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About this Issue

Statement of Purpose

The Rhodes Journal of Biological Science is a student-edited publication that recognizes the scientific achievements of Rhodes students. Volume XXXII marks the eleventh year since Mark Stratton and Dr. David Kesler brought the journal back into regular publication 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.

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Editorial Staff

Emily Lichtenberger ‘17 (Senior Editor) is a Biology major from Charlottesville, VA. On campus, Emily is one of the Animal Services Coordinators for the Kinney Program and had been a member of the Rhodes Equestrian team for the past two years. Emily is a member of Beta Beta Beta, Gamma Sigma Epsilon, and Phi Alpha Theta honor societies. Off-campus, Emily has a research internship at the Research & Conservation Lab at the Memphis Zoo. Last year she submitted and was awarded funding for a CAN grant to develop techniques to monitor hormones in snakes. She spent her past summer working on the research project. After graduation, Emily plans on applying to veterinary school.

Erin Deery ‘18 (Junior Editor) is a Biomathematics major with a minor in Religious Studies from Winter Park, FL. On campus, Erin does research in the mathematics department on the spread and control of the yellow fever virus and in the biology department on gap regeneration in Overton Park's old growth forest. She is an Innovation Fellow under the Mellon Grant and has worked with community partners like the Memphis Zoo and Overton Park Conservancy on digital projects. Additionally, Erin is a co-captain of the Rhodes Women's Basketball Team and throws the javelin for the Rhodes Track and Field Team. Off-campus Erin has completed the Summer Service Fellowship at the Mid-South Chapter of the American Red Cross and currently interns in the Epidemiology and Cancer Control Department at St. Jude Children's Research Hospital. Erin plans to attend medical school and pursue a graduate degree in epidemiology following graduation.

Carolyn Dishuck ’17 is a Biology major with a minor in urban and community health from Tuscaloosa, AL. During her time at Rhodes, she has been involved in student government as a senator, was the president of Rhodes College Kappa Delta, and was the vice president of Gamma Sigma Epsilon. She is most proud of her work as an undergraduate researcher in Dr. Cafiero’s computational chemistry lab. For the past three years, she has focused on designing inhibitors for the enzyme LpxC. This year, Carolyn will complete her honor’s research thesis. Carolyn is also involved in the Lynx Club, Beta Beta Beta, Delta Epsilon Iota, Rho Lambda, Order of Omega, Omicron Delta Kappa, Mortar Board, and Phi Beta Kappa. Following graduation, Carolyn will move back to Alabama and begin medical school at the University of Alabama School of Medicine.

Sarah Ferguson ‘17 is a double major in Biology and Environmental Science from Lancaster, PA. She is currently involved in behavioral research with Dr. Sarah Boyle focusing on the meerkat, elephant and fishing cat populations located at the Memphis Zoo. Previously, Sarah interned at the Lancaster County Central Park at their environmental education center where she helped with programs, including nature walks and stream studies. Sarah plans to attend graduate school in conservation biology. On Rhodes’ campus, Sarah is a peer advocate and a member of the Beta Beta Beta honor society.

Tyler Harvey ‘17 is a senior Urban and Community Health major from Greenville, South Carolina. While at Rhodes, he has conducted research at St. Jude Children's Research Hospital and in the Biology Department. After graduation, he hopes to earn degrees in medicine and public health, with the goals of working internationally to eliminate health disparities in the future.

Rachel Bassett ’18 is a Biology major with a minor in Chemistry from West Palm Beach, FL. On campus, she is a Peer Academic Coach and a Rhodes College Diplomat. Rachel is a member of Chi Omega and is the Philanthropy Chair for the chapter. Rachel is also a member of the Beta Beta Beta Biology Honor society and Gamma Sigma Epsilon Chemistry Honor society. Rachel conducts research in the Department of Chemical Biology and Therapeutics at St. Jude Children's Research Hospital, studying how natural products can be used to treat resistant acute lymphoblastic leukemia. Following Rhodes, she would like to attend Pharmacy School at University of Tennessee Health Science Center."

Christopher Parish '18 is from Largo, FL, where he graduated from Indian Rocks Christian School. He is a junior Biology major with a minor in Chemistry. His current areas of research include biological phase separation in the nucleolus at St. Jude Children's Research Hospital in the Kriwacki Lab in the Structural Biology department in conjunction with the Rhodes-St. Jude Summer Plus Program and fungal cell wall synthesis interactions in the Jackson-Hayes lab at Rhodes College. He also works for the Intramurals Sports department and is involved in Reformed University Fellowship. He plans to attend pharmacy school

Walker Lee '19 is a sophomore Pre-Med student majoring in Biology and triple minoring in Chemistry, Urban and Community Health, and Religious Studies. He is a Biology Department Teaching Assistant and Animal Care Technician who is involved in behavioral research with Dr. Boyle at the Memphis Zoo. He is also a PA Leader, a Biology Department tour guide, a volunteer for the Humane Society of Memphis and Shelby County, the Event Chair for the A.S.I.A. Club, and an active member of Sigma Nu. His hobbies include TV/movies, gaming, reading, tennis, and spending time with his dogs.

Avian excreta allows for transmission of cryptococcosis

Spencer Regelson

Cryptococcosis is an infection of animals and humans caused by the Cryptococcus neoformans fungi. It is typically spread by spores in the air and infects the lungs before disseminating throughout the body. Once the infection reaches the central nervous system (CNS), it causes cryptococcal meningitis, inflammation of the membranes surrounding the spinal cord and brain known as meninges. While it can infect immunocompetent individuals, immunocompromised individuals, such as AIDS patients, are at particular risk of contracting the disease. C. neoformans can typically be isolated from bird excreta (Tay et al. 2005), which suggests that birds might be a susceptible host for its transmission. Therefore, areas with high bird concentrations can be dangerous for HIV patients (Wegener and Staib 1983).

INTRODUCTION

In addition to being associated with symptoms aforementioned, PTSD is also associated with high levels of cortisol in nearly all has found (Lucassen et al. 2006). While there is no verified single cause behind PTSD, one diagnostic tool which has surfaced in the search for PTSD's origin is neuroimaging. Neuroimaging of affective disorders usually centers on ventricular enlargement and smaller volumes in both the left and right hippocampus (Shin et al. 2006), with the strongest findings revolving around hippocampal size. Much of the research regarding the relationship between hippocampal volume and affective disorders directs its attention towards PTSD and disorders that often arise from chronic stress (Shin et al. 2006). Within this body of research, there have been several substantive links found between PTSD and stress hormones, namely glucocorticoids. It has been demonstrated in previous studies that higher glucocorticoid levels associated with stress have resulted in damage to hippocampal neurons or in extreme cases cell apoptosis (Gilbertson et al. 2002). This often results in much smaller hippocampal volume (Lucassen et al. 2001). Additionally, exposure to early stress has been shown to modulate the individual to display enhanced stress responsiveness. It has also been demonstrated that stress affects important developmental processes, including neurogenesis, myelination, and synaptic overproduction (Gilbertson et al. 2002).

Because of the complexity of many of these systems, the relationship between PTSD and the hippocampus has often been examined in specific increments. As a result, the study of this relationship has splintered into three main approaches of research: The first approach has and found differences in hippocampal volume between individuals suffering from PTSD and those not suffering from the disorder. In this subsection of study, experiments have focused on the negative correlation between hippocampal volume and the incidence or severity of PTSD (Brenmer et al. 1999; DeBellis et al. 2001). A second approach to studying PTSD has performed longitudinal studies to assess the long-term effects of chronic stress on hippocampal volume (Lucassen et al. 2006; Gurevich et al. 1990; Weiner et al. 1997). In this subsection of study, experiments have centered on the relationship between glucocorticoids and hippocampal atrophy, discovering that there is a positive correlation between chronic overabundance of glucocorticoids and hippocampal atrophy. The third approach has revolved around recurrence of PTSD and associated hippocampal volume of subjects. While this is the most recent area of study, it has been shown by experiments in this subsection of study that there is a higher trend of incidence of PTSD following recurring periods of chronic stress or exposure to trauma (Yehuda et al. 2006; Yehuda et al. 1993; Bremner et al. 1997).

The majority of this body of work holds a few key pieces of information. First, that there is a significant difference in hippocampal volume between groups of individuals with PTSD and groups of individuals without PTSD. Second, that there is an overwhelming trend towards a serious and sustained reduction in hippocampal volume of individuals suffering from PTSD (Lucassen et al. 2006). Finally, that the rate of recurrence for individuals previously diagnosed with PTSD increases dramatically as hippocampal volume decreases among subjects (Shin et al. 2006). As a result, many questions as to the directionality of the causality between these two variables have been left unanswered.

The question I pose is this: Given all we now know, why is PTSD associated with decreased hippocampal volume? In response, I posit that the relationship between PTSD and the hippocampus is both cyclic and bidirectional. In this relationship, hippocampal volume affects the predisposition of the subject to PTSD if that individual is exposed to severe and chronic stress or trauma. Following the initial incidence of PTSD, diagnosed or not, the subject becomes vulnerable to the destruction of hippocampal cells via atrophy. When this has sufficiently decreased the volume of the subject's

hippocampus, hippocampal volume directly contributes to a greater predisposition within the subjects of experiencing recurring PTSD. Therefore, PTSD is possibly related to hippocampal volume via a positive feedback loop comprising the above facets. In the following survey of literature surrounding this relationship, I will explore what is known about this subject and provide a simple model for the effect of PTSD on hippocampal volume.

HIPPOCAMPAL VOLUME AS A BIOLOGICAL INDICATOR OF VULNERABILITY TO PTSD

PTSD is unique in terms of many mental disorders in that it involves several feedback loops which can have drastic effects on the length and severity of the disorder. The stochastic nature of PTSD pathology is one of the root causes behind its' relatively late emergence as a recognized disorder (Blake et al., 1995). This stochasticity results from the wide variety of possible origins of the disease. Often, several factors will play into the development of PTSD. One such factor is the hippocampal volume of the individual diagnosed with PTSD (Brenmer et al. 1999; DeBellis et al. 2001). In several studies, hippocampal volume has been correlated with the incidence of PTSD among study subjects (DeBellis et al. 2001; Gilbertson et al. 2002). It has been found that the average hippocampal volume of subjects with exposure to traumatic events and resulting development of PTSD was significantly smaller than the average hippocampal volume of subjects without similar trauma and no incidence of PTSD. While this correlation is certainly strong enough to suggest that there is causation, the direction of this influence could flow from either way. In order to test the directionality of this phenomenon, several studies have performed measurements of hippocampal volume of subjects prior to chronic stress or trauma and again in the midst of the stressor. These experiments set out to prove that small hippocampal volume is a factor in the predisposition of individuals to initial incidence of PTSD.

One method of study which has been popular in research surrounding abnormal psychology, specifically PTSD, has been twin-studies. While the validity of this sort of study has been questioned at times, it remains a powerful tool in reducing confounding variables which might differentiate study subjects (Gilbertson et al. 2002). In one such study, researchers compared sets of identical twins to determine if there was evidence of smaller hippocampi indeed constitute a risk factor for the development of stress-related psychopathology (Gilbertson et al. 2002). One twin in each of the sets of twins had been exposed to high-severity combat during the Vietnam War (Gilbertson et al. 2002). The researchers found that severity of the disorder in subjects who had been diagnosed with PTSD was negatively correlated with the hippocampal volume of those patients (Gilbertson et al. 2002). Further, the researchers found that the twins of the combat veterans diagnosed with PTSD who had not been in combat showed hippocampal volumes comparable to their combat-exposed brothers (Gilbertson et al. 2002).

Additionally, their hippocampal volumes were significantly smaller than both the hippocampal volumes of the combat veterans without PTSD and their twins, who were unexposed to combat (Gilbertson et al. 2002). To control for brain volume differences between the two twins which might have come about following the exposure to chronic stress, the experimenters took measurements of either twin's total brain volume and volume of their amygdala (Gilbertson et al. 2002). This experiment was set up to demonstrate whether the resulting correlation of effects of stress-induced neurotoxicity (Gilbertson et al. 2002). The results strongly suggest that hippocampal volume was a precursor to PTSD in these individuals and is a veritable factor in the predisposition of the subjects to PTSD.

Another study that examined the effects on the hippocampus under stressful condition was performed by a think-tank for the Israeli military (Admon et al. 2009). Though this study does not directly reflect hippocampal volume, such studies are rare and it is worth nothing that the relationship between hippocampal plasticity and hippocampal volume has been researched extensively (McEwen 1998; McEwen 2001). In this research, the relationship has been found to have a strong positive correlation. In addition, it has been found that hippocampal plasticity decreases dramatically when hippocampal atrophy occurs (McEwen 2001). This suggests that the use of hippocampal plasticity as a surrogate for hippocampal size in the following study warrants full credibility.

In the study, 50 healthy recruits to the Israeli Defense Forces were examined before they entered the compulsory service within their respective units and again after they had experienced chronic stress within their combat units (Admon et al. 2009). In both examinations, the subjects were given an assay of questions and their brain responses were measured with an fMRI. The examinations took place at 2 time points, during their first week training and, again, 18 months later (Admon et al. 2009). At the time of their second examination, they were serving in combat units as paramedics, arguably one of the most stressful positions within a combat unit. In both of the examinations, they were asked questions about

their emotional experiences and their brain responses were measured in response to a plethora of stimuli (Admon et al. 2009). Over the duration of the 18 months, soldiers reported to the experimenters that there was a dramatic increase in symptoms that are clinically associated with stress (Admon et al. 2009). Further, there was a dramatic reduction in hippocampal plasticity as defined by the study, suggesting that there was serious damage to hippocampal size and efficacy (Admon et al. 2009).

INCIDENCE OF HIPPOCAMPAL ATROPHY DURING EXPOSURE TO CHRONIC STRESS

The story, however, does not end with predisposition in the relationship between hippocampal volume and PTSD. To fully understand the bi-directional flow of influence between these two factors, we must understand one of the most complex relationships within the endocrine system: the hypothalamic-pituitary-adrenal (HPA) axis. The terminal point in this axis is the adrenal gland, which secretes cortisol, a glucocorticoid common in humans (Lucassen et al. 2006). The system is necessary for homeostasis and yet, can have drastically damaging effects when unregulated (Lucassen et al. 2006). The axis is extremely sensitive to changes in the environment of animals and humans, even if those changes are routine. These changes often require the organism to modify itself in an attempt to adapt to the environment (Lucassen et al. 2006). This modification is called stress. Stress is broken down into two categories: chronic stress, which occurs over extended periods of time, and acute stress, which occurs in short spurts and specific time points (Lucassen et al. 2006). Either type of stress triggers the release of large numbers of glucocorticoids, acting on specific brain regions. It has been demonstrated that one such brain region, the hippocampus, is particularly sensitive to changes in the excretion of cortisol (Czeh and Lucassen 2007). In an attempt to measure the effects of this sensitivity, researchers have experimented extensively with apoptosis, or programmed cell death. Using the findings of these experiments, I will demonstrate that elevated levels of glucocorticoids decrease hippocampal size in individuals diagnosed with PTSD.

Apoptosis induced by glucocorticoid is initiated by the interaction of the glucocorticoid with its receptor. In fact, the amount of apoptosis which occurs within a given area of the brain is strictly dependent upon this factor. The level of glucocorticoid receptor expression is a critical determinant not only for cell apoptosis but generally for glucocorticoid sensitivity (McEwen 2001). When glucocorticoids are overabundant, they disrupt the negative feedback loop which regulates the release of cortisol. They accomplish this by no longer inhibiting the release of corticotrophin-releasing hormone (CRH) (McEwen 2001). CRH is a hormone which works along the HPA axis whose purpose is to allow cortisol to be released in response to stress or trauma (McEwen 2001). Because glucocorticoids disrupt the inhibitory mechanisms, the concentration of cortisol becomes even greater, further contributing to its release. This finding has been replicated numerous times (Lucassen et al. 2006; Gurevich et al. 1990; Weiner et al. 1997), most notably in experiments conducted with veterans (Seckl and Meaney 2006).

In one experiment involving transgenic mice, sufficient glucocorticoid receptor levels were necessarily maintained for a significant amount of time in order to induce cell death by glucocorticoids (Reichardt et al. 1998). This finding suggest that while single-incidents of acute stress are not sufficient for cell apoptosis, recurrent incidents of acute stress or chronic stress are certainly sufficient to induce cell apoptosis. This finding further reinforces the general understanding of glucocorticoids: as essential for survival though dramatically maladaptive when prolonged (Reichardt et al. 1998). While several studies have examined hippocampal atrophy and found strikingly similar results, the data are mixed as to where specifically this atrophy occurs. Much of this occurs because different experiments perform different sampling techniques for the hippocampus and, as such, do not all look at identical regions. Some research suggests that the dentate gyrus is the seat of this atrophy (Lucassen et al. 2006; Swaab et al. 2005), while some suggest that overabundance of glucocorticoids simply suppresses neurogenesis (McEwen 2001), and still others suggest that the atrophy is selectively in the CA1 (Sapolsky 2000; Sicotte et al. 2008) or CA3 pyramidal neurons (Watanabe et al. 1992; McEwen, 2001). In the latter of the findings, the atrophy occurred primarily in the apical dendrites and not basal dendrites (Watanabe et al. 1992). This finding raises questions into the cause behind this selective atrophy, though to date they have been left unanswered.

