RJBS

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# About this Issue

**Statement of Purpose**

The Rhodes Journal of Biological Science is a student-edited publication which recognizes the scientific achievements of Rhodes students. Volume XXVII marks the sixth year since the journal was brought back into regular publication by Mark Stratton and Dr. David Kesler in 2006. Founded as a scholarly forum for student research and scientific ideas, the journal aims to maintain and stimulate the tradition of independent study among Rhodes College students. We hope that in reading the journal, other students will be encouraged to pursue scientific investigations and research.


Editorial Staff.....4

Student Contributors.....5

Influenza
Benjamin Brady.....6

What in God's Name is the Religious Brain? The Neurological Correlates of Religion and Spirituality
Lindsey Akers.....11

Poor Physical Function among Childhood Brain Tumor Survivors
Lan Tran et al.....19

Behavioral Changes in Adult Rodents Infected with *Toxoplasma gondii*
Laura Wagner.....29

MyoB is Required for Cytokinesis in *Aspergillus nidulans*
Xiao Wang.....33

Reading in Technicolor: Proposed Neural Mechanisms for Grapheme-Color Synesthesia
Grace Mosley.....37



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**Alex Yu '13** is a junior Biology major. In addition to serving as an editor for the RJBS for the 2012 issue, he also serves as the Sou'Wester news section editor, as a Kinney MED Coordinator, and is a member of BBB Biology Honor Society. In his spare time, he enjoys cooking, writing, and catching up on television shows. Currently applying to medical school with an interest in reproductive endocrinology, he hopes to begin in the fall of 2013 and start his journey to becoming a doctor.

**Liz Karolczuk '14** is a Neuroscience major and Religious Studies minor from Crystal Lake, IL. She is a Bonner Community Service Scholar and spends her time as a Labor and Delivery Unit assistant at the MED. She co-founded a program at Evergreen Aftercare called Science Fridays, where she strives to build a passion for science through experiments and lesson plans with elementary school children. Liz is on the Executive Board of GlobeMed, an organization on campus that advocates for social justice through global health education and activism. Liz also serves as the Community Service Coordinator for her sorority, Chi Omega. Last summer, she was an intern for the

American Red Cross in Chicago, IL and last fall she traveled to New Orleans to rebuild homes through Rhodes Rebuilds. This summer she will be volunteering in an orphanage for two months in Accra, Ghana for her Bonner International Summer of Service. In her free time, Liz enjoys watching documentaries, going to concerts, and playing tennis. After graduation, she plans on attending medical school and pursuing a career bridging global health policy and clinical medicine.

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# Influenza

Benjamin Rush Brady
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**Introduction**

*Background*

The influenza virus infects roughly 20 percent of the world's population each year (Lee et al. 2011). As such, the virus has remained one of the world's leading causes of viral illness since its description as an emerging disease in the latter half of the 12th century (Webby and Webster 2001). Arguably the major cause of influenza's continued success is unsurprisingly due in part to its structure. As an RNA virus, influenza is more susceptible than other types of viruses to mutations in its genetic material, leading to the formation of new strains. Influenza is categorized based upon the classification of the two host-determining surface proteins, hemagglutinin and neuraminidase. Each subtype of influenza is labeled based upon the structure of these proteins (hemagglutinins 1-15 and neuraminidases 1-9). Mutations in the structure of these proteins lead to the level of effectiveness of the virus in the host population. These mutations enable the virus to avoid the acquired human immune responses and to successfully infect new hosts. This process is known as antigenic drift and is a common phenomenon (Webby and Webster 2001). While less common but of greater concern is the process of antigenic shift (Webby and Webster 2001). This process occurs when two different strains of the influenza virus infect a common host, leading to the rearrangement of their genetic material. This rearrangement, in turn, produces a novel virion that can infect its host population largely unimpeded, leading to infections of pandemic proportions (Webby and Webster 2001).

Specifically, the 20th century witnessed three extreme cases of pandemic influenza outbreaks occurring in 1918, 1957, and 1968 (Guan et al. 2009). Beginning in 1918, over 50 million deaths were recorded over a two-year period as a result of the H1N1 subtype of influenza (Rossman and Lamb 2011). While controversial, the contemporary literature suggests that this particular subtype of influenza was directly derived from an avian precursor (Taubenberger et al. 2006). In 1957, most of Asia was infected by the H2N2 subtype of influenza which has since been characterized as the result of antigenic shift. It has been argued that this particular subtype was the result of genetic mixing between the H1N1 strain and the novel H2 and N2 proteins derived from Asian avian sources (Kilbourne 2006). Similarly, the 1968 pandemic of Hong Kong was caused by the H3N2 virus again as the result of genetic mixing of the H2N2 subtype with a novel H3 protein gene (Guan et al. 2009).

Importantly, the 21st century has also been subject to influenza outbreaks. In 2009, a pandemic outbreak of a novel H1N1 subtype emerged in the United States and Mexico (Guan et al. 2009). This subtype of influenza was antigenically distinct from seasonal H1N1 and humans were largely immunologically unprepared—over 22 million people were infected (Guan et al. 2009). This virus, unlike its 1918 avian predecessor, had its roots in a swine lineage. Currently, three major influenza subtypes are circulating in humans (seasonal H1N1, H3N2, and the more frighteningly pandemic H1N1) (Guan et al. 2009). Based on previous behaviors, it is likely that these strains will undergo further antigenic reassortment, thus leading to novel subtypes that could potentially cause new pandemics (Guan et al. 2009).

Due to influenza's unique structure and ensuing function, it has been an extremely successful virus and is still categorized as an emerging disease as a result (Webby and Webster 2001).

*Structure*

Influenza is an enveloped, negative-strand RNA virus containing eight RNA segments (Rossman and Lamb 2011). Covered with spike glycoproteins, influenza has two major surface proteins that largely determine its host specificity: hemagglutinin (HA) and neuraminidase (NA). Hemagglutinin is responsible for mediating viral entry into host cells while neuraminidase facilitates, and in most cases allows for, the release of viral progeny (Rossman and Lamb 2011). Thus, it is important to understand hemagglutinin's role in determining the host range and tissue tropism of particular influenza subtypes (Zambon 2001). In addition to both of these essential proteins is a third, equally essential, membrane protein. This protein, the M2 protein, is an ion channel that deals both with viral entry and release by allowing protons to enter the virion and dissociate the ribonucleotides from other structural proteins, allowing for their subsequent entry into the host cell (Rossman and Lamb 2011). M1 proteins, mentioned above, provide the influenza virus with its structural integrity and bridge the lipid membrane and the core of the virus (Rossman and Lamb 2011). (Figure 1)

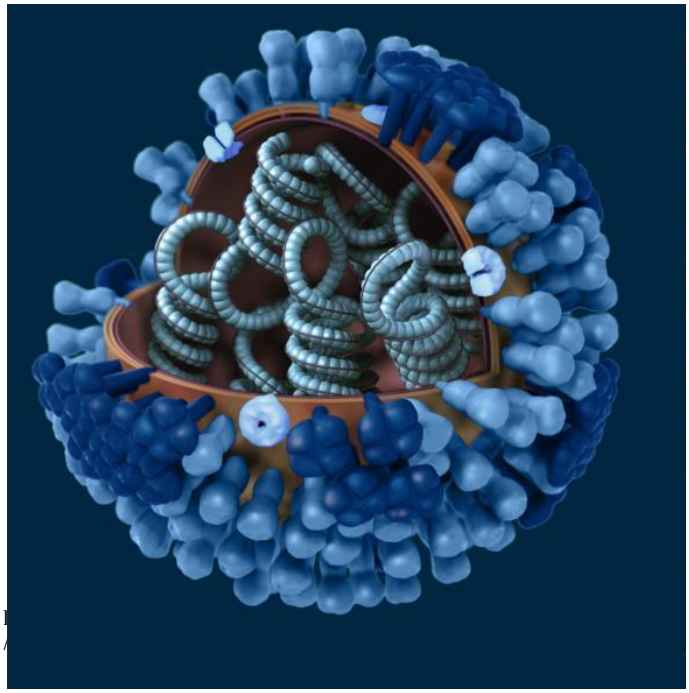

**Figure 1.** The basic structure of a spherical influenza virus particle. http://phil.cdc.gov/Phil/details.asp

As a pleiomorphic virus, influenza forms spherical and filamentous virions that are both roughly 100 nanometers in diameter and length, respectively. It is important to note that most studies have found that when the virus is isolated from lung and respiratory tract sections, the filamentous virions are present whereas the spherical form of the virus is largely used in laboratory settings (Nakajima et al. 2010). While most of what we know about the structure and function of the influenza virus is largely the result of spherical virions, more research into exactly how the filamentous virions differ is necessary (Rossman and Lamb 2011). Interestingly, both forms of the virus are similarly infectious and contain roughly similar ratios of viral proteins (Roberts et al. 1998).

Structured in this way, the influenza virus exhibits a fascinating and unique mechanism for adsorption, entry, replication, and release of virion progeny within the host cell.

*Pathogenesis*

The hemagglutinin glycoproteins of influenza, when in contact with host cells, bind specifically to host cell surface receptors that contain sialic acid (Zambon 2001). Once bound, the hemagglutinin protein mediates the fusion of the viral membrane and the cellular membrane via endocytosis (via clathrin-coated pits) (Fodor and Brownlee 2002). Once inside the cell, the endosome begins to acidify in an attempt to digest the molecule (Goodsell 2006). This acidification causes the hemagglutinin molecule to change in conformation, exposing a section of the protein that is termed the fusion peptide. This fusion peptide then quickly attaches itself to the endosomal membrane and "zips" the viral particle toward the membrane, leading to the fusion of the two membranes (Söllner 2004) (Figure 2). Once these membranes are in place but before fusion, the M2 proton channels enable protons to rush into the virus particle, acidifying its interior. Upon the acidification of its contents, the M1 proteins dissociate from the genetic material, allowing it to flow freely into the host cell after fusion where it is transported to the nucleus for replication (Zambon 2001).

Within the nucleus, the virus's ribonucleoproteins are transcribed by the supplied viral RNA-dependent RNA polymerase into mRNAs (Fodor and Brownlee 2002). This process creates a template to generate subsequent vRNAs, which in turn serve as templates for cRNA strands, required for replication. As an RNA virus, influenza is dependent upon the machinery within the host cell (Fodor and Brownlee 2002). Interestingly, influenza also inhibits the translation of the host's genetic material and induces preferential viral protein synthesis (Fodor and Brownlee 2002).

The export of the newly synthesized genetic material of influenza viral progeny and its association with structural M1 proteins is not well understood and is hotly contested (Fodor and Brownlee 2002). What is understood, however, is that the M1 protein is a central determinant in viral budding (Fodor and Brownlee 2002). Once the genetic material moves to the cell membrane it is enclosed by an envelope containing the hemagglutinin and neuraminidase glycoproteins, as well as the M2 proton channels.

Finally, the neuraminidase glycoproteins cleave the sialic acid-containing receptors from the newly synthesized hemagglutinin glycoproteins, thus preventing the accumulation of unreleased virion progeny within the host cell (Fodor and Brownlee 2002). (Figure 3)

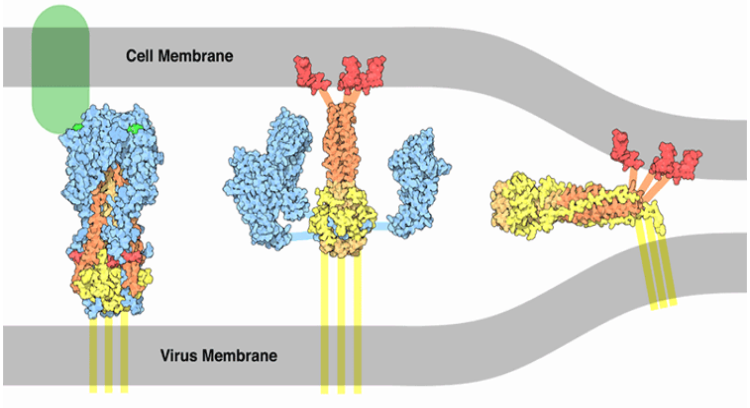


**Figure 2**. The three conformations of hemagglutinin. From left to right: initial conformation, unzipped acidic conformation exposing red fusion peptide, zipped conformation. Söllner 2004.

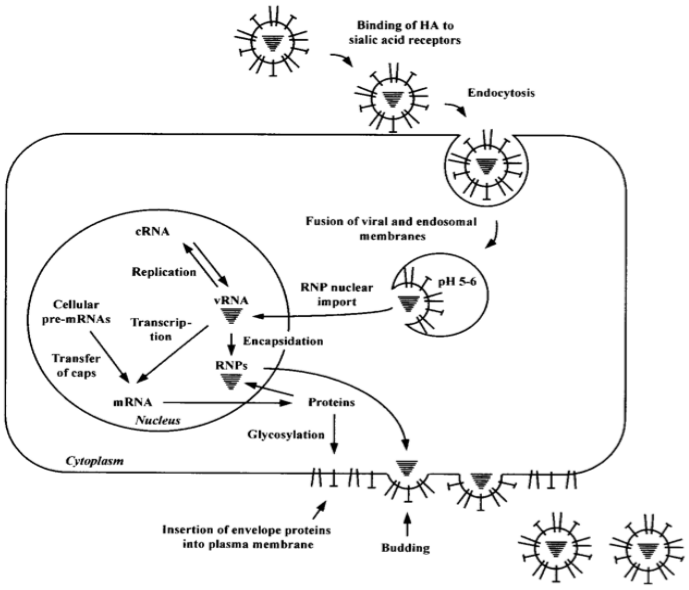


**Figure 3**. Mechanism of infection of influenza virus. Adsorption, entry, replication, assembly, release. Fodor and Brownlee 2002.

### *Clinical Manifestations*

Those infected with influenza will exhibit a range of clinical symptoms after a one to four day incubation period. The symptoms of acute infection include an abrupt onset of fever, chills, headache, cough, malaise, myalgia, sore throat, among others, and the severity of each symptom varies with each subtype (Zambon 2001). The replication site of influenza in humans also varies, but the respiratory tract appears to be the most common site of viral replication (Zambon 2001). Symptoms also vary according to the patient's age. It appears to be the case that young children and elderly adults are the most susceptible hosts (Bennet 1973).

### *Current Issues*

Currently, antiviral strategies are the most compelling, and indeed the most necessary topic of interest in the field of influenza research. Because of influenza's susceptibility to antigenic drift and shift, vaccinations often prove obsolete (Lee et al. 2011). Specifically, changes in the subtype after a vaccination has been put into circulation cause new outbreaks of novel strains. In a more perfect world, vaccinations would simply be remade after a new subtype has been identified, but practically speaking, the process by which vaccinations are created is vastly expensive and time consuming making this strategy less efficient and minimally effective (Lee et al. 2011).

Over the years, there have been two major classes of anti-influenza serums: neuraminidase inhibitors and adamantanes (Hedlund 2010). Neuraminidase inhibitors inhibit the neuraminidase glycoprotein from cleaving the sialic acid-containing receptors from the viral hemagglutinin glycoprotein spikes prior to the release of virion progeny, making the spread of the virus impossible (Hedlund 2010). Adamantanes, however, function to block M2 ion channels, thus preventing the dissociation of the M1 structural proteins from the genetic material. This process, in turn, inhibits the release of the genetic material into the host cell, rendering replication impossible (Hedlund 2010).

To date, the increased resistance (90% by 2005) and unfavorable side effects (insomnia, confusion, hallucinations, ataxia, difficulty in concentration, depression, dizziness, etc.) caused by adamantanes has led to the increased use of neuraminidase inhibitors (Hedlund 2010). Unfortunately, the increased use of these inhibitors has also led to the increased resistance of influenza to these drugs (Hensley et al. 2011). It is thus imperative that new antiviral strategies are developed hastily and effectively in order to prevent further outbreaks of pandemic proportions.

