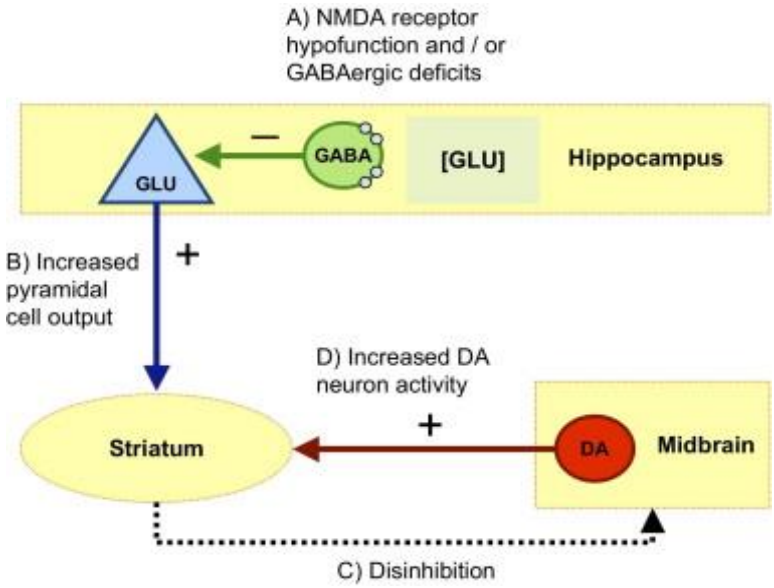

Fig. 3 A theorized pathway by which hippocampal glutamatergic hypofunction causes a striatal hyperdopaminergic state.
From Stone 2010

levels was confirmed behaviorally, as NMDA hypofunction mice exhibited increased locomotor activity, a common sign of a hyperdopaminergic state, which could be reversed with the dopamine receptor antagonist Haloperidol. In addition, the NMDA hypofunction mice had markedly decreased learning abilities, analogous to cognitive impairments found in schizophrenia (Miyamoto 2001).

In 2010, a study translated findings from the earlier research into human studies. Individuals who exhibited classic prodromal symptoms of schizophrenia were classified as having an at-risk mental state for psychosis (ARMS). These individuals were given clinical assessments, and their levels of hippocampal glutamate and striatal dopamine were measured. The ARMS individuals had a significant negative correlation between the glutamate and dopamine levels ($p=0.031$) that was not present in healthy volunteers ($p=0.89$). When compared with clinical evaluations, lower hippocampal glutamate was related to speech abnormalities ($p=0.059$), and excess striatal dopamine was significantly correlated with thought abnormalities ($p=0.025$). From these results, coupled with evidence from earlier investigations (as presented in earlier sections), a theoretical pathway took shape (Fig. 3) (Stone 2010).

This pathway theorizes that the lack of GABAergic inhibitory activity in the hippocampus caused by the inhibition of a7-nAchRs and NMDA hypofunction allows glutamatergic neurons to enter a hyper-excitable state. This causes increased glutamate release, which, in the striatum, results in increased dopaminergic activity as dopaminergic neurons in the midbrain become disinhibited (Stone 2010). Coupled with the massive increase in dopamine synthesis in toxoplasma-positive schizophrenic individuals, this causes a hyperdopaminergic state and significant increases in dopaminergic activity in the brain, leading to psychosis.

Toxoplasma gondii:

Toxoplasma gondii is a protozoan parasite, meaning it is a single-celled eukaryotic organism, in the phylum Apicomplexa, classified by the use of intracellular microfilaments for motility, in the Coccidisina subclass, which is characterized by the existence of an oocyst (a cyst containing the zygote form of a parasite) stage essential to the life-cycle of the parasite. The definitive hosts, or the hosts in which a parasite reaches sexual maturity, of *T. gondii* are the members of the Felidae family, however, it can infect a myriad of species outside its normal hosts including birds, mammals, and reptiles (Dubey 2010).

Toxoplasma gondii causes the disease toxoplasmosis, which is only symptomatic in 10-20% of infected individuals, and is zoonotic meaning it can pass from animals to humans. The most common infection route for human toxoplasmosis is through the consumption of oocysts, the free-living, spore filled cyst life-stage of the parasite, in contaminated meat, vegetables, or water (Fig. 4) (Jones 2010, Lass 2012). Other, less common, routes of infection include consuming raw or unpasteurized dairy, blood transfusions, organ transplantation, sexual transmission, or transplacental infection (Riemann 1975, Seigel 1971, Derouin 2008, Arantes 2009, Foulon 1994).

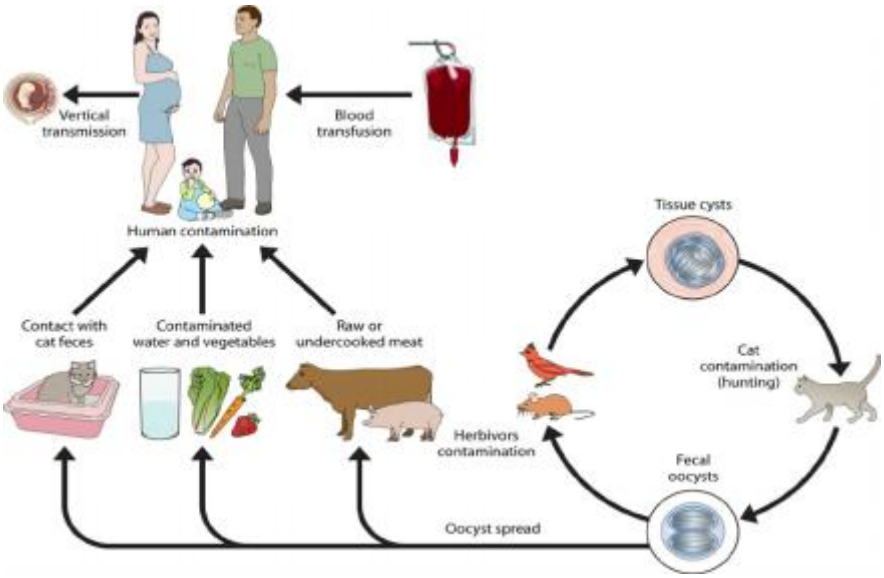

Fig. 4 *T. gondii* life cycle
From Esch and Petersen 2013

The most common symptom of acute infection is lymphadenopathy; however, other symptoms may include a maculopapular rash, malaise, fever, night sweats, myalgia, and atypical lymphocytosis. In rare cases, severe symptoms including encephalitis, myocarditis, polymyositis, pneumonitis, or hepatitis may occur in healthy individuals (Bonametti 1995, Hill 2002).

Toxoplasma encephalitis is a common cause of death in infected immunocompromised individuals, as the inability to maintain the chronic immune response that keeps *T. gondii* in its encysted form causes the cysts to grow and burst, releasing large stores of parasites into the tissue resulting in massive immune infiltration. Once freed from the cysts, the parasites revert back to the active phase and feed on the surrounding tissue, leading to the formation of focal necrotic lesions with inflammatory infiltrates, edema, and hemorrhage (Renold 1992, Magnerou 2012).

The outcome of transplacental infection depends greatly upon when in the pregnancy infection occurs. If infection occurs in early pregnancy, a hallmark triad of hydrocephalus, chorioretinitis, and intracranial calcifications normally result in miscarriage. If the infection occurs later in pregnancy, the newborn will likely be asymptomatic at birth, though symptoms may emerge months to years later. The most common effect of transplacental toxoplasmosis is chorioretinitis which can cause blindness in one or both eyes. Other, less common effects include hepatomegaly, splenomegaly, maculopapular rash, lymphadenopathy, hyperbilirubinemia, anemia, or thrombocytopenia. Behavioral changes including mental retardation, autism, Down syndrome, and mental disorders including schizophrenia have also been documented in individuals infected either prenatally or postnatally (Sensini 2009).

Epidemiology of *T. gondii*:

It is estimated that one-third of the human population is infected with *Toxoplasma gondii*. Rates of infection increase with age, and the highest rates of infection occur in lower socioeconomic classes and in areas of poor sanitation (Montoya & Liesenfeld 2004). Brazil has the highest rate of infection worldwide, with an average seroprevalence rate of 70-80%, with some areas reporting an infection rate as high as 97%. Seroprevalence is consistently high in

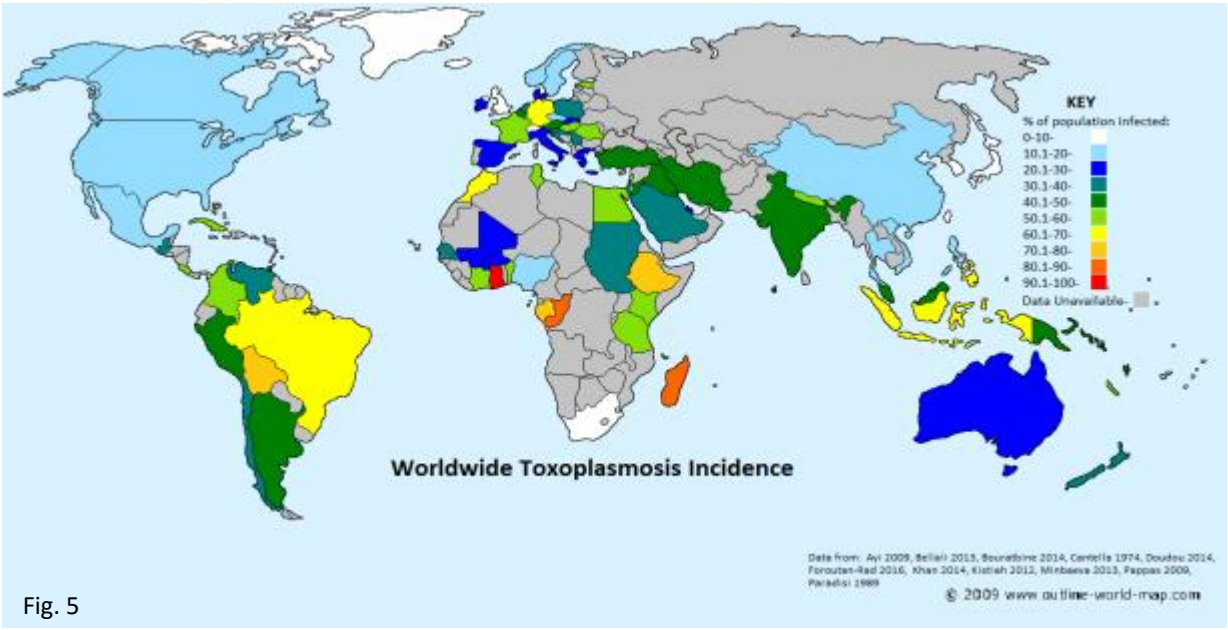

Fig. 5

South America and the Caribbean, with rates ranging from 40-80% due to favorable environmental conditions for parasite growth and dissemination. Infection rates in the United States range from 11-15%. Canada, at large, reports an infection rate of 16.8-18.6%, though Inuit populations who regularly consume seal and caribou meat had an infection rate of 59.8%.