However, there have also been studies which have suggested that hippocampal volume is not correlated with PTSD. In one such study, 37 individuals who had been exposed to severe trauma underwent extensive psychometric testing, structured clinical interviews, and MRI scans to measure the volume of several areas, notably the hippocampus (Bonne et al. 2001). These tests were administered at 1 week following the trauma and again at 6 months following the trauma, at which point PTSD was clinically diagnosed in 10 of the subjects (Bonne et

al. 2001). The subjects with clinically diagnosed PTSD and those without PTSD did not have significant differences in either left or right hippocampal volume. This finding was consistent at both 1 week and 6 months (Bonne et al. 2001). Additionally, there was no reduction in volume among the subjects diagnosed with PTSD between their initial scan at 1 week and at 6 months (Bonne et al. 2001).

In studies such as this, a different hypothesis for hippocampal atrophy has been suggested. This hypothesis is that the atrophy occurs primarily from the overabundance of a specific type of glucocorticoid receptors, not the overabundance of glucocorticoids themselves. A notable point about this study, however, is that all 37 subjects were taken from a sample of individuals who underwent acute stress (Bonne et al. 2001). This is a common theme in research which purports to have found no veritable hippocampal atrophy in PTSD subjects (Bonne et al. 2001; Katz et al. 1981; Bryant et al. 1998). As noted above, acute stress is dramatically different form chronic stress, primarily because the overabundance of glucocorticoids must be in effect for a long duration of time in order to have the consequences described above (Lucassen et al. 2006).

PRIOR HIPPOCAMPAL ATROPHY AS A BIOLOGICAL VULNERABILITY IN RECURRING PTSD

It has already been demonstrated that the initial incidence of PTSD is strongly correlated with hippocampal volume. Additionally, it has been demonstrated that when individuals undergo severe and chronic stress, cell apoptosis occurs. This reduction in hippocampal volume creates a set of circumstances which are strikingly similar to those which produced the initial incidence of PTSD or PTSD-like symptoms. Therefore, it only follows that absent a wave of neurogenesis in patients with PTSD the disorder would become cyclic. In short, decreased hippocampal volume in subjects previously exposed to severe trauma leaves those subjects more susceptible to incidence of PTSD. To demonstrate this, I will focus primarily on experiments studying the effect of recurrent cycles of chronic stress on the development and severity of PTSD. While some of the following studies do not directly examine hippocampal size, the previous assertions within this paper, that small hippocampal volume is a factor in the predisposition of individuals to initial incidence of PTSD and that elevated levels of glucocorticoids decrease hippocampal size in individuals diagnosed with PTSD, have sufficiently demonstrated this point.

In one such study, pre-military trauma was correlated with adult psychopathology in Vietnam veterans. Researchers compared 38 veterans with PTSD and 28 veterans without PTSD with respect to the amount of childhood trauma or sexual abuse and general pre-military trauma (Yehuda et al. 1993). Vietnam veterans in the study who were diagnosed with PTSD had a higher rate of pre-military or childhood abuse or trauma than did those without PTSD at more than 3 times the rate (Yehuda et al., 1993). Even after controlling for the difference in severity of combat between the two groups of veterans, it was determined that the study subjects with PTSD had a significantly higher rate of all pre-military traumatic events than the group without PTSD (Yehuda et al. 1993). These findings explicitly correlate pre-military stress and trauma with development of PTSD following chronic stress later in life. Further, these findings strongly suggest that the proven reduction in hippocampal volume following chronic stressors like childhood abuse reduces the subjects' allostatic load, resulting in increased susceptibility to PTSD.

In yet another longitudinal study, which compared the symptoms of holocaust survivors with and without post-traumatic stress disorder (PTSD), the effects of recurring stress were analyzed. In this study, symptoms and other behavioral tendencies were measured at two 10 year intervals for both the control and experimental groups. The subjects consisted of 40 holocaust survivors (Yehuda et al. 2007). There was a general reduction in the symptoms of PTSD over time in both groups, though this was not striking. What was interesting, however, is that in 10% of the subjects, new instances of PTSD were seen to develop between the two intervals (Yehuda et al. 2007). The assessments showed that there was a general trend towards increase in trauma-related symptoms over the time between the two intervals in persons who were not previously diagnosed with PTSD (Yehuda et al. 2007). This suggests that PTSD cannot be readily analyzed from a single diagnostic status at a single time, but rather has the ability to wax and wane in the face of factors such as neurogenesis and increase or reduction in allostatic load (Yehuda et al. 2007).

Finally, there is a study which brings together several facets of this paper into a single demonstration. In this study, researchers compared adult survivors of childhood abuse, previously diagnosed with PTSD, to matched controls with respect to hippocampal volume (Brenmer et al. 1997). In order to measure the volume of each subject's hippocampus, MRI was used. All subjects were adults aged 18-56 and of the 34 samples 17 were survivors of childhood abuse and 17 remaining subjects were combat-exposed but healthy (Brenmer et al. 1997). The control and experimental groups were with respect to age, sex,

race, which of their hands was dominant, the size of their bodies and height of their bodies, the number of years which they had abused alcohol, and the length of their formal education (Brenmer et al. 1997). All of the subjects in the study met the criteria for clinical PTSD diagnosis from events secondary to childhood abuse (Brenmer et al. 1997). The findings of the neuroimaging were that the subjects diagnosed with PTSD, compared to the control subjects, had a 12% smaller hippocampus, though either group had roughly comparable volumes of the temporal lobe, amygdala, and caudate (Brenmer et al. 1997). These findings strongly suggest that the recurrent cycles of chronic stress predispose individuals to the development of PTSD.

CONCLUSIONS

In conclusion, it seems evident from the above studies that hippocampal volume is neither simply the cause nor the effect of chronic stress. Rather, it seems that the relationship between hippocampal volume and PTSD is one of bidirectional causality. Where many studies have concluded that there is a decrease in hippocampal volume stemming from chronic stress or trauma, they are correct. However, where many separate studies have concluded that decreased hippocampal volume results in an adverse reaction to chronic stress or trauma, they are equally correct.

Because the hippocampal volume clearly regulates the magnitude of the predisposition within the subject to PTSD, the directionality is resounding from hippocampal volume to PTSD. Since the mechanisms which produce and sustain PTSD contribute to destruction of hippocampal cells, thereby decreasing volume, directionality of causation is equally obvious as moving from incidence of PTSD to hippocampal volume. Given the above evidence I can resolutely say that the relationship between hippocampal volume and PTSD is bi-directional. Each mechanism acts on the other to create a positive feedback loop which has can easily spiral out of control. Further, because each system has no obvious method of self-regulation whereby one of these systems ceases to contribute to the other, the feedback has a deadly potential within the subject.

This finding is consistent with previous studies regarding cortisol and its relationship with several other neurotransmitters to convey specific messages. One such neurotransmitter is epinephrine, which combines its efforts with cortisol in order to create memories which are outstanding in some way, usually which deviate strongly from the norm (Talarico and Rubin 2003). These memories called to as flash-bulb memories and help us to avoid items which pose a serious danger in the future (Talarico and Rubin 2003). From this perspective, a smaller hippocampus can be a profound biological vulnerability. The veterans studied, while in combat, have requirements to survive. One such requirement is a faster and more accurate memory recall. Because this feedback is dependent on the ability of the hippocampus to function seamlessly, atrophy in this region is detrimental to the soldier's ability. Further, a reduction in volume in the hippocampus can readily cause the soldier to cease to inhibit irrelevant information, instead choosing to incorporate it into perception. The impending overload from this sequence of events is obvious and one can easily see how this could cause a state of panic in the soldier.

While it is wise to be hesitant about claims that seem to have overarching and closed explanations regarding any given phenomenon, I am quick to note that the above evidence is likely only part of the explanation for the incidence of PTSD. Surely, given the wide variety of disorders which deal with allostatic load and resulting endocrine malfunction in the brain there are numerous factors which contribute to the development many of the symptoms which we have ascribed to our understanding of PTSD. Over the past several years, numerous researchers have begun to think of stress-related disorders as a multi-faceted spectrum, similar to autism or other behavioral abnormalities.

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Activation energies for the sulfation of Ligands in sulfotranserfase enzyme 1A1

Danielle Wilson, Larryn Peterson, Mauricio Cafiero

*Here, we studied the substrate selectivity of the sulfotransferase enzyme (SULT1A1) by the kinetics of proton abstraction in the active site. The sulfotransferase enzymes (SULTs) catalyze the addition of a sulfuryl group to a variety of small molecules, including neurotransmitters and xenobiotics. This reaction can activate, deactivate, or change their pharmacokinetic behavior of bio-active molecules. A set of dopamine analogs with substituents in the 6-position were chosen for study. The addition of the sulfuryl group to the ligand depends on deprotonation of a phenol group on the ligand. Thus, the activation energies for proton abstraction from the ligand to the histidine residue were calculated for all ligands using the computational model chemistry M062x/6-31++g**. Our results show a strong dependence of the activation energy of the proton transfer on the substituent in the 6-position.*

INTRODUCTION

Sulfotransferase 1A1 (SULT1A1) is an enzyme that is part of a larger enzyme family, Sulfotransferase (SULT) enzymes, which are important in the regulation of neurotransmitters and hormones and in the excretion of xenobiotics (Gamage et al. 2006). Although SULT enzymes have similar sequence, structure, and function, each has a unique selectivity and is expressed in different parts of the body (Gamage et al. 2006). SULT enzymes are divided into two families by their location in the cell. Members of the first family are located in the membrane of the Golgi apparatus and typically have peptides, proteins, and lipids as their substrates. The second class is made are cystolic and have steroids and neurotransmitters as their substrate (Gamage et al. 2006). The enzyme of focus in this paper, SULT1A1, belongs to the second family, and is mainly located in the liver but also appears in other areas of the body (Gamage et al. 2006). This family of enzymes catalyzes the sulfonation of hydroxyl and monoamine groups from 3'-Phosphoadenosine-5'-phosphosulfate (PAPS) (Gamage et al. 2006). The addition of the sulfuryl moiety is important for elimination of molecules as it increases the solubility of the molecule and decreases activity (Gamage et al. 2006). Thus, the SULT enzyme family is important in the detoxification of drugs, ultimately leading to their elimination through the body (Gamage et al. 2006). The enzymes accomplish this by the addition of a sulfuryl group ($-SO_3$) once a proton has been abstracted from a hydroxyl group on the substrate.

In our studies, we used derivatives of dopamine as the substrate for SULT1A1 in order to understand the substrate selectivity. Understanding the selectivity of SULT1A1 is helpful to drug design, as the enzyme's activity results in excretion of the drug. Therefore, drugs to which SULT1A1 has a higher affinity towards will be excreted from the body more readily. The dopaminergic derivatives used were 6-hydroxydopamine, 1,2,3,4-tetrahydroisoquinoline-6,7-diol, 6-nitrodopamine, 6-cyanodopamine, 6-ethenyldopamine, 3,4-dihydroxybenzonitrile, 2-(3,4-dihydroxyphenyl) acetonitrile. These derivatives, shown in **Figure 1**, were chosen because of their similarity to xeno and endobiotics that are sulfated by SULT1A1 and because of their varying range of polarization across the ring.

Dopamine has two hydroxyl groups located on the ring, position 3 and position 4. Therefore, SULT1A1 can deprotonate the hydroxyl group and attach the sulfuryl moiety in two positions. Within the active site, Histidine108 abstracts the proton from the substrate in either position 3 or position 4. This process can be seen in **Figure 2**.

The focus of this paper is the study of the substrate selectivity of SULT1A1 based upon the deprotonation of the substrate between position 3 and position 4. In order to study this, we will determine the transition state energy of the deprotonation of the substrate by SULT1A1 in both the 3 and the 4 position using dopamine and the series of dopaminergic derivatives. We believe that there will be a significant difference in transition state energy between the ligand deprotonated in the 3 and 4 position. This difference would be evidence of a higher percentage of sulfonated ligand in a particular position.

COMPUTATIONAL DETAILS

We performed a surface scan in order to locate the transition state energy of the proton transfer from the ligand to His108 by moving the proton from the ligand to the histidine a total distance of 1.3 angstroms in 0.1 angstroms increments and finding the energy at each step. The energy of the reactants (the start of the curve), subtracted from the energy at the top of the resulting curve (see **Figure 3**) is the activation energy of the reaction. We completed this scan using M062X/6-31++g** (Zhao and Truhlar 2006) in implicit solvent (Tomasi 2005),

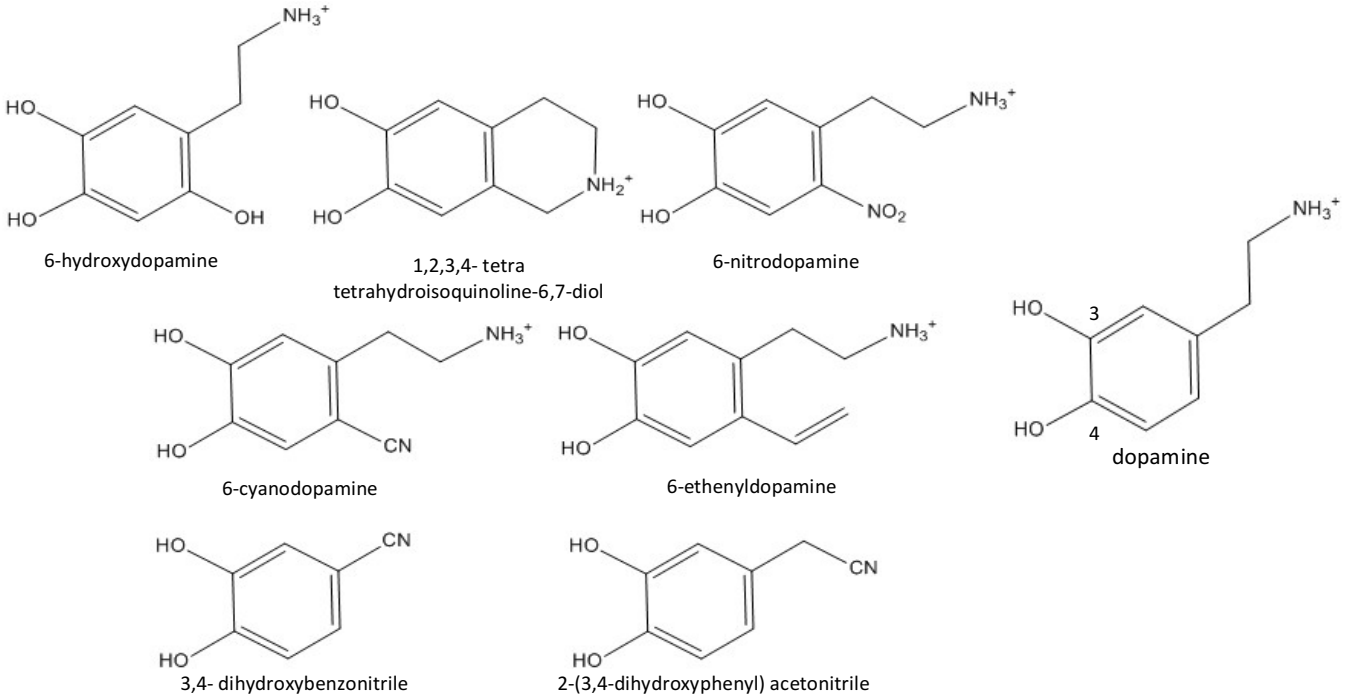


Figure 1. Dopaminergic derivatives and dopamine. This image shows the dopaminergic derivatives and dopamine used in our studies.

meaning that an electric field was set up around the molecule to mimic the effect of water. We obtained the original structure of the histidine complex with ligands from the crystal structure (Lu et. al 2005). M062X was used because it is computationally efficient and provides accurate values for energies of non-bonded molecules. We used Gaussian 09 in order to view and edit the histidine complex (Frisch et al. 2009).

RESULTS

We calculated the transition state energy of deprotonation from the 4 position on dopamine and a total of seven dopaminergic derivatives.. The values of the transition state energies can be seen in **Table 1.** All of the transition state energies were located at the proton 2.06719 angstroms away from the ligand, except 3,4- dihydroxybenzonitrile, which was 1.96719 angstroms from the ligand.
Additional calculations with the deprotonation by His108 in the 3 position were obtained using 6-hydroxydopamine and 6-nitrodopamine. These calculations will be used for the comparison between transition state energy of ligand deprotonation in the 3 position and the in the 4 position. These values can be seen in **Figure 3**.

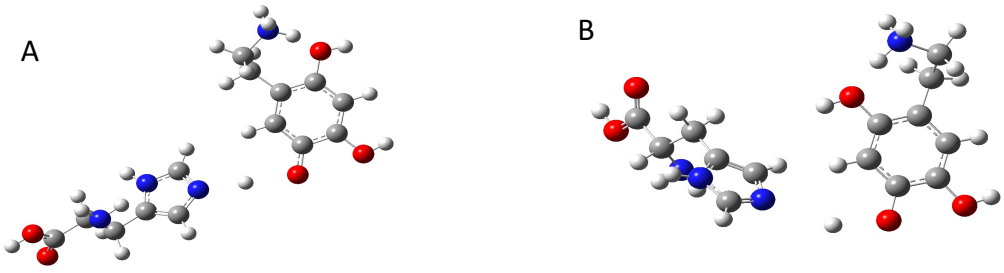


Figure 2: Transition state energy of deprotonation of 6-hydroxydopamine. A shows the position of the transition state of deprotonation the ligand from position 3. B shows the position of the transition state of deprotonation of the ligand from position 4

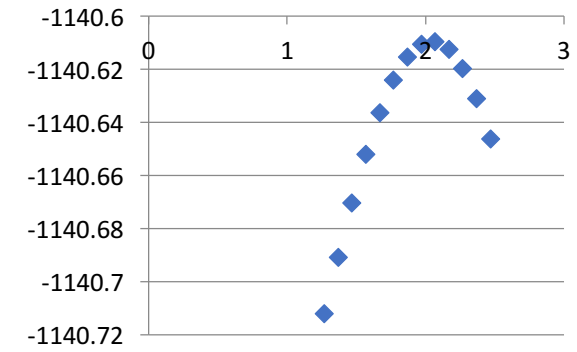
Ligand	Transition State Energy kcal/mol
Dopamine	77.2
6-hydroxydopamine	64.3
1,2,3,4-tetrahydroisoquinoline-6,7-diol	77.0
6-nitrodopamine	74.3
6-cyanodopamine	70.0
6-ethenyldopamine	78.1
3,4-dihydroxybenzonitrile	72.9
2-(3,4-dihydroxyphenyl) acetonitrile	62.9

Table 1. Transition State Energy of proton transfer in position 4 of ligands. The calculations were obtained from a surface scan to determine the highest energetic point of the proton transfer.