### Discussion

Most of the current research centers around two areas of study: antiviral strategy development and the study of influenza's mechanism of action and evolution. Because of the current issues revolving around influenza and its rapid rate of mutation, new antiviral strategies are of the essence. As mentioned earlier, nearly all infective influenza subtypes are resistant to the drugs currently available to the human population (Lee et al. 2011). In an attempt to remedy this precarious situation, researchers, with the aid of studies revealing more clearly influenza's mode of action, are beginning to develop antiviral strategies in the laboratory. In one recent study, researchers attempted to take the information that they had learned from previously successful antiviral strategies with HIV and apply it to influenza. Specifically, these researchers used peptides in order to freeze the hemagglutinin structure in an intermediary conformation, halting it from "zipping" the viral membrane to the host membrane in the later stages of infection. Peptides correspond to a variety of viral fusion proteins (as was the case in their previous study with HIV), and can bind to their conformational changes (Lee et al. 2011). However, this strategy for inhibiting viral infection is not without its shortcomings. These peptides have a very short half-life and a relatively low potency *in vivo*, calling for some alteration. As such, the researchers utilized the same strategy that they used when researching HIV—they used a cholesterol-conjugated, membrane-targeted peptide derived from the corresponding amino acid sequences of the hemagglutinin structure after its conformational change is triggered, but before it is able to bring the two membranes together. The cholesterol, when linked to the peptide, allows for it to be trafficked into the endosomal compartment with the influenza virus particle and greatly increases the peptides' half-life *in vivo*. Essentially, this peptide binds to the newly exposed fusion peptide before it can attach to the host's endosomal membrane (Lee et al. 2011). Upon experimentation, they found that peptides not tagged with cholesterol had little to no effect on influenza's infectivity, as those virus particles were clearly able to enter the host cells. However, when visualizing the results of the cholesterol-linked peptides, we see that membrane fusion is inhibited, thus halting infection altogether (Figure 4). They

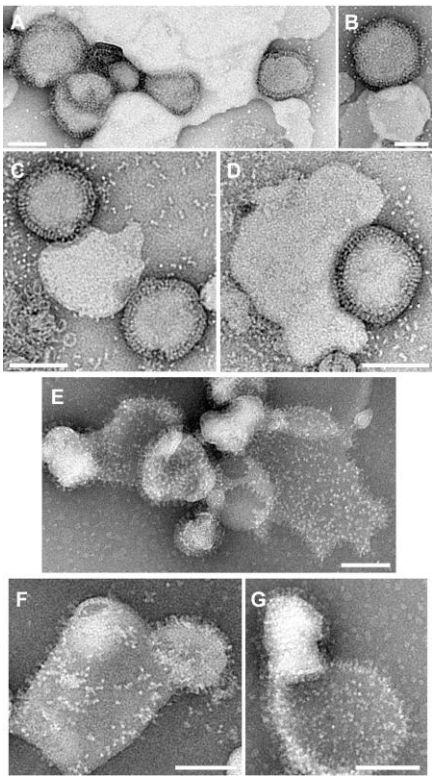


**Figure 4**. A-D: virus particles treated with cholesterol-conjugated peptides—no membrane fusion. E-G: virus particles not treated—membrane fusion ensues. Lee et al. 2011.

also used different sequences in order to create differently sized peptides, but found that the longest peptide coding for the fusion peptide region of the hemagglutinin was primarily responsible for inhibition. This research is invaluable, largely because of its novel role in halting infection at a time when we are nearly out of antiviral strategies, but also because this technique has been shown to work across multiple viral species, perhaps suggesting that the future could hold a single, but broadly effective antiviral strategy.

Related and equally as valuable is a study that examines the antigenic variation and drug resistance of influenza A virus. In studying two neuraminidase inhibitors (oseltamivir and zanamivir), the researchers set out to look at why the virus becomes resistant to neuraminidase inhibitors. While it is mostly the case that increased drug use leads to increased resistance, epidemiological studies suggest that there are more factors that need to be taken into account (Hensley 2011). For the purposes of this study, and for a better understanding of influenza, it is important to note that the hemagglutinin and the neuraminidase of a single virus particle must be compatible with each other (Hensley 2011). While this remains unclear, it has been shown that incompatible glycoproteins inhibit influenza's infectivity (Yang et al. 1997). Thus, once a single glycoprotein (either HA or NA) mutates, the other must compensate in order to ensure the virus's fitness (Hensley 2011). Here, the researchers were specifically interested in whether or not changes made in the hemagglutinin directly cause compensatory neuraminidase mutations in order to maximize viral fitness (Hensley 2011). They found that these changes in the hemagglutinin structure did indeed alter the neuraminidase structures in such a way that it promoted resistance to the drugs to which the virus was exposed. The researchers argue that their results may explain the poor correlation found between increased drug resistance and the increased use of certain drugs, also explaining in part the increased antigenic drift found within influenza A (Hensley 2011).

In better understanding both influenza's mechanism of action and its molecular structure, researchers will be better prepared for potential outbreaks in the near future.

**Summary**

To echo Webby and Webster 2001, it certainly seems to be the case that influenza continues to be an emerging disease. Whether new research is unveiling the rather mysterious mechanisms of the virus's entry into or exit out of the host or discovering new means of antiviral strategies, influenza certainly is at the forefront of viral research. While the circumstances of influenza's popularity may not be favorable (imminent pandemics, impossibly intricate mechanisms), they definitely place the influenza virus in a haunting perspective.

Hopefully, with new developments in technology and methodology will come new antiviral strategies that can somehow sidestep the pattern of viral resistance currently exhibited by the virus. If not, new strategies will be forced to develop in order to keep up with an ever-evolving disease. Also, by continuing to study the pathogenesis of influenza at the molecular level, researchers can better understand how to interrupt these processes, thereby inhibiting the spread and infectivity of the virus (Rossman and Lamb 2011).

In addition to these more direct antiviral strategies, indirect strategies need to be adopted as well. These types of strategies would require the separation of reservoir species from potential host species in environments such as open-air poultry markets of Asia (Webby and Webster 2001). While these solutions are not as immediate, they are nonetheless preventative measures that would initiate the subtle inhibition of novel subtypes of influenza.

With the research noted above, the future of antiviral strategies looks promising, and with more time and effort, the ultimate eradication of an otherwise formidable virus is becoming a very real possibility.

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# What in God's Name is the Religious Brain? The Neurological Correlates of Religion and Spirituality

Lindsey Akers
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*Religion is extremely prevalent among human beings on a global scale. With the majority of the world subscribing to some form of religion, the question arises about whether there is something neurologically unique to humans that allows for religious thought. This paper investigates the relevant literature on the neurological correlates of religion, spirituality, and mysticism and demonstrates that religion and the religious experience is an accumulation of many different neurological processes – both primal and higher order. Because of this, it is unlikely that humans possess a single element of the brain that is responsible for religion. Future research and areas of interest should delve deeper into the neurological differences between the religious and the nonreligious, taking into account the variety of regions and processes involved in religious thought.*

Religion is and has been one of the most predominant forces in the modern and antiquated world – whether it be western religion, such as Christianity, which has a following of roughly 2.1 billion adherents according to the world census figures, or other globally known religions like Islam, Hinduism, Paganism, and yes, even Scientology. Regardless of whether or not one wishes to argue for or against the existence of a god or deity, it is undeniable that religion is a human universal. In fact, atheism makes up only 2-2.5% of the world population, meaning that the remaining 98% adheres to or practices some form of religion or spirituality. While the religions or the belief systems tend to differ from location to location, and can even differ within a single religion (the different factions of Christianity, for example), they all possess underlying commonalities that make them so relatable. It is no coincidence that the majority of world cultures have an origin story, or some form of praise, ritual or homage to a higher power(s), and the reason for this falls into the field of neuroscience.

What is it that makes humans so prone to some form of belief or spirituality? As far as we know, religion is a behavior that is distinctly human. It has been postulated that the evolution of the human brain, and the psychological development of our higher cognitive functions and their interactions with our more primal brain, has given humans a propensity for religious thought (Boyer, 2008), (Hinde, 2003). While the research itself is controversial, there is a growing body of evidence for the neural correlates of religion and spirituality. Evidence however does not tend to support the idea of a God Module, an area of the brain that is specifically devoted to religiosity; in fact, most evidence tends to point at quite the opposite.

Religiosity and the human tendency to believe and to attempt to make sense of the world, to rationalize the more illogical aspects of religion, seem to be associated with numerous different regions of the brain (Harris, et al., 2009), (Kapogiannis, et al., 2008), and possibly even involve hemispheric dominance (Kurup & Kurup, 2003). Many of these regions are areas that are associated with the more "every-day" aspects of our higher cognitive function: frontal lobe and temporal lobe regions such as our love for another, our understanding of social boundaries, our understanding of Theory of Mind and concept of The Other as well as The Self, general belief and disbelief (Harris, et al., 2009), among other cognitive processes (Kapogiannis, et al., 2009).

The variety of religiosity has led to investigation of a variety of brain regions as potential correlates of religious activity. As previously mentioned, many of the areas deal with the higher cognitive capacities of humans, such as self-reference or emotional understanding, and even simple belief and disbelief of statements/concepts (Harris, et al., 2008), (Harris, et al., 2009), (Kapogiannis, et al., 2009) while others deal with more primal functions, such as addiction and habituation (Schjodt, U., et al. 2008). It is not simply brain regions that are investigated though, some have even proposed that specific neurotransmitters contribute to religious experiences. Brain chemistry can play an integral part in our religious tendencies. Dopamine and serotonin have been prime suspects for investigation as both seem to be correlated with religious experiences and religious activity, but some evidence also attempts to place glutamate into the equation (Newberg & Iverson, 2003). Many cases of abnormally high religiosity can be linked to irregularities in dopaminergic systems, such as with cases of schizophrenia, obsessive compulsive disorder, temporal lobe epilepsy, and others (Harris, et al., 2009), (Previc, F.H., 2006). Regarding serotonin, many religious or spiritual rituals/experiences often involve the use of hallucinogenic drugs that act on serotonergic systems (Borg, J., et al., 2003), (Harris, et al., 2009). In addition, it has even been shown that serotonin levels, at least in animal models, tend to increase during dying (Wutzler, et al., 2011) and may contribute to the "out of body" and spiritual/religious aspect of many reported near death experiences. Obviously, it is not simply abnormalities that allow for religious thought, but investigation of these abnormalities allows for a better understanding of how exactly the function of the brain is associated with religious belief and the propagation of religion in individuals without drastic brain abnormalities. Religion and spirituality are associated with the functionality of specific regions and neurotransmitters of the brain and their interaction

with other areas of the brain. Areas involved tend to mediate emotional, habitual, social, and higher order processes such as Theory of Mind, belief, and others, and the neurotransmitters dopamine, serotonin, and possibly glutamate play a key role in religious experience. The development of our brains, our psychology, and our neurotransmitter chemistry has left us susceptible to religious thought.

Religion and spirituality most obviously involve some form of belief and belief system. To have religion is, in and of itself, to believe the religion and its dogmas to be true. Atheism on the other hand is quite the opposite, and is disbelief and the rejection of religious ideas as being true. The mechanisms of belief therefore play an important role in the neurological aspect of religiosity and spirituality. A good place to start would be to first investigate more broadly and look at the psychological aspect of belief. Studies suggest that belief comes more "naturally" than disbelief (Harris, et al., 2008), (Mitchell, et al., 2011) and many philosophers, such as Baruch Spinoza (1982) have proposed the idea that belief is a natural phenomenon that occurs simultaneously with comprehension of a statement, whereas disbelief takes extra cognitive effort. In support of this theory, response times for belief of a statement are much faster than for disbelief of a statement (Harris, et al., 2008), (Harris, et al., 2009). On the neurological level, Harris and his colleagues found that belief and disbelief of general statements have a large amount of regional overlap, and it is centered mostly in the frontal lobe. There is, however, a significant difference in activity within the ventromedial prefrontal cortex (VMPFC), an area that deals heavily with knowledge and its emotional salience (as this region also activates with areas in the limbic system), reference of knowledge to the self, and the modification of behavior in response to knowledge or reward. When a statement is believed, activity in the VMPFC increases, while activity decreases during disbelief. However, it should be noted that for this specific study, belief and disbelief were analyzed without regard to religion. Statements of general facts and ethical situations were proposed, and the research showed that there were very similar patterns of activation for both types of statements, implying that belief of a statement, regardless of its content, is associated with activation in the VMPFC and its interconnected limbic regions. If emotional processing is involved within the VMPFC for belief and disbelief regardless of whether the statement itself is neutral or emotionally involved, then it would be logical to assume that there would be similar results for statements regarding religious belief and disbelief. The role of the VMPFC in religious belief has been investigated as well.

Activity within the medial prefrontal cortex (including the VMPFC) has been shown in processing religious belief (Young & Saxe, 2008) along with activity in the temporo-parietal junction and precuneus for encoding and integrating beliefs (Young & Saxe, 2008). And in a follow up to their own study, Harris and his colleagues (2009), results similar to those for the study on nonreligious belief and disbelief were found. In subjects reading statements that were both religious and nonreligious in nature, the same regional overlap within the frontal lobe was shown, and the same activational differences were found within the VMPFC. (Harris, et al., 2009) For statements of disbelief (both for religious and nonreligious statements), there was a decrease in activation in the VMPFC, whereas for belief, activation in this area increased. It is again important to note that these differences were found regardless of whether or not the statement dealt with religion or simply a neutral fact. These results pose the association that the analysis of religious statements, just like the analysis of neutral or ethical statements, involves some form of frontal lobe processing with the belief-response being the faster response and having more medial prefrontal cortex activity.

Going beyond simple belief and disbelief, other research has attempted to map out the neural networks involved with religious thought and religious experiences. It should not be particularly surprising that much of the network that has so far been discovered involves areas that mediate our understanding the self and our understanding of the "Other", (Kapogiannis, et al., 2009), (Young & Saxe, 2003) along with areas that handle emotional involvement and emotional salience (Kapogiannis, et al., 2008). The temporal lobe has been a suspect for belief (Britton & Bootzin, 2004), (Young & Saxe), and while it is heavily involved, as was previously mentioned, it is not the sole contender as the prefrontal cortex, in tandem with the limbic system within the temporal lobe, is greatly involved in religious thought and experience. The aforementioned studies support this, but other researchers have shown similar occurrences for a variety of different views of religiosity.

Research has attempted to demonstrate a possible neural map for religion. Kapogiannis and his colleagues conducted a functional MRI study investigating the levels of activation with regards to different aspects of religion. By developing a tentative psychological map for religion, they investigated the brain's activity in response to this psychological map. It should not be surprising that psychological aspects of religion that deal with Theory of Mind and agency were correlated with activity in brain regions that deal with Theory of Mind, empathy, and agency (Kapogiannis, et al., 2008), (Schulte-Ruther, et al., 2007): areas such as the medial prefrontal cortex, the human mirror neuron system, temporo-parietal regions and others (Kapogiannis, et al., 2008), (Young & Saxe). Doctrinal knowledge of the belief system correlated with regions dealing with abstract concepts and metaphorical meaning, and experiential knowledge engaged occipital lobe networks that deal with visual and motor imagery and memory (Kapogiannis, et al., 2008). These are all regions that have been previously mentioned, and these are regions that will be seen later as they are common areas involved in the religious experience.

However, the activation involved with actual religious practice, religious activity, and identification of self via religion is not the only factor in religiosity. There is also evidence of regional cortical volume variability that correlates with varying levels of religiosity (Kapogiannis, et al., 2009) in that depending on the level of religiosity, the volume of specific cortical structures will vary as well. For example, an intimate relationship with a personal god correlates with an increased volume of the R middle temporal gyrus (R-MTG)

and an increased volume of the L precuneus (Kapogiannis, et al., 2009). The relative volume of these regions, much like the activation shown in previous studies, appears to play some role in the higher order aspects of religion.

A common theme has begun to emerge relating religious or spiritual thought to both higher order and primal regions of the brain. The aforementioned study identified regions that are involved with processes that could be considered both higher order and primal. The higher order functions of the R-MTG tend to deal with a concept of self and the understanding of and even simply the perception of the other (Kapogiannis, et al., 2009) along with speech (McGuire, et al., 1995) and episodic memory (Cavanna & Trimble, 2005), while the more primal functions tend to deal with simple auditory processing. Some studies have even suggested that deficits in the MTG could contribute to the presence of auditory hallucinations (McGuire, et al., 1995), a trait often found in schizophrenia. The L precuneus deals heavily with self-processing, self-consciousness and the mental representations of the self. (Cavanna, et al., 2005). With increased activation in these areas, it is not difficult to understand why they are potentially correlated with religious thought, and more specifically, involved with having a close relationship with god. Their heavy involvement in Theory of Mind and with understanding the self and self-representation proposes them as regions that represent god or a higher power to the individual in a way that relates this supernatural being to the self. From increased volume or activation in these regions, one might expect to witness a better "understanding" of god, and therefore a closer relationship with a personal god.