Infection rates in Europe, Asia, and Africa vary wildly depending on immigration, diet, and availability of data (Fig. 5) (Pappas 2009).

The overall trend shows a gradual decrease in cases over the years, with the fewest cases occurring in cold climates and in areas where sanitary water and food sources are readily available. However, the severity of the disease also depends on the strain of *Toxoplasma gondii* in the region, with Type I being the most virulent strain by far, followed by the most common strain, Type II, and the least virulent strain being Type III which is predominantly found in animals (Howe & Sibley 1995). Infections in Europe and North America are mainly caused by the Type II strain, acquired through the consumption of contaminated food. In sub-Saharan Africa, Asia, and South America, Type I, III, and numerous atypical strains are prevalent (Ajzenberg 2002, Ajzenberg 2009, Shwab 2014). These strains vary in their effects and virulence, which will be described in detail in a later section.

Toxoplasmosis Treatments:

For the last century, toxoplasmosis has been regarded as an inert and harmless disease. As such, drug development for the treatment of toxoplasmosis has not been a profitable industry and the treatments available for the disease have not changed significantly in the last 30 years. Trimethoprim and Pyrimethamine are the two anti-protozoal drugs that are the most active against toxoplasma. Pyrimethamine is 20 to 50 times as effective as Trimethoprim *in vivo* and is the preferred toxoplasma drug (van der Ven 1996). The second drug that is always included in toxoplasma treatment is a sulfa antibiotic. Sulfadiazine is normally used in combination with pyrimethamine while sulfamethoxazole is paired with trimethoprim. This combined therapy multiplies the activity of the individual drugs and effectively kills the parasite. The recommended course of medication is a minimum of six weeks to ensure that no cysts remain (van der Ven 1996). If toxoplasma is acquired during pregnancy and the infection is diagnosed early enough, the macrolide antibiotic spiramycin may be used to prevent the transplacental infection of the fetus. (Couvreur 1988).

Since the disease has, until recently, been thought of as a benign condition, the treatment of toxoplasmosis has remained both lengthy and expensive. Given the flood of new data correlating the disease with numerous mental disorders, drug development may become necessary as treating the disease becomes an essential part of psychiatric therapies. Due to the length of treatment required to fully eliminate the presence of parasites, and the issues with drug compliance present in the schizophrenic population, attempts to treat schizophrenic

individuals with anti-protozoal therapies present a real challenge to the medical community, and as such few studies have been performed on the efficacy of this treatment.

Human Infection and Immunity:

Toxoplasma gondii has four distinct life stages: tachyzoite and bradyzoite stages which occur in all hosts and merozoite and sporozoite stages which only occur in the definitive host. Tachyzoites are the rapidly dividing stage of *T. gondii* that are responsible for dissemination throughout bodily tissues during the acute stage of infection. Tachyzoites convert into bradyzoites, which are slow replicating and are enclosed within cysts that can live in tissue indefinitely during the chronic stage of infection. Merozoites are responsible for giving rise to cells involved in sexual reproduction and recombination in the definitive host, and sporozoites are slow growing organisms contained within membrane bound structures called oocysts that are released into the environment and burst upon ingestion by an intermediate host (Dubey 2009).

After ingestion, oocysts burst following membrane erosion by acid in the stomach and small intestine. Within 30 seconds of rupture, sporozoites infiltrate enterocytes and travel to the lamina propria, where they infect a multitude of cells and divide rapidly (Cohen 2015). After infecting a host cell, the parasite is left in a parasitophorous vacuole which prevents the host cell, including phagocytic cells, from eliminating the parasite (Lang 2007). Secretions from club-shaped organelles called rhoptries assist in this process, helping to create the parasitophorous vacuole and recruiting the host cell mitochondria and ER to synthesize nutrients and numerous factors to alter host gene expression, tailoring the immune responses to promote parasite survival (Lebrun 2007).

Toxoplasmosis Infection and Colonization of the Brain:

In order to gain access to the brain through the blood brain barrier, toxoplasma employs a tactic known as the "Trojan horse" which involves hiding within a host cell to bypass gating mechanisms that would bar the parasite from entering in its free form (Randall 2011). Experimentally, it was concluded that the most likely cells employed by toxoplasma are monocytes or dendritic cells (Courret 2006). In order to reach the brain, toxoplasma causes increased synthesis and secretion of GABA in host dendritic cells, as confirmed in a 2012 study of infected mouse dendritic cells ($p<0.0001$). This causes the cell to enter a hyper-migratory state, which allows toxoplasma parasites to disseminate rapidly to target tissues, including the CNS, without the influence of chemoattractants (Fuks 2012). The exact route by which

toxoplasma enters the brain inside its host cell is not yet understood, though it is possible that the parasite takes advantage of the systemic immune response to pass through the blood brain barrier during a time when it has increased permeability to allow the entrance of immune cells (Lachenmaier 2011).

Once inside the brain, toxoplasma begins to divide within the host cell, forming rosettes of dividing tachyzoites. Once the host cell is inundated with tachyzoites, it will burst, releasing the parasites into the neural tissue, at which point they will infect astrocytes, microglia, and neurons. Histological studies have shown that cysts form in neurons with a 2 to 3-fold higher rate to that of astrocytes, and can form anywhere in the neuronal cell (Carruthers 2007). Studies have found cysts in the cell bodies, axons, and dendrites of neurons. After activation via the immune response, astrocytes and microglia are able to clear the parasites, while neurons remain infected chronically due to the lack of intracellular mechanisms to inhibit parasite growth, as well as a lack of MHC I which would normally signal CD8+ T cells to recognize and eliminate the infection (Blanchard 2015).

The effects of toxoplasma infection in the brain appear rapidly after the initial invasion. The first effect is the mounting of a massive Th1 immune response by the host, given the host is immunocompetent, which begins with infiltration of brain tissue by activated CD4+ and CD8+ T cells. CD8+ T cells recognize and eliminate infectious cells presenting toxoplasma antigens and release a number of immune mediating cytokines. The most important of these during acute toxoplasma infection are (1) INF-ɣ which, through over 200 downstream actions including the upregulation of the kynurenine pathway, causes the parasites to encyst and enter their slow-growing phase, and (2) TNFα and perforin, which keep the parasites from re-entering the active stage (Carruthers 2007).

The body is unable to fully clear parasitic cysts due to the manipulation of gene expression by toxoplasma that counteracts the immune response at every stage by (1) downregulating the expression of proinflammatory cytokines including IL-12 and TNF-α, (2) preventing NF-kB from translocating to the nucleus, and (3) causing phosphorylation of STAT-3 further reducing TNF-α and IL-12 levels. The parasite also upregulates anti-inflammatory mediators including (1) IL-10, which decreases the T cell response and deactivates macrophages, and (2) TGF-β, which reduces TNF-α and INF-ɣ production. Additionally, it (1) blocks the upregulation of MHC II that allows CD4+ T cells to recognize infected cells, (2) inhibits the creation of the anti-parasitic molecule nitric oxide(NO), and (3) blocks apoptosis in the host cell via inhibition of cytochrome-c release from the mitochondria and upregulation of anti-apoptotic factors (Lang 2007).

***Toxoplasma gondii* Mediated Behavioral Changes:**

Toxoplasmosis research conducted in the last 30 years has found evidence in support of the theory that toxoplasma infection causes behavioral changes in its human host in otherwise mentally healthy individuals. In a study published in 1996, 224 men and 170 women, composed of Charles University staff and students and patients at various Prague hospitals, were given personality tests measuring 16 factors. Of the participants, 63 men and 40 women tested positive for *T. gondii* antibodies. The behavioral shifts in infected individuals differed based on sex. Infected women scored significantly higher in the categories dealing with (1) affection and easy-going personality, (2) self-assurance and security, and (3) self-sufficiency and resourcefulness as compared to uninfected female controls. Infected men scored significantly higher in (1) disregarding of rules, (2) suspiciousness or jealousy, (3) insecurity and guilt, and (4) social group dependency as compared to uninfected male controls (Flegr 1996). Confirming these results, 502 male soldiers were given personality assessments. 154 of the men were toxoplasma-positive, and their tests revealed significant increases in anxiety, depression, phobia, hysteria, and neuroticism as compared to uninfected men (Flegr 2013). Furthermore, a 2006 study found that infected men scored lower in (1) relationships and warmth with friends, (2) self-control during the experiment, (3) appearance, (4) planning, and (5) clothes tidiness. Infected women once again scored significantly higher in self-control than their uninfected counterparts (Lindová 2006). A study of 1,755 children between the ages of 12 and 16 found that the 7.7% of children who were seropositive performed significantly worse than uninfected controls on tests of reading skills ($p=0.029$) and memory capacity ($p=0.017$) (Mendy 2015).