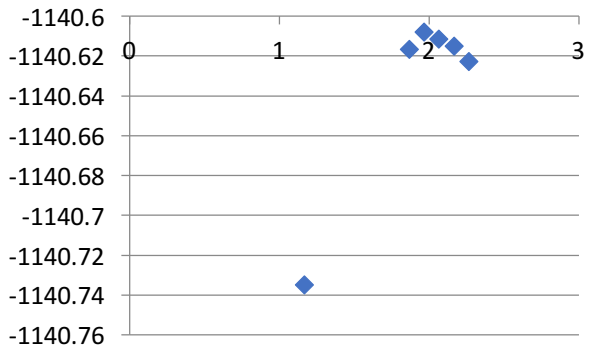
CONCLUSIONS

In comparing the transition state energy of proton transfer in position 4 on dopamine and the seven dopaminergic derivatives, we found that the lowest transition state energy was in 2-(3,4-dihydroxyphenyl) acetonitrile and the highest was in 6-ethenyldopamine. Dopamine had the second highest transition state energy of deprotonation from the 4 position. Based on this data, we predict that SULT1A1 has the highest affinity for 2-(3,4-dihydroxyphenyl) acetonitrile. We found that the ligands that were the least electron withdrawing had the highest activation energies. This is because electron withdrawing substituents weaken the oxygen and hydrogen bond on the hydroxyl groups, making deprotonation easier.

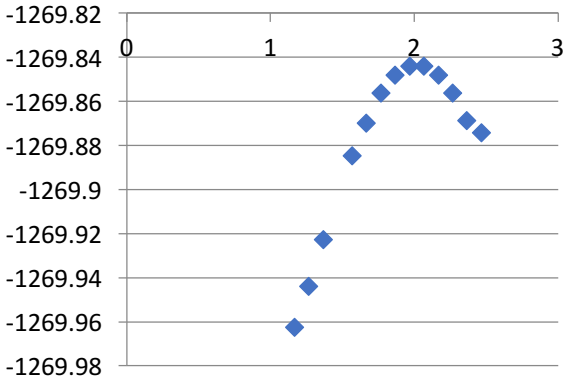
In comparing the transition state energy of proton transfer between position 3 and position 4 in 6-hydroxydopamine and 6-nitrodopamine, we determined the lowest energy to be in 6-nitrodopamine deprotonated in position 3. However, the transition state energy for deprotonation in 6-hydroxydopamine was lower when deprotonated in position 4. Therefore, we are unable to conclude whether deprotonation in either the 3 or the 4 position results in a smaller transition state energy. Further research will be completed in order to determine this trend.



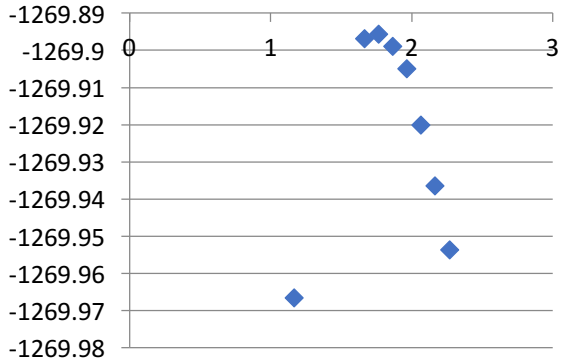
A. 6-hydroxydopamine with proton transfer from position 4. Transition state energy is 64.3 kcal/mol.



B. 6-hydroxydopamine with proton transfer from position 3. Transition state energy is 79.8 kcal/mol.



C. 6-nitrodopamine with proton transfer from position 4. Transition state energy is 77.0 kcal/mol.



D. 6-nitrodopamine with proton transfer from position 3. Transition state energy is 44.5 kcal/mol.

Figure 3. Transition state energies for proton transfer from position 3 and 4 in 6-hydroxydopamine and 6-nitrodopamine. Graphs B and D do not include all of the points as graphs A and C as the transition state was found to occur when the proton was 2.06719 angstroms from the ligand. Therefore, we only calculated the energies ranging from 1.86719 to 2.26719 angstroms from the ligand.

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Hippocampal volume: reduction in post-traumatic stress disorder

Andrew Williams

Post-Traumatic Stress Disorder (PTSD) is an affective psychiatric disorder characterized by paranoia, recurrent memories of the trauma, isolation, and avoidance of emotional trigger, in which nearly all patients have higher-than-average mortality at earlier ages than non-diagnosed counterparts (Lucassen et al, 2006). In the study of causes behind PTSD, hippocampal size has become every important in understanding the development of the disorder. The body of study regarding this relationship holds a few key pieces of information. First, there is a significant difference in hippocampal volume between groups of individuals with PTSD and groups of individuals without PTSD. Second, there is an overwhelming trend towards a continued reduction in hippocampal volume of individuals suffering from PTSD following initial development of the disorder (Lucassen et al, 2006). Finally, the rate of recurrence for individuals previously diagnosed with PTSD increases dramatically as hippocampal volume decreases among subjects (Shin et al, 2006). Each element of this relationship between these three bodies of research has not been studied to the extent of determining specific mechanisms for why the given correlations exist. However, I assert that the relationship between PTSD and the hippocampus is the following: Hippocampal volume affects the predisposition of a subject to PTSD which causes a decreased volume of the hippocampus and contributes to the incidence of cyclic relapses for the subject. This model will help us to develop more advanced and effective means of preventing the disorder, managing the disorder, and rehabilitating individuals with PTSD.

INTRODUCTION

In addition to being associated with symptoms aforementioned, PTSD is also associated with high levels of cortisol in nearly all has found (Lucassen et al. 2006). While there is no verified single cause behind PTSD, one diagnostic tool which has surfaced in the search for PTSD's origin is neuroimaging. Neuroimaging of affective disorders usually centers on ventricular enlargement and smaller volumes in both the left and right hippocampus (Shin et al. 2006), with the strongest findings revolving around hippocampal size. Much of the research regarding the relationship between hippocampal volume and affective disorders directs its attention towards PTSD and disorders that often arise from chronic stress (Shin et al. 2006). Within this body of research, there have been several substantive links found between PTSD and stress hormones, namely glucocorticoids. It has been demonstrated in previous studies that higher glucocorticoid levels associated with stress have resulted in damage to hippocampal neurons or in extreme cases cell apoptosis (Gilbertson et al. 2002). This often results in much smaller hippocampal volume (Lucassen et al. 2001). Additionally, exposure to early stress has been shown to modulate the individual to display enhanced stress responsiveness. It has also been demonstrated that stress affects important developmental processes, including neurogenesis, myelination, and synaptic overproduction (Gilbertson et al. 2002).

Because of the complexity of many of these systems, the relationship between PTSD and the hippocampus has often been examined in specific increments. As a result, the study of this relationship has splintered into three main approaches of research: The first approach has and found differences in hippocampal volume between individuals suffering from PTSD and those not suffering from the disorder. In this subsection of study, experiments have focused on the negative correlation between hippocampal volume and the incidence or severity of PTSD (Brenmer et al. 1999; DeBellis et al. 2001). A second approach to studying PTSD has performed longitudinal studies to assess the long-term effects of chronic stress on hippocampal volume (Lucassen et al. 2006; Gurevich et al. 1990; Weiner et al. 1997). In this subsection of study, experiments have centered on the relationship between glucocorticoids and hippocampal atrophy, discovering that there is a positive correlation between chronic overabundance of glucocorticoids and hippocampal atrophy. The third approach has revolved around recurrence of PTSD and associated hippocampal volume of subjects. While this is the most recent area of study, it has been shown by experiments in this subsection of study that there is a higher trend of incidence of PTSD following recurring periods of chronic stress or exposure to trauma (Yehuda et al. 2006; Yehuda et al. 1993; Bremner et al. 1997).

The majority of this body of work holds a few key pieces of information. First, that there is a significant difference in hippocampal volume between groups of individuals with PTSD and groups of individuals without PTSD. Second, that there is an overwhelming trend towards a serious and sustained reduction in hippocampal volume of individuals suffering from PTSD (Lucassen et al. 2006). Finally, that the rate of recurrence for individuals previously diagnosed with PTSD increases dramatically as hippocampal volume decreases among subjects (Shin et al. 2006). As a result, many questions as to the

directionality of the causality between these two variables have been left unanswered.

The question I pose is this: Given all we now know, why is PTSD associated with decreased hippocampal volume? In response, I posit that the relationship between PTSD and the hippocampus is both cyclic and bidirectional. In this relationship, hippocampal volume affects the predisposition of the subject to PTSD if that individual is exposed to severe and chronic stress or trauma. Following the initial incidence of PTSD, diagnosed or not, the subject becomes vulnerable to the destruction of hippocampal cells via atrophy. When this has sufficiently decreased the volume of the subject's hippocampus, hippocampal volume directly contributes to a greater predisposition within the subjects of experiencing recurring PTSD. Therefore, PTSD is possibly related to hippocampal volume via a positive feedback loop comprising the above facets. In the following survey of literature surrounding this relationship, I will explore what is known about this subject and provide a simple model for the effect of PTSD on hippocampal volume.

HIPPOCAMPAL VOLUME AS A BIOLOGICAL INDICATOR OF VULNERABILITY TO PTSD

PTSD is unique in terms of many mental disorders in that it involves several feedback loops which can have drastic effects on the length and severity of the disorder. The stochastic nature of PTSD pathology is one of the root causes behind its' relatively late emergence as a recognized disorder (Blake et al., 1995). This stochasticity results from the wide variety of possible origins of the disease. Often, several factors will play into the development of PTSD. One such factor is the hippocampal volume of the individual diagnosed with PTSD (Brenmer et al. 1999; DeBellis et al. 2001). In several studies, hippocampal volume has been correlated with the incidence of PTSD among study subjects (DeBellis et al. 2001; Gilbertson et al. 2002). It has been found that the average hippocampal volume of subjects with exposure to traumatic events and resulting development of PTSD was significantly smaller than the average hippocampal volume of subjects without similar trauma and no incidence of PTSD. While this correlation is certainly strong enough to suggest that there is causation, the direction of this influence could flow from either way. In order to test the directionality of this phenomenon, several studies have performed measurements of hippocampal volume of subjects prior to chronic stress or trauma and again in the midst of the stressor. These experiments set out to prove that small hippocampal volume is a factor in the predisposition of individuals to initial incidence of PTSD.

One method of study which has been popular in research surrounding abnormal psychology, specifically PTSD, has been twin-studies. While the validity of this sort of study has been questioned at times, it remains a powerful tool in reducing confounding variables which might differentiate study subjects (Gilbertson et al. 2002). In one such study, researchers compared sets of identical twins to determine if there was evidence of smaller hippocampi indeed constitute a risk factor for the development of stress-related psychopathology (Gilbertson et al. 2002). One twin in each of the sets of twins had been exposed to high-severity combat during the Vietnam War (Gilbertson et al. 2002). The researchers found that severity of the disorder in subjects who had been diagnosed with PTSD was negatively correlated with the hippocampal volume of those patients (Gilbertson et al. 2002). Further, the researchers found that the twins of the combat veterans diagnosed with PTSD who had not been in combat showed hippocampal volumes comparable to their combat-exposed brothers (Gilbertson et al. 2002).

Additionally, their hippocampal volumes were significantly smaller than both the hippocampal volumes of the combat veterans without PTSD and their twins, who were unexposed to combat (Gilbertson et al. 2002). To control for brain volume differences between the two twins which might have come about following the exposure to chronic stress, the experimenters took measurements of either twin's total brain volume and volume of their amygdala (Gilbertson et al. 2002). This experiment was set up to demonstrate whether the resulting correlation of effects of stress-induced neurotoxicity (Gilbertson et al. 2002). The results strongly suggest that hippocampal volume was a precursor to PTSD in these individuals and is a veritable factor in the predisposition of the subjects to PTSD.

Another study that examined the effects on the hippocampus under stressful condition was performed by a think-tank for the Israeli military (Admon et al. 2009). Though this study does not directly reflect hippocampal volume, such studies are rare and it is worth nothing that the relationship between hippocampal plasticity and hippocampal volume has been researched extensively (McEwen 1998; McEwen 2001). In this research, the relationship has been found to have a strong positive correlation. In addition, it has been found that hippocampal plasticity decreases dramatically when hippocampal atrophy occurs (McEwen 2001). This suggests that the use of hippocampal plasticity as a

surrogate for hippocampal size in the following study warrants full credibility.

In the study, 50 healthy recruits to the Israeli Defense Forces were examined before they entered the compulsory service within their respective units and again after they had experienced chronic stress within their combat units (Admon et al. 2009). In both examinations, the subjects were given an assay of questions and their brain responses were measured with an fMRI. The examinations took place at 2 time points, during their first week training and, again, 18 months later (Admon et al. 2009). At the time of their second examination, they were serving in combat units as paramedics, arguably one of the most stressful positions within a combat unit. In both of the examinations, they were asked questions about their emotional experiences and their brain responses were measured in response to a plethora of stimuli (Admon et al. 2009). Over the duration of the 18 months, soldiers reported to the experimenters that there was a dramatic increase in symptoms that are clinically associated with stress (Admon et al. 2009). Further, there was a dramatic reduction in hippocampal plasticity as defined by the study, suggesting that there was serious damage to hippocampal size and efficacy (Admon et al. 2009).

INCIDENCE OF HIPPOCAMPAL ATROPHY DURING EXPOSURE TO CHRONIC STRESS

The story, however, does not end with predisposition in the relationship between hippocampal volume and PTSD. To fully understand the bi-directional flow of influence between these two factors, we must understand one of the most complex relationships within the endocrine system: the hypothalamic-pituitary-adrenal (HPA) axis. The terminal point in this axis is the adrenal gland, which secretes cortisol, a glucocorticoid common in humans (Lucassen et al. 2006). The system is necessary for homeostasis and yet, can have drastically damaging effects when unregulated (Lucassen et al. 2006). The axis is extremely sensitive to changes in the environment of animals and humans, even if those changes are routine. These changes often require the organism to modify itself in an attempt to adapt to the environment (Lucassen et al. 2006). This modification is called stress. Stress is broken down into two categories: chronic stress, which occurs over extended periods of time, and acute stress, which occurs in short spurts and specific time points (Lucassen et al. 2006). Either type of stress triggers the release of large numbers of glucocorticoids, acting on specific brain regions. It has been demonstrated that one such brain region, the hippocampus, is particularly sensitive to changes in the excretion of cortisol (Czeh and Lucassen 2007). In an attempt to measure the effects of this sensitivity, researchers have experimented extensively with apoptosis, or programmed cell death. Using the findings of these experiments, I will demonstrate that elevated levels of glucocorticoids decrease hippocampal size in individuals diagnosed with PTSD.

Apoptosis induced by glucocorticoid is initiated by the interaction of the glucocorticoid with its receptor. In fact, the amount of apoptosis which occurs within a given area of the brain is strictly dependent upon this factor. The level of glucocorticoid receptor expression is a critical determinant not only for cell apoptosis but generally for glucocorticoid sensitivity (McEwen 2001). When glucocorticoids are overabundant, they disrupt the negative feedback loop which regulates the release of cortisol. They accomplish this by no longer inhibiting the release of corticotrophin-releasing hormone (CRH) (McEwen 2001). CRH is a hormone which works along the HPA axis whose purpose is to allow cortisol to be released in response to stress or trauma (McEwen 2001). Because glucocorticoids disrupt the inhibitory mechanisms, the concentration of cortisol becomes even greater, further contributing to its release. This finding has been replicated numerous times (Lucassen et al. 2006; Gurevich et al. 1990; Weiner et al. 1997), most notably in experiments conducted with veterans (Seckl and Meaney 2006).

In one experiment involving transgenic mice, sufficient glucocorticoid receptor levels were necessarily maintained for a significant amount of time in order to induce cell death by glucocorticoids (Reichardt et al. 1998). This finding suggest that while single-incidents of acute stress are not sufficient for cell apoptosis, recurrent incidents of acute stress or chronic stress are certainly sufficient to induce cell apoptosis. This finding further reinforces the general understanding of glucocorticoids: as essential for survival though dramatically maladaptive when prolonged (Reichardt et al. 1998). While several studies have examined hippocampal atrophy and found strikingly similar results, the data are mixed as to where specifically this atrophy occurs. Much of this occurs because different experiments perform different sampling techniques for the hippocampus and, as such, do not all look at identical regions. Some research suggests that the dentate gyrus is the seat of this atrophy (Lucassen et al. 2006; Swaab et al. 2005), while some suggest that overabundance of glucocorticoids simply suppresses neurogenesis (McEwen 2001), and still others suggest that the atrophy is selectively in the CA1 (Sapolsky 2000; Sicotte et al. 2008) or CA3 pyramidal neurons (Watanabe et al. 1992; McEwen, 2001). In the latter of the findings, the atrophy

occurred primarily in the apical dendrites and not basal dendrites (Watanabe et al. 1992). This finding raises questions into the cause behind this selective atrophy, though to date they have been left unanswered.