However, a decrease in volume in the L precuneus and the L orbitotemporal cortex correlated with a greater fear of god's anger (Kapogiannis, et al., 2009). These are regions, the L precuneus especially, that deal with emotional regulation and the inhibition of emotional reactions. In addition, the orbitotemporal cortex tends to deal with personalization (information about the self and about others and faces), reinforcement and reward along with sensory information and even abstract reinforcers (like losing or winning) (Rolls, E.T., 2004). It is again understandable going by this evidence why there might be a correlation here, in that a decrease in volume implies a decrease in emotional inhibition, resulting in a heightened fear of god's anger rather than a closer, more loving relationship with a personal god.

Religiosity too has been shown to correlate with hippocampal volume, in that decreased volume of the hippocampus is associated with increased levels of religiosity (Owen, et al., 2011). The hippocampus is involved in a myriad of different functions, such as learning and memory, emotional context and cortical arousal due to its communication with the prefrontal cortex, the amygdala and other limbic regions. Because of these associations, it is not unreasonable to think that the hippocampus may too play a role in religiosity. In individuals with refractory epilepsy, subjects that displayed hyper-religiosity showed smaller right hippocampal volume (Wuerfel, et al., 2004). An important distinction of this study, too, was that the amygdala did not show any correlation in volume with religiosity, but instead correlated with the more aggressive and psychosis-based aspects of refractory epilepsy (Wuerfel, et al., 2004), suggesting that the degradation of the hippocampus, while perhaps not causal, is at the very least highly associated with the intensification of religiosity. However, much of the research revolving around hippocampal involvement in religiosity surrounds the region with relation to the effect of epilepsy: a disorder that is commonly associated with hyperreligiosity, possession, and intense spirituality.

There is other evidence though, for non-epileptic patients, demonstrating a similar phenomenon regarding decreased hippocampal volume and religiosity. As one ages, there is an overall atrophy in the brain, and in a large sample of aging adults of different levels of religiosity (ranging from highly religious to having no religious affiliation), hippocampal atrophy was investigated with relation to different aspects of religiosity. The different religious aspects that were investigated dealt with frequency of public and private religious activity, the subject's religious affiliation, and whether or not they had self-reportedly experienced some form of intense or life-changing religious experience. Results demonstrated greater hippocampal atrophy in subjects who were part of "religious minority" groups, such as being a Born-Again Protestant, or someone with no religious affiliation (Owen, et al., 2011). As the hippocampus is involved in the stress response, it is possible that the increased levels of stress elicited by being part of a religious minority have increased the rate of atrophy in such subjects (Yoshifumi, et al., 1992). Results also showed increased atrophy in the hippocampus in subjects who reported having life-altering religious or spiritual experiences in the past (Owen, et al., 2011), suggesting that increased hippocampal atrophy may be directly related to religious experiences or to the stress induced by being members of a religious minority.

While the aforementioned research shows that hippocampal atrophy and decreased hippocampal volume is associated with hyper-religiosity, there is also evidence for the opposite effect in individuals who actively meditate. Rather than decreased activity or decreased hippocampal volume, as is seen for higher levels of religiosity, instead there is an increase in volume or an increase in activity in the hippocampus (and other regions such as the temporal gyrus and prefrontal regions, an area mentioned previously) during mindful meditation (Holzel, et al., 1007), (Lazar, et al., 2000). These few studies show again that while certain regions can be narrowed down, there is still a good amount of variability given the levels of religiosity and the type of religiosity (fundamentalism, born again, or spiritual meditation).

The volume of these different regions, however, is not the only factor that plays a role. As previously discussed, activation is critical, and blood flow is an important factor in relative activation. Evidence tends to show that during more complex spiritual tasks, using meditation for experienced meditators as an example again, there is an increase in the amount of blood flow and activation within the frontal lobes with a relative decrease in activation and metabolism in the other regions, such as the occipital and superior parietal lobes (Newberg, et al., 2001). This is not unusual as during any cognitive task that requires concentration, there will more than likely be an increase in activity within the frontal lobes as

well. However, it is important to reinforce that in the Newberg, et al. study, that while there was not necessarily a significant decrease in other regions of the brain, there was still a trend of deactivation in these other regions related to the increased activity in the frontal lobe region. There was still a significant correlation between increased frontal lobe activity and a decrease in other cortical regions, such as the superior parietal lobe, which is an area that is involved with spatial processing and that also interacts significantly with the prefrontal cortex (Cohen, et al., 1995). This seems to support the idea that this trend of decreased activation in the parietal lobe and ergo its decreased interaction with the prefrontal cortex lends to a more "altered" state of being – a contribution to perhaps the transcendental and subjective, personalized experience of deep meditation. This idea of subjective, transcendental experiences is an important factor in religious thought, and it will be addressed later, as well.

Much of what has been discussed so far has dealt with higher order cognition and their various regions. However, it is not solely higher order cognition and function that is associated with religious experience. Some evidence has even suggested that certain religious behaviors, such as fasting or ritualized prayer, can activate areas and processes associated with reward and habituation, both of which are lower order neurological functions. Fasting is a component that is prevalent in many religions such as Islam, Christianity, Buddhism, Mormonism, Judaism, and in many other religions in which the individual abstains from food or drink for a certain amount of time as a means of piety or duty to their religion. While the restriction of food does not initially appear to be at all rewarding, there is evidence showing that fasting causes an increased release of endogenous opiates in the brain (Molina, et al., 1995), perhaps as a means of dealing with the stress that is induced by restriction of food as some evidence has shown that endogenous opiates are released in response to stress (Willer, et al., 1980). In an animal model, research found that brain morphine levels increased roughly 5-fold after merely 24 hours of fasting (Molina, et al., 1995). As religious fasting takes place over the course of a certain number of hours, depending on the specific religion's dogmas, it is not unreasonable to think that release of endogenous opiates possibly contributes to perpetuation of the fasting behavior.

Ritualization and routine is an important component of religious activity – whether it is fasting, participating in sacred ceremonies or simply saying prayers every night before bed, religion and ritualization go hand in hand. Because of this, an area of interest has been the striatum, a region that has been implicated in reward, addiction, and goal-driven behavior (Everitt & Robbins, 2005), (Gerdeman, et al., 2003). Two specific subdivisions of the striatum have been investigated - the ventral and dorsal regions. The ventral striatum tends to be involved with evaluation of reward as it has connections to the limbic system and ventral frontal areas of the brain, while the dorsal striatum is more heavily involved in actual conditioning and learning in regards to goals and expectations of reward in the future (Delgado, 2007), (Tricomi, et al., 2004). The dorsal striatum, and the caudate nucleus within it, is also thought to be involved in social learning (Delgado, 2007). During ritualized prayer in those that self-reportedly pray regularly, there is a significant increase in caudate activity (Schjodt, et al., 2008). In fact, the more rigid the structure of the prayer, such as saying the Lord's Prayer as opposed to saying simply an unscripted personal prayer, the greater the caudate activity. (Schjodt, et al., 2008). However, when the same experiment was done with individuals who were self-reportedly religious but did not regularly or frequently pray, there was no observed increase in caudate or striatal activation (Schjodt, et al., 2008). These results imply that there is a level of reward-based habituation, and perhaps even addiction, that is associated with ritualized religious activity.

The involvement of the striatal reward system also suggests the contribution of dopamine in religious behavior and activity. The striatal regions have many dopaminergic neurons that extend to it and these areas are heavily involved in dopaminergic reward pathways (Everitt & Robbins, 2005), (Gerdeman, et al., 2003). It is well known at this point that dopamine plays a key role in addiction (Kelley & Berridge, 2002) – especially regarding physiologically addictive drugs such as cocaine, as they almost all act on the dopaminergic reward system (Kelley & Berridge, 2002). It should not be surprising to see that these dopaminergic neural pathways are spread across the frontal and mesocortical regions of the brain such as the substantia nigra, the ventral tegmental area, dorsal and ventral striatal regions, the caudate nucleus and nucleus accumbens among many others (Everitt & Robbins, 2005), (McNamara, 2006), (Rolls, 2004). The caudate nucleus exhibits increased activation with regards to ritualized prayer, implying some form of dopaminergic reward that could be the result of a habituated religious behavior.

But it is not simply reward that implicates this increase in activation and increased reception of dopamine in religiosity. Some research has suggested that a polymorphism of the dopaminergic gene DRD4 plays a part in spiritual acceptance (Comings, et al., 2000) and research investigating mental disorders such as schizophrenia or obsessive compulsive disorder tends to show an association between religiosity and the presence of those disorders as both schizophrenia and obsessive compulsive disorder have been associated with hyper-religiosity, religious delusions, and religious obsessions (Abramowitz , et al. 2004), (Harris, et al., 2009), (Siddle, et al., 2002). It is not surprising that religious delusions, obsessions or simply hyperreligiosity might develop in cases of schizophrenia or obsessive compulsive disorder as these disorders are marked by oversensitivity to dopamine (Owen, et al., 1978) or elevated levels of dopamine (Previc, 2006). In addition to these two disorders, temporal lobe epilepsy has also been associated with oversensitivity to dopamine (Previc, 2006), (Rocha, et al., 2011) and is often associated with increases in religiosity and increased numbers of religious experiences (Devinsky, et al., 2008). Historically, individuals with temporal lobe epilepsy have been viewed as being connected with the supernatural realm, the demonic forces, and as even having been blessed with the disorder by the gods (Devinsky, et al., 2008), (Previc, 2006), (Saver & Rabin, 1997). This evidence supports the idea dopamine plays an important role in religion and spirituality and that increased

levels of dopamine or increased sensitivity to dopamine relates to increased religiosity or religious experiences.

While increased religiosity can be seen with increased dopamine, there is also evidence that supports the opposite: that decreased amounts of dopamine correlate with reduced levels of religiosity (McNamara, et al., 2006). Fairly recent evidence from a pioneering group of researchers has shown that individuals with Parkinson's Disease, a disease marked by low levels of dopamine, show a blunted religiosity and decreased frequency of prayer or meditation (McNamara, et al., 2006). Some suggest that this decreased amount of dopamine contributes to decreased activity in the striatal regions (Schjodt, et al., 2008) and frontal lobe regions (McNamara, et al., 2006), which thereby affects the motivational aspect of repeated religious behavior (Schjodt, et al., 2008).

However, dopamine is not the only neurotransmitter that plays a role in the religious experience. Serotonin is also a key chemical in religious thought, experiences, and behavior. Serotonergic neurons extend to numerous regions of the brain such as the limbic system, the striatum, and the neocortex (Borg, et al., 2003), which are all regions that have previously been implicated in religious experiences. Some evidence even shows that genes that encode for serotonin transporters are correlated to spiritual acceptance on a personality scale (Nilsson, et al., 2007). Individuals with this personality trait also have been shown to have decreased binding potential for serotonin in dorsal raphe nuclei, the hippocampus and the neocortex (Borg, et al., 2003). It is, however, unclear whether or not this should be interpreted as a high release or a low release of serotonin, as research tends to support either interpretation (Borg, et al., 2003), however, further expansion may clarify the subject.

Serotonin is also linked with many hallucinogenic drugs, as hallucinogens such as LSD, psilocybin (magic mushrooms), mescaline, and others tend either to block or mimic serotonin at its receptors (McNamara, et al., 2006). It is no surprise that many of these hallucinogens often result in some form of spiritual experience for the user; in fact, many religious groups use hallucinogens as a part of their religious/spiritual ceremonies (Halpern, et al., 2005), (Metzner, 1998), while some individuals merely use it recreationally. These hallucinogenic drugs can elicit mystical or spiritual experiences (Goodman, 2002) that often carry significant personal meaning to the user (Griffiths, et al., 2006), and in tests using psilocybin, this personal significance can persist for a long period of time, even up to 14 months after the use of the drug itself (Griffiths, et al., 2011). According to the Hazardous Substances Data Bank, LSD can be up to 100 times as potent and long-lasting as psilocybin and up to 4000 times as effective as mescaline at producing hallucinogenic effects (HSDB, 2011), suggesting that the spiritual experiences elicited by a drug as potent as LSD could be more vivid, longer-lasting, and personally significant than those from a drug such as psilocybin.

There is, however, some evidence suggesting that for some dissociative hallucinogenic drugs, such as ketamine or phencyclidine, glutamate, rather than serotonin, might be implicated in the spiritual experience of meditation (McNamara, 2006), (Newberg & Iverson, 2003). According to Newberg & Iverson (2003) in their investigations on meditation, glutamate levels in the prefrontal cortex increase with meditation. They propose that the excess free glutamate in the prefrontal cortex can cause a limitation in the production of a specific enzyme responsible for converting the NMDA receptor antagonist NAAG into glutamate, resulting in excess NAAG in the prefrontal cortex. NAAG functions much like some dissociative hallucinogens like ketamine, phencyclidine, or nitrous oxide (Jevtovic-Todorovic, V., et al., 2001), (Newberg & Iverson, 2003), suggesting that the dissociative/transcendental aspects of meditation could be functionally similar to the dissociation/mysticism elicited by these types of hallucinogenic drugs.

The transcendental aspect of spirituality goes beyond meditation and the use of hallucinogenic drugs. Near death experiences (NDE) are significant aspects of the overall religious or spiritual experience. A near death experience can be defined as: an intense, significant, spiritual and sometimes pleasurable experience by an individual that is in a state close to death (Moore, 2001). These experiences may include seeing the afterlife, going through a tunnel or towards a bright light, out-of-body experiences (OBE), distortion of time, distortion of reality, odd visual perception, a review of the individual's life, or peacefulness and euphoria (Moore, 2001), (Saavedra-Aguilar & Gomez-Jeria, 1989). The Gale Encyclopedia of Psychology (2001) states that as many as 1 in 5 Americans have reportedly had a near death experience and have reported experiences similar to the aforementioned description. Given the reports of near death experiences, it is obvious that there is a very spiritual and transcendental component to them, and this can be correlated to neurological happenings near the time of death. Individuals who have reportedly had near death experiences tend to exhibit more temporal lobe epileptic-like symptoms such as sleepwalking or heightened olfactory sensitivity (Britton & Bootzin, 2004). In addition to the aforementioned symptoms, these individuals when compared to control subjects also exhibit more temporal lobe epileptic-like symptoms as a result of direct temporal lobe stimulation: symptoms such as experiencing intense emotional feelings or experiencing unusual visual, olfactory or auditory sensations and perceptions (Britton & Bootzin, 2004). This relation to temporal lobe epilepsy is familiar, as temporal lobe epilepsy has been heavily associated with religious or spiritual experiences and heightened levels of religiosity.

On a molecular level, researchers Newberg & Iverson, mentioned earlier, suggest too that the NMDA receptor agonists that are associated with dissociative hallucinogenic drugs and the transcendental aspects of meditation can create experiences that resemble other mystical experiences such as out-of-body or near death experiences (Newberg & Iverson, 2003). This proposition suggests that near death experiences are related chemically to the mystical, out of body or transcendental experiences associated with the same systems involved in the effects of hallucinogenic drugs and heavy meditation.

Serotonin and serotonergic binding potential have already been linked to spirituality and spiritual acceptance (Borg, et al., 2003), (Nilsson, et al., 2007) and to the

experiences caused by hallucinogenic drugs (Goodman, 2002), (Griffiths, et al., 2006), (McNamara, 2006), but new evidence suggests that this neurotransmitter could also be linked to near death experiences (Wutzler, et al., 2011). In dying rats, research has shown that brain levels of serotonin increase 3-fold from baseline levels during dying (Wutzler, et al., 2011). Since serotonin is a known mood regulator (Schloss & Williams, 1998), it is proposed that its presence during the process of death could be a means of making death easier (Wutzler, et al., 2011). Additionally, because of this association, its presence during dying could be responsible for the euphoric or pleasant feelings experienced in close-to-death states, and it could account for the transcendental aspects, similar to those of hallucinogenic drugs or deep meditation, of near death experiences.