Another study subjected 69 men and 47 women, 60 total of whom were toxoplasma-positive, to three 1-minute tests in which the subjects had to press a button every time a black square appeared on a white screen. Individuals with toxoplasma were markedly slower in the second and third tests, which indicates that toxoplasmosis may have an effect on reaction times (Havlicek 2001). This result was corroborated in a 2013 study in which 85 men and 61 women responsible for traffic accidents that they could have prevented, with no alcohol in their systems, were tested for toxoplasmosis. The control group consisted of 230 men and 216 women from the same area. Individuals involved in traffic accidents had significantly higher rates of toxoplasmosis ($p<0.0001$) (Flegr 2013). In addition, studies have shown that both men and women infected with *T. gondii* show a decrease in novelty seeking behavior, as well as an increase in extroversion, a result further correlated by a study of 807 male soldiers, in which infected men showed significantly less novelty seeking behavior and markedly decreased

intelligence and working memory as opposed to controls (Skallová 2005, Lindová 2012, Flegr 2003, Pearce 2014).

These studies indicate that, even without a diagnosable mental disorder, toxoplasmosis may cause changes in the brain that alter aspects of an individual's personality. However, these changes in behavior may be mild and unremarkable in the lives of infected individuals. The same cannot be said of those for whom infection with toxoplasma results in the emergence of a diagnosable mental illness.

A study published in 1953 found a correlation between mentally ill patients and toxoplasmosis, with the incidence of infection rising with age (Burkinshaw 1953). Since then, toxoplasmosis has been positively correlated with many mental disorders including: bipolar disorder, obsessive-compulsive disorder, and addiction (Fekadu 2010). However, the most convincing and thorough causative evidence exists linking toxoplasmosis and schizophrenia (Sutterland 2015).

Toxoplasmosis link to Schizophrenia:

Toxoplasmosis has one of the highest odds ratios of the known risk factors for schizophrenia. Odds ratios measure the odds of having a disease (schizophrenia) after being exposed to a factor (toxoplasma) compared to the odds of having the disease without exposure to toxoplasma. An odds ratio of greater than one confirms that two factors are associated with one another. The higher the odds ratio, the less likely the correlation is coincidental and more likely that a causative relationship exists (Bland 2000). The odds ratio of toxoplasma and schizophrenia far exceeds even that of genetic factors, which until recently has been thought to be the main causative agent of the disease. The relationship between the two disorders has been well studied and established via a multitude of different approaches. The most obvious approach being the prevalence of toxoplasmosis within the schizophrenic population in comparison to the unaffected population. In 2007, a meta-analysis compiled data from 46 studies that tested individuals with schizophrenia for toxoplasmosis. Of the 46 studies, 23 met the criteria of a clear schizophrenia diagnosis as outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM), the inclusion of a control group, and the use of one of the accepted methods of testing for toxoplasmosis infection. The combined 23 studies tested 3,873 individuals with schizophrenia and 7,046 controls and found an odds ratio of 2.73 with a 95% confidence interval. The studies were gathered from 17 countries worldwide in various languages (Torrey 2007). The same researchers published an updated study in 2012 that compiled data, using the same criteria, from papers published after 2007. The data returned an

odds ratio of 2.71. The combined 38 total studies tested 6,058 schizophrenic individuals and 8,715 healthy controls, and found an odds ratio of toxoplasmosis in schizophrenic patients of 2.73 (Torrey 2012). Another meta-analysis, published in 2015, compiled data from studies published in English, French, Dutch, and Spanish. Out of 2,866 studies, 50 were included in the odds ratio analysis. In the 50 studies, 12,009 schizophrenic individuals and 71,441 healthy volunteers were tested. An overall odds ratio of 1.81 was found between toxoplasmosis and schizophrenia. When the data was further narrowed to individuals with *T. gondii* antibody titers in the upper 75th percentile, the odds ratio increased to 1.85. When the data was further narrowed to individuals in the 90th percentile for *T. gondii* antibodies as compared to controls, the odds ratio dramatically increased to 2.27 (Sutterland 2015). In 2014, a study was published that estimated the population attributable fraction (PAF) for schizophrenia with toxoplasmosis as an aggravating factor. The population attributable fraction estimates the percent of cases of a disease that would not occur if a particular risk factor did not exist. It was determined that an estimated 21.4% of schizophrenia cases would not exist if toxoplasmosis infections were eliminated as a contributing factor. Due to variances in data and regional differences, this number could be as high as 30.6% or as low as 13.7%. If this is a correct estimate, it means that an estimated 355,000 cases of schizophrenia could theoretically be cured or improved upon over a single human lifetime simply by treating the underlying toxoplasmosis infection (Smith 2014).

Additional evidence includes higher rates of schizophrenia among populations with higher rates of toxoplasmosis. In France, the rate of toxoplasmosis infections in pregnant women is between 45 and 55%, while Britain reports a rate of less than 10% (Berger 2009, Pappas 2009). Rates of schizophrenia diagnoses are much higher in France as compared to Britain and continue to increase. Britain has much lower rates of diagnosis and has seen a decline in schizophrenia cases over time (van Os 1993). Similarly, Rural areas in Ireland have extremely high rates of toxoplasmosis infection, with one study finding positive titers in 73.5% of individuals in Northern Ireland (Stanford 1990). Rural areas in Ireland also report the highest rates of schizophrenia in the world, with a lifetime expectancy of 4% for men and 3.2% for women, compared to a worldwide average of 1% (Torrey 1984).

A 2012 study compared the brain matter volumes of schizophrenic individuals, toxoplasma-infected schizophrenic individuals, and healthy individuals with and without toxoplasma. All of the schizophrenic subjects were in chemical remission, meaning they currently do not display symptoms. Toxoplasma-positive healthy controls did not differ significantly from toxoplasma negative healthy controls. Individuals with schizophrenia who did were not infected with toxoplasmosis had slightly reduced grey matter volume only in the

temporal lobe region as compared to healthy controls. The only group that had significant changes in brain volume were toxoplasma-positive schizophrenia patients as compared to all other groups, including those with toxoplasmosis or schizophrenia alone. These individuals had significant, bilateral decreases in grey matter in the cerebellum, middle and posterior cingulate gyri, precentral gyri, caudate nucleus, thalamus, occipital cortex, and the temporal and mediotemporal regions including the hippocampus and parahippocampal gyri (Horacek 2012).

Current therapies for schizophrenia treatment include typical antipsychotics, the most common of which used today are thiothixene, haloperidol, thioridazine, chlorpromazine, loxapine, and fluphenazine, and for individuals who are treatment-resistant or experience extrapyramidal side effects, atypical antipsychotics, the most commonly prescribed of which are clozapine, olanzapine, ziprasidone, risperidone, aripiprazole, and quetiapine. Typical antipsychotics act mainly on dopamine receptors, though many act on a wider range of receptors leading to extrapyramidal side effects, while atypical antipsychotics act mainly on dopamine and serotonin receptors, but may also act on a wider range of yet unknown additional receptors (Marder & Stroup 2016).

In recent years, it was discovered that many of the above drugs may have anti-protozoal effects. This could indicate that some of the success of these drugs could be due to a reduction in parasite load in toxoplasma-positive schizophrenia patients. The first study to discover this association was performed in 2003. Four commonly used mood stabilizers and eight antipsychotic drugs were tested *in vitro* against toxoplasma-infected human fibroblasts. Each drug was administered at various concentrations ranging from 0-100 or 0-320μg/mL, from which the minimum inhibitory concentration (the point at which parasite growth is first inhibited) and the minimum cytotoxic dose (the point at which the human cells begin to die) were determined. Then the therapeutic index (minimum cytotoxic dose/ minimum inhibitory dose) was calculated and compared to the therapeutic index of Trimethoprim. All of the drugs showed some activity against toxoplasma parasites with the exception of Lithium. Of these drugs, Haloperidol, free Valproic acid, and Sodium Valproate had greater therapeutic indices than Trimethoprim, a commonly used anti-protozoal drug. Risperidone and Fluphenazine also had moderate anti-protozoal activity (Jones-Brando 2003). A 2006 study tested these claims *in vivo* by treating toxoplasma infected rats with the recommended rodent model dose of Haloperidol, Valproic acid, or trimethoprim and measured both behavioral and histological changes. Haloperidol performed as well as the standard treatment at reducing behaviors associated with toxoplasmosis, including decreasing the amount of time the rats spent in areas

that contain cat urine. Both Haloperidol and Valproic acid markedly decreased the number of infected glial and neural cells in histological sections of brain tissue (Webster 2006).

It has been theorized that the mechanism by which antipsychotics work at preventing or treating toxoplasma infections is through inhibition of the movement of calcium through ion channels on the parasite membrane. Since *T. gondii* requires the uptake of calcium to invade host cells, antipsychotics may prevent the initial invasion of host cells in the acute phase of the disease (Song 2004, Webster 2006). In a 2008 study, investigators inoculated mice with the parasite and waited for the chronic stage of the disease to develop. The mice were then given varying doses of Valproic acid with the highest dose approaching the LD50 of the drug. No reduction in tissue cysts was observed, which could mean that the drug was not effective or that the drug may only be effective at preventing cellular invasion or preventing the conversion of the parasite between the tachyzoite and bradyzoite stages (Goodwin 2008). A study performed in 2011 tested the effectiveness of three mood stabilizers (Fluphenazine, Thioridazine, and Trifluoperazine) against Clozapine and Haloperidol. Previous studies showed that Clozapine has a low rate of toxicity against toxoplasma, whereas Haloperidol has a very high level of activity against toxoplasma. The experiments tested the effectiveness of the drugs against toxoplasma-infected human fibroblast cells *in vitro*. All three of the tested drugs showed high anti-toxoplasma activity. Fluphenazine had an inhibitory concentration of 1.7 μM, Thioridazine 1.2 μM, and Trifluoperazine 3.8 μM. Clozapine did not show any inhibitory activity, and since drug concentrations below its minimum inhibitory concentration of 15 μM were used, Haloperidol likewise did not show any activity (Goodwin 2011).