However, there have also been studies which have suggested that hippocampal volume is not correlated with PTSD. In one such study, 37 individuals who had been exposed to severe trauma underwent extensive psychometric testing, structured clinical interviews, and MRI scans to measure the volume of several areas, notably the hippocampus (Bonne et al. 2001). These tests were administered at 1 week following the trauma and again at 6 months following the trauma, at which point PTSD was clinically diagnosed in 10 of the subjects (Bonne et al. 2001). The subjects with clinically diagnosed PTSD and those without PTSD did not have significant differences in either left or right hippocampal volume. This finding was consistent at both 1 week and 6 months (Bonne et al. 2001). Additionally, there was no reduction in volume among the subjects diagnosed with PTSD between their initial scan at 1 week and at 6 months (Bonne et al. 2001).

In studies such as this, a different hypothesis for hippocampal atrophy has been suggested. This hypothesis is that the atrophy occurs primarily from the overabundance of a specific type of glucocorticoid receptors, not the overabundance of glucocorticoids themselves. A notable point about this study, however, is that all 37 subjects were taken from a sample of individuals who underwent acute stress (Bonne et al. 2001). This is a common theme in research which purports to have found no veritable hippocampal atrophy in PTSD subjects (Bonne et al. 2001; Katz et al. 1981; Bryant et al. 1998). As noted above, acute stress is dramatically different form chronic stress, primarily because the overabundance of glucocorticoids must be in effect for a long duration of time in order to have the consequences described above (Lucassen et al. 2006).

PRIOR HIPPOCAMPAL ATROPHY AS A BIOLOGICAL VULNERABILITY IN RECURRING PTSD

It has already been demonstrated that the initial incidence of PTSD is strongly correlated with hippocampal volume. Additionally, it has been demonstrated that when individuals undergo severe and chronic stress, cell apoptosis occurs. This reduction in hippocampal volume creates a set of circumstances which are strikingly similar to those which produced the initial incidence of PTSD or PTSD-like symptoms. Therefore, it only follows that absent a wave of neurogenesis in patients with PTSD the disorder would become cyclic. In short, decreased hippocampal volume in subjects previously exposed to severe trauma leaves those subjects more susceptible to incidence of PTSD. To demonstrate this, I will focus primarily on experiments studying the effect of recurrent cycles of chronic stress on the development and severity of PTSD. While some of the following studies do not directly examine hippocampal size, the previous assertions within this paper, that small hippocampal volume is a factor in the predisposition of individuals to initial incidence of PTSD and that elevated levels of glucocorticoids decrease hippocampal size in individuals diagnosed with PTSD, have sufficiently demonstrated this point.

In one such study, pre-military trauma was correlated with adult psychopathology in Vietnam veterans. Researchers compared 38 veterans with PTSD and 28 veterans without PTSD with respect to the amount of childhood trauma or sexual abuse and general pre-military trauma (Yehuda et al. 1993). Vietnam veterans in the study who were diagnosed with PTSD had a higher rate of pre-military or childhood abuse or trauma than did those without PTSD at more than 3 times the rate (Yehuda et al., 1993). Even after controlling for the difference in severity of combat between the two groups of veterans, it was determined that the study subjects with PTSD had a significantly higher rate of all pre-military traumatic events than the group without PTSD (Yehuda et al. 1993). These findings explicitly correlate pre-military stress and trauma with development of PTSD following chronic stress later in life. Further, these findings strongly suggest that the proven reduction in hippocampal volume following chronic stressors like childhood abuse reduces the subjects' allostatic load, resulting in increased susceptibility to PTSD.

In yet another longitudinal study, which compared the symptoms of holocaust survivors with and without post-traumatic stress disorder (PTSD), the effects of recurring stress were analyzed. In this study, symptoms and other behavioral tendencies were measured at two 10 year intervals for both the control and experimental groups. The subjects consisted of 40 holocaust survivors (Yehuda et al. 2007). There was a general reduction in the symptoms of PTSD over time in both groups, though this was not striking. What was interesting, however, is that in 10% of the subjects, new instances of PTSD were seen to develop between the two intervals (Yehuda et al. 2007). The assessments showed that there was a general trend towards increase in trauma-related symptoms over the time between the two intervals in persons who were not previously diagnosed with PTSD (Yehuda et al. 2007). This suggests that PTSD cannot be readily analyzed from

a single diagnostic status at a single time, but rather has the ability to wax and wane in the face of factors such as neurogenesis and increase or reduction in allostatic load (Yehuda et al. 2007).

Finally, there is a study which brings together several facets of this paper into a single demonstration. In this study, researchers compared adult survivors of childhood abuse, previously diagnosed with PTSD, to matched controls with respect to hippocampal volume (Brenmer et al. 1997). In order to measure the volume of each subject's hippocampus, MRI was used. All subjects were adults aged 18-56 and of the 34 samples 17 were survivors of childhood abuse and 17 remaining subjects were combat-exposed but healthy (Brenmer et al. 1997). The control and experimental groups were with respect to age, sex, race, which of their hands was dominant, the size of their bodies and height of their bodies, the number of years which they had abused alcohol, and the length of their formal education (Brenmer et al. 1997). All of the subjects in the study met the criteria for clinical PTSD diagnosis from events secondary to childhood abuse (Brenmer et al. 1997). The findings of the neuroimaging were that the subjects diagnosed with PTSD, compared to the control subjects, had a 12% smaller hippocampus, though either group had roughly comparable volumes of the temporal lobe, amygdala, and caudate (Brenmer et al. 1997). These findings strongly suggest that the recurrent cycles of chronic stress predispose individuals to the development of PTSD.

CONCLUSIONS

In conclusion, it seems evident from the above studies that hippocampal volume is neither simply the cause nor the effect of chronic stress. Rather, it seems that the relationship between hippocampal volume and PTSD is one of bidirectional causality. Where many studies have concluded that there is a decrease in hippocampal volume stemming from chronic stress or trauma, they are correct. However, where many separate studies have concluded that decreased hippocampal volume results in an adverse reaction to chronic stress or trauma, they are equally correct.

Because the hippocampal volume clearly regulates the magnitude of the predisposition within the subject to PTSD, the directionality is resounding from hippocampal volume to PTSD. Since the mechanisms which produce and sustain PTSD contribute to destruction of hippocampal cells, thereby decreasing volume, directionality of causation is equally obvious as moving from incidence of PTSD to hippocampal volume. Given the above evidence I can resolutely say that the relationship between hippocampal volume and PTSD is bi-directional. Each mechanism acts on the other to create a positive feedback loop which has can easily spiral out of control. Further, because each system has no obvious method of self-regulation whereby one of these systems ceases to contribute to the other, the feedback has a deadly potential within the subject.

This finding is consistent with previous studies regarding cortisol and its relationship with several other neurotransmitters to convey specific messages. One such neurotransmitter is epinephrine, which combines its efforts with cortisol in order to create memories which are outstanding in some way, usually which deviate strongly from the norm (Talarico and Rubin 2003). These memories called to as flash-bulb memories and help us to avoid items which pose a serious danger in the future (Talarico and Rubin 2003). From this perspective, a smaller hippocampus can be a profound biological vulnerability. The veterans studied, while in combat, have requirements to survive. One such requirement is a faster and more accurate memory recall. Because this feedback is dependent on the ability of the hippocampus to function seamlessly, atrophy in this region is detrimental to the soldier's ability. Further, a reduction in volume in the hippocampus can readily cause the soldier to cease to inhibit irrelevant information, instead choosing to incorporate it into perception. The impending overload from this sequence of events is obvious and one can easily see how this could cause a state of panic in the soldier.

While it is wise to be hesitant about claims that seem to have overarching and closed explanations regarding any given phenomenon, I am quick to note that the above evidence is likely only part of the explanation for the incidence of PTSD. Surely, given the wide variety of disorders which deal with allostatic load and resulting endocrine malfunction in the brain there are numerous factors which contribute to the development many of the symptoms which we have ascribed to our understanding of PTSD. Over the past several years, numerous researchers have begun to think of stress-related disorders as a multi-faceted spectrum, similar to autism or other behavioral abnormalities.

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Factors that influence agonistic social behaviors in *Phoenicopterus chilensis* and *minor*

Layla Jubrial- Jaber, Maggie Wang

Agonistic behaviors have been observed across many captive and wild species of flamingos (Phoenicopterus) in a variety of settings. The present study examined the extent to which various social factors (e.g. species type, food competition, and captive or wild origin of birth) influenced agonistic behaviors in Chilean and lesser flamingos. Forty-six flamingos at the Memphis Zoo were observed at 2- minute scan sampling intervals. Observers made note of the agnostic behaviors displayed between species and where in the exhibit (at a feeder or not at feeder) the behavior was exhibited for one hour. For an additional hour, one wild and captive flamingo was observed continuously and agonistic behaviors exhibited were recorded. The study occurred over 5 weeks, between 1-4 pm on Wednesdays. Results showed that the species of flamingos did influence agonistic social behavior between Chilean and lesser flamingos, with Chilean flamingos exhibiting more agonistic behavior than lesser flamingos. These results show that the species of flamingo can influence agonistic behaviors. Agonistic behavior displayed around feeder sites differed from agonistic behavior displayed outside of the feeders, with agonistic behavior being displayed more at feeder locations than outside of the feeder locations. These results show that food competition can influence agonistic behavior in flamingos. The origin of birth did not influence agonistic behavior in flamingos. This study overall can aid in helping zoologists better understand what can influence agonistic behaviors in flamingo populations in zoos and aid in allowing zoos to help reduce agonistic behavior displays in exhibits.

INTRODUCTION

Of the six species of flamingos that currently inhabit the earth, the Chilean flamingo (*Phoenicopterus chilensis)*, a New World species, lives in northern and southern parts of Chile (Tobar et al. 2014), while the lesser flamingo (*Phoenicopterus minor)*, an Old World species, lives in sub-Saharan countries such as Kenya and Tanzania (Kaggwa et al. 2013). Lesser flamingos have been observed to have the highest population numbers in the wild during years when the cyanobacteria *Arthrospira* was abundant compared to the population of lesser flamingos during years when the cyanobacteria *Arthrospira* was less abundant; however, they will feed on other cyanobacteria as well (Kaggwa et al. 2013). Chilean flamingos in the wild have been observed to typically eat some species of invertebrates, such as copepods and polychaetes, and protists such as foraminifera, (Tobar et al. 2014) which they eat via filter feeding. For the American flamingo, (*Phoenicopterus ruber)*, the typical feeding times of the flamingo flock in the wild has been reported to be around early morning and late afternoon; however, these feeding times can vary (Bildstein et al. 1991). In flocks that include two individual captive species of flamingos, Chilean and American flamingos, it was observed that the two species, which sometimes ate at the same feeding trays, most often ate in separated flocks at separate feeding trays (members of the same species feeding together at a tray) (Bildstein et al. 1993). It was also observed that increasing the presence of the American flamingos in the pool led to the American flamingos spending longer times feeding at trays while the Chilean flamingos spent more time filter feeding in the water around feeding trays (increasing the number of Chilean flamingos did not reverse these results), suggesting that the presence of the American flamingos, which are much larger on average than Chilean flamingos, impacted feeding behavior of Chilean flamingos in captive populations (Bildstein et al. 1993).

In the wild, flamingo species are not known to have any observed dominance structure due to the lack of research done on wild flamingos (King 2000); however, captive American flamingos have been observed to display a dominance structure that persists throughout the season (Hughes 2015), which may suggest differences in social behaviors among captive and wild species of flamingos. It has been suggested that dominance structures in captive flamingos can both increase the amount of agnostic behaviors displayed within the flock while also having the potential to negatively impact more subordinate species members. (Hughes 2015). One study on the agonistic behaviors of captive Chilean flamingos found no sex-specific differences with aggression, but found that older individuals exhibited aggressive behaviors at higher rates than those of younger flamingos (Perdue et al. 2011).

In order to better understand social behavior in flamingos, we asked the following question: What factors influence social behaviors in flamingos? We tested the following three hypotheses regarding social behavior in flamingos. First, we hypothesize that the species of flamingos influences agonistic behaviors within the group. It is predicted that if the different species affect agonistic behavior, then Chilean

flamingos will display more agonistic behaviors towards the Lesser flamingos as they are larger in size. Second, we hypothesize that competition for resources affects agonistic behavior in captive flamingos. If competition influences agonistic behavior in flamingos, then it is predicted that agnostic behavior will increase in areas near food feeders, where competition for resources is greater. Third, we hypothesize that the origin of birth (in captivity or in the wild) affects agonistic behavior in flamingos. If the origin of birth influences agonistic behaviors, then it is predicted that flamingos born in the wild will display more agonistic behaviors than flamingos born in captivity. The null hypothesis is that agonistic behaviors in flamingos are not influenced by any social factors. Understanding the social dynamics of flamingo species can aid zoologists and researchers in the husbandry and breeding techniques used among flamingos currently in captivity at zoos as well as in the wild (King 2000). Agonistic behaviors can have negative impacts on flamingos (Hughes 2015), so by addressing and understanding agonistic behaviors within a species, researchers can better work to remove such behaviors from the environment.

METHODS

Study subjects and location

We studied a group of 46 flamingos of two different species (Chilean and lesser) at the Memphis Zoo during the fall months for two hours once a week (C. Hesch, pers. comm.). The population consisted of 31 Chilean flamingos (15 females, 16 males) and 15 lesser flamingos all of which were male. Of the Chilean flamingos, 22 were born in captivity, and nine were born in the wild; all of the lesser flamingos were born in the wild. Both species lived in the same exhibit and were on display at the same times during this project. The flamingo exhibit consisted of three feeding sites, which remained at the same location except during week three of data collection, a night house, and a flamingo pool (Figure 1).

Data Collection

Using binoculars and standing against the fence surrounding the exhibit, we observed the flamingos for two hours once a week (between 1-4 pm), collecting data on agonistic behaviors as well as nonsocial behaviors displayed by the flamingos; these nonsocial behaviors were not specified but grouped together under "other" or "eating and drinking" (Table 1**)**. Data were collected using scan sampling methods at two-minute intervals (Martin and Bateson 2007) for one hour. Throughout this hour the observer made note of which social behavior was being exhibited, where in the exhibit it was observed (either at the feeding site or not at feeding site), and which flamingo(s) species was doing the behavior. In order for a flamingo to be considered at a feeding site the flamingo needed to be able to access the feeder directly with its neck. The species of flamingo was identified via either their identification tag number or visual identification markers. For instance, Chilean flamingos have grey legs and all black beaks whereas lesser flamingos have pink legs and beaks with a pink spot near the front of the beaks (Grineld 2007). We then observed a single flamingo (one researcher observing a captive born or wild born individual each) for one hour using continuous behavioral sampling (Martin and Bateson 2007), recording all instances of social behavior the single flamingo exhibited. A total of 4 lesser flamingos and 6 Chilean flamingos were studied to determine if origin of birth influenced agonistic behaviors. To make data collection easier, the flamingo exhibit was split in half (between feeders two and three diagonally); one observer monitored one half of the exhibit while the other observer monitored the other half of the exhibit. Both observers collected data using the same methods of sampling. A possible point of conflict during the study was that the lesser flamingo population at the Memphis Zoo consisted only of males, which may impact data if there is gender specific social behavior. Also, feeder 2 was shifted slightly toward the center of the pool on day 3 (week 3) of data collection, but later returned back to its original position, which may have impacted data collection.

Analysis

Two sets of data for the five weeks were included for data analysis in order to test if the type of species influenced agonistic behavior in flamingos. We summed up the total number of agonistic behavioral scans for each of the Chilean and lesser flamingos separately for each week along with the total number of all behaviors displayed (solitary and social/ agonistic). We then calculated the ratio of behavioral scans for lesser flamingos to the behavioral scans displayed in all to get the percent of time lesser flamingos spent engaged in agonistic behaviors. We then calculated the ratio of behavioral scans for Chilean flamingos to the behavioral scans displayed in all to get the percent of time Chilean flamingos spent engaged in agonistic behaviors A Shapiro-Wilk test and Levene's test were run to test for homogeneity of variance and normal distribution of data. Data were found to have a normal distribution and have a homogeneity of variance ($p>0.05$), and so data were determined to be parametric and so a parametric t- test was used to

compare if the species of flamingos influenced agonistic behavior.

Four weeks of data collected for an hour each week were used to assess if competition influenced agonistic behavior in flamingos. The data from week one were eliminated from the study because the percent of agonistic behaviors at feeders and outside of the feeders resulted in a negative value, probably due to human error/overlap from the observers in counting the same flamingos twice. We summed up the total number of agonistic behavioral scans at all three feeders for each week along with the total number of all behaviors displayed (solitary and social/ agonistic) at locations elsewhere in the exhibit (i.e. on land or in the pool). We then calculated the ratio of behavioral scans for flamingos at the feeder to the behavioral scans displayed in all to get the percent of time flamingos spent engaged in agonistic behaviors at feeders. We then calculated the ratio of behavioral scans for flamingos at locations other than the feeder to the behavioral scans displayed in all to get the percent of time flamingos spent engaged in agonistic behaviors outside of feeder locations. A Shapiro-Wilk test and Levene's test were run to test for homogeneity of variance and normal distribution of data. Data were found to not have a normal distribution ($p<0.05$), and so data were determined to be nonparametric and a Mann Whitney U statistical test was run to determine if competition influenced agonistic behavior of flamingos.