Taking all the present evidence into account, it is difficult, nigh impossible, to point to a single region of the brain or a single neurotransmitter that is responsible for religious tendencies in human beings. As far as anyone is aware, religion and spirituality are traits unique to humans. However, it is apparent that many of the neurological aspects that contribute to and correlate with religion are present in animal models as well, suggesting that our unique evolution of higher order processes has geared us towards religious thoughts. The more antiquated, evolutionarily older regions and neurotransmitters of the brain – such as our limbic systems, or neurotransmitters like dopamine, or serotonin – contribute heavily to the entire religious experience, but at the same time, higher order processes and more advanced cognition play a critical role in our perception, understanding and further propagation of religion and spirituality. Given the research and the evidence at this time, it is unlikely that such a thing as a God Module exists in humans. Despite the fact that religion is uniquely human, it is in the integration, activation, and deactivation of numerous regions, neurotransmitters, and processes that allows for the religious experience on the whole.

If one were to investigate religion from the perspective that there was a single neurological item that accounted for religion in human beings, then future research would be quite simple. The religious and the spiritual would have God Module X and the nonbelievers, the remaining 2% of the world population, would not have God Module X. However, in light of the presented evidence, it is clear that religion is just not that simple. Given all of the different neurological processes that contribute to the religious experience, future research would benefit from investigating how different the religious and nonreligious populations are. Is there a key difference in the neuroanatomy and cortical volume of believers and nonbelievers? Or are there drastic differences in the levels and mechanisms of specific neurotransmitters in the religious and nonreligious? These questions have gone largely unasked in most of the present research. Further investigation could elucidate why only a small percentage of individuals have rejected religious thought, and why such a large percentage of the world population subscribes to religion. Such questions could be answered on a very philosophical or evolutionary level; however, the neurological aspects still play a critical role. It is essential to consider the numerous neurological regions, activations and neurotransmitters that contribute to religious thought when researching religion in the brain, and when comparing believers and nonbelievers in future investigations.

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# Poor Physical Function among Childhood Brain Tumor Survivors

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*The purpose of this study was to evaluate fitness in survivors of childhood brain tumors (BT), and determine if specific physical impairments contributed to poor fitness. Participants were survivors of childhood BT, >18 years of age and 10+ years from diagnosis. We measured fitness with the six minute walk test, balance with computerized dynamic posturography, strength with isokinetic dynamometry, and peripheral neuropathy with the modified total neuropathy score. Those who scored in the lowest 10th percentile when compared to population norms were classified as having poor fitness and were reported as percentages. General linear regression, adjusted for age, gender, height and weight were used to evaluate associations between weakness, impaired balance, neuropathy and poor fitness. Among 124 survivors of childhood BT (mean age 27±5 years, 57% male), 73% had poor fitness, 55% poor balance, 89% poor knee strength, 50% poor ankle strength and 11% neuropathy. Adjusted mean walk distances were less in those with quadriceps weakness (550.6±124.6 vs. 464.9±177.2 meters) and neuropathy (560.0±134.3 vs. 455.5±130.9 meters) than in those without these impairments. Intervention for lower extremity weakness may constitute a necessary component of any fitness training for childhood BT survivors.*

**Introduction**

Central nervous system (CNS) cancers are the second most common cancer in children in the United States, representing just over 20% of all malignancies during childhood [1]. In 2010, there were 4,030 new cases of pediatric brain and other CNS tumors in children between the ages of 0-19, including 2,880 who were younger than 15 years [2]. Incidence rates are slightly higher in males when compared to females, and slightly higher among white children when compared to black children [3].

Mortality following a diagnosis of a CNS tumor in childhood continues to decline. The five year survival rate among those 0-19 years of age now approaches 75%, up from 58% in 1977 [3]. Survival does not differ by sex or race [4]. Mariotto et al [5] estimated that there were 51,650 survivors of childhood brain cancers as of January 2005 in the United States.

There are more than 100 different types of brain tumors in children, but the majority of tumors diagnosed include medulloblastoma, primitive neuroectodermal tumor (PNET), ependymoma and gliomas [6]. Location and histology of each tumor helps to predict tumor behavior and guide treatment [6]. See Table I for the distribution of the most common types of tumors among all childhood CNS cancers.

**Treatment options and their late effects**

There are three components of treatment for brain tumors: chemotherapy, surgery, and radiation [7]. Patients with medulloblastoma and astrocytoma usually receive a combination of modalities to treat the tumor [6]. Neurocognitive and functional limitations are commonly present in this population, and could be the result of direct neurologic damage caused by the tumor and its removal, the long-term toxicity of chemotherapy, and/or the effects of radiation on the developing nervous system [8-10].

*Surgery*

Surgical resection is generally the first form of treatment and is essential for early management. It is necessary to establish a diagnosis, relieve hydrocephalus, and achieve maximal tumor resection [7,11-13]. Surgery is generally safe when the objective is reducing tumor size [6]. Survival and quality of life have improved due to gross total resection in children with cerebellar tumors [14].

*Late Effects of Surgery*

Effective as it may be, surgery is not without cost. It may cause significant late effects that may reduce quality of life and hinder physical function into adulthood. In a recent report by Küpeli et al. [15], 25% of children who underwent surgery for tumor resection in the posterior fossa developed loss of speech associated with decreased muscle tone, unsteadiness, emotional labiality and irritability, or posterior-fossa mutism syndrome. Common complications of posterior fossa tumor surgery include long tract deficits, hemiparesis, sensory abnormalities, deep venous thrombosis, and pulmonary embolism [16], which may limit daily function and physical activity.

Long-term psychological and physical complications from surgery vary depending on the location of the primary tumor. For example, some children who underwent surgery in the cerebellum developed severe neurocognitive and balance disorders [17,18]. Fifty-two percent of children who received a surgical intervention for craniopharyngiomas were severely obese as a result of hypothalamus-injury induced hyperphagia [19]. Long-term impairments may cause reduction of lower limb strength, abnormalities in sensation and balance, and poor overall physical function [20].

*Chemotherapy*

Chemotherapy is an essential component of treatment for patients with brain tumors. Chemotherapy consists of a combination of drugs that may be given by mouth, or injection into a vein, intrathecal space or muscle [6]. It is well documented that chemotherapeutic agents typically eliminate tumor cells by a mechanism involving programmed cell death [21]. In addition to surgery and radiation, chemotherapy has been used to successfully reduce the size of a tumor [6].

*Late Effects of Chemotherapy*

Though the benefits of chemotherapy tend to outweigh the risks, chemotherapy has many adverse side effects that may hinder proper function immediately and later in life [6]. Conventional chemotherapeutic drugs are well known to cause neuropathy syndromes [22-24]. Peripheral neuropathies generally affect the distal parts of the extremities, characterized by a "stocking and glove" phenomenon, and create sensations of numbness or tingling [23]. These sensations may make daily tasks difficult to complete without assistance, and may significantly reduce physical capacity [25].

Muscle strength deficits have also been reported with the use of chemotherapy in brain tumor survivors [26,27]. Some chemotherapy agents have such adverse effects they must be eliminated or greatly reduced in dosage to prevent individual toxicity [27]. Refer to Table II for common chemotherapeutic drugs used to treat brain tumors and commonly reported complications.

*Radiation*

Radiation therapy uses high-energy particles that interact with DNA, causing ionization damage, which shrink and eliminate cancer cells [28]. Specific doses of radiation depend on the tumor type, and are usually given post surgery with chemotherapy to target any remaining tumor, and to prevent relapses [6]. For example, medulloblastoma patients typically receive a dose of 23.4 Gray (Gy) of craniospinal radiation and 55.8 Gy boost to the posterior fossa [12].

*Late Effects of Radiation*

The delayed effects of radiation can lead to severe and irreversible neurological consequences, especially during neural tissue development [29]. Long-term effects of radiation may include endocrine complications (growth deficiencies), neurologic problems (muscle weakness and balance deficits), or sensory deficits (deafness, cataracts, and blindness) [30-34]. These impairments could cause significant difficulties performing activities of daily living and participating in physical fitness activities.

In a study by Meacham et al. [35], female brain tumor survivors treated with radiation were more likely to be underweight (body mass index (BMI) <18.5 $kg/m^2$) when compared to population norms. Galanos et al. [36,37] revealed that those with a low BMI had an increased risk for both early mortality and functional difficulties with activities for daily living. Being underweight may lead to a significant reduction in strength and thus, a reduction of functional capacity.

Chronic inflammations by radiation-induced osteoradionecrosis and significant cardiac dysfunction are reported as complications of radiation therapy [23,38]. Balance deficits, along with neurocognitive disorders, have also been reported in brain tumor survivors treated with radiation [8,39]. Decreased cardiovascular function and poor balance may contribute to a sedentary lifestyle.

Due to numerous late effects of brain tumors and their treatments, our aim was to determine associations between specific physical impairments and overall fitness. We aimed to determine patients' functional capacities by measuring strength, neuropathy, balance, and six minute walk (6MW) distance. We hypothesized that lower strength scores and higher modified total neuropathy scores will positively correlate with the distance that the patient will walk during his or her 6MW test. We also hypothesized that men would walk significantly farther than females.

## Methods

### Participants

Brain tumor survivors <21 years of age who were treated between 1962 and 2001 were recruited randomly from the clinical populations at St. Jude Children's Research Hospital (SJCRH). Participants were $\geq$18 years old, had to be at least a ten year alumnus, were willing to return to SJCRH for on-site evaluations, and were willing to comply with guidelines of St. Jude domiciliary care facilities. All participants signed informed consent prior to the study. Individuals receiving treatment for an active tumor were not eligible.

### Functional Assessments

Each patient completed performance based measures to quantify their physical abilities. Standard measures of body composition, tests of peripheral nervous system integrity, joint flexibility, muscular strength, balance, fitness and overall motor performance were included in the assessment.

*Body composition*

Height was measured in centimeters on a wall mounted standiometer. Weight was measured without shoes on an electronic scale in kilograms. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared [40]. The measure of BMI has been shown to be a strong predictor of overall mortality [41].

*Peripheral sensation*

Peripheral sensation was evaluated with the modified Total Neuropathy Score (mTNS). This tool was developed to evaluate peripheral neuropathy in individuals following administration of neurotoxic chemotherapy agents and is as sensitive to deficits in sensation evaluated with the parent instrument, the Total Neuropathy Score (TNS) [42]. The TNS has been validated and shown to be a reliable measure of peripheral nerve function by Cornblath et al. (R=0.966) [42] . The mTNS includes all items on the TNS except for nerve conduction velocity testing. The mTNS is a composite scale

(0-24 points) that includes patient reports of sensory and motor symptoms, pin sensibility, quantitative vibration thresholds using a biothesiometer, strength using manual muscle tests, and deep tendon reflexes [43]. The mTNS has been used to discriminate between cancer patients who had undergone completed taxane therapy and healthy controls [44].

*Biodex*

Lower extremity strength values were evaluated with the Biodex System III Pro (Biodex Medical, Shirley, NY). Participants were secured on a padded seat with manufacturer provided positioning straps at the chest, waist, and thigh. Peak torques were recorded during isokinetic contractions (60°, 180°, and 300° per second for the knee; 60° and 90° per second for the ankle). Calibration was performed before each session according to manufacturer's instructions. Care was taken to align the anatomical axis of the joint with the mechanical axis of the dynamometer before all tests. One practice trial was performed before each set of testing, followed by five (60° $sec^{-1}$), ten (180° $sec^{-1}$), or fifteen (300° $sec^{-1}$) maximal voluntary movements. Torque was corrected for gravitational moments of the lower leg and the lever arm. Rest periods of two minutes were allowed between trials to minimize fatigue [45]. Peak torque values from knee speeds of 180° $sec^{-1}$ and ankle speeds of 60° $sec^{-1}$ were chosen for analysis as these particular speeds mimic the function during the stance phase of walking [46,47]. Feiring et al. [48] demonstrated good reliability ($p<0.05$) of the Biodex by test-retest measures of peak torque and single repetition work. Normative values and ranges for healthy adults are provided by the equipment manufacturer (using previously published data) [49,50].

*Sensory organization test (SOT)*

Balance was measured using the sensory organization test (SOT) on a computerized dynamic posturography system (SmartEquitest, Neurocom International). The SOT evaluates three systems that contribute to postural control: somatosensory, visual, and vestibular [51] . In a study by Buatois et al. [52], the SOT was determined to be a more sensitive tool to identify those at high-risk of recurrent fall compared to other clinical balance tests. The SOT has been shown to exhibit good reliability in a population of older subjects [53].

All participants were required to wear a safety harness to prevent falls. Participants were instructed to stand as still as possible during the entire test, and to follow verbal instructions. Participants stood barefoot on dual force plates which can be inclined up or down in an anterior-posterior direction to provoke ankle motion. A colored visual screen surrounded the participant on three sides, and can also move in an anterior-posterior direction. The participant stood in an upright position, with the medial malleolli positioned directly over the axis of rotation of the force plates. Visual and kinesthetic inputs were manipulated to create six different conditions, each of which was tested with three 20 second trials. During testing, the force plates continuously recorded the participant's center of pressure. In conditions one and two, participants were asked to stand with their eyes opened or closed, respectively, while the screen and force plates remained stationary. During condition three, participants' eyes remained open while only the screen moved when motion was detected from the participant. Conditions four, five and six were identical to one, two, and three, with the addition of movement of the force plates when motion was detected. A difference score was computed from the normal range of anterior-posterior sway (12.5 degrees) and the maximum range of sway of the participant on each trial; that score is expressed as a percentage [53]. The higher the score, the less the participant swayed. A trial ending in a fall (injury prevented because of the safety harness) was scored as a zero [54]. A composite score from all six conditions was used to characterize balance [54].

*Six Minute Walk*

Participants completed the modified Cooper test, or the six minute walk (6MW) test, to evaluate cardiorespiratory performance [55,56]. Studies have reported significant correlations between the 6MW and peak oxygen consumption ($VO_2$) [57,58]. Participants wore a heart rate monitor in order to observe heart rate during the test. Using the Borg's rate of perceived exertion (RPE) scale, participants were instructed to rate how hard they felt they were working on a scale of 6-20 [59]. Participants were instructed to walk in the middle of a 3.05 meter wide rectangular corridor (39.65 meters long) as fast as possible for six minutes. The total distance was recorded in meters. Heart rate and RPE value were recorded pre-test, at two minute intervals during the test, and post-recovery. In a study by Pollentier et al. [60], the 6MW test was shown to have good overall reliability and validity and a significant ability to predict functional capacity. Many studies have also shown significant correlations ($p<0.05$) between RPE and heart rate in various groups of healthy participants and patients with cardiovascular disorders [59,61,62]. The 6MW distance was found to be an accurate estimate of functional status of patients, and is sensitive to clinical signs of disease or to risk facts for cardiovascular disease [55].

**Statistical Analysis**

Descriptive statistics were calculated to describe the study participants. Means and standard deviations were calculated for age, 6MW distance, expected walk distance [63], quadriceps and plantar flexion strengths, SOT composite scores, and mTNS scores. A frequency procedure was used to calculate percentages for poor walk distance, plantar flexion strength, quadriceps strength, SOT composite score and mTNS score. A general linear regression, adjusted for age, gender, height, and weight, was used to evaluate the associations between muscle weakness, impaired balance, neuropathy and poor fitness. SAS version 9.2 (SAS Inc., Cary, NC) was used for all analyses.

**Results**

**Participants**

Refer to Table III for participant characteristics. One hundred and twenty four survivors participated in the study. Study participants were 55.6% male, 80.6% white, had a mean age of 26.0 years (range 19-53), had a mean age at

diagnosis of 8.6 years (range 0-21), and had a median age since diagnosis of 18.2 years (range 11-41).

**Functional Outcomes**

Those who scored in the lowest $10^{th}$ percentile when compared to population norms were classified as having below expected results, and are presented in percentages. Refer to Figure 2.

Some participants were unable to complete all tests due to cognitive, physical, or visual limitations, or self-reported pain.

Walk distance was evaluated in 115 participants. Mean walk distance (515.3 ± 116.6 meters) was below what was expected for a healthy population (693.3 ± 57.3 meters) [63]. Of the 115 participants, 72.6% had walk distances that were below expected for their age and gender [63].

Quadriceps strength was evaluated in 112 participants. Mean quadriceps strength was 91.6 ± 36.7 N·M. Of the 112 participants, 88.7% had quadriceps strength that was below expected for their age and gender [50]. Plantar flexion strength was evaluated in 111 participants. Mean plantar flexion strength was 48.4 ± 29.3 N·M. Of the 111 participants, 54.8% had plantar flexion strength that was below expected for their age and gender [50].

Neuropathy was evaluated in 121 participants. The mean mTNS composite score was 2.4 ± 2.7. Of the 121 participants, 11.7% had a higher neuropathy score than expected for their age and gender [44].