Surprisingly, only one documented study, from Ethiopia, has been published that attempted to treat or lessen the severity of symptoms of toxoplasma-positive schizophrenia sufferers using anti-protozoal therapy. The study performed in 2010 conducted a double-blind placebo-controlled study that included 90 male patients with schizophrenia who were otherwise healthy. 10 patients were negative for toxoplasmosis and 80 patients were positive for the disease. Patients were given either 200mg/day of the anti-protozoal drug Trimethoprim or a placebo, and were monitored to make sure their current schizophrenia medications were taken on schedule. All of the patients improved dramatically in their symptom severity scores over the six-month period in which the study was conducted, though no data was taken to show if toxoplasma titers had improved (Shibre 2010). This study unfortunately has many issues. It is likely that prior to the study many patients had not strictly adhered to their medication regimen, and thus, it was the supervision and consistency in taking their prescribed medications that may have caused such a significant improvement in symptom severity. More problematic, however,

is that a study published in 1996 that tested various anti-protozoal therapies against toxoplasmosis in both its chronic and acute phases, found that out of all current treatments, Trimethoprim is by far the least effective by a factor of 25 to 50 times. In addition, the study found that Trimethoprim is far more effective when coupled with Sulfamethoxazole, which is how it is generally formulated to treat toxoplasma. Finally, the minimum inhibitory plasma concentration of Trimethoprim is 5-10mg/L, which can only be achieved via a dosing regimen of 20mg/kg/day. Ethiopia is one of the ten countries the World Health Organization lists as having the lowest BMIs worldwide with an average male weight between 53 and 55 kg. This means that, in order for the Trimethoprim to have any effect on parasite load, each man would need to receive a dose of 1060mg/day to 1100mg/day, a full 860-900mg more than they received during the experiment. Had this experiment used a more effective drug or a much higher dose it could have provided evidence as to whether anti-protozoal drugs could be used as a treatment for toxoplasma-positive schizophrenia patients, but as published this study neither supports nor refutes this hypothesis.

The theory that individuals with schizophrenia could experience lessened severity of symptoms and increased quality of life through the treatment of toxoplasmosis and thus removal of a probable contributing factor is not without merit. However, these trials would not be an easy task given the difficulty in finding patients who are schizophrenic, representing 1% of the population, and toxoplasma-positive, in developed countries with well-funded scientific research institutions. Tests for toxoplasmosis are rare, and tend to occur only in those who are symptomatic and have ready access to healthcare facilities. The treatment for toxoplasmosis is lengthy and expensive, which would make clinical trials difficult in a population, schizophrenic individuals, that already tend to have decreased rates of compliance with drug regimens. Additionally, the relatively low rate of these disorders in developed countries and the difficulty in maintaining regular employment in the schizophrenic population means that funding for this type of research is scarce at best. The evidence about the link between toxoplasma and schizophrenia indicates that treatment of the underlying parasitic infection may possibly improve schizophrenia symptoms, but research in this area would be difficult to perform.

Toxoplasma Strain Hypothesis:

Depending upon the method of sequencing there is widespread disagreement between researchers about the number of distinct strains of toxoplasma. Early studies employing RT-PCR discovered 15 genotypes representing three distinct strains, Type I, II, and III (Fig. 6) (Howe 1995). Since that time, additional strains with atypical genetics have been found in Asia,

Africa, and South America (Shwab 2014). Recently, new and more precise methods of sequencing, and the identification of variations in homology within classifications have led to numerous attempts to reclassify toxoplasma as having anywhere from as few as 2 strains to as many as 189 strains (Ajzenberg 2002, Shwab 2014). However, the original three strains and various outlying atypical strain classifications are currently the most widely used and studied.

The knowledge of the variations in virulence and effects of the different strains predates the division of common toxoplasma genotypes into the three strains used today. In 1992, a study published by the Sibley lab found that the most virulent strains of toxoplasma all belong to a single clonal lineage, which was later reclassified as Type I *Toxoplasma gondii* in a 1995 paper published by the same lab (Sibley 1992). This paper also documented the fact that Type II strains are the most common cause of toxoplasma infection among individuals in the United States, while Type I infections are highest in individuals with congenital infections (Howe 1995).

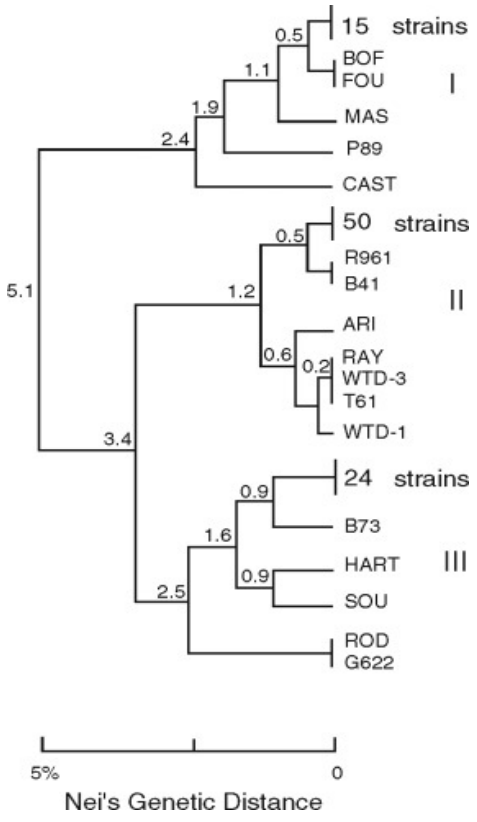

Fig.6 Phylogenetic groupings of the three main *Toxoplasma gondii* strains. From Howe & Sibley 1995

A paper published in 2002 found that Type II infections are also the most common strain in humans in Europe. The study found that, out of 86 isolates, 84.88% were Type II, 8.14% were Type I, and 2.33% were Type III. The remaining 4.65% of the isolates had an atypical genotype. Type III is mainly found within animals, though human infection is possible (Ajzenberg 2002). In South America, anywhere from three to over a hundred atypical strains can be found. South America also has the largest percentage of symptomatic cases, with toxoplasma being one of the leading causes of congenital blindness in the area. Despite the vast diversity of strains throughout the continent, there is little evidence for the existence of the Type II strain that is common in other regions (Shwab 2014). This data supports the theory that Type I toxoplasma and genetically similar atypical toxoplasma strains seem to be the cause of many of the issues related to symptomatic toxoplasma infections, including schizophrenia.

In recent years, the variations between the three strains of *Toxoplasma gondii* have been studied more thoroughly, and although the strains share up to 98% of their genes, their disease-

causing potential could not be more different. Type I has been found to be far more virulent than either of the other strains. From the first stage of infection, the strains differ not in basic pathology, but in the severity of the acute stage and the ferocity of the immune response. Type I causes an immensely powerful immune response, with a (T helper) Th1 response that is often deadly in rodent models. Type I and II also elicit a much higher macrophage response than Type III, and Type I causes a larger neutrophilic response than either of the other strains. After the initial infective stage, Type I creates far more cysts within the host tissues, with an experimental splenic cyst count of 6.7 log parasites/g of spleen tissue. Compared to the other two strains, Type II strains elicit a log parasite count/g of 3.6 in the spleen, which is 1000x less than Type I, and Type III induce a log parasites/g count of 2.4 which is 20,000x less than Type I (Hill 2012).

Not only is Type I toxoplasma highly correlated with symptomatic infections and a highly virulent pathology, but recent studies have found that Type I is the most likely candidate for being a contributing factor in schizophrenia due to genetic changes caused by the parasites.

The Xiao lab is at the forefront of these discoveries, starting with a 2009 paper that tested the sera of 219 mothers who had given birth to offspring who developed psychoses at a later date, and 613 mothers who had children with no apparent psychoses. Testing revealed that the control group had an infection percentage of 35.1%, while the latent-psychoses group had an infection percentage of 41.6%. Of the 41.6%, 12.3% were Type I, 6.8% were Type II, 2.7% were Type III, 13.2% were unclassifiable, and 6.4% indicated an infection with more than one strain. The controls had infection rates of 7.1% with Type I, 6.8% Type II, 3.6% Type III, 12.5% unclassifiable, and 5.2% reacted with more than one strain and were classified as atypical. Adjusting for age, sex, and race, the highest odds ratio between the toxoplasma strains and psychoses is Type I, with an odds ratio of 1.94. This ratio is comparable to the odds ratio of genetic factors and schizophrenia, which is 1.99. When the type of psychoses was limited to affective psychosis, which is characterized by drastic changes in mood accompanied by detachment from reality, the odds ratio of Type I toxoplasma infection as compared to controls was 5.24 (Xiao 2009). Indeed, the odds ratio of Type I infections as correlated with psychosis may in fact be even higher because individuals can be infected with multiple strains of toxoplasma at once, meaning a percentage of the unclassifiable and atypical strains in the latent-psychoses group could actually be experiencing effects caused by Type I or a genetically similar strain of toxoplasma.

***Toxoplasma gondii* and Regulation of Possible Schizophrenia-related Genes:**

To determine why the odds ratios of Type I toxoplasma as correlated to schizophrenia were far higher than that of any other strains, the Xiao lab examined the effects of each of the three strains in the acute stage on gene expression in neuroepithelial cells. To identify genes affected by each toxoplasma strain, each strain was tested against 43,004 transcripts on a microarray. Type I affected the largest number of transcripts, modifying 3.3% or 1,423 of those tested. In comparison, Type II only modified 0.4% and Type III modified 1.1% of transcripts. Changes in gene expression were much more prevalent in all strains in Refseq genes, or genomic sequences that correspond to well-characterized genes. Type I downregulated 726 and upregulated 396 genes, Type II downregulated 24 and upregulated 54 genes, and Type III downregulated 147 and upregulated 197 genes. Type I and Type III had the largest overlap of affected genes with both strains affecting 119 of the same genes. All three strains significantly upregulated seven of the same RefSeq genes, three of which are relevant to parasite survival and proposed schizophrenia mechanisms. One of the three genes affected is VIPR2 which has a role in GABAergic transmission, which could be a contributing factor to the deficits in learning and memory observed in infected individuals and is a gene of interest in schizophrenia research. LOC645317 encodes a protein that, when downregulated, allows the intrinsic apoptotic pathway to initiate. As it is constitutively upregulated in host cells infected with toxoplasma, it contributes to the anti-apoptotic effects of the parasite. The third gene, ALG1 assists in forming N-glycosylation intermediates which are scavenged by toxoplasma and are essential for infection.