Five weeks of data, one hour of data collection each week, were included for data analysis in order to test if the origin of birth influenced agonistic behavior in flamingos. We found the sum of the total number of agonistic behavioral scans for both the wild born and captive born flamingos separately for each week along with the total number of all behaviors displayed (solitary and social/ agonistic). Then we calculated the ratio of behavioral scans for wild born flamingos to the behavioral scans displayed in all to get the percent of time wild born flamingos spent engaged in agonistic behaviors. Then we calculated the ratio of behavioral scans for captive born flamingos to the behavioral scans displayed in all to get the percent of time captive born flamingos spent engaged in agonistic behaviors (solitary and social/ agonistic). Data were found to have a normal distribution and a homogeneity of variance ($p>0.05$), and so data were determined to be parametric and so a parametric t- test was used to compare if the origin of birth influenced agonistic behavior.

SOLITARY BEHAVIOR		
Eating/ Drinking	ED	Eating from the food buckets or drinking from the water, or both
SOCIAL BEHAVIORS		
Walking	W	Moving away from or towards an animal due to proximity (does not include solitary walking)
AGONISTIC BEHAVIORS		
Chasing	C	One flamingo following another flamingo by rapidly walking
Feather Ruffling	FR	Feathers lifted when in contact with another flamingo as a sign of aggression
Head Tossing	HT	Moving and shaking head back and forth rapidly in response to another flamingo.
Beak- Body Touching	B	Aggressive touching with the beak of one bird to the body of another flamingo.
Beak- Beak Touching	BB	Aggressive touching with the beak on one bird to the beak of another bird.
Body- Body Touching	BT	Aggressive touching with the body of one bird to the body of another bird.
OTHER		
Out of Sight	O	Flamingo is not seen by observer.
Other	OT	Other non social behaviors, including solitary walking, grooming, standing, and resting.

Table 1. Ethogram

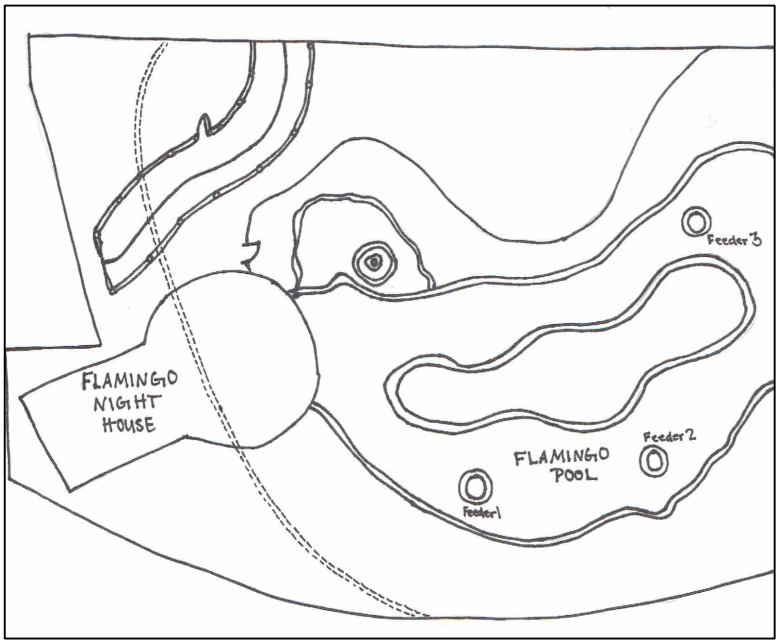

Figure 1. Exhibit Map

RESULTS

The species of flamingo did influence the social behavior of flamingos (Fig. 2). The Chilean flamingo displayed agonistic behavior for a greater percentage of scans (mean ± standard error) (31.5 ± 3.1 %) than did the lesser flamingos (9.63 ± 1.9 %). The species of flamingo was found to influence agonistic behavior (t_8=-5.951, $p < 0.001$).Excel, I conducted a paired t-test to compare percent modified land cover within each protected area to the 10 kilometer region surrounding each protected area.

Competition was found to influence agonistic behavior in flamingos. There was an increase in agonistic behavior displayed (mean ± standard error) around the feeder sites (40.0 ± 1.8 %) compared to other sites in the exhibit (1.31 ± 0.59 %) (U=0.0, N_1=4, N_2=4, p=0.029).

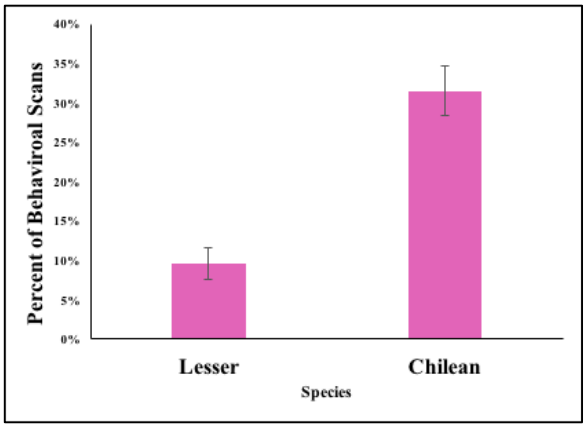

Figure 2. Agonistic Behaviors (mean ± standard error) of lesser (9.63 ± 1.9 %) and Chilean flamingos (31.5 ± 3.1 %) at the Memphis Zoo. Chilean flamingos displayed more agonistic behavior than lesser flamingos displayed (t_8=-5.951, $p < 0.001$).

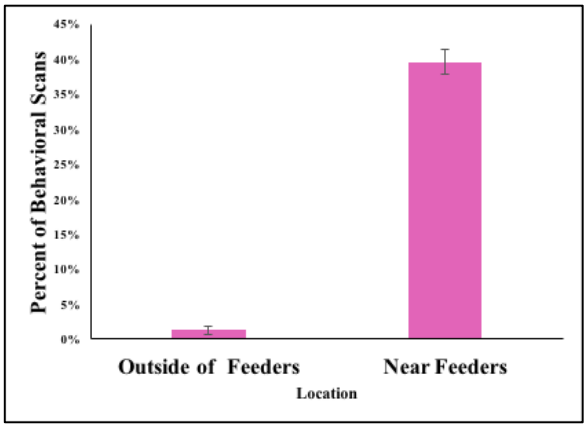

Figure 3. Agonistic Behavior (mean ± standard error) of flamingos at feeders (40.0 ± 1.8 %) or outside of the feeders (1.31 ± 0.59 %). Flamingos near the feeders exhibited more agonistic behavior than flamingos outside of the feeder location (U=0.0, N_1=4, N_2=4, p=0.029).

The origin of birth did not influence agonistic social behavior in flamingos (Fig 4). Flamingos born in the wild displayed (mean ± standard error) agonistic behavior for a larger percentage of behavioral scans (39.5 ± 7.6 %) than the captive born flamingos (48.3 ± 8.4 %) but did not differ in the time spent engaged in social agonistic behaviors (t_8= 0.755, p = 0.472).

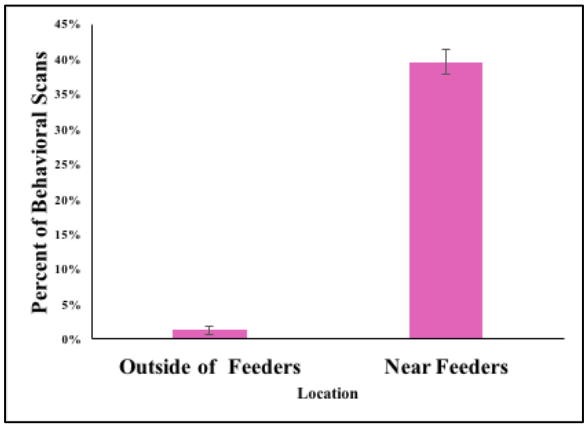

Figure 4. Agonistic Behaviors (mean ± standard error) of wild (39.5 ± 7.6 %) and captive (48.3 ± 8.4 %) flamingos at the Memphis Zoo. Wild flamingos did not significantly differ in the amount of agonistic social behavior displayed compared to captive flamingos (t_8= 0.755, p = 0.472).

DISCUSSION

Interspecific differences were found to have an influence on agonistic behavior in flamingos. The Chilean flamingos spent a greater percentage of behavioral scans engaged in agonistic behaviors than did the lesser flamingos. This finding is consistent with Bildstein et al. (1993), who found that the larger size species of flamingos did hold dominant positions and displayed more agonistic behaviors. While there has been limited research completed on interspecific agonistic relationships between flamingos, there have been observations of spatial segregation of wild populations of Chilean, Andean, and James flamingos when grouped together as well as differences in where each species chose to forage (Mascitti and Castanera 2006). These findings suggest that flamingos of different species prefer to remain separate from each other. Since the flamingo populations at the Memphis Zoo are mixed, agonistic behaviors may be being displayed due to the inability of flamingos to separate into individual populations. Current research remains divided about dominance structures with captive flamingo populations; research has shown that American flamingos do display a dominance hierarchy within the captive population, especially during breeding season (Hughes et al. 2015), while Chilean flamingos have been found not to display any dominance hierarchy within the captive population (Farrell et al. 2000). These studies suggest that flamingos of different species can exhibit different behaviors related to dominance; however, no research was found on whether or not a dominance hierarchy would exist in interspecific populations of flamingos. A possible way to further expand this study would be to look at dominance structures in interspecific species of flamingos in order to determine if the increase in agonistic behaviors displayed correlated to a dominance hierarchy in the flamingo species. Also, future research could look at whether the agonistic behaviors displayed by the flamingos were displayed to other members of the same species or between species. By studying whether or not agonistic behavior was displayed between members of the same species or across species, researchers can better design exhibits to minimize agonistic encounters or separate flamingo species to minimize agonistic behaviors.

Competition for resources was found to influence agonistic behavior in flamingos. Flamingos displayed more agonistic behavior in feeder areas compared to areas elsewhere in the exhibit. A similar study also found that agonistic behavior increased near feeder areas especially in areas where resources are limited (Farrell et al. 2000). Also, flamingos that most commonly feed at feeders have been shown to influence the amount of access and time other flamingos of the same species who filter feed have to those feeders (Farrell et al. 2000). Competition for resources in flamingos could be further studied to see if there was species-specific competition around the feeders. A possible avenue of research would be to look at agonistic behavior beyond food competition. Flamingos that were pair-bonded were the targets of more aggressive behaviors from other flamingos (Royer and Anderson 2014), suggesting that competition for mating rights can influence the display of agonistic behavior in flamingos. Looking at a wide range of sources for competition can provide a more complete analysis about how competition can influence agonistic behavior displays in flamingos. By studying food competition more closely, researchers and zoologists can better design exhibits so that the degree of competition is lowered (i.e. more feeders, increased space, etc.). One potential problem that occurred during the study was that on week 3 of data collection feeder two was moved closer to feeder 3. The movement of the feeder could have impacted how many flamingos had access to the feeder; however, the movement of the feeder did not appear to impact the results. In future studies, it may be necessary to speak with zoo exhibit

coordinators and make them aware of the study so that we can ensure the exhibit remains unchanged throughout the study.

Flamingos born in captivity and flamingos born in the wild did not differ in their displays of agonistic behavior, thus not supporting the hypothesis that origin of birth would influence agonistic behaviors in flamingos. One study has found that the behavior of captive flamingos is closely related to that of wild flamingos (Rose et al. 2014), however studies analyzing the difference between captive and wild flamingos is lacking (Bildstein et al. 1993). The findings of our study suggests that there is no difference between flamingos born in the wild compared to those flamingos born in captivity. These results can be used as a basis for comparing and correlating behavior in captive flamingo flocks to wild flamingo flocks, thus allowing researches to make better behavioral comparisons to be made about flamingos in nature. A possible avenue of research would be to look at when flamingos born in the wild were brought into captivity. By assessing the time in which a flamingo was brought into captivity could aid in understanding if agonistic behavior displays are learned in captivity or if they are due to various environmental factors.

One of the biggest limitations of this study was tracking the flamingos during the scan sampling. The flamingos were constantly moving, making it difficult to accurately count the agonistic behaviors during the observation. The duration of this study could also be a limiting factor of the findings; increasing the number of data collection days could potentially alter the findings of the paper and make them more precise and applicable to research in the field. Another point of conflict may be that the lesser flamingo population consists of all males, which may have impacted data if there were sex related differences in social behavior.

CONCLUSIONS

The present study found that interspecific interaction influence agonistic behavioral displays between Chilean and lesser flamingos. Food competition was also found to influence agonistic behavioral displays, while the origin of birth was not found to significantly influence agonistic behavior in flamingos. These findings allow researchers to better understand the behavior of flamingos in captivity and can aid zoos in finding ways to reduce agonistic behavioral displays in captive animal populations.

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Hidden rhythms of life: understanding circaseptan rhythms and their commonality in diverse life forms

Erika McCormick

The week is a crucially and globally shared experience among humans. During the billions of years that life has been evolving on this Earth, a geomagnetic week has been consistently shaping physiological processes. The field of chronobiology examines how time structures interact with biological entities, and recently there has been an increased interest in about-weekly, or circaseptan rhythms. An increasing amount of evidence supports the hypothesis that these circaseptan rhythms are endogenous, and potentially a shared feature among diverse life forms. These rhythms can be observed in a variety of organisms, but the evolutionary history of this biological rhythmicity continues to be explored and is largely unknown. Understanding circaseptan rhythms and other chronobiological phenomena is crucial for advancements in pharmacology, medicine and health outcomes, and the prospect of space colonization.

THE WEEK IS A CRUCIALLY AND GLOBALLY SHARED EXPERIENCE AMONG HUMANS

Weekly rhythms predetermine the days associated with working and rest: the dread associated with Mondays, and the anticipation of a Friday night. In monotheistic religions, the seven-day week has unique significance, created in order to distinguish between the common days of work and a mystical, extraordinary day of worship and rest. Religions such as Judaism, Christianity, and Islam find meaning and coherence in the sharing of a similar weekly schedule of worship and work.

Additionally, the week takes on different forms as one examines civilizations throughout history. For example, the ancient Mayan and Aztec peoples had many contrasting weekly cycles in their society, requiring multiple-week calendars. As general societal emphasis on spirituality has decreased and the importance of industrialization and secularization has increased, the ancient weekly structure was repurposed into the current weekend and work week dichotomy (Ayers et al. 2014).

Scholars and medics have noticed a peculiar connection between seven-day intervals and fluctuations in disease for thousands of years. Hippocrates, an influential ancient Greek physician, was one of the first to acknowledge the social and health-related effects of seven day cycles (Mikulecky 2014). Despite many observations hinting at an innate bodily response to weekly periodicity, this prospect remained absurd until the emergence of chronobiology led to speculation about the interplay between the week and human physiology.

CHRONOBIOLOGY

As organisms on Earth, we are exposed to many time cycles: the day, the week, the month, the year, and so on. These time structures are called chronomes, and the study of how these time structures interact with and affect biological substance is known as chronobiology (Halberg et al. 2004). However, our social lives and innate bodily rhythms do not exist as compartmentalized entities, but instead interact in intricate ways to regulate bodily activity.

Generally, bodies are synchronized by three determinants: a solar clock of varying light and temperature that coordinates things such as sleep and wakefulness, a social clock that governs work and social interactions, and an innate, internal biological clock that is encoded into the genome and controls physiological processes (Mehling and Fluhr 2006).

During the billions of years that life has been evolving on this Earth, a geomagnetic week has been consistently shaping physiological processes. This weekly rhythmicity has been written into our DNA over time, and now is proven to be genetically acquired (Mikulecky 2014). Genes such as “clock”, “period” and “frequency” have been identified as partial regulators of the innate biological clock (Mehling and Fluhr 2006).

With these advances in chronobiology, an increased interest has surrounded about-weekly, or circaseptan rhythms. Until fairly recently, any patterns in human weekly cycles were attributed to the effects of growing up in a socially constructed week. Yet, evidence strongly supports the presence of innate circaseptan cycles that are not necessarily correlated with the social or religious weeks. One researcher even went as far as to propose that ancient humans actually formulated the social week with a seventh day of rest because of the actions of a subconscious drive from innate weekly rhythmicity (Derer 1960).

Some circaseptan rhythms are highly influenced by external forces such as lunar cycles,

work schedules, and solar activity. However, there is an increasing consensus that many near-weekly patterns observed in organisms are endogenous, or genetically fixed, and occur independently from outside forces (Haus 1999). Many different genetic pathways show evidence of circaseptans, and these rhythms manifest in a multitude of physiological processes.

CIRCASEPTAN RHYTHMS

Intrinsic circaseptan rhythms are nearly ubiquitous among life on Earth. In fact, weekly patterns have been observed in organisms with no cognitive ability, eliminating the idea that all circaseptan rhythms are results of socialization to the communal week. The presence and persistence of these weekly rhythms in life on Earth is fascinating and quite beautiful. From a unicell's metabolic activity to the complex immune responses of human beings, these mysterious and unchanging patterns appear to be a common thread shared among diverse forms of life.

Acetabularia is a charismatic group of green algae found in subtropical waters. Affectionately nicknamed the "mermaid's wine glass," these gigantic unicellular organisms are shaped like an umbrella and live in marine habitats. Remarkably, a study done in 1986 yielded clear evidence that a circaseptan rhythmicity was present in the growth of these algal cells (Schweiger et al, 1986). This is fascinating because the presence of circaseptan rhythms in a unicellular organism such as *Acetabularia* hints these patterns may have originated in a more distant ancestor than originally expected.