Balance was evaluated in 108 participants. The mean SOT composite score was 66.6 ± 14.6. Of the 108 participants, 54.8% had balance that was below expected for their age [54].

**Association Outcomes**

A general linear regression, adjusted for age, gender, weight and height was used to evaluate associations between outcomes. See Figure 2.

There was a significant difference ($p<0.0001$) between the distance males (481.6 ± 23.7 meters) and females (533.9 ± 25.4 meters) walked.

There was a significant difference ($p<0.0001$) between the walk distances of those with poor quadriceps strength (464.9 ± 17.0 meters) and those with normal quadriceps strength (550.7 ± 33.3 meters).

There was a significant difference ($p<0.0001$) between the walk distances of those with suspected neuropathy (455.5 ± 35.9 meters) and those with neuropathy scores within normal limits (560.0 ± 15.0 meters).

**Discussion**

The principle findings of this study were that brain tumor survivors with poor quadriceps strength and abnormal neuropathy scores had reduced physical function. Females walked significantly farther than males. A majority of the participants exhibited poor walk distance, poor quadriceps and plantar flexion strength, and poor balance.

Our finding of the association between poor quadriceps strength and reduced physical function is consistent with much of the recent literature. Ness et al. [64] demonstrated that acute lymphoblastic leukemia (ALL) survivors who had knee extension strength less than or equal to -1.3 standard deviations below population expected walked slightly, but not significantly, shorter distances in two minutes when compared to those with knee strength greater than -1.3 standard deviations below the population norms. Though ALL survivors were examined in this study, decreased functional abilities were still observed, possibly due to similar treatments to that of brain tumors (i.e. cranial radiation). Three studies that examined an elderly population found an association between weak quadriceps strength, reduced walking velocities and increased functional limitations [65-67]. We obtained similar results to studies that examined the elderly, indicating that our survivors may be at a higher risk for declines in physical function that would normally accompany aging.

Premature aging is a unique phenomenon that is sometimes observed in individuals receiving long-term treatment for a chronic condition. Radiation damage to living tissues is similar to the mechanism of aging and can result in reduced physical function that should be seen in the older population, but were already being exhibited in our young cancer survivor population [68]. Richardson [68] reported that premature aging is mainly due to the reduced ability for the DNA to repair itself after damaging ionizing radiation. Alkylating agents are known to cause irreparable damage to DNA causing those cells to undergo apoptosis and contribute to aging [69,70]. Our results would suggest that relatively young brain tumor survivors exhibit decreased physical function, similar to that of elderly adults. The long-term impacts of premature aging of childhood cancer survivors are still unknown and should be considered in regards to early morbidity.

The strong association found between abnormal neuropathy scores and poor physical function is supported by previous research. Some investigators have determined that average self-selected walking speeds were significantly reduced in patients with diabetic peripheral neuropathy when compared to controls, and gait was characterized by marked decreased speed and stride length [71-73]. Manor et al. [74] found that those with peripheral neuropathy walked slower than the control group during the 6MW test ($p<0.001$). Over forty percent of our brain tumor population received either cisplatin or vincristine, which have been shown to cause peripheral neuropathy [22-24]. Only 11.7% of the participants exhibited abnormal neuropathy scores at the time of the assessment. This suggests that chemotherapy-induced peripheral neuropathy may only account for a slight reduction in physical function in our population.

In our study, females were found to walk farther than their male counterparts during the 6MW test. Jones et al. [75] determined that males self-reported a reduction in exercise behaviors after a brain tumor diagnosis, which could explain the decreased 6MW performance of males in our study. Warner et al. [76] showed that boys diagnosed with malignancies other than brain tumors (acute myeloid leukemia, non-Hodgkin's lymphoma, Wilms' tumor, neuroblastoma, yolk sac, rhabdomyosarcoma, Hodgkin's

lymphoma) had significantly reduced peak $VO_2$ compared to controls. Similar to our study, over half of the participants in Warner's et al. [76] study received cranial radiation, suggesting that this treatment may have a negative effect on cardiorespiratory function in numerous cancer types. In contrast to our findings, studies reported that female survivors of brain tumors and ALL were at the highest risk for motor impairment when compared to male survivors, and at the greatest risk for physical impairments when compared to controls [77-79]. Gerber et al. [80] reported reduced walking velocities during the 6MW test in only the female pediatric sarcoma survivors who exhibited limb weakness, however the sample size of this study was relatively small (N=32) and comparison of tumor types and locations between genders was not reported. Brain tumor survivors have been documented to exhibit and report significant motor impairments [26,77], in line with our results. We would expect for brain tumor survivors to have a more limited exercise regimen because they may have more pronounced motor deficits due to the direct infiltration in the brain as opposed to other cancers that do not affect the nervous system so invasively.

Just over half of our population exhibited poor balance, which is consistent with the literature. Syczewska et al. [81] assessed balance and found that over half of the 41 CNS tumor survivors had poor balance. Packer et al. [26] surveyed 1,607 brain tumor survivors and determined 49% had coordination problems, including balance. Lannering et al. [82] revealed, through neurological examinations, that motor dysfunction—especially balance—was prevalent in 25% of the brain tumor participants. Brain tumor survivors will often exhibit poor balance due to the treatment with cranial radiation that may have damaged the inner ear and thus the vestibular system which could potentially disrupt proper coordination.

Lower limb strength deficits were very prominent in our population. Brussel et al. [25] and Hovi et al. [83]determined that survivors of childhood ALL had knee extension strength significantly lower than population norms. Hartman et al. [84] determined that peripheral muscle strength was reduced in the long-term in children treated for ALL, Wilms' tumor, B non-Hodgkin's lymphoma and malignant mesenchymal tumors. Although the diagnosis groups vary in the aforementioned literature, similarities in treatment could play a role in the consistency of results. In addition, Nadeau et al. [85] found that stroke patients may be limited in gait speed due to weakness in plantar flexor muscles. Decreased lower limb strength can affect gait velocity and is prevalent in many clinical populations, including brain tumor survivors. An increase in sedentary lifestyle due to disease can negatively impact physical function.

Over 70% of our population received cranial radiation. Nearly nine out of ten patients exhibited poor leg strength, suggesting cranial radiation may have a significant effect on muscular strength. Ness et al. [64] determined that females treated with cranial radiation were found to have mean knee extension strength values over 58 Newtons lower than those who did not receive radiation. Helseth et al. [86] found that of 111 medulloblastoma survivors, all but three cases had major permanent functional deficits that were also associated with radiation therapy. Survivors treated with hematopoietic stem cell transplant with total body radiation were more likely to report physical limitations [87]. Cancer survivors may have comparable strength deficits due to similar treatment regimens with radiation therapy.

Poor physical function exhibited by our participants is supported by many other reports that include survivors of various cancer types. Boys and girls who survived ALL had significantly reduced peak $VO_2$, and impaired pulmonary function and exercise capacity when compared to controls [25,76,88]. Odame et al. [89] reported reduced physical fitness in brain tumor survivors treated with radiation therapy. In contrast, Bosma et al. [90] found that physical functioning of long-term glioma survivors reached levels comparable to that of a healthy population. This may be inconsistent with our results because Bosma's [90] long-term survivors were only a few years from diagnosis whereas our participants were over eighteen years from diagnosis; suggesting that with increasing time, physical function may decline.

Brain tumor survivors are documented to have numerous cognitive and psychological issues [91]. These problems may have a negative impact on daily and physical function. Several studies have demonstrated that performance tasks were impaired in brain tumor survivors with a low intelligence quotient [92-94]. In addition, Dennis et al. [92] reported a strong association between neurological impairment and reduced independence with daily living skills ($p<0.01$). Though we did not assess intelligence, this may be a contributing factor to the results of our population.

**Conclusions**

We acknowledge that our study has potential limitations. First, our study population was small. With only one hundred and twenty four brain tumor survivors, we may be over generalizing our results to the whole brain tumor survivor population. By including any type of brain tumor, instead of a selective group of brain tumors, we made our results more universal to brain tumors as a whole. Another possible limitation was that we had a relatively young population of participants (mean age 26.0 years). Enright et al. [63] advised to use caution when applying the 6MW prediction equation to non-Caucasians and those younger than 40 years and older than 80 years. Other studies that used this reference equation used an older population of participants, mean age ranging from 57.7 years to 73.2 years [95-97].

In summary, brain tumor survivors tend to have poor physical function. Although these results are not unexpected, this study has made clear an association between gender, quadriceps strength and neuropathy and overall physical function. Rehabilitation and exercise interventions during and post-treatment for brain tumor survivors may improve physical function, and should be considered in future research.

## Appendix

| Tumor type | Common location | Percentage of all childhood brain tumors |
|---|---|---|
| Medulloblastoma/PNET | Posterior fossa/Cerebellum | ≈ 35% |
| Ependymoma | Posterior fossa | ≤10% |
| Astroglial | Varies | ≤50% |
| Craniopharyngioma | Suprasellar | ≈2-3% |

**Table I.** Distribution of Childhood Brain Tumors[1]

| Chemotherapeutic Drug | Side Effects |
|---|---|
| Cisplatin | Neurotoxicity (peripheral neuropathies and/or bone marrow suppression) |
| Vincristine | Neurotoxicity, peripheral neuropathy being most problematic in adolescents, "foot drop", and/or auditory damage |
| Etoposide | Nausea and vomiting, alopecia, low blood counts, low blood pressure, and/or second malignancies |
| Cyclophosphamide | Nausea and vomiting, alopecia, myleosuppression, sterility, and/or second malignancies |

**Table II**. Chemotherapeutic Drugs and Associated Side Effects[1]

| | N=124 | |
|---|---|---|
| | N | % |
| Males | 69 | 55.6 |
| Race | | |
| White | 100 | 80.6 |
| Non-white | 24 | 19.4 |
| | | **Range** |
| Median age (years) | 26.0 | 19-53 |
| Median age since diagnosis (years) | 18.2 | 11-41 |
| Median age at diagnosis | 8.6 | 0-21 |
| Diagnosis group | | |
| Medulloblastoma/PNET | 37 | 29.8 |
| Astroglial | 59 | 47.6 |
| Ependymoma | 15 | 12.1 |
| Craniopharyngioma | 10 | 8.06 |
| Other | 3 | 2.4 |
| Tumor location | | |
| Posterior fossa | 44 | 35.5 |
| Suprasellar region | 10 | 8.06 |
| Cerebellum | 10 | 8.06 |
| Optic nerve/chiasm | 12 | 9.6 |
| Other | 48 | 38.7 |
| Surgery | | |
| Biopsy | 41 | 33.1 |
| Resection: near total | 3 | 2.4 |
| Resection: gross total | 66 | 53.2 |
| Radiation | 91 | 73.4 |
| Chemotherapeutic agents | | |
| Vincristine | 27 | 21.8 |
| Cyclophospamide | 31 | 25.0 |
| Cisplatin | 29 | 24.2 |
| Etoposide | 30 | 24.2 |
| Carboplatin | 16 | 12.9 |

**Table III.** Characteristics of Study Participants

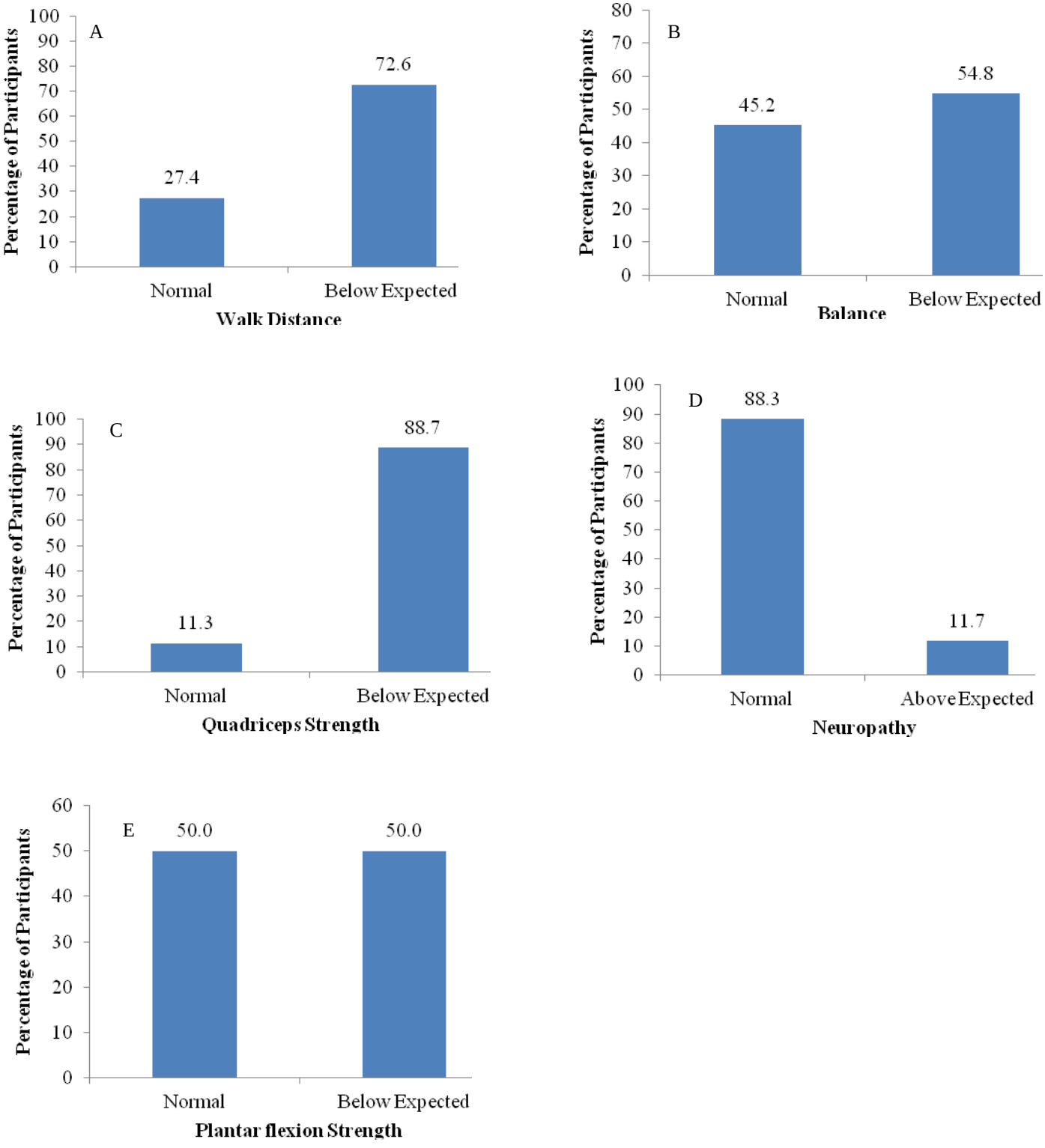


**Figure 1.** Percentages of participants that scored either below expected or within expected for the norms for (A) walk distance, (B) balance, (C) quadriceps strength, (D) neuropathy, and (E) plantar flexion strength.

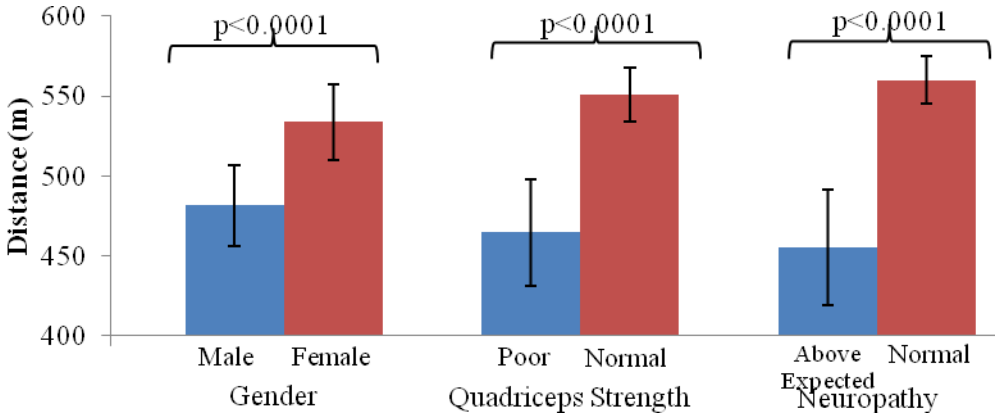


**Figure. 2.** Association betwween 6MW distance and gender, quadriceps strength and neuropathy. Means and standard error for six minute walk distance are adjusted for age, gender, weight, and height. Females (533.9 meters) outperformed males (481.6 meters), those with normal quadriceps (550.6 meters) outperformed those with poor quadriceps strength (464.8 meters) and those with minimal neuropathy (560.0 meters) outperformed those with above expected neuropathy (455.5 meters).