Using the Gene Ontology database, researchers sought to determine the processes affected by each of the three strains and found that:

> "In Type I strain-infected cells, GO analysis revealed a marked effect in processes related to reproduction, response to stimulus, motility, metabolism, homeostasis, the central nervous system, inflammation, apoptosis, behavior, and transport. In Type II strain-infected cells, overrepresented ontologies were found to be related to circadian rhythm, growth, and signaling. Analysis in Type III strain-infected cells revealed an overrepresentation of processes related to nucleic acid metabolism, protein targeting, transport, cellular response to stimulus, protein localization, gene expression, localization, metabolism, nuclear export, and signaling." (Xiao 2011)

Through this screening, 76 of the genes affected by Type I toxoplasma were found to be involved in nervous system development with 23 genes directly affecting brain development. This could explain some of the correlation found between toxoplasma infection during

pregnancy and increased rates of disorders relating to the brain including autism, psychosis, and behavioral changes. A further 31 affected genes play a role in nerve impulses, of which ten genes correspond to action potential regulation. The remaining 21 genes play a role in synaptic transmission and plasticity, which are key areas of study in learning and memory and schizophrenia research. Type I toxoplasma also causes the dysregulation of multiple metabolic pathways responsible for synthesizing amino acids, carbohydrates, and lipids, possibly to increase its nutrient supply. In addition, Type I downregulates many genes involved in the immune response including the downregulation of pro-inflammatory cytokines and apoptotic factors, and the upregulation of anti-inflammatory cytokines.

Type III toxoplasma had the second largest pool of dysregulated genes, most of which were upregulations of genes responsible for amino acid, lipid, and nucleotide metabolism. It is theorized that this may be a compensatory stress response to scavenging of nutrients by intracellular parasites. Additionally, genes related to inflammatory responses and apoptosis were downregulated.

Type II affects the smallest number of genes, most of which are related to growth, circadian rhythms, and signaling. The largest change caused by Type II infections is the upregulation of prolactin genes. Excess hormone prolactin is secreted as a stress response and may have a protective effect against toxoplasma infection, but can cause a number of other effects in the body. Prolactin affects the Na+/K+ balance in cells, growth and development especially as it relates to cell proliferation and maturation, endocrine and metabolic effects, reproduction and nurturing of young, immunostimulatory effects, analgesic effects, and disruption of normal circadian rhythms. Most notably, however, excess prolactin has major effects on the nervous system, which may explain the behavioral changes found in the majority of individuals with toxoplasmosis, who are statistically more likely to be infected with Type II. These behavioral effects in toxoplasma-positive individuals may be mediated via prolactin-related increases in the rate of dopamine turnover. Type II toxoplasma also suppresses the host immune response (Xiao 2011).

In 2013, the Xiao laboratory released another paper that focused exclusively on genes dysregulated by toxoplasma that are involved in regulating neurotransmitter and neuropeptide systems. Microarrays performed to gauge the levels of transcriptional expression related to neurotransmitter receptors found no significant dysregulation in Type II infected cells. Type III infected cells had altered transcription of GRIN2A (an NMDA receptor subunit), ADRB3 (adrenergic receptor beta-3), and ADRA1A (adrenergic receptor alpha 1-A), however, when researchers attempted to validate these results with quantitative real-time PCR (qPCR), the

alterations in transcription were not statistically significant. Type I infected cells had alterations in DRD1 (dopamine receptor 1), GRIN2A, and HTR1D and HTR3E (serotonin receptor subunits). The mRNA transcriptional expression changes were confirmed using qPCR, and it was determined that all four genes were significantly downregulated. Western blot analyses were then performed to determine the effect of each gene on protein levels. The largest change was found in DRD1 which was 61% lower in Type I infected cells as compared to controls. The second most significant change was in GRIN2A which had decreased by 48%. HTR3E protein expression had decreased by 37% and HTR1D results were inconclusive. In summary, these findings suggest that Type I toxoplasma is implicated in the downregulation of NMDA, dopamine, and serotonin receptors.

Microarrays and qPCR were also performed to determine any changes in ligand-receptor systems for neuropeptides, nucleotides, and lipids. Type I modulated receptors containing the neuropeptide PTGER4 (prostaglandin E receptor 4) and two neuropeptides: PROK2 (prokinectin 2) and TAC1 (tachykinin precursor 1). The expression of TAC1, which is well established as playing a major role in mood disorders including depression and anxiety, was upregulated by 2300% with an increase in protein expression of 18%, while PROK2 protein levels were upregulated 1200%. PROK2 normally affects circadian rhythms, and protein overexpression could cause abnormalities in the circadian cycles. Type III and Type II showed alterations in several genes, but none significantly changed protein expression.

Finally, all three strains were tested to determine if any of the major genes involved in neurotransmitter pathways were affected by the parasite. Type I was found to cause a 213% increase in SLC1A3 protein levels and a 51% decrease in MAOA protein levels. SLC1A3 encodes a high affinity glutamate transporter that removes glutamate from the synaptic cleft, therefore the increase in SLC1A3 levels is likely a result of increased glutamate in the synaptic cleft. Monoamine oxidase A (MAOA) plays a crucial role in catalyzing the oxidative deamination of monoamines, and downregulation of the enzyme leads to excess amounts of monoamines in neurons, including dopamine, serotonin, norepinephrine and other monoamines, and is determined to be contributing agent in many psychological disorders. Type III toxoplasma increased the levels of the protein TDO2 by 53%. TDO2 is the enzyme that catalyzes the rate limiting step of the kynurenine pathway (Xiao 2013).

These results suggest that Type I toxoplasma could be the cause of the high odds ratio correlating schizophrenia and toxoplasmosis infections. It also may provide an answer to the ever-present question about why toxoplasmosis causes mental disorders in only a fraction of the total individuals infected. The effects of Type II toxoplasma on prolactin levels and Type III

toxoplasma on TDO2 levels could explain the behavioral changes that have been observed through experimentation on otherwise healthy individuals. Given that research into the strain hypothesis of toxoplasmosis and mental disorders is still in its infancy, few correlational studies have taken each strain into account and no studies have sequenced the genetics of an individual with both conditions *in vivo*. To conclusively prove causation, thousands of toxoplasma-positive and negative schizophrenia patients and controls would need to be diagnosed with one of the three strains and have their genomes sequenced. Given that strain variation is rarely taken into account, the odds ratios between schizophrenia and specific toxoplasma strains could be much higher or lower than those previously reported, and far more research needs to be conducted examining the effects of individual strains.

Kynurenine Pathway:

One of the main questions in schizophrenia research is how glutamate receptor hypofunction occurs. In the case of toxoplasma-positive schizophrenic individuals, the answer lies in a pathway that, though still largely unknown, is gaining recognition as being of vital importance in normal neurological function. The kynurenine pathway is responsible for metabolizing 95% of the tryptophan that enters the body. Under normal physiological conditions in the brain, tryptophan is metabolized into either serotonin and melatonin, or NAD/H via the kynurenine pathway (Vécsei 2013). The kynurenine pathway was discovered in the late 1800's and the kynurenine metabolites formed by the pathway were able to be synthesized by the early 1900's (Homer 1914). These metabolites were initially thought to be inert, although this was found to be false in the 1980's when it was discovered that seizures induced by one of the metabolites, quinolinic acid (QUIN), could be subdued by administering another of the metabolites, kynurenic acid (KYNA) (Foster 1984). These two metabolites must always remain carefully balanced to avoid devastating effects.

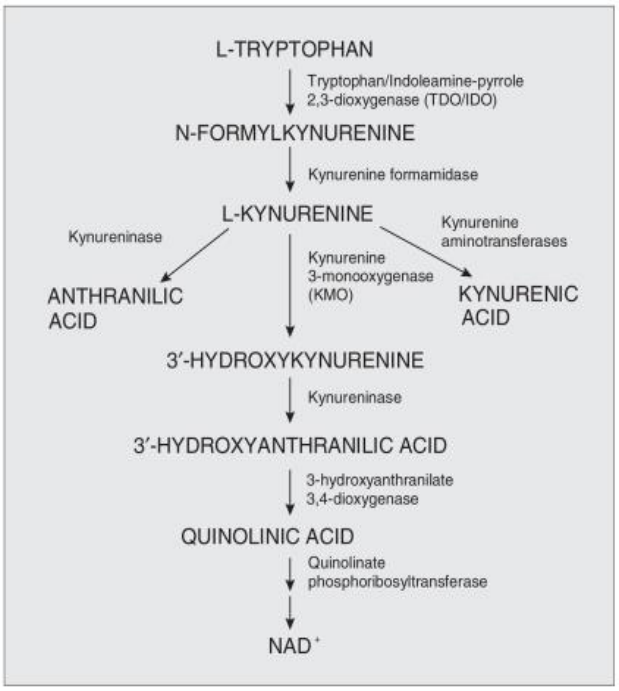

Fig. 7 The Kynurenine Pathway
From Andreassen & Jönsson 2012

The kynurenine pathway begins when the indole ring of tryptophan is opened to create formyl-kynurenine which undergoes rapid hydrolysis to create kynurenine. This is the rate-limiting step of the pathway and is catalyzed by one of two enzymes: TDO2 or IDO. The pathway then branches to either create KYNA via transamination by a family of enzymes known as kynurenine aminotransferases (KATs), or QUIN through the enzyme kynurenine 3-monooxygenase (KMO), which will go on to become NAD or NADH (Fig. 7) (Moroni 2012, Vécsei 2014).

KYNA and QUIN are diametrically opposed forces in the brain. Kynurenic acid is an NMDA receptor antagonist, whereas QUIN is an NMDA receptor agonist capable of causing excitotoxicity through excess glutamate release (Kessler 1989). Elevated KYNA has been implicated in numerous psychological disorders including schizophrenia, and QUIN has been implicated in numerous neurodegenerative disorders including Parkinson's disease and Huntington's disease (Stone 2001).