Even plants follow circaseptan rhythms. In a study done at the University of Antwerpen in Belgium, water uptake of bean seeds was monitored at noon every day for two years. Water uptake is crucial in both a dormant seed and a germinating one. The results showed a clear circaseptan rhythm, where minimum water uptake values occurred every seven days. The researchers believe that the rhythmicity in seeds they observed is endogenous (Spruyt et al. 1987). Additionally, this rhythm corresponds to the phases of the lunar cycle. It is thought that fluctuating water uptake according to lunar phases may maximize the durability of the plant and minimize herbivory (Koukkari and Sothern 2007).

Circaseptan rhythms were observed in a specific chicken antibody IgY found in egg yolks, which provides the developing egg with a passive immunity from its mother (He et al. 2014). Researchers focused on the variation in IgY concentration in the egg yolks of 4 chickens over a 60-day period. There was a seven-day fluctuation in concentrations of yolk IgY that was exhibited in all cases, confirming a circaseptan rhythm of yolk IgY (He et al. 2014). Since IgY is an antibody and plays a role in chicken immunity, the weekly fluctuations of this substance correspond with a broader pattern involving circaseptans and the immune system. Circaseptan rhythms have been identified in various species' immune responses, including in humans. This will be explored further in the next section about circaseptans in humans.

The beautiful blue luminescence of *Gonyaulax polyedra,* a motile dinoflagellate, can be seen in warm coastal waters across the globe. Interestingly enough, the luminescence of this organism follows a weekly circaseptan rhythm (Yang et al. 2007). It is predicted that the visible bioluminescence may hint at an underlying weekly mechanism in the communication of these dinoflagellates (Cornelissen et al. 1986).

Circaseptan rhythms in honey bees (*Apis mellifera)* were investigated using one bee colony during a period with no change in queen (Mikulecky and Bounjas 1997). This effectively means that all of the bees sampled were identical twins, possessing all of the same genes. Honey bee biochemistry has been identified as closely related to the phases of the moon, and observations have been made about worker bee flights and their correlation with lunar activity. The manifestation of a physiological weekly rhythm was found in the fluctuating concentrations of phospholipids, molecules involved in structural and metabolic processes in cells, in the hemolymph of worker bees (Mikulecky and Bounjas 1997).

Trehalose and glucose were also two components that exhibit a circaseptan concentration rhythmicity (Mikulecky and Bounjas 1997). These compounds are a circulating reserve for energy in these bees, and thus it would make sense that as flight patterns and activity levels change with lunar phases, the energy circulating in the hemolymph would fluctuate in similar ways (Mikulecky and Bounjas 1997).

In their natural habitat, beach beetles (*Chaerodes trachyscelides* Wihte) are nocturnal feeders. Beetles intentionally situate themselves near the debris band created on the beach through the accumulation of washed-up algae and seaweed. They burrow and hide under the sand during the day, and then feed at night on the algal band. However, tidal changes mean the debris band is not always present at the same location. This dynamic habitat forces the beach beetle to move up and down the beach in accordance with tides in order to stay near its food source (Meyer-Rochow and Brown 1998).

The weekly tidal changes in the beach beetle habitats clearly led to a selection for circaseptan behaviors in this species. In one study, the activity of

captive beach beetles was recorded over 29 days (Meyer-Rochow and Brown 1998). In a controlled environment of constant darkness (simulating their nocturnal activity period) and sand to burrow in, the captive beetles still exhibited a circaseptan rhythmicity in their behavior. About every seven days, the beetles started their nocturnal activity at an earlier time and were active longer, representing their substantial weekly move to follow their food source up and down the beach (Meyer-Rochow and Brown 1998).

In a study conducted by Schweiger et al., mice (*Mus musculus)* were infected with malaria in a laboratory setting (19986). Tracking their mortality, scientists found mice were inclined to die either 7, 14, or 21 days from initial infection (Schweiger et al. 1986). This is another example of weekly rhythm in immune response and this type of pattern has been observed in malaria, the common cold, and other illnesses in humans.

BUT WHAT ABOUT HUMANS

The biological week has also manifested in many innate aspects of human physiology. Rates of heart attack and stroke vary at about-weekly rates (Mehling and Fluhr 2006). By understanding the ways in which health outcomes and biological rhythms interact, the chronorisk of a disease can be estimated. A chronorisk is relevant when a health phenomenon varies in patterned ways. Just as the likelihood of death in mice injected with malaria is dependent on the timing of initial exposure to the pathogen, it is possible that humans also have cyclic and predictable risks of contracting diseases and have differential health outcomes dependent on timing of infection (Schweiger et al. 1986).

Chronorisk of Stroke

In a study done at the University of L'Aquila in Italy, 667 cases of stroke in humans were examined. Some of these strokes were a result of blood flow being cut off from the brain (cerebral infarction), and others were caused by a burst blood vessel in the brain (cerebral hemorrhage), both leading to significant brain damage in the area. Despite the very different origins of stroke in these two cases, both demonstrated a circaseptan rhythm (Pasqualetti et al. 1990).

A pattern showed that the highest risk of stroke was on Saturday evening, and some researchers were able to predict with high confidence that the stroke would likely occur sometime between Saturday and Monday (Pasqualetti et al. 1990). This circaseptan pattern may correspond to the social week, indicating changes in behavior from the busy work week to the weekend, or vice versa, may be a contributing factor to increased risk of stroke. However, in a database that contains the daily incidence of ambulance calls in Moscow over a period of three years, calls related to strokes had highest incidences during mid-week, with the lowest incidence over the weekend (Cornelissen et al. 1993). These discrepancies in results can partially be attributed to various causes of strokes, each with their own patterns of incidences (Johansson et al. 1990).

Stroke is a disease with a very high chronorisk, and besides exhibiting weekly risk fluctuations, it also varies in patterns of likelihood on a daily and yearly basis. According to one study, stroke is most likely to occur early in the morning and in the winter (Pasqualetti et al. 1990). Through examination of when a person will have the highest risk of disease, better treatments can be given and extra precautions can be taken during peaks of maximal incidence.

Transplant Rejection and Immune Response

Additionally, weekly rhythmic patterns impact the human immune system in profound ways. For example, many cell-mediated immune responses in the human body demonstrate circaseptan rhythms, such as some macrophage, cytotoxic T-cell, natural killer cells, and cytokines. All of these cell types work together to eliminate pathogens from the body and maintain a healthy system (Haus 1999).

In cases of transplant rejection, the human immune system attacks itself. Rejection of a transplant occurs because the body recognizes the donor cells as foreign and carries out an immune response as if the transplanted tissue were an antigen. The circaseptan rhythmicity of the immune system translates into a weekly pattern in transplant rejection. Weekly rhythms are involved in many aspects of the transplant recovery process including patterns in tissue regeneration, remedying the effects of immunosuppression, and the aforementioned immune responses by cells. For instance, in studies done on human kidney transplants, the most common rejection episodes occurred about a week after the transplant. It is interesting to note that the transplants occurred at many different days of the week, thus the circaseptan rhythm existed independently of the social week (Haus 1999).

By understanding the mechanisms of the immunological circaseptan tendencies, anti-transplant rejection therapies may be better customized and more effective in reducing the negative side effects of transplants.

Heart Rate

It is generally known that humans have innate 24-hour rhythms, called circadian rhythms, dominating many bodily processes. A more obvious example of this would be the sleep cycle, which fluctuates daily based on an endogenous pattern

encoded into the human genome. Typically, human heart rate is thought to be strongly regulated by circadian rhythms. However, an intriguing contradiction to this pattern exists in studies on humans in the neonatal stage and in a Persistent Vegetative State.

A study was conducted on neonatal patients where heart rate was measured at term of the pregnancy, immediately after birth. The heart rate of the infant during its first week of life was characterized by a circaseptan rhythm. By the second month of life, the typical dominance of a 24-hour rhythmicity of heart rate had replaced the 7-day rhythm in the infant (Watanabe et al. 2002). The presence of an about-weekly pattern in newborn human infants provides further evidence to the hypothesis that these circaseptan rhythms are encoded into our genome and not acquired based on socialization—a baby has no understanding of a social week. Similar results were found in patients in the Persistent Vegetative State. Patients in PVS show no higher brain function or responsiveness, and are kept alive only through medical intervention. Remarkably, the blood pressure and heart rate of these patients also exhibited a strong circaseptan rhythmicity (Guan et al. 2011).

These findings have led researchers to hypothesize that circaseptan rhythms are innate and that there is some kind of down-regulation of these rhythms occurring in a human lifetime. One proposed solution is the activity of the suprachiasmatic nuclei. The suprachiasmatic nuclei are located in the hypothalamus of the brain and are known to regulate circadian rhythms and physiological processes (Mehling and Fluh 2006). If these nuclei are the main endogenous pacemakers in the body, it is plausible that they are responsible for the prominence of circadian rhythms over circaseptan rhythms during lifetime. In the Persistent Vegetative State, the suprachiasmatic nuclei no longer have control because of a lack of functional brain activity. Thus, the circaseptan rhythms are unsuppressed and the typical domination of circadian rhythms is no longer is observed (Guan et al. 2011).

EVOLUTION OF CIRCASEPTAN RHYTHMS

The focus then shifts to the function of these widely prevalent about-weekly rhythms. Within a species, there would be an understandable benefit to having synchronized growth cycles, patterns of luminescence, and metabolic activities among its individuals. Having coordinated processes ensures order and harmony, both between entities and in the physiological processes of one organism. For example, it is crucial that all of the cells involved in immune responses in a human body are integrated into one effective retroaction.

But why has the week been chosen, passed down through many rounds of natural selection and evolution into species today?

Recent advances have been made in understanding the role of biological rhythms in the field of chronoastrobiology. Chronoastrobiology is the study of how our biological time structures interact with the universe, specifically geomagnetic and solar forces (Halberg et al. 2004). The forces that act on species today, such as lunar cycles and the geomagnetic pull of the Earth, vary at about-weekly rates and have acted on life forms since their beginnings on this planet.

All life shares a common ancestor in the distant past. This ancestor most likely lived between 3.5 to 3.8 billion years ago and was aquatic. In fact, for billions of years the only life on Earth was single celled organisms living in oceans. Synchronizing metabolic processes to the lunar cycles or geomagnetic fluctuations may have proved advantageous for aquatic ancestral species.

A clear example is the case of the Beach Beetle, in which the circaseptan behaviors of chasing a food source up and down the beach according to tides confers an adaptive advantage to those who possess it. The orbit of the moon affects tidal levels on Earth and varies at about-weekly rates. If an individual has an innate weekly movement cycle, it is more likely to effectively eat and thus becomes more likely to survive. The circaseptan behavior is an advantageous adaptation to the beetle's changing tidal environment (Meyer-Rochow and Brown 1998).

However, the evolutionary history of circaseptan rhythms is still poorly understood and other circaceptan tendencies have less obvious advantages for their proprietors. While one can observe these patterns in diverse life forms today, it is possible that these rhythms evolved convergently, or that circaseptan rhythms do not have a common origin, but instead have manifested in diverse species as an adaptation to a shared magnetic and solar environment.

It becomes nearly impossible to identify the effects of solely genetic components of biological rhythms because life on Earth is constantly exposed to a geophysical environment. Using the knowledge accumulated about the history of life and the known benefits of having these synchronized processes, potential hypotheses have been formulated.

Some evidence hints circaseptan rhythms may have evolved independently in various species throughout all of life (Young and Kay 2001). Yet, the prevalence of the biological week in such diverse species—from water uptake in bean seeds to the

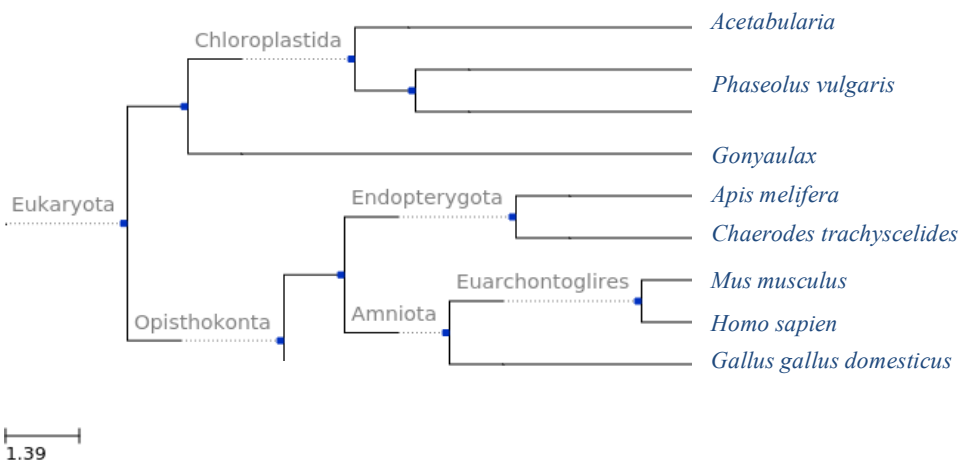

Figure 1. Phylogeny created using Phylotastic. Phylogenies show the evolutionary relationships between species. Each node represents a common ancestor. Plants and algae (*Chloroplastida*), insects (*Endopterygota*), and various vertebrate species (included in *Amniota*) all demonstrate circaseptan rhythms. Since such a broad range of Eukaryotes demonstrate these rhythms, it can be hypothesized that a common ancestor of all of these species, indicated by the first blue node at the beginning of the phylogeny, is where circaseptan rhythms first evolved.

complex flight patterns of honey bees—also indicates that these rhythms may have originated in ancestral species and could be a shared character of life. The prominence of circaseptan rhythms has been identified in unicells, crayfish, rats, pigs, and human babies (Halberg et al. 2004). Commonality in the physiological processes of such diverse species suggests an ancestral species likely developed an about-weekly pattern over time, and that having genes for this pattern was beneficial in their environment.

For example, many organisms demonstrate circaseptan rhythms in processes involving growth and immune response, which may have a common origin in an ancestor (Haus and Smolensky 2009). Operating under this assumption, it becomes plausible that the manifestation of the biological week in the genome occurred by natural selection in an antecedent species, and the circaseptan rhythm is a remnant conserved by evolution.

Since the ancestor of all of these species was likely a unicellular organism living in the deep sea, where there is no known cycles of light and darkness, it may have been beneficial to adopt some type of external synchronizer (King 2004). For many terrestrial species, light is a major regulator of activity, and cycles between light and dark are how humans construct a day, week, month, and year (Mistlberger and Skene 2004). For a hypothetical ancestral species living in darkness, fluctuations in geomagnetic pull or other non-photic cycles may have served as the main synchronizing factor. The ancestor would have benefitted from having cyclic activity and integrated processes in an otherwise dark and relatively unchanging environment.

Ancestral species with the genes for circaseptan rhythms may have survived longer and better, creating more offspring and having a greater fitness for their environment. In this way, circaseptans may simply be a vestigial, or non-functional trait left over from an adaptive advantage in the past. The basic cellular weekly rhythmicity may have evolved in an ancestor, but the diversity of circaseptans suggests that various life forms have evolved specific circaseptans independently.

AN EXPERT OPINION: DR. GERMAINE CORNELISSEN

Germaine Cornelissen is a professor at the University of Minnesota in the Department of Integrative Biology and Physiology. Additionally, she is the director of the Halberg Chronobiology Center, devoted to pioneering research in chronobiology and applying findings to improving cardiology and treatments of cardiovascular health problems. Dr. Cornelissen has done extensive research on circaseptan rhythms and their effects on chronomedicine (*Halberg Chronobiology Center*, Web Page).

In speaking about chronobiology, Dr. Cornelissen recommends considering all of the rhythms that make life possible including high-

frequency brain waves, the cardiac and respiratory cycles, about 90-min REM/NREM sleep cycles, and the "lower-frequency components" such as the week and year. She suggests different frequencies "address different needs to support important physiological functions to sustain life" (Cornelissen, personal correspondence). All of the rhythms in the body work together to keep the organism alive and functioning, and thus it is often difficult to determine the adaptive function of one specific rhythm, such as the week, because it exists in accordance with various other frequencies in the body.

However, circaseptans are found most prevalently in "growth, regeneration, and response to a single stimulus" (Cornelissen, personal correspondence). Examples she cited were birth, surgery, change in lighting regimen, and balneotherapy (special disease treatment using mineral springs). Among these phenomena, she notes that the artificial social week is an important synchronizer. However, there are cases when the time of stimulus sets the circaseptan rhythm, regardless of the day of the week (such as likelihood of kidney transplant rejection), and the fact also exists that neonatal blood pressure and heart rate are synchronized in about-weekly periods solely based on time of birth, not day of the week. Situations such as these negate the idea that circaseptan patterns exist only because of the social week.

According to her, "circaseptans may also constitute a bridge between circadians and about-monthly rhythms to handle tasks that needed more than a day but not as long as a month" (Cornelissen, personal correspondence). They may be an evolutionary adaptation to synchronizing these intermediate tasks. While the social week serves as a synchronizer in humans, there is also a presence of circaseptans in unicellular organisms and aquatic algae that clearly do not engage in human social behaviors. Thus, the possibility is raised that circaseptans initially developed by being synchronized to the lunar phases of the moon, or potentially the geomagnetic pulls of the Earth's magnetic field, which follow approximately a 27-day cycle. Dr. Cornelissen has found correlations in her research between neonatal blood pressure and the local geomagnetic indices.

Geomagnetic pulls could be sensed in the oceans, and ancient organisms may have evolved according to this magnetism. The commonality of all life means that if an ancestor of many extant species today had developed these rhythms in response to their geomagnetic environments, these circaseptans may be a vestige that still exists in many species today.