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# Behavioral Changes in Adult Rodents Infected with *Toxoplasma gondii*

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*Toxoplasma gondii is a common parasite that has the ability to manipulate its intermediate host in order to successfully transfer to a definitive host in the Felidae family. The species in the superfamily Muroidea are common intermediate hosts for T. gondii and are often used to study the behavioral changes in infected individuals. The parasite is able to manipulate the neophobic behavior of the rodents causing them to approach cat odor more frequently. This behavior would benefit the parasite by influencing the rodents to more readily approach the definitive hosts, cats. Uninfected rodents normally avoid cat odor due to predation risks. The learning capacity of the infected rodent is also modified, suggesting that the parasite can influence the ability of the rodent to recognize new stimuli. This alteration influences parasite transmission by altering the ability of the rodent to recognize a new environment, placing it at a greater risk for predation. The activity level of infected individuals is also increased which would benefit the parasite by making rodents an easier target for predators. Cats target moving objects in open areas causing more active rodents to be more susceptible to being eaten. All of these behavioral modifications are critical in understanding the mechanism by which the parasite affects the intermediate host. These results can then help to explain the behavioral changes seen in humans infected by T.gondii.*

**Introduction**

*T. gondii* is an intracellular protozoan that is one of the most common parasites found in the world (Webster 2007). Members of the Felidae family are the definitive host for the parasite, meaning that the parasite needs this host in order to complete its life cycle through reproduction (Webster 2007). All other endothermic mammals are intermediate hosts for the parasite. In the United States, it is estimated that at least 20% of people have been infected with *T. gondii* and contracted the disease toxoplasmosis. Toxoplasmosis is a disease which causes flu-like symptoms in most primary infections. However, the disease can also cause inflammation of the brain and infections in major organs in individuals with a suppressed immune system (Webster 2007). In countries such as China and India, the percentage of humans infected is much higher with somewhere between 50-80% of the population infected (Webster 2007). Humans can contract the parasite by eating undercooked meat with *Toxoplasma* cysts or fecal-contaminated fruit and vegetables. Also cleaning cat litter that contains cysts and then not thoroughly washing ones hands can cause transmission (Arling 2009). The parasite also can cross the placenta of a pregnant woman and pass on the disease to the fetus making this parasite extremely dangerous for a pregnant woman and the fetus (Bogtish et al. 2005).

*T.gondii* undergoes many developmental stages in its life cycle. A cat can become contaminated by ingesting a rodent that has *Toxoplasma* cysts. These cysts erupt in the stomach of the feline and release bradyzoites into the intestines. These bradyzoites invade tissue cells and begin reproduction (Bogtish et al. 2005). The bradyzoites also differentiate into either gametocytes or tachyzoites. The gametocytes fuse together to form a zygote which then becomes a cyst. These cysts are passed through fecal matter, and the cycle repeats when a rodent ingest the cysts (Bogtish et al. 2005). Other endothermic mammals such as cattle are also potential intermediate hosts. Humans are likely to ingest cysts in undercooked meat, because cattle are common intermediate hosts. (Bogtish et al. 2005). Due to the high prevalence of toxoplasmosis in humans, research on the effects of the parasite on intermediate hosts is an important area of study.

In order for the parasite to pass from an intermediate host, such as the rodent, it would be beneficial for the parasite to alter the intermediate host's behavior in order to ensure successful transmission to a definitive host. The parasite's ability to alter the host's behavior is known as the manipulation hypothesis (Berdoy et al. 2000). This paper will examine the evidence supporting *T. gondii's* ability to manipulate the host's neophobic behavior, learning capacity, and activity level.

**Neophobic Behavior**

One prediction of the manipulation hypothesis states that the parasite affects the aversion of rodents towards felines (Webster 2007). In order to measure if infected rats had a lower aversion to cat odor, Berdoy et al (2000) tested infected and control rats' response to different odors. Control rats that were injected with saline and not cysts showed a high perception of predation risks towards the cat. As shown in Figure 1, infected rats visited the cat odor a significantly higher number of times than control rats, suggesting that the parasite subtly alters the cognitive response of rats towards cat odor. This subtle alteration would aid the parasite's ability to successfully enter a definitive host and complete its life cycle. The infected rats responded the same to all other odors tested in the experiment. These results showed that the parasite does not affect the olfactory faculties but instead alters predation risk perception. Berdoy et al. (2000) discusses the importance

of these results in exploring the alteration of anti-psychotic drugs on infected rodents.

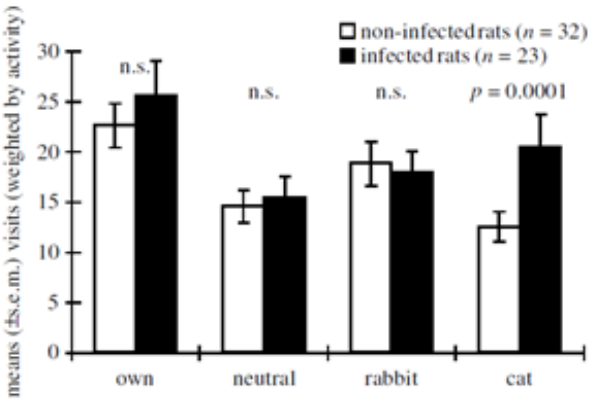


**Figure 1.** The mean number of visits of infected rats versus non-infected rats when approaching its own smell, a neutral odor, rabbit odor, and cat odor. Infected and non-infected rats differ in cat odor where predation risks are much greater. **(Berdoy et al. 2000**)

Anti-psychotic drugs used for mood stabilizers in humans have been shown to alter the parasite's ability to manipulate the behavior of infected rodents (Webster et al. 2006). In an experiment set up similarly to Berdoy et al. (2000), Webster et al. (2006) tested the hypothesis that anti-psychotic drugs, used to treat schizophrenia, alleviate induced behavioral alterations caused by *T. gondii* in rodents. Rats were infected and either treated with the haloperidol (HAL), valproic acid (VAL), or primethamine with Dapsone (PD). Another group was infected with *Toxoplasma* cysts and then untreated. The control group was not infected with cysts and treated with the three drugs. The drugs showed significantly different results between infected and uninfected individuals. PD and HAL in infected rats reduced the time spent in the cat area, suggesting the drugs had a positive effect on the infected rats' response to the cat area. The opposite occurred in uninfected rats that were treated with PD, HAL, and VAL. These drugs increased their likelihood to enter the cat area. In the discussion, Webster et al. (2006) states that the side effects of PD, HAL, and VAL on uninfected rats could be due to neuromodulation of dopamine levels. However, more experiments need to be conducted to understand the full extent of the side effects in uninfected intermediate hosts (Webster et al. 2006).

The studies discussed above have shown that the parasite is able to manipulate the cognitive processes of the rat by blocking the innate aversion of rats for cat urine. Webster et al. (2006) and Berdoy et al. (2000) maintained a set amount of cat urine through the experiment. When looking at different amounts of stimulus, Vyas et al. (2007) looked at various dosages of bobcat urine. As seen in Figure 2, the experiment showed that the effects of neophobic behavior are dependent on the amount of stimulus given during the experiment. An intermediate dose of bobcat urine produced the greatest behavioral change because a low dose would not be beneficial for the parasite, because there is a chance that there is not a definitive host present. High doses could cause innate aversion to the odor by the intermediate host.

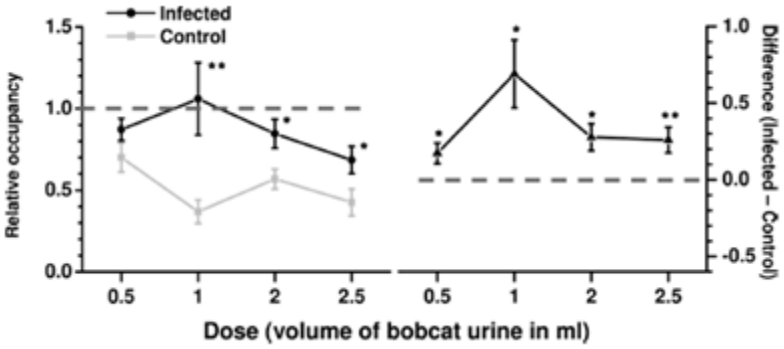


**Figure 2.** The graph above shows the results of relative occupancy of infected rats versus volume of bobcat urine. The intermediate dose of bobcat urine showed the greatest amount of time spent near the odor **(Vyas et al. 2007)**.

**Learning Capacity**

Studies looking at neophobic behavior alteration in rats agree that the parasite decreases the infected rats' fear of predation through subtle modification. Because *T. gondii* is able to modify its host behavior, the next area to study is the ability of the parasite to affect the learning capacity of host. The general prediction is that the parasite alters learning capacity of the rat because it can alter its learned fear of cats (Hodkova et al. 2007). However, the results from multiple experiments have yet to show a consensus on whether *T. gondii* can affect the learning capacity of the host.

Initial studies conducted by Hodkova et al. (2007) showed that mice with latent toxoplasmosis showed a lower learning capacity than the uninfected mice. In order to test the learning capacity of the mice, two experiments were set up. The first test was called the static rod test which timed the mice's ability to reach one end of the rod platform to the other with other rods placed in the direct path. The second test, the 8-arm radial maze test, consisted of a maze with 8 ends. Food was located at each end. The experiment monitored how long it took for them to find the food. In the 8-arm radial maze test and the static rod test, the *Toxoplasma*-infected mice performed worse than the control group, suggesting that the infection impaired the mice's ability to learn a new maze. However, control and infected mice showed a decrease in time spent in the maze between day one and day two showing that *Toxoplasmas gondii* had no effect on memory. The experiment does not establish if infected mice have impaired learning ability or a slower recognition of new stimuli (Hodkova et al 2007).

Gulinello et al. (2010) found evidence that contradicts the results from Hodkova (2007). Gulinello et al. (2010) conducted behavioral assays and histopathology of the brain of adult mice seven weeks after initial exposure to *T. gondii*. After euthanizing 10 infected mice and 5 control mice, brain samples were evaluated for damage and inflammation. The mice infected with *T. gondii* had significant alterations in

sensorimotor functions. On a balance beam, infected mice had more slips and falls then the control mice. Also, infected mice moved slower. The balance and locomotion deficits in the infected mice coincide with the increase predation risk seen in the neophobic behavior alteration (Gulinello et al. 2010).

When Gulinello et al. (2010) examined the cognitive functions such as learning and memory, the infected mice had normal cognitive function. The mice were tested for spatial learning and for new stimuli recognition. Figure 3 shows that infected and control mice show similar responses to both learning and new stimuli. These results are contradictory to previous studies mentioned in this paper. Gulinello et al. (2010) mentioned this contradicting evidence and suggested that the difference is due to food consumption. Hodkova et al. (2007) restricted the food before the mice ran the maze assay, which could have influenced their learning capacity.

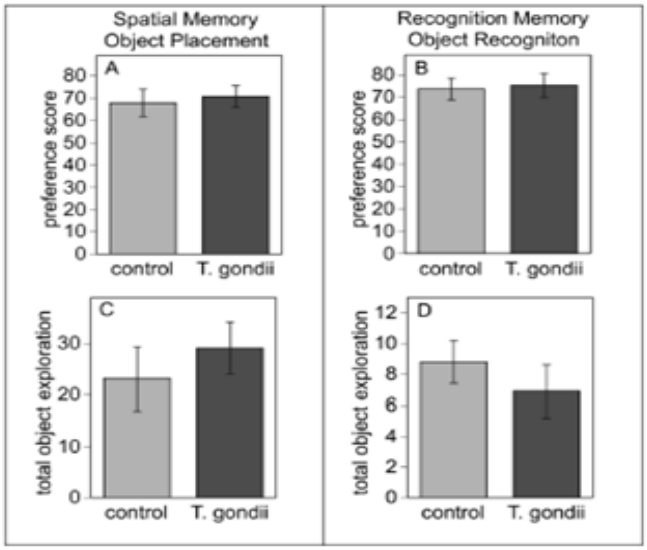


**Figure 3.** No cognitive deficits were found between infected and non-infected mice. Spatial and recognition memory was not significantly different between the control and infected mice **(Gulinello et al. 2010).**

Webster (2007) noted that rats and mice have different susceptibility to *T.gondii* in laboratory settings. Mice have a higher infection rate than rats, indicating that results for learning capacity could vary depending on species used in the experiment. Webster (2007) suggested that rats are the better test subjects because of the lower infection rate. Hodkova et al. (2007) and Gulinello et al. (2010) used mice in their experiments, which could account for the contradicting evidence because the mice have a higher susceptibility to infection (Webster 2007).

**Activity Levels**

When Gulinello et al. (2010) tested the speed of locomotion of the mice, the experiment was also looking at the activity levels of infected versus non-infected individuals. Cats are more attracted to moving and exposed prey (Gulinello et al. 2010). Because *T. gondii* can manipulate the intermediate host so that it is more vulnerable to predators, Hodkova et al. (2007) predicted that activity levels of infected individuals would increase because cats are more attracted to moving objects. Hodkova et al (2007) tested infected mice and non-infected mice by placing them on running wheels and then timed to see which mice covered the most distance and ran the fastest. The infected mice covered larger distances and stayed in motion for longer periods of time. One hypothesis explaining the increased time spent in motion addresses levels of dopamine concentrations. According to Hodkova et al. (2007), increased wheel running activity also increases dopamine concentrations. Infected mice have lower dopamine activity, indicating that these mice need more activity to reach the same level of endorphins as controls (Hodkova et al. 2007).

Webster (2006) found similar results when exploring anti-psychotic drugs on treated and untreated infected rats. Infected rats that were not treated with the drugs showed the most activity. Uninfected, treated rats showed an increase in activity as well. These results suggest that the anti-psychotic drugs make uninfected individuals act like a rat that has been exposed to *T. gondii* (Webster et al. 2006). Because these drugs alter the behavior of uninfected rats, it calls into question how these drugs could potentially react in humans who had previous exposure to toxoplasmosis.

Hrdáá et al (2000) monitored the activity level of mice in an open field. Three weeks post infection, a 30% decrease of activity was noted in *Toxoplasma*-infected mice. After six weeks, activity level returned to normal. When compared to the weight of the mice, the activity levels coincided with a decrease in weight suggesting that the lethargy of the mice was due to acute illness and not the parasite. These results contradict previous studies (Webster 1994) on the activity level of infected individuals. Hrdáá et al. (2000) noted the differences in the study's results and the results from Webster (1994). However, Webster (1994) only looked at latent infection usually between 14 to 28 weeks post infection. As the infection progresses, more cysts embed into tissues in the brain and muscle. Therefore, an acute and latent infection should show variation in activity level modification because the number of cysts embedded in tissue would affect the amount of damage caused by inflammation (Hrdáá et al. 2000).

**Mechanisms**

The mechanism by which *T. gondii* alters the intermediate host's behavior is unknown. Webster (2007) suggested that neuromodulation is responsible for the alteration. As reported by Berdoy et al (2000), N-methyl-D-aspartic acid (NMDA) receptors in the brain caused rats to approach cat odors more frequently similarly to how rats react when infected with *T. gondii*. NMDA is an amino acid derivative that is important in operant behavior, suggesting that this acid is used in learning manipulation (Berdoy et al. 2000). As mentioned above, dopamine also plays a role in the locomotion and learning behavior of the rat. When Webster et al. (2006) used haloperidol to treat infected individuals, the infected rats reacted similarly to control mice. Haloperidol is a

dopamine antagonist which shows that dopamine could contribute to behavioral manipulation in the intermediate host (Webster et al. 2006).

Learning the mechanism by which *T.gondii* manipulates its intermediate hosts, will lead to scientists having a better understanding of how the parasite affects its human intermediate host. In a study conducted by Arling et al. (2009), people who had attempted suicide exhibited higher *T. gondii* antibody IgG levels. The results from Arling et al (2009) suggested a correlation between individuals who had been previously exposed to toxoplasmosis and suicide. If the parasite is able to manipulate the rodents' behavior, predictions have been made that it can do the same thing to other intermediate hosts including humans (Arling et al. 2009). Vyas et al. (2007) describes rodents' behavior as self-destructive due to their lack of fear of predation. If the parasite benefits from self-destructive behavior through increase in transmission in rodents, the parasite could affect a human's brain in a similar way (Arling et al. 2009).