The kynurenine pathway performs another essential function in the body by acting as a valuable defense mechanism against foreign organisms establishing systemic infections. The increased activation of the kynurenine pathway results in the depletion of tryptophan in body tissues, thereby preventing invading organisms from obtaining the nutrients necessary for survival. Unfortunately, this is not the case with *T. gondii*, as the cystic form decreases parasite metabolism and allows it to survive the immune response and slowly grow within tissues through the production of encysted bradyzoites (Blanchard 2015). Initially, toxoplasmosis infection increases both QUIN and KYNA to 50x their normal concentrations in the acute phase of the infection. After the infection enters the chronic stage, KYNA remains elevated in the brain, though at lower concentrations than during the acute phase (Notarangelo 2014). QUIN levels return to normal after the parasites enter the chronic stage. This could be due to the lower Km of the rate limiting enzyme in the branch of the kynurenine pathway responsible for QUIN synthesis, KMO, which causes it to become saturated at lower concentrations than KAT II, the enzyme responsible for KYNA synthesis. Alternatively, evidence has been found that schizophrenic individuals have up to 36% lower rates of KMO activity in areas of the prefrontal cortex, causing the kynurenine pathway to shift towards the KYNA producing branch, creating an insurmountable imbalance in the KYNA: QUIN ratio (Sathyasaikumar 2011).

Kynurenine Pathway and Schizophrenia:

One 2001 study measuring the levels of kynurenine metabolites and their effects on the central nervous systems of schizophrenic individuals found that KYNA levels are significantly

greater in the prefrontal cortex of schizophrenic individuals as opposed to their unaffected counterparts (Schwarcz 2001). Another study from 2001 measured KYNA levels in 28 schizophrenic males and 17 healthy volunteers. Of the schizophrenic individuals, 25 were first-episode, drug-naïve patients. Healthy volunteers averaged 0.97±0.07 nM KYNA while the schizophrenic individuals averaged 1.67±0.27 nM with a much wider range of concentrations, the highest of which was 6.8nM (Erhardt 2001). In 2005, earlier findings were verified by a study that measured KYNA levels in 90 male schizophrenic individuals of whom 37 were first-episode and drug-naïve. A further 19 were drug-free for up to a year at the time of testing, and the remaining 34 were on various antipsychotic medications at the time of collection. These results were compared against 49 healthy male controls. The overall results from the experimental group returned an average KYNA level of 1.45±0.10 nM as compared to 1.06±0.06 nM for the controls. The results for the subgroups returned an average of 1.53±0.19 nM for drug-naïve, first-episode men and 1.53±0.17 nM for individuals currently taking antipsychotic medications. Interestingly, the drug-free group did not have significantly elevated KYNA levels with a 1.16±0.10 nM average. In this study, a positive correlation was found between KYNA levels in schizophrenic men and age, while no such correlation existed in the healthy population (Nilsson 2005). Women were not included in any of the above studies.

A study in 2014 expanded this experiment to include levels of QUIN in 22 Swedish schizophrenic patients, 13 male and 9 female, and 26 healthy controls (18 men and 8 women). All of the schizophrenic individuals were taking the drug Olanzapine. One schizophrenic patient was removed from the study due to a QUIN concentration of 85.1nM, most likely a result of contamination. There were no statistically significant differences between the QUIN levels of the experimental and control groups. However, in the schizophrenic group, the QUIN/KYNA ratio was decreased with a $p=0.057$, which when adjusted for age and gender resulted in a statistically significant $p=0.027$. Tryptophan levels were similar between both groups, KYNA was much higher in the schizophrenic group with a mean of 57.2±3.5 nM as compared to the controls with 37.3±4.3 nM per 50μL sample, and kynurenine was elevated in the schizophrenic group at 2.1±0.2nM vs 1.6±0.1nM in the control group (Kegel 2014).

All of the above studies provide substantial evidence that KYNA is elevated in schizophrenic individuals, while QUIN remains at physiological concentrations. None of the above studies included data about the status of participants with regard to toxoplasmosis infection or titers. Recent studies have begun to study the correlation between toxoplasmosis infection and KYNA levels. In one of the earliest studies, five mice were inoculated with 20 *T. gondii* cysts and sacrificed 6 weeks later after establishing chronic infections. Tissue samples

revealed a large number of activated glial cells, predominantly astrocytes, and a seven-fold increase in KYNA in infected mice (p= 0.01) as compared to controls (Guidetti 2006). The next study examined the brains of 15 schizophrenic individuals and 14 healthy control brains. In the schizophrenic tissue, KYNA was elevated in all regions of the brain (p=0.001), TDO2 activity was 61% higher than controls, IDO activity was 61% higher than controls, and KMO activity was 26% lower than controls (Schwarcz 2007).

Since the publication of these studies, multiple labs have successfully replicated these results and expanded upon their findings. One such study, published in 2013, inoculated mice with *T. gondii* cysts and measured the levels of KYNA at 0, 8, and 28 days, simulating the conditions prior to infection and during the acute and chronic phases. The study found that after 8 days both KYNA and QUIN levels were elevated in the serum, both of which returned to normal levels by day 28. After 8 days, tryptophan levels were reduced by 40%, while kynurenine levels were significantly increased in the brain. After 28 days, tryptophan levels had returned to normal, but kynurenine had increased 20-fold while QUIN, KYNA, and 3-HK had increased 50 fold. After 56 days, 3-HK and KYNA levels were still elevated, though to a lesser extent than at 28 days. This study also treated a separate infected group with anti-protozoal drugs. This group saw marked (p<0.001) decreases in 3-HK and KYNA levels after 28 days (Notarangelo 2014). Another article, published in 2012, studied the effects of acute toxoplasma infection on tryptophan metabolism and oxidative stress in mice. Mice were inoculated with *T. gondii* tachyzoites and after 72 hours of exposure, replicating the acute phase of the disease, the mice were sacrificed. At this time blood and urine were collected and levels of kynurenine and tryptophan were measured. As expected tryptophan decreased significantly while kynurenine levels increased significantly, with the ratio of kynurenine to tryptophan increasing in infected mice with a p= 0.002 (Engin 2012).

Toxoplasmosis and Schizophrenia interactions mediated by the Kynurenine Pathway:

The correlation between toxoplasma infection, increases in kynurenine metabolite concentrations, and their possible link to schizophrenia is a fairly recent discovery, and as such, only one study has been published that takes all three variables into account. A collaborative study compiled data from multiple medical and research facilities in six US states as well as universities in Austria and Germany. The purpose of the research was to evaluate the levels of kynurenine in toxoplasma-positive schizophrenic individuals and determine if a correlation exists between elevated kynurenine levels and a history of non-fatal self-directed suicidal

violence (NF-SDSV). The study took blood samples from 950 schizophrenic individuals and assessed both their symptom severity and history of NF-SDSV. Blood samples were assayed to determine kynurenine and tryptophan concentrations and toxoplasma infection status. The strains of toxoplasma were not determined. Participants were grouped into those with kynurenine levels in the upper 25th percentile and the lower 75th percentile. Both toxoplasma seropositivity and increased kynurenine levels were positively correlated with age, though older individuals tended to score lower in negative schizophrenia symptoms. Individuals who tested positive for toxoplasmosis and were in the upper 25th percentile for kynurenine were significantly more likely to have a history of NF-SDSV with an odds ratio of 1.63 ($p=0.048$), as opposed to seronegative individuals with an odds ratio of 0.75 ($p=0.170$). After adjusting for numerous factors including age, duration of illness, sex, level of education, etc., the odds ratio for seropositive individuals increased to 1.95 ($p=0.014$) compared to the negative group with an adjusted odds ratio of 0.81 ($p=0.371$) (Olaoluwa 2016).

This study is the first to take into account all three variables: toxoplasmosis infection status, schizophrenia, and kynurenine levels when assessing the incidence of NF-SDSV to occur. However, there are issues with this study, as it attempts to compile all four experimental conditions into single data points and loses much of the quantitative data in the process. The paper never gives the exact ranges of kynurenine in the negative or positive group, nor does it take into account toxoplasma titers, which have been shown in the past to have a measurable effect on symptom severity. The strain of toxoplasma present in the infected individuals was not determined, which, as previously noted, could be the determining factor in illness severity. Without these measurements, it is difficult to draw viable conclusions from these analyses.

Increases in KYNA are well documented in the CNS of both schizophrenia and toxoplasma-positive individuals, and the effects an overproduction of this metabolite has on neurologic function are complex and are not yet fully understood. In addition to the function of KYNA as an antagonist for NMDA, AMPA, and α7-nAchRs, KYNA also acts as an agonist for GPR35, which is a member of the family G-protein coupled receptors (GPRs), which contain 7-transmembrane domains and include 5,000 unique receptors. GPRs are thought to act as the targets of over half of the drugs currently on the market (Guo 2008). In 2006, a large screen was carried out in an attempt to find a ligand that specifically acted upon GPR35, and KYNA was identified as an agonist for the receptor which is involved in calcium mobilization. No other kynurenine metabolite was able to activate GPR35 (Wang 2006). A 2008 study by the NIH found that KYNA-bound GPR35 receptors specifically inhibit voltage-dependent calcium channels in sympathetic neurons (Guo 2008). The effect of this receptor could provide a

mechanism to explain the functional silencing of up to 78% of toxoplasma infected neurons. In a 2012 study, it was found that the tachyzoite stage of toxoplasma causes some neurons to become hyper-responsive to glutamate and fail to cease Ca2+ influx after stimulus is removed, while others become hypo-responsive to glutamate and Ca2+ levels do not increase upon stimulation. However, the more worrying effect of cerebral toxoplasmosis is that the bradyzoite form may functionally silence infected neurons over time, as indicated by a Thallium autometallography study, in which thallium ions, which act as an analogue to potassium, are introduced to the brain and uptake by neurons is able to be monitored. In this study, it was found that, though cysts decrease in number over time, the number of infected neurons that stop importing thallium, and thus have ceased to function, increases over time as only 40% of infected neurons were silenced at day 30, which had increased to 78% by day 60 (Haroon 2012).