In her research, Dr. Cornelissen has observed the presence of circaseptans in unicells, crayfish, fish, and various mammals. Although she and many others still struggle to understand the present adaptive advantage of having these rhythms in many living species, she has experimentally found that organisms operate in seven day intervals.

For example, three different studies were done on growth of *Acetabularia*, longevity of face flies, and survival of a worm. Twenty-four hour environmental synchronizers, such as light, were altered so that the organisms were exposed to different lighting regiments. For example, organisms were first synchronized to a regimen of 12 hours of light, followed by 12 hours of darkness. While one group remained on this control lighting regimen, experimental groups underwent 6-hour shifts from the 12:12 regimen at intervals that raged between 4 and 11 days for the groups. It was found that some shift intervals had better outcomes for the species than others, and the best and worse shift intervals were typically separated by 7 days. This is an interesting finding among a diverse sampling of species, and suggests that regardless of adaptive advantage, circaseptans are deeply engrained into the genome (Cornelissen, personal correspondence).

THE IMPORTANCE OF CIRCASEPTAN RHYTHMS

Chronobiology is a rapidly expanding field that is instrumental in better understanding pharmacology and disease treatment. Applications of chronobiology can improve the timing of medicine and better the likelihood of certain health outcomes (Mehling and Fluhr 2006). For example, understanding the weekly-timing of immune response to kidney transplants can improve anti-rejection therapies and ensure that transplants are more successful in the future (Haus 1999).

Just as the human genome has been entirely sequenced and largely standardized for medical treatment and understanding of the interplay between DNA and illness, some researchers have stressed the importance of having a standardized chronome for humans (Halberg et al. 2004). If there was a standard text with all known biological processes and their frequencies, it could greatly benefit medicine because it is a waste of time and resources for researchers to continually be rediscovering the same patterns in individual chronomes. Additionally, it is the idea that essential functions of the human body such as heart rate and immune responses are influenced and coordinated in part by Earth's geomagnetic field means that a human living outside of this field may become unsynchronized. Although the absence of a geomagnetic field may not impair certain endogenous

rhythms, it is important to research and comprehend the physiological effects of living in outer space before humans attempt to colonize other planets (Halberg et al. 2004).

Circaseptan rhythms orchestrate many biological processes that maintain the rhythmicity and order of life. Understanding this largely mysterious phenomena can provide another window into discerning the way life has evolved on Earth, and the ways in which organisms interact with their physical and geophysical environments. These fascinating and mysterious rhythms are at work constantly, and further research is crucial in this field of biology.

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The glucocorticoid theory of hippocampal atrophy in depression

Ritika M. Mazumder

Major depressive disorder (MDD) is a common, severe illness and its most prominent neurological marker is hippocampal atrophy. Although various studies have documented this finding, the specific cause of hippocampal volume loss in depression is uncertain. However, recently, MDD has been linked to the dysfunction of the HPA axis, suggesting that it is a stress-related disorder that results in an elevated level of circulating glucocorticoids (GC). Cushing's syndrome, which also results in an elevation of GC, has been correlated with hippocampal atrophy, suggesting that high GC exposure is somehow involved in the process of hippocampal volume loss in MDD as well. Even though so far only correlational human studies exist in this field, various animal models have shown that long-term overexposure to GC has adverse effects on hippocampal morphology. Additionally, since both the under and over expression of GC receptors result in depression-like symptoms, it is evident that GC play an important role in the development of MDD. Therefore, it is discernible that GC are responsible for the hippocampal atrophy associated with MDD, although further research with human participants is required in order to solidify this conclusion.

INTRODUCTION

A depressive episode consists of depressed mood, loss of interest and enjoyment in usual activities, and reduced energy among other factors. Such an episode can be a part of major depressive disorder (MDD), which is defined by symptoms such as suicidal tendencies, diminished concentration, fatigue, and weight change for at least two weeks (Belmaker and Agam 2008). A CDC survey in 2006 and 2008 found that an estimated 9.0% of the U.S. population met the current depression criteria while an estimated 3.4% met the criteria for MDD (Centers for Disease Control and Prevention 2010). This disease is not only common but can also have a severe negative impact on daily activities and overall life expectancy. The traditional neurobiological understanding of MDD stems from the monoamine hypothesis (Czéh and Lucassen 2007). This hypothesis states that the pathophysiological basis of depression is the imbalance or depletion of neurotransmitters such as serotonin, norepinephrine, and dopamine. The dominant treatment options for depression at present, namely selective serotonin reuptake inhibitors (SSRIs), are also based on this monoamine etiology of the disease (Delgado 2000). Contemporary theories of the neurobiological basis of depression, however, focus on changes in cellular structure, function, and plasticity (Czéh and Lucassen 2007).

The most prominent and repeated neurological marker of MDD is volume loss in the hippocampus (Czéh and Lucassen 2007). The hippocampus is a temporal lobe structure, which plays an important role in learning and memory. Therefore, hippocampal volume loss often results in cognitive deficits. In addition to such functional consequences, hippocampal atrophy is also one of the primary markers of Alzheimer's disease and schizophrenia. Furthermore, it may be the cause of reoccurring seizures in temporal lobe epilepsy (Anand and Dhikav 2012). Therefore, understanding hippocampal atrophy can improve our understanding of and approach to many other neurological disorders.

Although, it is uncertain why the hippocampus is so vulnerable to such volume loss, various mechanisms have been implicated in causing hippocampal atrophy. For example, recent studies now associate MDD with a maladaptive response to stress, which is related to impaired functioning of the hypothalamic-pituitary-adrenal axis (HPA axis). This dysfunction results in hormonal imbalances, specifically elevated levels of glucocorticoids like cortisol. Glucocorticoids (GC) are a type of steroid hormones released by the adrenal glands and are involved in the metabolism of glucose. Cortisol is a stress-related GC, which is often used to quantify a stress response (Sapolsky 2001; Greabu et al. 2006). While it is unclear if the hormonal imbalance precedes the development of MDD or vice versa, elevated levels of GC in depression have devastating effects on the hippocampus, facilitating the progression of the disease (Magariños et al. 1999; Villanueva 2013).

The hippocampus is highly influenced by stress and is susceptible to atrophy under various neurological conditions (Dhikav and Anand 2007). This is most likely due to the structure of the hippocampus, which consists of a variety of cells organized in a layered fashion. These cells comprise the highest concentration of GC receptors compared to other brain regions

(Spolsky 2001; Anand and Dhikav 2012). Moreover, research shows that Cushing's syndrome (CS), a condition that consists of chronically elevated blood cortisol levels, also results in hippocampal atrophy, which has been linked to the loss of short-term memory (Magariños et al. 1999). Additionally, there have been various reports of chronic stress resulting in altered hippocampal connectivity including decreased synaptic density in the dentate gyrus (Anand and Dhikav 2012).

However, while there is convincing evidence in favor of GC being responsible for hippocampal volume loss, it is still a controversial topic. This is especially true because the only human study conducted in this area, did not reveal any major morphological changes or signs of neuronal cell death in the hippocampus of subjects with either MDD or with long-term GC treatment (Muller et al. 2001). Due to the overwhelming evidence on the contrary, however, this review will demonstrate that glucocorticoids are responsible for the hippocampal atrophy associated with depression.

In this review, the relationship between hippocampal atrophy and depression will be established, followed by the mechanism of GC-related damage in neurons. Next, the effects of GC exposure will be described in order to illustrate that GC has adverse effects on the hippocampus. This will be followed by the introduction of the GC receptor hypothesis. Next, the validity of rodent models for humans will be evaluated before concluding that GC is the most probable cause of hippocampal atrophy in depression even though further human research is necessary for a definitive answer.

HIPPOCAMPAL ATROPHY IN MDD

The loss of hippocampal volume has been associated with MDD for quite some time now. This idea became well established in the late 20th century when the Sheline et al. study used magnetic resonance imaging to compare the hippocampal grey matter volume of patients with a history of MDD (but no current depressive symptoms) to their age, education level, and height matched controls (Sheline et al. 1996). The subject pool only consisted of women in order to minimize gender differences in brain size and since the patients were not experiencing depression at the time of the study, the effects seen were most likely a result of chronic as opposed to acute GC exposure. The overall brain volume, defined by all brain tissue including gray and white matter of the cerebral hemispheres, was also measured. Both left and right hippocampal volumes were significantly smaller in the group with a history of MDD compared to the control group (fig. 1). However, there was no difference in the overall brain volume of the two groups. Furthermore, this study found that the amount of time with MDD was inversely correlated with hippocampal volume (fig. 2). Altogether, this study established the association between MDD and hippocampal volume loss (Sheline et al. 1996).

Since then, hippocampal atrophy in association with depression has been a popular field of study, and various studies have continued to solidify the findings of Sheline et al. (1996). For example, one study consisted of both male and female subjects experiencing either a first or recurrent episode of MDD also showed a loss in hippocampal volume compared to their age and gender matched control (Stratmann et al. 2014). Another study found a significant 19% decrease in hippocampal volume of MDD patients in remission compared to healthy case-matched controls. Again, no difference was found between other brain areas or the overall brain size (Bremner et al. 2000).

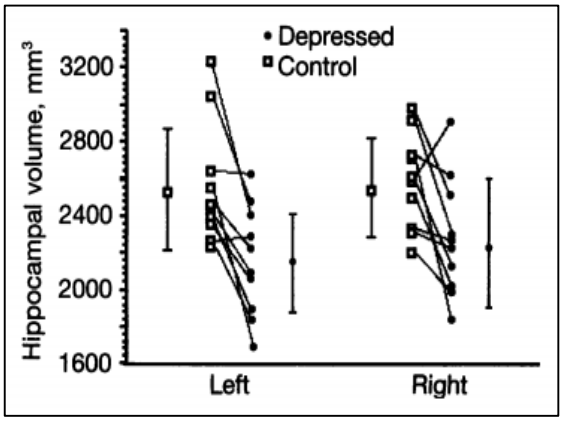

Figure 1: Left and right hippocampal volumes are lower for the depressed subjects (dots) than their matched controls (squares) (Sheline et al. 1996).

Despite the overwhelming amount of evidence demonstrating the link between hippocampal atrophy and depression, it is still uncertain whether the low volume is a facilitator or a consequence of MDD. However, there is little evidence for the former (Sapolsky 2001). Additionally, a recent finding suggests that a smaller hippocampal volume is a biological marker of depression as opposed to a vulnerability marker for it (Chan et al. 2016).

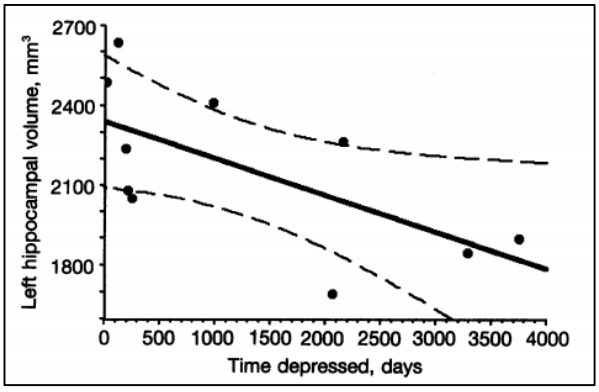

Figure 2: Left hippocampal grey matter volume is correlated with number of days spent with MDD (Sheline et al. 1996).

Consequently, the neurobiological basis of this hippocampal atrophy is highly related to stress since MDD is a stress-related disorder (Sapolsky 2001). The first possible mechanism of hippocampal volume loss is through the retraction of dendritic process since the administration of exogenous corticosterone in rats resulted in a decrease in apical dendrite length and number of branch points in CA3 pyramidal neurons (Woolley et al. 1990). A second mechanism of atrophy could be the inhibition of neurogenesis in MDD due to stress as concluded by a study performed on adult tree shrew (Gould et al. 1997). Finally, a third reason for the volume loss could be neurotoxicity or endangerment of neurons that are chronically exposed to GC. In other words, long-term exposure to GC makes the neurons vulnerable and less likely to survive trauma that would otherwise not cause much damage. This neuron loss could also account for hippocampal shrinkage in MDD, suggesting that multiple mechanisms collectively influence atrophy (Sapolsky 1999). Therefore, MDD is a stress-related disorder and hippocampal volume loss is its neurobiological marker, which probably arises as a result of excess GC exposure.

GLUCOCORTICOIDS ARE RESPONSIBLE FOR THE HIPPOCAMPAL ATROPHY IN MDD

GC mechanism of action

GC mechanism for hippocampal atrophy consists of two different aspects. The first aspect involves GC being directly toxic to neurons through the accumulation of cytosolic calcium while the other aspect includes GC increasing hippocampal vulnerability towards different types of trauma. As a direct effect, GC increases free cytosolic calcium concentration in hippocampal neurons by increasing the mobilization of calcium into the cell and decreasing its ability to leave the cell. GC does this by preventing the transcription of the calcium-ATPase pump whose primary function is to remove excess cytosolic calcium (Bhargava et al. 2000; Lee et al. 2002). The increase in cytosolic calcium leads to the over activation of calcium-based enzymes, which results in adverse effects such as protein misfolding and cytoskeletal degradation. These effects cumulatively lead to neuronal death (Lee et al. 2002).

The second and more indirect GC mechanism for hippocampal atrophy is based on the overexposure to glutamate. In the presence of trauma such as ischemia, seizure, hypoglycemia, etc., GC exposure increases the accumulation of extracellular glutamate. At basal levels, glutamate accumulated in the synapse binds to the NMDA receptors, allowing the mobilization of calcium to facilitate learning and memory processes. However, when excess glutamate is accumulated in the synapse, like with GC exposure during trauma, once again excess calcium is mobilized into the cell resulting in neuronal death (Lee et al. 2002).

GC overexposure and hippocampal atrophy

There is overwhelming evidence suggesting that there is hippocampal atrophy in MDD, and GC exposure appears to be the most probable cause (Sheline et al. 1996). For example, people who suffer from Cushing's syndrome experience elevated levels of endogenous GC for various reasons for extended periods of time. Furthermore, there is an inverse relationship between cortisol levels and hippocampal volume in CS patients (Starkman et al. 1992). This information about hippocampal morphology in CS can facilitate an understanding of GC-related atrophy in MDD as well. However, most human data in this field is correlational and therefore, causation cannot be established. On the other, animal models have proven useful in doing determining a causal link.

While the exact mechanism that causes hippocampal volume loss is still unclear, many studies have found evidence of alterations in the hippocampal morphology that may contribute to the overall volume loss. For example, since GC are some of the most powerful inhibitors of neurogenesis, the decrease in neurogenesis in the

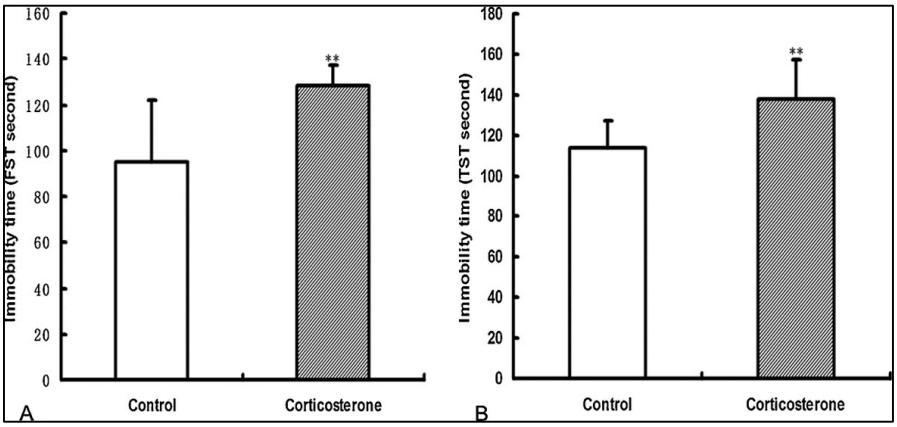

Figure 3: Immobility time was higher for the corticosterone group (CORT) compared to the control in the forced swim task (FST) and the tail suspension task (TST) (Zhang et al. 2015).

dentate gyrus of different species has been shown to play a role in hippocampal shrinkage (Gould et al. 1997; Gould et al. 1998; Pham et al. 2003). Additionally, numerous rodent studies have found that long-term exposure to GC can result in dendritic retraction in CA3 as well as CA1 pyramidal neurons (Woolley et al. 1990; Magarinos et al. 1996; Chez and Lucassen 2007). Finally, long-term exposure to GC can lead to neurotoxicity, which may contribute to hippocampal shrinkage by making neurons vulnerable and less likely to survive insults that would otherwise not be as detrimental (Sapolsky 1999).

While most studies have focused on changes in neuronal morphology in order to understand GC-related hippocampal volume loss, a recent rodent study focuses on the effect of GC on astrocytes. This is a result of type II GC receptors expressed here, which make astrocytes a major target for GC-related control (Vielkind et al. 1990).

Furthermore, astrocytes are not only the most abundant cells in the central nervous system but they also protect, nourish, and repair neurons. Thus, astrocyte activity is greatly linked with neuron activity. In this rodent study, subcutaneous corticosterone, a stress-related GC in mice, was administered to the CORT group daily for four weeks while the control group received a placebo injection. At the end of four weeks, the mice participated in forced swim tasks and tail suspension tasks. The CORT group was immobile for a longer time in both tasks compared to the control group. These results suggest that long-term GC exposure resulted in depressed-like symptoms in the rodents (fig. 3). Afterwards, the animals were sacrificed and immunohistochemistry and stereological techniques were used to assay the number, somal volume, and protrusion length of hippocampal astrocytes (marked by the presence of glial fibrillary acidic protein or GFAP) along with the overall hippocampal volume. The study found that the number of GFAP-positive astrocytes, somal volume, protrusion length, and overall hippocampal volume were lower in the CORT group compared to the control group (fig. 4). Furthermore, the hippocampal volume was correlated with each of the other three astrocyte measures (fig. 4) (Zhang et al. 2015). This study not only demonstrates the adverse effect of long-term GC exposure on hippocampal volume, but also sheds light on one of the ways in which this process occurs, since a decrease in hippocampal astrocyte plasticity appears to play a role in the mechanism of GC-related hippocampal atrophy. Therefore, various mechanisms such as dendritic retraction in pyramidal neurons, reduction in neurogenesis in the dentate gyrus, neurotoxicity and changes in astrocyte plasticity can all contribute to the hippocampal shrinkage due to an overexposure to GC.