**Conclusion**

*T. gondii* has the ability to manipulate the behavior of rodents through neophobic behavioral alteration, learning capacity deficiency, and increased activity levels. These alterations benefit the parasite by exposing the intermediate host to its predator usually in the Felidae family which is the definitive host for *T. gondii*. Other intermediate hosts such as cattle and humans are not usually prey for species in the Felidae family. Therefore, it would seem reasonable that most of the behavioral changes seen in intermediate hosts are seen in rodents, because rodents are commonly preyed upon by the definitive host. Once the parasite is ingested by the feline, the life cycle of the parasite can be completed. All three of the behavioral modifications seen in rodents suggest that the manipulation is not due to damaging of the brain but due to the highly specific, subtle modifications of sensorimotor pathways. The parasite has evolved in order to be better adapted to its environment by manipulating the intermediate hosts.

One area of research in behavioral modification that needs to be studied is multiple exposures to *T. gondii*. This parasite is able to enter an intermediate host multiple times causing multiple infections (Bogtish et al. 2005). Occasionally in a human, the symptoms for the toxoplasmosis aren't even noticed until the second exposure (Bogtish et al. 2005). Therefore, further studies should look at how the behavior of the intermediate hosts varies depending on primary or secondary infection.

Also, an experiment that investigates behavioral changing in acute versus latent stages would be beneficial in understanding consistent behavioral changes. The studies presented in this review were for either acute or latent but never both infections. Sometimes there were contradictions to the data presented in each study due to changing of species of rodents. Studies comparing a similar experiment, except changing acute infections to latent infections, could help to iron out the inconsistencies in the results.

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# Myob is Required for Cytokinesis of *Aspergillus nidulans*

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## Introduction

Cytokinesis in filamentous fungi requires the assembly and constriction of a ring of filamentous actin at division sites, a process that highly resembles cytokinesis in animal cells (Harris, 2001). The assembly of the actin ring is best characterized in fission yeast *Schizosaccharomyces pombe*, which requires that nodes of the motor protein myo2p localize to the division site and capture actin filaments nucleated by the formin Cdc12p. Following this, the myosin nodes condense along with the filamentous actin to form an actomyosin ring (Pollard 2010). Compared to animal cells, fungal cytokinesis is complicated by septation, a process of constructing cross-walls at the division sites, in which the deposition of chitin needs to coordinate with the contraction of an actin/myosin ring (Harris, 2001). It has been reported that in fission yeast, the absence of myo2p blocks separation of daughter cells but does not prevent the formation of septa (Kitayama, 1997), suggesting that myo2p is dispensable for septation in fission yeast.

Previously, a randomly mutagenized strain of filamentous fungi *Aspergillus nidulans* (strain RCH2) with septation defects was shown to have zero recombination with a strain having a GFP-labeled *myoB* allele, the *A. nidulans* orthologue of *S. pombe myo2*. (T. W. Hill, unpublished data). In this paper, it is shown that the RCH2 mutation is a missense mutation in the converter domain of *myoB*. Unlike the case in fission yeast, myosin is essential for septation in *A. nidulans*. This evidence indicates that *A. nidulans* primarily utilizes a highly conserved MyoB-dependent septation mechanism.

## Materials and Methods

### Sequencing and Characterizing the *myoB* Gene

The target gene (*myoB*, AN4706) was amplified by Polymerase Chain Reaction (PCR) from genomic DNA of the RCH2 mutant strain with primers designed to cover the entire coding region plus ca. 70 bp of 5′ and 3′ untranslated regions. PCR products were purified using a Qiagen PCR purification kit unless otherwise specified. Dideoxy chain termination sequencing was carried out as describe in Olsvik et al. (1993) through contract with the University of Tennessee Center for the Health Sciences using 19 sequencing primers with a spacing of ca. 450 bp designed to give redundant coverage. The resulting sequence was compared with an online fungal genome database (Eurofungbase) using the NCBI BLASTn program (Zhang et al., 2000), and nBLASTp for translated amino acid sequence (Altschul, et al., 1997). The multiple sequence alignment was performed by ClustalX (Larkin et al., 2007) and NCBI Conserved Domain Search (Marchler-Bauer et al. 2011).

### Cloning of wild type *myoB* and Transformation of the RCH2 strain with $myoB^{wt}$

The wild type *myoB* gene plus the 980 bp of 5′ untranslated region was amplified by PCR from genomic DNA of wild type strain A28, with *BamH*I (GGATCC) and *Kpn*I restriction sites (GGTACC) introduced at the 5′ and 3′ ends of the PCR product respectively. The product was purified using the Qiagen Gel Extraction Kit. The purified PCR product and vector pRG3 were double-digested with *BamH*I and *Kpn*I and ligated together using T4 DNA ligase (NEB) overnight at 16 °C. The resulting pRG3-*myoB* construct was cloned and purified. Protoplasts of the RCH2 strain were transformed with the final construct according to standard procedures. Transformants were examined microscopically for restoration of wild type septation.

### GFP and mRFP Labeling

GFP- or mRFP-encoding gene sequences were PCR-amplified along with selective markers, and combined using fusion PCR (Szewczyk et al., 2006) with 1 kbp PCR-amplified copies of the sequences flanking the stop codon of *myoB*. The resulting fusion PCR product, when transformed into wild type strain A1145, was designed to cause replacement of the *myoB* stop codon with the gene sequence encoding a GFP or mRFP gene product, allowing expression of a GFP-labeled or mRFP-labeled protein chimera. A corresponding procedure was used to GFP-label the *myoB* allele in the RCH2 mutant strain.

## Results

### Characterization of RCH2 phenotype and Complementation

At all growth temperatures, wild type *A. nidulans* hyphae contain abundant septa of uniform thickness extending fully across the width of the hyphae (Fig. 1A). At lower temperatures (e.g., 28 °C), septation in strains bearing the RCH2 mutation occurs at low frequency and results in reduced rate of successful septation (Fig. 1B). At 42 °C, septation is completely blocked (Fig. 1C).

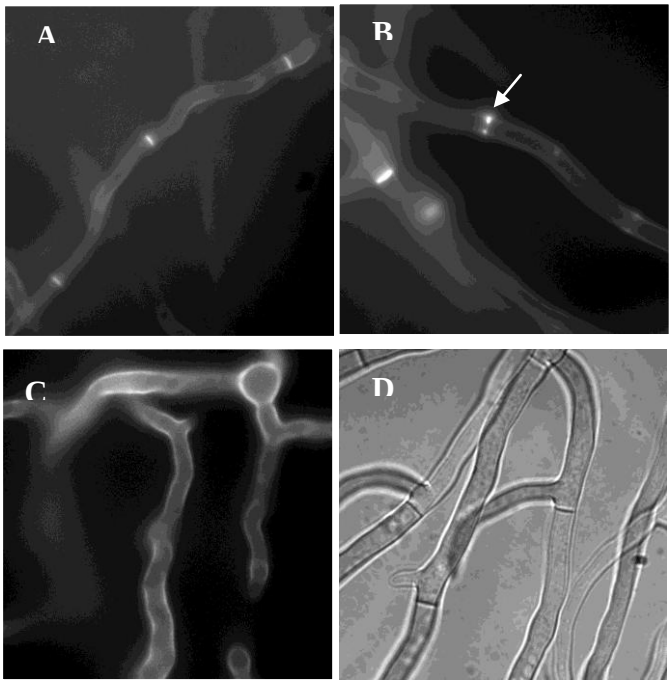


**Figure 1**. Septation in wild-type, mutant, and complemented strains of *A.nidulans*. In A – C, septa are stained by the fluorescent dye CFW; in D septa are imaged by bright-field microscopy. **A)**: A28 (wild-type) at 28 °C. **B)**: RCH2 at 28 °C. Septation is not entirely blocked, but many septa are defective (arrow). **C)**: RCH2 at 42 °C. Hyphae are aseptate. **D)**: Complementation of septation defect in RCH2 mutant strain, transformed with *myoB*$^{WT}$ allele.

### Sequencing and Sequence Alignments

Because earlier work (T. W. Hill, unpublished data) had suggested the possibility that the RCH2 mutation might lie in the gene *myoB* encoding *A. nidulans* myosin II, the *myoB* RCH2 allele was sequenced to identify possible mutations. A single base substitution was identified (G2685A), which is predicted to result in a glycine-to-aspartate substitution at amino acid residue #843. A conserved domain search revealed that residue #843 lies in the converter subdomain of the myosin head domain. Protein sequence alignment with type II myosins from other fungal species, as well as representative plant and animal type II myosins, indicated that the glycine at this position is highly conserved (Fig. 2). No sequences in which this site is occupied by an amino acid residue other than glycine were found.

**A)**

625
SCLDEDCVMPKATDKSFTEKLHSLWDTKSTKYRASRLRQGFILTHYAAEVEYSTDGWLEK
684
685
NKDPLNDNITRLLASSQDNHIAALFSDCGNADEVDHPRSRVKKGLFRTVAQRHKEQLSSL
744
745
MNQLHSTHPHFVRCIIPNHKKRPKMLNAPLVLDQLRCNGVLEGIRIARTGFPNRLSFNEF 804
805
RQRYEVLCRDMPKSYMDGQSAARIMLQKLALDKAWFRVDRTKVFFRAGVLAELEEKRDE
L 864
865
IRTIMTRFQSVARGFVQRRISNKRLYRAEATHIIQHNFRAYLEMKANPWWRLFSRMKPLL
924
925
GETRTAQEVKRRDEKIKQLETKMKQDQSERQKVEEERRRAEIEIQRIQQTLESERALALD
984
1045
IQRLEAERKEMQQKLEDLEQKLLEAQSSASETENHMRELGQEVKMLQSHLSLKERKLQDL
1044

**B)**

| | | |
|---|---|---|
| | | # # ## ### |
| **RCH2-myoB** | 815 | MPKSYMD GQSAARIMLQKLALDKAWFRVDRTKVFFRAG 852 |
| N.crassa | 816 | MPKGYVE GQAAARIMLDRFGLDKSLYRVGLTKVFFRAG 853 |
| S.pombe | 708 | PTGTYVE SRRASVMILEELKIDEASYRIGVSKIFFKAG 745 |
| S.cerevisiae | 727 | STTTTFS.[6].TKQNCEFLLTSLQLDTKVYKIGNTKLFFKAG 771 |
| U.maydis(UM03286) | 698 | IPPGYMD GRKACQRMVEALELDKLTFKIGTSKIFFKAG 735 |

**C)**

| | | |
|---|---|---|
| | | # # ## ### # ### |
| G.gallus(2MYS_A) | 742 | KASEKLLGGG DVDHTQYAFGHTXVFFXAGLLGLLEEMR 779 |
| **A.nidulans(myoB-)** | 824 | SAARIMLQKL ALDKAWFRVDRTKVFFRAGVLAELEEKR 861 |
| A.irradians(IB7T) | 737 | TVSEKILAGL QMDPAEYRLGTTKVFFKAGVLGNLEEMR 774 |
| R.pipiens | 749 | KATEKLLGSL DIDHTQYKFGHTKVFFKAGLLGQLEEMR 786 |
| H.sapiens(myh9) | 738 | QACVLMIKAL ELDSNLYRIGQSKVFFRAGVLAHLEEER 775 |
| X.laevis(myh4) | 741 | KACEKLLGSI DIDHTQYKLGHTKVFFKAGLLGTLEEMR 778 |
| C.elegans(Myo3) | 749 | KASVGILDKI.[4].NLTDEEFKVGETKIFFKAGVLAKLEDLR 790 |
| A.thaliana(myosin2) | 651 | SVSIAVLKQY DVHPEMYQVGYTKLYLRTGQIGIFEDRR 688 |

**Figure 2**. Identification of the mutation. **A)** A single base pair mutation from G to A at 2685 is predicted to change the #843 residue from glycine (G) to aspartate (D). **B)** The *myoB* amino acid sequence of *A. nidulans* was aligned with the myosin II orthologues of fungi *Neurospora crassa, S. pombe, S. cerevisiae, and Ustilago maydis.* **C)** The part of converter domain (marked with #) where the mutation was found was aligned with myosin II orthologues in non-fungal species (chicken, bay scallop, frog, human, nematode, and *Arabidopsis*). Residues that match the *A.nidulans* converter sequence are highlighted.

### Localization of MyoB in wild-type and mutant strains

Fluorescence of the wild-type MyoB-GFP chimeric protein was observed at septation sites, forming ring structures (Fig. 3A). Time-course microscopy revealed that the MyoB ring constricts until it is condensed to a single spot at the

center of the nearly completed septum (Fig. 3C), after which the signal disperses into the cytosol. In comparison with wild-type, MyoB in the RCH2 strain still localizes initially at septation sites, but rings are typically abnormal (Fig. 3B) and cannot constrict uniformly to a central spot like wild-type MyoB does. Instead, ring constriction is aborted prematurely, and RCH2 MyoB disperses into the cytoplasm in the form of nodes (Fig 3D, 1 – 4).

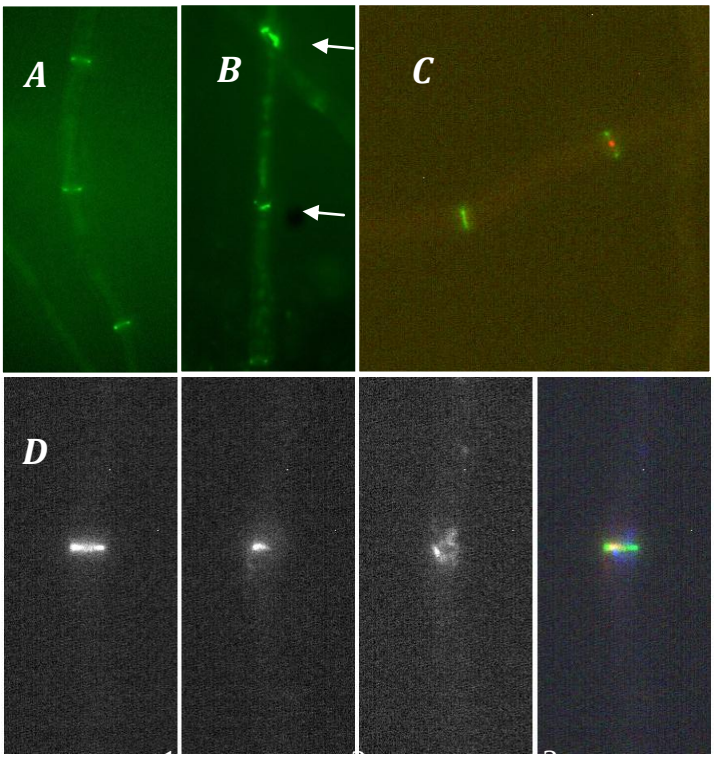


**Figure 3**. Localization of MyoB in RCH2 and A28 strains. **A)** GFP-tagged wild type MyoB at 28 °C showing normal ring structures. **B)** GFP-tagged MyoB in RCH2 at 28 °C showing aberrant ring formation (arrow). **C)** False color overlay showing progressive stages of constriction of a single wild type MyoB ring as observed in time-lapse microscopy. An early stage is shown in green, and the final stage of the same myosin ring is shown in red. **D.1 – D.3)** Time course showing stages in the constriction of a single MyoB ring in the RCH2 mutant strain. D.4 shows false color overlay of D.1 to D.3. The yellow color is a result of the colocalization of green (D1) and red (D2), demonstrating that MyoB in RCH2 does not condense to the center of nascent septum as in C, and that the ring disassembles before septation is completed.

***Discussion***

The results provide clear evidence that *myoB* in *A.nidulans* is critical for septation and cytokinesis. Previous genetic mapping studies in this lab revealed that the RCH2 strain has no recombination when crossed with a MyoB::GFP strain, suggesting that both the RCH2 mutation and the gene encoding MyoB may reside in the same chromosomal locus. Our sequencing of the *myoB* allele of the RCH2 mutant strain confirms the existence of a mutation in that locus. Because the glycine in the converter domain is highly conserved in all examined eukaryotes (fungi, plants, animals), this demonstrates that this residue is of great importance to myosin II function.