Significant Genes Affected by both Toxoplasmosis and Schizophrenia:

Since the advent of high-throughput genetic sequencing, researchers worldwide have been searching for specific genes that are abnormal in most schizophrenic individuals. As yet, no genes have been definitively shown to be the cause of the disease, though hundreds of genes have been identified as candidates. Patterns have begun to emerge between genes that are the most highly implicated in schizophrenia. These genes can be grouped into (1) genes that affect the availability of glutamate, (2) genes that affect the function of NMDA receptors, (3) genes that control AMPA receptor function, (4) genes that affect synaptic plasticity or dendrite formation, (5) genes that affect oligodendrocyte formation and viability, (6) genes involved in dopamine metabolism, release, or synthesis, (7) and genes that are related to oxidative stress (Carter 2006).

Many of the genes that are commonly found in individuals with schizophrenia are the same genes that are altered by toxoplasma Type I, or affect the same pathways. Of these genes, seven are particularly noteworthy in that they may provide an explanation as to why all individuals who are infected with Type I toxoplasmosis do not display schizophrenia symptoms. These mutations are present at birth, and it is through the actions of toxoplasmosis in conjunction with these genetic changes that schizophrenia could arise. These commonly mutated genes are GRIN2A, SLC1A3, DRD1, MAOA, VIPR2, CHRNA7, and KMO (Carter 2006, Xiao 2013).

GRIN2A is a gene that encodes the NR2A subunit in NMDA receptors. This gene has been shown to contain multiple repeats in schizophrenic patients, reducing its expression and decreasing the number of NR2A subunits produced. This leads to hypofunction of the NMDA

receptors in the brain (Liu 2015, Tang 2006). Toxoplasmosis Type I also targets GRIN2A, significantly downregulating its expression, and causing further hypofunction of NMDA receptors (Xiao 2013). As the expression levels of GRIN2A decrease, the severity of schizophrenia symptoms increase, thus the compounding effect of the preexisting GRIN2A mutation and further dysfunction caused by Type I toxoplasmosis could lead to the emergence of schizophrenia symptoms associated with glutamatergic hypofunction (Tang 2006).

SLC1A3 belongs to a class of genes known as the solute carrier family, which encode excitatory amino acid transporters, in this case Excitatory Amino Acid Transporter 1 (EAAT1). These transport proteins are responsible for removing glutamate from the synaptic cleft and have been found to be significantly increased in the brains of schizophrenic individuals (Smith 2001, Wilmsdorff 2013). This gene is also upregulated two-fold in neuroepithelial cells infected with Type I toxoplasmosis (Xiao 2013). An increase in expression of SLC1A3 could either cause an increase in glutamate uptake from the synaptic cleft, leaving a deficit in glutamate available to bind to NMDA receptors, or occur in response to a sustained, dramatic increase in glutamate in the synaptic cleft (Wilmsdorff 2013).

DRD1 is a gene that is responsible for the creation of D1 dopamine receptors, a decreased expression of which is implicated in increased risk of schizophrenia (Zhu 2011). Studies have shown that D1 receptors in the prefrontal cortex likely play a role in memory and learning through facilitating the excitatory actions of glutamatergic neurons, leading to an increase in long term potentiation, an essential process for learning and memory function (Williams 2006). In mouse studies where the function of D1 receptors were inhibited in the prefrontal cortex, mice showed decreased learning capabilities (Rinaldi 2007). Further studies employing rhesus monkeys as an animal model have corroborated these results. When monkeys were taught to perform a task and then given D1 antagonists, the accuracy with which they performed the task decreased, and the time it took to complete the task increased in a dose dependent manner (Sawaguchi 1991, Sawaguchi 1994). In humans, the association between mutations in the DRD1 gene causing decreased D1 receptor activity in the prefrontal cortex and working memory are consistent with findings in animal models. In a trial that tested the working memory of 139 schizophrenic patients that were grouped into individuals with mutations in the DRD1 gene that were homozygous, heterozygous, or had no mutation, individuals with the homozygous mutation showed significantly inferior performance as compared to the two other groups (Rybakowsi 2005). When PET scans were performed to determine D1 receptor concentration in the brains of 17 male schizophrenic individuals who were drug-naïve or at least two weeks free from drugs at the time of imaging, it was found that all of the schizophrenic subjects had

significantly decreased D1 concentration in the prefrontal cortex as compared to 18 healthy volunteers. The decrease in D1 receptor binding in the prefrontal cortex was positively correlated with both the severity of negative symptoms and worse performance on a test of working memory (Okubo 1997). Given that the worsening of negative symptoms and the decrease in working memory functions seem to be correlated with a decrease in DRD1 function in a dose-dependent manner, the presence of a preexisting DRD1 mutation coupled with the two-fold reduction in D1 receptors caused by downregulation of DRD1 by Type I toxoplasmosis could cause a severity of symptoms to a pathological degree (Okubo 1997, Xiao 2013).

MAOA encodes the enzyme Monoamine Oxidase A, which is a key enzyme responsible for the metabolism of serotonin, norepinephrine, and dopamine in the brain. While data is still inconclusive as to whether MAOA gene mutations are present in genomes of the majority of schizophrenic individuals, some studies have found a positive correlation between the two factors, and Type I Toxoplasma has been shown to cause a -1.96-fold change in protein expression (Li 2008, Liu 2015, Xiao 2013). Deficits in the production of this enzyme have been linked to aggressive and antisocial behaviors (Beach 2010, Reti 2011, McDermott 2013).

VIPR2 has been heavily implicated in the risk of schizophrenia, and has been experimentally shown to affect learning, memory, and normal circadian rhythm functions (Vacic 2011, Yuan 2014, Brown 2007). In mouse models with a deletion of the VIPR2 gene, conditioned fear responses were absent 48 and 72 hours after initial stimulus as compared to controls who continued to display fear responses up to 144 hours after initial conditioning (Chaudhury 2008). Further mouse studies found that deleting the gene VIPR2 or VIP, the gene encoding the neuropeptide that activates VIPR2, also causes mice to lose normal circadian rhythms, with the VIPR2-/- mice showing no pattern in circadian rhythms. In the VIP-/-, the addition of a VIPR2 agonist is sufficient to restore up to 71% of normal circadian rhythms (Aton 2005, Brown 2007). This gene was found to be altered by Type I toxoplasma, though data on changes in protein expression because of the alteration were inconclusive, thus more research into the effects of Type I toxoplasma on VIPR2 is necessary before drawing conclusions as to its effect (Xiao 2013).

CHRNA7 mutations are commonly found in schizophrenic individuals. Mutations in this gene cause a deficit in the number of α7-nAchRs, which have been implicated as one of the receptors that may play a role in the etiology of schizophrenia (Stephens 2009, Bakanidze 2013, Bertelsen 2015). Toxoplasmosis causes inhibition of α7-nAchRs through the binding of KYNA at higher than normal levels (Albuquerque 2009). Combined with the decrease in α7-nAchR levels caused by a CHRNA7 mutation, this could cause the function of the receptors to decrease to a

level that could cause schizophrenia symptoms in an individual who may have been affected to a lesser degree if toxoplasma were not present.

KMO is the rate-limiting enzyme in the branch of the kynurenine pathway that makes QUIN. Normally, QUIN is maintained in balance with KYNA under physiological conditions (Kessler 1989). However, chronic cerebral toxoplasmosis causes a chronic increase of KYNA, while QUIN levels return to normal after the acute stage ends (Guidetti 2006). This increase in only KYNA can be explained by a mutation in KMO that is found commonly in schizophrenic individuals (Andreassen 2012). A 2011 study found that, in the prefrontal cortex of schizophrenic individuals, KMO activity was reduced by 36% as compared to controls (Sathyasaikumar 2011). A mutation in the KMO gene was found to be correlated with decreased cognitive ability in humans in general, with schizophrenic individuals with the mutation having the worst performance of those tested (Wonodi 2014). Another study found that decreased KMO mRNA levels in the prefrontal cortex were correlated with psychosis in bipolar patients ($p=0.005$) and schizophrenic patients ($p=0.02$). Schizophrenic individuals had half the amount of prefrontal cortex KMO mRNA as compared to controls, which was positively correlated with increased KYNA levels (Lavebratt 2014). The presence of a mutation in KMO could explain why some, but not all, individuals infected with Type I toxoplasmosis exhibit symptoms of schizophrenia. If KMO activity is already decreased, infection with toxoplasma will cause an increase in KYNA that is not balanced by QUIN far greater than that of an individual with normally functioning KMO enzymes.

All of the above genes are implicated in the etiology of schizophrenia, and are additionally affected by chronic cerebral Type I toxoplasmosis. The compounding effect of toxoplasmosis infection, coupled with preexisting genetic mutations that predispose an individual to schizophrenia, could explain why some individuals with Type I toxoplasmosis exhibit schizophrenia symptoms while others do not. Schizophrenia has long been thought of as a disease which results from both genetic mutations and environmental factors. The actions of toxoplasmosis on the same genes or genes which directly affect the same pathways as those theorized to be affected in the pathology of schizophrenia could explain how infection with toxoplasmosis could act as a catalyst in the emergence of schizophrenia symptoms.

Mechanism Summary:

Toxoplasmosis affects around one-third of the world's population. Most of these infections are caused by the Type II strain, or the less common Type III strain, and are asymptomatic. Most of the remaining individuals are infected with the Type I strain. Type I

toxoplasma is far more virulent than the more common strains, causing a greater immune response and a 1,000x higher cystic load than Type II. Type I is most likely responsible for the high odds ratio between toxoplasmosis and schizophrenia discovered through testing of toxoplasma-positive and negative schizophrenic and healthy individuals.