GC underexposure and hippocampal atrophy

Since overexposure to GC causes hippocampal volume loss in animal models, it was first hypothesized that underexposure to GC would not be associated with atrophy. However, the hypothesized that underexposure to GC would not be associated with atrophy. However, the opposite was found to be true by a rodent study that compared the number of cells in hippocampal regions of adrenalectomized adult rats with the control group (Sapolsky et al.1991).

This was done by performing surgery on 10 mature rats to resect their adrenal glands (ADX) while 12 other rats underwent a sham surgery (control). Three months later, all animals were sacrificed in order to count the number of cells in the hippocampal region.

While this experiment consisted of two groups of rats, the results illustrate a comparison between three separate groups. During the experiment, a subset of the ADX rats exhibited attenuated weight gain (AW). This is suggestive of complete removal of both adrenal glands as well as the surrounding adrenal tissue, thus there was no exposure to GC.

On the other hand, the rest of the ADX rats had normal weight gain (NW), which suggests that even after the surgery some adrenal tissue was left behind. This finding signified that the rats still had minimal exposure to GC. The AW group had significantly lower number of cells in the hippocampus, especially the dentate gyrus, compared to the NW and control groups (fig.5). However, there was no difference in cell numbers of the NW and control groups (Sapolsky et al. 1991).

This study shows that similar to overexposure, underexposure to GC can also have adverse effects on neurons as seen by the AW group. However, the NW group was no different from the control, suggesting that a minimal exposure to GC prevented loss of neurons. This data presents an inverted U-function of GC exposure since either extreme of GC exposure is detrimental to hippocampal cells. However, some level of GC is needed for neuronal survival (Sapolsky et al. 1991).

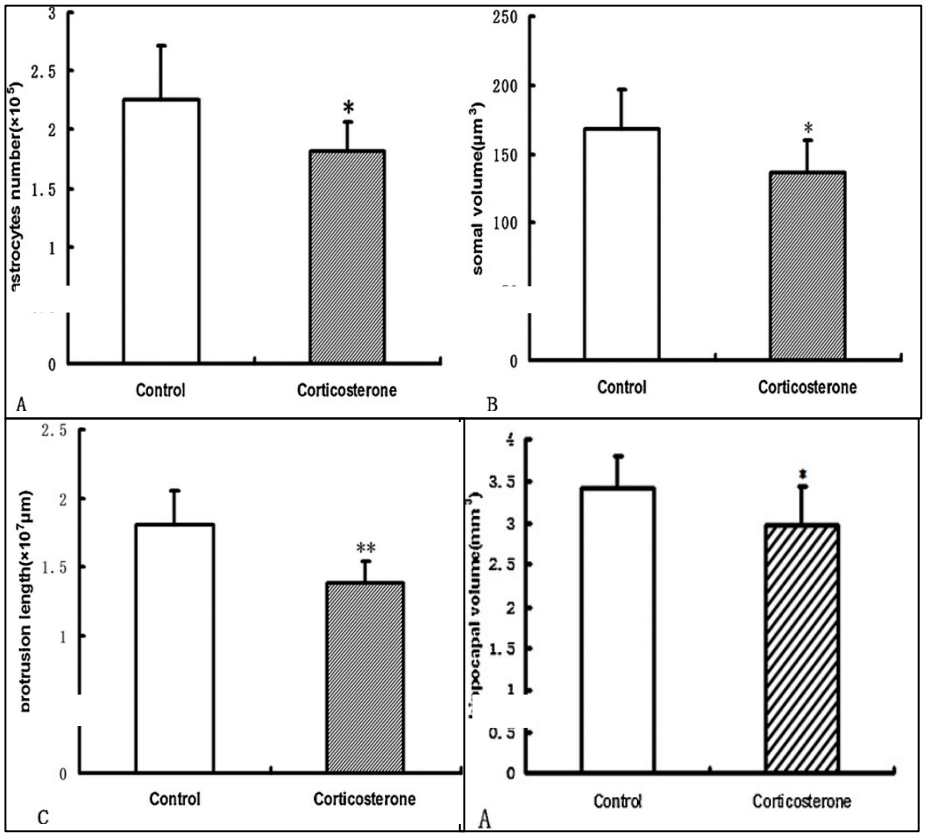

Figure 4: Coticosterone significant decreased the (top left) number of GFAP-positive astrocytes, (top right) somal volume, (bottom left) protrusion length of these astrocytes and (bottom right) the overall hippocampal volume compared to the control group (Zhang et al. 2015).

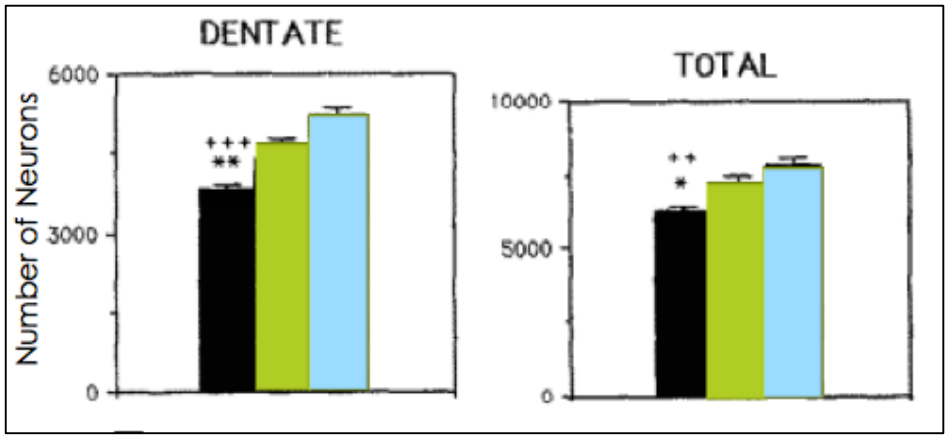

Figure 5: Black-AW, Green-NW, Blue-Control. Number of neurons were significantly lower in ADX rats with attenuated weight gain (AW) compared to NW and control in the dentate gyrus as well as the over all hippocampal tissue (Sapolsky et al. 1991).

The GC receptor hypothesis of stress and atrophy

As mentioned above, MDD is a stress-related disorder due to the dysfunction of the HPA axis, which results in an imbalance in the level of stress hormones called GCs. GCs act by binding to steroid hormone receptors of two kind: type I and type II. These receptors have different affinities for GCs and consequently play opposing roles in hippocampal plasticity. Type I receptors have a high affinity for GCs, meaning that the receptors are fully occupied at a basal concentration of circulating GC. Type II receptors, on the other hand, have a low affinity for GCs and only become fully occupied at an elevated circulating level of GCs, like during a stress response or in Cushing's syndrome. These receptors are responsible for producing a biphasic effect in response to GC levels, illustrated by an inverted U-function. For example, administering a type I agonist and type II agonist yield opposing effects on long-term potentiation in CA1 pyramidal neurons. While type I agonist increased long-term potentiation (LTP), type II agonist diminished it (Pavlides et al. 1995; Kim et al. 2015). Therefore, the opposing roles of the type I and type II receptors help modulate LTP. So, while high levels of GC have adverse effects on hippocampal neurons, basal levels of GC are necessary to maintain basic neuronal functions such as LTP. Furthermore, some studies claim that the difference in ratio of type I to type II receptors facilitated the HPA dysfunction, resulting in the development of MDD (Medina et al. 2013). For example, the disruption of type II receptors in the rat hippocampus and other forebrain structures in

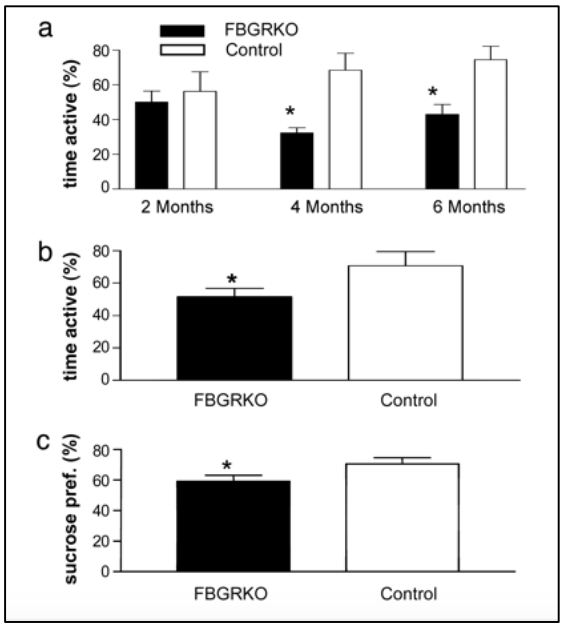

Figure 6: FBGRKO mice show increase in depression-like behaviors over time. (*a*) FBGRKO mice show significantly less activity in the forced swim test at 4 and 6 months of age (*b*) FBGRKO mice show significantly less activity in the in the tail suspension test at 6 months. (*c*) FBGRKO mice show significantly decreased sucrose preference (Boyle et al. 2005).

the glucocorticoid receptor knockout model (FBGRKO) resulted in an increase in MDD-like behavior. This was determined by recording the performance of the FBGRKO rats in forced swim and tail suspension tasks. Additionally, their sucrose intake was measured. The FBGRKO rats showed

decreased activity and sucrose intake compared to the control rats (fig. 6), suggesting depressive behavior (Boyle et al. 2005). At the same time, the overexpression of the type II receptor in the forebrain of mice also resulted in an increase in stress- and anxiety-like behavior (Wei et al. 2004). Therefore, it is evident that a change in the expression of GC receptors in either direction yields a depressive phenotype. Once again, the inverted U-function of stress, suggesting a biphasic effect, becomes apparent where either extreme of GC exposure has adverse effects on neuronal plasticity and facilitates the development of depression-related behavior.

VALIDITY OF THE GLUCOCORTICOID THEORY FOR HUMANS

Human post-mortem study

Most of the studies in this field have been conducted on animal models as opposed to human subjects. The studies that do involve human subjects have mainly been correlational. The first and one of the only human studies of relevance to this review assessed hippocampal morphology in the post-mortem human brain. This study consisted of three groups. The first group consisted of MDD patients, the second consisted of people who had been treated with GC long-term, and the third was the control group. The brains from all the subjects were dissected and a specimen from the hippocampal formation was used to conduct the study. This study claims to have found no evidence of any major morphological changes in the hippocampus. However, they did find increased GFAP immunoreactivity in the CA1 and CA3 regions, which is often accompanied by neuronal loss. Furthermore, this study did not assess overall hippocampal volume in the post-mortem brain of MDD patients. Thus, it is difficult to draw a direct conclusion about GC exposure and hippocampal volume loss in humans (Muller et al. 2001).

Therefore, even if the findings of this human study are credible, they simply suggest that mass-neuron loss is not the likely mechanism for GC-related hippocampal atrophy in MDD. However, other mechanisms such as dendritic retraction, inhibition of neurogenesis, and changes in astrocyte plasticity due to GC exposure may still play an important role in facilitating hippocampal atrophy associated with MDD.

SIMIARITIES BETWEEN THE RODENT AND PRIMATE HIPPOCAMPI

There is a scarcity of human data available in investigating the effect of GC on hippocampal shrinkage. Although numerous human correlational studies show the association between GC and hippocampal atrophy, like the memory-related cognitive deficits and changes in hippocampal morphology in Cushing's disease, causation has not been established in humans (Patil et al. 2007). Furthermore, while some studies have documented hippocampal atrophy due to GC exposure in non-human primates such as tree shrews, a large portion of the literature in this field is based on rodent models (Gould et al. 1997). For this reason, it is important to note the similarities between the primate and the rodent hippocampi, which make it possible to draw conclusions about the effect of GC on hippocampal atrophy in MDD based on casual relationships established by rodent models.

The rodent hippocampus has a high concentration of GC receptors, which makes their hippocampus sensitive and vulnerable to GC exposure. Similarly, the human hippocampus must also be highly influenced by GC since the highest concentration of GC receptors in the human brain exists in the hippocampus (Lee et al. 2002). Furthermore, non-human mammalian models have been used to understand the neuroanatomy of long-term declarative memory, which also involves the hippocampus (Clark and Squire 2013). Therefore, a rodent model of the hippocampus has been previously used to further the understanding of the primate and human hippocampi and its functions as cited throughout this article. Additionally, just like in the rodent hippocampus, there is evidence that excess GC exposure can have adverse effects in the human hippocampus. This effect has been observed in correlational studies conducted in patients who were either suffering from Cushing's syndrome, resulting in hypercotisolism, or patients who were administered long-term GC treatment. Both groups show selective loss of consolidation and retrieval of declarative memories, which suggests the presence of neuronal damage in these conditions in addition to the hippocampal shrinkage also associated with these conditions. Additionally, non-human primate studies have also documented neuronal damage in the hippocampus after experiencing long-term social stress or being exposed to GC (Uno et al. 1989; Sapolsky et al. 1990). Therefore, since the rodent hippocampus is similar to primates and has been used before as an animal model for the human hippocampus, the overwhelming body of evidence linking GC and hippocampal atrophy should be used to draw conclusions about the human hippocampal shrinkage in MDD.

CONCLUSION

MDD is associated with a maladaptive response to stress, which suggests that the

dysfunction of the HPA axis is involved. The dysfunction of the HPA axis results in elevated level of GC. GC act by binding to GC receptors, which are highly concentrated in the hippocampus (Sapolsky 2001). However, rodent studies show that an overexposure to GC, like in MDD, can not only facilitate depressive symptoms, but also affect hippocampal morphology, since GC easily influences this structure due to the abundance of GC receptors (Sheline et al. 1996). In vitro studies show that an overexposure to GC results in excess calcium mobilization into the cells and inhibits escape. The accumulation of cytosolic calcium leads to protein misfolding and cytoskeletal degradation, which ultimately has adverse effects on the cells and can even result in neuron death (Lee et al. 2002). These adverse effects most likely contribute to changes in hippocampal morphology through dendritic retraction, decrease in neurogenesis, and changes in astrocyte plasticity. These changes in neuronal morphology due to the overexposure to GC are therefore a probable cause for hippocampal shrinkage in MDD.

Interestingly, the underexposure to GC also has adverse effects on the hippocampus as seen in ADX rodents (Sapolsky et al. 1991). This suggests that GC has a biphasic effect on neurons. However, unlike overexposure of GC, there is no evidence that underexposure of GC is linked with depressive symptoms. This may be due to the opposing roles played by the type I and type II GC receptors, which help to modulate neuronal functions like LTP (Pavlides et al. 1995). Independent of the biphasic effect of GC, the link between overexposure and hippocampal atrophy is made clear with various rodent studies in addition to correlational human studies. While it is controversial to base the GC theory of hippocampal atrophy in MDD on the causation established in rodent studies, there are various similarities between the rodent and primate hippocampi that make it valid to draw conclusions about human processes from these animal models (Lee et al. 2002).

While the rodent studies demonstrate the validity of the GC theory, extensive human research will be required in order to confirm it. It is difficult and unethical to conduct experiments on humans in this case since it would require the purposeful induction of long-term stress. The studies conducted on patients with Cushing's and Addison's disease, however, may reveal critical information on the issue. Patients with Cushing's disease show over five-time increase in being diagnosed with MDD (Feelders et al. 2012). This provides an excellent model for studying the effect of excessive GC exposure in MDD. On the other hand, patients suffering from Addison's disease have adrenal insufficiency and would therefore sever as a human model to study the effect of GC underexposure. While the current status of the field illustrates that overexposure to GC appears to be the most probable cause of hippocampal volume loss in MDD, the models mentioned above can be tested in an attempt to further solidify the GC theory.

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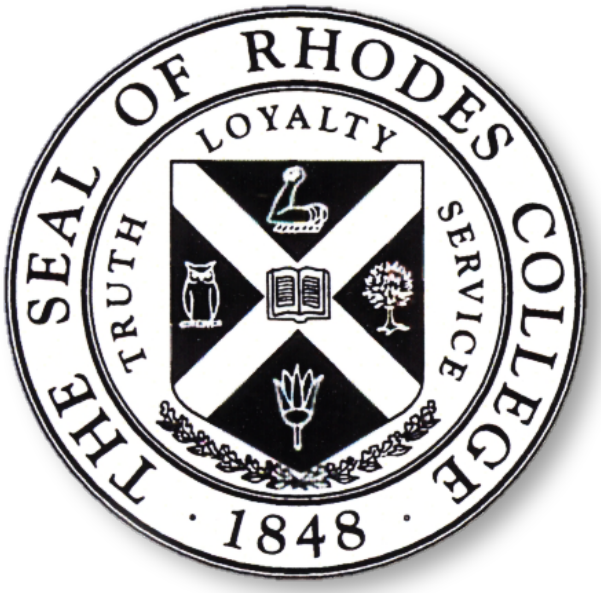

Rhodes College

—1848—

Department of Biology
2000 North Parkway
Memphis, TN 38112
www.rhodes.edu