By comparing to the structure of scallop myosin, the converter subdomain in *myoB* is located to residues F795 - R861 (Houdusse et al., 1999). Fig. 3 shows the corresponding residues in a 3D model of the 2MYS_A in *G. gallus* (NCBI Conserved Domain Database). Residue G843, having no side chains for H-bonding, is positioned next to the turning of the first strand of the beta-pleated sheet. The G843D mutation adds a hydrophilic side chain that contains 4 potential H-bonding sites to the residue, thus significantly alters the hydrophobicity of this residue. The substitution of a large charged aspartic acid residue for a small neutral glycine would be expected to have severe consequences for the structure and function of the protein.

Evidence from mutational studies on myosin-II for cardiomyopathy disease in humans suggests that the converter is the main site where the elastic distortion of myosin cross-bridge occurs, and mutations that reduce cross-bridge elasticity decrease the overall efficiency of myosin-II (Kholer 2002; Seebohm 2009). It has been shown in *Drosophila* that converter domains have naturally occurring isoforms that adapt to different kinetic requirements, and by experimentally altering the converter isoforms, the myosin cross-bridge kinetics changes accordingly. It was proposed that variations between converter isoforms affect the length of the actin-myosin cross-bridging cycle, which is determined by the attachment and/or detachment rate (Swank 2002). These studies demonstrate that the converter domain is critical for motor kinetics.

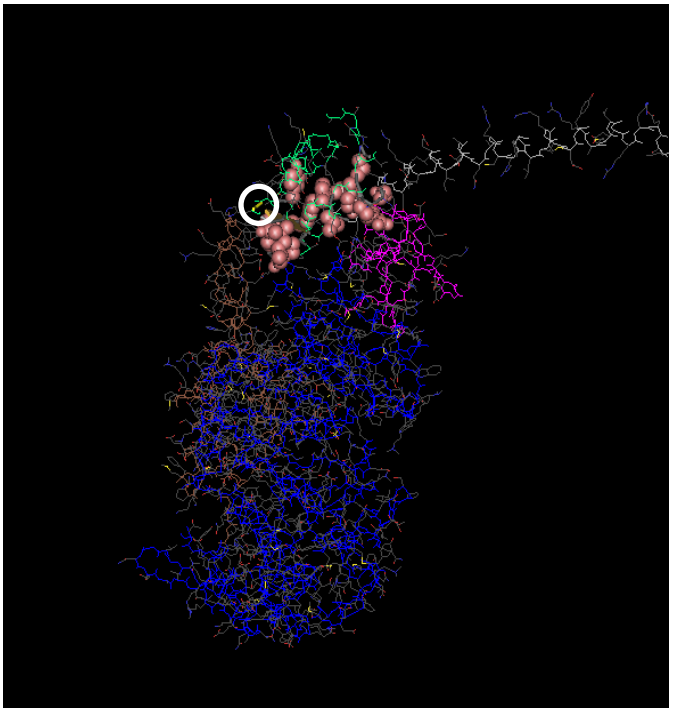

**Figure 4.** Structure of the myosin head group. The converter domain is highlighted in green. The corresponding residue of the G843 in *A.nidulans* is highlighted in yellow and circled. (NCBI's Entrez Structure database, cd01377)

The type II myosin for the contraction of the actomyosin ring has been identified in budding yeast (myo1p) and fission yeast (myo2p), and a recent investigation for the homologous myosin in filamentous fungi identified *myoB* in *Penicillium marneffei* (Canovas, 2011). In fission yeast the absence of myosin II does not prevent the formation of septa, but daughter cells cannot separate properly after septum is constructed (Kitayama 1997. It has been reported that in budding yeast there is an alternative cytokinesis pathway that is independent of myosin (Tolliday, 2003). Functionally, the role of myosin II in *A. nidulans* appears to be more similar to that of its homologue in *Penicillium marneffei*, the absence of which blocks septation, than to its homologues in either of these yeasts. Also, in *P. marneffei*, the formation of an actin ring is not affected in the absence of *myoB*, and the same result is observed in *A. nidulans* (T. W. Hill, unpublished observation). In the latter case, MyoB cannot localize to the septation site if actin polymerization is experimentally blocked, suggesting that the localization of MyoB is dependent on actin, but not the reverse in *A. nidulans*. Therefore, the simplest model accounting for the RCH2 phenotype is that the MyoB with reduced efficiency cannot seize and walk the F-actins extending from the nucleation site. However, it is still unknown how the actomyosin ring communicates to other components of the septation complex to signal the deposition of chitin.

***Acknowledgements***

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# Reading in Technicolor: Proposed Neural Mechanisms for Grapheme-Color Synesthesia

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Each individual has a unique perception of the world—but are some perceptions more unique than others? Recent research into a phenomenon known as synesthesia would argue that some people do in fact perceive the world in a much more unique manner than the average individual. For the synesthete, one stimulus may trigger multiple, distinct perceptions. In particular, there has been a large amount of research on a specific form of synesthesia known as grapheme-color synesthesia, in which the perception of letters or digits (graphemes) is strongly tied to the perception of color. Despite the high volume of research into synesthesia and its variants, the neural mechanism for this phenomenon is not very well understood. The two main theories for what causes synesthesia are a theory of decreased inhibition (known as the disinhibited feedback theory) and a theory of increased connectivity within specific brain regions (known as the cross-activation theory). Based on the research currently available, a modified version of the cross-activation theory known as the cascaded cross-tuning model is the most plausible mechanism for this phenomenon, although even this is not definitive.

But what exactly is synesthesia? Only recently has it been confirmed as an actual neurological condition—it has yet to claim a position the latest Diagnostic and Statistical Manual of Mental Disorders. Synesthesia is defined as a "perceptual phenomenon in which certain stimuli elicit a sensation in two or more sensual modalities" (Jäncke et al., 2009). That is, a stimulus that feeds through only one processing stream in the average individual will concurrently feed through another unstimulated stream in a synesthete (Brang et al., 2010). This can take the form of smells evoking the experience of shapes, tastes evoking tactile perception, letters or numbers eliciting the perception of distinct colors, or various other cross-sensory pairings. Of the numerous possible cross-pairings, those involving color are the most prevalent, or at least the most documented, making up between 80.6% and 95% of all synesthetic reports (Eagleman & Goodale, 2009). Specifically, grapheme-color synesthesia is one of the most studied varieties of color-linked synesthesia. This grapheme-hue pairing is not simply a memorized series on the part of the synesthetes—associations are seemingly effortless, highly consistent throughout a synesthete's life, and particularly specific (each letter or digit evokes a distinct hue) (Rouw & Scholte, 2007). Additionally, this behavior is not and cannot be learned, as it most often results from a developmental, and possibly genetic predisposition (Rouw & Scholte 2007). However, while grapheme-color synesthesia is not learned, increased amounts of exposure to graphemes can have an influence on the intensity of the synesthetic experience. According to Brang and his colleagues (2011), letters with a higher frequency of exposure often pair with more common color names. Additionally, they found that stronger responses are elicited from vowels than consonants as a result of their relative frequency (Brang et al., 2011).

When studying grapheme-color synesthetes, it is important to note that there are two discrete subtypes: projectors and associators. Associator synesthetes report that the associated color experience is a phenomenological experience (Brang et al., 2011). In other words, the grapheme evokes the concept of the color within the subconscious, without the synesthete actually having a conscious, visual perception of it. Of the two, this is the more prevalent subtype of grapheme-color synesthesia (Brang et al., 2011), which may be due to the fact that it is not as strong of a synesthetic experience as projector grapheme-color synesthesia. Projectors perceive synesthetic color as a part of their visual experience (Rouw & Scholte, 2007). Their synesthesia does not simply result in the concept of a distinct hue being associated with a specific grapheme, but also includes an actual visual perception of the color itself. The synesthetic color is typically perceived as though radiating from, or superimposed over the letters and numbers (Carriere et al., 2008). The differences between these two subtypes of grapheme-color synesthesia are significant not only in the location of the synesthetic color, but also within the brain structures involved. Diffusion tensor imaging has shown that projector synesthetes' brains are more hyperconnected in the early visual areas of the inferior temporal cortex than the associator synesthetes' brains (Rouw & Scholte, 2007). This suggests that there may be a gradient to the degree of grapheme-color synesthesia, with projector grapheme-color synesthesia representing the "stronger" form and associator grapheme-color synesthesia being the "weaker" form.

In order to better understand the proposed mechanics of color-grapheme synesthesia, one must first grasp how graphemes and colors are processed in non-synesthetes. In the analysis of visual stimuli, there are two distinct streams of processing: the dorsal stream, which processes the "where" of objects, such as location and movement, and the ventral stream, which analyzes the "what" aspects, such as form and color. The ventral stream is most significant to the study of grapheme-color synesthesia, as it is involved in both word recognition, which includes grapheme processing, and the processing and perception of color (Barnett et al., 2008). Within the ventral stream, the perception of color takes place in the V4 and V8 regions of the visual cortex. Specifically, V4 is responsible for a majority of color processing, including the phenomenon of "color constancy," in which a color's appearance is maintained regardless of the level of light.

Additionally, V8 regulates color perception and the memories of objects' colors. Graphemes, particularly letters and digits, are processed in the posterior temporal grapheme processing area (PTGA), which is a component of the larger lateral occipital complex (LOC) that processes a variety of shapes and forms. In non-synesthetes, graphemes and colors are processed independently, despite the proximity of these brain areas [see Figure 1]. In grapheme-color synesthetes, however, the brain areas responsible for color and grapheme processing interact, although how they do so is disputed (Carlson, 2011).

As mentioned earlier, one of the main theories for explaining the neural mechanism behind synesthesia is the theory of disinhibited feedback. This model suggests that there is no increased structural connectivity among the relevant brain areas (Eagleman & Goodale, 2009). Instead, the synesthesia results from the lack of inhibition of feedback from higher-level processing areas (Brang et al., 2010). Because synesthetes have weakened inhibition within this visual pathway, there may be excitation occurring in unstimulated brain regions after the initial stimulation of the synesthetically linked stimulated area [see Figure 2]. But how does this secondary excitation arise? According to Brang and his colleagues (2010), in this model, the sensory information spreads through multiple levels of the "visual hierarchy," arrives at a "convergence site," and then feeds back to V4 (Brang et al., 2010). Thus, the activation of V4 only occurs after substantial cortical processing, such as the processing of graphemes (Brang et al., 2010). This excitation is able to provide feedback to the unstimulated V4 because when the specific inhibitory interneurons are blocked, activity in one cortical area is able to spread more broadly, (Eagleman & Goodale, 2009) allowing the activation of PTGA to diffuse to the nearby V4.

The disinhibited feedback theory first arose as an explanation for some atypical acquired synesthesias (Brang et al., 2010), because the prior model of structural connectivity would be unlikely for synesthesia not present since early childhood. In addition to this initial reasoning, the theory is further supported by the fact that non-synesthetes can have synesthesia-like experiences during states of altered consciousness, such as during meditation, while falling asleep, or while using certain drugs (Eagleman & Goodale, 2009). It is highly unlikely that these experiences are solely a result of increased structural connectivity, as they occur in situations where consciousness is altered, and neural connectivity does not have the capability to rapidly change during these states. While these instances suggest that the disinhibited feedback theory describes the mechanism behind grapheme-color synesthesia, there is much opposition to this model.

The main theory in opposition to the disinhibited feedback theory is the cross-activation theory, which claims that instead of decreased inhibition, synesthesia results from increased structural connectivity between PTGA and V4 (Brang et al., 2010). This connectivity is believed to have a developmental origin that is strongly influenced by genetics. It is possible that insufficient pruning occurs during brain development in early childhood, causing the extra connections to exist throughout the individual's life (Eagleman & Goodale, 2009). Others suggest that instead of, or potentially in addition to the insufficient pruning of neurons, synesthesia may result from increased arborization, which is the additional formation of synaptic connections between related brain areas when the "regular" connections are initially being created in synesthetes' brains (Eagleman & Goodale, 2009). Because in this model the two synesthetically tied brain regions are physically connected, there is no time delay in activation as suggested by the disinhibited feedback model—PTGA and V4 are activated nearly simultaneously (Brang et al., 2010). Overall, this theory argues that the linkage between PTGA and V4 is purely physical in nature.

Due to the physical nature of the cross-activation theory, neuroimaging studies can be extremely useful in testing its validity. Rouw and Scholte (2007) used diffusion tensor imaging (DTI) to determine the extent of the neural connectivity between PTGA and V4. DTI utilizes a magnetic resonance signal to measure the diffusion of water within the brain, which can be used to determine the white matter structures in vivo. More coherent white matter leads to increased anisotropic (directionally dependent) diffusion (Rouw & Scholte, 2007). After quantifying the directionality of local structures by calculating fractional anisotropy (FA) values, it was observed that there was an increased level of connectivity in several distinct areas in synesthetes that was not found in non-synesthetes (Rouw & Scholte, 2007). Connectivity was greater in all synesthetes near the fusiform gyrus, which is located near PTGA and V4, and it was observed that those with the greatest FA values near the early visual areas were most likely to be projector synesthetes (Rouw & Scholte, 2007). In a repetition of the previous study, Jäncke and his colleagues (2009) determined that synesthetes had increased cortical volume, thickness, and surface area in the ventral visual stream, but did not have higher FA values in the fusiform gyri. Finally, magnetoencephalography, a neuroimaging technique that is capable of recording magnetic activity elicited by the firing of large numbers of neurons was used to determine whether the feedback inhibition theory or cross-activation theory better described synesthesia. This technique operates on a millisecond time scale and has sufficient spatial resolution to distinguish V4 from PTGA (Brang et al., 2010). This study showed that the excitation of V4 occurs nearly simultaneously with processing in PTGA, demonstrating that the disinhibited feedback theory is not a plausible model, as it requires a time delay between PTGA and V4 activation (Brang et al., 2010). While the cross-activation theory is now better supported than the disinhibited feedback theory, it still does not completely explain grapheme-color synesthesia.

Expanding off of the cross-activation theory, Brang and his colleagues (2010) have suggested the cascaded cross-tuning model to explain the mechanism behind synesthesia. According to this model, grapheme recognition results from activation spreading up a hierarchal network of visual processing—from least to most complex visual feature processing (Brang et al., 2010). Within the PTGA, the hierarchy of processing travels from the posterior region to the anterior region, indicating that feature-level processing occurs prior to letter level processing (Brang et al., 2010). This model does not depict a linear progression through the hierarchy, but

instead perception is described as being built up as it cycles from low-level areas, such as color and form, to higher-level cognitive areas and then back to the lower-level areas (Carriere et al., 2008). Interestingly, perception does not occur until the information has cycled through the hierarchy several times (Carriere et al., 2008). The strongest support for this theory comes from studies observing how synesthetes perceive similarly shaped letters. In their 2011 study, Brang and his colleagues determined that similarly shaped letters evoked similar synesthetic colors, with projectors demonstrating this effect more strongly than associators (Brang et al., 2011). This supports the cascaded cross-tuning model because shape and color are processed before higher-level grapheme concepts, and would therefore be more closely linked in grapheme-color synesthesia. Although this model appears to be the most accurate of the three proposed, it cannot be affirmed with certainty, as it is a very novel idea and has yet to be extensively studied.

Based on the current research, it is clear that the exact mechanism of grapheme-color synesthesia is far from being elucidated. But given the data available, it appears that the disinhibited feedback theory is not a viable explanation of synesthesia, as its necessary temporal segregation of PTGA and V4 activation have been shown via magnetoencephalography to not occur. Compared to the disinhibited feedback theory, the cross-activation theory is much more strongly supported. Diffusion tensor imaging has shown that increased physical connections between relevant brain areas do exist in grapheme-color synesthetes, and magnetoencephalography has demonstrated that PTGA and V4 are activated nearly simultaneously, affirming the mechanism proposed in this model. However, beyond the cross-activation theory, the recently proposed cascaded cross-tuning model appears to be the best estimate of the mechanism of grapheme-color synesthesia to date. It expands upon the basic framework of the cross-activation theory by adding the repeated cyclical feedback/feed-forward between PTGA and V4 that is used to explain why letters with similar shapes typically have similar synesthetic colors. While the cascaded cross-tuning model is clearly the strongest of the three current theories, it has yet to clearly become the definitive mechanism of grapheme-color synesthesia. Hopefully, with advances in neuroimaging techniques and other research methodologies, the details of synesthesia will be discovered and the mystery of grapheme-color synesthesia solved.

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