After infection with Type I toxoplasma, the parasite colonizes host cells systemically, especially those within the brain. Having passed the blood-brain barrier, tachyzoites will replicate and colonize neural cells, triggering a Th1 immune response. The immune response activates astrocytes and glial cells, which are able to clear the parasites while neurons remain infected. The Th1 response brings massive numbers of T cells into the brain, which release cytokines, the most important of which being IFN-γ. IFN-γ causes, among many other immune functions, a large increase in the amount of the rate limiting enzymes in the kynurenine pathway, TDO2 and IDO, in order to upregulate the degradation of tryptophan and starve the parasites. This causes the parasite to encyst and transitions the infection into a chronic state.

The kynurenine pathway remains constitutively activated, keeping parasites in their encysted form, and causing a marked increase in the concentration of KYNA with no compensatory increase in QUIN, possibly due to a mutation in KMO common to schizophrenia sufferers. At high concentrations, KYNA acts as an antagonist of NMDA and AMPA receptors causing hypofunction, which GABAergic neurons interpret as too little excitatory activity in the brain and lower inhibitory activity in response, leaving the excitatory activity unchecked. Experimentally, hypofunction of NMDA and AMPA receptors was found to lead to cognitive impairment and negative schizophrenia symptoms. KYNA also acts as an antagonist of α7-nAchRs which mediate the release of neurotransmitters in various areas of the brain. In the prefrontal cortex, α7-nAchRs control the presynaptic release of glutamate. When inhibited by KYNA, α7-nAchR mediated glutamate release decreases, contributing to the hypofunction of NMDA receptors via decreased ligands available for binding. In the hippocampus, α7-nAchRs control GABAergic activity, and when KYNA is at abnormally increased concentrations, α7-nAchRs are inhibited at a higher than normal levels. This inhibition, coupled with the prevalent CHRNA7 mutation that decreases the number of α7-nAchRs in the brains of schizophrenic individuals creates an additive effect. This significantly decreases GABAergic activity and leaves hippocampal pyramidal neurons in a hyper-excitable state which leads to impaired learning, memory, and sensory gating.

In addition to KYNA mediated mechanisms, toxoplasmosis also causes massive increases in L-DOPA in the CNS via two tyrosine hydroxylase enzymes secreted by the parasite. These large increases in L-DOPA are converted to dopamine in dopaminergic neurons, the highest concentrations of which are in the substantia nigra and the striatum. A dysfunctional signaling pathway beginning with decreased GABAergic activity in the hippocampus caused by KYNA binding to α7-nAchRs and NMDA receptors, causes hyper-excitable glutamatergic neurons, which lead to an increase in glutamate release in the striatum. This disinhibits dopaminergic neurons in the midbrain, causing increased dopaminergic activity in the striatal projections of the neurons. This, coupled with the high levels of dopamine synthesis, leads to an extreme hyperdopaminergic state. Hyperdopaminergic states are known to cause positive schizophrenia symptoms, predominantly those collectively labeled as psychosis. Lastly, some individuals may be predisposed to schizophrenia due to inherited genetic mutations. When combined with the genetic changes caused by Type I toxoplasmosis, this could have a compounding effect, creating a diagnosable disease state. The culmination of all of the dysregulation in neural pathways in addition to preexisting mutations can explain all of the symptoms that make up the disease schizophrenia through the influence of chronic cerebral toxoplasmosis (Fig 8).

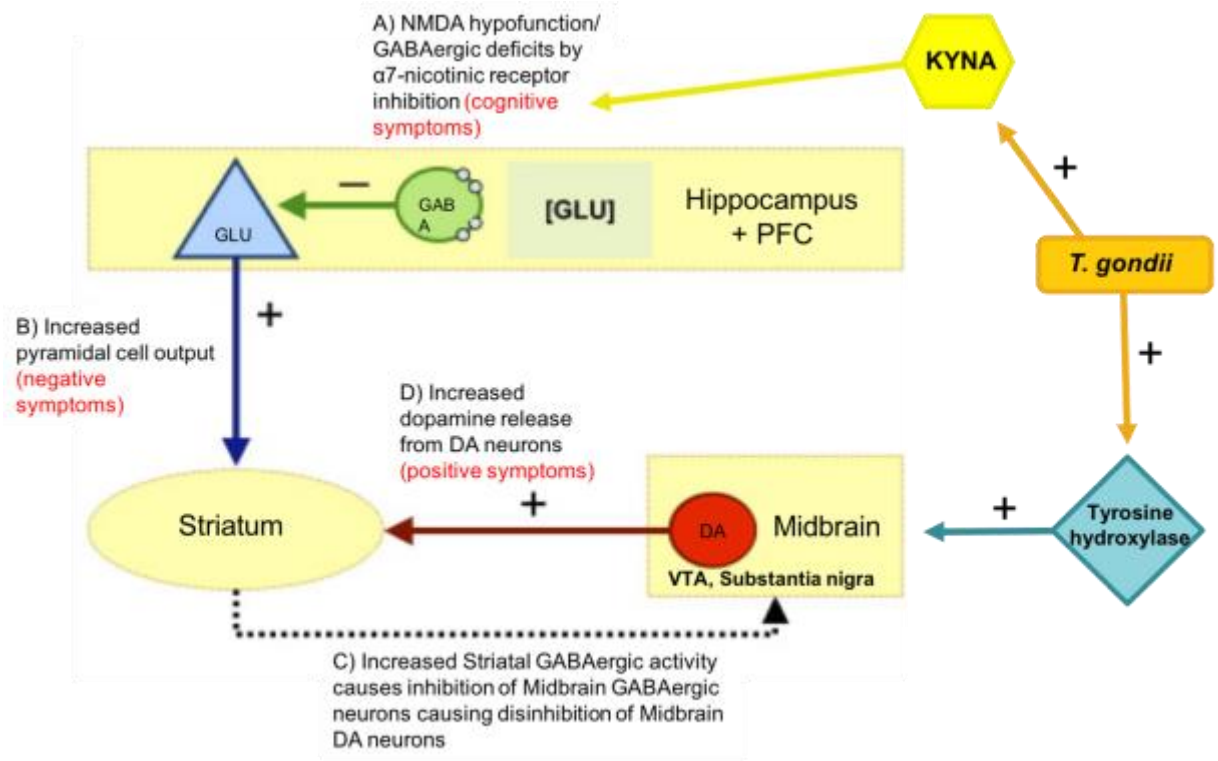

Fig 8

From Stone 2010 with alterations

Future Research:

Though some evidence and a promising model exist to explain toxoplasmosis as a contributing factor in the development of schizophrenia, the research into the connection is still in its infancy. In order to conclusively establish a link between the two conditions to the point that testing and treatment for toxoplasmosis becomes a standard step in schizophrenia management, there are a few crucial avenues that must be explored.

The first of these avenues is fairly simple and involves first testing schizophrenic individuals for toxoplasmosis and performing a psychological evaluation to determine symptom severity. These individuals would then be treated for the toxoplasmosis infection until no cysts remain, and would then be reevaluated. Of course, any trial which involves monitoring individuals with schizophrenia for proper adherence to medication regimens will cause all subjects to show improvement in symptoms, so to account for this false positive result, subjects would need to be tested again anywhere from six months to one year post study. This would ensure that (1) the toxoplasmosis infection was eliminated and has not recolonized and (2) that symptoms have actually improved and were not a result of medication regimen adherence. If patients showed a marked improvement in symptoms, it could be concluded that toxoplasmosis did cause a worsening of symptoms. In this case, treatment of the infection would be a worthwhile step in managing schizophrenia.

A second study that would provide evidence for the relationship between toxoplasmosis and schizophrenia would be the sequencing of genes from both schizophrenic and mentally healthy individuals with each of the two common strains of toxoplasmosis. The experiments performed by the Xiao laboratory (2009, 2011, 2013) provide promising data as to which genes involved in neural function are affected by each strain of toxoplasma, but these studies were performed *in vitro* on neuroepithelial cells in a petri dish. Without the effect of any of the complex influences exerted on cells *in vivo*, these results do not reflect what is actually taking place in the human brain. To test the actual genetic effects of toxoplasmosis, multiple brains from deceased individuals with Type I or II toxoplasmosis would need to have the mRNA profiles of infected cells sequenced and compared to the neural cells of healthy individuals. This would show which genes showed changes in expression and to what extent each common strain of toxoplasmosis affects neural genes. In addition, the mRNA profiles of infected neural cells of type I or II toxoplasma-positive schizophrenic individuals would need to be sequenced and compared to the profiles of uninfected schizophrenic individuals. The comparison of all of these data could provide promising avenues for research into specific genes that are affected by toxoplasma strains and are more prominent in toxoplasma-positive schizophrenic individuals,

as well as providing much needed data into the difference each strain has on neural genes *in vivo*.

A third study that would provide more evidence as to which strains of toxoplasma alter the risk of developing schizophrenia is an epidemiological survey that includes testing for strain variation. The combined meta analyses exploring the relationship between toxoplasmosis and schizophrenia tested over 10,000 schizophrenic individuals. However, none of these studies considered the effect of the three different strains on these odds. More studies should be performed testing for individual toxoplasma strains in both schizophrenic and healthy individuals. This data could provide a more concrete link between Type I toxoplasma and schizophrenia with a much higher odds ratio (as suggested in Xiao 2009), while potentially eliminating the common type II strain as a factor.

The fourth study would confirm or refute the findings of Haroon (2012), which found that after 60 days of infection with toxoplasma, 78% of neurons are functionally silenced. Given the large number and breadth of neural cells infected with toxoplasma, the functional silencing of such a high percentage of infected neurons seems implausible. If such a high number of neurons were functionally silenced, one would expect to see far more progressive cognitive decline to the degree that toxoplasma infection would mimic a neurodegenerative disease. Since one third of the world's population is infected with toxoplasma, and widespread advanced neurodegeneration is not nearly so prevalent, it seems there must be some other explanation. Repeating this study on a larger number of animals, with the three different strains, would help to confirm or contradict this research. It would also be important to visualize functional silencing in ways other than thallium uptake studies, such as electrophysiology to visualize cell polarization and evoked release, studies of synthesis of key cellular proteins, or measuring uptake of other ions like calcium to see if perhaps the method created a false positive result.

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